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*Full Length Research Paper*

# Modeling site-specific fertilizer recommendations for maize production in the Sudan savannah agro-ecology of Ghana

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**Maize (*Zea mays* L.) production in the Sudan savannah agro-ecological zone of Ghana is hindered by erratic rainfall and low soil fertility. This study was conducted to refine profitable fertilizer recommendations for maize production on a selected benchmark soil in Sudan savannah agro-ecological zone using Decision Support System for Agro-technology transfer (DSSAT). Maize variety *Obaatanpa* was used for the experiment and the rates of N P K nutrients evaluated were 0-0-0, 0-90-90, 40-90-90, 80-90-90, 120-0-90, 120-45-90, 120-90-90, 120-90-0, 120-90-45 and 160-90-90 kg ha$^{-1}$. Predictive ability of the model was tested and validated and the simulation of maize yields with nutrient rates. Results showed that treatment 160-90-90 was efficient for maize production in 2010 growing season and 120-0-90 was efficient for maize production in 2011 growing season. However, the use of treatment 120-90-90 was more preferred and sustainable option for maize production due to the available types and combinations of fertilizer in the Ghanaian market, affordability and prevalence of P-deficient soils. Model sensitiveness to N fertilizer rates should be reworked in order to make model predictions for treatments with low N rates more accurate.**

**Key words:** maize production, agro-ecological and fertilizer.

## INTRODUCTION

Maize is the third most cultivated field crop after wheat and rice in the world as well as in most parts of West Africa (Fosu et al., 2004). Maize is the most popular crop due to its high yield and easy to process at low cost compared to other cereal crops (Jaliya et al., 2008). In Ghana, maize is the major staple crop especially in the northern part where it is currently replacing sorghum and millet which were the major staple crops some years ago.

The major maize growing areas in Ghana are forest, savannah and transition agro-ecological zones. Ghana produces about 1,100,000 metric tons of maize annually over an area of 755,300 ha (SRID, MoFA, 2007). The average yield is 1.5 t ha$^{-1}$ compared to an immense potential yield of up to 7.5 t ha$^{-1}$ in the tropics if the crop is properly managed. The most limiting factors for maize production in these areas, especially the savannah agro-

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ecological zone are the erratic rainfall pattern and low soil fertility. The major causes of the low soil fertility are low application of external inputs, poor soil fertility management practices, continuous cropping on the same piece of land for a very long time and poor nature of soils. Average fertilizer application in Ghana is approximately 8 kg $ha^{-1}$ (FAO, 2005). FAO estimates show negative nutrient balance for all crops in Ghana despite that the escalating rate of soil nutrient mining is a serious threat to sustainability of agriculture. Improving soil productivity therefore is the key to reversing the negative trends in maize production in potential areas of Ghana.

The introduction of high yielding varieties alone has not solved the problem of low yields and sustainable increase in maize production. The use of old or blanket fertilizer recommendations in the Sudan savannah agro-ecological zone is not useful recently. The combination of these has not attained the maximum yield potentials. In spite of all these efforts, Ghana still needs to increase maize productivity while conserving the natural resource base and preventing further degradation that has characterized most soils in the country. Use of inorganic fertilizers is the core strategy to overcome soil fertility depletion through nutrient mining and soil degradation. Smallholder and resource poor farmers in Ghana appreciate the use of inorganic fertilizers but the problem associated with the use of these soil amendments is the inconsistency in the quantity to be applied.

There is also inadequate knowledge and inherent complexities about how the weather, soil and crop interact to affect crop production, which prompts many researchers to make use of models. Models also help in matching biological requirement of crops for achieving specified objectives faster than the traditional method which requires substantial time. The Decision Support Systems for Agro-technology Transfer (DSSAT) model has been used in most researches and it is able to approximate weather, soil and crop dynamics for a narrow range of factors that influence them under limited conditions (Hoogenboom et al., 2004, 2009). Therefore, there is a need to determine the most limiting nutrient requirement and develop new fertilizer recommendations for increased and sustainable production of maize in the Sudan savannah agro-ecological zone of Ghana. This study was to refine profitable fertilizer recommendations for maize on a selected benchmark soil of the Sudan savannah agro-ecological zone of Ghana. Another objective was to test and validate the CSM-CERES-Maize model in DSSAT.

## MATERIALS AND METHODS

### Description of the study area

This study was conducted in the Sudan savannah agro-ecological zone of the extreme north-east corner of Ghana. The area lies between latitude 10° 30' 11" and longitude 0° 1' 30" and covers an area of 1765 $km^2$ along Ghana-Burkina Faso border. It has an altitude of 200 to 400 m above sea level (Adu, 1969; Nyarko et al., 2008). The area experiences a unimodal rainfall season which extends from April to October, with the heaviest rainfall mainly occurring between June and October and the mean annual rainfall is 1365 mm usually recorded in August (Nyarko et al., 2008). The mean monthly minimum temperature ranges from 18.9 to 25.7°C and the mean monthly maximum temperature ranges from 32.4 to 38.6°C while the mean annual minimum and maximum temperatures are 22.3 and 34.3°C, respectively (Adu, 1969). The mean annual relative humidity for a day is about 40 to 50% (Adu, 1969) and the dominant soil in the study area is *Tanchera* series (Ferric Lixisol) (FAO, 2006).

### Field experiment and simulation study

A field experiment was conducted for 2 years during the rainy seasons of 2010 and 2011. A randomized complete block design with 4 replications and a plot size of 6.0 x 4.8 m was used. The treatments used in the experiments were 0-0-0, 0-90-90, 40-90-90, 80-90-90, 120-0-90, 120-45-90, 120-90-90, 120-90-0, 120-90-45 and 160-90-90 kg $ha^{-1}$. The maize variety used was *Obaatanpa* and was planted at a spacing of 80 x 40 cm.

In simulation studies, the treatments were developed to cover a range of management input files. These input files included experimental file which was created by inputting name and geographical position of the field, planting date, fertilizer application dates, five levels of N (0, 40, 80, 120, 160), three levels of P (0, 45, 90) and three levels of K (0, 45, 90). The source of N was urea, P was from triple super phosphate and K was obtained from muriate of potash. The soil file included the analytical characteristics of the soil of the study field such as particle size, pH, nitrate, ammonium, total N, available phosphorus, exchangeable potassium, organic carbon, bulk density, and volumetric moisture content. The weather file also consisted of precipitation, minimum and maximum temperatures and solar radiation of the study field from 1960 to 2050. Field results were used to calibrate the genetic co-efficient of maize and these model inputs were integrated to provide a framework for simulating and analyzing outputs.

## RESULTS

### Model calibration and validation

Evaluation of the DSSAT-CERES model involved comparing model outputs with real data and determination of suitability for an intended purpose of making site-specific fertilizer recommendations. The results of the thermal time from seedling emergence to the end of juvenile phase (P1 in degree days), photoperiod sensitivity coefficient (P2 in days), and thermal time from silking to physiological maturity (P5 in degree days) were collected. Other results were maximum kernel number per plant (G2), potential grain filling rate (G3 in mg $d^{-1}$) and thermal time between successive leaf tip appearances (PHINT in degree days). These results were all reported in two different years to be 380, 0.1, 750, 532, 8.0, 38.9 for 2010 growing season and 300, 0.1, 700, 693, 7.8, 56.8 for 2011 growing season, respectively. The CSM-CERES model was validated by comparing the observed field data with the simulated data for 2010 and 2011 growing seasons (Table 1). Results indicated that the normalized root

**Table 1.** Comparison between observed and simulated maize yield parameters (kg/ha) in two years (2010 and 2011) at Navrongo, Ghana.

| Variables | 2010 | | | 2011 | | |
|---|---|---|---|---|---|---|
| | Grain | Stover | Total biomass | Grain | Stover | Total biomass |
| [a]Obs | 1940 | 7635 | 10487 | 2385 | 5250 | 7638 |
| [b]Sim | 2280 | 9075 | 11268 | 2622 | 6351 | 8926 |
| [c]MD | 340* | 1440** | 781** | 236NS | 1101** | 1289** |
| [d]RMSE | 507.02 | 1622.41 | 855.17 | 435.09 | 1471.41 | 1696.05 |
| [e]R-Sqaure | 0.92 | 0.89 | 0.99 | 0.89 | 0.75 | 0.85 |
| [f]NRMSE (%) | 26.13 | 21.25 | 08.15 | 18.24 | 28.03 | 22.21 |

[a]Observed; [b]Simulated; [c]Mean Difference; [d]Root Mean Square Error; [e]Root Square; [f]Normalised Root Mean Square Error; NS= Not significant; *= Significant and **= Highly significant.

mean square error (NRMSE) between the observed and the simulated grain yield results for 2010 and 2011 were 26.13 and 18.24%, respectively.

### Maize performance

The mean difference between the observed and simulated grain yield was significant for 2010 and not significant for 2011 results based on t-test for paired sample analysis. The comparison between the observed and simulated data showed that the $R^2$ values were 0.99% for 2010 growing season and 0.89% for 2011 growing season. The $R^2$ value between the observed and the simulated result was 0.92% for 2010 season and 0.89% for 2011 season. The model showed a good simulation performance for both 2010 and 2011 growing season with $R^2$ values of 0.89 and 0.75% between the observed and simulated results, respectively. The NRSME between the observed and simulated were 08.15 and 22.21% for 2010 and 2011, respectively.

### Seasonal and biophysical analysis

The yields at maturity for the treatments for 2010 and 2011 growing seasons were used to run 90 years seasonal analysis. The biophysical analysis determined the minimum and maximum yields for the treatments during the 90 years. Treatment 160-90-90 gave the best yield among the treatments but was not significantly different from treatment 120-90-0, 120-0-90, 120-90-45, 120-90-90 and 120-45-90 during the 90 years seasonal analysis using 2010 growing season grain yield results. The minimum yield of 25% was above 2200 kg $ha^{-1}$ which was above 75% yield of the rest of the treatments and the maximum yield of above 3800 kg $ha^{-1}$. Treatment 0-90-90 had the least yield with a minimum of 640 kg $ha^{-1}$ and maximum yield of 1400 kg $ha^{-1}$. This showed the level of significance of N in the development and growth of maize. Cumulative probability of grain yield for using treatment 160-90-90 was high within 25 to 75% level (about 2550 to 3350 kg $ha^{-1}$) compared to the rest of the treatments which were within 75 to 100% level. This was due to a wide range of yield obtained of 25 to 75 % level of treatment 160-90-90.

Treatment 120-90-90 together with treatments 120-0-90, 120-45-90 and 120-90-45 gave the highest yield but were not significantly different from treatment 160-90-90, 80-90-90 and 120-90-0 for the 90 years seasonal analysis using 2011 growing season grain yield result (Table 2). Treatment 120-0-90, 120-45-90, 120-90-90 had a minimum up to 25% yield of about 4600 kg $ha^{-1}$ which was similar to 50% yield obtained in treatments 120-90-0, 120-90-45, and 160-90-90 and higher than 100% yield obtained from the rest of the treatments. Cumulative probability for using treatment 120-90-90 was within 0 to 25% level (about 2000 to 3100 kg $ha^{-1}$ and was the same for treatments 120-0-90 and 120-45-90. This was as a result of a wide range of grain yield obtained by 0 to 25% level of these treatments. The cumulative probability of treatments 120-0-90, 120-45-90 and 120-90-90 were better than the cumulative probability of the rest of the treatments.

### Economic and strategic analysis

Mean-Gini dominance analysis was performed to evaluate the economic strategies of the treatments for 50 years behind and 40 years ahead. The results showed that treatments 160-90-90 and 120-0-90 were the best fertilizer recommendations for economic strategic production of maize in the Sudan savannah agro-ecological zone of Ghana in 2010 and 2011 growing seasons respectively (Table 3, Figures 1 and 2).

## DISCUSSION

The model simulation of grain yield had similar trend to the observed field results for the 2010 and 2011 growing season respectively. The model showed a good

**Table 2.** Observed field and simulated maize yield parameters (kg ha-1) for 2010 and 2011 growing season at Navrongo, Ghana.

| Treatment | 2010 | | | | | | 2011 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grain yield | | Stover weight | | Total biomass | | Grain yield | | Stover weight | | Total biomass | |
| | Obs | Sim | Obs | Sim | Obs | Sim | Obs | Sim | Obs | Sim | Obs | Sim |
| 0-0-0 | 245 | 1196 | 5231 | 5543 | 5625 | 6676 | 544 | 738 | 1636 | 2840 | 2180 | 3548 |
| 0-90-90 | 230 | 1180 | 5135 | 5498 | 5523 | 6614 | 972 | 738 | 2178 | 2840 | 3151 | 3548 |
| 40-90-90 | 1505 | 1559 | 5594 | 7362 | 8278 | 8840 | 2200 | 1771 | 4432 | 6049 | 6632 | 7771 |
| 80-90-90 | 1730 | 2025 | 7500 | 9041 | 10137 | 10974 | 2711 | 3149 | 5419 | 8176 | 8130 | 11268 |
| 120-0-90 | 2730 | 2736 | 8919 | 10340 | 12725 | 12985 | 3095 | 3370 | 5542 | 7615 | 8637 | 10933 |
| 120-45-90 | 2230 | 2612 | 8897 | 10461 | 12047 | 12976 | 2703 | 3370 | 5720 | 7615 | 8423 | 10933 |
| 120-90-90 | 2590 | 2612 | 8513 | 10461 | 12031 | 12976 | 3370 | 3370 | 7613 | 7615 | 10983 | 10933 |
| 120-90-0 | 2020 | 2746 | 9025 | 10380 | 11738 | 13032 | 2931 | 3053 | 6566 | 5890 | 9497 | 8902 |
| 120-90-45 | 2595 | 2612 | 8387 | 10461 | 12281 | 12976 | 2772 | 3370 | 6645 | 7615 | 9418 | 10933 |
| 160-90-90 | 3525 | 3525 | 9281 | 11203 | 14488 | 14632 | 2567 | 3290 | 6758 | 7254 | 9324 | 10494 |
| Critical value for comparison | 949.3 | 949.3 | 1208.4 | 1208.4 | 286.6 | 286.6 | 863.0 | 863.0 | 2139.0 | 2139.0 | 2438.1 | 2438.1 |

Obs= observed field results; Sim= simulated yield results.

**Table 3.** Period in years (1960-2050) of the Mean-Gini dominance of seasonal partial budget analysis for different rates of NPK fertilizer at Navrongo, Ghana.

| Treatment | 2010 | | | 2011 | | |
|---|---|---|---|---|---|---|
| | E(x) (€) | E(x)-F(x) (€) | Efficiency | E(x) (€) | E(x)-F(x) (€) | Efficiency |
| 0-0-0 | 15.2 | -1.3 | NO | -22.1 | -3.4 | NO |
| 0-90-90 | 14.7 | -1.4 | NO | -83.9 | -95.8 | NO |
| 40-90-90 | 27.2 | 8.6 | NO | 38.4 | 13.1 | NO |
| 80-90-90 | 69.8 | 45.9 | NO | 196.3 | 154.3 | NO |
| 120-0-90 | 146.6 | 111.4 | NO | 247.0 | 191.3 | YES |
| 120-45-90 | 137.0 | 103.8 | NO | 235.4 | 179.7 | NO |
| 120-90-90 | 137.0 | 103.7 | NO | 225.5 | 169.9 | NO |
| 120-90-0 | 154.3 | 116.7 | NO | 227.3 | 173.7 | NO |
| 120-90-45 | 137.5 | 104.3 | NO | 227.5 | 169.0 | NO |
| 160-90-90 | 236.6 | 190.7 | YES | 181.8 | 127.3 | NO |

E(x) = Mean monetary return per hectare and F(x) = Gini coefficient.

performance as the $R^2$ value was close to 100% (Wilmott et al., 1985; Wallach and Goffinet, 1987). This also showed that the model performance in simulating the yield at maturity was in acceptable range for 2010 season and good range for 2011 season (Jamieson et al., 1991; Loague and Green, 1991). However, the model was very sensitive to fertilizer rates as the simulation of yields for treatments with no or low fertilizer rates especially N was not good compared to treatments with high fertilizers. The model underestimated yields at low N levels in 2010 as compared to overestimation of yield values at low N levels in 2011. Model sensitiveness to low N fertilizer rates should be reworked in order to make model predictions for treatments without N or low N levels more accurate.

The mean difference between observed and simulated by-product weight showed a highly significant difference for both 2010 and 2011 growing seasons using t-test for paired sample analysis. According to Wilmott et al. (1985) and Wallach and Goffinet (1987), any $R^2$ value between observed and simulated result close to 100% shows a good model simulation performance. The NRSME values between the observed and simulated results of 21.25% for 2010 growing season and 28.03% for 2011 growing season were within the acceptable range according to Jamieson et al. (1991) and Loague and Green (1991).

The simulation of top weight at maturity in general by the model, showed a similar trend as the observed field results for the 2010 and 2011 growing season. The mean difference between the observed and simulated top weight at maturity for the 2010 and 2011 growing seasons were highly significant using t-test for paired sample analysis. The $R^2$ values were in accordance with the findings of Wilmott et al. (1985) and Wallach and

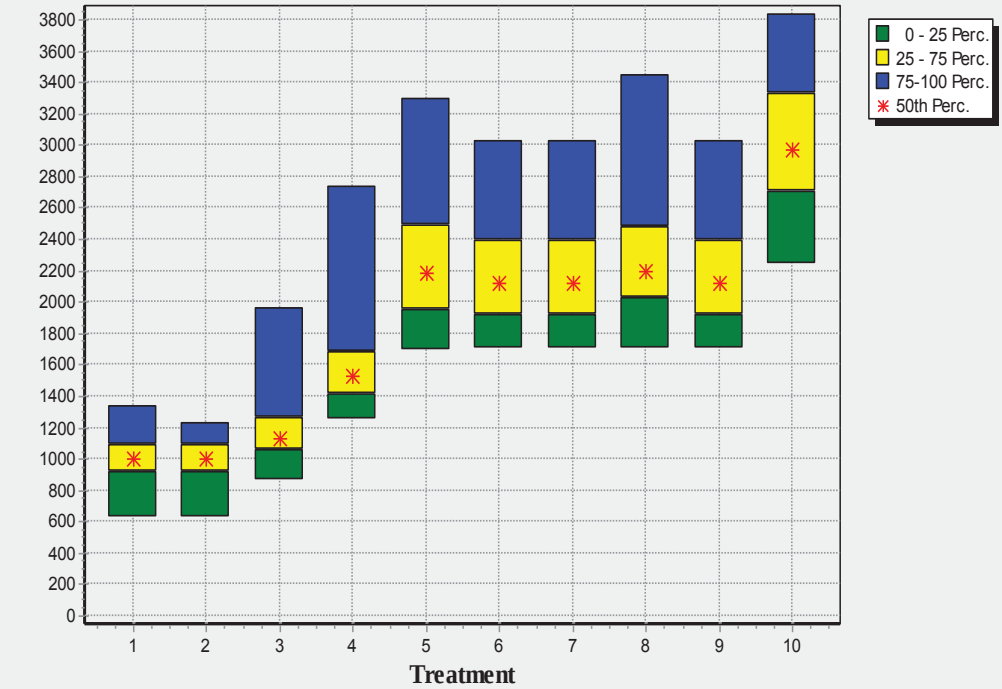


**Figure 1.** Maize yield as affected by different rates of NPK fertilizer for 90 years (1960-2050) seasonal and biophysical analysis using 2010 growing season grain yield result at Navrongo, Ghana.1 = 0-0-0; 2 = 0-90-90; 3 = 40-90-90; 4 = 80-90-90; 5 =120-0-90; 6 = 120-45-90; 7 = 120-90-90; 8 = 120-90-0; 9 = 120-90-45; 10 = 160-90-90 NPK kg/ha respectively.

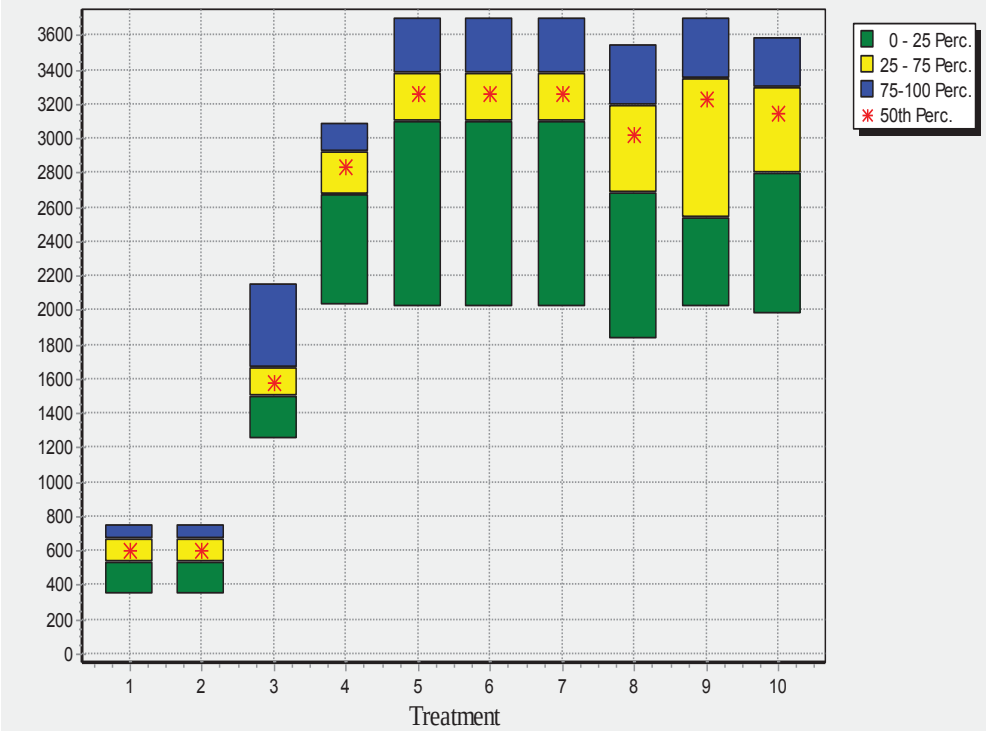


**Figure 2.** Maize yield as affected by different rates of NPK fertilizer for 90 years (1960-2050) seasonal and biophysical analysis using 2011 growing season grain yield result at Navrongo, Ghana. 1 = 0-0-0; 2 = 0-90-90; 3 = 40-90-90; 4 = 80-90-90; 5 =120-0-90; 6 = 120-45-90; 7 = 120-90-90; 8 = 120-90-0; 9 = 120-90-45; 10 = 160-90-90 NPK kg/ha respectively.

Goffinet (1987) that $R^2$ value between observed and simulated result close to 100% shows a good performance of the model. The values of NRSME showed an excellent model performance for 2010 season and acceptable model performance for 2011 season in simulating top weight in comparison with the observed top weight (Jamieson et al., 1991; Loague and Green, 1991).

The average performance of the Mean-Gini Dominance for the 2 seasons showed that treatment 160-90-90 had the highest return per hectare followed by treatment 120-0-90 and 120-45-90 respectively. This was due to the high monetary return obtained by these treatments from the difference between mean monetary return per hectare and Gini-coefficient. However, high cost of fertilizer, availability of fertilizer in the market and poor nature of soils make treatment 120-80-90 more economical for sustainable production of maize on Tanchera series (Ferric Lixisol, FAO, 2006) in the Sudan savannah agro-ecological zone of Ghana.

The model was helpful in making decision for refining fertilizer recommendation for the Sudan savannah agro-ecological zone. Dzotsi et al. (2003) and Soler et al. (2007) also depicted that CERES-Maize in DSSAT could successfully be used to predict the future crop yields under different management practices, and select the best one for sustainable production of maize and other crops.

## Conclusion

Maize grain yield was affected by different rates of fertilizers. The model predictions were generally very good and were in the same trend as the observed field results. This suggests that the model can be used as a tool for developing site specific fertilizer recommendations for improved maize and other crops production in similar agro-ecological zones of Ghana. The model could be rerun with many years of generated weather data to compare different N management strategies for improving fertilizer efficiency given the uncertainty of weather.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# *Stay-green* effects on adaptability and stability in wheat

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**The delayed leaf senescence (*stay-green*) has been frequently attributed to significant yield gains in different crops, especially under stress conditions. The goal of the study was to elucidate if the delayed leaf senescence of the wheat plant through the expression of the *stay-green* character brings effective contributions to adaptability and stability parameters for yield and weight of a thousand grains, aiming for genetic gains in the selection of more stable and productive genotypes. The experiment was conducted on the years 2003, 2004 and 2005 under field conditions. The experimental design was random blocks with three repetitions involving lines selected for high grain yield and distinct maturation groups (presence and absence of *stay-green*). The delayed leaf senescence in wheat had a strong contribution on the stability and increment in grain yield, especially in unfavorable years due to reduced precipitation. However, no changes were detected for the character weight of a thousand grains. Therefore, the introduction of the *stay-green* character into elite lines and cultivars represent a promising strategy for more stable and higher yielding wheat genotypes.**

**Key words:** *Triticum aestivum* L., delayed senescence, grain yield, weight of thousand grains.

## INTRODUCTION

Earth climate changes have set bigger challenges for plant breeders searching for superior genotypes (Araus et al., 2008). Beyond genetic gains for high yield and grain quality, maintaining yield stability by the reducing of losses by environmental stresses has received great attention (Oliveira et al., 2011). Among the strategies, the introduction of *stay-green* (delayed leaf senescence at late grain filling stage) character has shown promising results in several species. Strong evidences of the greater adaptability of *stay-green* genotypes under

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drought stress have been reported for *sorghum* (Kassahun et al., 2010), maize (Costa et al., 2008) and wheat (Izanloo et al., 2008). An evaluation of 936 wheat lines detected a significant association between tolerance to stresses by high temperatures and *stay-green*, indicating the delayed leaf senescence as a criterion for heat tolerance selection (Kumari et al., 2007). Plants with the character *stay-green* showed a lower reduction of the photosynthetic active area close to the physiological maturity (Ahlawat et al., 2008). Thus, delaying leaf senescence closer to physiological maturity stages may promote higher translocation and grain filling rates with direct reflex on yield components (Kumar et al., 2010).

Strong G x E interaction effects on grain yield has been reported for the wheat plant, making it difficult to select superior performance genotypes (Sanchez-Garcia et al., 2012). The analysis of adaptability and stability is essential for the partitioning of this interaction, allowing the identification of cultivars with predictable behavior and responsive to changes in environment (Franceschi et al., 2010). Among the various methods available for this determination, those based on regression of phenotypic values in relation to an environmental index have been the most widely used. Among those, is the model proposed by Eberhart and Russell (1966), commonly reported for cereals (Crestani et al., 2010). From regression models, the termed traditional method has also been used to estimate the stability with the advantage of application to a limited number of environments (Cruz et al., 2004). It is important to combine the adaptability and stability analysis via regression with other methods such as traditional, strengthening the inferences to be drawn against the predictability of genotypes (Silva and Duarte, 2006).

The contribution of genes that promote delayed leaf senescence of the plant may represent an effective mechanism that favors grain filling, adding benefits related to the higher yield stability. Thus, the aim of this study was to elucidate if the delayed leaf senescence in wheat by the expression of *stay-green* character brings forward effective contributions to the parameters of adaptability and stability in grain yield and thousand grain weight, longing for the possibility of genetic gain in selection of more stable and productive genotypes.

## MATERIALS AND METHODS

The populations were formed by crossing the genetic constitutions of wheat TB438 (bearer of character "*stay-green*") and TB188 (synchronized maturation) comprising lines selected by the breeding program of Embrapa Clima Temperado (Pelotas/RS) obtained via recurrent selection with several promising lines that had the character *stay-green* and others revealing synchronized senescence. In 1998, crosses were made between these lines and the $F_1$ generation was obtained and used to obtain the two backcrosses and the $F_2$ population. These lines were advanced for many generations ($F_n$) in order to reach high grain yield and differences in maturity, that is, *stay-green* (SG) and synchronized (SZ). Moreover, the backcrosses, $RC1F_1$ (P1 // P1/P2) and $RC2F_1$ (P2 // P1/P2), respectively, were subjected to self-pollination and selected for the presence and absence of the *stay-green* character until highly homozygous.

It is noteworthy that in these populations the selection also involved the simultaneous analysis for high grain yield and presence/absence of *stay-green* character. In 2002, 14 *stay-green* (SG30, SG39, SG47, SG53, SG65, SG71, SG74, RC1SG32, RC2SG34, RC2SG40, RC2SG46, RC2SG54, RC2SG62, RC2SG67), 16 synchronized (SZ31, SZ37 SZ49, SZ57, SZ69, RC1SZ43, RC1SZ45, RC1SZ55, RC1SZ58, RC1SZ68, RC1SZ72, RC1SZ76, RC2SZ35, RC2SZ42, RC2SZ56, RC2SZ61) were obtained and compared to parental lines TB438 and TB188.

The lines that were conducted in 2003, 2004 and 2005 in the experimental field located in Capão do Leão County, Rio Grande do Sul State, Brazil. And the soil is classified as Red Yellow Podzolic unit in Mapping Pelotas, which its U.S. equivalent is Typic Hapludalf (USDA, 2010). The County is situated at 31°52'00'' S lat and 52°21'24'' W long at an altitude of 13.24 m, with an average annual rainfall of 1280.2 mm.

A randomized complete block design with three replications was used, and each experimental unit consisted of five lines with three meters in length and spacing of 0.2 m, in a seeding rate of 300 viable seeds $m^{-2}$. The fertilization and liming were made based on recommendations for wheat to an expected crop yield around 2 t $ha^{-1}$. Other cultural practices such as weed control, diseases and pests were performed according to the indications for the crop.

This study evaluated the grain yield (GY, kg $ha^{-1}$) obtained by the yield value of each plot and scaled to a hectare, weight of a thousand grains (WTG, g), by the count of 250 grains in each plot with subsequent weighing and multiplied by four. Data were subjected to variance analysis (ANOVA) and comparison of means between wheat lines contrasting for the maturation in different growing seasons. After, it was determined by traditional phenotypic stability method based on environmental variation for each genotype, recommending that those who showed the smallest mean square values are considered stable (Cruz et al., 2004). Also, adaptability and stability parameters were obtained using the Eberhart and Russell (1966) method, based on linear regression using the mathematical model $Y_{ij} = \beta_{oi} + \beta_{1i}I_j + \delta_{ij} + \bar{\varepsilon}_{ij}$, where $Y_{ij}$ is the average of genotype i at environment j; $\beta_{oi}$: overall average genotype i; $\beta_{1i}$: linear regression coefficient which measures the response of the genotype to variation of the environment; $I_j$: environmental index coded ($\sum_j Ij = 0$); $\delta_{ij}$: deviations from regression; and $\bar{\varepsilon}_{ij}$: average experimental error. The genotype predictability was obtained with the determination coefficient ($R^2$). All analyzes were performed using the GENES software (Cruz, 2006).

## RESULTS AND DISCUSSION

The genotype versus year (GxY) analysis indicated a significant source of variation for grain yield (GY) therefore a comparison of means was performed seeking to decompose the interaction effects (Table 1). The GY average for the lines TB438, SG65, SG74 and RC1SZ72 did not differ between years, suggesting a trend towards stability. However, despite the stability shown by the genotype RC1SZ72, it expressed a lower average grain yield than the lines carrying the *stay-green* character in different years, showing superiority of genotypes with

**Table 1.** Mean performance of wheat lines from different maturity groups in grain yield (GY, kg ha$^{-1}$) harvested in 2003, 2004 and 2005 in Capão do Leão County, RS, Brazil.

| Character | Mean square | | | | General | CV |
|---|---|---|---|---|---|---|
| | Genotype (G) | Year (Y) | GxY | Error | Mean | (%) |
| GY | 699850* | 16201278* | 307039* | 61214 | 2005 | 22.34 |

| Lines | GY mean values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2003 | | | 2004 | | | 2005 | | Mean | | | |
| TB438$^{gg}$ | A | 2233$^{+}$ | c | A | 2042 | c | A | 2126 | a | 2134 | a | |
| SG30 | A | 2414 | c | A | 2241 | b | B | 1774 | a | 2143 | a | |
| SG39 | A | 2734 | b | A | 2760 | a | B | 1552 | b | 2349 | a | |
| SG47 | A | 2740 | b | B | 2210 | b | B | 2230 | a | 2394 | a | |
| SG53 | A | 3103 | a | B | 2443 | b | B | 2215 | a | 2587 | a | |
| SG65 | A | 2311 | c | A | 2523 | b | A | 2124 | a | 2319 | a | |
| SG71 | A | 3055 | a | A | 2763 | a | B | 1612 | b | 2477 | a | |
| SG74 | A | 2407 | c | A | 2379 | b | A | 2137 | a | 2308 | a | |
| RC1SG32 | A | 2609 | b | A | 2902 | a | B | 1322 | c | 2278 | a | |
| RC2SG34 | A | 2611 | b | B | 1562 | d | B | 1629 | b | 1934 | b | |
| RC2SG40 | A | 2524 | b | A | 2787 | a | B | 948 | c | 2086 | a | |
| RC2SG46 | A | 2922 | a | B | 1756 | d | B | 1507 | b | 2062 | a | |
| RC2SG54 | A | 2434 | c | B | 2074 | c | B | 1983 | a | 2164 | a | |
| RC2SG62 | A | 2941 | a | B | 2249 | b | C | 1711 | b | 2300 | a | |
| RC2SG67 | A | 2533 | b | B | 1923 | c | B | 1585 | b | 2014 | b | |
| TB188$^{gz}$ | A | 2523 | b | B | 1331 | d | B | 992 | c | 1615 | b | |
| SZ31 | A | 2426 | c | B | 1601 | d | B | 1535 | b | 1854 | b | |
| SZ37 | A | 2659 | b | B | 1578 | d | B | 1277 | c | 1838 | b | |
| SZ49 | A | 2160 | c | B | 1406 | d | B | 1389 | b | 1652 | b | |
| SZ57 | A | 2091 | c | B | 1488 | d | B | 1539 | b | 1706 | b | |
| SZ69 | A | 2500 | b | C | 1415 | d | B | 1879 | a | 1931 | b | |
| RC1SZ43 | A | 2281 | c | A | 2376 | b | B | 1262 | c | 1973 | b | |
| RC1SZ45 | A | 2140 | c | B | 1493 | d | B | 1548 | b | 1727 | b | |
| RC1S Z55 | A | 1907 | c | A | 1634 | d | B | 1166 | c | 1569 | b | |
| RC1SZ58 | A | 2137 | c | A | 2008 | c | B | 1437 | b | 1861 | b | |
| RC1SZ68 | A | 2331 | c | A | 2524 | b | B | 1978 | a | 2278 | a | |
| RC1SZ72 | A | 1802 | c | A | 1692 | d | A | 1661 | b | 1718 | b | |
| RC1SZ76 | A | 2225 | c | B | 1705 | d | B | 1315 | c | 1748 | b | |
| RC2SZ35 | A | 2431 | c | B | 1555 | d | B | 1448 | b | 1811 | b | |
| RC2SZ42 | A | 2141 | c | B | 1823 | c | B | 1509 | b | 1824 | b | |
| RC2SZ56 | A | 1992 | c | B | 1644 | d | B | 1318 | c | 1651 | b | |
| RC2SZ61 | A | 2092 | c | A | 2051 | c | B | 1414 | b | 1852 | b | |
| Overall Mean | A | 2419 | | B | 1998 | | C | 1598 | | 2005 | | |

$^{+}$ Means followed by the same letter in the row and column do not differ at 0.05 probability by the Scott & Knott test, * significant at 0.05 probability by the F test; $^{ns}$ not significant by F test; MS = mean square; CV = coefficient of variation; SG = *stay-green* trait; SZ = synchronized trait; $^{gg}$ and $^{gz}$ = *stay-green* parents and synchronized, respectively.

delayed senescence. Inconsistencies were found between high yield and stability in lines of *Triticum durum* L., confirming the need for the simultaneous assessment of these characters in order to release new superior genotypes (Pedro et al., 2011). Environments of greater instability are more amenable to G x E analysis, favoring the identification of responsive and stable genotypes (Crestani et al., 2010).

In the analysis of GY per year of cultivation (Table 1), a higher average yield was observed in 2003 (2419 kg ha$^{-1}$), proved to be a favorable year for the crop. Four lines with high GY, all carrying the *stay-green* character, were ranked together and statistically superior to others. In 2004, there was an average GY lower than in 2003, but

**Table 2.** Monthly precipitation for the years 2003, 2004 and 2005 and expected for the months of June to December, including the relationship between occurred rainfall and the regular for each month (RP), registered in the agrometeorological Pelotas station (EAP Capão do Leão, RS, Brazil).

| Month | 2003 | | 2004 | | 2005 | | Ratio |
|---|---|---|---|---|---|---|---|
| | mm | RP (%) | mm | RP (%) | mm | RP (%) | Mm |
| June | 246.2 | 233 | 57.7 | 55 | 28.0 | 26 | 105.7 |
| July | 97.4 | 67 | 95.6 | 65 | 42.2 | 29 | 146.0 |
| August | 93.4 | 79 | 94.4 | 80 | 101.6 | 86 | 117.7 |
| September | 115.5 | 93 | 90.3 | 73 | 241.6 | 195 | 123.7 |
| October | 48.8 | 48 | 112.0 | 111 | 93.3 | 93 | 100.7 |
| November | 103.2 | 104 | 91.5 | 92 | 23.7 | 24 | 99.5 |
| December | 76.3 | 74 | 28.6 | 28 | 54.6 | 53 | 103.2 |

Source: EAP (2014).

within the expected average of 2 t $ha^{-1}$. Accordingly, some of the highest averaging genotypes were also from the delayed maturation type. In 2005, the low average rainfall between the months of November and December were decisive for the lower production values, including restrictive conditions during grain filling (Table 2).

This condition enabled a grain yield of 1598 kg $ha^{-1}$ (Table 1). The reduced availability of water towards the end of the grain filling stage changes the plant relationships between source and drain, the concentration of reactive oxygen (Chen et al., 2010) and accelerates senescence and photoassimilate accumulation (Samarah et al., 2009), causing yield losses.

In more restrictive conditions (2005), nine genetic constitutions showed superior performance in GY, seven carrying the *stay-green* character, including one parent and two expressing synchronized maturation (Table 1). Some delayed maturation genotypes showed, in these conditions, GY values greater than 2 t $ha^{-1}$, strengthening the evidence that the *stay-green* character favors a greater ability to stress tolerance of reduced water availability in wheat, agreeing with other reports (Adu et al., 2011). A strong relationship in maize chlorophyll content and delayed leaf senescence with a higher yield stability under more restrictive water supply (Messmer et al., 2011). In the analysis involving three year average GY for each genotype (Table 1), near all the *stay-green* genotypes showed the better performance. On the other hand, in the synchronized group, only RC1SZ68 showed superior behavior. Wheat genotypes with delayed senescence indicate significant gains in GY, bringing great prospects for increasing production efficiency by modification of plant photosynthetic ability (Parry et al., 2011).

In the WTG analysis (Table 3), both simple effects such as interaction were statistically significant. Similar to GY, WTG also showed greater magnitude of the mean square for the year of cultivation, indicating the most significant source of variation in character changes (Table 3). Similar results were obtained by G x E analysis in oat (Crestani et al., 2010). A large number of lines showed unchanged average values in WTG over the years of assessment (Table 3), indicating stable behavior of delayed senescence *stay-green* genotypes. In 2003, a favorable year for wheat cultivation, there was a predominance of synchronized maturation genotypes and only three *stay-green* genotypes showing high WTG (Table 3). In this condition, a similar behavior was observed in the parents. In 2004, similar results were observed, with the highest WTG values predominantly displayed by lines of the synchronized maturation group. Of the seventeen lines of superior performance, six were *stay-green* and eleven synchronized.

Also, the synchronized displayed higher WTG expression when compared to the delayed senescence parent (Table 3). Thus, in favorable years (2003 and 2004) little contribution from the *stay-green* character to WTG was observed, prevailing a superiority of synchronized senescence genotypes. These results disagree with the literature regarding contributions of *stay-green* to grain filling (Ahlawat et al., 2008). In wheat, genetic stability for high WTG and the availability of photoassimilates next to anthesis may favor other components connected to yield (Silva et al., 2005). The low performance of *stay-green* lines for WTG may be results of negative relationship among weight average of grains and number of grains per ear, seen that in favorable environments the presence of *stay-green* character improve also the fertility of spikelets, specially of those presents in the base and apical of the ear, where the grains are smaller, reducing to WTG (Luche et al., 2013). Also, positive effects on the number of fertile flowers and spikelets and ear grains have been reported (Silva et al., 2005; Ahlawat et al., 2008). In 2005, the year with highest water stress conditions (Table 2), an opposite behavior was observed in lines with the different maturity groups (Table 3). The *stay-green* genotypes

**Table 3.** Mean performance of wheat lines from different maturity groups for weight of a thousand grains (WTG, g) harvested in 2003, 2004 and 2005 in Capão do Leão County, RS, Brazil.

| Character | Mean square | | | General | CV |
|---|---|---|---|---|---|
| | Genotype (G) | Year (Y) | GxY | Error | Mean | (%) |
| WTG | 17.95* | 195.67* | 17.36* | 4.73 | 34.69 | 6.27 |

| Lines | WTG mean values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2003 | | | 2004 | | | 2005 | | | Mean | | |
| TB438[gg] | B | 34.30[+] | b | B | 32.68 | b | A | 37.15 | a | 34.71 | a |
| SG30 | A | 33.29 | b | A | 34.65 | b | A | 32.85 | b | 33.60 | b |
| SG39 | A | 29.94 | c | A | 33.20 | b | A | 32.83 | b | 31.99 | b |
| SG47 | B | 34.03 | b | B | 33.00 | b | A | 37.33 | a | 34.79 | a |
| SG53 | B | 33.77 | b | A | 38.77 | a | B | 35.66 | a | 36.07 | a |
| SG65 | A | 38.33 | a | B | 31.82 | b | A | 37.00 | a | 35.72 | a |
| SG71 | A | 34.76 | b | A | 34.37 | b | A | 36.11 | a | 35.08 | a |
| SG74 | B | 32.69 | b | A | 35.60 | b | A | 37.66 | a | 35.32 | a |
| RC1SG32 | A | 33.20 | b | A | 35.74 | b | A | 31.36 | b | 33.44 | b |
| RC2SG34 | A | 33.31 | b | A | 36.58 | a | A | 33.20 | b | 34.36 | b |
| RC2SG40 | A | 32.28 | b | A | 34.69 | b | A | 33.31 | b | 33.43 | b |
| RC2SG46 | A | 36.57 | a | A | 37.57 | a | A | 34.91 | a | 36.35 | a |
| RC2SG54 | B | 32.33 | b | A | 36.73 | a | B | 33.55 | b | 34.20 | b |
| RC2SG62 | B | 33.16 | b | A | 38.12 | a | B | 32.96 | b | 34.75 | a |
| RC2SG67 | A | 39.64 | a | A | 39.00 | a | B | 32.01 | b | 36.88 | a |
| TB188[gz] | B | 32.41 | b | A | 36.96 | a | A | 36.71 | a | 35.03 | a |
| SZ31 | B | 29.91 | c | A | 37.28 | a | B | 31.71 | b | 32.97 | b |
| SZ37 | B | 33.02 | b | A | 37.28 | a | B | 32.80 | b | 34.37 | b |
| SZ49 | A | 37.30 | a | A | 36.13 | b | B | 32.76 | b | 35.40 | a |
| SZ57 | B | 32.68 | b | A | 37.82 | a | B | 31.11 | b | 33.87 | b |
| SZ69 | B | 28.75 | c | A | 33.34 | b | A | 33.33 | b | 31.81 | b |
| RC1SZ43 | A | 35.94 | a | A | 34.21 | b | B | 28.55 | b | 32.90 | b |
| RC1SZ45 | A | 33.42 | b | A | 35.01 | b | B | 30.45 | b | 32.96 | b |
| RC1S Z55 | A | 35.80 | a | A | 38.18 | a | B | 32.26 | b | 35.41 | a |
| RC1SZ58 | B | 33.85 | b | A | 38.97 | a | B | 34.21 | b | 35.68 | a |
| RC1SZ68 | B | 30.86 | c | A | 40.00 | a | A | 37.06 | a | 35.97 | a |
| RC1SZ72 | A | 36.88 | a | A | 36.60 | a | B | 32.25 | b | 35.24 | a |
| RC1SZ76 | A | 38.79 | a | A | 35.78 | b | A | 34.75 | a | 36.44 | a |
| RC2SZ35 | A | 33.30 | b | A | 34.60 | b | A | 32.70 | b | 33.53 | b |
| RC2SZ42 | B | 32.31 | b | A | 38.66 | a | B | 34.03 | b | 35.00 | a |
| RC2SZ56 | B | 34.38 | b | A | 38.57 | a | B | 31.13 | b | 34.69 | a |
| RC2SZ61 | A | 39.34 | a | A | 40.53 | a | B | 34.56 | a | 38.14 | a |
| Mean | B | 34.05 | | A | 36.33 | | B | 33.70 | | 34.69 | |

[+] Means followed by the same letter in the row and column do not differ at 0.05 probability by the Scott & Knott test. * significant at 0.05 probability by the F test; [ns] not significant by F test; MS = square mean; CV = coefficient of variation; SG = *stay-green* trait; SS = synchronized trait; [gg] e [gz] = *stay-green* parents and synchronized, respectively.

showed higher values for WTG. These results coupled with genotype performances in the overall average between years (Table 3) appear to show the greatest effect of *stay-green* on WTG is more evident under conditions of environmental restrictions, enhancing the tolerance to abiotic stresses. Results were also observed in other species (Izanloo et al., 2008; Adu et al., 2011).

Seeking to strengthen the evidence and hypotheses, the results of adaptability and stability analyses through simple regression, proposed by Eberhart and Russell (1966) and stability of the traditional method were obtained (Table 4). The variable GY in the *stay-green* character genotypes showed higher values than on those with synchronized maturation. Moreover, WTG means were equivalent for both groups of maturation. This reinforces the hypotheses reported by other researchers,

**Table 4.** Mean values ($\beta_0$), adaptability parameters ($\beta_1$) and stability ($S^2d$) and determination coefficient ($R^2$) by Eberhart & Russell and through the Traditional Method (NDE) on grain yield (GY, kg $ha^{-1}$) and weight of a thousand grains (WTG, g) on wheat lines of distinct groups of maturation.

| Lines | GY | | | | | WTG | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\beta_0$ | $\beta_1$ | $S^2d$ | $R^2$ | NDE | $\beta_0$ | $\beta_1$ | $S^2d$ | $R^2$ | NDE |
| TB438$^{gg}$ | 2133 a | 0.13* | -8077 $^{ns}$ | 83 | 27500$^{ns}$ | 34.71 a | -1.35* | 1.28 $^{ns}$ | 72 | 15.36* |
| SG30 | 2143 a | 0.78$^{ns}$ | -4326 $^{ns}$ | 93 | 329548* | 33.59 b | 0.65$^{ns}$ | -1.56 $^{ns}$ | 98 | 2.65$^{ns}$ |
| SG39 | 2349 a | 1.43$^{ns}$ | 245598* | 72 | 1429714* | 31.99 b | 0.60 $^{ns}$ | 3.30 $^{ns}$ | 63 | 9.53$^{ns}$ |
| SG47 | 2394 a | 0.63 $^{ns}$ | 27699 $^{ns}$ | 73 | 270700* | 34.79 a | -1.22* | 2.60 $^{ns}$ | 69 | 15.36* |
| SG53 | 2587 a | 1.09 $^{ns}$ | 7678 $^{ns}$ | 93 | 639097* | 36.07 a | 1.55 $^{ns}$ | 1.44 $^{ns}$ | 76 | 19.11* |
| SG65 | 2319 a | 0.22* | 42822 $^{ns}$ | 81 | 119565$^{ns}$ | 35.72 a | -2.29* | 0.74 $^{ns}$ | 90 | 35.43* |
| SG71 | 2477 a | 1.75* | 113058* | 89 | 1746157* | 35.08 a | -0.49* | -0.87 $^{ns}$ | 78 | 2.51$^{ns}$ |
| SG74 | 2308 a | 0.33* | -12360 $^{ns}$ | 82 | 65882$^{ns}$ | 35.32 a | -0.05 $^{ns}$ | 10.90* | 57 | 18.72* |
| RC1SG32 | 2277 a | 1.55* | 583926* | 57 | 2118685* | 33.44 b | 1.47 $^{ns}$ | -0.69 $^{ns}$ | 91 | 14.51* |
| RC2SG34 | 1934 b | 1.21 $^{ns}$ | 177892* | 71 | 1033253* | 34.36 b | 1.34 $^{ns}$ | -1.51 $^{ns}$ | 99 | 11.10$^{ns}$ |
| RC2SG40 | 2086 a | 1.90* | 744560* | 61 | 2968835* | 33.43 b | 0.71 $^{ns}$ | -0.74 $^{ns}$ | 71 | 4.37$^{ns}$ |
| RC2SG46 | 2062 a | 1.73* | 108952* | 89 | 1711585* | 36.35 a | 0.81 $^{ns}$ | -0.62 $^{ns}$ | 74 | 5.40$^{ns}$ |
| RC2SG54 | 2164 a | 0.55 $^{ns}$ | -9418 $^{ns}$ | 90 | 170281$^{ns}$ | 34.2 b | 1.47 $^{ns}$ | -0.05 $^{ns}$ | 85 | 15.48* |
| RC2SG62 | 2300 a | 1.50* | -17855 $^{ns}$ | 99 | 1140015* | 34.74 a | 2.04 $^{ns}$ | -1.44 $^{ns}$ | 99 | 25.59* |
| RC2SG67 | 2013 b | 1.16 $^{ns}$ | -10153 $^{ns}$ | 98 | 692953* | 36.88 a | 1.60 $^{ns}$ | 23.73* | 49 | 53.64* |
| TB188$^{gz}$ | 1615 b | 1.87* | 90511* | 91 | 1939534* | 35.03 a | 0.93 $^{ns}$ | 14.52* | 48 | 29.47* |
| SZ31 | 1854 b | 1.09 $^{ns}$ | 69808* | 82 | 739497* | 32.97 b | 2.52* | 2.09 $^{ns}$ | 88 | 44.23* |
| SZ37 | 1838 b | 1.69* | 72306* | 91 | 1583982* | 34.36 b | 1.76 $^{ns}$ | -1.50 $^{ns}$ | 99 | 19.11* |
| SZ49 | 1651 b | 0.95 $^{ns}$ | 65526* | 78 | 582131* | 35.4 a | 0.64 $^{ns}$ | 7.85* | 55 | 16.62* |
| SZ57 | 1706 b | 0.68 $^{ns}$ | 47758* | 69 | 335239* | 33.87 b | 2.45* | -1.33 $^{ns}$ | 99 | 36.97* |
| SZ69 | 1931 b | 0.77 $^{ns}$ | 371299* | 33 | 888512* | 31.81 b | 0.73 $^{ns}$ | 10.31* | 55 | 21.06* |
| RC1SZ43 | 1973 b | 1.23 $^{ns}$ | 233489* | 67 | 1143172* | 32.9 b | 1.11 $^{ns}$ | 23.35* | 57 | 44.89* |
| RC1SZ45 | 1727 b | 0.73 $^{ns}$ | 58145* | 70 | 385815* | 32.96 b | 1.36 $^{ns}$ | 1.59 $^{ns}$ | 71 | 16.10* |
| RC1S Z55 | 1569 b | 0.90 $^{ns}$ | -12906 $^{ns}$ | 97 | 421187* | 35.41 a | 1.82 $^{ns}$ | 2.68 $^{ns}$ | 76 | 26.61* |
| RC1SZ58 | 1860 b | 0.85 $^{ns}$ | 14670 $^{ns}$ | 87 | 416894* | 35.68 a | 1.97 $^{ns}$ | -1.02 $^{ns}$ | 97 | 24.46* |
| RC1SZ68 | 2277 a | 0.42* | 72614* | 39 | 230094* | 35.97 a | 2.16 $^{ns}$ | 23.04* | 44 | 65.33* |
| RC1SZ72 | 1718 b | 0.17* | -19464 $^{ns}$ | 92 | 16528$^{ns}$ | 35.24 a | 1.02 $^{ns}$ | 7.71* | 51 | 20.23* |
| RC1SZ76 | 1748 b | 1.11 $^{ns}$ | -18486 $^{ns}$ | 99 | 626210* | 36.44 a | -0.22* | 7.03* | 42 | 13.20$^{ns}$ |
| RC2SZ35 | 1811 b | 1.20 $^{ns}$ | 72120* | 84 | 873797* | 33.53 b | 0.67 $^{ns}$ | -1.51 $^{ns}$ | 96 | 2.83$^{ns}$ |
| RC2SZ42 | 1824 b | 0.77 $^{ns}$ | -20378 $^{ns}$ | 99 | 299254* | 35.00 a | 2.13 $^{ns}$ | 1.53 $^{ns}$ | 85 | 32.42* |
| RC2SZ56 | 1651 b | 0.82 $^{ns}$ | -20398 $^{ns}$ | 99 | 340502* | 34.69 a | 2.47* | 1.29 $^{ns}$ | 90 | 41.73* |
| RC2SZ61 | 1852 b | 0.82 $^{ns}$ | 42052* | 78 | 433369* | 38.14 a | 1.64 $^{ns}$ | 7.38* | 55 | 29.91* |

$^{+}$ Means followed by the same letter in the row and column do not differ at 0.05 probability by the Scott & Knott test, * significant at 0.05 probability by the F test; $^{ns}$ not significant by F test; MS = Square Mean; DF = degrees of freedom; CV = coefficient of variation; SG = *stay-green* trait; SZ = synchronized trait; $^{gg}$ and $^{gz}$ = *stay-green* parent and synchronized, respectively.

indicating the superiority of *stay-green* genotypes in yield of grain goes beyond the greater capacity of grain filling, having an influence on other characters also linked to the formation of yield components (Silva et al., 2005; Ahlawat et al., 2008). The lines studied for GY indicated that the synchronized genotypes accounted for the majority of individuals with wide adaptability ($\beta_1$=0), showing greater responsiveness to environmental improvements. Moreover, the *stay-green* parent showed specific adaptability to the unfavorable environment, ranking on top in GY, unlike the parent synchronized, adjusted to favorable environments, but among the lowest in the character expression. However, TB438, SG65 and SG74 showed significantly lower adaptability values ($\beta_1$ <1.0), which, associated with phenotypic stability, discloses independently of method used, lines of great potential as sources for alleles for high GY and stability in unfavorable environments.

For WTG, the numbers of individuals with wide adaptability from different maturation groups were similar. The *stay-green* parent showed specific adaptability to harsh environments and was also included in the group

expressing the best averages. On the other hand, the synchronized parent showed general adaptation, was also represented the group that expressed the highest values in character. Therefore, the similarity in WTG mean between the parents and the proportions of lines between maturity groups possibly indicate that the contribution of *stay-green* also affects other important characters in the increase of grain yield (Table 4). Stability parameters in the method of Eberhart and Russell ($S^2d$ and $R^2$), the majority of exhibit delayed senescence genotypes were in stable expression of GY. It is noteworthy stability observed for who shows more permanent green plant next harvest parent, unlike what happened to the parent of synchronized pattern. In the analysis involving the traditional method (NDE) for the same character, there was a reduction in the number of phenotypic stability genotypes, showing that all tested lines, four belonged to the *stay-green* group and only a trend with the synchronized maturation. In a general analysis, involving the two observation methods, TB438, SG65, SG74 and RC2SG54 genotypes were those that showed the stability and effectiveness fully belong to *stay-green* ripening group. Even were included that expressed the best medium and limited adaptability to harsh environments group. We highlight the RC2SG54 lineage, because in addition to expressing high average with stability, was the one who showed ability to adapt front to GY expression in different years of assessment. Thus, maintenance of the photosynthetic active machinery for favoring higher chlorophyll concentration in tissues, provides maintenance of grain filling, providing greater stability in yield and grain yield, particularly under stress conditions (Izanloo et al., 2008).

Regarding WTG (Table 4), the adaptability parameter ($\beta_1$) from the Eberhart and Russell method was found to be similar between the two maturity groups, with the *stay-green* parent indicating adaptability to restricted environments and the synchronized with wide adaptation. It is highlighted by this method ($S^2d$ and $R^2$), a slight tendency to stability in a larger number of lines representing the *stay-green* group. In the mean square stability analysis, six *stay-green* (SG30, SG39, SG71, RC2SG34, and RC2SG40 RC2SG46) and only one synchronized (RC1SZ72) line were stable. It is noteworthy that for WTG, the RC2SG46 genotype showed both high adaptability and stability by both methods.

The traditional method was more conservative than the Eberhart and Russell, detecting a lower number of stable genotypes. On the other hand, the study of correlations between methods for estimating stability and adaptability, indicated a high positive (0.83) correlation between the traditional method and proposed by Eberhart and Russell (1966) and Silva and Duarte (2006). However, the model in the Eberhart and Russell (1966) method takes into account a higher number of parameters for the analysis, defining an ideal genotype as one which displays high yield ($\beta_0$), regression coefficient equal to 1.0 ($\beta_1$= 1.0) and regression deviation equal to zero ($S^2d$ =0), in other words, high average values for the character, wide adaptability and stability, respectively (Cruz et al., 2004).

## Conclusions

Delayed senescence wheat plants have higher stability and grain yield, especially in unfavorable years marked by reduced precipitation. However, no detectable influence is found on weight of a thousand grains. The introduction of the *stay-green* character into superior cultivars and lines represents a promising strategy to obtain more stable and productive wheat genotypes.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Identification and control of fungi causing fruits rot in pipiana pumpkin (*Cucurbita argyrosperma* Huber)

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**Severe rot symptoms were observed in samples collected during 2012 in a small farm in Cocula, Guerrero, Mexico. One oomycete and two deuteromycetes fungus were isolated from collected symptomatic fruits. In order to carry out the molecular analysis by the amplification of the internal transcribed spacer region (ITS) two representative isolates of each fungus were chosen. The pathogenicity of each isolate was verified with pumpkin fruit inoculation with fungus inoculants and fruits sprinkled only with sterile distilled water as control. The control fruit remained healthy while the fruits which were inoculated with the pathogens were observed to have injuries and rot symptoms five days after inoculation. From the fruits showing injuries and rot symptoms, the oomycete and fungus were re-isolated. Based on the isolation, morphological, and molecular identification, as well as on pathogenicity tests, *Phytophthora capsici* Leon, *Rhizoctonia solani* Kühn, and *Sclerotium rolfsii* Sacc., were determined to be the causal agents for rot of the pumpkin fruits. Additionally, five fungicides and the biocontrol agent *Trichoderma asperellum* were assessed against the above fungus in pumpkin fruits in a greenhouse. The fungicides propamocarb + fosetyl-Al, metalaxyl + Chlorothalonil, quintozene, and the biocontrol agent *T. asperellum*, delayed the presence of *P. capsici*, *R. solani*, and *S. rolfsii* for 6.00, 4.67, and 5.83 days, respectively.**

**Key words:** *Cucurbita argyrosperma*, soil pathogens, fruit rot, fungicides, *Trichoderma asperellum*.

## INTRODUCTION

Mexico is one of the most important diversity centers of the Cucurbitaceae family, which includes 118 genus and 825 species, which have had a very important role in culture and economy among the different social stratus. The pipiana pumkpin (*Cucurbita argyrosperma* Huber) is an economically important crop in Guerrero state, south

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of Mexico. During 2011, 5,742 ha were grown (SIAP, 2013), and just in the northern part of the state, over 20 family businesses producing the typical food “green mole” are located. The pumkpin seed is the main raw material for making this type of food, as well as the main ingredient in making typical candies such as “palanqueta”, and “jamoncillos”. Guerrero is one of the main seed-producer states nationwide. In some regions of the state this crop is grown in heavy, clay, flat and bad drained soils. During the summer the weather is warm and there is high relative humidity which, in combination with the habit of creeping growth and undetermined crop, it generates a micro-weather with favorable conditions for the development and infection of phytopathogenic fungi inhabiting the soil, which has not been formally identified, and which causes fruit rot, decrease in efficiency and in producers’ economical incomes (Ayvar-Serna et al., 2007). In the productive zone of Cocula, Guerrero, Mexico, severe fruit rot symptoms in pumpkin fruits have been observed. In the region of study and related to pumpkin do not exist information about the diseases management based on biological agents or chemical control. However, in other crops there are several studies of biocontrol for the same pathogens associated with pumpkin rot fruits, for instance the use of *Trichoderma asperellum* against *Sclerotium rolfsii* in onion (Guzmán-Valle et al., 2014), *Rhizoctonia solani* in rice (*Oryza sativa* L.) (Chen et al., 2015), *Phytophthora capsici* in pepper (Segarra et al., 2013), as well as *Bacillus subtilis* and *Streptomyces* spp., against *S. rolfsii*, *R. solani* y *P. capsici* (Rodríguez-Villarreal et al., 2014; Khabbaz et al., 2015). Similarly, related to the control with chemical products, Gisi and Sierotzki (2008) reports the use of propamocarb, fosetyl-Al, metalaxyl, chlorothalonil against oomycetes; Mendoza-Zamora (1990) applied Benomyl and quintozene (PCNB) against *R. solani* and *S. rolfsii*; while Bokshi et al. (2007) reports the use of iodine in the control of true fungi. Because of what has been mentioned, the objectives of this study were (i) to identify the agents causing rot by morphological, molecular, and pathogenic analysis, and (ii) to assess chemical and biological products for the control of the pathogens involved.

## MATERIALS AND METHODS

### Sample collecting

#### *Experimental site*

During August and September 2012, pipiana pumpkin fruits showing different symptoms and rot levels were collected from the experimental field of Colegio Superior Agropecuario del Estado de Guerrero (CSAEGro), located in the county of Cocula, north of Guerrero state, Mexico, at 18° 19’ NL and 99° 39’ WL, at 640 m above sea level (masl). The weather is Aw0, which belongs to warm - sub humid, with rains in the summer, and an annual average temperature of 26.4°C, an average of the coldest month (December) is 23.4°C. The temperature oscillation from one month to another is 5 to 7°C. The annual average precipitation is 767 mm (García, 2005).

#### *Vegetal material*

The sample size and kind of sampling were carried out under the methodology proposed by Pedroza-Sandoval (2009), by a systematic sampling of transect in W, collecting 10 fruits 30 days after the starting of the fruiting, of the creole genotype “Apipilulco”. The symptoms considered were: caved watery injuries color brown; dry brown spongy putrefactions with a white halo; watery caved spots with a white mycelium growth in the inferior and superior part of the fruit; fruits with nest-type white cottony mycelium (where the fruit is in contact with the ground) and with sclerotia (Zitter et al., 2004).

#### *Isolation and purification*

There were some 1 $cm^2$ tissue cuts performed to the fruits showing rot signs and symptoms. These cuts were obtained from the transition zone between the healthy tissue and the sick tissue. The tissue fragments were sanitized using sodium hypochlorite at 1.5% for two minutes; they were rinsed three times with sterile distilled water and then were laid on drying paper for two minutes. One hundred tissue samples were grown, each 5 of them were placed in Petri boxes with growing medium potato-dextrose-agar (PDA) and agar-vegetable juice (V8-Agar). The boxes were incubated at 24°C under continuous black light conditions. The most frequent fungi colonies were transferred to Petri boxes in PDA and V8-Agar growing medium. Mono-zoosporic and hypha tip cultures were obtained according to the methodology described by Fernández-Herrera et al. (2013).

#### *Morphological identification*

Out of the fruit re-isolated pathogens, pure cultures were obtained and preparations with glycerol from the asexual reproduction structures (sporangium) and mycelium were done. The preparations were analyzed in light microscopy at 400X. In this analysis, the size, septa, shape of sporangium and hyphae were registered. The identification was performed by comparing the morphological structures of each fungus, following the keys of Sneh et al. (1991), Barnett and Hunter (1998) and Gallegly and Hong (2008).

#### *Pathogenicity test*

The isolated fungus were inoculated in 20 fruits of the creole genotype Apipilulco. The fruits were previously decontaminated with sodium hypochlorite at 1.5% for two minutes and then were rinsed with sterile distilled water, letting them dry for 5 min. Four fruits were used for each isolation. The inoculant of each fungus was adjusted at $4\times10^6$ zoospores $mL^{-1}$ for isolation 1; and at $8\times10^5$ and $5\times10^6$ colony forming units (CFU) $mL^{-1}$ for isolations 2 and 3, using a Neubahuer chamber. Each fruit was inoculated through spraying 2.5 ml and adding 2 ml of surfactant Tween 20° per liter of sterile distilled water were added. The suspension was applied using a manual sprayer over the pumpkin fruits until runoff point. Four fruits were used as control, which were sprayed using only sterile distilled water. All the fruits were stored in a humid chamber for seven days at 22 ± 2°C and with 100% relative humidity. Once the inoculated fungus showed the same symptomatology than the initially collected tissues, re-isolates were obtained out of the inoculated organism, following the methodology described by

**Table 1.** Treatments used in the greenhouse experiment for each group of pathogens: Oomycete and true fungi.

| Treatment | Pathogen | |
|---|---|---|
| Number | Oomycete | True fungi |
| 1 | *Trichoderma asperellum* strain CSAEGro | *Trichoderma asperellum* strain CSAEGro |
| 2 | PHC® Biopak-F® (*Bacilus* spp., *Streptomyces* spp., *Trichoderma* spp.) | PHC® Biopak-F® (*Bacilus* spp., *Streptomyces* spp., *Trichoderma* spp.) |
| 3 | PHC® RootMate® (*Trichoderma virens* strain G-41) | PHC® RootMate® (*Trichoderma virens* strain G-41) |
| 4 | Q 2000 (free iodine) | Q 2000 (free iodine) |
| 5[a] | Previcur® energy (propamocarb + fosetyl-Al) | Benomil (benomyl 50%) |
| 6[a] | Ridomil gold® Bravo (metalaxyl + chlorothalonil) | Pentaclor* 600 F (quintozene: PCNB 46%) |
| 7 | Control | Control |

[a]: All treatments were the same for both Oomycete and true fungi, except 5 and 6 treatments.

Núñez-Rios et al. (2013). The pathogenicity test was performed twice.

***Molecular identification***

For the DNA extraction and performance of PCR two representative isolates of each fungus were used. For isolation 1, a mono-zoosporic culture was obtained (Quesada et al., 2011), from which five 1-centimeter-diameter discs were cut and put into Petri boxes with 20 ml of sterilized water. The pathogen (oomycete) was incubated at 25 ± 1°C and after four days, the isolations that had produced sporangia were changed at 12°C for 15 min for releasing zoospores. Out of the zoospores solution, one drop was taken and poured on a plate full with growing medium V-8 Agar. It was distributed uniformly and incubated at 25 ± 1°C in a dark place. It was observed 24 and 48 h later in order to detect germinated zoospores (Gallegly and Hong, 2008). Some isolated colonies were taken in order to get the mono zoosporic cultures. Finally, for isolations 2 and 3, a culture of hypha tip was obtained (Okubara et al., 2008; Le et al., 2012). Out of the fungi developed in PDA growing medium, a portion was taken including both medium and mycelium. It was then put into a test tube along with 20 ml sterile water, and shaken strenuously. Afterwards, some serial dilutions with 5 dilution orders were performed. One drop out of dilutions $10^{-4}$ and $10^{-5}$ were put into boxes with PDA medium, uniformly distributed and incubated at 25°C for 24 h. The fungal growth was cut and re-isolated again in PDA for one week (Fernández-Herrera et al., 2013).

The DNA extraction was done from 50 to 100 mg of mycelium for each isolation, using the DNeasy Plant Mini Kit™, following the manufacturer´s procedure (Qiagen, 2012). The procedure was performed 4 times for the oomycete and each fungus. Universal PCR reactions were performed with ITS-1fu 5'-tccgtaggtgaacctgcgg-3' and ITS-4 5'-tcctccgcttattgatatgc-3' primers (White et al., 1990), which amplify two internal intergenic spacers (IGS) and the gene 5.8S of the ribosomal RNA, generating a product between 500 and 900 bases pairs (bp).

This practice was carried out with a reaction mixture in a 25 µl volume, whose final components were: 1X, reaction buffer, 2 mM $MgCl_2$, 200 nM dNTP's of each one, 20 pmoles of each primer, and one unit of Taq DNA polymerase (Promega). The thermal program consisted in keeping a temperature of 94°C for 2 min, followed by 35 cycles at a 94-55-72°C temperature for 30-30-60 s, and a final extension of 5 min at 72°C temperature. The products of the PCR reactions were separated through electrophoresis in agarose gels at 1.5%, and the bands were observed in an ultraviolet light trans-illuminator UVP brand. The amplified fragments by PCR were directly sequenced and the results were compared against the available sequences in the Genbank of the National Center for Biotechnology Information (NCBI).

***Greenhouse control test***

For the greenhouse test, unripe pumpkin fruits of genotype Apipilulco were used (directly from the field) weighing approximately 150 g. Six commercial fungicides were assessed for the three pathogens. Two groups were formed based on the type of fungus, the oomycete was assigned to the first group, and the true fungi – deuteromycetes- were assigned into the second group (Table 1). The evaluated variable was the number of days to the pathogen presence (DPP), determining in how many days the colonies would arise on the fruits once the treatments and inoculation of the pathogen were applied. The treatments were applied using a Kwazar manual sprinkler, whose capacity is 1 L. The surface of the fruits were sprinkled using a calibrated quantity of 300 L $ha^{-1}$ of water. There was a gap of 12 h before the products were allowed inside (FRAC, 2011), then, 2.5 ml of the settled concentration of each pathogen was sprinkled.

***Experimental design and data analysis***

The greenhouse experiment was established under a completely randomized design with four replicates. The experimental unit consisted of four fruits. The treatments evaluated are described in Table 1. Data were statistically analyzed through analysis of variance and a multiple means comparison procedure by using Tukey's HSD test with a significance level of 5%. All the statistical analysis were performed using the software Statistical Analysis System (SAS, 2013).

## RESULTS AND DISCUSSION

### Isolated fungi from fruits with rot symptoms

From the colony developed in PDA and V8-Agar were obtained five isolations. During the sampling in field it was observed that the rot due to the oomycete and one of the deuteromycetes fungus was more common among unripe fruits when there were some conditions like high humidity and precipitation alternated with drought periods. However, the fact that they can harm ripe fruits is not discarded. The second detected fungus was

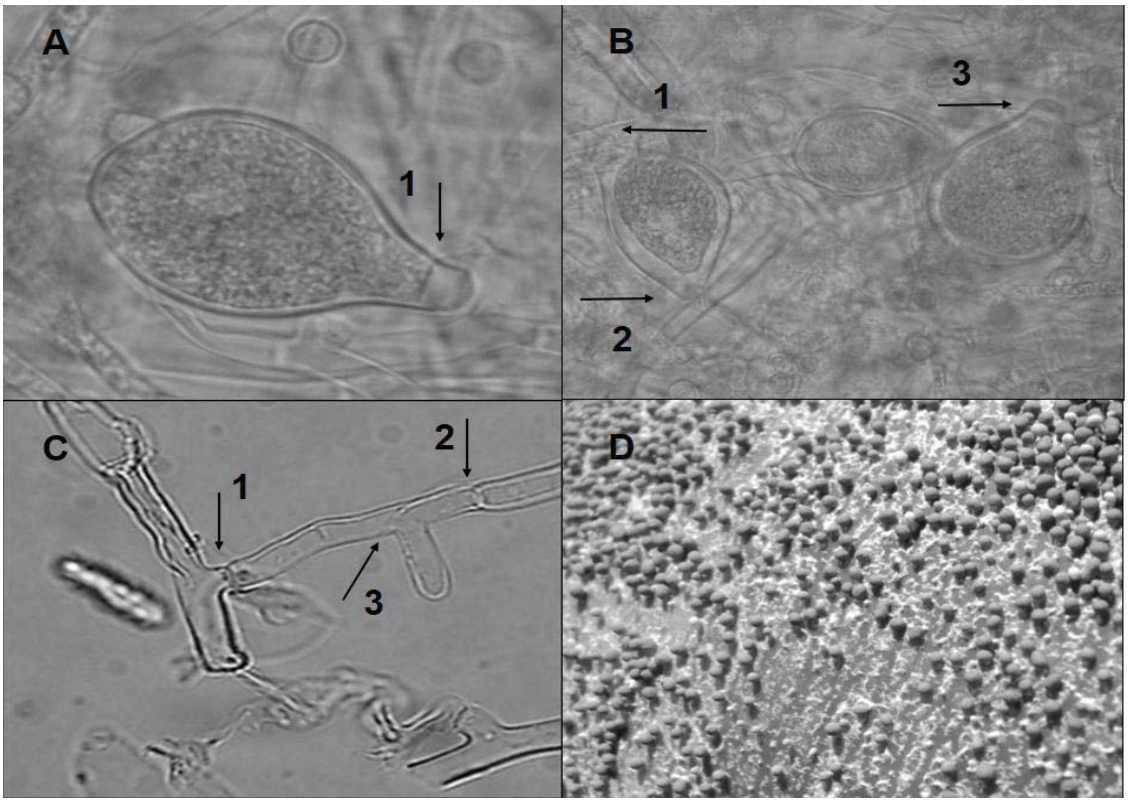


**Figure 1.** Morphological characteristics of *P. capsici*, *R. solani* and *S. rolfsii*. A(1) and B(3) one papillae per sporangium; B(1) and B(2) sporangium with two papillae of *P. capsici*; (C1) constriction, (C2) septa, and (C3) right angle branching out of *R. solani*; (D) sclerotia of *S. rolfsii*.

observed when the foliage decreased and there was more light impact on the ground which made the temperature increase as well. It can harm juicy ripeness fruits but it can also harm unripe fruits. Such results are agreed with what is reported by Zitter et al. (2004).

## Morphological identification

Three species were identified: **Isolation 1**. This fungus showed sporangia 20 - 50 × 15 - 42.5 µm, whit only one papillae and/or with two papillae, of size 6.02 to 7.05 µm wide, and 1.2 to 6.0 µm depth. All the morphological characteristics observed concur with the descriptions by Gallegly and Hong (2008) for *P. capsici* (Figure 1A and B). **Isolation 2**. The characteristics were, hyphae 5 to 8 µm wide, hyphae branching out at a right angle position approximately, with a constriction in the branching out joints, close to their place of origin. Another characteristic was sclerotia showing a 1 to 3 mm diameter, typical of *R. solani* (Sneh et al., 1991) (Figure 1C). **Isolation 3**. This fungus showed main hyphae with a 4.5 to 8.0 µm diameter, with ring-shape joints which allow secondary and tertiary 2.0 to 4.5 µm-diameter-hyphae arise, and 0.5 to 2.0 mm spherical sclerotia. Due to the characteristics found, it was identified as *S. rolfsii* (Barnett and Hunter, 1998) (Figure 1D).

## Pathogenicity test

Five days after inoculation with a zoospores' suspension of *P. capsici* and CFU of *R. solani* and *S. rolfsii*, all fruits showed rot, in addition to plentiful fungus mycelium. Among the symptoms presented, there were; caved watery putrefactions with white mycelium growth (Figure 2A); caved watery injuries brown color (Figure 2B); dry spongy putrefactions with a white halo (Figure 2C). The control fruits were free of disease (Figure 2D). *P. capsici, R. solani* and *S. rolfsii* were re-isolated from the symptomatic fruits (Figure 2E, F and G).

## Molecular identification

When comparing the obtained sequences in the current work against the available sequences in the GenBank, some similarities between 99 and 100% with previously reported sequences were found. This fact corroborates the veracity of the morphological identification. The

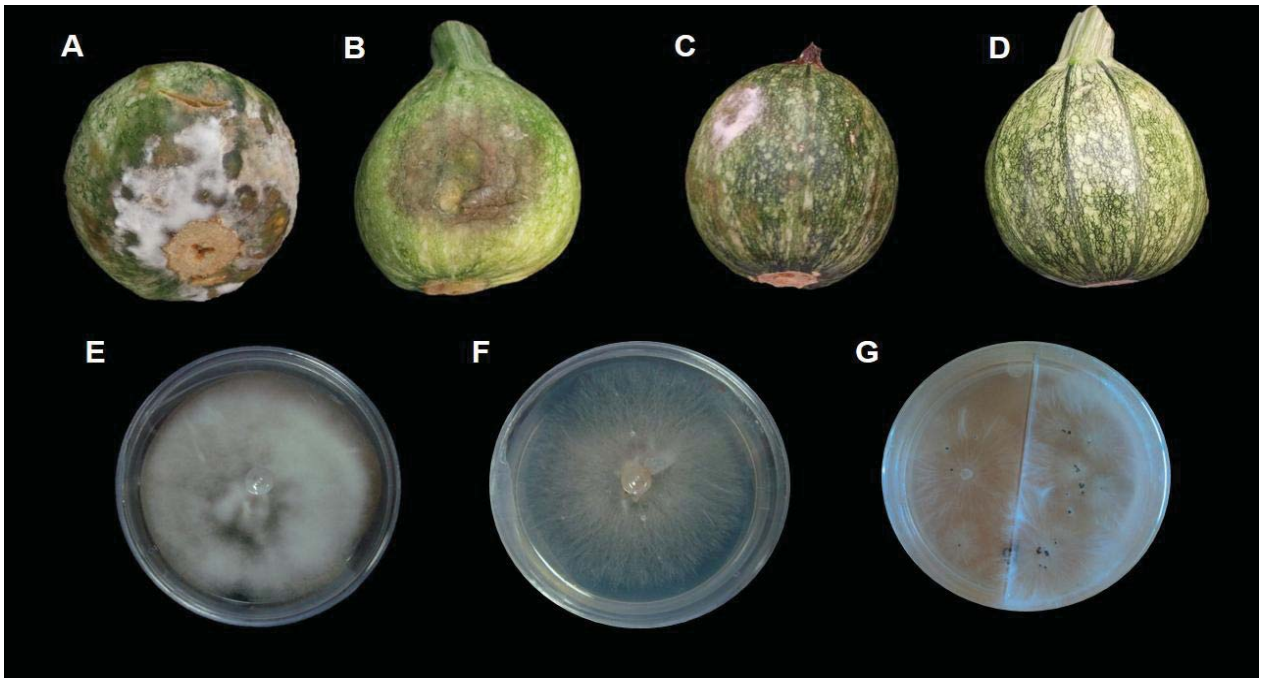


**Figure 2.** Symptomatology induced by the inoculation of *P. capsici, R. solani* and *S. rolfsii* in pumpkin fruits. Isolates from pumpkin fruits showing rot symptoms. A) watery caved putrefactions with white mycelium growth; B) watery caved injuries color dun to brown; C) Dry and spongy putrefactions with a white halo; D) Control fruit without inoculation; E) *P. capsici* colony 4-day grown in vegetable juice-agar (V8-Agar); F and G) *R. solani* and *S. rolfsii* colonies grown in PDA medium.

sequence of the three species found in this current work were deposited in the GenBank under the following accession numbers: KJ652220, for *P. capsici*; KJ652221, for *R. solani*, and KJ652222, for *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. There was coincidence in the morphological and molecular identification for the three species. The DNA sequence of *P. capsici* had a similarity of 99%, with over 100 sequences of the GenBank, but the maximum score (max score) was obtained through two sequences (AJ854286.1 and AJ854287.1) whose strains were isolated in Italy, from *Cucurbita pepo* L., a specie belonging to the same genus of the pipiana pumkpin in this study.

## Greenhouse control test

### *Days after the pathogen presence*

This characteristic is important, since once the pathogen presence has been identified in unripe fruits, these fruits are harmed completely and consequently lost. For *P. capsici* highly significant differences ($P<0.0001$) among the treatment effects were found; in the multiple means comparison the active ingredients propamocarb + fosetyl-Al (T5: Previcur® energy), and metalaxyl + Chlorothalonil (T6: Ridomil gold® Bravo) delayed the appearance of *P. capsici* at day 6, whereas the control treatment was differentiated from all other treatments, and the pathogen appeared 2.82 days after inoculation (Figure 3). For *R. solani* this variable showed significant differences ($P=0.0156$) for the treatments applied. *T. asperellum* (T1), free iodine (T4) and quintozene PCNB (T6) were the best treatments, which delayed the appearance of the pathogen at day 4.67, 4.42, and 4.42, respectively. The control treatment was differentiated from all the products, the pathogen appeared 3.17 days after inoculation (Figure 3). Regarding the *S. rolfsii* case, high significant differences were detected ($P=0.008$); the quintozene was the best treatment, it extended the presence of pathogen colonies up to 5.83 days while the *T. asperellum* and PHC® Biopack did not differentiated each other (Figure 3).

In relation to *P. capsici*, Hu et al. (2007) point out that propamocarb and fosetyl-Al have a good protective and curative action against oomycetes because they inhibit the oospores production in *Phytophthora* spp. This has been supported with what has been found in *P. capsici* in the current research. Additionally, it has been reported that fosetyl-Al has a high degree of systemic activity and efficiency which is generally superior against oomycetes showing a good control (Dufour and Corio, 2013; Gent et al., 2010). It has also been observed that metalaxyl reduces the progress of diseases caused by the oomycetes (Álvarez-Romero et al., 2013). Mihajlović et al. (2013) studied different specific fungicides against oomycetes and it was found that fosetyl-Al had a control

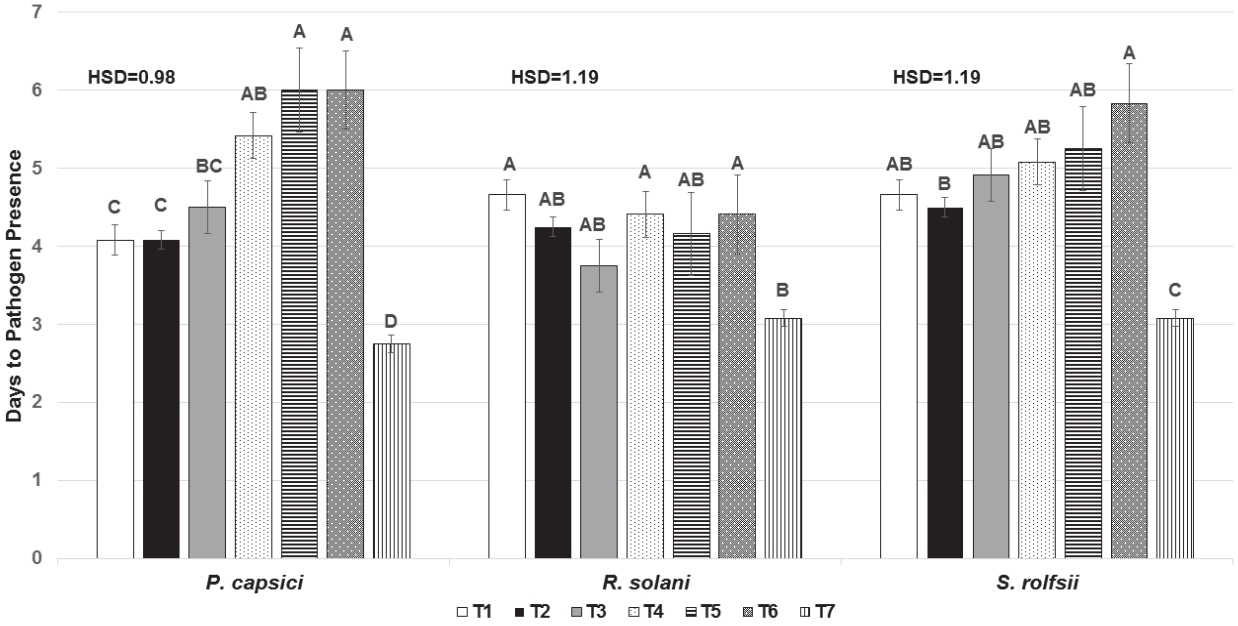


**Figure 3.** Chemical and Biological control of *P. capsici, R. solani* and *S. rolfsii* in pumpkin fruits in the greenhouse experiment. T1: *Trichoderma asperellum* strain CSAEGRO; T2: PHC® Biopak-F®; T3: PHC® Root Mate®; T4: Q 2000; T5[a]: Previcur® Energy/*Benomil*; T6 [a]: Ridomil Gold® Bravo SC/*Pentaclor* 600F*; T7: Control. DPP: Days to the pathogen presence. [a]: for treatments T5 and T6, the fungicide before slash and in roman letters corresponds to the treatment applied to *P. capsici*, while the fungicide after slash and in italic letters corresponds to the treatment applied to *R. solani* and *S. rolfsii* . HSD: Tukey's Honest Significant Difference at 5% significance level.

efficiency of 97.5% over the control of *Pythuim aphanidermatum* (Edson) Fitzp. The multi-site fungicide Chlorothalonil turned out to be one of the most effective treatments in the range of fungicides assessed in combination with metalaxyl. This is because it is a highly toxic compound over oomycetes, as Gisi and Sierotzki (2008) report.

Regarding to *R. solani,* Amrutha et al. (2014) point out that the key factors that contribute to antagonistic effect of *Trichoderma* are: its quick growth, production of metabolites antimicrobials, and physiological characteristics (El-Katatny and Emam, 2012). Vargas-Hoyos et al. (2012) assessed several isolations of *Trichoderma in vitro* and *in vivo* in greenhouses as biocontroller agents of *R. solani* and *S. rolfsii* and the specie *T. asperellum* had the best control over these pathogens. Likewise, Asad et al. (2014) and De França et al. (2014) studied the biocontroller effect of several species of *Trichoderma* and it was found that *T. asperellum* was one of the most effective for *R. solani* control. It is generally accepted that the fungi toxicity of this compound is due to the peroxidation of lipids in the membranes, the effectivity of *T. asperellum* was confirmed in this research based on the good control obtained. Bokshi et al. (2007) in a study in *Cucumis melo* L. using iodine against *Fusarium* sp., *Alternaria* sp. and *Rhizopus* sp. to prevent rotting of postharvest fruits, they found that using hot iodine as post-harvest treatment, most of the pathogens causing rot were controlled. Mendoza-Zamora (1990) found that quintozene affected the integrity of the membrane, cell walls and mitochondria of phytopathogenic fungi, decreasing sclerotia and infectious propagules' formation. Finally, in relation to *A. rolfsii*, Cavallo et al. (2005) assessed the effect of the PCNB in combination with carboxin+tiram, and they found that such active ingredient was which had more control of true fungi in peanut (*Arachis hypogaea* L.). Chastagner (2002) reports PCNB as an efficient fungicide for *Sclerotium rolfsii* Sacc. var. *delphinii* (Welch) and *Rhizoctonia tuliparum* Whetzel & Arthur in ornamentals.

Although the previous information supports the results found in this research, it is necessary to continue assessing the active ingredients in the strains of isolated pathogens from pumpkin, to design an integrated management process and to validate it in both greenhouse and field conditions.

## Conclusions

The morphological and molecular identification, as well as the pathogenicity tests confirmed that *P. capsici*, *R.*

*solani*, and *S. rolfsii* were the agents causing the fruits rot in pipiana pumpkin (*C. argyrosperma*) in Cocula, Guerrero, Mexico. The propamocarb + fosetyl-Al, metalaxyl + Chlorothalonil fungicides, quintozene, and the biocontrol agent *T. asperellum* delayed the *P. capsici*, *R. solani* and *S. rolfsii* presence at 6.00, 6.00, 4.67, and 5.83 days, respectively, compared to around 3 days for the control treatment.

## Conflict of Interest

The authors have not declared any conflict of interest.

## ACKNOWLEDGEMENTS

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**Abbreviations: ITS,** Internal transcribed spacer region; **IGS,** internal intergenic spacer; **NCBI,** National Center for Biotechnology Information; **DNA,** deoxyribonucleic acid; **RNA,** ribonucleic acid; **PCR,** polymerase chain reaction; **PCNB,** Pentachloronitrobenzene; **CFU,** colony forming units.

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*Full Length Research Paper*

# Soil DNA isolation to use in polymerase chain reaction (PCR) amplification

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**The polymerase chain reaction (PCR) is a relatively simple technique that amplifies a DNA template to produce specific DNA fragments *in vitro*. Basic PCR is commonplace in many molecular biology labs where it is used to amplify DNA fragments and detect DNA or RNA sequences within a cell or environment. The method is rapid, cost efficient, and when combined with suitable internal controls can be applied to the detection and quantification of specific soil organisms or pathogens on a large-scale basis. In the present study, we have adapted this approach to soil samples, providing for a simple extraction protocol which can be used directly with PCR amplification without additional DNA purification**

**Key words:** DNA extraction, polymerase chain reaction (PCR), soil organisms.

## INTRODUCTION

Polymerase chain reaction (PCR) is a revolutionary method developed by Kary Mullis in the 1980s. PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. Polymerase chain reaction (PCR) has rapidly become one of the most widely used techniques in molecular biology and for good reason: it is a rapid, inexpensive and simple means of producing relatively large numbers of copies of DNA molecules from minute quantities of source DNA material--even when the source DNA is of relatively poor quality.

The PCR is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size (Cheng et al., 1994). PCR is being applied more often to the assay of microorganisms in the environment, including soils (for a review, Steffan et al., 1991). The simplicity of this technology, together with its potential to detect small numbers of target organisms without a need for the culturing of cells, easily makes it an important method for monitoring pathogens and indicator bacteria. Despite this potential, technical limitations have continued to limit the large-scale use of PCR with soil samples primarily because extraction techniques have been labor

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intensive and often unreliable. While debate regarding the potential for genetic exchange in soils has continued for more than two decades and genetically engineered organisms are being released ever more frequently, the quantification of genetic transfer and our knowledge of the fate of genetic materials in soils remain surprisingly limited. Such questions underline the need to develop more-effective large-scale methods which can be efficiently applied when many samples must be evaluated. Subsequently modified the procedure by using polyvinylpyrrolidone to remove soil organic matter from the cell preparations and repetitive cesium chloride density gradient centrifugation to purify the DNA. While these approaches have been effective, they remain very labor-intensive. Methods also have been developed specifically for use with PCR amplification. For example, Pillai et al. (1991), developed a method to separate bacterial cells by modified sucrose gradient centrifugation, but again the approach is too labor-intensive for wide-scale application. The direct extraction of DNA from soil organisms without prior purification or culturing clearly would provide an attractive alternative.

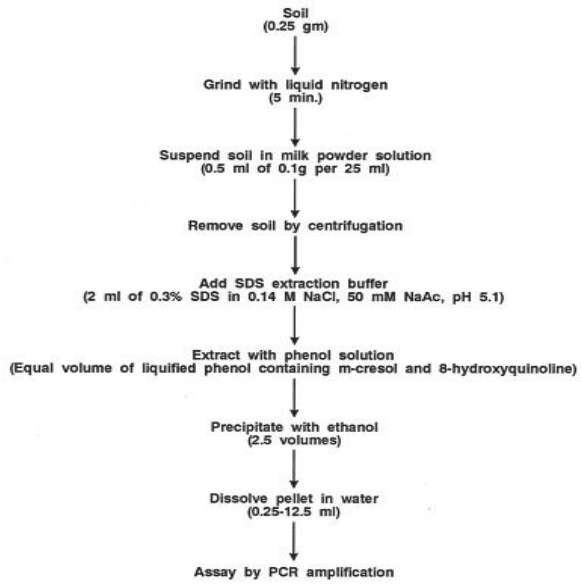


**Figure 1.** Outline of direct soil DNA extraction protocol.

## MATERIALS AND METHODS

### Organisms and plasmids

Three types of DNA were used as targets for PCR amplification: purified *Verticillium dahliae* genomic DNA, an internal control template cloned in pTZ19R (Hu et al., 1993), and purified *V. dahliae* microsclerotia, kindly provided by G. Lazarovits. For genomic DNA, mycelia were grown without light in Czapek's broth Tuite (1969), at 228°C with shaking, and the DNA was extracted by the hexadecyl trimethylammonium bromide (CTAB) method of Rogers and Bendich (1985), as previously described by Hu et al. (1993). The plasmid control template DNA was prepared as described by Holmes and Quigley (1981). Both types of DNA were purified further by CsCl density gradient centrifugation (Radloff et al., 1967), and the amount of DNA was determined at *A*260 with the assumption that 1 unit of double-stranded DNA at *A*260 is equivalent to 50 mg/ml.

### Extraction of DNA from soil

The optimized protocol developed in this study was based on previously described direct extraction methods for plant tissues containing *Verticillium* pathogens (Nazar et al., 1991). As indicated in Figure 1, in the basic procedure, 0.25 g of soil sample is ground with liquid nitrogen by using a mortar and pestle for about 5 min or until a fine powder remains. The powdered soil is suspended in 0.5 ml of skim milk powder solution (0.1 g of milk powder in 25 ml of $H_2O$) by vigorous vortexing; for quantitative assays, internal control template DNA (usually 500 pg) is also added at this time. The soil and debris are removed by centrifugation at 48°C (12,000 3 g, 10 min), and the supernatant is mixed with 2 ml of sodium dodecyl sulfate (SDS) extraction buffer (0.3% SDS in 0.14 M NaCl, 50 mM sodium acetate [pH 5.1]) by vortexing. An equal volume of water-saturated phenol solution (Steele et al., 1965), is added; the phases are mixed by intermittent vortexing for 2 min at room temperature and then separated by centrifugation (12,000 3 g, 10 min).

### PCR amplification of soil DNA extracts

Five microliters of DNA extract was assayed; usually, the extract was first diluted 50-fold to reduce or avoid inhibiting substances. PCR amplification normally was conducted by using 50 ml. The PCR reaction mixture containing PCR buffer (normally, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl [pH 9.0], 0.1% Triton X-100), 0.1 mg of bovine serum albumin (BSA) per ml, 0.2 mM each deoxyribonucleotide triphosphate, 12.5 pmol of each *V. dahliae* specific oligonucleotide primer (Nazar et al., 1991), 2 U of *Taq* DNA polymerase (Promega Corp., Madison, Wis.), and the DNA extract. The primers were synthesized by using a Cyclone Plus automated oligonucleotide synthesizer (Milligen/Biosearch, Milford, Mass). The amplification was performed in a programmable block (Pharmacia Biotech, Uppsala, Sweden) by using 30 reaction cycles, each consisting of a 1-min denaturation step at 948°C, a 1 min annealing step at 608°C, and a 2 min elongation step at 728°C. For nested PCR amplifications, the first amplification was carried out with a second set of oligonucleotide primers (CTCATAACCC TTTG TGAACC and CCGAGGTCAACCGTTG CCG), with target sites external to the standardized *V. dahliae*-specific primers which are used in the second amplification phase.

The products of PCR amplification were analyzed after fractionation by agarose gel electrophoresis. Usually, 5 ml of the PCR reaction mixture was mixed with 2 ml of loading dye (5% SDS, 25% glycerol, 0.025% bromophenol blue), heated to 658°C for 1 to 3 min, and loaded on a 2% horizontal slab gel (McDonnell et al., 1977). When the dye marker was approaching the bottom of the gel slab, the gel was stained for 40 min with ethidium bromide (0.5 mg/ml), rinsed with water (Sharpe et al., 1973), and visualized with a UV transilluminator (300 nm). For quantitative measurements, a charge-coupled device camera imaging system and Molecular Analyst/PC software (Bio-Rad Laboratories, Hercules, Calif.) were used to capture the image and to calculate the band intensities.

## RESULTS AND DISCUSSION

In this study, three potential problems were considered in

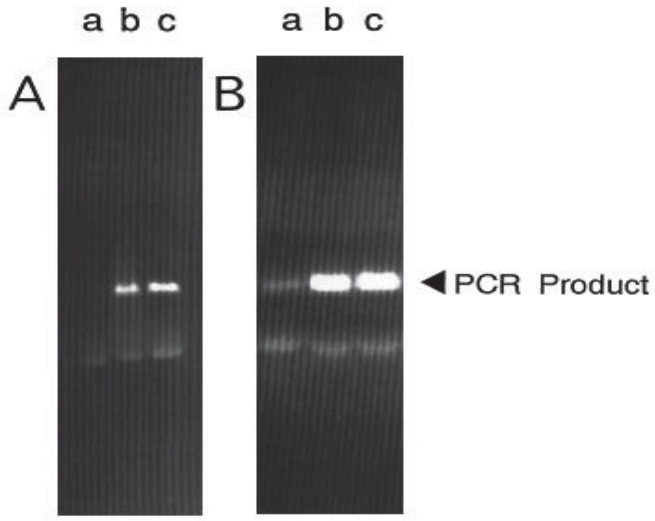


**Figure 2.** PCR amplification of direct soil extracts. (A) Two farm soil samples from different regions containing 2 pg/g of control template DNA were extracted as described in Figure 1 without skim milk powder, PCR amplified, and fractionated by agarose gel electrophoresis (lanes a and b). A reaction mixture containing an equivalent amount of purified template DNA is included in lane c. (B) A third soil sample containing control template DNA also was extracted, and both undiluted (lane a) and 50-fold diluted (lane b) extracts were PCR amplified and fractionated. A reaction mixture containing an equivalent amount of purified template DNA is included in lane c as an uninhibited control reaction and marker for the 294-bp product.

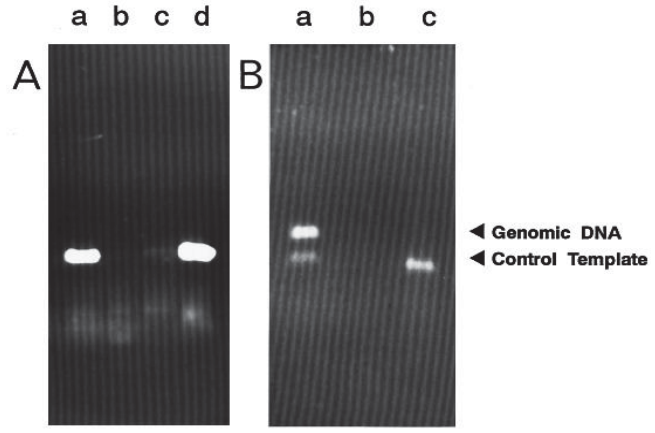


**Figure 3.** Effect of additional treatments on the extraction and PCR amplification of soil DNA. (A) A liquid nitrogen-ground uninfected farm soil sample containing control template DNA (2 pg/g of soil) was treated further by vortexing with glass beads (lane a), by microwave heating (lane b), or by brief boiling (3 times) (lane c) before extraction and PCR amplification as described in the legend to Figure 2. A reaction mixture containing an equivalent amount of purified template DNA is included in lane d. (B) A *V. dahliae*-infected farm soil sample containing control template DNA (4 mg/g of soil) was ground with liquid nitrogen and extracted with SDS-phenol (lane a) or alkaline SDS-phenol (lane b) and PCR amplified. A reaction mixture containing an equivalent amount of purified template DNA is included in lane d.

the application of PCR amplification to soil samples: DNA losses due to degradation and adsorption as well as reaction inhibiting contaminants. In parallel, an attempt was made to maximize the simplicity of any extraction procedure. Because cell or DNA purification steps are especially labor-intensive, direct extraction after cell grinding in liquid nitrogen was adapted as a minimal method for cell disruption and DNA extraction. Previous experience had indicated that grinding in liquid nitrogen was entirely sufficient to disrupt both plant and fungal tissues (Nazar et al., 1991). In the present study, the soil actually provided additional abrasion in the cell disruption process and the use of liquid nitrogen allowed cell disruption under temperature conditions which minimized nucleic acid degradation.

Usually the nucleic acid was extracted with SDS buffer phenol (Steele et al., 1965), a very common nucleic acid extraction procedure for biochemical or genetic analyses which also had proven to be effective with plant and fungal tissues (Nazar et al., 1991). As illustrated by the examples shown in Figure 2 (gel A), when a target organism or an internal control template was added to soil samples such basic extracts often could not be adequately amplified directly (lane a), but some signal was occasionally detected even without dilution (lane b). In many cases the signal strength could be increased significantly by further dilution of the extract (Figure 2, gel B) presumably because levels of inhibiting substances are reduced and PCR amplification remains sufficiently sensitive to permit the detection of target DNA. Because more-drastic disruption methods or conditions have previously been used for soil extracts, additional treatments also were examined; they included the use of an alkaline SDS extraction buffer, often used for DNA preparations (18); vortexing with glass beads; microwave heating or freeze-thawing to disrupt the cells (Steffan et al., 1988), (Smalla et al., 1993); and additional extraction with acetone or acetonitrile to remove substances which may interfere with PCR amplification. As illustrated in the examples shown in Figure 3, none of these treatments was found to be beneficial and most actually reduced the signal or even eliminated it entirely. For example, an alkaline SDS buffer (gel B, lane b) resulted in much higher levels of inhibition, presumably because additional inhibitors were extracted under alkaline conditions, and boiling (gel A, lane b) led to much higher losses presumably because the DNA was degraded or adsorbed. Whatever the mechanism, these methods were not helpful and were not incorporated in the standardized protocol. Because soil sample signals often remained lower than those of equivalent DNA controls even when inhibiting substances were not detected, further efforts were made to eliminate losses due to absorption or degradation. In many biochemical studies of various nucleic acids, adsorption and degradation are often minimized through the addition of nucleic acid carrier or other polyvalent polymers. In hybridization

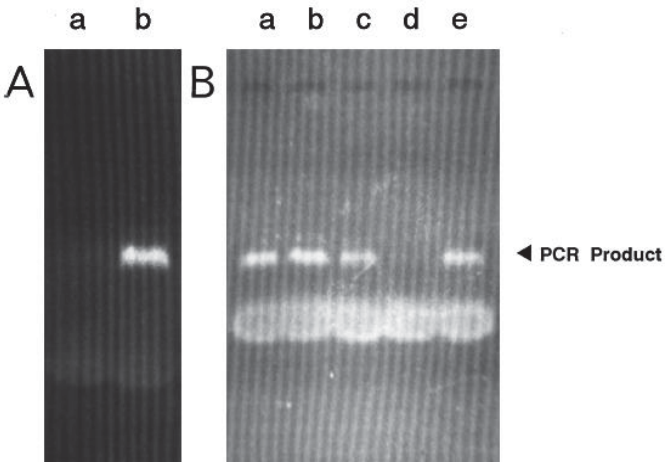


**Figure 4.** Effect of Denhardt's solution on extraction and PCR amplification of soil DNA. Denhardt's solution (A) or the individual constituents (B) were added to 0.25 g of liquid nitrogen-ground soil containing 0.5 mg of target DNA, and the mixture was extracted with SDS-phenol as described in the legend to Figure 2. The extracted DNA was dissolved in 250 ml of water, and 5 ml aliquots of 50-folddiluted extract were PCR amplified before fractionation by agarose gel electrophoresis. (A) For the complete Denhardt's solution, lanes a and b represent extracts without and with Denhardt's solution, respectively. (B) For the individual components, lanes a to c represent extracts with 1% bovine serum albumin, 1% Ficoll, or 1% polyvinylpyrrolidone, respectively. Lanes d and e represent reaction mixtures with no macromolecular carrier added and with only an equivalent amount of purified target DNA, respectively.

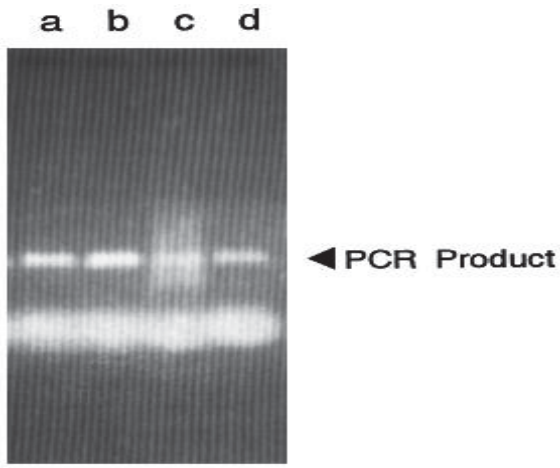


**Figure 5.** Effect of skim milk powder on extraction and PCR amplification of soil DNA. Skim milk powder solution was added to 0.25 g of liquid nitrogenground soil containing 0.5 mg of target DNA, and the mixture was extracted with SDS-phenol as described in Figure 1. The extracted DNA was dissolved in 250 ml of water, and 5 ml aliquots of 50-fold-diluted extract were PCR amplified before agarose gel fractionation. Lanes a to c represent extractions with 0.01, 0.1, and 1 g of milk powder per 25 ml of water, respectively, and lane d contains a reaction mixture with an equivalent amount of purified target DNA.

analyses, for example, Denhardt (1966), addressed this problem by incorporating a mixture of three carrier macromolecules: 1% BSA, 1% Ficoll (Pharmacia Biotech Inc., Uppsala, Sweden), and 1% polyvinylpyrrolidone, commonly referred to as Denhardt's solution. To evaluate the possibility that such a solution or one of the constituents might significantly reduce or eliminate losses due to degradation or adsorption, the three components, both as a complete mixture and as individual components, were added to the soil immediately prior to the extraction buffer. As illustrated in Figure 4, all were often found to significantly improve the signal strength, and when the PCR product yield was compared with the yield of control reactions containing only equivalent amounts of target DNA (gel B, lane e), the recovery was clearly high, with little loss of target DNA. In fact, a slight increase in signal strength was often observed (e.g., gel B, lane b), possibly because the carriers further stabilize the *Taq* DNA polymerase or enhance the reaction by some other means. Because the constituents of Denhardt's solution are relatively expensive and not always readily available, a more common carrier was examined, namely, skim milk powder. This substance has also been reported to be effective as a carrier in reducing background signals and clearly would be inexpensive and readily available. As shown in Figure 5, with the same soil sample as used in Figure 4, the results were again very satisfactory, with 0.1 g of milk powder per 25 ml of $H_2O$ being an optimized concentration (lane b). Without milk powder virtually no signal was observed (Figure 4). Lower concentrations often resulted in a reduced signal strength (e.g., in lane a with 0.01 g of milk powder the signal is reduced by 42%), and higher concentrations resulted in streaking (e.g., lane c). Furthermore, as shown in Figure 6, when applied to typical farm soils from six diverse regions of Iran, a signal was sometimes detectable without dilution, but the signal strength was always strong when the extracts were diluted 50-fold prior to PCR amplification. As shown in Figure 7, the standard protocol (gel A) was equally successful with sand and fine gravel (lanes a, c, e, and g), but only trace or no signals were observed with clay (lanes b and f). As also shown in Figure 7, this problem could be partially overcome with the use of higher milk powder concentrations (gel B). Although quantitative analyses could remain a problem with clay samples, the target DNA was detectable. Because control template DNA was used in developing the extraction protocol, key experiments were also repeated with microsclerotia, a highly resistant storage form of *V. dahlia* which is commonly found in soils. As illustrated in Figure 8, the conclusions were the same for both standard and nested PCR amplification. The genomic DNA signal was relatively weak with the standard amplification protocol (gel A, lane a) but much stronger after nested PCR amplification (gel B, lane a).

As previously noted by other investigators (Haqqi et al., 1988), the application of nested PCR provides for a

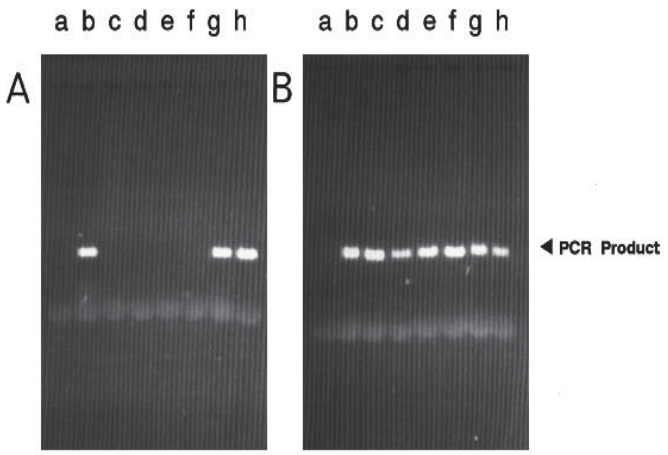


**Figure 6.** Extraction of DNA from soils of diverse origins. Target DNA was added to six different farm soil samples (lanes b to g) from diverse areas in Canada, and 0.25 g samples containing 0.5 mg of target DNA were extracted with SDS-phenol as described in Figure 1. Undiluted (A) and 50-fold-diluted (B) extracts were PCR amplified, and the reaction products were fractionated by agarose gel electrophoresis. Reaction mixtures with no extract (lanes a) and an equivalent aliquot of target DNA (lanes h) are included.

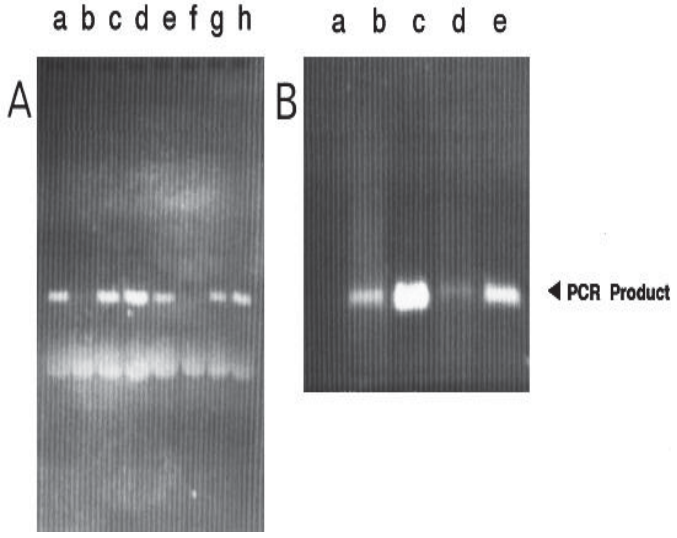


**Figure 7.** Extraction and PCR amplification of DNA from shoreline samples. (A) Control template DNA was added to sand, clay, or gravel taken from a lake shoreline, and 0.25-g samples containing 0.5 mg of control template DNA were extracted with SDS-phenol as described in Figure 1. Undiluted extracts (lanes a toc) and 50-fold-diluted (lanes e to g) extracts were PCR amplified before fractionation by agarose gel electrophoresis. Reaction mixtures containing equivalent amounts of purified target DNA are included (lanes d and h, respectively). (B) A clay sample of milk powder containing target DNA was further extracted by using 1 g/25 ml of water, and undiluted (lane b) or 50-fold-diluted (lane d) aliquots were PCR amplified. Reaction mixtures containing equivalent amounts of purified target DNA are included (lanes c and e, respectively), and a reaction mixture without extract is included (lane a).

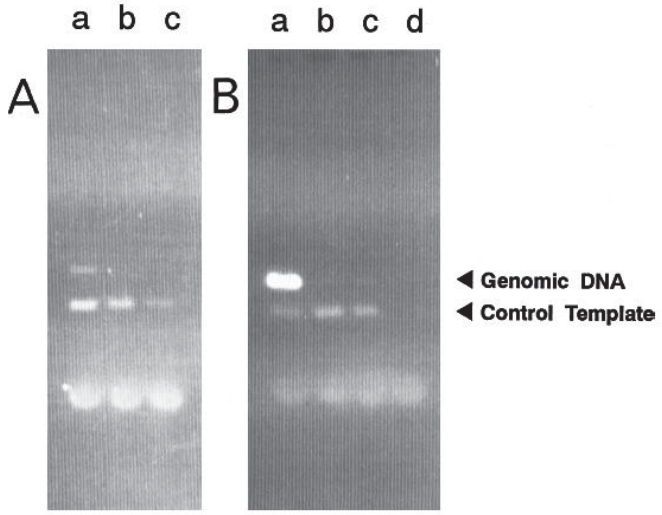


**Figure 8.** Extraction and PCR amplification of DNA from soil containing microsclerotia of *V. dahliae*. An internal control template was added to farm soil (0.25 g) containing 1 mg of microsclerotia which was extracted as described in Figure 1 and PCR amplified by using a standard (A, lane a) or nested (B, lane b) PCR protocol before fractionation by agarose gel electrophoresis. With 30 cycles of *V. dahliae*-specific amplification (lane a), a soil extract without microsclerotia and a reaction mixture with an equivalent aliquot of control template are included (lanes b and c, respectively). By nested PCR with two 30-cycle amplifications (lane a), a reaction mixture with only the second phase of *V. dahliae*specific amplification and one containing an equivalent aliquot of control template are included (lanes b and c, respectively). Lane d contains a reaction mixture without extract.

more dramatic level of sensitivity and permits much higher levels of dilution and diagnostics which should be able to detect almost any level of microbe activity in soil samples. In summary, therefore, a rapid and cost-effective method to extract DNA directly from soil samples which can be utilized with PCR amplification to effectively detect specific soil organisms has been developed. Many PCR-based assays for specific organisms have already been developed and many more are certain to follow. The extraction procedure which is defined by this study should be applicable to many, if not all, of these specific assays, providing for accurate and efficient monitoring of these target organisms in soil. Extracts from samples containing large amounts of clay are less effective, but qualitative analyses are possible and the use of internal control templates should permit quantitative analyses as well.

## CONCLUSION

### Limitations of PCR and RT-PCR

The PCR reaction starts to generate copies of the target sequence exponentially. Only during the exponential

phase of the PCR reaction is it possible to extrapolate back to determine the starting quantity of the target sequence contained in the sample. Because of inhibitors of the polymerase reaction found in the sample, reagent limitation, accumulation of pyrophosphate molecules, and self-annealing of the accumulating product, the PCR reaction eventually ceases to amplify target sequence at an exponential rate and a "plateau effect" occurs, making the end point quantification of PCR products unreliable. In summary, The discovery in 1976 of Taq polymerase -a DNA polymerase purified from the thermophilic bacterium, *Thermus aquaticus*, which naturally lives in hot (50 to 80°C (122 to 176 °F)) environments (Chien et al., 1976). Such as hot springs- paved the way for dramatic improvements of the PCR method. the genetic differences observed through RFLP analysis of the PCR amplified nuclear rDNA IGS region and mitochondrial SSU rRNA gene indicated intraspecific variability in *V. dahliae*, separating isolates from olive from those in other hosts. Further research, using a more representative set of isolates, including cross pathogenicity studies with all isolates, and full length sequencing of PCR products, will be necessary to determine if intraspecific groups continue to correlate with the plant hosts.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Postharvest conservation of cherry tomato with edible coating

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**This study aimed at evaluating quality maintenance in cherry tomatoes covered with edible films of yam starch and glycerol. Cherry tomatoes were acquired in the region of Viçosa (State of Minas Gerais, Brazil) and carried to UFV's Centreinar Laboratory. The tomatoes were washed and sanitized, then immersed in three suspensions of yam starch and glycerol: I - 7.5% starch and 30% glycerol, II - 7.5% starch and 40% glycerol and III - 7.5% starch and 50% glycerol, beyond tomatoes that were not immersed, at an average temperature of 25°C. Later, the solution was dried at a temperature approaching environment temperature (25 ± 3°C). The tomatoes coated with film were kept in a controlled environment of 25°C and 70% relative humidity, for 18 days. We performed analyses of loss in mass, total soluble solids, total titratable acidity and firmness every other day, beyond initial and final quantification of phenolic compounds, antioxidant activity and lycopene content in the coated and uncoated tomatoes. Coating with 7.5% yam starch and 30% glycerol promoted higher stability for the loss in mass, soluble solids/total titratable acidity ratio, phenolic compounds, antioxidant activity and lycopene content in relation to the freshly harvested fruit, since it got 46 and 18% less of loss in mass and soluble solid/total titratable acidity ratio, respectively, indicating the slower maturation of the fruit. Therefore, it was efficient for preserving the shelf life and quality of the cherry tomato.**

**Key words:** Nutritional quality, antioxidants, perishable, shelf-life, postharvest, *Lycopersicum esculentum*.

## INTRODUCTION

Brazilian tomato (*Lycopersicum esculentum*) cultivars can be divided in five groups: Santa Cruz, Salad or Kaki, Saladinha, Saladete or Italian, and Cherry. Cherry tomato, known by the Brazilian consumer market since the 1990s, is mainly characterized by its sensory properties, like excellent taste and attractive, uniform red coloration. Recently, there has been an increasing demand for this fruits, which is used in the decoration of

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dishes, as well as in restaurants (Alvarenga, 2004; Rocha et al., 2009).

Tomato has the status of functional food as it contains antioxidant substances like vitamin C, lycopene (Lyc) and phenolic compounds, which have a preventive role, especially against certain types of cancer and chronic non-communicable diseases (George et al., 2004). The fruit of the tomato plant has a high water content and is subject to variations of temperature and relative humidity of its environment, being thus a highly perishable fruit. Loss in water causes loss in mass and appearance of the fruit (Chiumarelli and Ferreira, 2006). Low relative air humidity results in higher weight loss, withering and nutritional losses (Kader, 1986). Therefore, biodegradable packaging can be used to slow down abovementioned alterations and, consequently, to increase the fruit's shelf life. The quality of coated tomatoes has been evaluated according to some parameters: acidity, soluble solid content, sugar content, Lyc content, appearance, texture, taste, size and succulence (Monteiro et al., 2008).

Application of edible coatings to fruits promotes the formation of a cover with partial filling of the stomata and lenticels, thus reducing moisture transfer (transpiration) and gas exchanges (respiration), which allows, in principle, to extend the fruit's life (Assis et al*.,* 2009). Biodegradability is also a big advantage of edible coatings which needs to be highlighted (Luvielmo and Lamas, 2012). Among the compounds most used to make edible coatings are the polysaccharides (starch and its derivatives, pectin, cellulose and its derivatives, alginate and carrageenan), which, alone or in combination with other compounds (proteins, lipids), are used profitably to put in practice the specific characteristics of each compound class (Luvielmo and Lamas, 2012). Yam has 84.94% starch (Daiuto and Cereda, 2006), which favors the use of this tuberous root for extraction of starch and, consequently, exploitation of this compound in the making of edible films.

Glycerol is a hydrophilic plasticizer widely used in the preparation of biodegradable films, since it interacts with the starch chains, increasing molecular mobility and, consequently, hydrophilicity and flexibility of the plastic films (Mali et al*.,* 2004). Edible packaging films must provide good food protection without losing quality through handling, and must also be flexible enough to adapt to possible food deformations, without mechanical damages (Alves, 2005).

Edible films were made with 4% yam starch and 1.3 and 2.0% glycerol to extend the shelf life of strawberries stored at 4°C and 85% relative humidity. Packaging significantly reduced fruit deterioration by comparison with the treatment without coating (Mali and Grossmann, 2003).

Experiments with starch as the main component in the making of edible films have found positive effects. Castriini et al. (2010) evaluated the influence of cassava starch coating at 1, 3 and 5%, in the maturation of entire papayas, during 14 days of storage. This research used cassava starch formulations, and the 3 and 5% coatings reduced the loss in mass, keeping the green coloration during storage.

Trigo et al. (2012) applied edible coating made with 3% rice starch, 0.5% sodium alginate and 0.25% carboxymethyl cellulose to minimally processed papayas stored at 5°C and 90% relative humidity, and observed positive effects of the coatings by the 12th and 15th day for the preservation of the useful life of this fruit.

In this study, we expected the interaction between starch and glycerol to form solutions capable of maintaining the quality of the cherry tomato for longer, preserving traits like reduced mass loss, reduced fruit maturation and better preservation of the functional characteristics, like Lyc content, antioxidant and polyphenol. The objective of this study was to evaluate the postharvest quality of the cherry tomato coated with different edible films made of yam starch and glycerol, stored at 25°C and 80% relative humidity for 18 days.

## MATERIALS AND METHODS

About three hundred cherry-type tomatoes, *L. esculentum*, in maturation stage with more than 90% red content, visually quantified, just harvested, acquired directly from a producer of the region of Viçosa (State of Minas Gerais) were carried in plastic trays to the Centreinar laboratory of the Federal University of Viçosa. The fruits were selected in function of size, color and lack of damage, washed in running water and sanitized with a cooled 200 mg $L^{-1}$ sodium hypochlorite solution for 15 min under room conditions (Prates and Ascheri, 2011).

Starch extraction, elaboration of the filmogenic solution and selection of the treatments used in the present study were made in accordance with Reis et al. (2013). The following three treatments were used: T1 (7.5% yam starch + 30% glycerol), T2 (7.5% yam starch + 40% glycerol), and T3 (7.5% yam starch + 50% glycerol). The control treatment (T0) was realized without immersion of the fruits in the filmogenic solution. After the mixing of starch, glycerol and distilled water, the solutions were warmed up on a hotplate, for 4.5 min at 90°C and were left to settle until reaching a temperature close to room temperature (25 ± 3°C), being monitored by means of a digital skewer thermometer. Each tomato was immersed in the filmogenic solution for 5 min, suspended by tweezers, then disposed on expanded polystyrene trays and kept under conditions close to room conditions, at 25°C and 70% relative humidity, until drying of the coating, for about 30 min (Prates and Ascheri, 2011).

The experimental unit, represented by the tray, consisted of ten fruits. The twenty trays, 3 treatments + control (5 replications each) containing the fruits with fixed and dried coverings were stored for 18 days in a B.O.D. incubation chamber (Marconi, MA415, Brazil), at a temperature of 25 ± 1.0°C and 80 ± 5% relative humidity.

The firmness of the fruits was evaluated by means of compression assays, carried through with a Universal Testing Machine, TA.HD Texture Analyser, (Stable Micro Systems, United Kingdom), also known as texturometer, equipped with the software Texture Expert for Windows® with a load cell of 500 N. For the test, we used a circular flat plate probe with 100 mm in diameter and at a test speed of 0.02 m $min^{-1}$ (Van Dijk et al*.,* 2006; Batu, 2004). Obtaining the compression curves of the product (force in function of the deformation), the values of the maximum force sustained by the fruit were determined, measured in newton (N).

The chemical analyses was based on the methodologies

recommended by the Adolf Lutz Institute (Brasil, 2005). Before each chemical analysis, the fruits were washed with distilled water, then dried at room temperature to remove the coatings. The fruits were manually kneaded and filtered. The total soluble solid content was determined by means of a digital refractometer (Ceti, Belgium), with 0.1 precision and direct reading, using one or two pulp drops. The total titratable acidity was obtained by titration with a 0.1 mol $L^{-1}$ sodium hydroxide solution. The loss in mass was evaluated in all storage periods using a digital scale (Scientech, AS-210, United States), with $10^{-6}$ kg precision, and the results being expressed in percentage.

The analyses of phenolic compounds and antioxidant activity used a crude extract from the samples submitted to extraction with methanol 50% (v/v), followed by extraction with acetone 70% (v/v), according to the methodology described by Rufino et al. (2007). The determination of the phenolic compound content was based on the Folin-Denis colorimetric method. The total phenolic values were expressed as equivalents of Gallic acid (mg Gallic acid equivalent (GAE) for 100 g sample, in wet base) (AOAC, 2000).

The evaluation of the antioxidant activity was made by the DPPH method (2,2-diphenyl-1-picrylhydrazyl), according to the methodology described by Duarte-Almeida et al. (2006) and Andrade et al. (2007), with a reaction time of 0.5 h, absorbance being measured at 517 nm with a spectrophotometer UV-VIS (E225 D, single-beam, United States ). DPPH ethanol solution (1 M) was used as control. To evaluate the mentioned activity, the inhibition percentage was obtained according to Equation (1):

$$AA = \frac{Abs_c - Abs_a}{Abs_c} \times 100 \quad (1)$$

Where: AA is the antioxidant activity (%); $Abs_c$ is the reading of the absorbance of the control, and Absa is the reading of the absorbance of the sample.

The Lyc content was quantified according to the methodology described by Leão et al., (2006). The extract obtained with the described methodology was transferred to a volumetric flask of 100 ml, whose volume was completed up to 80 ml and then taken for reading to a spectrophotometer UV-VIS (E225 D, single-beam , United States). In the specific wave length for Lyc, 470 nm. Equation (2) was used to calculate the Lyc content in µg $g^{-1}$:

$$Lyc = \frac{A \times V \times 10^{-6}}{A_{1cm1\%} \times M} \times 100 \quad (2)$$

Where: A is the absorbance measure; V is the final solution volume (L); $A_{1cm1\%}$ is the pigment extinction coefficient in a specific solvent = 3450; and M is the mass of the sample (kg) (in wet base).

The experiment used a completely randomized split plot in time design, for the following variables: loss in mass, soluble solid ratio, total titratable acidity and firmness. With regard to total phenolic content, antioxidant activity and Lyc content, since the analysis was carried out solely at the beginning and at the end of the storage, only the completely randomized design was used. All analyses were carried out in five replications.

The plots were formed by the glycerol concentration used in each treatment, and the subplots by the storage times of the fruits. Three treatments of the fruits with filmogenic solution coating (T1, T2 and T3) and a treatment without coating (C = control) were contemplated. Ten storage periods were considered (0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 days). The results were submitted to variance analysis at 5% probability ($p \leq 0.05$). To describe the characteristics of the samples in function of the storage periods, we carried out first and second degree regression analyses, the choice of the best model being made through observation of the significance of the F test for each model at 5% level. To make the statistical analyses, the software SAS - Statistical Analysis System, version 9.1, licensed to the Federal University of Viçosa was used (SAS, 1999). The degree of adjustment of the regression model considered the magnitude of the determination coefficient ($R^2$), the magnitude of the relative average error (P) and the standard deviation of the estimate (SE). The relative average error and the standard deviation of the estimate for each model was calculated using Equations (3) and (4), respectively.

$$P = \frac{100}{n} \sum_{i=1}^{n} \frac{|Y - Y_0|}{Y} \quad (3)$$

$$SE = \sqrt{\frac{\sum_{i=1}^{n} (Y - Y_0)^2}{GLR}} \quad (4)$$

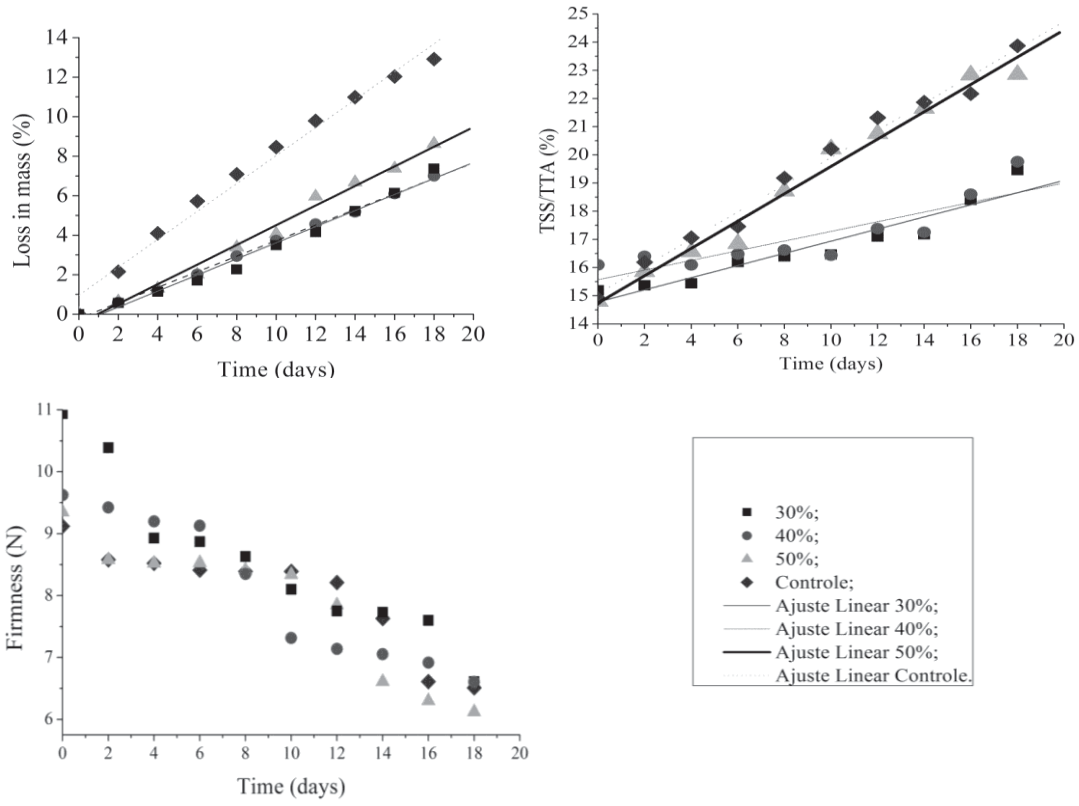
Where: Y is the experimentally observed value, $Y_0$ is the value calculated by the model, n is the amount of experimental observations, and GLR is the number of degrees of freedom of the model.

## RESULTS AND DISCUSSION

The interaction between glycerol variation and storage time was significant (Figure 1A; $p \leq 0.01$) for the variables loss in mass and ratio (Table 1). At 18 days of storage, tomatoes treated with 30, 40 and 50% glycerol had a reduction of loss in mass of, 46, 43 and 33% respectively, compared with those that got no filmogenic solution, that is, being thus the treatment with 30% glycerol with the smallest loss in fresh mass (Figure 1A, $p \leq 0.01$).

Pereira et al. (2006) evaluated the effect of cassava starch coating (1, 2 and 3%) on the loss in mass of the 'Formosa' variety of papaya and detected that the treatments had no effect on that variable. However, Scanavaca Júnior et al. (2007) confirmed that edible coatings with 1, 2 and 3% cassava starch were efficient in reducing the loss in mass of the 'Surpresa' mango variety stored at room temperature.

In the present study, 7.5% yam starch reduced the loss in mass of the tomatoes, regardless of the glycerol amount. This can be associated with the low water solubility index of yam starch (1.78%; Reis et al., 2010), when compared to the 'Manteiga' variety of cassava starch (4.52%; Nunes et al., 2009), since the loss in water can be lower in coatings that use low water solubility starch. When comparing the adopted treatments, one notices that the plasticizer had some effect on the loss in mass of the tomatoes. According to Mali et al. (2004), glycerol is a hydrophilic plasticizer that is much used in the making of biodegradable films. This hydrophilicity causes bigger interaction with water and therefore higher solubility of the coating, which explains that the treatment with higher glycerol percentage causes a greater loss in mass when compared with the other treatments (except for the control treatment). However, the loss in mass of all the treatments with coating was much lower than that of the control, showing that coating is an effective way of slowing down this parameter in the cherry tomato.




$LM_C = 0.96 + 0.71D^*$; $R^2 = 99.24\%$; P = 2.53%; SE = 0.31;
$LM_{30\%} = -0.21 + 0.39D^*$; $R^2 = 99.87\%$; P = 0.13%; SE = 0.02;
$LM_{40\%} = -0.45 + 0.40D^*$; $R^2 = 99.13\%$; P = 0.96%; SE = 0.12;
$LM_{50\%} = -0.47 + 0.49D^*$; $R^2 = 99.38\%$; P = 1.03%; SE = 0.13;
$TSS/TAA_C = 14.74345 + 0.48473D^*$; $R^2 = 98.97\%$; P = 10.22%; SE = 0.89.
$TSS/TAA_{30\%} = 14.78267 + 0.2154D^*$; $R^2 = 90.52\%$; P = 6.02%; SE = 0.60;
$TSS/TAA_{40\%} = 15.56909 + 0.17155D^*$; $R^2 = 86.54\%$; P = 15.44%; SE = 0.99;
$TSS/TAA_{50\%} = 15.08 + 0.48258D^*$; $R^2 = 99.30\%$; P = 9.53%; SE = 0.70;
Where: * is significant at 5% probability for test F; $LM_c$ is the loss of mass of the treatment without covering; $LM_{30\%}$ is the loss in mass of the treatment with 30% glycerol; $LM_{40\%}$ is the loss in mass of the treatment with 40% glycerol and; $LM_{50\%}$ is the loss in mass of the treatment with 50% glycerol; $TSS/TAA_c$ is the ratio between TSS and TTA of the treatment without covering; $TSS/TAA_{30\%}$ is the ratio between TSS and TTA of the treatment with 30% glycerol; $TSS/TAA_{40\%}$ is the ratio between TSS and TTA of the treatment with 40% glycerol; $SA_{50\%}$ is the ratio between TSS and TTA of the treatment with 50% glycerol and; D is the storage in days.

**Figure 1.** A. Loss in mass; 1-B. Ratio of total soluble solids (TSS) and total titratable acidity (TTA) and 1-C Firmness of the cherry tomatoes submitted to the treatments without coating, with 50, 40 and 30% glycerol with 7,5% yam starch during the storage time for 18 days at 25 ± 1°C (Figure 1-A. Loss in mass; 1b. Value of total soluble solids (TSS) and titratable acidity (TTA) and 1-C Firmness of cherry tomatoes subjected to uncoated treatments, 50% 40 and 30% glycerol for 18 days at 25 ± 1°C). Viçosa, Centreinar-UFV. 2010.

Higher values of TSS/TTA were found in the treatment without coating over time, meaning that the treatment with 30 and 40% glycerol slowed down the maturation of the evaluated tomatoes, followed by the treatment with50% plasticizer (Figure 1B, $p \leq 0.01$). The studied relationship got lower values for the treatments with 30 and 40% plasticizer, due to the influence of the plasticizer. There was no visual difference between the treatment with 50% glycerol and the treatment without coating. The total soluble solid content (TSS) and the total titratable acidity (TTA) determine the TSS/TTA ratio for fruits (°Brix/% citric acid). Thus, a high value of the ratio indicates a mild flavor, whereas low values indicate an acid flavor (Bolzan, 2008). The firmness data of the analyzed tomatoes did not follow any trend to linear or quadratic regression adjustments. It was found that, in all the treatments, firmness diminishes with conservation time (Figure 1C).

Pereira et al. (2006) evaluated the maturation at room temperature of 'Formosa' papaya fruits coated with edible cassava starch-based film, which was applied through immersion of the entire fruits in suspensions of 1, 2 and

**Table 1.** Total phenolic compounds, antioxidant activity and lycopene content of tomatoes freshly harvested and after 18 days without coating and with different treatments with different glycerol content (30, 40% and 50%) in the coating. Viçosa, Centreinar-UFV. 2010.

| Treatment | Total phenolic compounds (mg EAG100 $g^{-1}$) | Antioxidant activity (%) | Lycopene (µg $g^{-1}$) |
|---|---|---|---|
| Freshly harvested | $278^{a*}$ ± 3.9 | $41^{a}$ ±2.2 | $29^{a}$ ±3.3 |
| 7.5% starch + 30% glycerol | $213^{b}$ ±4.2 | $36^{ab}$ ±2.7 | $25^{b}$ ±2.1 |
| 7.5% starch + 40% glycerol | $158^{c}$ ±2.8 | $33^{b}$ ±3.4 | $23^{c}$ ±4.0 |
| 7.5% starch + 50% glycerol | $101^{d}$ ±2.7 | $25^{c}$ ±2.9 | $19^{d}$ ±3.2 |
| Without coating | $48^{e}$ ±3.3 | $20^{c}$ ±4.1 | $14^{e}$ ±4.2 |

*Same letters in the column do not differ statistically at the level of 5% probability.

3%. Results showed that the coatings with 1 and 3% extended the useful postharvest life by four days, maintaining the quality. The treatments slowed down the maturation of the fruits, whose alterations of skin color,pulp firmness, soluble solids and titratable acidity were significantly slower than in the untreated fruits. The same alterations were perceived in the present study.

With time fixed and treatment type analyzed, it was found that treatments, including control, differ during the conservation time. In the analyses of phenolic compounds and lycopene, at 18 days of storage, tomatoes treated with 30, 40 and 50% glycerol, without coating and freshly harvested.

In the analysis of phenolic compounds, lower values were detected in the treatments at 18 days of storage, in relation to the initial average value of the fruit (Table 1). As for the fruits that received no coating, the total phenol content decreased 83% in relation to the initial time. On the other hand, the treatments with 30, 40 and 50% decreased by 24, 44 and 64%, respectively.

Rocha and Silva (2011) evaluated the total phenol content in conventional (Alambra cultivar) and organic (Debora cultivar) tomatoes by the Folin-Ciocalteau method and found values of 59.89 and 45.89 mg 100 $g^{-1}$, respectively. Such values are lower than those of the cherry tomato in the present study. Phenolic compounds in fruits and vegetables can have beneficial effects for eliminating free radicals (Chun et al., 2003). So phenolic compounds can help to protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002). For analysis of the antioxidant activity, the treatment with 30% glycerol did not differ from the freshly harvested tomato nor from the treatment with 40% glycerol, but comparison of the freshly harvested tomato and the tomato treated with 40% glycerol showed a difference significant at 5% probability for the same analysis. There was a reduction of 12, 40, 20 and 51 51, 40, 20 and 12% of the antioxidant activity in the treatments with 30, 40, 50% glycerol and without coating, respectively, in relation to the freshly harvested cherry tomato. Thus, the treatment with 30% glycerol got the value of antioxidant activity closest to the freshly harvested cherry tomato, that is, the best preservation of the activity was observed in this treatment (Table 1).

Robles- Sánchez et al. (2013) studied the content of phenolic compounds with antioxidant capacity of the fresh-cut Kent mangoes coated with sodium alginate, which when compared with the treatment they received no coating showed the same behavior for the two analyzes. The same authors have also included in ascorbic acid and citric acid coating, which increased both the phenolic content as the antioxidant activity of the sleeves in court.

Researches point out that the antioxidant activity of food is related to the phenolic compound content (Martinez-Valverde, 2002; Cheung et al., 2003). Monteiro et al. (2008) analyzed the antioxidant activity of the Italian tomato and found values of 8.65 and 5.94% for tomato pulp and for the entire tomato, respectively, values lower than those we got in this study (Table 1). Lycopene content diminished by 14, 21, 35 and 48% in the treatments with 30, 40, 50% glycerol and without coating stored for 18 days, respectively, in relation to the freshly harvested tomato. We believe that the coating formed a wall and minimized the lycopene oxidation in relation to the uncoated tomatoes, since, according to Baldwin (1999), edible coatings have the capacity to reduce the dehydration and oxidation of the coated product that, consequently, harm its color, taste and texture.

Lycopene appears currently as one of the most powerful antioxidant substances, being recommended to prevent carcinogenesis and atherogenesis by protecting molecules as lipids, low-density lipoproteins (LDL), proteins and DNA (Shami and Moreira, 2004).

Malacrida et al. (2006) reported reduction in lycopene accumulation in tomatoes during the normal maturation and Perkins-Veazie and Collins (2004) pointed out that the lycopene total content was not significantly modified in any cultivar stored for two days at 2°C. The treatment with 30% glycerol got closer values of phenolic compounds, antioxidant activity and lycopene in relation to the initially analyzed fruit. This means that the treatment with coating was the one that best kept the characteristics of the freshly harvested fruit, and it may be considered the most efficient treatment for the conservation of the cherry tomato, since it got the lowest

loss in mass, the lowest ratio of total soluble solids and total titratable acidity (showing lower maturation of the fruit, even if the results did not follow any trend for the variable firmness) in comparison to the other treatments used. Moreover, visually, this treatment delayed the appearance by 8 days in relation to the control.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Environmental constraints and sustainability of dairy cattle farms in the suburban area of the city of Blida (Mitidja, Algeria)

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**The purpose of this study was to assess the agro-ecological sustainability of dairy cattle farms in the suburban area of the city of Blida. An investigation was carried out on 19 farms. The study area, located in the Mitidja plain, is well known for its farming tradition and has suffered over the last decades from countless environmental constraints. The assessment tool used is the *IDEA* method (Indicateurs de Durabilité des Exploitations Agricoles or Farm Sustainability Indicators). The agro-ecological scale comprises three components: domestic diversity, organization of space and farming practices, summing up 18 indicators. Analysis of the results showed that the surveyed farms are characterized by a low agro ecological sustainability (45.97/100) mainly explained by the limited diversity of perennial crops, lack of crop rotation, poor use of space and water, many failures in the management of fodder resources, the non-protection of soil resources, and high energy dependency.**

**Key words:** Assessment, sustainability, indicateurs de durabilité des exploitations agricoles (IDEA) method, dairy cattle, Mitidja.

## INTRODUCTION

Mitidja, long coastal plain of 1400 $Km^2$ located at the centre of Northern Algeria is one of the country's most fertile plains. Its economic and social importance is measured by the extent of its surfaces, to the actual labour and production values. The four departments who administer (Algiers, Boumerdes, Blida and Tipaza) those areas produce more than half of Algeria's citrus production and 20% of rosacea. This plain also houses 75% of tree seedlings production nurseries and horticultural plants in Algeria.

As an alternative for the cultivation adopted in the 70s, following the uprooting of vines in Mitidja, was to create a dairy shed. Thus, an import of high production cows program has been implemented which has resulted in significant growth of this speculation, hence the name of dairy shed attributed to this plain. Competition for farmland is considerable, especially since the 90s urban pressure becoming stronger.

This fast and diffuse urban growth disrupts the structures of this peri-urban agriculture. The land needs to meet urban socioeconomic demand, make difficult farming in this plain which became the place of projection cities of Algiers, Blida, Boumerdes and Tipaza. Farms and especially cattle farms meet several constraints

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that result from evolution characterized by deficiencies in the management of space, higher pressure on the natural environment and strong competition between sectors and stakeholders, economic and social.

To this can be added the combination of other phenomena such as inadequate farming practices, land fragmentation and pollution. These are all contributing factors to the deterioration of the environment and lead us to question the agroecological sustainability of these cattle farms. Various methods based on a quantification of sustainability indicators have been designed to assess the sustainability concept (Biewinga and Van Der Bijl, 1996; De Koning et al., 1997; Rossing et al., 1997).

The *IDEA* method (Indicateurs de Durabilité des Exploitations Agricoles or Farm Sustainability Indicators) (Vilain, 2008) was selected. This method was chosen for its ease of implementation and adaptability to a survey in limited time. It allows us to draw up an inventory of farms regarding the environment as part of this study to assess the agroecological sustainability of 19 dairy cattle farms in the peri-urban area of the city of Blida.

## MATERIALS AND METHODS

The analysis of the agro-ecological sustainability of dairy cattle farms in the peri-urban area of the city of Blida was performed using the *IDEA* method (Farm Sustainability Indicators) (Vilain, 2008). IDEA is based on the assessment scores that establish an overall performance of the farm, from 42 indicators. It assumes that it is possible to quantify the various characteristics of farming systems by assigning a numerical score, and then aggregate the information obtained to get a score or overall performance. The aggregation is based on a rating between 0 and 100, each of the following three scales: i) Agro ecological sustainability that analyzes the ability of a system to combine local resources and includes 18 indicators describing three components: Diversity (4 indicators), Organization of space (7 indicators) and Farming practices (7 indicators); ii) Socio territorial sustainability measures the insertion of farms in its territory and includes 18 indicators describing three components: Quality of products and land (5 indicators), employment and services (6 indicators), Ethics and human development (7 indicators); and iii) Economic sustainability that helps to understand the economic performance beyond the short term and economic uncertainties; and includes six indicators describing four components: Economic viability (2 indicators), independence (2 indicators), transferability (1 indicator) and efficiency (1 indicator). The overall performances of each scale of sustainability are independent and cannot be added. In this study, the choice to address only the agro ecological dimension whose objectives refer according to Viaux (1999) to the principles of integrated farming in order to as low as possible ecological cost is based on the observation made by Imache et al. (2010) which show that agriculture in the Mitidja plain suffers many disadvantages mainly related to a massive and uncontrolled urbanization. This phenomenon creates environmental problems such as land fragmentation, destruction of irrigation systems, shrinkage of grazing areas, trampling plots, vehicle traffic, pollution... which are all factors that threaten sustainability of farms in the plain.

This study is based on surveys carried out in January, February and March, 2012 in 19 farms over 7 municipalities of the *Wilaya* of Blida. Sample selection criteria are based on the dairy vocation of the farm, availability and collaboration of farmers and the need to cover a wide range of farms in terms of herd size, farming land and productions. The raw data was collected using a questionnaire, inspired by the IDEA method and included 64 questions. Afterwards data was processed to calculate the indicators of agroecological sustainability. Information obtained was entered into an Excel spreadsheet to create a database file on which the analyses were performed using Excel (2007) for the descriptive statistics (mean and standard deviations) and SPAD software to build a typology based on a factor analysis of multiple correspondences and hierarchical cluster analysis.

## RESULTS AND DISCUSSION

### Descriptive analysis of farms

The average agricultural area (useable agricultural area; and forage area) is 13.51 ± 11.06 ha. Standard deviations are significant reflecting a wide variability between farms. The Useable Agricultural Area (UAA) of our sample is strongly related to their legal status. Thus, 78% of the farms holding above 10 ha of UAA are owned by collective farms (EAC) and individual farms (EAI) all from the dismantling of the old state-managed farms as a result of the land reform of 1987. Forage crops are present in all surveyed farms with an average area of 7.96 ha of which 22.64% is irrigated. Forage crops were clover (17 farms), oats (13 farms), sorghum (9 farms), maize (4 farms), alfalfa (1 farm) and barley (1 farm). 52% of the farms grow fodder alone. Farms that combine forages and fruit growing stood at 47% of the sample while only 15% of farms grow cereal crops, in addition to the forage and fruit.

The cattle were reared at only 79% of farms. Sheep and goat farming was only present in three farms with a herd size not exceeding 10 heads. One farm practiced turkey farming with 2 flocks / year. There was a significant difference in herd size between the farms. This ranged from 5-144 heads for total cattle and 4-64 heads for the dairy cows. The average of Livestock Units (LU) on the farms totaled 4.79LU / ha of forage area. It appears lower than that reported by Bekhouche (2011) for the same area or 5.44 LU/ but is higher than 2.13 LU / ha observed by Bouzida (2008) for farms of Tizi Ouzou region.

The grazing pattern is not common in the study area since it concerns only 21% of farms. Finally, over 74% of farmers surveyed use to purchase, in addition of the concentrate, roughage mainly oats and vetch hay and straw which shows the growing gap between the livestock needs and production permitted by these farms.

### Typology of farms

Figure 1 shows the projection of the 17 variables characterizing farm structure (Land capital, Livestock number, Labour, Irrigation, Crops,..) in the main plane of the MCA (Multiple Correspondences Analysis) retaining the first two factors accounted for 28.72% of the total

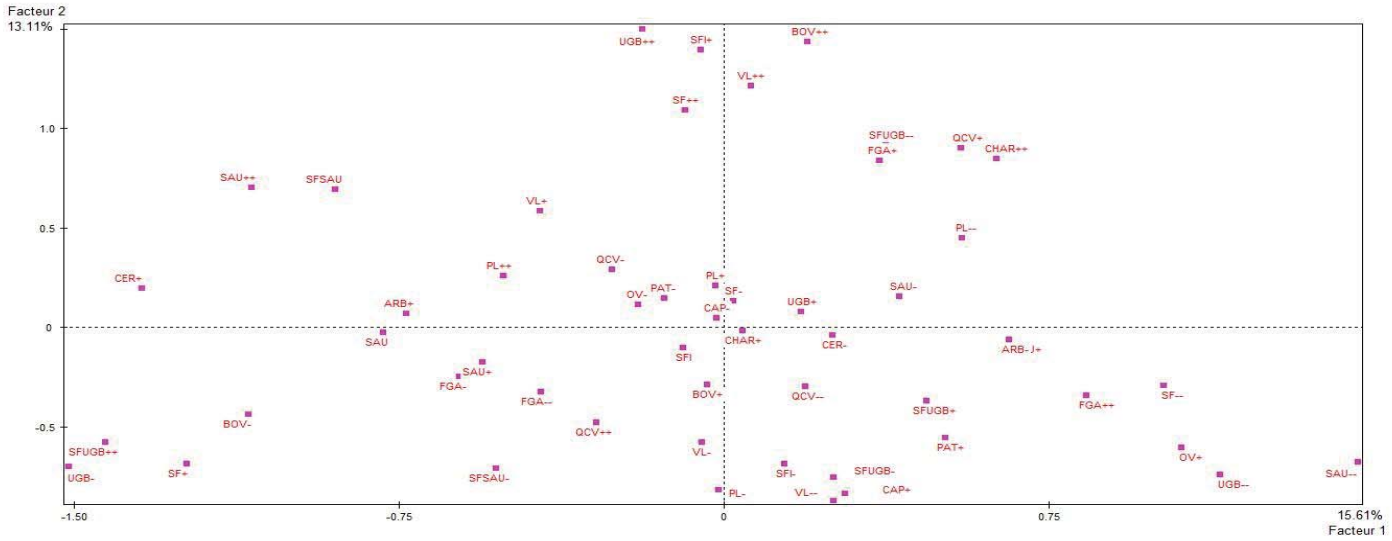


**Figure 1.** Projection of the variables in the plane 1-2 of the MCA.

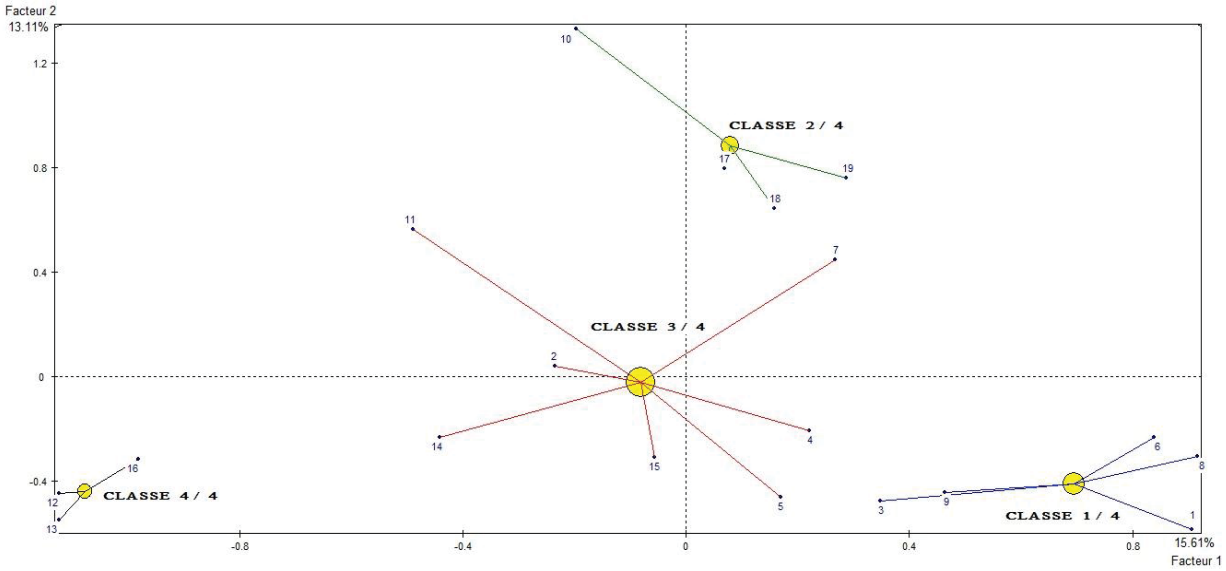


**Figure 2.** Paragons of the four cluster groups.

variability. Statistical analysis identified four groups (Figure 2):

**Group 1 (small farms with relatively high stocking):** Consisted of 5 farms (1, 3, 6, 8 and 9) is characterized by low UAA (3.75 ha) and by low forage acreage (2.20 ha). This group comprised the farms with a lower number of cattle and dairy cows respectively 8.00 ± 1.73 and 4.60 ± 1.67 heads but with a livestock load reaching 5.90 ± 5.99 LU / ha.

**Group 2 (medium-sized farms with high stocking):** Consists of four farms (10, 17, 18 and 19) which are characterized by a relatively average UAA and forage areas respectively 14.44 ± 16.56 and 8.50 ± 7.19 ha. This group is characterized by the presence of irrigated fodder production and herd size for cattle and dairy cows with respective averages of 76 and 38 animals per farm which implies a high stocking rates averaging 9.88 LU ± 7.74/ ha of forage grown on the farm.

**Group 3 (medium-sized farms with low stocking):** Consists of seven farms (7, 4, 5, 7, 11, 14 and 15) with an average UAA of 16.75 ± 8.03 ha of which 10.89 are reserved for fodder crops. Cattle number is on average 9.88 ± 7.74 heads which results in a low stocking rate of

**Table 1.** Notes of agro-ecological sustainability.

| Components | Indicators | Scores | Bounds (Min-Max) | Percentage of the theoretical maximum score (%) |
|---|---|---|---|---|
| Diversity | A1. Diversity of annual and temporary crops | 6.68±1.57 | 0-14 | 47.71 |
| | A2. Diversity of perennial crops | 3.31±4.11 | 0-14 | 23.64 |
| | A3. Animal diversity | 08±2.43 | 0-14 | 57.14 |
| | A4. Enhancement and conservation of genetic resources | 00±0.00 | 0-6 | 00 |
| | **Total of Component** | **17.68±4.83** | **0-33** | **54.51** |
| Organisation of space | A5. Cropping patterns | 0.36±0.53 | 0-8 | 04.50 |
| | A6. Dimension of fields | 4.42±1.02 | 0-6 | 73.66 |
| | A7. Organic matter management | 1.78±0.63 | 0-5 | 35.60 |
| | A8. Environmental buffer area | 1.15±1.07 | 0-12 | 09.58 |
| | A9. Contribution to environmental issues of territory | 00±0.00 | 0-4 | 00 |
| | A10. Enhancement of space | 1.05±1.68 | 0-5 | 21.00 |
| | A11. Fodder area management | 0.21±0.42 | 0-3 | 07.00 |
| | **Total of Component** | **8.97±1.52** | **0-33** | **27.18** |
| Farming Practices | A12. Nitrogen balance | 3.52±3.47 | 0-8 | 44.00 |
| | A13. Effluent processing | 02±0.00 | 0-3 | 66.66 |
| | A14. Pesticides | 9.36±2.99 | 0-13 | 72.00 |
| | A15. Veterinary treatment | 1.78±0.42 | 0-3 | 59.33 |
| | A16. Soil resource protection | 0.52±0.50 | 0-5 | 10.40 |
| | A17. Water resource management | 0.89±1.00 | 0-4 | 22.25 |
| | A18. Energy dependence | 0.94±1.50 | 0-10 | 09.40 |
| | **Total of Component** | **19.01±6.10** | **0-34** | **55.91** |
| | **Total** | **45.97 ±5.03** | **100** | **45.97** |

2.72 ± 1.55.

**Group 4 (large farms with low stocking):** Includes three farms (12, 13 and 16) is characterized on the one hand, the relative importance of the UAA with an average of 21.00 ± 10.44 ha of which nearly half (10 ha) is restricted to forage and, secondly, by the small size of cattle population is on average 12 heads per farm which translates into a very low stocking rate (1.02± 0.28). This group is also characterized by the presence of the orchard occupying an average of 11 ha of UAA.

## Analysis of the agro-ecological sustainability

Scores on the scale agro-ecological sustainability vary from 34 to 60% with an average of 45.97% of the theoretical maximum (Table 1 and Figure 2). This value confirms those reported by Bekhouche (2004) and Bekhouche-Guendouz (2011) for the same study area 45, 14 and 45.20% respectively. It is against much lower than those recorded by Yakhlef et al. (2005) and Far (2007) for dairy cattle farms in the semi arid region of Setif 70.00 and 67.6% respectively. Benatellah (2007) obtained a higher value for the livestock farms of the Algiers suburban area or 55.70%.

The relatively low Agro-ecological sustainability of these farms is caused by zero or very low scores assigned to 11 of the 18 indicators informed (Figure 2). This is: 1) diversity of perennial crops (score:3.31±4.11), and 2) enhancement and conservation of genetic resources (score: 0) of diversity component, 3) cropping patterns (score: 0.36±0.63), 4) organic matter management (score:1.78±0.63), 5) environmental buffer area (score:1.15±1.07), 6) contribution to environmental issues of territory (score:0), 7) Enhancement of space (score : 1.05 ± 1.68) 8) management of forage area (score: 0.21±0.42), of the space organization component, 9) soil resource protection (score: 0.52±0.50), 10) water resource management (score: 0.89±1.00) and 11) energy dependence (score: 0.94±1.50) of the farming practices component. The zero score recorded by all farms is explained by the lack of specifications which the farmers undertake to respect and protect the natural heritage while the zero score for development and conservation of genetic resources indicator is caused by the total

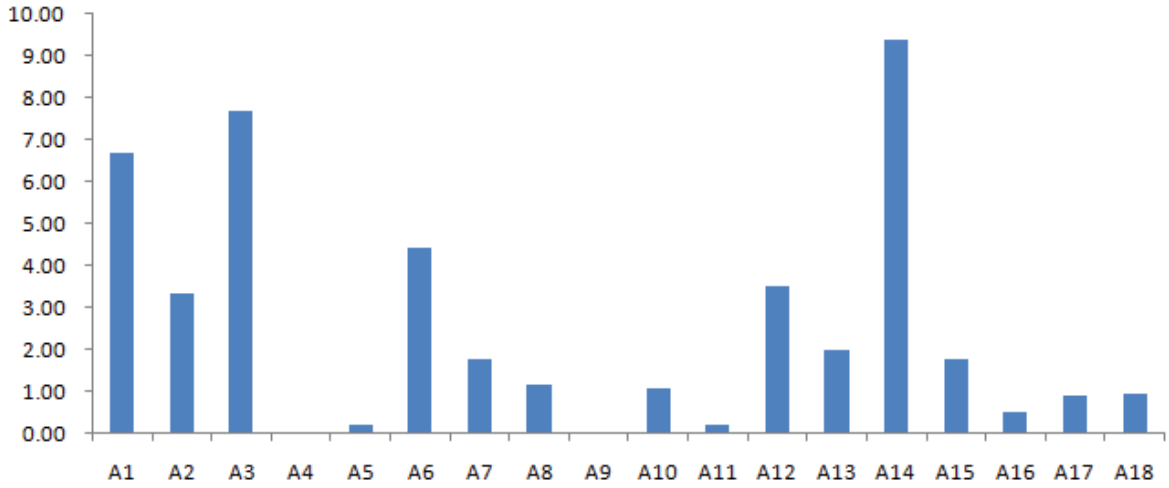


**Figure 3.** Average values of indicators.

absence of any regional, rare or endangered variety or breed (Figure 3).

The indicator scoring the diversity of perennial crops was low because the occurrence of permanent or temporary grasslands over 5 years of age is very small or more often absent because of the low UAA of farms and lack of water resources and irrigation equipment. The very low score for the cropping patterns indicator refers to the fact that 60.5% of surveyed farms do not practice crop rotation and 10% spent more than 50% of the cultivated surface to the main annual crop (oats). The survey results showed that the surface of environmental buffer area on farms was often lacking due to the absence of rivers, forest areas and small dams. It was also noted that the majority of farmers did not provide for erosion control measures. 50% of farmers use manure while others prefer to sell or exchange with mowed grass from orchards. There is an absence of slurry barn systems in the region. In addition, the use of nitrogen catching crops is scarce, and there is no compost is made from crop residuals. The low score for the protection of soil indicator is related to the lack of soil protection techniques such as mentioned by in the questions from IDEA (Vilain, 2008) (no-tillage technique, straw burning). Soil protection is limited in the majority of farms to a few trees as windbreaks. However, the practice of tillage is systematic in all surveyed farms as the regional soil type (heavy soils) requires loosening of the soil. The amount of irrigation on the farms depends on surfaces, water resources, the crop type and technical and financial resources available to farmers. The low score recorded by this indicator is due to the lack of use of waste water systems and the use of exhaustible water resources such as drillings with a depth of over 110 m, while the law limits their depth to 90 m. The energy dependence of the surveyed farms is very high. Fossil fuel oil consumption per hectare as much as 500 L can be explained by the lack of renewable energy sources (wind, solar ...).

Only two indicators have high scores. This is dimension of fields of space organization and pesticides of farming practices component. According to Mesli (2007), the majority of farms in Algeria are small with average size of about 6 ha which explains the score of 4.42±1.02 points on average a maximum of 6 assigned to the dimension of fields indicator. Finally, the survey revealed a low use of pesticide (fruit and vegetable crops) due to high cost price of chemicals.

## Analysis of the sustainability of identified livestock farms types

Group 4 shows the highest level of agro-ecological sustainability (48.29±5.03 points) thanks to the better score recorded by the space organization component (12.32±1.53 points) (Table 2). The scores recorded by the indicator A10 (enhancement of space) explains the good performance of this component. Indeed, the stocking is an important element that provides information on the balance between the number of animals and forage areas that supplies them. The standard is around 1 to 2 Livestock Units per hectare of forage area (LU / MFA) is most often not met due to the small size of farm land. However, this group had the lowest score for sustainability regarding farming practices component mainly because of low ratings assigned to the indicators: A16 (soil resource protection), A17 (water resource management) and A18 (energy dependence).

The sustainability scores of the groups 2 and 3 are not statistically different, 45.75±5.44 and 45.31±5.87 points respectively. The sustainability level of the group 1 is lower; 43.6±3.36 points. These three groups are penalized by poor scores recorded by the space

**Table 2.** Notes of agro-ecological sustainability of identified livestock farms types.

| Groups | Diversity | Organisation of space | Farming practices | Agro-ecological sustainability |
|---|---|---|---|---|
| 1 | 15.20±3.42 | 7.40±2.30 | 21.00±5.29 | 43.60±3.36 |
| 2 | 19.20±7.33 | 8.25±1.89 | 18.00±7.70 | 45.75±5.44 |
| 3 | 16.17±4.15 | 8.70±2.56 | 20.44±6.75 | 45.31±5.87 |
| 4 | 21.66±2.31 | 12.32±1.53 | 14.31±4.72 | 48.29±5.03 |

organization component mainly because of the dominance of monoculture whose consequences are lack of pastures, poor management of farm land with simplified crop rotations. This diversity of livestock farming systems is also reported by Bekhouche (2011) for the dairy basin of Annaba. The author indicated that livestock systems encountered prefer the simplified crop rotations while sustainable farming systems seeking rather complex rotations (Vilain, 2008). The implementation of the IDEA method is a diagnostic and assessment tool has enabled an inventory of the current situation of cattle farms surveyed in the viewpoint of sustainability and highlighted the strengths and the weaknesses of different livestock systems identified.

## Conclusion

The analysis of the agro-ecological sustainability of 19 dairy cattle farms in the suburban area of the city of Blida showed a variety of results. With an average of 45.97% of the theoretical maximum, the surveyed farms are below the threshold of sustainability of agro-ecological scale. Farms sustainability is based on livestock farming systems that are influenced by production region in which they are located. Thus, these livestock farming systems are characterized by an increased fragmentation of land, undeveloped fodder crops, improper use of concentrate, an appeal to the market to purchase a portion of roughage and very important competitiveness in water between livestock, home consumption, more profitable crops and industrial use.

If cattle occupy a strategic position in the agricultural and economic development of the Mitidja plain, sustainability seems to be compromised in the medium and long term by a set of environmental constraints such as the reduction of agricultural land, water resources and poor organization of space. Methodologically, the method does not purport to be perfect because the relationship between livestock and its context are little discussed. Furthermore, several indicators seem to lack precision in their methods of determining or overestimate the scores scales. Thus, in addition to the changes to be made to some indicators, it should also include other indicators such as urban and industrial expansion at the expense of agricultural land and give more importance to the water availability and origin factor.

Thus, in view of future validation of this method in the context of the Algerian agriculture, it is necessary in the component “improvement” to put into action a group of researchers, experts and farmers to study in detail the amendments to the IDEA matrix. These amendments must cover both the choice of indicators and ratings that variables that make up each indicator. However, this method is functional and allows operational approach for environmental constraints designed to educate farmers and policy makers to the concept of sustainability and the concept to better take into account the protection of natural environments.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Yield, quality and profitability of rice (*Oryza sativa* L.) varieties grown in the eastern Himalayan region of India

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**Rice is a major staple food for over 3 billion people, representing the major carbohydrate and even protein source in South Eastern Asia, but also in Africa. Unfortunately, rice is a poor source of many essential micronutrients. Thus, a rice-based diet is the primary cause of micronutrient malnutrition throughout much of the developing world. Iron, zinc, and vitamin A deficiencies are common in rice-consuming regions. These deficiencies account for decreased work productivity, reduced mental capacity, stunting, blindness, increased child mortality, and elevated morbidity and mortality in general. Therefore, an experiment was conducted to study the performance of seven improved rice varieties introduced in the Eastern Himalayan Region of India with a local variety considering yield, grain quality for human nutrition and economic benefit under lowland condition. The highest grain yield was recorded in paddy variety, RC Maniphou 7 (5.3 t ha$^{-1}$) followed by RC Maniphou 11 (5.2 t ha$^{-1}$) over the indigenous paddy variety, Daramphou. These paddy varieties recorded 81 and 79% higher grain yield over the local variety and found to be highest profitable as compare to the other variety under the study. However, the grain nutritive value was found to be higher in the paddy variety RC Maniphou-5 followed by RC Maniphou-4. So, agronomic biofortification of rice with micronutrient might be an effective component of a food system strategy to reduce micronutrient malnutrition in rice eating populations. Evaluation of regression factor scores through principal component analysis using grain yield, grain quality and economic benefit has proved the superiority of RC Maniphou-7, RC Maniphou-10 and RC Maniphou-11 over the other improved and local varieties for the foothills of eastern Himalayan region.**

**Key words:** Rice, yield, grain quality, economic benefit.

## INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple food for more than half of the world population (Sasaki and Burr, 2000). It provides around 21% of dietary energy and 15% of protein to global population (IRRI, http://irri.org/about-rice/rice-facts/rice-basics). It is the most important food grain crop in the north eastern hill agro-ecosystem of India in the eastern Himalayan region. It is occupying 3.5 million ha in the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, which accounts for more than 80% of the total

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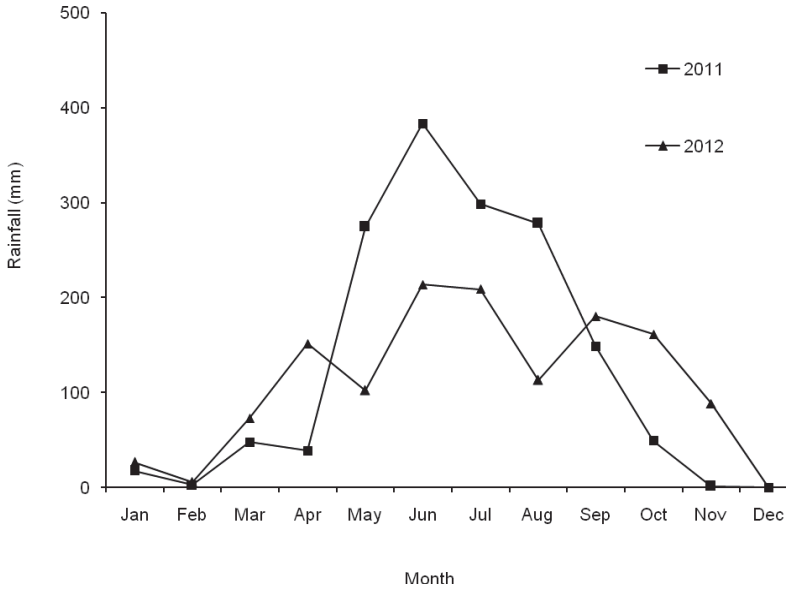


**Figure 1.** Distribution of monthly rainfall of the experimental site during 2011-2012.

cultivated area of the region (http://www.rkmp.co.in). The eastern Himalayan region of India is considered to be one of the hot pockets of rice genetic resources in the world and a potential rice-growing region with extremely diverse rice growing conditions as compared to other parts of the country. Being the secondary centre of origin of rice, the region is rich in diverse germplasm that shows the distinctness amongst them. Selection made unknowingly by various ethnic groups inhabiting at different altitudes and climatic situations, practicing different forms of cultivation might have also contributed to some extent towards the diversity of rice crop in this region (Hore, 2005). Although, during the post-green revolution period due to introduction of improved varieties, rice yield in north eastern region of India has been almost doubled, there is further scope for increasing its productivity, which remains much below the national productivity. Again, rice grain protein, available starch, dietary fiber, vitamin and macro- and micronutrients have wide variability among the cultivars (Eggum et al., 1993; Graham et al., 1999; Meng et al., 2005). Improved rice (*Oryza sativa* L.) varieties introduced in the eastern Himalayan region of India need to be assessed for their yield performance, nutritional quality and economic profitability. Therefore, it is desirable to have information about the rice varieties grown in a region with regards to their yield performance, nutritional quality and economic benefit. In this context, the present investigation has been under taken to compare yield, quality of grain for human nutrition and profitability of some of the improved rice varieties vis-à-vis a local variety grown under lowland rain fed condition of the eastern Himalayan region of India.

## MATERIALS AND METHODS

### Experiment

Field experiment was conducted during two consecutive years (2011 and 2012) at the Research Farm of ICAR Research Complex for NEH Region, Manipur, India (25.45° N, 93.53° E, 295 m above mean sea level). The site is representative of the foot hills of eastern Himalayan Region and falls under sub-humid region. It receives an average annual rainfall of 1433 mm (mean of the two years) of which 70% occurs during the rice growing season (June to October) (Figure 1). It also experiences mean annual daily minimum and maximum temperature of 21 and 31°C, respectively during the rice season. The experimental soil (0 to 0.15 m depth) is clay loam in texture, acidic in reaction (pH 5.15) and oxidizable organic carbon, available nitrogen, available phosphorus and available potassium are 46.5 g $kg^{-1}$, 238 mg $kg^{-1}$, 9 mg $kg^{-1}$ and 156 mg $ka^{-1}$, respectively. Eight rice varieties (seven improved varieties released from ICAR Research Complex for NEH Region, Manipur such as RC Maniphou 4, RC Maniphou 5, RC Maniphou 6, RC Maniphou 7, RC Maniphou 10, RC Maniphou 11, RC Maniphou 12 and one indigenous local variety Daramphou) were grown in randomized block design (5 x 5 m plot size). The varieties were replicated thrice. The crop was sown at the 1$^{st}$ fortnight of June and harvested at physiological maturity in October. The crop was grown with recommended agronomic package of practices including fertilization, weeding and pest control. Grain and straw yield of rice was recorded at harvest and representative grain samples were collected for analysis.

### Grain analysis

Nitrogen concentration in grain was determined by micro-Kjeldahl digestion and distillation (Nelson and Sommers, 1973). For determination of P, K, Fe, Mn, Cu and Zn plant samples were ashed in a muffle furnace at 550°C for 3 h and subsequently extracted with 2 N HCl. Then the extract was analyzed for P (vanadomolybdate yellow color method; Jackson, 1973), K, Fe, Mn,

**Table 1.** Formulae and units of different economic parameters.

| Parameter | Formula | Unit |
|---|---|---|
| Gross return | Grain yield value + straw yield value | INR $ha^{-1}$ |
| Net return | Gross return – cost of cultivation | INR $ha^{-1}$ |
| Benefit to cost ratio | Gross return/cost of cultivation | -- |
| Crop profitability | Net return/crop growth period in days | INR $ha^{-1}$ $d^{-1}$ |
| Market production efficiency | Economic yield/crop growth period in days | kg $ha^{-1}$ $d^{-1}$ |
| Biological production efficiency | Biological yield/crop growth period in days | kg $ha^{-1}$ $d^{-1}$ |

Currency: one US Dollar = 55 Indian Rupees (INR), Grain price = 10800 INR $Mg^{-1}$, Straw price = 1200 INR $Mg^{-1}$, Growth period of RC Maniphou 4, RC Maniphou 5, RC Maniphou 6, RC Maniphou 7, RC Maniphou 10, RC Maniphou 11, RC Maniphou 12 and Darmaphou = 110, 115, 125, 135, 125, 125, 125 and 135 days, respectively.

**Table 2.** Yield attributing characters and yield of rice varieties.

| Treatment | Plant height (cm) | Number of tillers per plant | Panicle length (cm) | 1000 grain weight (g) | Harvest index (%) | Grain yield (t $ha^{-1}$) | Straw yield (t $ha^{-1}$) |
|---|---|---|---|---|---|---|---|
| RC Maniphou 4 | 84.5 | 13.4 | 21.3 | 28.93# | 39# | 3.9 | 6.0 |
| RC Maniphou 5 | 82.6 | 15.2 | 21.1 | 29.47# | 40# | 4.1 | 6.1 |
| RC Maniphou 6 | 95.3 | 11.3# | 18.2 | 29.03# | 39# | 4.8# | 7.3 |
| RC Maniphou 7 | 99.1 | 17.5 | 24.5# | 29.50# | 42 | 5.3 | 7.8# |
| RC Maniphou 10 | 123.3 | 11.2# | 23.0# | 30.67 | 39# | 4.9# | 7.5 |
| RC Maniphou 11 | 113.7 | 10.3# | 22.2# | 27.87 | 39# | 5.2 | 7.8# |
| RC Maniphou 12 | 78.6 | 10.4# | 22.5# | 33.67 | 39# | 4.2 | 6.3 |
| Daramphou | 144.2 | 9.6# | 20.6 | 32.90 | 36 | 2.9 | 5.1 |
| SEm± | 1.86 | 0.83 | 0.75 | 0.37 | 0.73 | 0.07 | 0.11 |
| LSD ($p$ = 0.05) | 5.71 | 2.53 | 2.30 | 1.13 | 2.24 | 0.23 | 0.34 |

Harvest index (%) = (economic yield/biological yield) x 100, #indicates the values are statistically at par with each other.

Cu and Zn (atomic absorption spectrometry, PerkinElmer). Protein and starch content in grain was analyzed in FOSS grain analyzer.

#### Quality index (QI)

Grain quality was assessed using linear indexing technique to integrate different parameters important for human nutrition, such as N, P, K, Fe, Cu, Zn, Mn, starch and protein content in rice grain. In this technique, each observation was divided by the highest observed value of that parameter so that the highest observed value received a score of one. Values of each of the selected quality parameters were summed up to get QI for each variety. The higher the index score better is the crop quality for human nutrition.

#### Economic analysis

Gross return, net return, benefit to cost ratio, crop profitability and production efficiencies were determined according to the procedures presented in Table 1. The analysis of variance for the effects of year, variety and year x variety interaction was computed using biomass yield, yield attributing characters and grain nutrient content of rice as dependent variables. Yield, yield attributes and nutrient content were not significantly influenced either by year or by year x variety interaction, which indicated that treatment effects were consistent across years. Therefore, data were pooled and presented across the years.

The relative strength of all the eight rice varieties were compared by employing non parametric evaluation of regression factor scores through principal component analysis (PCA) using yield, QI and BCR as goal variables. In this screening technique all components corresponding to eigen values more than one have been considered. The relative size of the eigen value associated with a particular component indicated the relative contribution of concerned component to the total variance of original data set. Regression scores were then ranked from 1 to 8 for the highest score as 1 and the least one as 8. Statistical analyses of the data were done using SPSS 17.0 (SPSS Institute Inc.).

## RESULTS

### Yield attributes

The results indicated that yield attributes of rice varieties respond differently under the same growing conditions (Table 2). Significant variations ($p$=0.05) in plant height, number of effective tillers per plant, panicle length, test weight of grain (1000 grain weight), grain, straw yield and harvest index were observed among the rice varieties

**Table 3.** Grain quality parameters and quality index of different rice varieties.

| Treatment | Starch | Protein | Nitrogen | Phosphorus | Potassium | Iron | Copper | Zinc | Manganese | Quality index |
|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | | | | | (mg kg$^{-1}$) | | | | |
| RC Maniphou 4 | 67.9# | 8.4# | 1.33# | 0.35# | 0.34 | 13.7 | 2.0# | 17.0# | 22.3# | 8.1 |
| RC Maniphou 5 | 66.4# | 8.5# | 1.43# | 0.36# | 0.38# | 18.0 | 2.0# | 17.6# | 17.4 | 8.3 |
| RC Maniphou 6 | 58.6 | 7.8 | 1.32# | 0.36# | 0.39# | 14.3 | 1.8# | 14.3$ | 22.3# | 7.8 |
| RC Maniphou 7 | 63.5# | 8.8# | 1.47# | 0.33 | 0.34 | 12.2 | 1.9# | 14.2$ | 13.1 | 7.5 |
| RC Maniphou 10 | 50.1 | 8.7# | 1.46# | 0.35# | 0.40# | 12.7 | 1.4 | 14.4$ | 20.3# | 7.5 |
| RC Maniphou 11 | 49.3 | 6.6 | 1.21 | 0.42 | 0.42 | 9.3 | 1.2 | 14.6$ | 6.4 | 6.2 |
| RC Maniphou 12 | 57.3 | 8.4# | 1.41# | 0.36# | 0.46 | 11.7 | 1.9# | 13.7$ | 22.7# | 8.0 |
| Daramphou | 68.7# | 4.8 | 0.80 | 0.31 | 0.41# | 9.3 | 0.4 | 10.4 | 16.0 | 5.7 |
| SEm± | 2.37 | 0.23 | 0.07 | 0.02 | 0.01 | 1.95 | 0.12 | 0.35 | 1.03 | |
| LSD (p = 0.05) | 7.3 | 0.7 | 0.20 | 0.05 | 0.03 | NS | 0.4 | 1.1 | 3.2 | |

NS, not significant at p = 0.05.# and $ indicates the values are statistically at par with each other.

tested (Table 2). The highest (144 cm) and lowest (78 cm) plant height were recorded in Daramphou and RC
Maniphou 12, respectively. The highest number of effective tillers per plant (17.5), panicle length (24.5 cm) and harvest index (42%) were observed in RC Maniphou – 7 over the other varieties and control under the study. However, in the case of 1000 grain weight, the highest (33.7 g) and lowest (27.9) values were recorded in RC Maniphou 12 and RC Maniphou 11, respectively. This might be due to genetic makeup as well as effect of favourable environment on the paddy varieties under the study. As plant height is an important morphological character because of its relationships with light interception efficiency, lodging and dry matter accumulation. The number of effective tillers per plant is also important for higher productivity, because more number of effective tillers results more the numbers of panicle per unit area. Significant variation in number of effective tillers per plant, number of grains per panicle, grain size might result significant variation in rice grain yield.

## Yield

Grain and straw yield of rice varied significantly ($p$=0.05)
among the varieties (Table 2). The highest grain and straw yield was recorded in RC Maniphou 7 (5.3 t ha$^{-1}$ and 7.8 t ha$^{-1}$), which was statistically at par with RC Maniphou 11 (5.2 and 7.8 t ha$^{-1}$, respectively). These two varieties resulted in 81 and 79% higher grain yield, over the local variety, Dharamphou. Higher grain yield in the above mentioned varieties are due to higher values in one or more yield attribute(s), such as number of effective tillers per plant, panicle length, 1000 grain weight and/or harvest index. As varietal evaluation of any crop based on their yield performance is important because yield varied significantly among the varieties when grown under similar environment. The improved rice varieties produced 34 to 83% higher grain yield than Dramphou with the same management practices and performance of RC maniphou 7 and RC Maniphou 11 was better among the improved ones (Table 2). Farmers in the foothills of eastern Himalayan region, India grow mostly traditional cultivars of rice and get only about 1.57 t/ha grain ha$^{-1}$ (http://www.rkmp.co.in) across the growing conditions of lowland and upland including shifting cultivation. Whereas, under lowland condition the average yield ~ 2.0 tha$^{-1}$, which is much below (<50%) the average yield of improved varieties obtained in this study (4.6 t ha$^{-1}$). Thus, there is a great potential to increase rice yield in north eastern states of India with adoption of suitable cultivar along with judicious agronomic management practices.

## Nutritive quality of rice

Rice is a major source of dietary protein and nutrients for most of the rice growing Asian countries. Rice varieties showed significant ($p$=0.05) variation in starch and protein content of grain. The local variety Daramphou recorded the highest starch content (68.7%) over the other varieties (Table 3). This might be due to its genetic makeup. Starch content in RC Maniphou

**Table 4.** Economics and crop profitability of different rice varieties.

| Treatments | Cost of cultivation (INR) | Gross return (INR) | Net return (INR) | Benefit to cost ratio | Crop profitability (INR $ha^{-1}$ $d^{-1}$) | Market production efficiency (kg $ha^{-1}$ $d^{-1}$) | Biological production efficiency (kg $ha^{-1}$ $d^{-1}$) |
|---|---|---|---|---|---|---|---|
| RC Maniphou 4 | 20920 | 49320 | 28400 | 2.4 | 258 | 35 | 90 |
| RC Maniphou 5 | 20920 | 51600 | 30680 | 2.5 | 267 | 36 | 89 |
| RC Maniphou 6 | 20920 | 60600 | 39680 | 2.9 | 317 | 38 | 97 |
| RC Maniphou 7 | 20920 | 66600 | 45680 | 3.2 | 338 | 39 | 97 |
| RC Maniphou 10 | 20920 | 61920 | 41000 | 3.0 | 328 | 39 | 99 |
| RC Maniphou 11 | 20920 | 65520 | 44600 | 3.1 | 337 | 42 | 104 |
| RC Maniphou 12 | 20920 | 52920 | 32000 | 2.5 | 256 | 34 | 84 |
| Daramphou | 20920 | 37440 | 16520 | 1.8 | 122 | 21 | 59 |

4 (67.9%), RC Maniphou 5 (66.4%) and RC Maniphou 7 (63.5%) was comparable with the local variety, Dramphou. All the rice varieties under the study recorded higher protein content over the local one. The highest protein content was recorded in the paddy variety RC Maniphou 7 (8.8%) followed by RC Maniphou 10 (8.7%). The above mentioned two varieties recorded 84 and 82% higher protein content, respectively, over the local variety. Protein, which is a key factor influencing the eating quality of rice varies with the environment and types of soil. The levels of nutrient concentration in grains of rice varieties as observed in the present investigation have also been reported by elsewhere. Both macronutrient (N, P and K) and micronutrient (Cu, Zn and Mn) concentration in grain recorded significant ($p$=0.05) variations among the rice varieties (Table 3). The lowest values of grain nutrient content were recorded in the local variety, Daramphou. The highest values of grain N (1.47%), P (0.42%), K (0.46%), Fe (18.0 mg $kg^{-1}$), Cu (2.0 mg $kg^{-1}$), Zn (17.6 mg $kg^{-1}$) and Mn (22.7 mg $kg^{-1}$) were observed in RC Maniphou 7, RC Maniphou 11, RC Maniphou 12, RC Maniphou 5, RC Maniphou 4 and 5, RC Maniphou 5 and RC Maniphou 4, respectively. The QI values of rice grain for human nutrition was significantly high (>7.5) in all the improved varieties with the exception of RC Maniphou 11 (6.2) over the local one (5.7) (Table 3). This might be suitability of the said variety in North Eastern Himalayan.

## Economic benefit

All the improved varieties under the study recorded higher net return, benefit to cost ratio, crop profitability, market production efficiency and biological production efficiency over the local variety Daramphou (Table 4).

The highest net return (INR 45,680) and benefit to cost ratio (3.2) was observed in RC Maniphou 7, but RC Maniphou 11 recorded the highest values of crop profitability (357 INR $ha^{-1}$ $d^{-1}$), market production efficiency (42 kg $ha^{-1}$ $d^{-1}$) and biological production efficiency (104 kg $ha^{-1}$ $d^{-1}$). This is due to the fact that RC Maniphou 11 has shorter crop duration (125 days) as compared to RC Maniphou 7 (135 days).

## Ranking of varieties

The results of PCA considering yield, QI and benefit cost ratio showed that the highest rank (regression factor score 0.970) was assigned to RC Maniphou 7 (Table 5). This indicates that RC Maniphou 7 is the best among the rice varieties compared followed by RC Maniphou 10, RC Maniphou 11, RC Maniphou 6, RC Maniphou 5, RC Maniphopu 9, RC Maniphou 12 and Daramphou in decreasing order of ranking.

## DISCUSSION

The results indicated that yield attributes of rice varieties respond differently under comparable growing conditions (Table 2). Similar values of yield attributes of rice genotypes have also been reported by Ojha (2006, 2010) and Fageria (2004). Plant height is an important morphological character because of its relationships with light interception efficiency, lodging and dry matter accumulation (Fageria et al., 2006). Generally, higher the panicle length higher will be the

**Table 5.** Screening of the rice varieties through regression factor score.

| Treatment | Grain yield (Mg ha$^{-1}$) | Crop quality index | Benefit to cost ratio | Regression factor score | Rank |
|---|---|---|---|---|---|
| RC Maniphou 4 | 3.9 | 8.1 | 2.4 | -0.351 | 6 |
| RC Maniphou 5 | 4.1 | 8.3 | 2.5 | -0.105 | 5 |
| RC Maniphou 6 | 4.8 | 7.8 | 2.9 | 0.556 | 4 |
| RC Maniphou 7 | 5.3 | 7.5 | 3.2 | 0.970 | 1 |
| RC Maniphou 10 | 4.9 | 7.5 | 3.0 | 0.604 | 2 |
| RC Maniphou 11 | 5.2 | 6.2 | 3.1 | 0.602 | 3 |
| RC Maniphou 12 | 4.2 | 8.0 | 2.5 | -0.066 | 7 |
| Daramphou | 2.9 | 5.7 | 1.8 | -2.210 | 8 |
| Statistics of variance of PCA | | | | | |
| Eigen value | | 2.12 | | | |
| % variance explained | | 70.59 | | | |

number of grain, which ultimately causes higher yield. The number of effective tillers per plant is also important for higher productivity, because more number of effective tillers results more the numbers of panicle per unit area. Ojha (2006) reported that significant variation in number of effective tillers per plant, number of grains per panicle, grain size might result significant variation in rice grain yield. The information on dry matter partitioning to grain (harvest index value) is important in understanding productive capacity of a cultivar (Sheng and Hunt, 1991). Such information should permit better analysis and interpretation of the results and also to better understanding of processes and resources exploitation in crop production (Williams et al., 1996). Based on the observed yield attributing characters RC Maniphou 7 was found to be superior to the other varieties compared. This might be due to better response of this variety over the others under similar agro-ecological situation.

Varietal evaluation of any crop based on their yield performance is important (Baishya et al., 2010), because yield varied significantly among the varieties when grown under similar environment. The improved rice varieties produced 34 to83% higher grain yield than Dramphou with the same management practices and performance of RC maniphou 7 and RC Maniphou 11 was better among the improved ones (Table 2). Farmers in the foothills of eastern Himalayan region, India grow mostly traditional cultivars of rice and get only about 1.57 t grain ha$^{-1}$ (http://www.rkmp.co.in) across the growing conditions of lowland and upland including shifting cultivation. Whereas, under lowland condition the average yield ~ 2.0 t ha$^{-1}$, which is much below (<50%) the average yield of improved varieties obtained in this study (4.6 t ha$^{-1}$). Thus, there is a great potential to increase rice yield in north eastern states of India with adoption of suitable cultivar along with judicious agronomic management practices.

Rice is a major source of dietary protein and nutrients for most of the rice growing Asian countries. Protein, which is a key factor influencing the eating quality of rice (Adu-Kwarteng et al., 2003), was considerably high (>7.0%) in all the improved varieties except RC Manipho 11 (6.6) and significantly low in the local variety (4.8%) (Table 3). Reports of such variation in protein content due to varietal influence are not uncommon (Adu-Kwarteng et al., 2003; Perez et al., 1996). A comparison of concentration of seven nutrient elements indicates that all the released varieties are superior in terms of grain nutrient content over the local one. The levels of nutrient concentration in grains of rice varieties as observed in the present investigation have also been reported by elsewhere (Wissuwa et al., 2008; Pooniya and Shivay, 2013). The order of concentration of nutrients across the cultivars was N > K > P > Mn > Zn > Fe > Cu, which is almost similar to that reported by Fageria and Knupp (2013). Among micronutrients, Fe and Zn deficiency occur in both crops and humans (Hotz and Brown, 2004; Sperotto et al., 2010). Rice provides energy to almost half of the world's population and is also a poor source of essential micronutrients particularly Fe and Zn (Bouis and Welch, 2010). Thus, growing rice varieties rich in grain micronutrient content, particularly Fe and Zn, has great potential to mitigate widespread micronutrient deficiencies in humans. Low protein content in grain of RC Maniphou 11 was the cause for its relatively low nutritive value among the improved cultivars. However, based on nutrient (N, P, K, Fe, Cu, Zn and Mn), starch and protein content in grain, the improved cultivars were much superior to the local one for human nutrition and RC Maniphou 4 and 5 were the beast among the improved ones.

Considering yield performance, nutritive quality of grain and economic benefit RC Maniphou 7 was found to be the best among the eight varieties compared followed by RC Maniphou 10, RC Maniphou 11, RC Maniphou 6, RC Maniphou 12, RC Maniphou 5, RC Maniphou 4 and Daramphou in deceasing order of superiority (Table 5). Therefore, from yield, quality and profitability point of view

farmers of this region have a choice to select one improved variety instead of growing traditional one. For obtaining higher yield and monetary benefit they can grow any one of RC Maniphou 7, RC Maniphou 10 and RC Maniphou 11. However, if anyone is concern about nutritive value, he can opt for either RC Maniphou 4 or RC Maniphou 5, which give > 40% higher grain yield than the local variety.

## Conclusions

Significant differences were obtained among the eight rice varieties for all the parameters compared. Yield, nutritive quality and profitability were better in the improved cultivars than the local one. This is obvious because breeding efforts are made towards attaining food and nutritional security through development of improved crop varieties. Crop improvement also aims to make crop production economically viable. The results of this study prove superiority of RC Maniphou 7 and other improved varieties over the local cultivar in foothills of eastern Himalayan region.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Controlled environmental conditions on germination of bermudagrass seeds

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***Cynodon dactylon* (L.) Pers., widely known as bermudagrass, is a cosmopolitan species used to form lawns, what provides aesthetic effects in parks and gardens, but also composes pastures and sports fields, such as golf and football. The use of seeds for the formation of new lawns is a common practice in Europe and in the United States, and is currently considerably expanding also in Brazil. It is important to understand the ideal environmental conditions for seed germination of each species, or cultivar. The aim of this study was to evaluate effects of salinity, temperature, light, substrate water contents, and sowing methods on germination of two bermudagrass cultivars: Princess 77 and Riviera. Three experiments, arranged in factorial schemes, were conducted: Experiment 1. Five salt concentrations (0, 25, 50, 75, and 100 mM) x two salt sources [sodium chloride (NaCl) and potassium chloride (KCl)]; Experiment 2. Three temperatures (constant at 30°C, alternating at 20 to 30°C, and alternating at 20 to 35°C) x presence or absence of light (8 h of light and 16 h of darkness, and total darkness); and Experiment 3. Four substrate water contents (25, 50, 75 and 100% of the substrate water retention capacity) x two sowing methods (in sand, and on sand surface). Germination percentage and germination rate were evaluated. Germination of Princess 77 was more effective in the absence of NaCl and KCl; at 20 to 35°C, either in the light or darkness; and at around 50% of the substrate water retention capacity, sown either in sand or on sand surface. Germination of Riviera seeds was more effective in the absence of NaCl and presence of KCl; at 20 to 35°C, in the light; and at 100% of the substrate water retention capacity, sown on sand surface.**

**Key words:** Poaceae, *Cynodon dactylon*, Princess 77, Riviera, salinity, temperature, light, substrate water content, sowing method.

## INTRODUCTION

*Cynodon dactylon* (L.) Pers., widely known as bermudagrass or silk grass, is a perennial cosmopolitan species that hybridizes either naturally or artificially, and produces gray-green short blades, usually 2 to 15 cm long, with jagged edges; its erect stems can grow up to 30 cm height (Walker et al., 2001). It may be considered

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a weed, forage, or ornamental (Vilela et al., 2005). Lawns, which are formed by grass species, usually provide aesthetic effects in parks and gardens, but also compose pastures and sports fields, such as golf and football. Furthermore, grasses may act, among others, on slope stabilization and erosion control (Freitas et al., 2012; Raven et al., 2001).

Use of seeds for the formation of new lawns is a common practice in Europe and in the United States, and is currently considerably expanding also in Brazil. Because of sporting events carried out in the country in 2014 and, also, to be accomplished in 2016, there is even greater expectation of increased market due to investments towards the necessary infrastructure (Godoy et al., 2012), what includes the formation of new lawns or renovation of existing ones.

Grasses can self-propagate by seeds, rhizomes, stolons, and tillers. Although plants from vegetative propagation show better initial development, this method has several agricultural limitations, such as higher dissemination of pests and diseases, elevated demand of propagation material from extensive areas, great need of hand labor, and high costs, apart from faster perishability of vegetative materials. The use of seeds, thus, is preferable whenever possible (Carmona et al., 1998; Evers and Parsons, 2010).

Germination usually depends on seed both endogenous and exogenous characteristics (Santos et al., 2004); therefore, it is essential to understand the ideal environmental conditions for each species, that vary according to each region, such as water, temperature, and light which, according to Santos et al. (2004), are considered the most important ones, besides salinity and soil/substrate moisture.

High salt contents in the soil, for instance, especially sodium chloride (NaCl), may inhibit germination mainly due to the osmotic effect. Although some plants are able to osmotically adjust themselves to maintain growth and turgor (Alshammary, 2012), an increase in salt concentrations greatly enhances the percentage of abnormal seedlings, due to the salt toxic action on germinating seeds (Lima et al., 2005), apart from affecting plant establishment and productivity
(Ortiz et al., 2014). Furthermore, grasses may differ in their capacity to tolerate salinity at the germination stage (El-Keblawy et al., 2011).

This study aimed to evaluate the effects of salinity, temperature, light, substrate water content, and sowing method on seed germination of two bermudagrass cultivars: Princess 77 and Riviera.

## MATERIALS AND METHODS

Three experiments with two bermudagrass cultivars, Princess 77 and Riviera, were conducted from May to June 2012, in the Seed Laboratory of the Department of Crop Production at the College of Agricultural and Veterinary Sciences of the State University of São Paulo (FCAV/UNESP), campus in Jaboticabal, Brazil.

The experimental design for Experiment 1, which tested the salinity effect on seed germination, was entirely randomized, with 10 treatments arranged in a 5 x 2 factorial scheme: five salt concentrations (0, 25, 50, 75 and 100 mM) x two salt sources [sodium chloride (NaCl) and potassium chloride (KCl)]. There were four replications of 100 seeds, totaling 4,000 seeds. The NaCl electrical conductivity of those concentrations was, respectively, 0.59, 2.10, 3.48, 5.23 and 8.00 dS $m^{-1}$; for KCl concentrations, it was, respectively, 0.59, 2.34, 4.25, 6.20 and 8.20 dS $m^{-1}$.

The experimental design for Experiment 2, which tested the effect of temperature and light on seed germination, was entirely randomized, with six treatments arranged in a 3 x 2 factorial scheme: three temperatures (constant at 30°C, alternating at 20 to 30°C, and alternating at 20 to 35°C) x two light regimes (8 h of light and 16 h of darkness, and total darkness). There were four replications of 100 seeds, totaling 2,400 seeds.

The experimental design for Experiment 3, which tested the effect of substrate water contents and sowing methods on seed germination, was entirely randomized, with eight treatments arranged in a 4 x 2 factorial scheme: four water contents (25, 50, 75 and 100% of the sand water retention capacity) x two sowing methods (in sand, and on sand surface), with four replications of 100 seeds, totaling 3,200 seeds.

Seeds were sown on sand surface in plastic boxes (11 x 11 x 3.5 cm), with the exception of Experiment 3, which seeds were also sown in sand. The plastic boxes remained in translucent plastic bags of low density polyethylene, and were placed in an incubator under the alternating temperatures of 20 to 35 and 20 to 30°C for Experiment 1 and 3, respectively; a photoperiod of 8 h of light and 16 h of darkness was settled, according to the recommended for *Cynodon dactylon* seeds in the Rules for Seed Analysis (Brasil, 2009). For Experiment 2, besides those mentioned, the temperature of 30°C and light regime of total darkness were also tested, as specified.

Substrate was weighted daily for water replacement, what was performed whenever it showed, for Experiments 1 and 2, 50% of its water retention capacity, which was calculated before the beginning of the experiment. For Experiment 3, water replacement was performed whenever necessary to maintain the sand water content of each treatment (Brasil, 2009). Germination was recorded daily for 28 days. Percentage of normal seedlings that were either equal to or higher than 2 mm were noted. Germination percentage and germination rate were determined as described by Maguire (1962). Germination data were arcsine $(x/100)^{1/2}$ transformed before the variance analysis. Means of the resulting values were compared by the Tukey test at 1 and 5% probability. Polynomial regression analysis was also performed to verify the effect of the tested salinity and substrate water contents on Princess 77 and Riviera bermudagrass seed germination.

## RESULTS

We verified, from the results of Experiment 1, that there was a significant interaction among salt concentrations and sources for germination percentage and germination rate for both bermudagrass cultivars (Table 1). However, at the concentrations of 0, 25, and 50 mM, there were no significant differences among salt sources for both germination percentage and germination rate of Princess 77 seeds, but at the concentrations of 75 and 100 mM, there was greater germination percentage and faster germination when seeds were submitted to the KCl treatment in comparison with NaCl. Even with the KCl superior results at higher concentrations when compared

**Table 1.** Variance analysis of germination percentage (%G) and germination rate (GR) of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different salt concentrations and sources.

| Princess 77 | | | | | | |
|---|---|---|---|---|---|---|
| **Variation sources** | **DF** | | **%G** | | **GR** | |
| Salt concentrations (SC) | 4 | | 13.02** | | 70.83** | |
| Salt sources (SS) | 1 | | 6.01* | | 8.79** | |
| SC x SS | 4 | | 7.67** | | 8.79** | |
| Residue | 30 | | | | | |
| CV (%) | | | 5.40 | | 8.68 | |
| | | **%G** | | | **GR** | |
| **Salt concentrations** | **NaCl** | | **KCl** | | **NaCl** | **KCl** |
| 0 mM | (49.90)[1] | 58.50[2a] | (49.90) | 58.50 [a] | 14.43 [a] | 14.43 [a] |
| 25 mM | (49.03) | 57.00 [a] | (47.15) | 53.75 [a] | 10.77 [a] | 9.85 [a] |
| 50 mM | (46.72) | 53.00 [a] | (43.99) | 48.25 [a] | 8.85 [a] | 9.68 [a] |
| 75 mM | (42.70) | 46.00 [b] | (44.57) | 49.25 [a] | 8.21 [b] | 10.83 [a] |
| 100 mM | (38.34) | 38.50 [b] | (44.57) | 49.25 [a] | 6.51 [b] | 8.12 [a] |

| Riviera | | | | | | |
|---|---|---|---|---|---|---|
| **Variation sources** | **DF** | | **%G** | | **GR** | |
| Salt concentrations (SC) | 4 | | 43.07** | | 103.45** | |
| Salt sources (SS) | 1 | | 438.97** | | 219.57** | |
| SC x SS | 4 | | 65.79** | | 77.20** | |
| Residue | 30 | | | | | |
| CV (%) | | | 3.57 | | 5.92 | |
| | | **%G** | | | **GR** | |
| **Salt concentrations** | **NaCl** | | **KCl** | | **NaCl** | **KCl** |
| 0 mM | (60.44)[1] | 75.50[2a] | (62.06) | 78.00 [a] | 15.84 [a] | 15.84 [a] |
| 25 mM | (58.69) | 73.00 [a] | (61.57) | 77.25 [a] | 12.60 [a] | 12.03 [a] |
| 50 mM | (47.86) | 55.00 [b] | (61.53) | 77.25 [a] | 10.08 [b] | 11.88 [a] |
| 75 mM | (46.00) | 51.75 [b] | (65.32) | 82.50 [a] | 8.69 [b] | 13.97 [a] |
| 100 mM | (35.05) | 33.00 [b] | (64.16) | 81.00 [a] | 3.97 [b] | 13.93 [a] |

**Significant at 1% probability; *Significant at 5% probability. [1]Data transformed to arcsine $(x/100)^{1/2}$; [2]Non-transformed data. Means followed by the same letter in the line do not differ from each other by the Tukey test at 5% probability.

with NaCl, germination percentage of Princess 77 seeds linearly decreased with increasing salt concentrations of both NaCl and KCl. The highest seed germination percentage found for this cultivar was observed in the absence of NaCl and KCl: 49.9% for both salts (Figure 1). The germination rate also decreased with increasing salt concentrations of both NaCl and KCl.

Similarly to what happened to Princess 77 seeds, there were no significant differences among lower concentrations (0 and 25 mM) of both salt sources for Riviera. However, at the concentrations of 50, 75, and 100 mM, there were greater germination percentage and faster germination when seeds were submitted to KCl in comparison with NaCl (Figure 1).

Germination percentage of Riviera seeds decreased with increasing concentrations of NaCl; the highest percentage, 60.44%, was observed under salt absence. However, there was a little gain (from 78 to 81%) on seed germination of this cultivar with increasing concentrations of KCl (Figure 1). Germination rate of this cultivar also linearly decreased with increasing NaCl concentrations, but under KCl, the rate increased from 50 mM, showing decreasing values under lower levels. Although the statistical analysis has shown that there were no differences for germination percentage and germination rate when Riviera seeds were submitted to KCl concentrations (Table 1), the generated regression equation (Figure 1) indicated that the germination rate had a little gain under the salt absence (15.30) when compared with results from the 100 mM KCl concentration (13.37). However, Figure 1 also shows that these values tend to increase if seeds were submitted to KCl concentrations higher than 100 mM.

Regarding the effect of temperature and light on seed germination (Experiment 2), there was a significant interaction among factors for germination percentage and germination rate for both bermudagrass cultivars (Table 2). The highest germination percentage and fastest

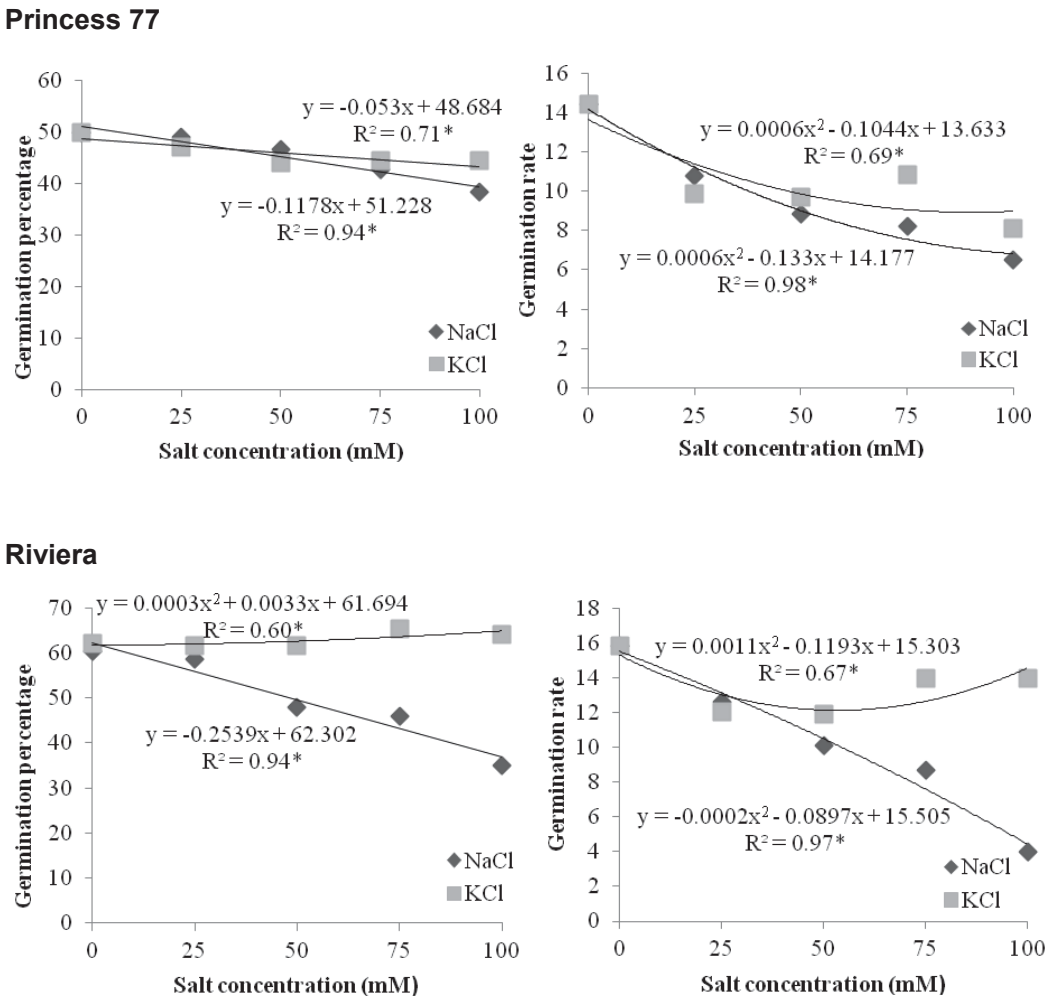


**Figure 1.** Germination percentage and germination rate of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different salt concentrations and sources. *Significant at 5% probability.

germination of Princess 77 seeds were observed at the alternating temperature of 20 to 35°C, either in the light or darkness. For Riviera, highest germination percentage and fastest germination were also found at the alternating temperature of 20 to 35°C, but only when seeds were submitted to the light regime.

For Experiment 3, there was a significant interaction among substrate water contents and sowing methods for germination percentage and germination rate for both Princess 77 and Riviera bermudagrass cultivars (Table 3).

High germination percentages of Princess 77 seeds occurred: when sown either in sand or on sand surface, at 25 and 50% of the substrate water retention capacity; on sand surface, at 75% of the substrate water retention capacity; and in sand, at 100% of the substrate water retention capacity (Table 3). Regarding the germination rate, seeds presented much faster germination when sown: either in sand or on sand surface, at 25 and 50% of the substrate water retention capacity; and in sand, either at 75 or 100% of the substrate water retention capacity.

Seed germination percentage and germination rate of Princess 77, when sown on sand surface, increased up to 53 and 46% of the substrate water retention capacity, respectively, when it started to decrease (Figure 2). When seeds were sown in sand, germination percentage linearly decreased with increasing substrate water content; the germination rate, on the contrary, increased according to the increment in the substrate water content up to 100% of its retention capacity.

Riviera seeds presented increasing germination percentages and germination rates when sown in sand, up to 55 and 57% of the substrate water retention capacity, respectively (Figure 2). When it reached 100% of its retention capacity, higher germination percentage occurred on sand surface, but there was no difference for the germination rate between sowing methods (Table 3). Seeds sown on sand surface, therefore, showed

**Table 2.** Variance analysis of the germination percentage (%G) and germination rate (GR) of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different temperatures and light regimes.

| Princess 77 | | | | | | |
|---|---|---|---|---|---|---|
| **Variation sources** | **DF** | | **%G** | | **GR** | |
| Temperature (T) | 2 | | 138.02** | | 90.32** | |
| Light (L) | 1 | | 10.47** | | 8.90** | |
| T x L | 2 | | 21.15** | | 29.12** | |
| Residue | 18 | | | | | |
| CV (%) | | | 4.44 | | 8.86 | |
| | | **%G** | | | **GR** | |
| **Temperatures** | **Light** | | **Dark** | | **Light** | **Dark** |
| 30 °C | (30.16)[1] | 25.25[2cB] | (39.37) | 40.25 [bA] | 4.72 [cB] | 9.06 [bA] |
| 20-30 °C | (44.14) | 48.50 [bA] | (41.26) | 43.50 [bA] | 7.86 [bA] | 7.33 [cA] |
| 20-35 °C | (49.90) | 58.50 [aA] | (51.07) | 60.50 [aA] | 12.19 [aA] | 11.21 [aA] |

| Riviera | | | | | | |
|---|---|---|---|---|---|---|
| **Variation sources** | **DF** | | **%G** | | **GR** | |
| Temperature (T) | 2 | | 473.78** | | 143.99** | |
| Light (L) | 1 | | 1,719.71** | | 581.79** | |
| T x L | 2 | | 40.81** | | 65.93** | |
| Residue | 18 | | | | | |
| CV (%) | | | 7.14 | | 16.26 | |
| | | **%G** | | | **GR** | |
| **Temperatures** | **Light** | | **Dark** | | **Light** | **Dark** |
| 30 °C | (28.95)[1] | 23.50[2cA] | (0.00) | 0.00 [bB] | 4.33 [bA] | 0.00 [bB] |
| 20-30 °C | (33.52) | 30.50 [bA] | (9.19) | 2.75 [bB] | 4.79 [bA] | 0.58 [bB] |
| 20-35 °C | (62.07) | 78.00 [aA] | (21.54) | 13.50 [aB] | 12.59 [aA] | 1.82 [aB] |

**Significant at 1% probability. [1]Data transformed to arcsine $(x/100)^{1/2}$; [2]Non-transformed data. Means followed by the same lower case letters in the column and same upper case letters in the line do not differ from each other by the Tukey test at 5% probability.

decreasing germination percentage and germination rate up to 51 and 46% of the substrate water retention capacity, respectively, when started to increase according to the greater increment in moisture up to 100% of the substrate water retention capacity (Figure 2).

## DISCUSSION

The NaCl treatment negatively affected germination of both Princess 77 and Riviera cultivars; on the other hand, KCl promoted a positive effect on Riviera seeds, what was not observed for Princess 77. The salt source, therefore, did influence the salinity tolerance behavior of those cultivars as, according to Ortiz et al. (2014), each source has chemical differences that may affect seed germination differently even when the osmotic potentials are similar. Also, Zhou and Xiao (2010), when studying the effects of specific ions on germination of sunflower seeds, concluded that germination is influenced not only by salt concentration (or osmotic potential) but also by ion nature in the salt solution and its interactions.

In accordance with these results, Zapryanova and Atanassova (2009), when studying the salinity tolerance of *Tagetes patula* and *Ageratum mexicanum* cultivated in pots, concluded that substrate salinity did inhibit plant growth; with increasing NaCl concentrations, the flowering period of both species decreased from 54 to 23 days for *T. patula*, and from 71 to 28 days for *A. mexicanum*. The inhibitory effect was best expressed in plants treated with 2% NaCl.

Some plants, however, do benefit from high salinity levels during germination, what provides greater adaptability to salinity during the remainder of their life cycles (Viana et al., 2004). Also, salt effects depend on other factors, such as plant species, cultivar, and phenological stage, apart from salt source, intensity and duration of salt stress, crop management, irrigation, and climatic conditions (Tester and Davénport, 2003). Coan et al. (2008), for instance, found that salinity levels up to 6.0 dS $m^{-1}$ did not restrict seedling emergence of both Mirage bermudagrass and *Lolium perenne* seeds.

Each species, and even cultivar, therefore, responds differently to substrate salinity, so that it is important to correctly select the cultivar or species according to soil or substrate conditions, thus aiming at normal seedling

**Table 3.** Variance analysis of the germination percentage (%G) and germination rate (GR) of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different substrate water contents (% substrate water retention capacity) and sowing methods.

| Princess 77 | | | | | | |
|---|---|---|---|---|---|---|
| **Variation sources** | **DF** | | **%G** | | **GR** | |
| Water retention capacity (W) | 3 | | 12.59** | | 8.87** | |
| Sowing methods (SM) | 1 | | 9.33** | | 36.18** | |
| W x SM | 3 | | 31.41** | | 24.12** | |
| Residue | 24 | | | | | |
| CV (%) | | | 5.46 | | 14.88 | |
| | | **%G** | | | **GR** | |
| **Water retention capacity** | **On sand** | | **In sand** | | **On sand** | **In sand** |
| 25% | (40.40)[1] | 42.00[2a] | (41.26) | 43.50 [a] | 5.59 [a] | 6.10 [a] |
| 50% | (42.70) | 46.00 [a] | (42.42) | 45.50 [a] | 7.54 [a] | 6.38 [a] |
| 75% | (44.57) | 49.25 [a] | (38.78) | 39.25 [b] | 4.38 [b] | 6.50 [a] |
| 100% | (28.97) | 23.50 [b] | (37.90) | 37.75 [a] | 1.90 [b] | 7.72 [a] |

| Riviera | | | | | | |
|---|---|---|---|---|---|---|
| Variation sources | DF | | %G | | GR | |
| Water retention capacity (W) | 3 | | 0.75[ns] | | 21.89** | |
| Sowing methods (SM) | 1 | | 182.40** | | 431.21** | |
| W x SM | 3 | | 78.45** | | 75.71** | |
| Residue | 24 | | | | | |
| CV (%) | | | 10.33 | | 14.43 | |
| | | **%G** | | | **GR** | |
| **Water retention capacity** | **On sand** | | **In sand** | | **On sand** | **In sand** |
| 25% | (11.70)[1] | 4.25[2b] | (23.13) | 15.50 [a] | 0.62 [b] | 2.08 [a] |
| 50% | (9.90) | 3.00 [b] | (27.07) | 20.75 [a] | 0.47 [b] | 4.14 [a] |
| 75% | (10.76) | 3.50 [b] | (26.55) | 20.00 [a] | 0.57 [b] | 2.72 [a] |
| 100% | (22.71) | 15.00 [a] | (14.40) | 6.25 [b] | 1.57 [a] | 1.57 [a] |

[ns]Not significant; **Significant at 1% probability. [1]Data transformed to arcsine $(x/100)^{1/2}$; [2]Non-transformed data. Means followed by the same letters in the line do not differ from each other by the Tukey test at 5% probability.

emergence stand.

The results from the temperature x light experiment endorse the indicated in the Rules for Seed Analysis (Brasil, 2009) that recommends such alternating temperature (20 to 35°C) for the germination of bermudagrass seeds. Evers and Parsons (2010) also found the alternating temperature of 25 to 35°C to be the most appropriate for germination of common bermudagrass, generating better results of both germination percentage and germination rate. On the other hand, the Rules for Seed Analysis (Brasil, 2009) also indicates the alternating temperature of 20 to 30°C, which was not the most effective for the studied cultivars, evidencing the importance of reviewing those tests for new released cultivars.

Seeds of some species show better germination behavior when subjected to alternating temperatures, what mimics the natural fluctuations encountered in the environment; however, according to Lima et al. (1997), there are also species which seed germination is favored when submitted to constant temperatures. Moreover, El-Keblawy et al. (2011) mention that there are species which response to light during germination is linked to temperature, what is confirmed by the interaction among temperatures and light regimes found in this study.

According to Orozco-Segovia and Vasquez-Yanes (1992), seeds may be classified according to their germination behavior to light. They are called positive photoblastic when exposure to light is a condition for germination; negative photoblastic, when germination is inhibited by light; and non-photoblastic, when germination happens regardless light presence. Seeds of Princess 77 bermudagrass are non-photoblastic, as they germinated either in the light or darkness. However, Riviera seeds were considered positive photoblastic, since germination occurred only when submitted to the light regime.

Regarding Experiment 3 and the tested substrate water contents and sowing methods, Evers and Parsons (2010)

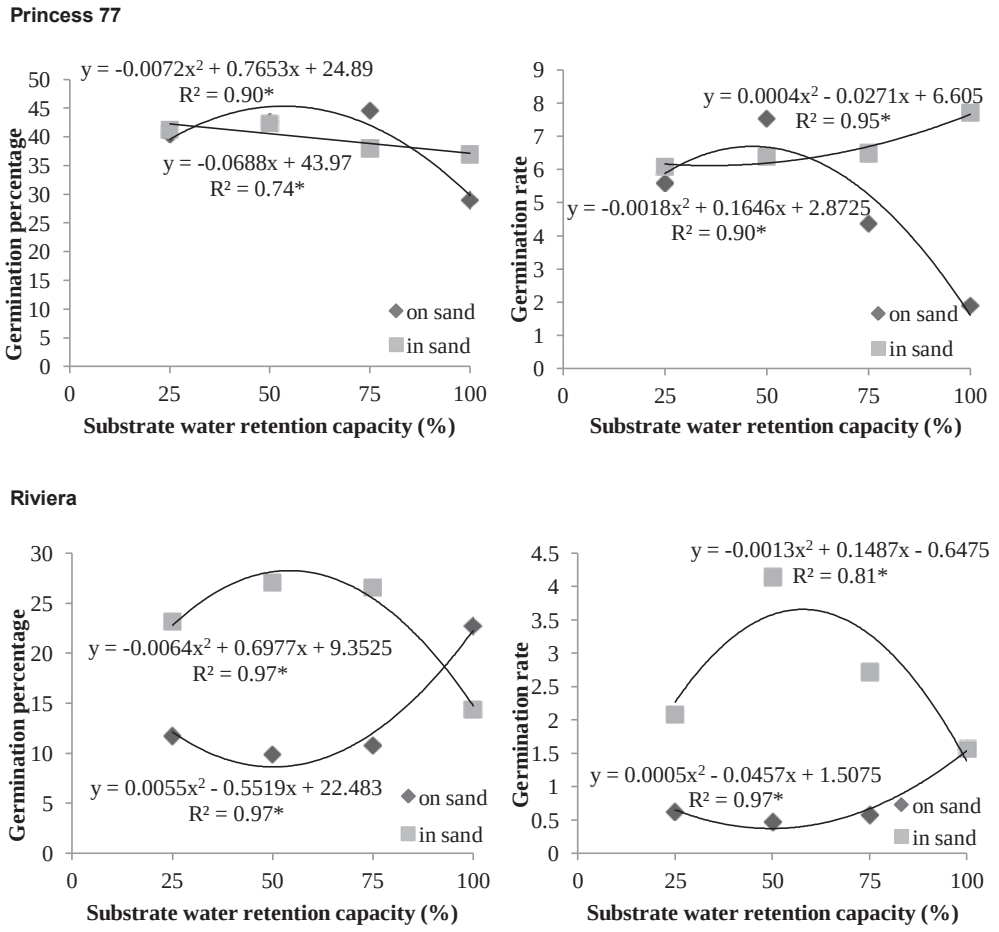


**Figure 2.** Germination percentage and germination rate of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different substrate water contents (% substrate water retention capacity) and sowing methods. *Significant at 5% probability.

mention that, for best results, bermudagrass seeds should be sown on the substrate surface, so that the seed is not placed too deep to have its germination hampered. However, at least for Princess 77, seed location in relation to the substrate did not seem to interfere much. For this cultivar, the substrate water content was the limiting factor for seed germination.

According to Piana et al. (1994), the substrate water content level that most favors seed germination of many species ranges from 40 to 60% of its water retention capacity. For bermudagrass cultivars, the Rules for Seed Analysis (Brasil, 2009) indicate 50% of the substrate water retention capacity as the ideal moisture level, which falls around the most suitable percentage for both Princess 77 and Riviera seeds.

## Conclusion

Seed germination of Princess 77 bermudagrass (*C. dactylon*) was more effective in the absence of NaCl and KCl; at the alternating temperature of 20 to 35 °C, either in the light or darkness; and at around 50% of the substrate water retention capacity, when sown either in sand or on sand surface.

Seed germination of Riviera bermudagrass (*C. dactylon*) was more effective in the absence of NaCl and presence of KCl; at the alternating temperature of 20 to 35°C, in the light; and at 100% of the substrate water retention capacity, when sown on sand surface.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Effect of weather parameters on activity of chiku bud borer, *Anarsia achrasella* Bradley on sapota

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**An experiment on effect of weather parameters on activity of sapota bud borer, *Anarsia achrasella* Bradley on sapota was carried out under middle Gujarat conditions at Anand Agricultural University, Anand. The higher population (6.00 to 17.50 larvae /50 twigs) and bud damage (14.50 to 36.11%) of *A. achrasella* were found during January to May. The correlation between bright sunshine hours and larval population (r=0.500) as well as bud damage (r=0.559) was highly significant and positive. The morning relative humidity, evening relative humidity, mean relative humidity, evening vapour pressure, rainy days and rainfall were highly significant and negative with larval population of *A. achrasella* and its damage in sapota orchard. Mean temperature, morning vapour pressure, mean vapour pressure and wind speed had significant negative correlation with bud damage. Regression analysis of larval population indicated those bright sunshine hours, wind speed, mean temperature, evening vapour pressure and morning relative humidity, the partial regression coefficients were found significant except wind speed and coefficient of determination ($R^2$) was 0.39. Whereas regression analysis of bud damage showed that wind speed, evening vapour pressure and morning relative humidity were non-significant, the partial regression coefficients were found significant and coefficient of determination ($R^2$) was 0.45.**

**Key words:** Weather parameters, *Anarsia achrasella,* activity, sapota.

## INTRODUCTION

Sapota is a vital fruit crop. It is widely grown in Maharashtra, Gujarat, Karnataka, Tamil Nadu, Kerala, Punjab and Hariyana state of India (Anonymous, 2009). Of the various factors limiting the yield of fruits, damage caused by insect pests is pertinent. Sapota tree is attacked by more than 25 insect pests (Butani, 1979). For the management of sapota bud borer, *Anarsia achrasella* Bradley, it is prime need to know occurrence and economic status of insect pests. Considering the economic importance of the pest and lacunae in the information regarding activity and its relationship with different weather parameters, the present study was

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carried out under middle Gujarat conditions.

## MATERIALS AND METHODS

The study on effect of weather parameters on activity of *A. achrasella* was carried out at Anand Agricultural University, Anand (Gujarat), India for two years that is, 2007-2008 and 2008-2009. In order to study the population dynamics of *A. achrasella* five trees were randomly selected and kept free from insecticidal application. From each tree ten twigs (20 cm length) were selected randomly for recording the observations of larval population as well as number of healthy and damaged buds due to *A. achrasella*. The observations were recorded at fortnightly interval. To determine the influence of various physical factors of environment in causing population fluctuation of the pest and its damage, the larval population data were correlated with different meteorological parameters and the statistical analysis was worked out through correlation and regression analysis.

## RESULTS AND DISCUSSION

Data (Tables 1 and 2, Figures 1 and 2) indicated that infestation of *A. achrasella* was found more or less throughout the year. The higher (6.00 to 17.50 larvae /50 twigs) population of larvae and its damage on buds were noticed during January to May (14.50 to 36.11%). Its activity was medium during October to December, whereas it was less active during monsoon (June to September). The pest caused as high as 31.65% infestation on buds in south Gujarat (Patel, 1981 and Jhala et al., 1986) and 35.07% in Junagadh area of Saurashtra (Anonymous, 1986). The infestation of *A. achrasella* persisted almost throughout the year with a single peak in May (Parvathi and Belavadi, 1994). The infestation of the pest on bud remained throughout the year in widely grown variety Kalipatti and remained higher during second fortnight of February to June (Desmukh, 2001). Sushil and Bhatt (2002) reported higher incidence (> 15%) of bud borer during February - May and reached its peak (27.19%) on buds during May. Thus, the present findings are almost tally with the earlier reports.

The correlation between bright sunshine hours and larval population (r=0.500) as well as bud damage (r=0.559) was highly significant and positive. The minimum temperature, morning relative humidity, evening relative humidity, mean relative humidity, evening vapour pressure, rainy days and rainfall had highly significant negative association with larval population of *A. achrasella* and its damage in sapota orchard. The mean temperature, morning vapour pressure, mean vapour pressure and wind speed had non-significant negative correlation with larval population but significant negative correlation with bud damage. Maximum temperature had non-significant positive association with larval population of *A. achrasella* and its damage on sapota orchard. The *A. achrasella* incidence on buds had significant negative correlation with minimum temperature and relative humidity (Anonymous, 1998). The infestation of *A. achrasella* had significant positive correlation with maximum temperature and significant negative correlation with relative humidity (Desmukh, 2001). Sushil and Bhatt (2002) reported that bud borer infestation had significant positive correlation with maximum temperature.

The regression equation fitted to the data by taking larval population of *A. achrasella* (Y) as dependent variable and meteorological parameters as independent variables is

$$Y = 11.2838 + 1.2435^{**} BSS + 0.5184^{ns} WS + 0.8362^{*} MT - 1.8569^{**} VP_2 - 0.5711^{**} RH_1 + 0.9435^{**} \quad (R^2 = 0.39).$$

Where, Y = Larval population, BSS = Bright sunshine hours, WS = Wind speed, MT = Mean temperature, $VP_2$ = Evening vapour pressure, $RH_1$ = Morning relative humidity

All the significant partial regression coefficients were found highly significant except the regression coefficient of mean temperature. The partial regression coefficient of wind speed was found non significant. The model was explained very low variation (39%) existing in number of larva even though the most of partial regression coefficients were found significant. Regression analysis was carried out using the stepwise regression method by taking percentage of bud damage due to *A. achrasella* (Y) as dependent variable and meteorological parameters as independent variables. The coefficient determination ($R^2$) was computed and resultant regression model fitted as:

$$Y = 48.2213 + 1.4030^{*} WS - 1.5122^{**} VP_2 - 0.1282^{*} RH_1 \quad (R^2 = 0.45)$$

Where, Y = Bud damage (%), WS= Wind speed, $VP_2$ = Evening vapour pressure, $RH_1$= Morning relative humidity.

Partial regression coefficients of wind speed, evening vapour pressure and morning relative humidity were found significant and coefficient of determination ($R^2$) was 0.45, it indicated that total variation in bud damage due to *A. achrasella* was explained about 45% variation. It was lower, even though all the partial regression coefficients were found significant.

### Conclusion

The environmental factors *viz.,* bright sunshine hours and maximum temperature were played an important role on activity of *A. achrasella* larval population as well as its damage on buds of *A. achrasella* on sapota. This information generated in present study would be helpful in developing efficient pest management strategies to combat *A. achrasella* on sapota orchard and thereby

**Table 1.** Population fluctuation of *A. achrasella* and its damage in sapota orchard.

| Month and week | Standard meteorological week | Larvae/50 twigs | | | Bud damage (%) | | |
|---|---|---|---|---|---|---|---|
| | | 2007-2008 | 2008-2009 | Mean | 2007-2008 | 2008-2009 | Mean |
| April I | 14 | 11 | 10 | 10.50 | 30.08 | 33.51 | 31.80 |
| II | 15 | 10 | 11 | 10.50 | 32.15 | 29.50 | 30.83 |
| III | 16 | 12 | 11 | 11.50 | 29.62 | 37.30 | 33.46 |
| IV | 17 | 15 | 20 | 17.50 | 34.07 | 38.14 | 36.11 |
| V | 18 | 10 | 11 | 10.50 | 32.25 | 33.00 | 32.63 |
| May I | 19 | 2 | 18 | 10.00 | 27.44 | 23.30 | 25.37 |
| II | 20 | 5 | 17 | 11.00 | 26.52 | 30.18 | 28.35 |
| III | 21 | 8 | 14 | 11.00 | 24.02 | 26.20 | 25.11 |
| IV | 22 | 10 | 7 | 8.50 | 27.54 | 23.72 | 25.63 |
| June I | 23 | 4 | 5 | 4.50 | 21.46 | 14.20 | 17.83 |
| II | 24 | 8 | 5 | 6.50 | 27.01 | 8.80 | 17.91 |
| III | 25 | 1 | 9 | 5.00 | 18.80 | 19.60 | 19.20 |
| IV | 26 | 3 | 0 | 1.50 | 12.10 | 9.20 | 10.65 |
| July I | 27 | 2 | 2 | 2.00 | 16.66 | 19.40 | 18.03 |
| II | 28 | 1 | 0 | 0.50 | 13.20 | 14.10 | 13.65 |
| III | 29 | 2 | 1 | 1.50 | 9.90 | 12.00 | 10.95 |
| IV | 30 | 1 | 0 | 0.50 | 7.45 | 8.40 | 7.93 |
| V | 31 | 0 | 1 | 0.50 | 3.32 | 6.85 | 5.09 |
| August I | 32 | 3 | 1 | 2.00 | 7.40 | 6.40 | 6.90 |
| II | 33 | 1 | 0 | 0.50 | 5.55 | 5.40 | 5.48 |
| III | 34 | 0 | 0 | 0.00 | 6.65 | 2.77 | 4.71 |
| IV | 35 | 0 | 1 | 0.50 | 1.20 | 10.25 | 5.73 |
| September I | 36 | 0 | 0 | 0.00 | 4.80 | 4.34 | 4.57 |
| II | 37 | 4 | 8 | 6.00 | 8.55 | 28.24 | 18.40 |
| III | 38 | 3 | 7 | 5.00 | 6.33 | 27.29 | 16.81 |
| IV | 39 | 15 | 0 | 7.50 | 25.60 | 5.68 | 15.64 |
| October I | 40 | 14 | 1 | 7.50 | 29.55 | 12.72 | 21.14 |
| II | 41 | 13 | 4 | 8.50 | 27.44 | 10.12 | 18.78 |
| III | 42 | 14 | 1 | 7.50 | 33.45 | 12.33 | 22.89 |
| IV | 43 | 15 | 0 | 7.50 | 27.10 | 9.47 | 18.29 |
| V | 44 | 11 | 0 | 5.50 | 27.01 | 7.37 | 17.19 |
| November I | 45 | 11 | 6 | 8.50 | 33.51 | 31.39 | 32.45 |
| II | 46 | 9 | 2 | 5.50 | 34.80 | 12.84 | 23.82 |
| III | 47 | 11 | 1 | 6.00 | 25.37 | 10.29 | 17.83 |
| IV | 48 | 4 | 1 | 2.50 | 27.50 | 13.50 | 20.50 |
| December I | 49 | 10 | 0 | 5.00 | 22.25 | 6.89 | 14.57 |
| II | 50 | 18 | 0 | 9.00 | 27.76 | 7.00 | 17.38 |
| III | 51 | 20 | 1 | 10.50 | 28.53 | 6.52 | 17.52 |
| IV | 52 | 12 | 6 | 9.00 | 14.60 | 15.67 | 15.14 |
| January I | 1 | 16 | 7 | 11.50 | 14.40 | 14.70 | 14.55 |
| II | 2 | 11 | 14 | 12.50 | 24.50 | 31.92 | 28.21 |
| III | 3 | 18 | 15 | 16.50 | 29.60 | 20.14 | 24.87 |
| IV | 4 | 10 | 13 | 11.50 | 22.50 | 24.73 | 23.62 |
| V | 5 | 4 | 8 | 6.00 | 19.76 | 12.84 | 16.30 |
| February I | 6 | 11 | 12 | 11.50 | 14.60 | 21.13 | 17.87 |
| II | 7 | 10 | 13 | 11.50 | 16.60 | 33.51 | 25.06 |
| III | 8 | 6 | 14 | 10.00 | 38.25 | 29.32 | 33.79 |
| IV | 9 | 6 | 11 | 8.50 | 37.82 | 28.27 | 33.05 |
| March I | 10 | 15 | 12 | 13.50 | 41.71 | 26.06 | 33.89 |
| II | 11 | 10 | 14 | 12.00 | 33.73 | 27.94 | 30.84 |

**Table 1.** Contd.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| III | 12 | 10 | 8 | 9.00 | 31.91 | 20.74 | 26.33 |
| IV | 13 | 13 | 13 | 13.00 | 35.94 | 27.91 | 31.93 |

**Table 2.** Correlation coefficient between weather parameters and *A. achrasella* on sapota.

| Weather parameter | *A. achrasella* | |
|---|---|---|
| | Larva | Bud damage (%) |
| Bright sunshine hours, hrday$^{-1}$ (BSS) | 0.500** | 0.559** |
| Maximum temperature, °C (MaxT) | 0.140 | 0.038 |
| Minimum temperature, °C (MinT) | -0.259** | -0.498** |
| Mean temperature, °C (MT) | -0.106 | -0.313** |
| Morning relative humidity, % ($RH_1$) | -0.274** | -0.321** |
| Evening relative humidity, % ($RH_2$) | -0.431** | -0.588** |
| Mean relative humidity, % (MRH) | -0.0421** | -.0544** |
| Morning vapour pressure, mm of Hg ($VP_1$) | -0124 | -0.234* |
| Evening vapour pressure, mm of Hg ($VP_2$) | -0.419** | -0.631** |
| Mean vapour pressure, mm of Hg (MVP) | -0.179 | -0.312** |
| Rainy days | -0.332** | -0.403** |
| Wind speed, kmhr$^{-1}$ (WS) | -0.085 | -0.229* |
| Rainfall, mm (RF) | -0.314** | -0.361** |

*Significant at 5% level, **Significant at 1% level.

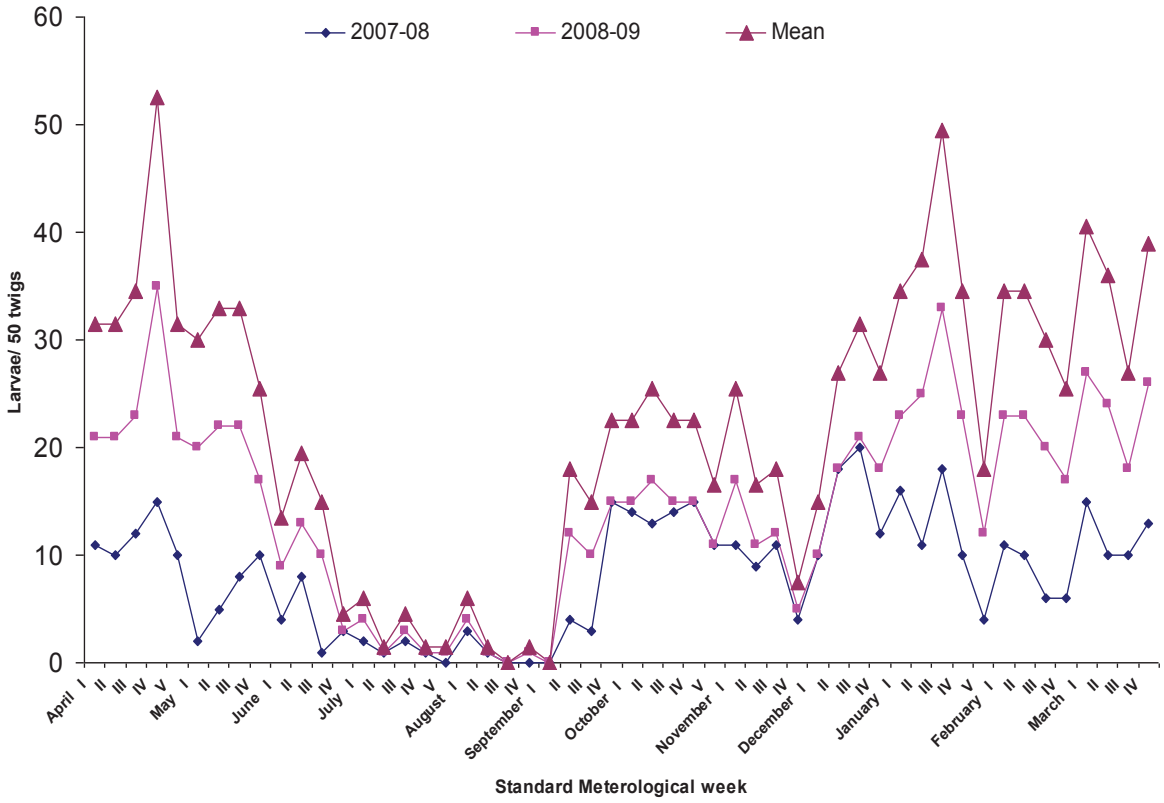


**Figure 1.** Population fluctuation of *A. achrasella* in sapota.

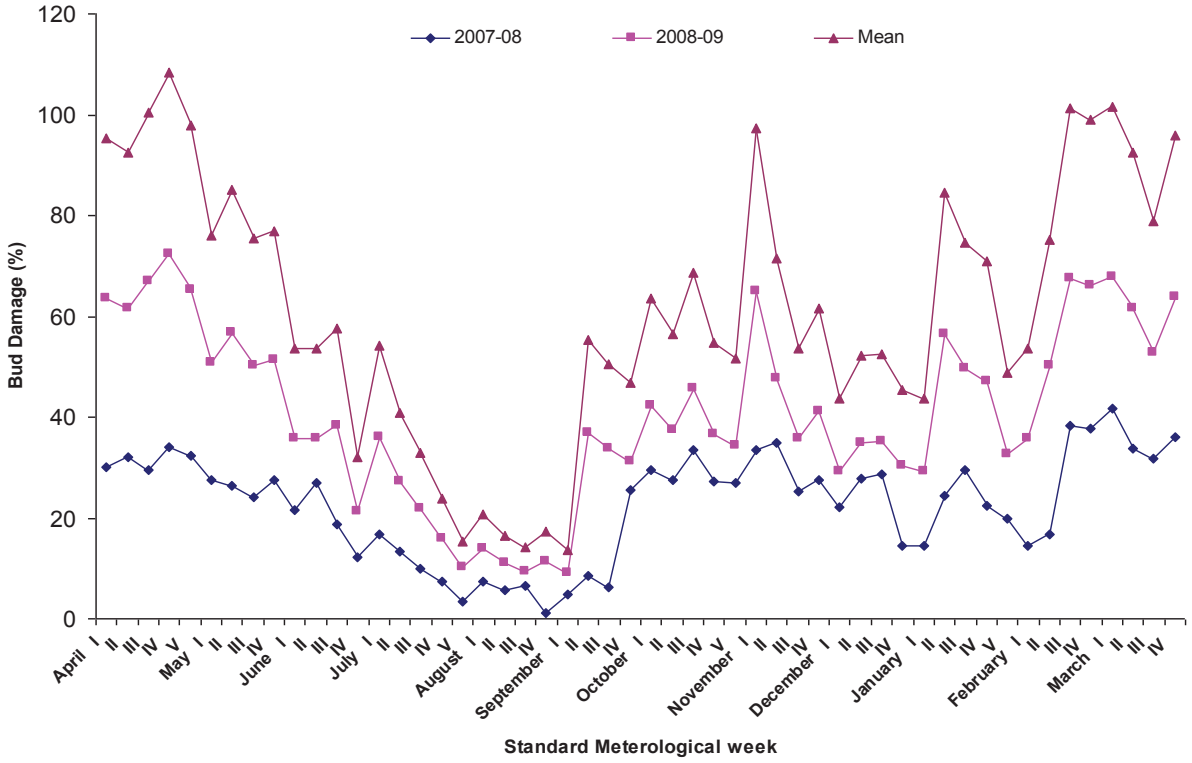


**Figure 2.** Bud damage by *A. achrasella* in sapota.

increase the productivity of sapota besides safety to the environment.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Management of Nguni goats to control gastrointestinal parasites and anthelmintic resistance at KwaMthethwa and Owen Sitole College of Agriculture area

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**Management techniques that can assist in treating only parasite infested animals can reduce anthelmintic resistance arousing from frequent dosing of all animals. This study aimed to investigate the application of FAMACHA© with no formal training of resource-poor farmers to identify anaemic or helminthes infested animals. Two sites (KwaMthethwa (KM) village and Owen Sitole College of Agriculture (OSCA) farm) with 40 animals each of mixed sex were used in this study. The animals grazed on natural pasture during the day and housed in Kraal at night. FAMACHA© chart scored animals on eye colour; 1 (Red, non-anaemic and acceptable), 2 (red-pink, non-anaemic and border line), 3 (pink, mildly anaemic and dangerous), 4 (pink-white, anaemic and fatal) or 5 (porcelain white, severely)). Eye scoring was over four seasons (autumn, winter, spring and summer) alongside faecal egg count (FEC) as a positive control to FAMACHA© diagnosis method. Anaemic animals varied (P<0.05) between the sites, 76.72, 40.34 and 49.37% for KM, OSCA and KM+OSCA, respectively. Comparison of FAMACHA© with FEC showed that only 61.53, 33.5 and 40.12% of the animals were anaemic at KM, OSCA and KM+OSCA, respectively, due to false positive results. Spring, autumn and summer were identified as seasons for frequent monitoring due to higher (P<0.05) gastrointestinal parasite especially *Trichostrongylus axei and Moniezia expansa*. Approximately 80% of all anaemic animals were identified by no skilled resource-poor farmers using FAMACHA©. This reinforces the economic (cheap) importance and reliability of FAMACHA© chart in parasite resistance management but emphasized on formal training as 20% false negative anemic animals is a lot for a resource-poor farmers.**

**Key words:** FAMACHA©, Anthelmintic, resistance, faecal egg count, Nguni goat.

## INTRODUCTION

The world's goat population is estimated to be 876 million with 276 million coming from Africa where about 6.2 million is produced by South Africa according to the Food and Agriculture Organization of the United Nations Statistical database (FAOSTAT, 2011). However, the goat population in South Africa has been declining (from

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2008 (6.5 million), 2009 (6.4 million), 2010 (6.3 million) to 2011 (6.2 million)) which is a major concern especially to the rural communities where it serves as a major source of income or protein supply in diets (FAOSTAT, 2011). Therefore, it is possible to postulate that by 2025 the goat population will be less than 5 million in South Africa. These are significant and alarming statistics especially with the growing demand for animal protein (Mmbengwa et al., 2008) by the ever increasing human population (48.6 (2008) to 51.8 (2011) million people) in South Africa (STATSA, 2011). No specific reason has been associated to the decrease in goat production though different researchers have advanced the following reasons; low research on goats as their contribution towards formal national economy is assumed to be less, lack of etiology of diseases (internal and external) prevalence, inaccessibility of conventional knowledge and information among small-scale farmers, minimal resources (land and capital), poor food security and informal labour. Goat production is bound to decline without any intervention especially among the small scale farmers in South Africa who owns about 50% of the total goats population (Shabalala and Mosima, 2002). KwaZulu-Natal has the second largest human population (10.3 million) and the third largest goat population in South Africa, with the indigenous Nguni breed as majority (Slippers et al., 2006). This breed is preferred by most resource-poor farmers because of its resistance to some diseases and parasites, high conception rates, lower mortality rate, hardiness, adaptability and good mothering ability (Barry and Godke, 2001). Though the Nguni breed is superior over the other goat species, health issues such as worms, diarrhea, poor condition, external parasites, fertility, abortion, eyes problems are still a major task to eradicate.

Infection of the gastrointestinal tract by helminthes (nematodes) has been coined as a major limiting factor in goat production particularly among resource-poor farms (Hoste et al., 2002). Anthelmintic resistance on both commercial and some resource-poor small ruminant farmers has been reported in South Africa (Spickett et al., 2012; Van Wyk and Bath, 2002; Vatta et al., 2002). However, anthelmintic resistance prevalence has been reported to be higher in goats than in sheep (Hoste et al., 2002). Therefore alternative methods for worm management in other to reduce worm resistance are vital as goat production is no longer cost effective (millions of Rands are spent on drugs that parasite population has already developed resistance). The treatment of only sick animals has been suggested as it favours unselected worms coming from untreated animals (Wyk and Bath, 2002). FAMACHA© system (grading animal according to their anaemic level) and body condition scores has been used by trained participants to identify sick (infested with *Haemonchus contortus*) animals (Spickett et al., 2012; Van Wyk and Bath, 2002). The results demonstrated benefits such as reduction in treatments, lower selection pressure on helminthes for anthelmintic resistance and discriminate animals with variable ability to cope with infection but demanded more work on the application of FAMACHA© chart on goats (Leask et al., 2012). Though some of the resource-poor farmers are gradually getting access to anthelmintic drugs (subsidized) due to government intervention, wastage is still a major problem as all animals are often treated at once instead of selecting for sick animals. Management methods for sick animal identification such as FAMACHA© has shown 93% success after training (Reynecke et al., 2011) but can it work for resource-poor farmers without any formal training? This study aimed to use the FAMACHA© chart as a tool to control parasite infestation and to manage anthelmintic resistance in goats of resource-poor farmers at KwaMthethwa (KM) village and Owen Sitole College of Agriculture (OSCA) of the province of KwaZulu-Natal in South Africa. Fecal egg loads were counted as a positive control for the FAMACHA© diagnostic method (Quijada et al., 2012). It was hypothesized that no skill resource-poor farmers will not be able to manage sick animals on their farm with the FAMACHA© chart.

## MATERIALS AND METHODS

### Experimental sites, animals, nutrition and sampling

The study sites were KwaMthethwa (KM) village and Owen Sitole College of Agriculture (OSCA), located at the north coast of KwaZulu-Natal Province, South Africa. KM is located at 28°31'S Latitude and 31°51'E Longitude while OSCA bearings are 28°45'S Latitude and 31°53'E Longitude both of which have an annual rainfall of 900 mm and an average temperature of 26°C. Forty Nguni goats in each area KM (belonging to four resource-poor farmers) and OSCA (owned by government for student research) were monitored (Mitchell, 1982). In both areas the animals were allowed to graze during the day on communal or natural pastures (under the supervision of a shepherd) and housed in kraal at night. Animal sampling was carried out monthly in four seasons; summer (October-January), autumn (February-April), winter (may-July), and spring (August-September) from 2012-2013. Sex (male and female), ages (between 1-4 years calculated from number of incisors (Mitchell, 1982) and Alba-Hurtado et al. (2010) ) and weight of goats were some of the independent variables considered. All animals were ethically treated as per the University of Zululand Ethical Committee research guide manual (S590904).

### Depicting anaemia level by FAMACHA© chart

FAMACHA© chart is an eye colour based stratification method, with five colour categories of the conjunctival mucous membrane from bright red to pale as an indicator of anaemia (Malan and Van Wyk, 1992; Van Wyk and Bath, 2002). The lower eyelid mucous membrane of each goat were examined and compared to a laminated colour chart picture of eye with five different levels of anaemia and assigned a score of either 1 (Red, non-anaemic and acceptable), 2 (red-pink, non-anaemic and border line), 3 (pink, mildly anaemic and dangerous), 4 (pink-white, anaemic and fatal) or 5 (porcelain white, severely). Animals with scores of 3-5 were advised for treatment. FAMACHA© chart level rating at KM was done by the farmers while at OSCA it was done by the Shepard. All

**Table 1.** FAMACHA© diagnosis of anaemic animals and reliability assessment.

| Sites | FAMACHA© Diagnosis (%) | | Comparing FAMACHA© against egg count diagnosis (%) | | | |
|---|---|---|---|---|---|---|
| | Anaemic | Non anaemic | Positive | Negative | False positive | False negative |
| KM | 76.92 | 23.01 | 61.53 | 7.69 | 12.82 | 17.96 |
| OSCA | 40.34 | 59.67 | 33.5 | 24.58 | 8.47 | 33.48 |
| KM+OSCA | 49.37 | 50.63 | 40.12 | 20.38 | 9.55 | 29.94 |
| SEM | 2.61 | 1.98 | 2.03 | 4.01 | 3.12 | 2.25 |
| P value | * | * | * | * | * | * |

*P<0.05.

the farmers received equal advised from researcher on anaemia rating using FAMACHA© chart.

#### Determination of faecal egg counts per gram of faeces

Faecal egg count (FEC) was carried out to investigate any correlations with FAMACHA rating of animal's health by resource-poor farmers. One gram of faeces collected from the rectum was dissolved in 40 ml of sugar solution and allowed to stand for five minutes. Faecal egg count was counted using a modified McMaster Technique because it estimates egg count per gram (EPG) of faeces (Hansen and Perry, 1994). The number of nematode eggs in both wells of the McMaster chamber was multiply by 133 ([40 ml ÷ (0.15 ml x 2) = 133]) to get EPG. Generally, 500 EPG has been suggested for treatment by many authors in order to decrease pasture contamination and prevent the development of subclinical diseases (Stephen et al., 2003). However, subclinical symptoms development would depends on a lot of factors such as animal's genetic resistance, age, state of nutrition and specific worm species (Stephen et al., 2003). Although FAMACHA© chart is often linked to the wireworm (*Haemonchus contortus*) infection, these study considered total egg counts including wireworm when relating faecal egg counts to FAMACHA scores. Because pathogenicity count is greatly influenced by species type and number, total egg counts greater than 2000 were considered as potential anaemic counts.

*Trichostrongylus axei* and *Moniezia expansa* were monitored throughout the season by observing the diagnostic features of their eggs under the microscope as described by Thienpont et al. (1979). *M. expansa* identification was much easier due to its diagnostic features peculiarity whereas *T. axei* was a bit difficult due to its similarities with *Strongyles* spp under the microscope. Means of FEC and number of anaemic animals estimates were subjected to analysis of variance (ANOVA) using the general linear model of SAS (2002). Student Newman-Keuls' test was used to compare means.

## RESULTS

FAMACHA© scores showed that the number of anaemic animals were higher (P<0.05) in KM than at OSCA or KM+OSCA (Table 1). Non anaemic animals were lower (P<0.05) in KM than at OSCA and KM +OSCA. The results obtained from comparing clinical FAMACHA© scores to faecal egg counts showed that the percentage of anaemic animals were still higher in KM than at OSCA or combined sites. False negative results were prevalent (P<0.05) at OSCA than KM while false positive results were prevalent at KM when compared to OSCA.

Faecal egg count varied (P<0.05) between seasons with the highest counts recorded in spring and autumn, followed by summer and least in winter (Table 2). Animal sex had an effect on FEC as a higher number of eggs (P<0.05) were observed in males than in females (Table 2). The number of FEC was also influenced by age as the number of eggs increased (P<0.001) with animal age. The least number of FEC were observed in Nguni goats of 1 year and below while the highest were observed in goats of 5 years and beyond. Generally, FEC increased with increased in FAMACHA© scores but for the FAMACHA© score of 5 that was the same with 4.

The highest (P<0.05) number of *T. axei* and *M. expansa* eggs in Nguni goats were recorded in spring and summer for both KM and OSCA (Table 3). *T. axei* and *M. expansa* egg counts in Nguni goats were lower in winter than autumn for both KM and OSCA.

## DISCUSSION

Most studies in the literature utilize people with formal or partial training on FAMACHA© chart (Leask et al., 2012; Papadopoulos et al., 2012) while this study used resource-poor farmers with no form of training, which is equivalent to buying off the counter. The reason behind this trial was based on the fact that formal training by Non-Governmental Organizations or local government programmes rarely reaches these farmers in the remote areas. Application of FAMACHA© chart by resource-poor farmers with no formal training was able to identify anaemic (sick) animals in this study which is similar to the results obtained by Ribeiro et al. (2012). The animals at KM were heavily infested with helminthes as shown by the higher number of anaemic animals compared to OSCA. The lower percentage of anaemic animals at OSCA was associated to frequent dosing when compared to KM. Animal identification results by FAMACHA© chart were similar to those obtained by other researchers but with either partial or formal trained staff (Van Wyk and Bath, 2002; Vatta et al., 2011). The correlation between FAMACHA© and FEC diagnosis was quite high with an $R^2$ value of 0.91. When FEC was used as a positive control to FAMACHA© chart, it was found that 17.96, 33.48 and 29.94% of anaemic animals at KM,

**Table 2.** Prevalence of gastrointestinal parasites in Nguni goats at KAM and OSCA.

| Source of variation | level | Mean FEC (±SD) | Coefficient of variation |
|---|---|---|---|
| Season | Autumn | 3817±178 | 0.031 |
| | Winter | 3538±140 | 0.026 |
| | Spring | 3817±160 | 0.028 |
| | Summer | 3684±138 | 0.025 |
| Sex | Male | 3777±114 | 0.020 |
| | female | 3378±192 | 0.038 |
| Age | 1 | 2474±190 | 0.051 |
| | 2 | 3777±246 | 0.043 |
| | 3 | 4376±206 | 0.031 |
| | 4 | 4562±276 | 0.040 |
| FAMACHA© | 1 | 1500±420 | 0.112 |
| | 2 | 2001±200 | 0.043 |
| | 3 | 3244±164 | 0.028 |
| | 4 | 3777±206 | 0.036 |
| | 5 | 4376±280 | 0.043 |
| Effects | Sex | * | |
| | Age | *** | |
| | FAMACHA© | ** | |
| | Season * Sex | ns | |
| | Season * Age | ns | |
| | Season * FAMACHA© | * | |
| | Sex * Age | *** | |
| | Sex * FAMACHA© | *** | |
| | Age * FAMACHA© | ns | |
| | Season * Sex * FAMACHA© | ns | |
| | Season * Age * FAMACHA© | ns | |
| | Sex * Age * FAMACHA© | * | |

FEC = faecal egg count, EPG = egg count per gram, *P<0.05, **P<0.01, ***P<0.001.

**Table 3.** Effect of season on *T. axei* and *M. expansa* egg counts in Nguni goats raised at KAM and OSCA.

| Site | Season | Mean weight (kg) | *T. axei* (mean counts) | *M. expansa* (mean counts) |
|---|---|---|---|---|
| OSCA | Autumn | 33.2 | 1120 | 1680 |
| | Winter | 27.4 | 1500 | 1020 |
| | Spring | 24.8 | 1940 | 2640 |
| | Summer | 28.1 | 1860 | 2840 |
| KM | Autumn | 29.9 | 1920 | 1820 |
| | Winter | 30.7 | 1180 | 1620 |
| | Spring | 23.5 | 3300 | 2300 |
| | Summer | 24.1 | 2240 | 2614 |
| SEM | | 6.74 | 1110 | 1092 |
| P-value | | * | * | * |

*P < 0.05.

OSCA and KM+OSCA, respectively, were false negative. Though the numbers of false positive animals were lower compared to false negative, false negative is still worth improving as one dead animal is a lot to resource-poor

farmers. The false negative and positive results were associated to no formal or very little training of the resource-poor farmers rather than reliability of the FAMACHA© chart. Even when formal training on FAMACHA© system has been used in trials, only 93% of anaemic animal identification has been achieved (Vatta et al., 2011). Therefore the result obtained in this study is important but can be improved by formal training of rural farmers. If only two people are properly trained in each community, they can pass on the technology easily rather than sending a few professionals who will not be able to reach the needy (Vatta et al., 2011). A high false positive result implies that animals that were not sick will be treated which comes at a price. Firstly, anthelmintic drug wastage on treating animals that are not sick and secondly anthelmintic resistance will be prevalent because of frequent dosing (Hoste et al. 2002). Under-dosing which promotes anthelmintic resistance may also be problematic in false negative animals because of incomplete treatment of parasite especially when a general deworming programme is being followed. Incomplete treatment implies exposure of parasites to low doses of anthelmintic drugs hence the development of parasite resistance (Degen et al., 1995; Jabbar et al., 2013).

The results from FEC shows that autumn, spring and summer are the periods to constantly observe the animals for parasite infestation than winter. Similar studies emphasizing on frequent observation and treatment of sick animals during spring and autumn has also been reported as breeding conditions are suitable (Githigia et al., 2001; Reynecke et al., 2011). The incidence of helminthes in male and female goats were similar to those obtained by Asanji and Williams (1984). An explanation of male prevalence to anthelmintic over female goats where not clear but has been associated to the following factors; sex of the host, genetic composition, environmental factors, feeding food habits of the host or the host parasite interaction. It has also been observed that females are more susceptible to infection during pregnancy and peri-parturient period because of weaker immune system and stress (Dagnachew et al., 2011; Khan et al., 2010). FAMACHA© chart seasonal egg counts and *T. axei* and *M. expansa* egg counts per gram of sample also demonstrated that gastrointestinal parasitic infestation was prevalent in spring and summer than winter. Therefore animals should be frequently checked and treated for any helminthes infection during those periods. It is also worth mentioning that parasite infestation varied with age of animal with the least egg counts observed in the youngest animals. This was contrary to the results obtained in most literature where younger animals are more infested with helminthes due to weaker immune response (Dagnachew et al., 2011; Khan et al., 2010). The hypothesis was therefore rejected as anaemic animals were identified by farmers with no formal training.

## Conclusion

It was concluded that FAMACHA© chart is a good and an economical tool to manage anthelmintic resistance by resource-poor farmers but can be a better management tool if strategies on formal training are properly outlined. Spring, autumn and summer were confirmed as the seasons for frequent checks on the conjunctival mucous membrane (helminthes infestation) to reduce pathogenicity of egg counts**.** Future studies on expanded projects with formal training of field workers that can educate more resource-poor farmers in the remote areas can assist in decreasing the number of false negative anaemic animals identified in this study.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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# Free radical scavenging properties and their relationship with bioactive compounds content of dehydrated calyces of roselle (*Hibiscus sabdariffa* L.)

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**Roselle (*Hibiscus sabdariffa* L.) also called roselle fruit or flower of jamaica is a plant used in the traditional medicine due to its wealth of bioactive compounds. These compounds confer beneficial health benefits on it in aqueous infusions prepared with the blossoms of the jamaica flower. In the present study, we determined the antioxidant activity of 64 roselle varieties and quantified the following bioactive compound contents: phenolic; monomeric anthocyanins, and ascorbic acid. The results show that highest antiradical scavenging activity and reductor capacity belonged to varieties with dark-red calyces. Similarly, we found that the bioactive compound concentration increased as the pigmentation of the fresh calyces intensified. Finally, our results demonstrated that the aqueous extracts' antioxidant activity is correlated with the bioactive compound concentration, this correlation greater with the content of ascorbic acid.**

**Key words:** Roselle, antioxidant activity, phenolic compounds, monomeric anthocyanins.

## INTRODUCTION

Bioactive compounds promote beneficial effects on health, and among these we find their action as antioxidants. Free radicals (FR) are substances generated by the aerobic metabolism, reactions with

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drugs or with environmental agents. When the cellular levels of these species overcome an organism's antioxidant defense system, a condition appears that is denominated oxidative stress (OS), which can cause cell damage, trigger physiological disorders, and favor the appearance of health problems such as cancer, cardiovascular diseases, and degenerative and inflammatory diseases. Bioactive compounds are the product of plant-related metabolism and are characterized by promoting beneficial effects in an organism; among these effects, we find inhibiting or delaying the reaction of FR on biological structures, inhibiting lipid peroxidation, and chelating heavy metal ions, and examples of bioactive compounds comprise phenolic compounds, pigments, and vitamins. Antioxidant substances represent one of the important mechanisms of defense against Free radicals (FR), but Endogenous Antioxidant Molecules (EAM) by themselves are not sufficiently effective for counteracting the damage caused by reactive oxygen species, particularly if the present lifestyle is taken into account: Smoking, drugs, alcohol, unbalanced diet, contamination and inadequate solar exposure, all of which facilitate the formation of Free radicals (FR). Thus, increasing the dietary intake of antioxidants is of great importance for good health, as evidenced by studies that characterize the antioxidant activity of foods (Gregoris et al., 2013). At present, there is great interest in consuming antioxidants derived from natural sources, such as plants that have been utilized in traditional medicine due to their being rich in bioactive compounds (Mungole and Chaturvedi, 2011), and roselle (*Hibiscus sabdariffa* L.*)* is one of those cases. The calyces and flowers of roselle, or *jamaica*, as it is known in Spanish, contain alkaloids, ascorbic acid, β-carotene, citric acid, malic acid, protocatechuic acid, anthocyanins, quercetin, pectin, and polysaccharides. In addition, the extracts obtained from the calyces contain phytochemical compounds such as polyphenic acids, flavonoids, and anthocyanins (Maganha et al., 2010), which confer upon them properties that are beneficial for health, including antihypertensive and hypocholesterolemic characteristics (Hopkins et al., 2013), antibacterial properties (Yin and Chao, 2008), selective cytotoxic and apoptogenic activity (Khaghani, 2011), and even anticancerigenous activity (Lin et al., 2012). Anthocyanins present in the extracts confer on the latter 51% of their antioxidant activity (Tsai et al., 2002). The objective of this work was to characterize the antioxidant activity and concentration of the bioactive compounds of 64 varieties of roselle (*jamaica*).

## MATERIALS AND METHODS

The following reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): 1,1-Diphenyl-2-picrylhydrazyl (DPPH•); 2,6-Dichloro-indophenol (DCPI); sodium acetate; Ascorbic acid (AA), gallic acid; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); ferric chloride(III); potassium chloride; potassium hexacyanoferrate(III); potassium persulfate; Folin-Ciocalteu reagent; glacial acetic acid; oxalic acid; trichloroacetic acid; 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$); sodium carbonate, and anhydrous ethanol. A spectrophotometer UV-Vis BioTek PowerWave XS with microplates reader was used to measure absorbances in all techniques.

### Experimental determinations

#### *Reception and sample preparation*

We received 64 dehydrated calyces of varieties of roselle (*Hibiscus sabdariffa* L.) that had been cultivated in the experimental fields of the Academic Unit of Agriculture belonging to the Autonomous University of Nayarit (UAN), Mexico, during the period of July through December 2012. The dehydrated calyces were frozen at -18°C for their later lyophilization to constant weight. Once they were dried, they were ground until a fine powder was obtained. We performed aqueous extractions for each of the varieties, in triplicate, following the method proposed by Prenesti et al. (2007). The aqueous extract was stored at -18°C until time for its analysis. For determination of total monomeric anthocyanins, we followed the protocol described by Giusti and Wrolstad (2005).

#### *DPPH radical scavenging activity*

DPPH●-based antiradical activity was evaluated according to the procedure reported by Morales and Jiménez-Pérez (2001). The technique consisted of preparing a solution of DPPH● at a concentration of 74 $mg \cdot L^{-1}$ in ethanol and shaking it for 10 min. Later, we placed 100 µl of the samples in vials, added 500 µl of DPPH● solution, and shook vigorously; these vials were allowed to stand at room temperature during 1 h. After this time, the vials were centrifuged at 13,000 *g* for 5 min at room temperature and later, supernatant absorbance was measured at a wavelength of 520 nm. The scavenging capacity of the free radical DPPH• of the aqueous extracts was obtained from a standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and was expressed as Trolox-equivalent (TE) micromoles per gram of calyces dry base (d.b.) (µmol $TE \cdot g^{-1}$ calyces d.b.).

#### *$ABTS^{\bullet+}$ radical scavenging activity*

$ABTS^{\bullet+}$ stable cation radical scavenging capacity was determined according to the method reported by Re et al. (1999) and by Kuskoski et al. (2005). The $ABTS^{\bullet+}$ radical is obtained from the reaction of $ABTS^{\bullet+}$ (7 $mmol \cdot l^{-1}$) with potassium persulfate (2.45 $mmol \cdot l^{-1}$) at a 1:0.5 ratio (v/v) and incubated at 4°C under conditions of darkness during 16 h. Once formed, the $ABTS^{\bullet+}$ radical is diluted with ethanol until obtaining an absorbance value of between 0.70 (±0.1) and 754 nm. The samples (including the standardized curve) are adequately diluted with water until 20 to 80% inhibition of the initial $ABTS^{\bullet+}$ color is produced. Twenty microliter of the sample is added to 980 µl of the $ABTS^{\bullet+}$ dilution. Absorbance is measured at 754 nm at 7 min. The radical scavenging capacity of the free radical $ABTS^{\bullet+}$ of the extracts was determined by means of a standardized curve of AA and was expressed as TE of Ascorbic acid (AA) per g dry base (d.b.) calyces (mg $EAA \cdot g^{-1}$ calyces d.b.).

### Determination of the reduction capacity of the Fe(III) ion into Fe(II)

We determined this by the method of Hinneburg et al. (2006). The

method consisted of taking a 25 µl aliquot of each extract; mixed this with 63 µl of phosphate buffer (0.2 mol·l$^{-1}$, pH 6.6) and 63 µl of potassium hexacyanoferrate (III) [$K_3Fe(CN)_6$] at 1%. After to 30 min incubation at 50°C, we added 63 µl of chloroacetic acid at 10% and centrifuged this during 10 min at 13,000 *g*. Next, we added 63 µl of supernatant to 63 µl of water and 12.5 µl of ferric chloride. Absorbance was registered at 700 nm. The capacity to reduce the iron (III) was determined as mg acetic acid equivalents (AAE) per gram of dry base (d.b.) calyces (mg AAE·g$^{-1}$ of calyces d.b.) from a standard curve of AA.

**Determination of the concentration of total phenolic compounds**

This was determined according to the method of Stintzing et al. (2005), which utilizes the Folin-Ciocalteu reagent. The blue coloration is read at a wavelength of 765 nm and reflects the total amount of polyphenols, expressed as gallic acid equivalents (GAE). One hundred microliter of the sample was measured and we added 500 µl of Folin-Ciocalteu solution (1:10 in deionized water) and 400 µl of sodium carbonate solution (at 7.5%); then, the samples were vortex-shaken and incubated at room temperature during 30 min. Afterward, we measured absorbance at a wavelength of 765 nm. Total phenolic compound concentration was obtained from a standardized curve of gallic acid and was expressed as gallic acid equivalents (GAE) per gram of dry base (d.b.) calyces (mg GAE·g$^{-1}$ of calyces d.b.).

**Determination of the concentration of total monomeric anthocyanins**

Total monomeric anthocyanin content was determined by spectrophotometry according to the protocol described by Giusti and Wrolstad (2005), which utilized the differential pH. For analysis of the total monomeric anthocyanin content, we placed 50 µl aliquots of the filtered solution into two assay tubes and measured this with 450 µl of pH 1.0 and pH 4.5 buffer solutions, respectively (Dilution factor [DF] = 10). In both solutions, we measured absorbance at 520 and 700 nm wavelengths by Ultraviolet (UV)-Vis spectrophotometry. The content of the pigment was calculated as delphinidin-3-glucoside with the following formula:

$$CA=\frac{A*MW*DF*1000}{£*l}$$

in which CA = Concentration of total monomeric anthocynanins, mg delphinidin-3-glucoside (D3G) per liter, DF = Dilution factor, A = Absorbance, ($A_{520}$ pH 1 – $A_{700}$ pH 1) – ($A_{520}$ pH 4.5 – $A_{700}$ pH 4.5), l = length of the passage of light in a cell in cm (0.64 cm), £ = coefficient of molar absorbance 27,481 l·(mol·cm)$^{-1}$ for delphinidin-3-glucoside, and MW = Molecular weight of delphinidin-glucoside (465.2 g·mol$^{-1}$). Total monomeric anthocyanins concentration was expressed as mg delphinidin-3-glucoside (D3G) per gram of dry base (d.b.) calyces (mg D3G·g$^{-1}$ of calyces d.b.).

**Determination of ascorbic acid concentration**

We employed the colorimetric method described by Dürüst et al. (1997)and prepared the following solutions: 2,6 Dichlorophenol-indophenol (DCPI) disodic salt at 24 mg·L of distilled water, oxalic acid at 0.4% in distilled water, acetate buffer (composed of 3 g of sodium acetate, 7 ml of distilled water, and 10 ml of glacial acetic acid), and a base solution of 100 mg·L of ascorbic acid diluted in oxalic acid at 0.4%, to 100 µl of each of the samples plus 100 µl of acetate buffer and 800 µl of DCPI. After vortex-shaking of the samples, absorbance was read at 520 nm, utilizing as target oxalic acid at 0.4%. The ascorbic acid (AA) concentration in the extracts was determined by means of a standard curve and was reported as mg of AA per g calyces of dry base (mg AA·g$^{-1}$ calyces d.b.).

**Statistical analysis**

The results obtained of the different determinations were analyzed by determining their normality and homoscedasticity. After this, we carried out Analysis of variance (ANOVA) test by means of Tukey test ($p$ <0.05) for each of the determinations. We established correlations between the concentrations of bioactive compounds (total phenols, total monomeric anthocyanins, and ascorbic acid) and determinations of antioxidant activity. The data were compiled and organized employing Microsoft Excel 2010 and for statistical analysis, we utilized the Minitab ver. 16 program.

## RESULTS AND DISCUSSION

Concentrations of bioactive compounds and determinations of the antioxidant activity of the aqueous extracts of the varieties analyzed, grouped subjectively by the color of the fresh calyces, are depicted in Table 1. We observed that, even with variations in the color groups, varieties in groups with greater pigmentation presented a greater concentration of anthocyanins than those that were less pigmented. Antioxidant activity, determined as free radical scavenging capacity and reductor activity, is directly proportional to the concentration of the bioactive compounds quantified: total monomeric anthocyanin phenols and AA.

The 64 varieties of roselle analyzed were classified into groups according to concentration of bioactive compounds. The results of this classification show that there is a positive correlation between the concentration of bioactive compounds quantified and the antioxidant activity of the aqueous extracts of roselle varieties analyzed.

Determination of antioxidant activity (by the three techniques employed), phenolic content, and that of the monomeric anthocyanins of the aqueous extracts of the roselle varieties analyzed show that the red varieties present greater activity and concentration of bioactives, while green varieties present less activity. The latter coincides with that reported by Juliani et al. (2009), who analyzed phenolic compounds, $ABTS^{\bullet+}$ cation scavenging capacity, and anthocyanin content in roselle varieties of different colors in Senegal, and the results of Christian and Jackson (2009), who determined the content of anthocyanins, phenolics, and the activity on DPPH• of three roselle varieties (dark red, red, and green). With regard to the aqueous extracts' AA content, the higher concentration is found in varieties with dark-toned calyces (red and purple) and the lower one, in the green-colored varieties; this differs significantly with that previously reported by Salinas-Moreno et al. (2012) and Babalola et al. (2001); both investigations concluded that light-toned calyces contained more AA than dark-toned

**Table 1.** Antioxidant activity and concentration of the bioactive compounds of 64 varieties of roselle (*jamaica*)

| Variety | DPPH• [μmol TE·g$^{-1}$ calyces d.b.] | ABTS•$^{+}$ [mg AAE·g$^{-1}$ calyces d.b.] | FRAP [mg AAE·g$^{-1}$ calyces d.b.] | TPC [mg GAE·g$^{-1}$ calyces d.b.] | TMA [mg D3G·g$^{-1}$ calyces d.b.] | AA [mg AA·g$^{-1}$ calyces d.b.] |
|---|---|---|---|---|---|---|
| **Green varieties** | | | | | | |
| UAN 4 | 68.1 ± 1.6 | 10.1 ± 0.1 | 10.4 ± 0.0 | 17.3 ± 0.5 | 0.0 ± 0.0 | 1.5 ± 0.1 |
| UAN $16_1$ | 52.2 ± 1.4 | 7.4 ± 0.2 | 9.2 ± 0.2 | 11.0 ± 0.0 | 0.0 ± 0.0 | 1.0 ± 0.1 |
| **Pink variety** | | | | | | |
| $8Q_8$ | 37.4 ± 0.3 | 5.9 ± 0.0 | 6.0 ± 0.1 | 8.9 ± 0.0 | 0.3 ± 0.0 | 1.0 ± 0.0 |
| **Red varieties** | | | | | | |
| Tempranilla flor | 65.3 ± 2.1 | 8.8 ± 0.1 | 7.8 ± 0.2 | 8.5 ± 0.0 | 2.6 ± 0.1 | 2.1 ± 0.1 |
| Colima | 76.3 ± 0.8 | 12.4 ± 0.4 | 11.8 ± 0.4 | 13.9 ± 0.3 | 4.4 ± 0.0 | 3.6 ± 0.1 |
| Criolla roja | 100.0 ± 1.1 | 13.0 ± 0.6 | 12.4 ± 0.2 | 16.4 ± 0.3 | 4.6 ± 0.0 | 3.6 ± 0.0 |
| Criolla rojo violeta | 75.1 ± 0.6 | 12.5 ± 3.0 | 9.0 ± 0.1 | 16.7 ± 0.0 | 4.0 ± 0.0 | 3.0 ± 0.0 |
| Criolla super precoz | 57.2 ± 1.2 | 6.9 ± 0.1 | 6.8 ± 0.0 | 7.7 ± 0.8 | 1.9 ± 0.0 | 1.9 ± 0.0 |
| Criolla Puebla precoz | 76.5 ± 0.0 | 11.8 ± 0.2 | 9.2 ± 0.1 | 10.1 ± 0.1 | 4.4 ± 0.2 | 3.1 ± 0.0 |
| Criolla precoz | 72.2 ± 3.1 | 12.8 ± 0.6 | 11.0 ± 0.1 | 12.3 ± 0.4 | 3.8 ± 0.1 | 3.0 ± 0.1 |
| UAN 6 Puga | 84.0 ± 0.8 | 13.4 ± 0.0 | 14.8 ± 0.0 | 11.4 ± 0.4 | 5.5 ± 0.1 | 3.5 ± 0.2 |
| UAN 6 Novillero | 68.3 ± 0.8 | 10.9 ± 0.1 | 13.8 ± 0.0 | 13.4 ± 0.1 | 3.6 ± 0.0 | 3.0 ± 0.0 |
| UAN $25_1$ | 66.7 ± 0.5 | 12.3 ± 0.2 | 13.4 ± 0.3 | 14.0 ± 0.0 | 3.8 ± 0.0 | 3.5 ± 0.1 |
| UAN 7 | 145.4 ± 6.1 | 11.8 ± 0.3 | 15.8 ± 0.0 | 10.7 ± 0.3 | 5.9 ± 0.1 | 4.8 ± 0.1 |
| Tempranilla roja | 68.7 ± 3.2 | 6.9 ± 0.8 | 10.6 ± 0.3 | 10.9 ± 0.0 | 3.0 ± 0.0 | 1.9 ± 0.2 |
| UAN 11 | 85.5 ± 0.3 | 14.1 ± 0.1 | 13.0 ± 0.0 | 15.6 ± 0.3 | 4.2 ± 0.0 | 4.1 ± 0.0 |
| UAN 8 | 76.1 ± 0.4 | 14.5 ± 0.3 | 15.9 ± 0.3 | 14.4 ± 0.4 | 4.0 ± 0.0 | 4.1 ± 0.0 |
| UAN 27 | 125.0 ± 1.2 | 19.7 ± 0.3 | 15.3 ± 0.1 | 13.1 ± 0.4 | 12.5 ± 0.1 | 5.7 ± 0.1 |
| UAN 15 | 69.5 ± 0.6 | 10.5 ± 0.1 | 9.3 ± 0.2 | 10.7 ± 0.1 | 3.9 ± 0.0 | 3.1 ± 0.0 |
| UAN 12 | 79.0 ± 2.4 | 11.4 ± 0.2 | 10.7 ± 0.2 | 14.5 ± 0.1 | 4.2 ± 0.0 | 3.4 ± 0.0 |
| UAN 22 | 77.0 ± 1.3 | 11.2 ± 0.0 | 10.0 ± 0.1 | 13.2 ± 0.1 | 3.4 ± 0.1 | 3.1 ± 0.1 |
| UAN $10_1$ | 99.1 ± 1.0 | 13.3 ± 0.4 | 10.2 ± 0.5 | 19.0 ± 0.3 | 3.7 ± 0.1 | 3.6 ± 0.1 |
| UAN 25 | 55.7 ± 1.1 | 10.1 ± 0.4 | 8.0 ± 0.4 | 8.3 ± 0.3 | 3.7 ± 0.0 | 2.6 ± 0.1 |
| UAN 30 | 82.8 ± 0.6 | 11.5 ± 0.2 | 10.2 ± 0.2 | 14.0 ± 0.2 | 3.6 ± 0.0 | 3.2 ± 0.0 |
| UAN 9 | 70.0 ± 1.4 | 9.3 ± 0.3 | 7.7 ± 0.3 | 10.4 ± 0.3 | 3.7 ± 0.0 | 3.3 ± 0.1 |
| UAN 16 | 81.3 ± 2.0 | 12.6 ± 0.4 | 10.4 ± 0.1 | 15.6 ± 0.5 | 4.3 ± 0.0 | 3.1 ± 0.0 |
| UAN $10_2$ | 86.5 ± 3.4 | 13.8 ± 0.3 | 12.0 ± 0.4 | 11.5 ± 0.6 | 3.6 ± 0.0 | 3.0 ± 0.0 |
| $2Q_2$ | 79.6 ± 0.4 | 13.4 ± 0.4 | 10.4 ± 0.2 | 10.1 ± 0.1 | 3.9 ± 0.0 | 3.7 ± 0.1 |
| 11 Coneja | 78.1 ± 0.5 | 12.1 ± 0.1 | 9.2 ± 0.0 | 15.0 ± 0.2 | 3.7 ± 0.1 | 2.9 ± 0.0 |
| $Q_{12}CR$ | 74.3 ± 0.1 | 8.3 ± 0.3 | 8.8 ± 0.0 | 12.4 ± 0.3 | 4.1 ± 0.0 | 3.2 ± 0.1 |
| **Red purple varieties** | | | | | | |
| Criolla Huajicori | 74.3 ± 3.9 | 11.0 ± 0.2 | 9.5 ± 0.0 | 11.3 ± 0.2 | 3.9 ± 0.0 | 3.1 ± 0.1 |
| Negra UAN | 92.7 ± 3.1 | 12.7 ± 1.4 | 11.8 ± 0.2 | 14.8 ± 0.1 | 11.0 ± 0.1 | 5.1 ± 0.0 |
| UAN 5 | 127.9 ± 0.0 | 11.8 ± 0.2 | 15.8 ± 0.7 | 18.1 ± 0.2 | 12.0 ± 0.1 | 6.3 ± 0.0 |
| UAN $6_1$ | 92.0 ± 3.6 | 12.2 ± 0.2 | 15.0 ± 0.1 | 14.8 ± 0.2 | 4.5 ± 0.2 | 3.3 ± 0.1 |
| UAN $16_2$ | 130.9 ± 0.5 | 19.8 ± 0.3 | 19.8 ± 0.2 | 18.8 ± 0.2 | 9.2 ± 0.1 | 5.9 ± 0.0 |
| Bellotuda | 78.9 ± 1.9 | 15.0 ± 0.1 | 13.5 ± 0.1 | 12.5 ± 0.2 | 5.1 ± 0.0 | 3.6 ± 0.1 |
| UAN 23 | 83.7 ± 0.1 | 14.0 ± 0.0 | 14.6 ± 0.0 | 13.7 ± 0.5 | 5.1 ± 0.1 | 3.7 ± 0.1 |
| UAN 24 | 96.2 ± 2.4 | 14.7 ± 0.9 | 14.3 ± 0.0 | 15.3 ± 0.8 | 6.7 ± 0.1 | 4.7 ± 0.1 |
| UAN 13 | 79.2 ± 1.7 | 16.8 ± 0.8 | 13.2 ± 0.1 | 12.4 ± 0.0 | 6.1 ± 0.1 | 2.0 ± 0.0 |
| UAN 26 | 93.3 ± 2.1 | 14.1 ± 0.3 | 10.4 ± 0.1 | 13.6 ± 0.2 | 6.9 ± 0.0 | 4.2 ± 0.0 |
| UAN $23_1$ | 94.3 ± 0.4 | 14.2 ± 0.2 | 10.0 ± 0.4 | 13.8 ± 0.1 | 5.3 ± 0.0 | 4.7 ± 0.0 |

**Table 1.** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| UAN $13_1$ | 67.4 ± 1.3 | 11.6 ± 0.4 | 9.4 ± 0.1 | 11.6 ± 0.6 | 3.4 ± 0.0 | 2.8 ± 0.0 |
| UAN $24_1$ | 95.5 ± 0.2 | 11.0 ± 0.2 | 9.6 ± 0.5 | 15.5 ± 0.2 | 6.6 ± 0.0 | 4.1 ± 0.1 |
| UAN 20 | 92.3 ± 0.5 | 13.7 ± 0.1 | 12.2 ± 0.0 | 15.7 ± 0.1 | 6.1 ± 0.0 | 4.4 ± 0.1 |
| UAN 29 | 142.9 ± 2.8 | 23.8 ± 0.3 | 17.0 ± 0.5 | 15.6 ± 0.5 | 11.2 ± 0.1 | 5.9 ± 0.1 |
| UAN 19 | 86.9 ± 1.1 | 12.3 ± 0.1 | 10.9 ± 0.0 | 15.0 ± 0.2 | 3.8 ± 0.0 | 3.0 ± 0.1 |
| $2MQ_2$ | 103.3 ± 0.8 | 14.2 ± 0.5 | 12.8 ± 0.2 | 10.2 ± 0.1 | 8.8 ± 0.0 | 5.0 ± 0.0 |
| $4Q_4$ | 135.4 ± 1.8 | 21.8 ± 0.3 | 15.0 ± 0.0 | 18.8 ± 0.0 | 10.2 ± 0.1 | 4.7 ± 0.1 |
| $7Q_7$ | 134.1 ± 3.1 | 22.9 ± 0.1 | 14.4 ± 0.8 | 19.3 ± 0.1 | 10.6 ± 0.2 | 6.5 ± 0.0 |
| Variedad 10 | 132.9 ± 2.4 | 20.4 ± 0.1 | 15.1 ± 0.1 | 16.3 ± 0.0 | 9.8 ± 0.0 | 5.6 ± 0.1 |
| **Purple varieties** | | | | | | |
| Criolla morada | 95.1 ± 0.9 | 12.2 ± 0.6 | 10.3 ± 0.1 | 15.9 ± 0.1 | 6.5 ± 0.0 | 4.8 ± 0.1 |
| UAN 31 | 92.3 ± 2.3 | 14.1 ± 0.1 | 13.8 ± 0.2 | 11.5 ± 0.1 | 11.2 ± 0.1 | 4.6 ± 0.0 |
| **Deep purple varieties** | | | | | | |
| Tempranilla negra | 73.5 ± 0.4 | 12.6 ± 0.1 | 9.5 ± 0.0 | 14.7 ± 1.0 | 6.2 ± 0.2 | 3.9 ± 0.0 |
| Yersey Acriollada | 119.2 ± 0.3 | 16.3 ± 0.6 | 14.6 ± 0.0 | 16.5 ± 0.1 | 10.0 ± 0.1 | 5.0 ± 0.0 |
| Negra Quiviquinta | 118.0 ± 1.1 | 22.0 ± 0.1 | 16.4 ± 0.2 | 17.9 ± 0.1 | 9.4 ± 0.1 | 6.4 ± 0.0 |
| China | 102.6 ± 0.8 | 14.7 ± 0.6 | 13.7 ± 0.3 | 18.3 ± 0.0 | 8.5 ± 0.0 | 4.7 ± 0.0 |
| Morada X Roja | 109.8 ± 1.8 | 17.8 ± 0.9 | 15.2 ± 0.8 | 15.1 ± 0.0 | 7.4 ± 0.0 | 5.3 ± 0.0 |
| UAN 21 | 147.4 ± 0.2 | 21.6 ± 0.8 | 21.3 ± 0.6 | 18.0 ± 1.0 | 13.1 ± 0.2 | 5.6 ± 0.1 |
| UAN 17 | 137.6 ± 2.2 | 23.7 ± 0.1 | 18.2 ± 0.4 | 21.0 ± 0.2 | 12.2 ± 0.0 | 6.4 ± 0.0 |
| UAN $12_1$ | 154.1 ± 0.4 | 23.4 ± 0.1 | 18.5 ± 0.0 | 22.1 ± 0.0 | 13.3 ± 0.0 | 6.4 ± 0.0 |
| UAN 18 | 100.9 ± 1.6 | 19.0 ± 0.3 | 11.6 ± 0.2 | 13.2 ± 0.3 | 10.7 ± 0.0 | 4.5 ± 0.0 |
| UAN $21_1$ | 60.1 ± 2.2 | 10.0 ± 0.2 | 8.7 ± 0.2 | 8.2 ± 0.4 | 3.4 ± 0.0 | 2.9 ± 0.2 |
| $6Q_6$ | 105.5 ± 0.8 | 15.6 ± 0.1 | 10.4 ± 0.2 | 6.9 ± 0.3 | 9.1 ± 0.0 | 5.4 ± 0.0 |
| Cruza negra | 141.5 ± 0.4 | 23.1 ± 0.1 | 19.7 ± 0.6 | 17.2 ± 0.5 | 14.9 ± 0.2 | 7.7 ± 0.1 |

calyces. The difference between the results reported previously and those of this work could be due to the extraction technique in these: Salinas-Moreno et al. conducted extraction at 92°C for 15 min, a longer time than that of this investigation. It was reported that AA is thermolabile, that its diminution depends on diverse factors (Munyaka et al., 2010), and that in dark hibiscus varieties, AA reduction is greater than in lighter toned varieties. However, a more recent investigation reported that green roselle varieties present a very much lower content of AA than the red varieties (Ademiluyi and Oboh, 2013).

Analysis of the correlation between the antioxidant activity of the aqueous extracts and their concentration of bioactive compounds indicates that there is a positive correlation between their concentration and antioxidant activity, determined by any of the three techniques utilized, even though the correlation coefficients among themselves are lower than those previously reported. Results of antioxidant activity according to the content of phenolic compounds are illustrated in Figure 1.

Anokwuru et al. (2011) reported that the content of TPC is strongly correlated ($r$ = 0.969) with antiradical scavenging capacity in DPPH•, a much greater correlation than that found in the present investigation ($r$ = 0.649; $p$ <0.05). Juliani et al. (2009) reported that between the TPC content and the antioxidant activity of roselle extracts, expressed as antiradical scavenging activity toward the $ABTS^{\bullet+}$ cation, and its anthocyanin coefficients of 0.74 and 0.39, respectively. Prenesti et al. (2007) reported that the phenolic compound content is found to be strongly related with the Briggs-Rauscher Antioxidant Index (BRAI), without indicating the correlation coefficient between these. The authors suggest that it is reasonable to suppose that the antioxidant power of roselle calyx infusions is related exclusively with their content of phenolic compounds. In this investigation, we found that the phenolic compound concentration possessed the lowest correlation with the extracts' antioxidant activity, while AA is that which exhibited the greatest correlation coefficients. The difference between the results of the investigations cited could be attributed to the amount of roselle varieties analyzed; while Prenesti et al. (2007) and Anokwuru et al. (2011) experimented with a sole variety of calyces, this investigation experimented with 64 varieties, which presents a high coefficient of variation in the concentration of bioactive compounds as well as in antioxidant activity.

Antiradical scavenging activity to the DPPH• and total

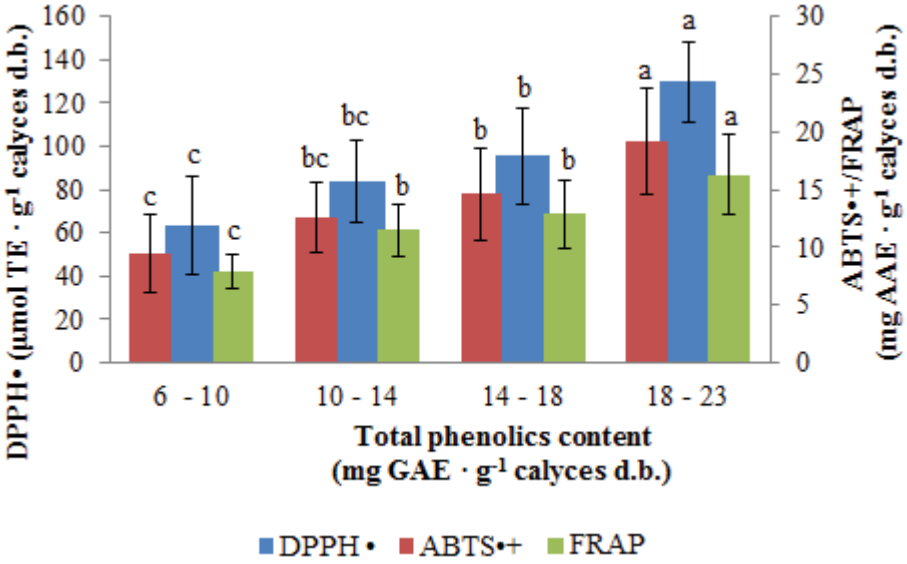


**Figure 1.** Antioxidant activity according to the total phenolic compound concentration of the 64 varieties of roselle (*jamaica*). Measurements without a letter in common are significantly different according to the Tukey test ($p < 0.05$).

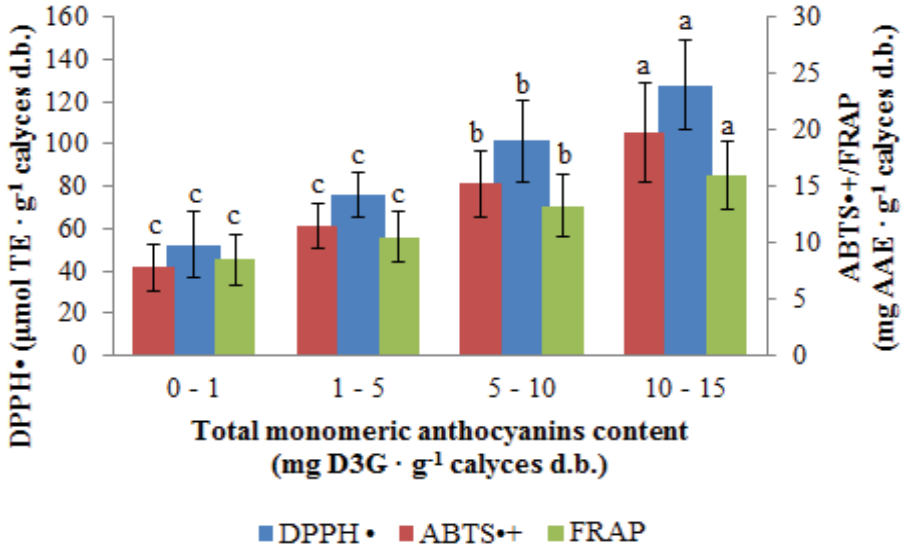


**Figure 2.** Antioxidant activity according to the total monomeric anthocyanin (TMA) concentration of 64 varieties of roselle (*jamaica*). Measurements without a letter in common are significantly different according to the Tukey test ($p < 0.05$).

phenolic concentration had a Pearson *r* correlation coefficient = 0.649 for the scavenging capacity of the ABTS•$^{+}$ cation: Coefficient *r* was 0.635, and for reductor activity, *r* = 0.625.

Figure 2 shows the values for determination of antioxidant activity according to total monomeric anthocyanin concentration. Correlation coefficients among the determinations were 0.867, 0.829, and 0.745 for the scavenging capacity of the DPPH• free radical, ABTS•$^{+}$ cation scavenging capacity, and reductor capacity, respectively.

Figure 3 shows the results of the determination of antioxidant activity according to the Ascorbic acid (AA) concentration. Correlation coefficients were 0.891, 0.827, and 0.763 for the 1-1-Diphenyl-2-picrylhydrazyl (DPPH•), 2-2'Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS•$^{+}$), and Ferric ion reducing antioxidant power (FRAP) assays, respectively.

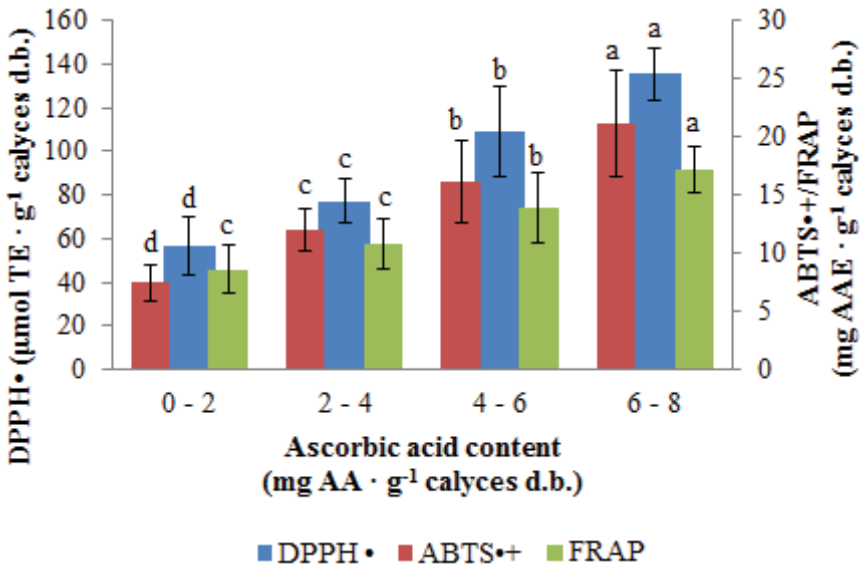


**Figure 3.** Antioxidant activity according to the ascorbic acid (AA) concentration of 64 varieties of roselle (*jamaica*). Measurements without a letter in common are significantly different according to the Tukey test ($p < 0.05$).

We performed a regression analysis to establish the relationship between the concentration of bioactive compounds analyzed and the antioxidant activity of the aqueous extracts of the 64 varieties of roselle (*jamaica*) subjected to study.

For the scavenging capacity of the DPPH• free radical, the regression equation was Equation (1):

$$DPPH\bullet = 19.4 + 1.62\ TPC + 2.62\ TMA + 8.6\ AA \quad (1)$$

The determination coefficient indicates that 83.9% of the capacity of the roselle aqueous extracts is explained from the concentration of Total phenolic compounds (TPC), or total monomeric anthocyanins (TMA), and of ascorbic acid (AA).

The scavenging capacity of the $ABTS\bullet^{+}$ cation of the aqueous extracts was defined as in Equation (2):

$$ABTS\bullet^{+} = 2.71 + 0.314\ TPC + 0.582\ TMA + 0.831AA \quad (2)$$

This equation determines that 75.6% of the capacity of the aqueous extracts of the roselle (*jamaica*) varieties subjected to study is due to the concentration of total phenolic compounds (TPC), total monomeric anthocyanins (TMC), and ascorbic acid (AA).

The reductor capacity of the Fe(III) ion into Fe(II) was found to be determined by the Equation (3):

$$FRAP = 3.68 + 0.276\ TPC + 0.298\ TMA + 0.739\ AA \quad (3)$$

64.7% of the reducing capacity of the aqueous extracts of the roselle (*jamaica*) varieties analyzed is due to the concentration of total phenolic compounds (TPC), total monomeric anthocyanins (TMA) and ascorbic acid (AA).

## Conclusions

Aqueous extracts of the roselle varieties analyzed present bioactive compound concentrations that confer upon these extracts the capacity of acting as antioxidants. Varieties of roselle with darkly pigmented calyces possess a greater capacity for free radical scavenging as well as for reducing oxidant molecules than light-toned roselle varieties. Due to its greater correlation coefficient, the concentration of ascorbic acid is a better predictor of the antioxidant activity of the aqueous extracts of the roselle varieties analyzed.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Collection and characterization of indigenous genotypes of Tikhur (*Curcuma angustifolia* Roxb.) under Bastar Plateau of Chhattisgarh

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**The investigation was undertaken during the year of kharif seasons 2010-11 and 2011-12 at Shaheed Gundadhoor College of Agriculture and Research Station (IGKV) Kumhrawand, Jagdalpur, Bastar (C.G.) India. Twenty indigenous genotypes of Tikhur (*Curcuma angustifolia* Roxb.) collected from thirteen districts of Chhattisgarh viz., Bastar, Korba, Dhamtari, Rajnandgaon, Surguja, Jashpur, Korea, Bilaspur, Kondagaon, Narayanpur, Kanker, Dantewada and Bijapur during March to June 2010. The experiment was aimed at collection, characterization and evaluation of 20 indigenous genotypes of Tikhur. The mean and range are estimated during characterization of 20 genotypes. The highest tall plant (115.70 cm), leaf length (53.78 cm), leaf breadth (20.92 cm), basal diameter of sucker in two directions (3.08 cm), leaf sheath length (49.86 cm) and maximum breath of lamina (12.34 cm) was recorded in genotype IGDMT-10-1. Maximum number of leaves per plant 18.97 and number of suckers in a clone (5.10) was recorded in genotype IGKOT-10-1. Maximum petiole length 21.70 cm was recorded in genotype IGSJT-10-1. The flowering was observed only in 14 genotypes out of 20 and fruit setting was absent. Highest frequency of colour of coma bracts (78.57%) was observed for pink tint. The genotypes IGKNT-10-1 took less time (160 days) for maturity as compared among 20 genotypes. The highest rhizomes yield (30.32 t/ha) and highest starch recovery (16.57%) was recorded in genotype IGSJT-10-2. Tasteless, fine and white colour of starch was observed from all 20 genotypes. The highest protein content (0.945%) in starch recorded in genotype IGSJT-10-4. Characterization of genotypes provided the information on morphological agronomic and biochemical aspects of the material that is essential for gene bank management and conservation of the Tikhur (*Curcuma angustifolia* Roxb.).**

**Key words:** Tikhur, *Curcuma angustifolia* Roxb., collection, characterization, rhizome yield, starch recovery.

## INTRODUCTION

Tikhur (*Curcuma angustifolia*; family Zingiberaceae) is a rhizomatous herb also known as white turmeric or East Indian Arrowroot. Its cultivation has now been undertaken by the farmers of Bastar on a large area. Tikhur is

cultivated as medicinal crop in many parts of the state under moist deciduous mixed and *sal* forest of Madhya Pradesh, Chhattisgarh and Jharkhand. Tikhur is also found in central province, Bihar, Maharashtra and Southern part of India. In undivided Madhya Pradesh, it is widely distributed in Bastar, Balaghat, Chhindwara, Surguja, Bilaspur, Raipur and Mandla districts (Kirtikar and Basu, 1918). The total collection of Tikhur rhizome as a minor forest produce in Chhattisgarh is 1,90.00 tonnes. Bastar and Bilashpur divisions are the major potential area of the state for Tikhur (Anonymous, 2005). Two types of Tikhur are found in the Bastar division; one with creamy white flowers and another having light pink coloured flowers (Singh et al., 1999).

Tikhur rhizomes are used as appetizer reducing burning sensations and stomach pains, removal of stone from kidney, useful for ulcer patient (Sharma, 2003) and rhizome pulp is used for treatment of headache as well as it gives cooling effect (Nag et al., 2006). The starch of Tikhur is used for the preparation of many sweet meals and herbal dishes like *halwa, barfi, jalebi etc.* It is used specially during fast (*Vrata, Upwas*). Farmers also prepare herbal drink "*sarbat*" through Tikhur starch during summer due to its cooling effect (Singh and Palta, 2004). Rhizome pulp is used as a remedy for headache, joint pains, jaundice and leucoria (Hemadri and Rao, 1984), while essential oil of Tikhur rhizome is used against tape worm (Benerjee and Nigam, 1978).

In the past, Tikhur was occurring to a large extent throughout the *Sal forest* of Chhattisgarh. But at present the unscientific manner of harvesting and over exploitation have brought its occurrence to the restricted patches. No research work has been carried out on collection, characterization and conservation of Tikhur to screen superior genotypes assessing its genetic diversity and variation etc.

The farmers of Chhattisgarh who reside in the vicinity of the forest, collect naturally grown Tikhur rhizomes as a minor forest produce and some farmers grow it commercially in their kitchen garden and *badi* farming system. Farmers grew unidentified locally available genotypes of Tikhur for rhizome production and processing of rhizomes through traditional method for starch extraction. Farmers' effort yielded less starch due to unrefined extraction process. Very little information is available regarding this crop especially on collection and characterization of Tikhur genotypes under agro-climatic condition of Chhattisgarh. These kinds of work would ensure *ex-situ* conservation of medicinal plants, besides the economical up-scaling of farmers and the augmentation of supply of raw material to pharmaceutical industries. The importance of the crop for people of Chhattisgarh prompted this investigation.

## MATERIALS AND METHODS

This research was conducted at Shaheed Gundadhoor College of Agriculture and Research Station (IGKV), Kumhrawand, Jagdalpur, Bastar, Chhattisgarh during *Kharif* seasons of 2010-11 and 2011-12. Twenty indigenous genotypes of Tikhur (*Curcuma angustifolia* Roxb.) collected (IGBT-10-1, IGKOT-10-1,IGDMT-10-1, IGDMT-10-2, IGMOT-10-1, IGSJT-10-1,IGJT-10-1,IGSJT-10-2, IGSJT-10-3, IGKT-10-1, IGSJT-10-4, IGBLT-10-1, IGBT-10-2, IGBT-10-3 (Local), IGNT-10-1, IGBT-10-4, IGBLT-10-2, IGKNT-10-1, IGDNT-10-1 and IGBJT-10-1) from thirteen districts of Chhattisgarh viz., Bastar, Korba, Dhamtari, Rajnandgaon, Surguja, Jashpur, Korea, Bilaspur, Kondagaon, Narayanpur, Kanker, Dantewada and Bijapur during March 2010 to June 2010. The passport data of collected Tikhur germplasm are given in Table 1. The collected rhizomes of the indigenous Tikhur genotypes was planted in Experimental Research Farm, AICRP Palm Experimental Field, Jagdalpur, Bastar, Chhattisgarh, during the period from June 2010 to November 2010 and June 2011 to November 2011. Characterization of Tikhur genotypes was done as per NBPGR descriptor.

The genotypes were grown randomly in single replication/block in a total of 20 plots of 3.0 m × 3.0 m each containing 75 plants per plot and spacing was 60 × 20 cm. The crop was grown under rainfed conditions. All the observations were taken from sprouting of rhizomes and up to maturity of crop for characterization. The mean and range are estimated during characterization of 20 genotypes of Tikhur.

## RESULTS AND DISCUSSION

The results of characterization and grouping of genotypes for different morphological characters based on characterization data as per NBPGR descriptor are presented in Tables 2 to 6. Fifty-seven characters of twenty genotypes of Tikhur were studied and characterized during 2010-2011 and 2011-2012 and grouped under the following categories for interpretation of results. Findings are discussed here with pooled data of two years.

### Leaf sucker and plant characters

The sprout colour was categorized into four groups with highest frequency observed in redish purple (35%). Leaf disposition pattern was categorized into three groups and highest frequency (50%) was observed as erect. Ventral leaf colour were categorized into two groups with dark green having the highest frequency (75%). Carpal leaf colour was categorized into two groups with highest frequency (70%) observed for light green. Spatial arrangement of veins on leaves were categorized into two groups and close arrangement of veins on leaves had the highest frequency (55%). Prominence of leaf venation was categorized into two groups with prominent

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**Table 1.** Collection details of indigenous genotypes of Tikhur (*Curcuma angustifolia* Roxb.).

| S/No. | Collection No. and Name | Collected by | Place of collection | Location of collection | Status of plant (Cultivated/Wild) | Collected material |
|---|---|---|---|---|---|---|
| 1. | IGBT-10-1 | Shri Deo Shankar & Shri Gundhar Bhardwaj | Village: Bhatagura, Jagdalpur, Bastar (C. G.) | N 19° 39.963' E 81° 13.197' H 507 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 2. | IGKOT-10-1 | Shri Deo Shankar & Shri D. P. Singh Tanwar | Shri Deepak Tanwar, Village: Rampur, Tahs-Katghora, Dist.- Korba (C. G.) | N 23° 01.432' E 82° 21.610' H 434 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 3. | IGDMT-10-1 | Shri Deo Shankar & Shri Asharam Netam | Shri Asharam Netam, Village: Dugali, Block: Nagari, Dist.- Dhamtari (C. G.) | N 20° 29.477' E 81° 52.166' H 445 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 4. | IGDMT-10-2 | Shri Deo Shankar & Shri J. L. Nag | Shri Lokeshwar Bhandari, Village: Mashulkhoi, Block: Nagari, Dist.- Dhamtari (C. G.) | N 20° 25.959' E 81° 43.228' H 459 m | Wild addible (Collected from *Forest*) | Rhizomes |
| 5. | IGMOT-10-1 | Shri Deo Shankar & Bhubaneswar Prasad Purame | Village: Mohla, Block: Mohla, District: Rajnandgaon (C. G.) | N 20° 29.477' E 81° 52.166' H 445 m | Wild addible (Collected from *Forest*) | Rhizomes |
| 6. | IGSJT-10-1 | Shri Deo Shankar | Shri Shiv Charan Singh,Village: Damodarpur, Block: Shankargarh, Tahs: Kusami (Samri), District: Surguja (C. G.) | N 21° 05.12' E 81° 02.21' H 432 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 7. | IGJT-10-1 | Shri Kawatch Bhagat & Shri Deo Shankar | Village: Bagicha, District: Jaspur (C. G.) | N 21° 58.359' E 83° 01.64' H 621 m | Cultivated (Collected from *Orchard*) | Rhizomes |
| 8. | IGSJT-10-2 | Shri Deo Shankar & Shri Khora Ram | Shri Khora Ram, Village: Kamari, Block: Shankargarh, Tahs: Kusami (Samri), District: Surguja (C. G.) | N 23° 17.497' E 83° 37.190' H 680 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 9. | IGSJT-10-3 | Shri Deo Shankar & Shri Iswar Prasad | Shri Iswar Prasad, Village: Kamari, Block: Shankargarh, Tahs: Kusami (Samri), District: Surguja (C. G.) | N 23° 17.469' E 83° 37.068' H 686 m | Cultivated (Collected from *Badi*) | Rhizomes |

**Table 1.** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| 10. | IGKT-10-1 | Shri Deo Shankar & Shri R. K. Patre | Shri Mohit Rajwade, Village: Kailaspur, Block: Sonhat, Tahs: Sonhat, District: Korea (C. G.) | N 23° 26.698' E 82° 28.844' H 761 m | Wild addible (Collected from *Forest*) | Rhizomes |
| 11. | IGSJT-10-4 | Shri Ramphal Singh & Shri Deo Shankar | Shri Ramphal Singh, Village: Sukhari, Block: Lakhanpur, Tahs: Ambikapur, District: Surguja (C.G.) | N 23° 05.982' E 83° 02.933' H 624 m | Cultivated (Collected from *Mango Orchard*) | Rhizomes |
| 12. | IGBLT-10-1 | Shri Prahlad Singh Kusaro & Shri Deo Shankar | Village: Mohli, Tahs: Kota, District: Bilaspur (C. G.) | | Wild addible (Collected from *Forest*) | Rhizomes |
| 13. | IGBT-10-2 | Shri Gundhar Bhardwaj and Shri Mangal Bagde | Shri Mangal Bagde, Village: Makdi, Tahs: Kondagaon, District: Bastar (C. G.) | N 19° 59.655' E 81° 36.362' H 632 m | Wild (Collected from *Forest*) | Rhizomes |
| 14. | IGBT-10-3 (Local) | Shri Gundhar Bhardwaj and Shri Mangal Bagde | Shri Gundhar Bhardwaj, Village: Dharmaur, Block: Tokapal, Tahs: Jagdalpur, District: Bastar (C. G.) | N 19° 13.696' E 81° 98.116' H 677 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 15. | IGNT-10-1 | Shri Gundhar Bhardwaj and Shri Pawan Kunwar | Village: Narayanpur, District: Narayanpur (C. G.) | N 19° 40.864' E 81° 14.572' H 507 m | Wild addible (Collected from *Bunds of pond*) | Rhizomes |
| 16. | IGBT-10-4 | Shri Gundhar Bhardwaj and Shri Bhubaneswar Majhi | Village: Machkot, Tahs: Jagdalpur, District: Bastar (C. G.) | N 19° 02.789' E 81° 57.041' H 559 m | Wild (Collected from *Forest*) | Rhizomes |
| 17. | IGBLT-10-2 | Shri Bodhram Paikra & Shri Deo Shankar | Village: Banabel, Block: Belgahana, District: Bilaspur (C. G.) | | Cultivated (Collected from *Badi*) | Rhizomes |
| 18. | IGKNT-10-1 | Shri M. R. Netam and Shri Deo Shankar | Village: Narharpur, Block: Narharpur, District: Kanker (C. G.) | N 20° 26.213' E 81° 43.429' H 698 m | Cultivated (Collected from *Badi*) | Rhizomes |
| 19. | IGDNT-10-1 | Shri M. K. Druv and Shri Mahadev Netam | Village: Binjam, Block: Geedam, District: Dantewada (C. G.) | N 18° 59.106' E 81° 05.707' H 512 m | Wild addible (Collected from *Forest*) | Rhizomes |
| 20. | IGBJT-10-1 | Shri Tankeshwar Nag, RAEO, Bhairamgarh | Shri Balram Kashyap, Village: Nelusnar, Block: Bhairamgarh, District: Bijapur (C. G.) | N 19° 39.963' E 81° 13.197' H 507 m | Wild addible (Collected from *Forest*) | Rhizomes |

**Table 2.** Morphological characterization of indigenous genotypes of Tikhur (*Curcuma angustifolia Roxb*) as per NBPGR descriptor.

| S/No. | A. Leaf, sucker and plant characters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Collection No. and Name | Sprout Colour | Leaf disposition pattern (Ho./SE./E.) | Ventral leaf Colour (G./D. G.) | Dorsal leaf Colour (Gr./D.Gr.) | Spatial arrangement of veins on leaves (Cl./D.) | Prominence of leaf venation (Less Pro./Pro.) | Plicate of leaves (L/M/H) |
| 1. | IGBT-10-1 | Reddish Purple | Erect | Green | Green | Distant | Less Prominent | Medium |
| 2. | IGKOT-10-1 | Reddish Purple | Semi erect | Green | Light Green | Distant | Less Prominent | High |
| 3. | IGDMT-10-1 | Blackish Purple | Semi erect | Dark Green | Green | Close | Prominent | Low |
| 4. | IGDMT-10-2 | Dark Purple | Semi erect | Dark Green | Green | Distant | Prominent | Low |
| 5. | IGMOT-10-1 | Reddish Purple | Semi erect | Green | Light Green | Distant | Less Prominent | Medium |
| 6. | IGSJT-10-1 | Dark Purple | Erect | Green | Light Green | Close | Prominent | Medium |
| 7. | IGJT-10-1 | Blackish Purple | Semi erect | Green | Light Green | Close | Prominent | Medium |
| 8. | IGSJT-10-2 | Light Purple | Erect | Green | Light Green | Close | Prominent | Medium |
| 9. | IGSJT-10-3 | Reddish Purple | Erect | Green | Light Green | Close | Prominent | Medium |
| 10. | IGKT-10-1 | Reddish Purple | Erect | Green | Light Green | Close | Less Prominent | Medium |
| 11. | IGSJT-10-4 | Dark Purple | Erect | Dark Green | Green | Close | Prominent | Medium |
| 12. | IGBLT-10-1 | Blackish Purple | Horizontal | Green | Light Green | Distant | Less Prominent | High |
| 13. | IGBT-10-2 | Dark Purple | Erect | Green | Light Green | Distant | Less Prominent | Medium |
| 14. | IGBT-10-3 | Reddish Purple | Erect | Green | Light Green | Distant | Less Prominent | Medium |
| 15. | IGNT-10-1 | Light Purple | Semi erect | Dark Green | Green | Distant | Prominent | Low |
| 16. | IGBT-10-4 | Reddish Purple | Semi erect | Dark Green | Green | Close | Prominent | Medium |
| 17. | IGBLT-10-2 | Dark Purple | Horizontal | Green | Light Green | Close | Less Prominent | High |
| 18. | IGKNT-10-1 | Dark Purple | Semi erect | Green | Light Green | Distant | Prominent | Low |
| 19. | IGDNT-10-1 | Light Purple | Erect | Green | Light Green | Close | Prominent | Medium |
| 20. | IGBJT-10-1 | Light Purple | Erect | Green | Light Green | Close | Prominent | Medium |

leaf venation having the highest frequency (60%). Plicate of leaves was categorized into three groups with medium plicate having the highest frequency (65%). The highest tall plant (115.70 cm), maximum leaf length 53.78 cm, leaf breadth 20.92 cm, highest basal diameter of sucker in two directions (3.08 cm), maximum leaf breadth (20.92 cm) and breath of lamina (12.34 cm) was recorded in genotype IGDMT-10-1 (Figure 1). Number of suckers in a clone (5.10) was recorded maximum in genotype IGKOT-10-1. Maximum petiole length was recorded as 21.70 cm in genotype IGSJT-10-1. Maximum number of leaves per plant 18.97 was recorded in genotype IGKOT-10-1. The much variability were observed in collected genotypes for leaf suckers and plant characters like plant height, sprout colour, ventral leaf colour, spatial arrangement of veins etc. and it might be due to genetic makeup of plant genotype which express their own characters. This was also reported by Latha et al. (1994), Naidu et al. (2000), Srivastava and Singh (2003) in turmeric.

## Flower characters

Flowering was observed only in 14 genotypes out of 20. Months of flowering were classified into three groups and highest frequency was observed for June month of flowering (85.71%). Colour of coma bracts were categorized into three groups and highest frequency 78.57% was observed for

**Table 3.** Morphological characterization of indigenous genotypes of Tikhur (*Curcuma angustifolia Roxb*) as per NBPGR descriptor.

| S/No. | A. Leaf, sucker and plant characters | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection No. and Name | Plant ht. up to the tip of leaves | No. of suckers in a clone | Length of petiole (cm) | Length of leaf (cm) | Breadth of leaf (cm) | Basal diam. of sucker in two direct. | Leaf sheath length LP (cm) | Breadth of lamina (cm) | No. of leaves per plant | Length of mother plant (cm) |
| 1. | IGBT-10-1 | 95.57 | 3.37 | 11.69 | 35.99 | 14.05 | 2.33 | 35.49 | 6.58 | 13.67 | 101.24 |
| 2. | IGKOT-10-1 | 86.35 | 5.10 | 10.77 | 39.59 | 15.74 | 2.55 | 40.95 | 6.72 | 18.97 | 133.24 |
| 3. | IGDMT-10-1 | 118.75 | 1.84 | 14.65 | 53.78 | 20.92 | 3.08 | 49.86 | 12.34 | 9.83 | 152.21 |
| 4. | IGDMT-10-2 | 107.00 | 1.87 | 13.81 | 43.89 | 13.92 | 1.94 | 27.19 | 7.03 | 9.30 | 105.03 |
| 5. | IGMOT-10-1 | 98.60 | 1.80 | 14.36 | 38.07 | 15.18 | 2.47 | 44.93 | 7.53 | 8.17 | 127.53 |
| 6. | IGSJT-10-1 | 108.62 | 2.90 | 21.85 | 38.29 | 14.48 | 1.88 | 34.63 | 12.30 | 10.17 | 132.01 |
| 7. | IGJT-10-1 | 77.69 | 2.80 | 20.69 | 34.70 | 14.82 | 2.33 | 40.09 | 7.05 | 8.70 | 136.61 |
| 8. | IGSJT-10-2 | 79.10 | 2.33 | 22.80 | 36.83 | 16.97 | 2.23 | 38.85 | 8.11 | 8.13 | 135.33 |
| 9. | IGSJT-10-3 | 66.85 | 1.97 | 14.51 | 32.00 | 17.55 | 2.20 | 36.51 | 12.04 | 8.04 | 121.85 |
| 10. | IGKT-10-1 | 86.73 | 2.00 | 16.68 | 39.98 | 13.59 | 1.80 | 36.90 | 6.81 | 9.07 | 114.65 |
| 11. | IGSJT-10-4 | 88.42 | 1.90 | 18.55 | 39.09 | 16.18 | 1.80 | 45.68 | 11.96 | 9.50 | 138.95 |
| 12. | IGBLT-10-1 | 74.14 | 2.60 | 11.20 | 27.28 | 16.49 | 2.50 | 35.29 | 10.73 | 9.17 | 117.90 |
| 13. | IGBT-10-2 | 76.39 | 2.37 | 17.01 | 32.89 | 15.90 | 1.77 | 45.70 | 11.90 | 8.37 | 141.66 |
| 14. | IGBT-10-3 | 66.45 | 3.37 | 19.02 | 29.85 | 17.29 | 2.30 | 44.13 | 12.17 | 11.10 | 140.18 |
| 15. | IGNT-10-1 | 102.94 | 1.97 | 9.41 | 39.48 | 18.21 | 2.58 | 45.11 | 10.94 | 9.17 | 141.83 |
| 16. | IGBT-10-4 | 99.75 | 3.13 | 13.15 | 44.07 | 14.56 | 2.27 | 40.17 | 8.48 | 12.40 | 135.01 |
| 17. | IGBLT-10-2 | 73.00 | 2.53 | 10.03 | 35.75 | 15.71 | 2.54 | 38.08 | 8.17 | 10.54 | 124.82 |
| 18. | IGKNT-10-1 | 77.59 | 2.30 | 14.93 | 36.77 | 18.61 | 2.16 | 44.78 | 12.45 | 10.67 | 140.41 |
| 19. | IGDNT-10-1 | 84.59 | 1.93 | 14.14 | 39.60 | 12.55 | 1.45 | 33.86 | 8.55 | 12.00 | 107.96 |
| 20. | IGBJT-10-1 | 77.14 | 1.97 | 16.67 | 32.75 | 13.22 | 1.79 | 34.45 | 8.48 | 9.43 | 116.66 |

pink tint colour (Figure 2). Flower bracts colour was categorized into two groups and light green colour had the highest frequency 85.71%. Shape of coma bracts observed ovate in all the 14 flowered genotypes. Flower bracts were categorized into two groups and lanceolate flower bract had the highest frequency 92.85%.

Fruit setting was absent in all the 14 genotypes which bears maximum peduncle length 15.20 cm and was recorded in genotype IGJT-10-1. The maximum spike with 13.6 cm was recorded in genotype IGBT-10-4. Maximum flower bract length 8.0 cm was recorded in genotype IGSJT-10-3. Maximum flower bract width 4.7 cm was recorded in genotype IGSJT-10-3. Maximum coma bract length 5.0 cm was recorded in genotype IGBT-10-4; maximum coma bract width 4.7 cm length was also recorded, and maximum flower tip was recorded as 27.4 cm in genotype IGSJT-10-3 as reported by Pant et al. (1998) in gladiolus.

The variation in flower characters might be due to genetic makeup of plant genotype which expresses their own character.

**Rhizome characters**

Number of mother rhizome per plant (2.63) was recorded highest in entry IGBT-10-2, as was also reported by Dutta et al. (2001) in turmeric. The maximum length of mother rhizome (6.11 cm) was observed in genotype IGNT-10-1. The maximum thickness of mother rhizome 3.82 cm was recorded in genotype IGDMT-10-1. Maximum number of primary finger rhizomes per plant (10.42) was recorded in genotype IGKOT-10-1. Maximum length of primary fingers (9.63 cm) was

**Table 4.** Morphological characterization of indigenous genotypes of Tikhur (*Curcuma angustifolia Roxb*) as per NBPGR descriptor.

| S/No. | B. flower characters | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection No. and Name | Months of flowering | Colour of Coma bracts (W/WG/LG/ LP tint/ P.tint) | Flower bracts colour (W/WG/ LG/LP tint/ P.tint) | Shape of coma bracts (Ov/Ob/ Elpt./lan./obla n./ linear) | Flower bract (Ov./ Lan.) | Fruit setting (Ab/ Pr) | Length of peduncle (cm) | Length of inflorescence (cm) | Width of spike (cm) | Flower bract length (cm) | Flower bract width (cm) | Coma bract length (cm) | Coma bract width (cm) | Length of flower up to tip (cm) |
| 1. | IGBT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 7.0 | 10.2 | 4.0 | 5.0 | 3.0 | 3.6 | 3.2 | 16.62 |
| 2. | IGKOT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 7.5 | 13.4 | 8.5 | 4.4 | 3.4 | 4.0 | 3.7 | 21.0 |
| 3. | IGDMT-10-1 | June | Light pink tint | Light green | Ovate | Lanceolate | Absent | 5.6 | 10.6 | 7.2 | 5.1 | 3.3 | 4.0 | 3.6 | 15.5 |
| 4. | IGDMT-10-2 | Sept. | Creamy white pink tint | Whitish green | Ovate | Lanceolate | Absent | 6.2 | 11.2 | 8.1 | 6.1 | 3.5 | 4.1 | 3.7 | 17.2 |
| 5. | IGMOT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 5.6 | 15.8 | 11.7 | 5.7 | 3.6 | 4.3 | 4.2 | 21.2 |
| 6. | IGSJT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 7.2 | 15.9 | 7.9 | 6.5 | 3.7 | 4.3 | 4.1 | 20.6 |
| 7. | IGJT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 15.2 | 14.3 | 9.0 | 6.2 | 3.8 | 3.2 | 3.8 | 23.3 |
| 8. | IGSJT-10-2 | June | Pink tint | Light green | Ovate | Ovate | Absent | 14.5 | 13.9 | 8.4 | 5.8 | 3.5 | 3.4 | 3.5 | 19.8 |
| 9. | IGSJT-10-3 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 8.5 | 14.6 | 9.1 | 8.0 | 4.7 | 4.3 | 4.6 | 27.4 |
| 10. | IGKT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 5.5 | 12.1 | 8.0 | 5.3 | 3.4 | 3.6 | 4.3 | 17.6 |
| 11. | IGSJT-10-4 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 3.8 | 16.9 | 7.0 | 7.6 | 4.2 | 3.4 | 4.2 | 16.5 |
| 12. | IGBLT-10-1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13. | IGBT-10-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14. | IGBT-10-3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15. | IGNT-10-1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16. | IGBT-10-4 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 9.2 | 13.6 | 13.6 | 5.5 | 3.2 | 5.0 | 4.7 | 27.3 |
| 17. | IGBLT-10-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 18. | IGKNT-10-1 | Oct. | Creamy white pink tint | Whitish green | Ovate | Lanceolate | Absent | 13.5 | 12.6 | 7.90 | 6.9 | 3.9 | 3.8 | 3.8 | 22.6 |
| 19. | IGDNT-10-1 | June | Pink tint | Light green | Ovate | Lanceolate | Absent | 9.3 | 16.2 | 10.2 | 4.8 | 3.6 | 4.4 | 4.4 | 25.6 |
| 20. | IGBJT-10-1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

recorded in genotype IGSJT-10-2. Maximum thickness of primary fingers (2.15 cm) was recorded in genotype IGBT-10-1. Maximum number of secondary finger rhizome per plant (10.90) was recorded in genotype IGSJT-10-3. Maximum length of secondary finger rhizome (6.14 cm) was recorded in genotype IGBT-10-3. The maximum thickness of secondary finger rhizomes (1.95 cm) and length of tertiary finger rhizomes (4.90 cm) was recorded in genotype IGDMT-10-2. Maximum number of tertiary finger rhizomes (7.0) was recorded in genotype IGBT-10-3. Similar findings

**Table 5.** Morphological characterization of indigenous genotypes of Tikhur (*Curcuma angustifolia Roxb*) as per NBPGR descriptor.

| S/ No. | C. Rhizome characters | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Collection No. and Name | No. of Mother rhizomes per plant | Length of Mother rhizome (cm) | Thickness of Mother rhizome | No. of Primary fingers | Length of prim. fingers (cm) | Thickness of prim. fingers (cm) | No. of second. finger rhizome per plant | Length of sec. fingers (cm) | Thickness of Sec. fingers (cm) | No. of tertiary fingers | Length of tertiary finger (cm) | Thickness of tertiary fingers (mm) |
| 1. | IGBT-10-1 | 1.40 | 5.42 | 3.48 | 6.77 | 7.92 | 1.86 | 6.94 | 4.62 | 1.83 | 4 | 1.15 | 4.60 |
| 2. | IGKOT-10-1 | 1.77 | 5.02 | 3.13 | 10.24 | 7.73 | 1.54 | 8.34 | 4.36 | 1.77 | 5 | 1.10 | 4.45 |
| 3. | IGDMT-10-1 | 1.54 | 5.07 | 3.83 | 6.37 | 7.79 | 1.81 | 10.70 | 4.96 | 1.61 | 6 | 1.25 | 4.05 |
| 4. | IGDMT-10-2 | 1.44 | 3.95 | 2.98 | 6.70 | 5.41 | 1.66 | 9.97 | 5.10 | 1.95 | 6 | 1.32 | 4.90 |
| 5. | IGMOT-10-1 | 2.07 | 4.05 | 3.14 | 8.17 | 7.43 | 1.87 | 9.20 | 4.65 | 1.80 | 6 | 1.20 | 4.52 |
| 6. | IGSJT-10-1 | 1.57 | 3.96 | 3.59 | 8.74 | 9.23 | 2.01 | 10.40 | 5.93 | 1.28 | 6 | 1.45 | 3.22 |
| 7. | IGJT-10-1 | 1.30 | 4.53 | 3.62 | 6.40 | 7.49 | 1.91 | 9.20 | 5.10 | 1.25 | 6 | 1.30 | 3.15 |
| 8. | IGSJT-10-2 | 2.13 | 4.29 | 3.19 | 10.87 | 9.63 | 2.29 | 8.77 | 5.79 | 1.21 | 5 | 1.50 | 3.02 |
| 9. | IGSJT-10-3 | 1.40 | 4.76 | 3.59 | 8.30 | 8.50 | 1.87 | 10.90 | 5.83 | 1.09 | 5 | 1.45 | 2.75 |
| 10. | IGKT-10-1 | 1.34 | 3.84 | 3.27 | 7.20 | 8.17 | 1.82 | 10.84 | 6.04 | 1.60 | 6 | 1.50 | 3.98 |
| 11. | IGSJT-10-4 | 1.90 | 4.56 | 3.26 | 8.97 | 8.49 | 1.89 | 8.04 | 4.36 | 1.03 | 5 | 1.12 | 2.55 |
| 12. | IGBLT-10-1 | 1.87 | 4.32 | 2.77 | 5.57 | 8.23 | 1.77 | 9.03 | 5.22 | 1.01 | 6 | 1.30 | 2.52 |
| 13. | IGBT-10-2 | 2.63 | 4.51 | 3.05 | 8.23 | 7.47 | 1.70 | 10.54 | 5.44 | 1.05 | 6 | 1.38 | 2.65 |
| 14. | IGBT-10-3 | 1.40 | 4.87 | 2.83 | 8.40 | 9.25 | 1.58 | 11.04 | 6.14 | 1.10 | 7 | 1.52 | 2.76 |
| 15. | IGNT-10-1 | 1.74 | 6.11 | 3.08 | 6.73 | 6.84 | 1.76 | 6.03 | 3.17 | 1.82 | 4 | 0.80 | 4.66 |
| 16. | IGBT-10-4 | 3.13 | 4.76 | 3.53 | 6.83 | 8.42 | 2.15 | 9.74 | 5.00 | 1.21 | 6 | 1.25 | 3.04 |
| 17. | IGBLT-10-2 | 1.60 | 4.24 | 2.86 | 5.90 | 7.32 | 1.52 | 6.27 | 4.67 | 0.99 | 4 | 1.18 | 2.50 |
| 18. | IGKNT-10-1 | 1.44 | 5.27 | 3.60 | 6.34 | 7.13 | 2.01 | 7.17 | 5.01 | 1.44 | 5 | 1.26 | 3.62 |
| 19. | IGDNT-10-1 | 1.34 | 4.48 | 3.54 | 6.17 | 7.13 | 1.71 | 5.14 | 4.65 | 1.14 | 3 | 1.20 | 2.82 |
| 20. | IGBJT-10-1 | 1.34 | 4.18 | 2.86 | 5.97 | 7.44 | 1.46 | 8.50 | 5.31 | 0.99 | 5 | 1.30 | 2.46 |

were given by Pathania et al. (1988), Sinkar et al. (2005) and Chaudhary et al. (2006) in turmeric.

## Rhizome, rhizome flesh and starch characters

All the 20 genotypes had stipitate or stalked rhizomes. The stipitate tubers help the plant in strong extra starch and water for regeneration immediately after the summer. The stipitate tubers vary in size shape and colour reported by Vimala and Nambisan (2010). Genotype IGKOT-10-1 had maximum length of stipitate rhizomes (5.27 cm). Maximum thickness of stipitate rhizome (2.74 cm) was recorded in genotype IGSJT-10-1. For leaf spot disease observation, genotypes were categorized into two groups, absence and presence of disease. The highest frequency was observed (70%) for absence of leaf spot disease. Fresh colour of rhizomes genotypes were categorized in three groups and cream fresh colour had the highest frequency (80%) which is the valuable character for the classification of the species (Figure 1). The highest rhizome yield (30.32 t/ha) was recorded in genotype IGSJT-10-2 and also recorded by Vimala and Nambison (2010) in *Curcuma malabarica*. For organoleptic taste of rhizomes, genotypes were classified into three groups and highest frequency was observed for mild bitter test (45%). For the shape of mother

**Table 6.** Morphological characterization of indigenous genotypes of Tikhur (*Curcuma angustifolia Roxb*) as per NBPGR descriptor.

| | | D. Rhizome, rhizome flesh and starch characters | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S/No. | Collection No. and Name | Presence of stipitate or stalked rhizome (P/A)* | Length of stipitate rhizomes (cm) | Thickness of stipitate rhizomes (cm) | Leaf spot disease (P/A)* | Fresh colour of rhizomes (C/PY/YC/)* | Organoleptic taste of rhizomes (MB/VB/W/B/SA) | Shape of mother rhizome (Sp/Ob)* | Aroma of rhizomes (Aro/SA/HA/NA)* | Days to maturity | Rhizome yield (t/ha.) | Starch recovery (%) | Organoleptic taste of starch | Colour of starch | Nature of starch (granular / fine/ sticky) | Protein content (%) in starch |
| 1. | IGBT-10-1 | P | 4.47 | 2.46 | P | C | MB | Sp | SA | 170 | 14.46 | 13.90 | Tasteless | White | Fine | 0.855 |
| 2. | IGKOT-10-1 | P | 5.27 | 2.01 | A | C | VB | Sp | HA | 171 | 13.57 | 12.99 | Tasteless | White | Fine | 0.915 |
| 3. | IGDMT-10-1 | P | 3.80 | 2.31 | A | P Y | W | Ob | NA | 174 | 16.82 | 12.17 | Tasteless | White | Fine | 0.78 |
| 4. | IGDMT-10-2 | P | 3.13 | 1.68 | A | PY | W | Ob | NA | 174 | 13.37 | 9.46 | Tasteless | White | Fine | 0.86 |
| 5. | IGMOT-10-1 | P | 5.20 | 2.34 | P | C | MB | Sp | SA | 169 | 15.09 | 13.08 | Tasteless | White | Fine | 0.815 |
| 6. | IGSJT-10-1 | P | 4.07 | 2.74 | A | C | MB | Sp | SA | 160 | 21.52 | 13.88 | Tasteless | White | Fine | 0.57 |
| 7. | IGJT-10-1 | P | 2.93 | 1.91 | A | C | W | Sp | NA | 167 | 21.18 | 12.81 | Tasteless | White | Fine | 0.69 |
| 8. | IGSJT-10-2 | P | 4.27 | 1.84 | P | C | MB | Sp | SA | 166 | 30.32 | 16.57 | Tasteless | White | Fine | 0.785 |
| 9. | IGSJT-10-3 | P | 4.00 | 2.69 | A | C | B | Sp | Aro | 167 | 20.24 | 11.87 | Tasteless | White | Fine | 0.76 |
| 10. | IGKT-10-1 | P | 3.60 | 1.41 | P | C | MB | Sp | SA | 168 | 17.87 | 13.72 | Tasteless | White | Fine | 0.41 |
| 11. | IGSJT-10-4 | P | 2.73 | 1.74 | A | C | B | Sp | Aro | 166 | 19.25 | 15.45 | Tasteless | White | Fine | 0.945 |
| 12. | IGBLT-10-1 | P | 5.13 | 1.81 | A | C | VB | Sp | HA | 166 | 17.46 | 15.52 | Tasteless | White | Fine | 0.645 |
| 13. | IGBT-10-2 | P | 4.80 | 1.44 | A | C | MB | Sp | SA | 167 | 19.73 | 12.34 | Tasteless | White | Fine | 0.685 |
| 14. | IGBT-10-3 | P | 3.93 | 1.69 | A | C | MB | Sp | SA | 161 | 20.12 | 11.23 | Tasteless | White | Fine | 0.755 |
| 15. | IGNT-10-1 | P | 2.53 | 1.89 | A | Y C | W | Ob | HA | 169 | 12.37 | 10.05 | Tasteless | White | Fine | 0.845 |
| 16. | IGBT-10-4 | P | 3.13 | 2.31 | P | C | M B | Sp | SA | 172 | 16.56 | 15.80 | Tasteless | White | Fine | 0.765 |
| 17. | IGBLT-10-2 | P | 4.93 | 1.36 | P | C | VB | Sp | HA | 171 | 8.11 | 13.60 | Tasteless | White | Fine | 0.695 |
| 18. | IGKNT-10-1 | P | 2.87 | 2.36 | A | P Y | SA | Ob | HA | 177 | 10.17 | 12.66 | Tasteless | White | Fine | 0.91 |
| 19. | IGDNT-10-1 | P | 2.20 | 1.49 | A | C | MB | Sp | SA | 170 | 12.20 | 10.84 | Tasteless | White | Fine | 0.68 |
| 20. | IGBJT-10-1 | P | 2.93 | 1.32 | A | C | B | Sp | Aro | 168 | 14.12 | 11.87 | Tasteless | White | Fine | 0.865 |

*P=Present, A=Absent, C=Cream, PY=Pale Yellow, YC=Yellowish Cream, MB=Mild Bitter, VB=Very Bitter, W=Watery, B=Bitter, SA=Slighty Acrid, Sp=Spherical, O=Oblong, Aro=Aromatic, SA=Slightly Aromatic, HA=Highly Aromatic, NA=Non Aromatic.

rhizome, genotypes were categorized into two groups and spherical shape had the highest frequency (80%). The root stock is the mother rhizome while its branches were divided into primary secondary and tertiary rhizomes. The root stock bears both sessile and stipitate tubers. It was also observed that the root stocks vary in shape from spherical to slightly conical; hemispherical and cylindrical was also reported by Vimala and Nambisan (2010) in starchy *curcuma species*. For aroma of rhizomes, genotypes were categorized into four groups and highest aromatic had the highest frequency (45%). The genotype IGKNT-10-1 took less time (160 days) for maturity among 20 genotypes. The highest rhizomes yield (30.32 t/ha) and starch recovery (16.57%) were recorded in genotype IGSJT-10-2; the findings correlate with that of Vimala (2002). Organoleptic tastes of extracted starch from all 20 genotypes were observed tasteless. Only white colour of starch was observed for all 20 genotypes (Figure 3). Nature of starch for all the 20 genotypes was fine. The highest protein content (0.945%) in starch was recorded in genotype IGSJT-10-4.

## Conclusion

The genotype IGDMT-10-1 was observed for tall


**(1) Plant and rhizome characters of genotype IGKOT-10-1**


**(2) Plant and rhizome characters of genotype IGDMT-10-1**


**(3) Plant and rhizome characters of genotype IGSJT-10-1**


**(4) Plant and rhizome characters of genotype IGSJT-10-2**

**Figure 1.** Specific characteristics of Tikhur (*Curcuma angustifolia* Roxb.) genotypes.

plant, maximum leaf length, leaf breadth, basal diameter of sucker in two directions, leaf sheath length and maximum breath of lamina. Genotype IGKOT-10-1 was observed for maximum number of leaves per plant and number of suckers in a clone. Maximum petiole length was recorded in genotype IGSJT-10-1. The flowering was observed only in 14 genotypes out of 20 and fruit setting was absent. Highest frequency of colour of coma bracts

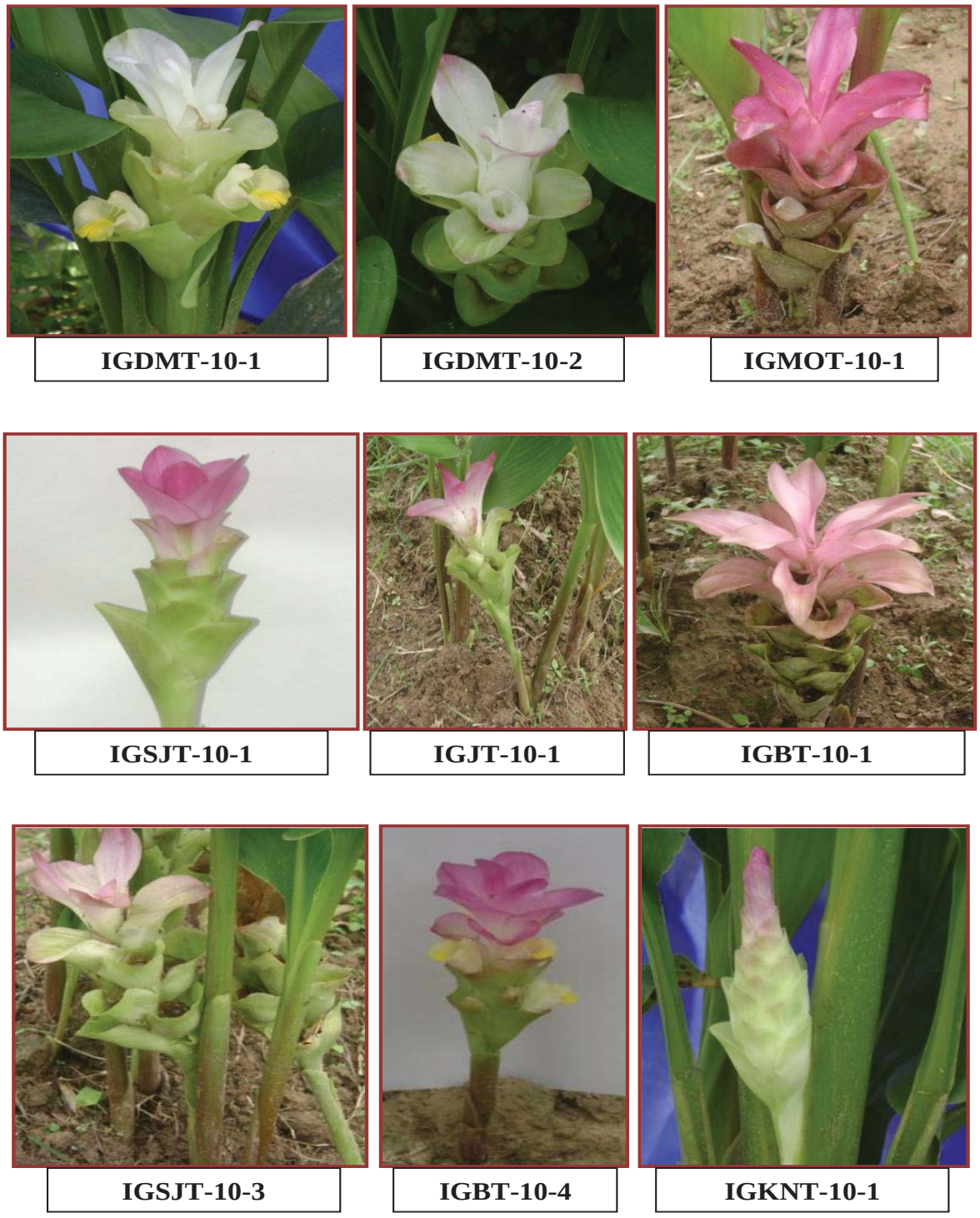


**Figure 2.** Flower characters of Tikhur genotypes.

was observed for pink tint. The genotypes IGKNT-10-1 was observed for early maturity as compared among 20 genotypes. The highest rhizomes yield (30.32 t/ha) and highest starch recovery (16.57%) was recorded in genotype IGSJT-10-2. Tastless, fine and white colour of starch was observed from all 20 genotypes. The highest protein content (0.945%) in starch was recorded in genotype IGSJT-10-4. Characterization of indigenous genotypes or germplasm of Tikhur (*Curcuma angustifolia* Roxb.) is very important for their evaluation, effective


**Figure 3.** Starch characters of Tikhur (*Curcuma angustifolia* Roxb.) genotypes ($T_1$-$T_{20}$).

management and subsequent utilization. The main objective behind evaluation is to isolate the potential donors for their effective utilization in subsequent breeding programme, such as for transferring the desirable trait in only standard variety, to make the genotype ideal.

## Conflict of Interest

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Diversity and agronomic status of tomato and pepper fruit pests in two agro-ecological zones of Southern Cameroon: Western Highland and the Southern Plateau of Cameroon

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**Tomato and pepper are two major market gardening crops in Cameroon. In order improve pest insects control strategies, we assessed their diversity and evaluated their impact on yield losses in two agro-ecological areas of southern Cameroon. To achieve this, estimations of damages by visual observations were done twice per month from March 2010 to February 2011, in trap gardens set up respectively at Koutaba (Western Highlands) and at Okola (Southern Plateau). During each sampling, all fruits present in the garden were counted, those attacked or fallen on the ground collected and incubated in the laboratory for pest identification needs. Seven insect pests species belonging to two orders were identified. Among them, *Dacus punctatifrons* (Diptera-Tephritidae) *and, Chrysodeixis chalcites* (Lepidoptera-Noctuidae) were recorded on tomato, *Ceratitis capitata* (Diptera-Tephritidae), *Chryptophlebia leucotreta and Leucinoides orbonalis* (Lepidoptera-Pyralidea) on pepper while *Spodoptera littoralis,* and *Helicoverpa armigera* (Lepidoptera-Noctuidae) were recorded on both plants species. Fruit loses related to insects' activities were greater in Koutaba (43-47%) than they were at Okola (28-33%). These rates varied with seasons. For instance, frequencies of fruits affected by *D. punctatifrons, C. capitata, C. leucotreta* were positively correlated to the abiotic factors, especially temperature and rainfall.**

**Key words:** Gardening, pest insect, damages, abiotic factors.

## INTRODUCTION

With the global economic crisis, subsequent structural adjustment and the crash of cash crops prices in developing countries in the late 1980s, rural populations and low-income workers of public and private sectors,

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who lost their jobs, converted to food crop production in various areas of Cameroon. This was especially the case in the Western Highlands of Cameroon and in the urban and peri-urban areas of the Center and Littoral Region, where large areas previously occupied by cocoa and coffee farms, are currently occupied by food crop plantations and market crop gardens. For a great part of the population; especially in these zones, market gardening is presently the main activity (Westphal et al., 1981; Kegne and De Jong, 2002).

The intensiveness of this activity has led to the development of a diversified fauna of pests whose damages are very harmful to crops and their yield (Novotny and Basset, 2005). Up to date, the only arms disposed by the Cameroonian producers for pest control are large spectrum of synthetic chemicals. Moreover, farmer's knowledge about these constraints is usually very poor; leading to the misuse of these chemicals. This can cause environmental pollution, accumulation of chemical residues in crops, selection of resistant insects, etc (Edwards-Jones, 2008). To solve this problem, researchers have undertaken several studies, including inventories of entomofauna in market gardening (Djiéto-Lordon and Aléné, 2002, 2006; Djiéto-Lordon et al., 2014; Mokam et al., 2014a), biological and behavioral studies of some insect pests (Tindo and Tamo, 1999; Okolle and Tonifor, 2005), laboratories and field tests of alternative methods to chemicals (Berry and Mansfield, 2006), cultural practice tests (Wood and Reilly, 2000), etc. All these studies were intended to find ways to reduce the chemicals used in pest control. In their inventory, Djiéto-Lordon and Aléné (2002, 2006) showed that in *Lycopersicon esculentum* Mill. (1754) and *Capsicum annuum L.* (1753), fruits were the most affected organs. Recently, several studies provided some important data on the biology and ecology of some pests (Djiéto-Lordon et al., 2014; Mokam et al., 2014b). Despite their predominant agronomic and economic importance in market gardening in Cameroon, few data are available on pest of both tomato and pepper in the Western Highlands and Southern Plateau Regions of the country.

Considering that climatic conditions (altitude, rainfall, temperature, hygrometry, and vegetation) often influence insect species richness, the following hypothesis was stated: The diversity and dynamism of pest's communities of the two Solanaceous plants may vary according to agro-ecological zones, and that the rate of fruit losses would be affected by variations of weather conditions.

The study aims to provide basic data on diversity and ecology of the main fruit pests of tomato and pepper plants in two major gardening basins of the country: The southern plateau and the Western Highlands of Cameroon. For this purpose, (i) an inventory of the fruit entomofauna of these plants was done in order to supply an annotated list of the carpophagous insects associated with tomato and pepper in the two Regions, (ii) damages due to the main fruit insect pests were assessed in the two sites and (iii) dynamics of fruit losses were surveyed.

## MATERIALS AND METHODS

### Study sites

Data collection was conducted from March 2010 to February 2011, in trap gardens settled in two sites located in two main gardening basin of Cameroon: (i) Okola (04°01'39, 0'' N; 011°23' 00, 1" E, altitude: 604 m) on the southern plateau, with bimodal rainfall regime (monthly rainfall varying between 415.6 and 7.2 mm, the mean value being 162.82 mm in 2010 and temperatures extending between 23 and 25.9°C, the mean value being 24.46°C; (ii) Koutaba (05°38'47, 9'' N; 010°48' 22, 2'' E, altitude: 1186 m) in the Western Highlands, with unimodal rainfall regime (monthly rainfalls varying between 315.4 and 0 mm, the mean rainfall being 130.04 mm in 2010 and temperature varying between 17.2 and 20.4°C, the mean value being 18.66°C). These two Regions are known to be pioneer in the market gardening practices in Cameroon (Westphal et al., 1981), and remain the main market crop gardening areas of the country. The two sites, characterized by the different altitudes and weather conditions presented different vegetation structures. The Okola's vegetation is a mosaic of more or less disturbed semi-decidious forest, cocoa based agroforestry systems and food crop farmlands. Here, cocoa plants were associated to various fruit trees including mango trees (*Mangifera indica)*, plum trees (*Dacryodes edulis*) etc. The main cropping system in this farmland is slashed and burned farming. Food crops include cassava (*Manihot esculenta*), corn (*Zea mays*), groundnut (*Arachis hypogaea*), bananas and plantain (*Musa* spp.) and market gardening lands of (pepper, tomato, green spices, and vegetables). The Koutaba's vegetation is composed of bushy savannah with remains of deciduous and gallery forests (Olivry and Chastanet, 1986). It is characterized by a mosaic of *Pennisetum purpureum* and *Imperata cylindrica* savannahs (Letouzey, 1963). According to the farmers, areas colonized by *Pennisetum purpureum* supported high fertile soils contrarily to those colonized by *Imperata cylindrica*. This site also holds coffee based plantations, fruit trees such as mango trees (*Mangifera indica*), avocado trees (*Persea americana*) and guava trees (*Psidium guajava*) and food crop farmlands.

For the assessment of the diversity of insects associated to the studied species, additional data were collected all around these two main sites.

### Experimental design

Each trap garden was composed of a total of 16 ridges (11 m long × 1 m wide) separated by 50 cm furrow set up following local technics. Poultry manure was used to enrich the soil, 8 ridges were used for tomatoes and 8 used to plant pepper. On each ridge, 20 seedlings were planted on two lines and spacing of 0.7 m between the plants of the same line and 0.6 m between the plants of two different lines. A total of 80 seedlings of each studied species were planted, and all of them were systematically examined during each sampling.

### Data collection

#### *Fruit loss and pest biodiversity*

Data collection started when the first fruits appeared. All the fruits present in the garden were counted and examined visually. Those fallen on the ground or with oviposition or larvae penetration holes

were collected for further incubation in the laboratory, following protocols of Djiéto-Lordon and Aléné (2006), Diamantidis et al. (2009) and Vayssières et al. (2002). For this need, we used plastic boxes (7 × 10 × 17 cm) previously containing a small quantity of sand for insect's pupation and covered with close mesh net to prevent the insects escape at emergence. Once emerged, insects were collected, counted and preserved in 70% ethanol. This was done twice per month alternatively on the two study sites. Advantages of these methods were the possibility to provide a quasi-exhaustive list of insects associated with different fruit species, to observe their activities, to establish their specificities and to assess their yield loss due to each pest species.

Determinations were done by using several identification keys: Delvare and Aberlenc (1989), Daly et al. (1998), Borror et al. (1976) for families and some genus of insects; Bezzi (1915) for syrphids species, Villiers (1952) for hemipterans; Goureau (1974) for coccinelids, White and Elson-Harris (2004) for fruit flies. These determinations were confirmed by the taxonomists of the faunistic laboratory of the CIRAD (Montpellier).

#### Climatic data

About weather conditions, data used in this study are from the National center of Meteorology of Yaoundé for Okola site and from the airport station of Koutaba for Koutaba site.

#### Data analysis

##### *Yield losses*

The yield loss due to a given pest ($T_{xi}$) was calculated by the following formula:

$$Txi = \frac{ni}{N}_{x} 100$$

Where (*ni)* is the number of fruits attacked by this pest, (*N)* the total number of fruits obtained with the whole harvest.

#### Evaluation of the sampling success

In order to evaluate the strength (S) of the sampling effort, the theoretical species richness (TSR) was calculated on the base of eight different non-parametric estimators: ACE (Abundance-based Coverage), Jack1 and Jack2 (first and second order Jack-knife), Chao1 and Chao2, ICE (Incidence based Cover Estimator), MMM (Michaelis Menten Mean) and Bootstrap Estimator, using EstimateS 8.2 software (Colwell, 2006; Magurran, 2004). The strength of sampling effort was expressed as the ratio:

$$S = \frac{OSR}{TSR} \times 100.$$

where OSR is the Observed Species Richness and TSR the Theoretical Species Richness.
The mean of the theoretical species richness obtained was compared to the observed species richness for each site. Also, rarefaction curve based on the evolution of species richness with increasing plot number was plotted.

#### Evaluation of species richness and diversity

To determine and compare species diversity within habitats (alpha diversity), the Shannon Wiener's index H' was used Barbault, (1997), in the formula: $H' = -\sum Pi \, Log_2 Pi$ ($0 \leq H \leq Log_2 S$); where *Pi* is the relative proportion of species *i* in a sample; S the number of species present in a sample. An equitability index was used to assess the number of individuals in the species $E = H'/Log_2 S$; ($0 \leq E \leq 1$).

#### Evaluation of fruit losses in relation to abiotic factors

Each month, amongst all mature fruits harvested on the two study sites, the attacked ones, recognized by the type of wounds seen on their surface, were grouped and counted. Then all the fruits with the same wounds or the fruits where the same insect species emerged were counted monthly. After that, the dynamics of monthly fruit loss were analyzed with respect to the environmental data.

#### Statistical analysis

Species richness was estimated using Estimates 8.2 software (Colwell, 2006). Communities comparison between the habitats were done using Shannon-Weiner index, equitability index of Pielou was used to appreciate equitability using Past Software and then the non-parametric correlation of Spearman was used, to analyze the relationship between the abiotic factors and fruit loss using the SPSS 17.5 software.

## RESULTS

### Fauna diversity and abundance

#### *Sampling success*

Species accumulation curves were calculated for each plant species for each site (Figure 1). In the two sites, the curves showed a similar pattern, with a species saturation plateau. This suggests a high sampling effort. The slope of the tomato's diversity curve at Okola (T. Okola curve) was similar to that of pepper at Okola (P. Okola), and the slope of pepper at Koutaba (P. Koutaba curve) is similar to that of tomato at Koutaba (T. Koutaba), indicating that, the increase of the new species number in the last five sampling were about 2 to 3 species in the Okola forest areas and only one species in the Koutaba savannah zone.

Virtually all the species were recorded after 5 to 6 sampling periods. So the data collected on each site were representatives of the community and may be used in community studies.

### Taxonomic composition of pest diversity

A total of eight insect species belonging to seven genus, five families and two orders were identified from a sample of 1669 specimen obtained from the incubations of fruits collected on tomato and pepper fruits at both Okola and Koutaba sites (Table 1). Among them, *Dacus* (*Dacus*) *punctatifrons* (Krasch, 1887), *D.* (*Dacus*) *bivittatus* (Bigot, 1858) were reared form tomato fruits whereas *Ceratitis capitata* (Wiedermann, 1824), *Leucinoides orbonalis* (Guenée, 1854) and *Cryptopheibia leucotrata* (Meyrick, 1913) were reared from the pepper fruits. *Helicoverpa armigera* (Hübner, 1808), *Spodoptera littoralis* (Boiduval,

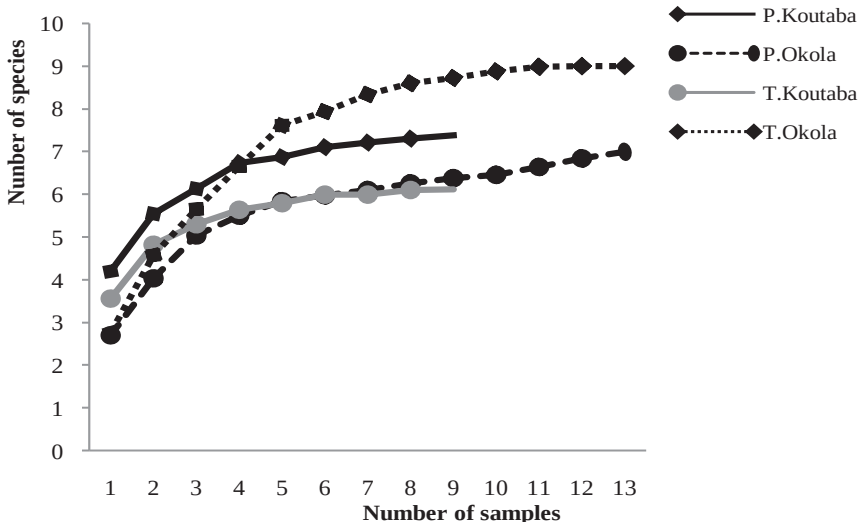


**Figure 1.** Species accumulation curves of occurrence of tomato (T) and pepper (P) pest at Okola and Koutaba.

**Table 1.** Absolute and relative abundances of pest species found in incubated boxes.

| Plant species | Pest family | Pest species | Okolani (%) | Koutabani (%) |
|---|---|---|---|---|
| *Lycopersicon esculentum* | Noctuidea | *Helicoverpa armigera (Hübner, 1808)* | 12(2.1) | 53(8) |
| | | *Spodoptera littoralis* (Boiduval, 1833) | 1(0.1) | 7(1) |
| | | *Chrysodeisis chalcites* (Esper, 1789) | 1(0.1) | 0(0) |
| | Thephritidae | *Dacus puctatifrons* (Karsch, 1858) | 557(97) | 587(89) |
| | | *Dacus bivittatus* (Bigot, 1858) | 0(0) | 11(1.6) |
| | Noctuidae | *Spodoptera littoralis*(Boiduval, 1833) | 0(0) | 4(2.19) |
| | | *Chrysodeixis chalcites*(Esper, 1789) | 1(0.01) | 0(0) |
| *Capsicum annuum* | Pyralidae | *Cryptophlebia leucotreta (*Meyrick, 1913) | 28(28) | 43(23.62) |
| | | *Leucinoide sorbonalis (*Guinée, 1854) | 9(9) | 0(0) |
| | Thephritidae | *Ceratitis capittata*(Weidemann, 1824) | 62(62) | 135(74.17) |

1833) and *Chrysodeixis chalcites* (Esper, 1789) were common to fruits of the two species.

## Species richness estimators

Based on eight different species richness estimators nine species were expected both on pepper at Okola and Koutaba and on tomato at Okola, while seven species were expected on Tomatoes at Koutaba (Table 2). Compared to the observed species richness, the sampling effort varied between 83.35 and 96.87%) (Table 2).

Considering estimators individually, the rates varied between 63 and 100%. Almost seven of the eight estimators showed highest estimated success of 100% on pepper in the two sites and on tomato at Koutaba. The lowest mean sampling success was (Jack2) 63% on pepper at Okala (Table 2).

## Pest abundance

Independent of the study site, fruit pests of tomato were dominant, by *D. punctatifrons*, with respectively 97% of the total community at Okola and 89 at Koutaba. It was followed by *H. armigera* with (2%) and (8%) respectively. On pepper community, the populations of *C. capitata* were the most important in the two study sites, 62% and 74.17% respectively, followed by *C. leucotreta* (Table 1). The pest community of Koutaba was more diversified than the one of Okola, the Shannon index was $H_1$'=0.992 and $H_2$'=0.666 respectively. The abundance distribution among species were more equitable in Koutaba than in

**Table 2.** Recorded and expected number of species as calculated with different species richness estimators.

| Variables | Samples | S.obs. | ACE | ICE | Chao 1 | Chao 2 | Jack 1 | Jack 2 | Bootstrap | MMMean | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| P.Koutaba | 8 | 7 | 7(100) | 8(87.5) | 7(100) | 7(100) | 9(77.77) | 9(77.77) | 8(87.5) | 10(70) | 9(87.56) |
| T.Okola | 13 | 9 | 9(100) | 9(100) | 9(100) | 9(100) | 9(100) | 9(100) | 9(100) | 12(75) | 9(96.87) |
| T.Koutaba | 8 | 6 | 6(100) | 6(100) | 6(100) | 6(100) | 7(85.71) | 8(75) | 6(100) | 7(85.71) | 7(93.30) |
| P.Okola | 13 | 7 | 8(87.5) | 8(87.5) | 8(87.5) | 8(87.5) | 9(77.77) | 11(**63.63**) | 8(87.5) | 8(87.5) | 9(83.35) |

The sampling success given as proportion of sampled species (Sobs) to the estimated species numbers are given in brackets. Maximum and minimum successes are indicated by bold numbers. P=pepper, T=tomato, S. obs = Species observed.

**Table 3.** Distribution of fruit loss relative abundance in relation to the pest species at Okala and Koutaba.

| Host plant | Pest species | Okola (%) | Koutaba (%) |
|---|---|---|---|
| | Healthy fruits | 66.6 | 50.24 |
| | FA *D. punctatifrons* | 19.34 | 24.8 |
| *L. esculentum* | FA *H. armigera* | 11.17 | 23.51 |
| | FA *C. chalcites* | 1.3 | 0.24 |
| | FA *S. litoralis* | 0.53 | 0.65 |
| | FA Other pest | 1.0 | 0.57 |
| | Healthy fruits | 52.97 | 71.69 |
| | FA *C. capitata* | 21.0 | 16.95 |
| | FA *C. leucotreta* | 22.32 | 9.44 |
| *C. annuum* | FA *L. orbonalis* | 1.06 | 0.07 |
| | FA *H. armigera* | 0.0 | 0.51 |
| | FA *S. litoralis* | 0.0 | 0.04 |
| | FA Other pest | 2.64 | 1.3 |

FA=fruit attacked by the pest species.

Okola; Equitability index of Pielou (J) was $J_1$=0.51 at Koutaba and $J_2$=0.32 at Okola.

## Yield loss

The two paired comparison of Wilcoxon shows that the yield loss for pepper and tomato were greater in Koutaba than in Okola (P<0.05). A total of 757 fruits of pepper were harvested at Koutaba, 356 fruits were damage which represents the yield loss of 47.03%. On the other hand at Okola, 2755 fruits were harvested and 780 showed traces of attacks which represented the yield loss of 28.31%. For tomatoes, 3264 fruits were harvested at Koutaba, 1624 were attacked, that is 49.76% of yield loss. At Okola, on 7693 fruits harvested 2565 were attacked, equal to 33.4% of yields loss (Table 3).

## Fruit losses dynamics in relation to abiotic factors

Considering the low rate of fruits affected by S*. littoralis C., chalcites, D. bivittatus, L. orbonalis* respectively, they were not taken into account in the following analysis as, they were considered as secondary pest of these crops. Pest species that had economic important damages were selected to analyze their potential correlation with weather factors (rainfall, Temperature and sunshine):

1. *C. leucotreta* damage has shown a positive and non-significant relationship with precipitation both at Koutaba and at Okola (Tables 4 and 5). Meaning that, the population of this pest was not significantly affected by rainfall. A similar result was obtained with temperature at Okola (r=0.142; P=0.662). Contrarily, a positive and significant correlation was obtained with temperature at Koutaba (r=0.681; P=0.03).
2. *Ceratitis capitata* damage showed a negative and non-significant correlation with precipitation on the two sites(r=-0.366; P=0.298 and r=-0.210; P=0.513). A similar result was obtained for temperature at Koutaba (r=-0.065; P=0.854); but not in Okola where a non-significant positive correlation was found (r=0.067; P=0.837). Globally, population fluctuations were not affected by temperature.
3. *D. punctatifrons* damage had a strong positive and significant correlation with precipitation (r=0.835;

**Table 4.** Result of correlation test between of climatic factors on pest insect damages at Koutaba Cameroon.

| Factor | | *Cryptophlebia leucotreta* | *Ceratitis capitata* | *Dacus punctatifrons* | *Helicoverpa armigera* |
|---|---|---|---|---|---|
| Rainfall | r-Value | 0.474 | -0.366 | 0.835** | -0.553 |
| | P-value Sig (2-tailed) | 0.166 | 0.298 | 0.003 | 0.097 |
| | N | 10 | 10 | 10 | 10 |
| Temperature | r-Value | -0.681* | -0.067 | -0.219 | -0.139 |
| | P-value, Sig (2-tailed) | 0.030 | 0.854 | 0.544 | 0.701 |
| | N | 10 | 10 | 10 | 10 |

*Correlation is significant at the 0.05 level, **correlation significant at the 0.01 level.

**Table 5.** Result of correlation test between climatic factors on insect pest damages at Okola Cameroon.

| Factor | | *C. leucotreta* | *C. capitata* | *D. punctatifrons* | *H. armigera* |
|---|---|---|---|---|---|
| Rainfall | r-value | 0.125 | -0.210 | 0.853** | -0.245 |
| | P-value, Sig (2-tailed) | 0.699 | 0.513 | 0.000 | 0.442 |
| | N | 12 | 12 | 12 | 12 |
| Temperature | r-value | 0.141 | 0.067 | 0.130 | -0.067 |
| | P-value, Sig (2-tailed) | 0.662 | 0.837 | 0.687 | 0.836 |
| | N | 12 | 12 | 12 | 12 |

*Correlation is significant at the 0.05 level, **Correlation significant at the 0.01 level.

p=0.003 and r=0.853; p=0.0001) in the two areas. This means, when the precipitation increases, the population of these pest increases. Temperature had a negative but non-significant correlation with the damage at Koutaba (r=-0.219; P=0.544); and a positive but also non-significant correlation at Okola (r=0.130; P=0.688).

4. *H. armigera* damage had a negative and non-significant correlation with precipitation on the two sites (r=-0.553; P=0.097 and r=-0.245; P=0.442). Temperature was negative and non-significantly correlated to the damage caused by *H. armigera* both at Koutaba (r=-0.139; P=0.710); and at Okola (r=-0.067; P=0.836).

## DISCUSSION

### Pest diversity

It is commonly accepted that species richness in a whole-community is very difficult to measure and usually requires the use of various sampling technics as well as theoretical estimators (Longino et al., 2002). In the present study, two main estimation tools were used: The species accumulation curves and non-parametric estimators (Longino et al., 2002; Gotelli and Colwell, 2013). The species accumulation curves showed that after 6 samplings, the rate of new species occurring in each community was very low. The pest insect communities found on almost all the localities within the study areas were very close, but significant differences occurred in the abundant distribution amongst species. For instance, numerical domination of *H. armigera* and *D. punctatifrons* populations were more obvious in the humid savannah of Western Highlands than in the forest zone of the southern plateau. Geographical variations of abundant distributions amongst communities with close species composition may be explained by a certain number of factors, including availability of food resources, constraints of natural enemies, weather condition or interspecific competitions (Futuyma, 2005; Molles, 2008). The lower abundance of pests observed in the forest zone may be related to higher diversity of potential host plants. Altieri, (1999) established that the level of internal functioning regulations in agro-ecosystems is largely dependent on the level of plant and animal diversity. The high abundance of *H. armigera* and *D. punctatifrons* in Highland can also be explained by weather conditions, the mean temperature is 18°C different from the temperature of forest area where the mean is about 24°C and moisture was about 80%, different to 75% in Okola. Altitude also influence either directly by the changing of weather conditions or indirectly through insect communities interactions (Hodkinson, 2005).Generally, biodiversity decreases with altitude (Nabors, 2004; Atalay, 2006). Also insect populations are affected by habitat disturbance Atalay (2006). For example in the

savannah area where bush fires are frequent, phytophagous insects tend to move in cultivated areas where moisture is constant and nesting sites available. Mac Arthur cited by Molles (2008) was one of the first ecologists to demonstrate and quantify the relationship between species diversity and environmental heterogeneity using the Shannon-Wienner index H'. The presence of encountered pest species in all examined habitats from 600 to 1200 m suggested that these pests can tolerate a high range of altitudes and other environmental conditions if their host plants are present.

**Yield loss**

The yield loss at Koutaba (47.03 and 49.76% on pepper and tomato respectively) were higher than those observed at Okola (28.31 and 33.4% on pepper and tomato respectively). This difference may be due to altitudes of the sites (600 m altitude and 1200 m respectively). Air temperature can influence the specific richness particularly the predators like ants that normally reduces the pest populations by feeding on their larvae. Cagnolo et al. (2002) demonstrated a decrease of certain species of insects on the altitudinal gradient. The difference in yield loss can be also due to surrounding vegetation; the first site is in a forest area with wild trees and the second is in a savannah dominated by mono-specific vegetation made of Graminaceae which are not usually used as food by the studied pest larvae (caterpillars or maggots). The forest vegetation which is more diversified than the savannah offers a larger spectrum of food resources to the phyllophages insects. Moreover, the physical environment of Okola offers more favorable micro -habitats for the development of many other organisms that could prevent outbreaks of pests on cultivated crops. In this forest vegetation, trophic network are more complex and it is well known in ecology that, the more trophic network is complex the better the agro-system is balanced (Russell et al., 2008). Mate-finding failure can also explain the different impacts of pest on the yield. Russell et al. (2008) demonstrate the negative effect of landscape fragmentation on animal mating. At Koutaba food resources are not diversified this may explain why the phyllophagous insects concentrate their reproduction on the agro system. Moreover the landscape was less fragmented and sexual mate easily met. Yield loss was important during our study period but some pests such as *D. punctatifrons* were particularly harmful to fruits 19.34 and 24.8% at Koutaba and Okola respectively. In the early 1990s Tindo and Tamo (1999) signaled *D. punctatifrons* as the major pest of tomato at Nkometou and Obala, two sites located in the same landscape as Okola. These authors mentioned that the rate of infested fruit increased from 9.8% in 1996 to 42.6-33% in 1997. The difference in the infestation rate can be due to the sampling periods. In fact, rainy seasons are favorable to the development of *D. punctatifrons*. The infestation rate was higher during the rainy season than in the dry season. However in the present study, infestation rate was evaluated throughout the year so the effect of the season was accumulated. As for *H. armigera* 11.17 and 23.51% fruit loss were obtained respectively in Okola and Koutaba. This pest was recorded as the main pest of tomato in northern India with a fruit yield loss of about 70% (Metha et al., 2010).

The season appeared to have an influence on infestation rate of our main pest. So, *H. armigera* appeared more active during the dry season than during the humid one. For *C. capitata* the fruit loss of 21 and 16.95%; were recorded respectively in Okola and Koutaba and for *C. leucotreta,* 22.32 and 9.44% respectively in Okola and Koutaba. Infestation rates of 14.86% for *C. leucotreta* and 6.71% for *C. capitata* were reported on the pepper in Yaoundé central Region (Djieto Lordon et al., 2014).

**Fruit loss dynamic in relation to abiotic factors**

Bateman (1972) demonstrated that the principal components of the life cycle of fruit pests are temperature, photoperiod, food, natural enemies and symbiosis. But none of these can stand out as a great important determinant of fruit loss. The contribution of each environmental factor varies with environmental and biotic conditions of the area. The main factor determining fruit loss in our study appeared to be the rainfall. It can be direct or indirect. The increase in damage due to *D. punctatifrons* by rainfall can be direct as rainfall induces a high level of moisture in the soil, enabling pupae to hatch easily. During dry period, the soil is compact; thus pupae become quiescent during a long period. In this form many pupae undergo dryness and die. Rainfall can also be indirectly involved in the increasing damage due to *D. punctatifrons* since the rainy season is the period when almost all plant species bloom, increasing the availability of food resources for adult flies (secretions, nectar, sap exuding from trunk, stem, leaf, rotting fruits) (Christenson and Foote, 1960). Adult flies can also feed on fruit injuries such as those caused by mechanical damage or biting holes due to some herbivorous insects. Adults *D. punctatifrons* were also observed flying around Aphids honeydew (*Macrosiphom euphorbiae and Aphis gossipy*) (pers. observ.). These different sources of insect food may help in egg maturation of the fruit flies. Christenson and Foote (1960) realized that honeydew secreted by homopterous insects provides hydrolyzed proteins, minerals, and vitamins that can be required by *D. dorsalis* for normal fertility and fecundity. The increase of fruits which are the breeding sites and food for insects' larvae were also proposed to explain increasing damage of *D. punctatifrons* during the rainy season, but this idea cannot be the best because in Cameroon gardening

practices are permanent and do not depend on climate.

Rainfall is negatively correlated with damages due to *H. armigera*. This may be due to the fact that females of this butter fly lay it is eggs on the external surface of fruits, and flowers. Thus, most of these eggs are destroyed by the rainfall before hatching. Amongst the larvae which successfully penetrated the fruit, many are drowned by water licking through the entrance hole.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Environmental and socio-economic effects of timber harvesting in Ebonyi State, Nigeria

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**This study was carried out to evaluate the socio-economic and environmental effects of timber harvesting in Ebonyi State, Nigeria. Three Local Government Areas (LGAs) were purposively selected from the timber producing areas of the state, and multi stage random sampling technique was employed to select a total of 160 respondents – composed of 50 randomly selected farmers from each of the three LGAs and 10 officials from the State Forestry Department. Primary data were used for the study. A structured questionnaire was used to collect information from the respondents. Data was subjected to statistical and econometric analysis which included percentages, frequencies and exploratory factor analysis. Environmental economic and social effects of timber harvesting observed from the study included: Silting of rivers and lakes, damaging of immature trees and non-wood forest products, loss of biodiversity, climate change/global warming, high cost of farm labour, disputes and crises over land and compensation, high cost of living, loss of forest land and increased cost of wood and timber products. Level of economic losses amounted to over 2000 trees per year from the forests excluding those harvested from free areas that were not officially on record. The study recommended that the Ministry of Agriculture/Environment and other related stakeholders should adequately sensitize the public on the long term implication of illegal logging on the environment and socio-economic well-being of the farmers in the concerned communities.**

**Key words:** Environment, economic, social, effects, timber harvesting.

## INTRODUCTION

Timber harvesting is the cutting down of wood from the wild and reserved areas for both domestic and commercial purposes. It should not be confused with illegal logging, which refers to the harvest, transportation, purchase or sale of timber in violation of laws. The harvesting procedure itself may be illegal including using corrupt means to gain access to forests; extraction without permission or from a protected area; the cutting of protected species; or the extraction of timber in excess of agreed limits. Throughout history, humans have manipulated natural resources to produce food. Although other products from the natural environment have been exploited- the rate of timber harvesting has accelerated significantly since the turn of the century. According to

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FAO (2012), the world has just less than 4 billion hectares of forest, covering about 20% of the world's land area. Surprisingly, the net forest loss remains 7.3 million hectares per year or 20,000 ha per day (Ajake and Enang, 2012). This is most serious in the tropics where over 2.5 billion people depend on the natural forest resources for variety of services (Tijani, 2007; Butler, 2012). In Central and West Africa, the tropical rainforest has been an important source of timber, as well as other valuable non-timber products (Keegan, 2011; Laird, 2008; Abayomi, 2001; Abayomi et al., 2002). Unfortunately, increased demand for timber resources and the technology adopted by man for their extraction has caused severe degradation of forest resources (Jimoh, 2001). At present, estimated timber losses in Africa (FAO, 2012) were observed to be higher than those of Latin America and the Caribbean. For instance, between 2000 and 2010, the continent lost about 5.2 million hectare of forest, accounting for about 52% of the global reduction of forest cover (FAO, 2012). To support this argument, Okonkwo et al. (2002) deduced that numerous unchecked activities including illegal logging have been taking place in the forest zones of Nigeria, ranking it second after Cameroun. Forest loss in Nigeria is put at an average of 400,000 ha per year, while afforestation has been only 32,000 ha yearly. The cumulative effect of these is that the continent has lost about 50 million hectare of forest in less than 100 years (Mmon and Mbee 2014). Furthermore, this could lead to desert encroachment, global warming, food chain depletion, destruction of soil structure, extinction of wildlife, draught and exposure of bush to burning (Rhett, 2005).

Consequently, majority of developing countries now have lower per capita income than when the decade began. According to Barbier (2005), rising poverty and unemployment have increased pressure on environmental resources as more people have been forced to rely more directly upon them. This assertion was emanates from the fact that poverty and environment are linked in a "downward spiral" approach in which poor people are forced to overuse environmental resources for their daily survival, and are further impoverished by the degradation of these resources (Cronin and Pandya, 2009; Todaro and Smith, 2009). The loss of timber resources beyond sustainable limit is a serious issue in Nigeria. The study therefore seeks to investigate the environmental, economic and social effects of timber harvesting in Ebonyi State. A proper understanding of resource-environment linkage is a good approach in developing effective public policy in retrospect to the myriads of harm that have been wittingly done to the ever-diminishing timber resources, while still observing environmental sustainability.

It has been observed that multinational companies perform most of the harvesting activities with little benefits reaching the local community. Now, because of their little or no effort in improving the lives of the host communities and with the attendant impoverished environment, they are faced with the challenges of environmental degradation and resources depletion. The adverse effects of industrial timber harvesting on the forest ecosystem have been acknowledged to include loss of biodiversity, exposure of soil to erosion and harsh weather, etc. (Reading et al., 2005; Obot, 2002). Moreover, there is lack of information on the volume of timber harvesting in Nigeria, effects of timber harvesting, and the level of economic loss sustained as a result of timber harvesting. So, the rate of timber harvesting could not be compared with the regeneration potential of the natural forest and the rate of plantation establishment. This would have formed the premise on which forestry planning and development should rest, like in the developed nations that have committed substantial amount of funds to monitor growth and timber harvesting in their natural and plantation forests. Consequently, this study specifically aims to: (i) Determine, from the perception of farmers, the possible consequences (effects) of illegal harvesting of timber; (ii) Estimate the level of economic losses incurred by illegal logging, and (iii) Make recommendations based on the findings, strategies that will ensure sustainable timber utilization over time.

## METHODOLOGY

### Theoretical framework

#### *Exploratory factor analysis*

The ultimate goal of factor analysis is to explain the covariance relationships among the variables in terms of some unobservable and non-measurable random factors. A wide range of dimension or multivariate variables may exist; therefore factor analysis aims at reducing the dimensionality or multi-variate data set to an orderly structure (Ashley et al., 2006; Ledyard and Robert, 1997). Factor analysis is a technique of describing groups of highly correlated variables by a single underlying construct or factor that is responsible for the observed correlations (Ashely, 2008), and once the groups of correlated variable are identified, they are interpreted and labeled.

There are methods of factor analysis which include common and principal component analysis. As reported by Wilkinson et al. (1996), most data sets under both methods of analysis lead to similar results. Exploratory factor analysis procedure using the principal component model with iteration and varimax rotation will be employed in grouping the effects of timber harvesting into major components. Only variables with factor loading of 0.30 and above will be used in naming the factor (Ashley et al., 2006). Also variable(s) that loaded in more than one factor will not be used. High reliability of factor analysis models in social science studies has widely been explored by several authors. Ashley et al. (2006) employed factor analysis to analyze education systems of 64 countries around the world while Okorji and Chukwuone (2000) applied factor analysis to determine constraining factors to community seed project in Enugu State, Nigeria. Agwu (2000) analyzed his data on extracting cowpea technology diffusion in Northeast Savanna Zone of Nigeria using Factor analysis; Kessler (2006) applied factor analysis in determining the decisive key factors influencing farm households' soil and water conservation investments in Netherlands.


**Figure 1.** Map of Ebonyi State showing the study areas. Source: EB-MANR, 2011.

**The study area**

The study was carried out in Ebonyi State, Nigeria. The state lies approximately within longitudes 7° 30`` and 8° 30`` East of the Greenwich Meridian and latitudes 5° 40`` and 6° 45`` North of the Equator. It is bounded in the North by Benue State, to the West by Enugu State, to the East by Cross River State and to the South by Abia State. Ebonyi State has a total of thirteen (13) Local Government Areas (LGAs) (Ebonyi State Government, 2009). By the 2006 population census, the population of Ebonyi State was put at 2.1 million (NPC, 2006). It has a total land area of about 5,935 $km^2$. The State is endowed with enormous mineral resources: Salt lakes at Uburu, Okposi and Oshiri; Zinc and lead deposits at Enyigba as well as Kaolin and Limestone at Ishiagu, Afikpo and Nkalagu (EB-SEEDS, 2004). Agriculture is a major occupation in Ebonyi State, with an estimated 85% of the population earning their living from one form of agriculture or another. Major food crops grown in large quantities include rice, yam, cassava, maize, cocoyam, cowpea and groundnut. cash crops such as oil palm, cashew, cocoa, rubber, etc are vigorously cultivated (Figure 1).

At present, the State has eleven officially developed forest reserves and many sacred grooves which protect her rich biodiversity. The Akanto game reserve (with an area of about 450 hectares) is a protected area where endemic wildlife species are conserved. The Ministry of Agriculture has planted over 6000; 5000 and 8000 seedlings of teak (*Tectonia grandii*) at Effium, Ovuum and Ozziza Reserves respectively; and has embarked on the forest reserve study of Federal Government of Nigeria aimed at the development of a forestry management plan (EB-MANR, 2011).

**Sampling procedure**

The sampling techniques adopted and utilized for selecting the respondents for the study were the multi-stage sampling technique. Out of the thirteen Local Government Areas (LGAs) in the state, 3 LGAs were purposively selected from the areas where forest reserve exists. Then, random sampling procedure was used to select 5 communities from each local government making a total of 15 communities for the study. From each sampled community, 10 farm-households were randomly selected to give a sum of 150 farmers. Also, ten (10) respondents were randomly selected from the Department of Forestry, Ebonyi State Ministry of Agriculture and Natural resources (EB-MANR, 2011). This gave a grand total of 160 respondents.

**Data collection and analysis**

Data for the study was collected from the primary source. This was done using a set of structured questions grouped in a pre-tested questionnaire. Information gathered included the perceived effects of timber harvesting on man and the environment. The questionnaire had four sections namely: Socio-economic characteristics of respondents, level of economic losses incurred as a result of timber harvesting, environmental, economic and social effects of timber harvesting constraints to effective timber management. The questionnaire was administered by the researcher with the assistance of enumerators who are familiar with the terrain and the people. In order to realize the specific objectives of the study, relevant analytical tools were employed. Principal component factor analysis model was used to realize objective (i) while descriptive statistics such as frequency and percentage were used to realize objective (ii). The principal component factor analysis model is specified as follows:

$Y_1 = a_{11}\, x_1 + a_{12}\, X_2 + \ldots\ldots\ldots + a_{1n}\, X_n$
$Y_2 = a_{21}\, x1 + a_{12}\, x_2 + \ldots\ldots\ldots\ldots + a_{2n}\, x_n$
$Y_n = an_1\, X + an_2\, X_2 + \ldots\ldots\ldots\ldots + a_{nn}\, X_n$
Where:

$Y_1$, $Y_2$…….$Y_n$ = Observed variable or consequences of timber harvesting; $a_1 - a_n$ = Factor loading or correlation coefficients, and $x_1$, $x_2$……..$x_n$ = unobserved underlying factors or consequences of

**Table 1.** Varimax Distribution of environmental, economic and social effects of timber exploitation.

| S/N | Resultant effect | Factor one environmental effect | Factor two social effect | Factor three economic effect |
|---|---|---|---|---|
| $V_o1$ | Silting of rivers and lake | 0.214 | 0.000 | - 0.003 |
| $V_o2$ | High cost of farm labour | - 0.165 | 0.977 | - 0.059 |
| $V_o3$ | Occurrence of disputes and crises over land and compensation | - 0.940 | 0.224 | - 0.134 |
| $V_o4$ | Damaging of immature trees and non- wood forest product | 0.101 | - 0.003 | - 0.022 |
| $V_o5$ | Loss of income and revenue by govt. | - 0.019 | 0.007 | - 0.005 |
| $V_o6$ | Reduction in soil fertility and croup output | 0.064 | - 0.031 | 0.006 |
| $V_o7$ | Loss of biodiversity | 0.605 | - 0.011 | - 0.010 |
| $V_o8$ | Loss of forestland | 0.010 | - 0.008 | 0.891 |
| $V_o9$ | Disappearance of forest cover | 0.014 | 0.016 | 0.017 |
| $V_o10$ | Rural-urban migration | 0.022 | - 0.011 | - 0.005 |
| $V_o11$ | High cost of living | - 0.143 | 0.103 | 0.974 |
| $V_o12$ | Increased cost of wood and timber products | 0.035 | - 0.007 | 0.912 |
| $V_o13$ | Climate change/global warming | 0.222 | - 0.014 | - 0.001 |

Source: Field Survey, 2014.

timber harvesting.

## RESULTS AND DISCUSSION

### Environmental, economic and social effects of timber exploitation

Here, the environmental and the socio-economic effects of timber harvesting in the study area were identified. The determined effects include, but not limited to: occurrence of disputes and crises over land/compensation, damaging of immature trees and non-wood forest products, loss of income and revenue by government, loss of biodiversity, disappearance of forest cover, increased cost of wood and timber products etc. This is summarized in Table 1.

Based on the results obtained from the exploratory factor analysis with decision score of 0.3, factors that loaded from 0.3 and above were noted to be areas of significant effects, while factors that loaded below 0.3 were noted to have less significant effects and as such, were ignored. Meanwhile, factors (effects) that loaded high were categorically grouped into three factor groups. Factor one was named environmental effects; factor two was named social effects, while factor three was labeled economic effects.

Those effects that loaded high under factor one (environmental factors) were: Silting of rivers and lakes (0.214); damaging of immature trees and non-wood forest products (0.101); loss of biodiversity (0.605) and climate change/global warming (0.222). This shows that over-reliance of the rural dwellers on timber and other forest resources have resulted to a heavy decline on the ecosystem functioning of the forest. On the other hand, effects that loaded high under factor two (social effects) were: High cost of farm labour (0.977); occurrence of disputes and crises over land/compensation (0.224); and High cost of living (0.103). It is quite evident from the result obtained that efforts made in utilizing forest resources unsustainably are self-defeating. It ends up impoverishing the people; and in most cases, hostilities arise due to unequal distribution of benefits. This agrees with Madukwe (2005), who opined that more often than known, timber exploitation, when left uncontrolled have fueled communal crises, crippling the economy. Also, effect that loaded high under factor three (economic factors) were: Loss of forest land (0.891); high cost of living (0.974) and increased cost of wood and timber products (0.912). These effects above indicated areas that significantly affected the study area in terms of their economic, social and environmental well-being.

### Assessment of economic losses arising from timber harvesting

The percentage distribution of the volumes of annual timber loss due to illegal logging is presented in Table 2. From the results obtained from the Staff of Forestry Department, Ministry of Agriculture, Ebonyi State; about 70% of the respondents agreed that over 2000 harvested trees ($574,000) disappear from the forest in the state illegally. This trend supports the findings of Macedo et al. (2012), that most tree disappearance in tropical forests are over 2000 trees annually unreplaced. Also, 40% of the respondents admitted that the volume of harvestable timber from the three forest reserves in the study area cannot be easily quantified. About 30% of the respondents reported that the annual timber loss was about 500 to 1000 trees ($143,000-$287,000). From the surveys gathered from various timber experts on the market price of mature harvested trees, a base price of

$287 was established as at the time of the study (depending on the specie and girth). This amounts to about 7% of annual budget of the State; hence, a huge loss on the economy.

## CONCLUSION AND RECOMMENDATIONS

Findings show that timber harvesting affects the environmental, economic and social well-being of the respondents in the study area. A lot of these effects are quite obvious such as increased draught, water stress, poor yield of crops arising from flooding, crisis, etc; whereas others like the much pronounced global warming and species extinction are likely to manifest in the future. Rational and efficient use of natural resources is the only means to sustain the long term availability of these resources while still improving human conditions. The great danger posed by uncontrolled/illegal logging is that it has inevitably served as a tool for sponsoring armed conflicts as was recently seen in Effium/Ngbo crisis that claimed many lives, loss of government revenue, climate change, loss of biodiversity which has often created an imbalance in ecosystem services and other related problems on the host communities. Although, farmers harvest these resources for immediate survival from poverty and hunger; but at the long run, has reinforced the scourge of hardship Thus, there is need for a holistic approach to resource management and sustainability. Hence, the study made the following recommendations:

1. Government should ensure that all timber harvesting companies – cooperative bodies and private individuals must acquire and tender their certification and license before entering the forest. This will go a long way in abating corrupt practices by potential users and timberland owners.
2. They should also impose stiff penalties on wood companies and individuals who harvest these resources beyond agreed limits. A more feasible way to achieve this measure is by utilizing trained personnel that will help enforce these rules and subsequent arrest of parties who may display criminal behavior.
3. The ministry of agriculture and other related stakeholders should adequately sensitize the public on the long term implications of illegal logging on the environment and socio-economic well-being of farmers in the concerned communities.

## Conflict of Interest

The authors have not declared any conflict of interest

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# Effect of different levels of NaCl and $Na_2SO_4$ salinity on dry matter and ionic contents of cowpea (*Vigna unguiculata* L. Walp.)

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**Salinity is an environmental stress that limits growth and development in plants. The present study was to assess the effect of salinity on dry matter and ionic contents in cowpea accessions. A pot experiment was carried out to investigate the effect of different levels of salinity on growth and ionic contents of cowpea plant. There were three levels of NaCl and $Na_2SO_4$ salinity which includes 0 (control), 50, 100 and 150 mM. Growth characters such as root and shoot dry weights decreased with increase in salinity levels. $Na^+$, $Cl^-$, $PO_4^-$, $SO_4^-$ and $Na^+/K^+$ concentrations increased with increase in salinity but $Ca^{2+}$, $K^+$ and $Mg^{2+}$ concentrations were lower as salinity levels increased. It is concluded that with increase in salinity levels there was a significant reduction in biomass production in cowpea plant. Pattern of accumulation of ions varied significantly.**

**Key words:** Salinity, concentrations, *Vigna unguiculata* (L.) Walp., stress.

## INTRODUCTION

Salinity is an environmental stress that limits growth and development in plants. It is consider a significant factor affecting crop production and agricultural sustainability in arid and semi arid region of the world reducing the value and productivity of the affected land (Munns, 2002). Salinity causes not only differences between the mean yield and the potential yield, but also causes yield reduction from year to year. It affects the plant growth directly through its interaction with metabolic rates and pathways within the plants. In the simplest analysis of the response of a plant to salinity stress, the reduction in shoot growth occurs in two phases: A rapid response to the increase in external osmotic pressure and a slower response due to accumulation of $Na^+$ in leaves (Munn, 2002). In the first, osmotic phase which starts immediately after the salt concentration around the root increases to the threshold 40 mM NaCl for most plant which is equivalent to 4 dSm (George, 2008). As a result, the rate of shoot growth falls significantly. The second is the ionic specific phase of plant response to salinity which start when salt accumulate to toxic concentration in the leaves causing necrosis and reducing photosynthesis area resulting in decline in growth (Munn, 2002; Bayuelo-Jimenz et al., 2012).

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**Table 1.** Effect of different concentrations of NaCl and $Na_2SO_4$ on biomass components of two accessions of *Vigna unguiculata* (L.) Walp.

| Salt | Conc. (mM) | RDW (g) | | SDW (g) | | TDW (g) | |
|---|---|---|---|---|---|---|---|
| | | TVu 11711 | TVu 15245 | TVu 11711 | TVu 15245 | TVu 11711 | TVu 15245 |
| NaCl | 0 | 1.20[a] | 1.13[a] | 8.19[a] | 6.20[a] | 9.39[a] | 7.33[a] |
| | 50 | 0.59[b] | 0.3[b] | 2.57[b] | 2.35[b] | 3.15[b] | 2.65[b] |
| | 100 | 0.28[c] | 0.27[b] | 1.74[b] | 0.88[c] | 2.02[b] | 1.14[c] |
| | 150 | 0.29[c] | 0.15[b] | 1.85[b] | 0.67[c] | 2.14[b] | 0.82[c] |
| $Na_2SO_4$ | 0 | 0.94[a] | 0.96[a] | 8.23[a] | 6.52[a] | 9.15[a] | 7.49[a] |
| | 50 | 0.46[b] | 0.39[b] | 2.74[b] | 1.99[b] | 3.20[b] | 2.38[b] |
| | 100 | 0.38[b] | 0.21[b] | 2.00[bc] | 1.63[b] | 2.39[bc] | 1.84[b] |
| | 150 | 0.20[c] | 0.15[b] | 1.52[c] | 0.9[b] | 1.73[c] | 1.05[b] |

RDW, Root dried weight; SDW, shoot dried weight; TDW, Total dried weight. Each value is a mean of three replicates. Values with the same superscript are not significantly different at P≥0.05 using Duncan's multiple range test (DMRT).

Cowpea (*Vigna unguiculata* L. Walp.) is an important grain legume crop used as a fodder crop for livestock (Zahedi et al., 2012) and as a cheap source of vegetable. It is consumed both as green pod and as dry seed. The fresh green pods of cowpea contain 85.9% moisture, 4.6% protein, 0.2% fat, 0.8% minerals, 2.0% fibre and 8.5% carbohydrates. The high protein content of cowpea makes it an important supply to the diet of many African people (Giami et al., 2001).

Many varieties are grown in tropical and sub-tropical agricultural areas of the world, where salinity is a yield-limiting factor (Zahedi et al., 2012). Cowpea is reported to have a good tolerance to heat and drought (Vasquez-Tello et al., 1990 cited in Zahedi et al., 2012), and it has a high yield potential under irrigation (Murillo-Amador et al., 2006). Salinity stress disturbs the uptake and accumulation of essential nutrients (Zhu, 2001).

In view of these studies, the principal objective to carry out the present study was to assess the effect of salinity on dry matter and ionic contents in cowpea accessions.

## MATERIALS AND METHODS

This study was carried out from February to April, 2013 in the green house at the Department of Botany, Faculty of Science, University of Ibadan and seeds of cowpea cultivars (namely; TVu 11711 and TVu 15245) were obtained from the International Institute of Tropical Agriculture (IITA), Oyo State, Ibadan. Cowpea seeds were planted in pots of 14 cm diameter and 18.5 cm depth; each pot was filled with 3.0 kg soil. Four seeds of each cultivar were sown in each pot. Four levels of NaCl salt (0, 50, 100 and 150 mM) were applied after 15 days of germination. The experiment was laid in completely randomized designed in triplicates. When the seedlings were well established (after 10 days) that is, when the first trifoliate leaf had reached its full size and the second trifoliate leaf was starting its development, thinning was carried out, leaving two plants per pot. Salt treatment commenced 5 days after plants were thinned. These involved the application of NaCl and $Na_2SO_4$ with varied equimolar concentration (0, 50, 100 and 150 mM). The treatments were applied twice a week except for the control (0 mM) that was watered regularly. Plants were harvested four weeks after treatment

Plants were uprooted carefully and washed in running tap water. Plant samples were placed in oven at 80°C. After 4-days shoot and root dry weights (g/pot) were estimated with the help of weighing balance at final harvest.

The dried ground plant material (0.1 g) was digested with sulphuric acid ($H_2SO_4$) and hydrogen peroxide $H_2O_2$ according to the method of Wolf (1982). The Ionic contents ($Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$) of the samples were determined by atomic absorption spectrophotometry. The $PO_4$ and $SO_4$ contents were estimated by colorimetric and turbidimetric methods, respectively.

The Chloride of the plant material (0. 1 g) was extracted in 10 ml distilled water at 80°C for 4 h. The $Cl^-$ content was analyzed by precipitation as AgCl and titrated according to Johnson and Ulrich (1959).

Data were subjected to analysis of variance, using Statistical Analysis System (SAS). Various treatment means were compared with Duncan's New Multiple Range (DMR) Test.

## RESULTS

Effect of varying concentrations of NaCl and $Na_2SO_4$ on biomass of two cowpea accessions is presented in Table 1. It was observed that depending on the increasing salt concentration, the fresh and dry weights of root and shoot of both accessions decrease from 0 to 150 mM (Table 1). Salt stress significantly reduced dry matter production. Total dry matter at the highest salinity (150 mM) was lower than the dry matter of plants grown in the control pots after 4 weeks of saline treatment. At the highest salinity treatment, TVu 11711 had greater dry weight than TVu 15245. Shoot dry weight was significantly reduced by salinity at $P<0.05$. Shoot dry weight at the highest salinity (150 mM) had the lowest values compared to non-stressed (0 mM) plants in both accessions (Table 1).

Salinity affected ions uptakes of the two cowpea accessions (Tables 2 and 3). shoot nutrient analysis of the two cowpea accessions indicated that $Na^+$, $Mg^{2+}$, $Na^+$, $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$ and $Na^+$: $K^+$ ratio significantly increased under saline condition ($p\leq0.05$) with increasing NaCl and Na2SO4 concentrations except K+, and Ca2+ (Tables 2 and 3). K+ uptake of salt stressed cowpea plants appeared to be differentially influenced by NaCl and

**Table 2.** Effect of varying concentrations of NaCl and $Na_2SO_4$ on inorganic ionic contents of leaves of two accessions of *V. unguiculata* (L.) Walp.

| Salt | Conc. (mM) | $K^+$ | | $Na^+$ | | $Ca^{2+}$ | | $Na^+$: $K^+$ | | $Mg^{2+}$ | | $PO_4^{3-}$ | | $SO_4^{2-}$ | | $Cl^-$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| NaCl | 0 | $1.00^a$ | $1.80^a$ | $1.99^c$ | $2.17^d$ | $6.26^a$ | $5.82^a$ | $2.06^c$ | $1.21^d$ | $0.95^a$ | $0.91^a$ | $1.93^d$ | $1.31^d$ | $2.00^d$ | $2.01^d$ | $1.69^d$ | $1.78^d$ |
| | 50 | $0.64^b$ | $0.84^b$ | $2.27^b$ | $3.07^c$ | $5.88^b$ | $5.29^b$ | $3.58^c$ | $3.69^c$ | $0.77^b$ | $0.88^a$ | $3.16^c$ | $3.02^c$ | $3.02^c$ | $2.64^c$ | $2.70^c$ | $2.995^c$ |
| | 100 | $0.42^c$ | $0.64^c$ | $2.41^b$ | $4.95^b$ | $5.30^c$ | $4.77^c$ | $4.28^b$ | $7.82^b$ | $0.75^b$ | $0.82^b$ | $4.29^b$ | $3.79^b$ | $3.84^b$ | $3.68^b$ | $3.35^b$ | $3.71^b$ |
| | 150 | $0.21^d$ | $0.44^d$ | $2.91^a$ | $6.11^a$ | $4.95^d$ | $4.33^d$ | $5.86^a$ | $3.97^a$ | $0.68^c$ | $0.79^b$ | $4.83^a$ | $4.18^a$ | $4.12^a$ | $4.13^a$ | $4.06^a$ | $4.18^a$ |
| $Na_2SO_4$ | 0 | $5.00^a$ | $2.00^a$ | $1.80^d$ | $1.99^d$ | $5.00^a$ | $4.72^a$ | $0.36^d$ | $0.99^d$ | $1.21^a$ | $0.92^a$ | $1.15^d$ | $1.86^a$ | $1.90^d$ | $1.83^a$ | $1.60^a$ | $1.62^d$ |
| | 50 | $1.55^b$ | $0.76^b$ | $2.01^c$ | $2.38^c$ | $4.15^b$ | $4.08^b$ | $1.31^c$ | $3.13^c$ | $1.13^a$ | $0.86^b$ | $1.60^c$ | $2.19^b$ | $2.08^c$ | $2.20^b$ | $2.63^b$ | $2.91^c$ |
| | 100 | $0.94^c$ | $0.61^b$ | $2.24^b$ | $2.85^b$ | $4.04^b$ | $3.36^c$ | $2.40^b$ | $4.71^b$ | $1.08^a$ | $0.83^c$ | $1.89^b$ | $2.86^c$ | $2.78^b$ | $2.91^c$ | $3.15^c$ | $3.84^b$ |
| | 150 | $0.74^c$ | $0.80^c$ | $2.60^a$ | $3.22^a$ | $3.76^c$ | $2.91^d$ | $3.55^a$ | $4.02^a$ | $0.89^b$ | $0.80^c$ | $0.19^a$ | $3.54^d$ | $3.10^a$ | $3.30^d$ | $3.91^a$ | $4.28^a$ |

Each value is a mean of three replicates. Values in the same column with the same letter(s) were not significantly different at P≥0.05 using Duncan's Multiple Range Test (DMRT). A, TVu 11711; B, TVu 15245

**Table 3.** Effect of varying concentrations of NaCl and $Na_2SO_4$ applications on inorganic ionic contents of stems of two accessions *V. unguiculata* (L.) Walp.

| Salt | Conc. (mM) | $K^+$ | | $Na^+$ | | $Ca^{2+}$ | | $Na^+$: $K^+$ | | $Mg^{2+}$ | | $PO_4^{3-}$ | | $SO_4^{2-}$ | | $Cl^-$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| NaCl | 0 | $0.81^a$ | $0.89^a$ | $1.04^d$ | $1.81^d$ | $4.48^a$ | $2.28^a$ | $1.29^c$ | $2.07^c$ | $0.83^a$ | $0.195^c$ | $1.41^d$ | $1.01^d$ | $1.60^d$ | $1.03^a$ | $1.50^d$ | $1.62^d$ |
| | 50 | $0.51^b$ | $0.62^b$ | $1.40^c$ | $2.08^c$ | $4.02^b$ | $1.30^b$ | $2.75^{bc}$ | $3.36^c$ | $0.80^a$ | $0.51^a$ | $2.20^c$ | $1.99^c$ | $1.91^c$ | $0.79^b$ | $2.60^c$ | $3.23^c$ |
| | 100 | $0.29^c$ | $0.58^b$ | $1.84^b$ | $3.83^b$ | $3.30^c$ | $0.93^c$ | $6.48^b$ | $6.73^b$ | $0.54^b$ | $0.35^b$ | $2.98^b$ | $2.98^b$ | $2.08^b$ | $0.63^c$ | $3.23^b$ | $4.67^b$ |
| | 150 | $0.15^d$ | $0.34^c$ | $2.14^a$ | $5.38^a$ | $3.02^d$ | $0.66^d$ | $15.43^a$ | $5.90^a$ | $0.39^c$ | $0.255^c$ | $3.84^a$ | $3.79^a$ | $2.48^a$ | $0.40^d$ | $4.01^a$ | $5.19^a$ |
| $Na_2SO_4$ | 0 | $2.85^a$ | $1.65^a$ | $1.11^d$ | $1.35^d$ | $3.35^a$ | $3.93^a$ | $0.39^c$ | $0.82^c$ | $0.61^a$ | $0.70^a$ | $0.89^d$ | $1.52^d$ | $1.33^d$ | $1.18^d$ | $1.27^d$ | $1.50^d$ |
| | 50 | $0.82^b$ | $0.56^c$ | $1.56^c$ | $2.13^c$ | $2.50^b$ | $3.08^b$ | $1.91^b$ | $3.81^b$ | $0.42^b$ | $0.55^b$ | $1.12^c$ | $1.70^c$ | $1.63^c$ | $1.49^c$ | $2.11^c$ | $2.07^c$ |
| | 100 | $0.83^b$ | $0.43^d$ | $1.81^b$ | $2.47^b$ | $2.09^b$ | $2.61^c$ | $2.18^b$ | $5.79^a$ | $0.26^c$ | $0.43^c$ | $1.28^b$ | $1.90^b$ | $1.89^b$ | $1.79^b$ | $3.18^b$ | $3.15^b$ |
| | 150 | $0.62^c$ | $0.73^b$ | $2.14^a$ | $3.11^a$ | $1.75^c$ | $2.33^d$ | $3.46^a$ | $4.31^b$ | $0.15^d$ | $0.31^d$ | $1.53^a$ | $2.07^a$ | $2.02^a$ | $1.99^a$ | $3.67^a$ | $4.05^a$ |

Each value is a mean of three replicates. Values in the same column with the same letter(s) were not significantly different at P≥0.05 using Duncan's Multiple Range Test (DMRT). A, TVu 11711; B, TVu 15245.

Na2SO4. NaCl application reduced the uptake of K+ in both leaf and stem. Similarly, the uptake of K+ content in the accessions treated with Na2SO4 was significantly reduced with increased in concentration. The amount of Na+ uptake increased markedly in response to increasing salt levels. Besides, increased amounts of Na+, the Na+/K+ ratio rose significantly by increasing salts concentrations in both accessions. Calcium uptake in leaf was also reduced by both salts in the accessions (Tables 2 and 3), to a great extent by Na2SO4, NaCl also followed the same trend but the rate of absorption was significantly lower than in Na2SO4 in the accessions with increased in concentrations. The uptake of magnesium was also affected by increasing concentration of salinity in the accessions in both NaCl and Na2SO4 (Tables 2 and 3). There was a

decreased in the content of magnesium from 0 - 150 mM of salt treatment and this decreased were reflected in the chlorophyll contents of both accessions in both treatments since magnesium is majorly responsible for chlorophyll formation in leaf. Leaf and stem phosphate and sulfate ion concentrations from control plants did not differ significantly among the different accessions, in both salt types (Tables 2 and 3). However, the uptake of SO42- and PO43- concentrations in the shoot from salt-stressed plants increased with increase in the salts concentrations from 0 - 150 mM of Na2SO4 and NaCl

## DISCUSSION

In the present study, salt stress caused a great reduction in fresh and dry weights of shoot and root of both cowpea accessions with the increase in saline contents. Reduction in plant growth as a result of salt stress had also been reported in several other plant species (Ashraf and O'leary, 1997).

The increase in $Na^+$ content and decrease in $K^+$ uptake disturbs ionic imbalance as observed in cowpea plant exposed to salt stress. The diminution of $K^+$ concentration in tissue may also be due to direct competition between $K^+$ and $Na^+$ at plasma membrane, inhibition of $Na^+$ on $K^+$ transport process in xylem tissues and/or $Na^+$ induced $K^+$ efflux from the roots. $K^+$ and $Ca^{2+}$ have been reported to be the major cautions in cell organization as well as the major contributors to osmotic adjustment under stress conditions in several plant species (Santos-Diaz and Alejo- Ochoa, 1994; Hirschi, 2004). In the present study, the level of $K^+$, $Mg^{2+}$ and $Ca^{2+}$ in the salt-stressed cowpea plants gradually decreased while that of $Na^+$ was dramatically increased. Increasing levels of NaCl induced a progressive absorption of Na and Cl in plant, agreeing with Taban et al. (1999) and Turan et al. (2007a, 2007b). Excessive $Na^+$ concentration in the plant tissue hinders nutrient balance, osmotic regulation and causes toxicity (Amdouni et al., 2014).

Accumulation of $Cl^-$ in the root tissue is disruptive to membrane uptake mechanisms, and these results in increased translocation of $Cl^-$ to the shoots (Turan et al., 2009). When NaCl was applied to the soil, the levels of K in plant were reduced in accordance with the antagonism between $Na^+$ and $K^+$ (Alberico and Cramer, 1993; Azevedo and Tabosa, 2000). Mansour (1997) showed that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions. The decrease in $K^+$ and $Ca^{2+}$ content under stress condition had been previously reported in other species particularly in the salt-sensitive lines (Lutts et al., 2004). According to Weimberg (1987) cited in Summart et al. (2010) high levels of $Na^+$ inhibit the $K^+$ uptake and as a result of this it causes an increase in the $Na^+/K^+$ ratio. According to Ahmed El Sayed (2011), $Na^+$ and $Ca^{2+}$ ions probably compete much more for common uptake sites. From the result obtained, it appears that $Na^+$ in combination with $SO_4^{2-}$ is more toxic than with $Cl^-$. Phosphate content was increased due to salt stress in both leaf and stem. The influence of salinity on phosphorus in phosphate uptake is controversial. A suppression of P uptake due to salt stress was been reported by Ahmed El Sayed (2011) whereas increased $PO_4$ content due to salt stress was reported by Garg et al. (2005). Indeed symptoms of $PO_4^-$ toxicity induced by salinity have been recognized by Nieman and Shannon (1976). According Ahmed El Sayed (2011) resistance to secondary salt induced stress in *Glycine falcata* Benth. was due to its ability to maintain a high P content in the presence of salt stress. It is possible that a high phosphate content in stem and leaf of salinized cowpea plant played similar role which corroborated with Silva et al. (2003) who reported an increase in leaf P concentration resulting from salt stress in mature and immature cowpea leaves. On the other hand, P accumulation in salt-stressed plants could be a consequence of reduced translocation associated with a decreased demand for growth.

Addition of NaCl and $Na_2SO_4$ had an adverse effect on the growth of cowpea plant. Salinity caused a significant effect on shoot fresh and dry weights. The reason for growth reduction in cowpea plant could be due to water shortage and ionic toxicity caused by salinity. Assessment of pattern of accumulation of toxic ions in a species is vital importance to understand, whether the species uses partial exclusion or inclusion mechanism for tolerating toxic ions present in its growth medium (Khalid et al., 2009).

## Conclusion

It is concluded that salinity had adverse effect on growth of cowpea plant. Growth was reduced with the increase in salinity levels. Its ionic contents also varied significantly under salt stress. So, salinity had adverse effects on plant life cycle. However, further research should be conducted in field in other to authenticate these findings.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Incidence and severity of potentially toxigenic *Aspergillus flavus* in maize (*Zea mays* L.) from different major maize growing regions of Uganda

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**Maize is grown in most regions of Uganda. Aflatoxin contamination of maize occurs in Uganda but there is lack of information on the distribution of *Aspergillus flavus* in major maize production regions of the country. The objective of this study was to determine the incidence and severity of *Aspergillus fla*vus in major maize growing regions of Uganda. Hierarchical sampling procedure was used to randomly collect maize samples from all different fields in 16 districts in two cropping seasons of 2013. Samples were assayed for *A. flavus* incidence and severity. Results revealed significant (P<0.001) variation among regions and districts within regions for *A. flavus* incidence and severity. The highest *A. flavus* incidence and severity were recorded for Pallisa (74.2% and 4.8, respectively), one of the leading maize producing districts in Uganda. Among regions, the highest *A. flavus* incidence and severity were registered in eastern region at 62.4% and 4.6 respectively. These results reveal the presence of *A. flavus* and provide information on its distribution in the key maize producing regions in Uganda. There is need for regular monitoring of aflatoxin levels in maize grain from the major maize production districts in Uganda in order to establish proper aflatoxin management guidelines.**

**Key words:** *Aspergillus flavus,* incidence, severity, Uganda, maize.

## INTRODUCTION

*Aspergillus flavus* is a saprophytic fungus that belongs to *Aspergillus* section *Flavi*. *A. flavus* produces a diverse array of secondary metabolites, among which the most important are mycotoxins. A type of mycotoxin produced

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by *A. flavus* that occurs frequently and is of agricultural and health significance is aflatoxin. Maize infection by *A. flavus* and subsequent aflatoxin accumulation, poses a serious threat to both human and animals because of aspergillosis and aflatoxicosis effects (Krishnan et al., 2009). *Aspergillus flavus* can invade several types of crops which include cereals, legumes, cotton, and nut trees as well as other crops either before or after harvest hence results into food and feed contamination due to aflatoxin accumulation (Cotty et al., 1994). Losses due to aflatoxin contamination are attributed directly to crop and livestock and from the cost of regulatory programs which are designed to moderate risks to human and animal health. Estimates by Food and Agricultural Organisation (FAO) indicate that up to 25% of the world's crops are affected by mycotoxins, with aflatoxins being the most notorious (Moreno and Kang, 1999, FAO, 2002). Aflatoxin contamination in feed for livestock and poultry may result into death, suppression of immune system, growth rates reduction, and losses in feed efficiency (Sisson, 1987, Kaaya and Warren, 2005). In crops, aflatoxin contamination results in lower yields for food and fiber crops (Simyung et al., 2013).

Maize is a major crop grown in most geographic regions of Uganda. In 2011, maize occupied 19% of the total land area under food crops in Uganda (Simyung et al., 2013). In 2011/12 the total maize production was 3,150,000 MT with total export of 787,000 MT totaling USD 46.9 million (MFPED, 2014). Marketing of maize in Uganda is hampered by contamination with different mycotoxins that reduce quality (Rodrigues and Naehrer, 2002). Uganda is characterized by diverse climate in the different agro ecologies, and this may result into variability among mycotoxin causing pathogens, including *A. flavus.*

In Uganda, aflatoxin contamination of maize has been reported (Kaaya and Warren, 2005, Simyung et al., 2013) but there is lack of information on the distribution of either *A. flavus* or other fungi of *Aspergillus* section *Flavi* across the major maize growing regions in Uganda. Comparisons of aflatoxin-producing potential among *Aspergillus* section *Flav*i communities from different maize growing regions is important for understanding population dynamics and suitable control measures for field reduction of pre-harvest aflatoxin contamination (Cotty, 1997; Horn and Dorner, 1999; Calvo et al., 1999). This study was conducted to establish the incidence and severity of *A. flavus* in all the major maize growing regions of Uganda.

## MATERIALS AND METHODS

### Survey and sampling strategy

Field survey were conducted to collect samples and determine the occurrences of the potential toxigenic strain of aspergillus flavus form maize in different maize growing districts in different regions of Uganda (Figure 1). The surveys were carried out during two cropping seasons: 2013A (March to July) and 2013B (August to December), which are characterized by longer and shorter rainy periods, respectively. Five districts in the western region (Hoima, Masindi, Kiryandongo, Kyenjojo, and Kabarole), four districts in the central region (Wakiso, Luwero, Mityana, and Mubende), two districts in the northern region (Lira and Oyam) and five districts in the eastern region (Iganga, Bugiri, Kumi, Pallisa, and Soroti) were surveyed. Districts were chosen to capture the different maize growing areas in Uganda with diverse cropping patterns and environmental conditions which have an influence on the occurrence of the fungus.

A three-level hierarchical sampling method was used to collect infected maize cob samples. This sampling method was used to facilitate capture of diversity of the fungus in different maize cobs by stratifying and focusing on collecting enough samples in fields within a districts and also representative districts with different regions. The samples were collected from different maize fields in each district within different regions in the country. Within each district 30 fields were selected. Within each fields a quadrant of 20*20 m was drawn and then on average 5 maize cobs were randomly sampled within the quadrant. All diseased maize cob samples were taken from all varieties in each farmer's fields in cases where farmers planted a mixture because of farmer's lack of knowledge of the varieties they cultivate. Climate data during the sampling period was accessed from the website (http://me.awhere.com/) (Table 1).

### Fungal infection assessment

Ears in a sample collected from each field were put in the paper bags to reduce the evaporation and also absorb all the moisture from the samples, then they were sun dried with in their respective paper bags (Figure 2) for 7 days and then shelled as a bulked. One hundred sun dried kernels from each sample from each location were assayed for fungal mold using direct plating technique for internal infestation (Zhang et al., 1997; Moreno and Kang, 1999). All the Kernels were surface sterilized for 1 min in 2.5% NaOCl, washed three times in sterile distilled water, and fifty kernels were plate Standard 90mm Petri Dish in two replicates on the surface of 1/4 strength Potato Dextrose Agar (PDA) containing 9.75 g/l Potato Dextrose Broth (Difco) and 20 g/l agar, amended with 2 ml/l lactic acid to suppress bacterial contamination. Plated kernels were incubated at 31°C for 3 days. All cultures that developed from the kernels were identified on the plates based macromorphological features which included; conidial and mycelial colour, colony diameter, reverse colony colour, presence of sclerotia (Klich, 2002). *Aspergillus* ear rot disease incidence and severity were assessed using percentage kernel infection method (Zhang et al., 1997). The number of infected kernels was counted and the percent kernel infection calculated Incidence of *A. flavus* in each sample was determined as:

$$\text{Incidence} = \frac{(\text{Number of kernels infected with } A.\,flavus) \times 100}{\text{Total number of kernels incubated}}$$

Severity of *A. flavus* infection in each sample was visually rated on a scale of 1-5 where 1 = 0-1%; 2 = 2-5%; 3 = 6-10%, 4 = 11-49%, and 5 = 50-100% (Corkidi et al., 2006).

### Statistical analysis

Incidence and severity data were subjected to analysis of variance using MINITAB version 14 (www.minitab.com.). The general linear model (GLM) option was used to ascertain the influence of region, district and field on incidence and severity of *A. fl*avus.

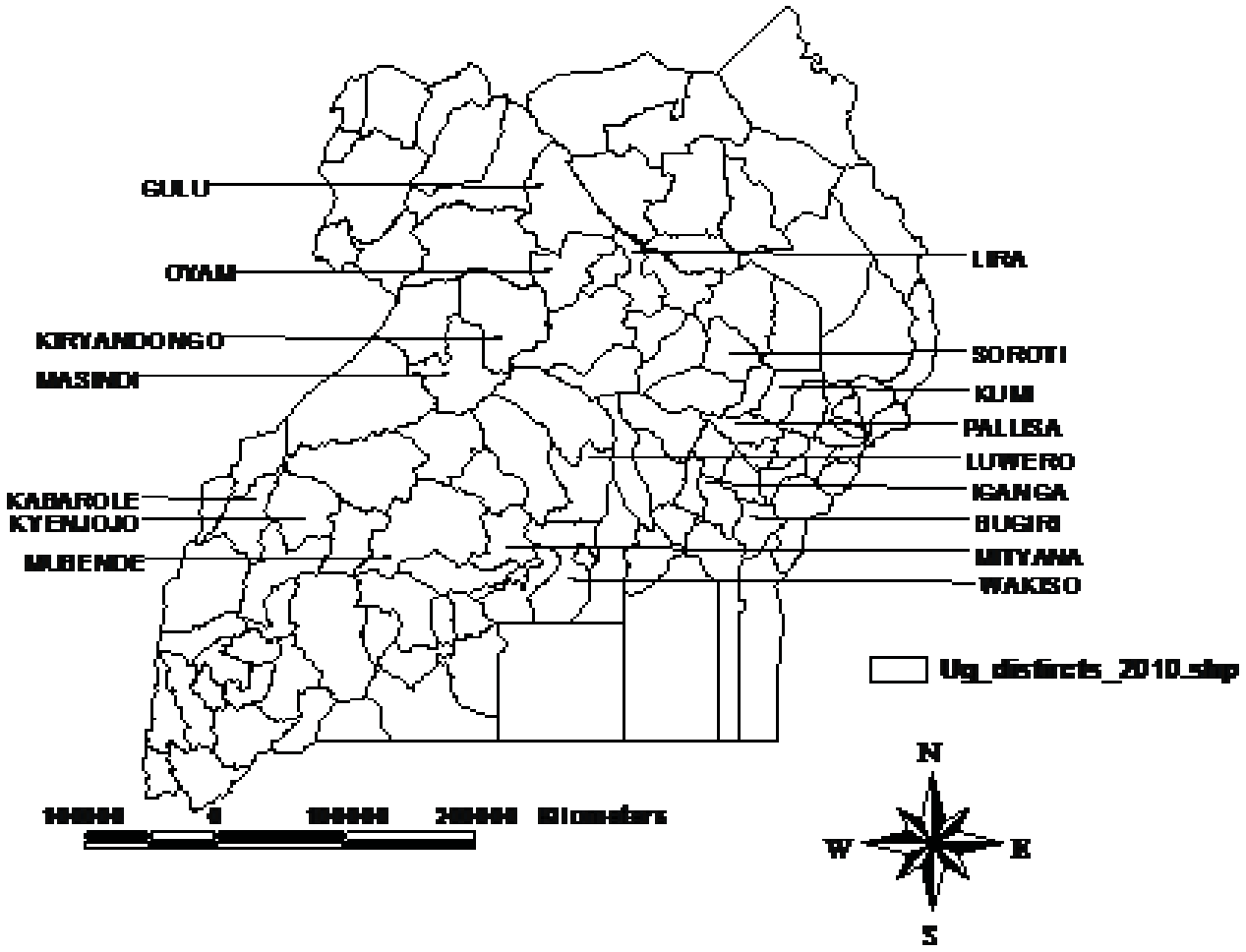


**Figure 1.** Map of Uganda showing sampled districts.

**Table 1.** Climatic conditions of districts in 2013 and number of samples collected per district.

| Districts | Annual precipitation (mm) | Temperature (°C) | | Relative humidity (%) | Number of samples collected |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Hoima | 608.0 | 19.6 | 27.7 | 71.4 | 15.0 |
| Masindi | 560.3 | 19.2 | 27.4 | 70.7 | 29.0 |
| Kabarole | 598.4 | 18.0 | 26.2 | 72.0 | 13.0 |
| Kyenjojo | 610.0 | 18.5 | 26.7 | 72.2 | 18.0 |
| Kiryandongo | 615.8 | 20.1 | 28.3 | 70.4 | 19.0 |
| Wakiso | 356.3 | 18.8 | 27.4 | 73.3 | 5.0 |
| Luwero | 484.8 | 19.4 | 27.9 | 73.1 | 17.0 |
| Mityana | 399.1 | 18.9 | 27.4 | 73.2 | 15.0 |
| Mubende | 804.6 | 18.2 | 26.6 | 72.5 | 18.0 |
| Lira | 687.6 | 20.0 | 28.6 | 68.6 | 23.0 |
| Oyam | 1120.6 | 20.3 | 28.7 | 68.6 | 14.0 |
| Iganga | 709.6 | 19.0 | 28.1 | 73.0 | 21.0 |
| Bugiri | 776.9 | 18.9 | 28.1 | 72.9 | 18.0 |
| Kumi | 527.5 | 19.2 | 28.4 | 70.2 | 13.0 |
| Pallisa | 535.7 | 19.1 | 28.3 | 71.2 | 12.0 |
| Soroti | 792.5 | 19.5 | 28.5 | 69.7 | 7.0 |

Source for weather data: http://me.awhere.com.


**Figure 2.** Sun drying of the field samples (A) and cultured maize grain on Potato Dextrose Agar (B).

**Table 2.** Effect of region, district, and field on *Aspergillus flavus* incidence and severity in maize samples from four regions in Uganda.

| Source | df | Incidence | | Severity | |
|---|---|---|---|---|---|
| | | Mean square | F value | Mean square | F value |
| Region | 3 | 7812.67 | 5.72*** | 20.09 | 18.83*** |
| District (Region) | 12 | 4168.58 | 3.05* | 7.23 | 6.78*** |
| Field (Region District) | 226 | 1107.79 | $0.81^{ns}$ | 1.92 | $1.80^{ns}$ |
| Error | 15 | 1364.87 | | 1.07 | |

*Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001.

## RESULTS

There was highly significant (P < 0.001) variation among regions for incidence and severity of *Aspergillus flavus* (Table 2). Incidence and severity of *Aspergillus flavus* differed significantly (P < 0.05 or P < 0.001) among districts within regions but not among fields with regions and districts. The mean incidence and severity of *A. flavus* in the districts were 48.3% and 3.9, respectively (Table 3). Among the districts, Pallisa had the highest incidence (74.2%) followed by Bugiri (73.9%) among the districts surveyed. These two districts also recorded some of the highest severity values of *A. flavus*. Kumi and Soroti districts had significantly lower incidence than both Pallisa and Bugiri that are in the same region (Table 3). Both Masindi and Hoima districts had significantly lower incidence than other districts in the same region. Wakiso district had the lowest incidence (7%) and least severity (2.0) of *A. flavus* among the districts in central region and overall. At the regional level, the highest *A. flavus* incidence (62.4%) was registered in the eastern region (Table 4). The eastern region had the two districts with the highest incidence. The northern region registered the lowest incidence (28.9%) but this did not differ significantly from the incidence recorded for the western region. Severity of *A. flavus* infection was highest in the eastern region (4.6) and lowest in the northern region (3.0).

## DISCUSSION

This study was conducted in the major maize growing regions and samples analyzed revealed occurrence of *A. flavus*. *A. flavus* was identified in all the samples collected in different districts of Uganda. These results indicate that *A. flavus* is widely distributed in the country and that most of the commonly grown maize varieties are susceptible to fungal spoilage and aflatoxin contamination. These results corroborate earlier reports that aflatoxin accumulation in different food and feed commodities are a major threat to not only maize production in the country but also to both human and animal health (Kaaya and Warren, 2005). In a study by Simyung et al. (2013), about 11% of samples collected from different districts of Uganda were contaminated with aflatoxin that ranged from 12.7 to 123.5 µg/Kg. In addition, 9% of samples exceeded the range of 4 µg/kg to

**Table 3.** *Aspergillus flavus* incidence and severity in maize samples from 16 districts of Uganda.

| Districts | Incidence (%) | Severity (1-5) |
|---|---|---|
| Hoima | 31.3 | 3.2 |
| Masindi | 16.7 | 2.4 |
| Kabarole | 60.0 | 4.2 |
| Kyenjojo | 58.1 | 4.4 |
| Kiryandongo | 63.9 | 4.5 |
| Wakiso | 7.0 | 2.0 |
| Luwero | 62.9 | 4.2 |
| Mityana | 55.7 | 3.6 |
| Mubende | 59.7 | 4.1 |
| Lira | 28.3 | 2.9 |
| Oyam | 30.0 | 3.3 |
| Iganga | 61.4 | 4.6 |
| Bugiri | 73.9 | 4.6 |
| Kumi | 48.5 | 4.7 |
| Pallisa | 74.2 | 4.8 |
| Soroti | 41.4 | 4.8 |
| **Mean** | 48.3 | 3.9 |
| **Standard error of difference (SED)** | 20.2 | 0.9 |

10 or 15 µg/kg as per the new European Union aflatoxin maximum level guidelines (EU, 2010). Because we could not obtain the names of the varieties planted by the farmers, it was not possible to ascertain whether high disease incidence was related to adoption of elite maize varieties or the use of older maize varieties in the study areas. The variability of both incidence and severity of *A. flavus* observed among district and regions may be attributed to the different weather patterns prevailing during the sampling periods.

During the study period, annual rainfall ranged from 356.3 to 1120 mm (Table 1) which was low compared to the normal rainfall amounts characteristic of Uganda which range from 1000 to 1400 mm, but the mean temperature was as high as 28°C with average relative humidity of up to 73.2%. Environment conditions characterized with high temperatures and drought stress have an impact on the physiology of both the host and fungus, with high temperature which favour fungal growth may affect the infected plant. In addition, there are compounds present within the kernel that are induced when infected by *Aspergillus* spp. which affect its development hence the low severity (Chanda et al., 2009, Atehnkeng et al., 2008). Studies have demonstrated that kernel pericarp wax has been associated with resistance to *Aspergillus* infection and inhibited growth (Gembeh et al., 2001; Brown et al., 2013). In addition Gas chromatography/mass spectroscopy (GC/MS) analysis of the whole was component showed a higher percentage of phenol-like compounds in the resistant genotypes than in the susceptible lines (Gembeh et al., 2001). Another study which examined kernel proteins revealed differences between genotypes resistant and susceptible to aflatoxins contaminations (Huang et al., 1997)

Earlier studies have indicated that *A. flavus* in maize may differed between the different agro-ecological zones due to the prevailing climatic conditions (Cotty and Jaime-Garcia, 2007), the cultivars grown in each zone, the cultural practices and / or the storage methods (Sétamou et al., 1997). Land management strategies and, particularly, crop rotation systems and factors such as genotype may influence crop infestation by Aspergillus section Flavi and the aflatoxin content of maize.

Although rainfall received was low but temperature were high during the seasons when this study was carried out (Table 1), it created an environment characteristic of warm and humid. Such conditions are conducive for *Aspergillus* growth. Similar results were reported for maize in the USA (Anderson et al., 1975) but are contrary to results by Sisson (1987) which revealed that minimum temperature above 21°C was negatively correlated with aflatoxin incidence in maize in the USA. *Aspergillus* strains have been reported to survive in a wider range of temperature from 19 to 35°C (Northolt and Van Egmond, 1981), although 28°C is the most conducive for aflatoxin production (Sanchis and Magan, 2004; Simyung et al., 2013). The mean temperature in the districts covered by the study was 28°C for most of the districts, and this probably enhanced the development of *A. flavus.* This is exemplified by the high incidence and severity in Pallisa district which experienced moderate rainfall, high temperatures (28°C) and high relative humidity (71%) (Table 1).

In addition to temperature, availability of moisture has a

significant impact on *A. flavus* growth and aflatoxin production. This combination of susceptible genotypes and good weather (warm-humid) supports high pathogen severity and incidence. Besides the climatic factors, the high incidence of *A. flavus* in samples from Pallisa, Bugiri, Kiryandongo, and Iganga, could be explained by the common farming practice of leaving maize in the field for more than three weeks after physiological maturity, which leads to increased incidence of *A. flavus* (Kaaya et al., 2005).

These results provide for the first time, key evidence about the presence and distribution of *A. flavus* in the key maize producing regions in Uganda. The implication of these results is that maize grain from these districts may place consumers at risk of exposure to aflatoxin. It is necessary to establish the levels of aflatoxin contamination in maize grain from farmers' fields and maize grain dealers in these districts and correlate these with the incidence and severity of *A. flavus.* This information will be useful for the formulation of guidelines on aflatoxin management in Uganda. From the earlier studies (Gardner et al., 1987; Campbell and White, 1995), combined use of management control strategies should be considered to reduce *Aspergillus* infection and hence aflatoxin control.

Incidences of over 50% in samples from central and eastern regions suggest that *A. flavus* occurrence is widespread in some of the important maize production regions in Uganda. Our study demonstrates presence of *A. flavus*, and these are useful resources for identifying and developing biological control technology to manage aflatoxins (Atehnkeng et al., 2008). However, more research is required to evaluate these atoxigenic strains for identifying a few effective and adapted atoxigenic vegetative compatibility groups that can be pursued in developing biological control technology in Uganda. Also further work is needed to understand the relationship between pathogen occurrence and aflatoxin levels along the maize supply chain.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Initial growth of eucalyptus plants treated with gibberellin

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**This study was aimed at studying the effect of gibberellin ($GA_3$) on the growth of Eucalyptus plants. The experiment was conducted with 90-day-old seedlings on full sun bench at Goiás State University, Brazil, following the completely randomized design in a 5 × 2 factorial arrangement (five *eucalyptus* samples and two treatments with gibberellins). The application of gibberellin to eucalyptus is a promising practice, as it significantly affects the vegetative growth and increases the stem biomass accumulation.**

**Key words:** Hormone, silviculture, early growth.

## INTRODUCTION

The ongoing increase in economic value and the scarcity of hardwoods have boosted the diversified use of eucalyptus (Souza et al., 2012). The productivity of plantations in Brazilian territory is superior to that of traditional countries such as Australia (center of the species origin), and its current situation provides opportunities for the consolidation of Brazil as one of the major powers in the world's forest-based industry. The forestry sector accounts for 3.5% of the country's Gross Domestic Product (GDP) and generates 4.7 million direct and indirect jobs (Abraf, 2013).

Notwithstanding Brazil's high potential in the forestry sector, the country can further expand production and transfer wealth to other segments of economy. And in order to remain competitive, carrying on this process in a consistent and sustainable manner, it requires the development of research aimed to increase productivity, which will open new markets, increase exports and simultaneously modernize and ensure high social and environmental standards to forestry activities (Ferreira et al., 2012).

Reducing the cutting age and increasing the biomass accumulation of eucalyptus forests will contribute to the sector development and to the growth of Brazil's participation in the world market. However, the production of trees suitable for short-time harvesting will certainly depend on physiological changes in the species and/or adoption of new management techniques.

The use of plant growth regulators in agriculture has been an important management technique, as it has

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increased the productivity of several species, still its application is not yet a common practice in cultures that have not reached high technological level.

There is great interest in understanding the interference of plant growth regulators in lignification, wood density, early growth and yield (Pereira et al., 2011), since their performance does not depend solely on chemical composition, but also on how they are "perceived" by target tissues. In other words, the same substance can cause different effects depending on the tissue or organ upon which it is acting, on hormone concentration, and on the time of development of the same tissue (Wei et al., 2012). The use of growth regulators to improve wood quality, induce flowering, slow down or accelerate the vegetative growth of several species has been a constant issue in scientific research (Doorn et al., 2011; Kiba and Sakakibara, 2010; Xiong et al., 2009; Pereira et al., 2011).

The current world scenario is characterized by increasing environmental pressure towards the exploitation of renewable energy sources rather than fossil fuels. And in this direction, scientific understanding of how physiological aspects guide the growth of the species will contribute to greater production of early growth plants. However, little is known about the biochemical action and physiology of growth regulators in eucalyptus. Therefore, the present study was designed to investigate the effect of gibberellin ($GA_3$) on eucalyptus plant growth, with a view to reducing the cutting age and accelerating biomass accumulation.

## MATERIALS AND METHODS

### Experimental design

The work was accomplished at Goiás State University, Ipameri Unit (17º43'19"S, 48º09'35"W, Alt. 773 m), located in the municipality of Ipameri, Goiás, a region with predominant Aw climate according to the Köppen classification. The experiment was carried out on a bench exposed to full sun, following the completely randomized design in a 5 × 2 factorial arrangement (five eucalyptus samples, including three clones from the crossing of *Eucalyptus grandis* x *Eucalyptus urophylla* "*Eucalyptus urograndis* GG100", "*Eucalyptus urograndis* H13" and "*Eucalyptus urograndis* Super Clone"; one clone derived from the crossing between *Eucalyptus urophylla* x *Eucalyptus camaldulensis* "*Eucalyptus urocan* 58", and one sample from seeds of *Eucalyptus citriodora* x two treatments with 100 and 0 mg $L^{-1}$ of gibberellins) and three replications. The eucalyptus seedlings were irrigated daily to maintain soil moisture near field capacity. At 90 days after germination the seedlings were transplanted and cultivated in six-liter pots with soil, sand and manure at 3:1:0.5 proportion, respectively. After substrate analysis, pH correction to 6.0 and fertilization were carried out. The foliar applications of $GA_3$ at 100 mg $L^{-1}$ concentration and 50 ml per plant were accomplished on April 28 and 30 and May 28 and 30, 2012 at 100, 101, 129 and 130 days after germination. The plants that did not receive gibberellin were sprayed with deionized water in similar amount to that used for the regulator application. Maximum uniformity was sought in the application of gibberellin via spraying on all leaf area using a metering valve attached to a backpack sprayer. At 160 days after emergence the plants were analyzed.

### Growth variables

The number of leaves and the stem length and diameter were measured between 8 and 10 am using a graduated ruler and calipers. The leaves, roots and stems were removed and put to dry in an oven at 72°C until steady dry mass state, and then weighed separately. Based on the dry mass data, the leaf mass ratio (LMR), root mass ratio (RMR), stem mass ratio (SMR), shoot/root system ratio (S/RS) and total biomass (TB) were calculated.

Fully expanded leaves were used to obtain the specific leaf area (SLA) and the leaf area (LA). Six discs with 14-mm diameter and known area were collected from each fully expanded leaf and dried at 70°C for 72 h, after which mass and SLA were measured. The LA was obtained by measuring the length and diameter.

### Photosynthetic pigments

In order to determine the total chlorophyll and carotenoid concentration values, leaf discs with known area were removed and placed in glass containing dimethyl sulfoxide (DMSO). Subsequently, extraction was accomplished in water bath at 65°C for one hour. Aliquots were extracted for spectrophotometric reading at 480, 649.1 and 665 nm. The contents of chlorophyll a (Cl *a*), chlorophyll b (Cl *b*) and carotenoids were determined according to the equation proposed by Wellburn (1994).

### Statistical procedures

The experiment was set up following the completely randomized design in a 5 × 2 factorial arrangement with three replications, and the data was submitted to variance analysis and Newman Keuls test for multiple comparisons of treatment averages using SISVAR 5.3 software (Ferreira, 2011).

## RESULTS

Statistical analysis showed significant differences between treatments and samples for all variables (Tables 1 and 2). Only for the reason of shoot/root system (S/RS) and because of the root mass was no interaction treatment x samples (Tables 3 and 4). The average leaf number values varied significantly among the samples, where the *Eucalyptus citriodora* species had the lowest number (55.16) compared to that of the other samples, which were similar. However, the *Eucalyptus urocan* 58 species presented a greater number of leaves (138.50) compared to the others.

There was statistical difference in the number of branches, where the *E. citriodora* sample had a smaller number (5.16) compared to the others, which had similar amounts. However, the *Eucalyptus urograndis* GG100 sample stood out (14.00) among the others, presenting the greatest numerical amount of branches. Regarding the plant height, all samples showed high growth, highlighting the *E. urograndis* GG100 hybrid, which differed significantly from the others, with 113.83-cm height. The samples treated with $GA_3$ were statistically superior in height (104.46) compared to those not treated with gibberellin (Table 1).

The samples did not show any significant differences in

**Table 1.** Summary of the variance analysis and mean square test for number of leaves, branches, plant height, stem diameter (Ø), leaf area (LA) and specific leaf area (SLA) of different eucalyptus samples treated with $GA_3$.

| Source of variation | DOF | Mean square | | | | | |
|---|---|---|---|---|---|---|---|
| | | No. of leaves | No. of branches | Height cm) | Ø (mm) | LA ($cm^2$) | SLA ($m^2kg^{-1}$) |
| Sample | 4 | 6335* | 70* | 1879* | 3.3 | 465* | 53 |
| Treatment | 1 | 710 | 0.30 | 13525* | 21* | 0.96 | 147 |
| Sample treatment* | 4 | 555 | 13 | 222 | 0.46 | 44 | 20 |
| Error | 20 | 551 | 6.5 | 104 | 1.2 | 101 | 47 |
| CV (%) | | 20 | 23 | 12 | 14 | 33 | 27 |
| **Treatment** | | | | **Averages** | | | |
| Without $GA_3$ | | $107.3^A$ | $11.00^A$ | $62.0^B$ | $6.8^B$ | $30.3^A$ | $23.2^A$ |
| With $GA_3$ | | $107.1^A$ | $11.2^A$ | $104.5^A$ | $8.5^A$ | $30.6^A$ | $27.6^A$ |
| **Sample** | | | | **Averages** | | | |
| *E.citriodora* | | $55.2^B$ | $5.2^B$ | $68.3^B$ | $6.6^A$ | $38.9^A$ | $22.2^A$ |
| *E.urograndis* GG100 | | $116.0^A$ | $14.0^A$ | $113.8^A$ | $7.5^A$ | $24.2^{AB}$ | $25.5^A$ |
| *E.urocan* 58 | | $138.5^A$ | $12.3^A$ | $75.6^B$ | $7.4^A$ | $20.3^B$ | $27.4^A$ |
| *E.urograndis* H13 | | $129.6^A$ | $12.3^A$ | $80.6^B$ | $8.3^A$ | $28.6^{AB}$ | $29.3^A$ |
| *E.urograndis* super clone | | $127.6^A$ | $11.6^A$ | $77.6^B$ | $8.5^A$ | $40.1^A$ | $29.3^A$ |

Values represent the arithmetic average. Averages followed by the same letter within each column do not differ among themselves, at 5% probability by Newman Keuls test. * = Significant.

**Table 2.** Summary of variance analysis and average test for shoot/root system ratio (S/RS), total biomass (TB), leaf mass ratio (LMR), stem (SMR) and root (RMR), leaf carotenoid concentration (Car) and total chlorophyll (Chl *a*+*b*) of different *eucalyptus* samples treated with $GA_3$.

| Source of variation | DOF | Mean square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | S/RS | TB (g) | LMR | SMR | RMR | Car ($g\ kg^{-1}$) | Chl *a*+*b* ($g\ kg^{-1}$) |
| Sample | 4 | 1.6 | 141* | 0.004* | 0.006 | 0.007* | 0.34* | 1.4 |
| Treatment | 1 | 0.05 | 51 | 0.15* | 0.14* | 0.0007 | 0.08 | 2.0 |
| Sample treatment* | 4 | 3.2* | 19 | 0.002 | 0.006 | 0.009* | 0.17 | 0.5 |
| Error | 20 | 0.95 | 39 | 0.0008 | 0.0012 | 0.002 | 0.12 | 1.1 |
| CV (%) | | 28 | 26 | 7 | 10 | 17 | 20 | 14 |
| **Treatments** | | | | | **Averages** | | | |
| Without $GA_3$ | | $3.4^A$ | $22.0^A$ | $0.48^A$ | $0.28^B$ | $0.23^A$ | $1.57^A$ | $7.53^A$ |
| With $GA_3$ | | $3.5^A$ | $24.6^A$ | $0.34^A$ | $0.41^A$ | $0.24^A$ | $1.68^A$ | $7.02^A$ |
| **Sample** | | | | | **Averages** | | | |
| *E. citriodora* | | $3.9^A$ | $15.0^A$ | $0.45^A$ | $0.34^B$ | $0.21^B$ | $1.4^B$ | $7.6^A$ |
| *E. urograndis* GG100 | | $3.9^A$ | $25.8^A$ | $0.39^B$ | $0.40^A$ | $0.21^B$ | $2.0^A$ | $7.6^A$ |
| *E. urocan* 58 | | $2.9^A$ | $23.0^A$ | $0.39^B$ | $0.32^B$ | $0.29^A$ | $1.6^{AB}$ | $7.6^A$ |
| *E. urograndis* H13 | | $3.3^A$ | $26.9^A$ | $0.41^B$ | $0.36^B$ | $0.23^{AB}$ | $1.7^{AB}$ | $6.6^A$ |
| *E. urograndis* Super Clone | | $2.9^A$ | $25.7^A$ | $0.41^B$ | $0.33^B$ | $0.26^{AB}$ | $1.4^{AB}$ | $6.9^A$ |

Values represent the arithmetic average. Averages followed by the same letter within each column do not differ among themselves at 5% probability by Newman Keuls test. * Significant.

the stem diameter (Table 1), however, there was a numerical variation of 1.82 mm between the *E. urograndis* Super Clone and the *E. citriodora* species samples. Regardless of the sample, the plants treated with $GA_3$ showed longer diameter (8.51 mm), with 1.68-mm increase compared to untreated plants (6.83 mm). The leaf area varied among the different samples, highlighting larger leaf area in *E. urograndis* Super Clone (40.16) and smaller leaf area in *E. citriodora* (20.37). However, the treated and untreated plants had almost the

**Table 3.** Breaking down of S/RS of different eucalyptus samples treated with $GA_3$.

| Sample | Without $GA_3$ spraying | With $GA_3$ spraying |
|---|---|---|
| *E. citriodora* | $3.30^{aA}$ | $4.67^{aA}$ |
| *E. urograndis* GG100 | $3.30^{aA}$ | $4.54^{aA}$ |
| *E. urocan* 58 | $4.01^{aA}$ | $1.81^{bB}$ |
| *E. urograndis* H13 | $3.46^{aA}$ | $3.17^{abA}$ |
| *E. urograndis* Super Clone | $2.79^{aA}$ | $3.08^{abA}$ |
| CV (%) | 28.51 | |

Different lowercase letters indicate significant differences between the samples; different capital letters indicate significant differences between treatments in each sample by Newman Keuls test at 5% significance level.

**Table 4.** Breaking down of RMR of different eucalyptus samples treated with $GA_3$.

| Sample | Without $GA_3$ spraying | With $GA_3$ spraying |
|---|---|---|
| *E. citriodora* | $0.230^{aA}$ | $0.183^{aB}$ |
| *E. urograndis* GG100 | $0.216^{bA}$ | $0.356^{aA}$ |
| *E. urocan* 58 | $0.233^{aA}$ | $0.190^{aB}$ |
| *E. urograndis* H13 | $0.223^{aA}$ | $0.240^{aB}$ |
| *E. urograndis* Super Clone | $0.266^{aA}$ | $0.250^{aB}$ |
| CV (%) | 17.55 | |

same leaf area, and they did not differ statistically. Regarding the specific leaf area there was no difference between the samples and treatments with $GA_3$ (Table 1).

The variance analysis and average test show interaction between the shoot/root system ratio (S/RS) factors, as shown in Table 2. In numerical values, the highest ratio was obtained in *Eucalyptus citriodora* (4.67) treated with $GA_3$, and the lowest ratio was recorded in *Eucalyptus urocan* 58 (1.81), and even with this difference in average values, there was no statistical difference between samples. However, *Eucalyptus urocan* 58 had the lowest values among the samples treated with $GA_3$.

The total biomass of samples differed statistically, and *E. citriodora* had the lowest total biomass (15.03). The leaf mass ratio was higher in plants not sprayed with $GA_3$, however, the plants treated with $GA_3$ showed higher stem mass ratio, with *E. urograndis* GG100 showing the highest numerical value. The root mass ratio showed slight variation between samples, with *Eucalyptus urocan* 58 presenting the highest numerical value in relation to the other samples, but without statistical difference between plants treated with $GA_3$ and untreated ones.

The samples differed statistically in carotenoid concentration, where the *E. urograndis*. GG100 sample presented higher average (1.975) than the others. There was no significant difference between samples treated with g $GA_3$ and untreated ones.

When the sample treatment results are broken down, it is noted that regardless of the eucalyptus sample, the highest numerical values were obtained in plants treated with $GA_3$ (Table 4).

Different lowercase letters indicate significant differences between the samples; different capital letters indicate significant differences between treatments in each sample by Newman Keuls test at 5% significance.

## DISCUSSION

Physiological analysis of the plants at an early stage helps to identify promising species with high productivity potential and contributes to the choice for suitable materials for the prevailing conditions (Peixoto et al., 2006). Originally the hybrid clones and *E. citriodora* showed marked differences in vegetative growth. The variations recorded in the samples growth and development are determined by genetic characteristics, however, the $GA_3$ applications intensified the vegetative growth and promoted significant changes in the allocation of assimilates and biomass partition, as discussed below.

The application of $GA_3$ did not cause significant changes in the vegetative growth of the studied samples. The similarity in the number of leaves, branches, leaf area, specific leaf area, biomass, leaf and root mass ratio, chlorophyll and total carotenoids between plants treated with $GA_3$ and untreated ones indicate that the time between regulator application and evaluations was short for vegetative growth and changes in anatomical variables, such as specific leaf area. Despite the lack of statistical difference, the trend of specific leaf area increase in plants treated with $GA_3$ is possibly related to higher leaf transmittance of solar radiation in the canopy. Increased leaf transmittance allows greater solar radiation distribution in the canopy, providing light energy to lower leaves, contributing to increased photosynthetic activity and biomass accumulation over time. Increases in the specific leaf area, and reductions in leaf thickness decrease absorption and increase leaf transmittance, changing the solar radiation distribution pattern in the canopy (Borges et al., 2014). Greater biomass in plants treated with $GA_3$, even with no statistical difference, is an indication that a longer time interval between application and evaluation is necessary to record any difference.

The notable changes in vegetative growth are due to the differences among the samples used. *E. urograndis* Super Clone stood out among the samples for presenting high number of leaves, large leaf area and specific leaf area, and great biomass. However, *E. urograndis* GG100 was the sample with the most desirable features for forestry due to its higher biomass distribution to the stem resulting in greater height and large stem diameter. Larger stem diameter in *E. urograndis* GG100 clones has

been observed in experiments and appears to be a significant variable of the sample (Pinto et al., 2011).

Greater plant height, stem diameter and stem mass ratio in plants treated with $GA_3$ indicate that the growth regulator caused changes in biomass distribution with greater assimilates allocation to the stem to the detriment of the root system and mainly of the leaves. The high stem growth in height and diameter is of great interest to forestry as it directly affects the eucalyptus early growth and wood quality and productivity. The application of $GA_3$ changes the vegetative growth, intensifying the development of shoots (Sponsel, 2006). The absence of difference in photosynthetic pigment concentration and leaf area is evidence that there was no change in the photosynthetic mechanism to maximize photosynthesis, the existing differences being related to changes in biomass distribution. These changes may be associated to the fact that hormones generally act upon cellular communication, since they are defined as “chemical messengers produced in a cell or tissue that modulate cellular processes in other cells, by interacting with specific proteins called receptors”, contributing to metabolic processes that allow the maintenance and growth of primary organs (Taiz and Zeiger, 2013).

Higher S/RS ratio in plants treated with gibberellin is because they allocate greater biomass to the stem, thus increasing the shoot mass. Lower RMR in treated plants is due to the same fact, as larger investment in the shoot took place at the expense of smaller biomass distribution to the root. Greater translocation of assimilates to the shoot can be beneficial under adequate soil fertility conditions because of higher stem and leaf development.

Biomass production is determined by the species genetic characteristics (Santana et al., 2002). The remarkable differences in vegetative growth indicate that the species have genetic variability. Regardless of the treatment with $GA_3$, the *E. citriodora* species showed less vigorous vegetative growth than the hybrids. The *E. urograndis* GG100 clone showed high biomass distribution to the stem, proving to be a promising material for future studies. The application of $GA_3$ in eucalyptus plants is a promising practice, as it significantly affects vegetative growth and enhances stem biomass accumulation (the element of economic interest).

## Conclusions

1. The application of $GA_3$ in *Eucalyptus* seedlings accelerates vegetative growth, changes biomass distribution and possibly interferes with the early growth and wood productivity.
*2. E. citriodora* was the sample with the least vigorous vegetative growth.
3. The *E. urograndis* GG 100 hybrid clone stood out from the others for showing the desirable features for forestry, such as greater stem mass ratio, height and stem diameter.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Short Communication*

# Electrolytic leakage as a tool to assess seedling health in pine

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**Electrolytic leakage in infected Blue pine (*Pinus wallichiana*) needles was monitored by measuring conductivity change in pine needles affected by Lophodermium needle blight disease. Electrolytic leakage in infected Blue pine needles varied significantly between severity categories. Highest electrolytic leakage of 345.33 µmoh/cm was noticed in pine needles having > 81% disease severity in comparison to unaffected check (181.33 µmoh/cm) indicating a positive relationship between disease severity and electrolytic leakage. The evaluation of electrolyte leakage appears to be rapid and accurate method for assessing the hardwood seedling health and physiological status.**

**Key words:** Electrolytic leakage, blue pine, needle blight.

## INTRODUCTION

The assessment of seedling health is an essential component of any reforestation or afforestation programme. The use of low quality seedlings may often result in decline in plant growth and establishment (Sampson et al., 1996). Therefore, it is imperative to identify the quality stocks showing vigorous growth attribute. Healthy vigorous seedlings grow at increasingly faster rates than the seedlings of low vigour (Burdett and Brand, 1990) and the plants raised from high quality saplings require less maintenance as well as exhibit more resistance to insects and diseases. Seedling vigour is associated with physiological activity of healthy tissue (Sampson et al., 1996).

Therefore, there is need for efficient practical methods to monitor the changes in physiological status of seedlings due to diseases. Electrolyte leakage determines the loss of electrolyte due to some interference in plant physiology and has successfully been employed in phyllosphere of sorghum for assessing the extent of disease infection (Balasubramanian, 1973) and for hardiness determine in conifers (Colombo et al., 1995; Bigras, 1997). Conifers are hardwood plants which often express disease symptoms very late rendering the disease management difficult. Therefore, the assessment of electrolyte leakage may help in providing some preliminary idea about pathpogenic interference even though the pathogen is latent. In the present communication, an attempt has been made to explore the possibility of using electrolyte leakage as a tool to assess disease through establishing a relationship between

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**Table 1.** Electrolytic leakage of Blue pine needles exhibiting blight severity of different categories.

| Needle blight severity categories | Electrolytic leakage (µmoh/cm) |
|---|---|
| Needles without any infection | 181.33 (13.46) |
| Needles with < 20% disease | 226.67 (15.05) |
| Needles with 21 – 40% disease | 253.00 (15.90) |
| Needles with 41 - 60% disease | 286.00 (16.91) |
| Needles with 61 - 80% disease | 302.00 (17.37) |
| Needles with > 81% disease | 345.33 (18.58) |
| CD (P = 0.05) | 0.32 |

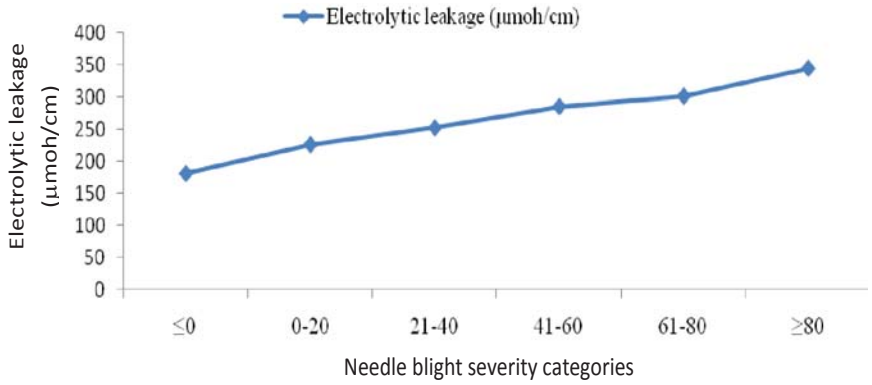


**Figure 1.** Effect of needle blight disease severity on electrolytic leakage.

needlecast disease severity and electrolyte loss.

## MATERIALS AND METHODS

The infected Blue pine (*Pinus wallichiana*) needles bearing needlecast symptoms of varying intensity category were collected from Forest Nursery, SKUAST-K, Shalimar, Srinagar (J&K) during September-October, 2010. Six of 10 year old Blue pine plants were randomly selected and tagged for scoring of the diseased needles under natural epiphytotic conditions. Scoring was done as per the 0-4 point scale given by Skilling and Nicholls (1974) wherein 0 = needles/whorls showing no disease symptoms, 1 = needles/whorls affected with 1 - 25% infection; 2 = needles/whorls affected with 26 - 50% infection; 3 = needles/whorls affected with 51 - 75% infection, and 4 = needles/whorls affected with 76 - 100% infection. The percent disease intensity was calculated as per the formula:

$$\text{Percent disease intensity} = \frac{\sum (n \times v)}{N \times 4} \times 100$$

Where, Σ = Summation; n = number of diseased needles/whorls in each category, v = numerical value of each category; N = number of needles/whorls examined, and 4 = maximum grade value. The loss of electrolytes due to disease was estimated by measuring the conductivity of leachates from the diseased pine needles as per Wheeler and Hanchey (1968). The infected needles were thoroughly washed with tap water, rinsed three times with distilled water and finally washed with deionized water. The needle sample (1 g each) was blotted dry and suspended in sterile double distilled water in 10 ml water. The suspended material was incubated at 25 ± 1°C for 24 h. The conductivity of bathing solution was measured with the help of a conductivity bridge having platinum electrodes PICO pH meter (Labindia Ltd.). The temperature of bathing solution during conductivity measurement was maintained at 25 ± 1°C. The results were expressed as specific conductivity (µmoh/cm) of the leachetes.

## RESULTS AND DISCUSSION

Electrolytic leakage in infected Blue pine needles was monitored by measuring the changes in the conductivity of needles affected by varied needle blight disease intensities. All the severity classes significantly varied in their electrolytic leakages (µmoh/cm). The highest electrolytic leakage of 345.33 µmoh/cm was noticed in pine needles having > 81% disease severity in comparison to control (181.33 µmoh/cm) (Table 1 and Figure 1). The electrolytic leakage in plants has previously been studied with respect to stress tolerance (McKay and White, 1997), cold hardiness (Burr et al., 1990) and dormancy status (Wilson and Jacobs, 2004), all of which are interrelated and depict cell damage due to loss of cell membrane integrity. The present study is first of its kind in conifer needles as well as with respect to forest diseases.

Grossnickle (2005) studied electrolytic leakages in

conifer roots for determining the physiological stress. Further, they suggested that root electrolytic leakage procedures could forecast overall seedling performance over a range of root damages and reported that root electrolytes are species-specific. The loss of electrolytic leakage observed in disease affected needles in the present study may be attributed to the membrane damage due to necrosis and higher absorption of electrolytes by diseased plants. Our findings are in corroboration with Campos et al. (2003) who reported that electrolytic leakage in plant membranes is often associated with increases in permeability and loss of integrity. Wheeler and Hanchey (1968) reported electrolytic leakage as a result of downy mildew disease in sorghum caused by *Sclerospora sorghi.* Pellizzari et al. (1976) have reported that electrolytic leakage from infected leaf tissues represents an early pathogenic event occurring during the hypersensitive response (HR) to viruses, bacteria or fungi. Water and electrolyte losses may explain the formation of necrotic local lesions, characteristic of HR, by assuming that they represent an early step of necrogenesis due to membrane damage, leading ultimately to cell death (Pellizzari et al., 1976; Goodman, 1972). The stock health quality tests that measure the functional integrity of seedlings may help to forecast their survival capability and growth under optimal conditions (Grossnickle and Folk, 1993).

The correlation between needle blight severity and electrolytic leakage was highly significant (P = 0.01) and positively correlated (r = 0.98). Also, the multiple regression analysis revealed that the unit change in disease severity exerted influence on electrolytic leakage upto the extent of 1.63 units in positive direction. The regression equation of $Y_{\text{Electrolytic leakage (µmoh/cm)}} = 197 + 1.63\ _{\text{Needle blight severity category}}$ and $R^2 = 95.5$ revealed that only 4.5% variation is due to other reasons than that of needle caste disease. Perusal of literature reveled that knowledge about electrolytic leakage due to needle blight disease is lacking and it signifies its importance of studies.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Sources and levels of glycerin for broilers from 22 to 35 days

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**The aim of this experiment was to evaluate the performance and carcass yield of broilers from 22 to 35 days old, receiving diets with different levels of soybean glycerin (SG) and semipurified glycerin (PURG). A total of 792 male broilers of the Cobb 500 strain, a 4x2+1 factorial arrangement was used designed in randomized blocks by the weight of the birds, with four inclusion levels (17.5, 35.0, 52.5 and 70, 0 g/kg) of two glycerins (SG and PURG) in the diets and one glycerin-free control treatment. Four replicates per treatment of 22 birds were used for each experimental unit. Feed intake and carcass yield were not influenced by the levels and sources of glycerin. There was an interaction between levels and sources of glycerin for the weight gain (WG) where a quadratic effect was observed for the SG and the largest WG obtained with the addition of 35.50 g/kg of SG. There was an interaction between glycerol levels and sources for feed conversion (FC) and a quadratic effect was observed for both sources; the level of 35.5 g/kg of SG promoted a better FC and the level of 39.44 g/kg of PURG promoted the worst FC. For the thigh and drumstick yield (TDY), there was an interaction between levels and sources of glycerin, and the inclusion of 40.21 g/kg of SG provided a lower TDY. There was also interaction for breast yield (BY), where the highest levels of BY were 35.15 g/kg of SG and 43.69 g/kg PURG. The percentage of abdominal fat (AF) was influenced only by the sources of glycerins, where the lowest percentage of AF was promoted by PURG. Regarding the control, the inclusion of 70 g/kg SG provided the worst PG, TDY and BY. In conclusion, the addition of 35.5 g/kg of SG and 70.0 g/kg PURG provides the best performance outcome of birds at 22 to 35 days old, within the evaluated levels.**

**Key words:** Poultry, commercial cuts, glycerol, abdominal fat.

## INTRODUCTION

Glycerin is a byproduct of biodiesel production, derived from vegetable oils and animal fats (Rivaldi et al., 2007). According to Swiatkiewicz and Koreleski (2009), from each 1000 kg of biodiesel produced, about 100 kg of glycerin is obtained.

In Brazil there is an increasing production of biodiesel which, according to the National Petroleum Agency (2013), the estimated production of biodiesel for 2012 is 2.72 billion liters. Therefore, there was a production of 272 million liters of crude glycerin only in Brazil. Thus,

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**Table 1.** Ingredient and calculated nutritional composition of the diets (g/kg as fed basis) during the period from 22 to 35 days of age.

| Ingredient | Control | Crude glycerin from soybean oil | | | | Semipurified glycerin† | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 17.5 | 35.0 | 52.5 | 70.0 | 17.5 | 35.0 | 52.5 | 70.0 |
| Corn | 617.39 | 597.48 | 577.55 | 556.37 | 534.60 | 597.44 | 577.47 | 556.77 | 535.13 |
| Soybean meal | 316.02 | 319.63 | 323.24 | 327.08 | 331.03 | 319.64 | 323.26 | 327.01 | 330.94 |
| Soybean oil | 29.53 | 29.91 | 30.29 | 31.09 | 32.11 | 29.80 | 30.06 | 30.58 | 31.42 |
| Glycerin | 0.00 | 17.5 | 35.00 | 52.50 | 70.0 | 17.50 | 35.00 | 52.50 | 70.00 |
| Dicalcium phosphate | 16.47 | 16.52 | 16.57 | 16.63 | 16.68 | 16.52 | 16.57 | 16.63 | 16.68 |
| Limestone | 8.36 | 8.32 | 8.28 | 7.61 | 6.63 | 8.32 | 8.28 | 7.87 | 6.98 |
| Common salt | 2.24 | 2.25 | 2.26 | 1.57 | 0.53 | 2.25 | 2.26 | 1.86 | 0.92 |
| DL-Methionine | 2.23 | 2.25 | 2.28 | 2.31 | 2.33 | 2.25 | 2.28 | 2.30 | 2.33 |
| L-Lysine HCl | 1.75 | 1.68 | 1.62 | 1.55 | 1.48 | 1.68 | 1.62 | 1.55 | 1.48 |
| L-Threonine | 0.36 | 0.35 | 0.35 | 0.35 | 0.34 | 0.35 | 0.35 | 0.35 | 0.34 |
| Calcium chloride | 0.00 | 0.00 | 0.00 | 0.89 | 2.22 | 0.00 | 0.00 | 0.53 | 1.73 |
| Sodium bicarbonate | 3.61 | 2.06 | 0.51 | 0.00 | 0.00 | 2.20 | 0.80 | 0.00 | 0.00 |
| Lasalocid | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Mineral supplement‡ | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin supplement● | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Choline chloride | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Zinc bacitracin | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Calculated nutrient composition (g/kg as fed basis)** | | | | | | | | | |
| ME (MJ/kg) | 12.98 | 12.98 | 12.98 | 12.98 | 12.98 | 12.98 | 12.98 | 12.98 | 12.98 |
| Crude protein | 197.27 | 197.27 | 197.27 | 197.27 | 197.27 | 197.27 | 197.27 | 197.27 | 197.27 |
| Glycerol► | 0.00 | 12.25 | 24.5 | 36.75 | 49.00 | 13.88 | 27.76 | 41.64 | 55.52 |
| Calcium | 8.24 | 8.24 | 8.24 | 8.24 | 8.24 | 8.24 | 8.24 | 8.24 | 8.24 |
| Available Phosphorus | 4.11 | 4.11 | 4.11 | 4.11 | 4.11 | 4.11 | 4.11 | 4.11 | 4.11 |
| Sodium | 20.50 | 20.50 | 20.50 | 20.50 | 20.50 | 20.50 | 20.50 | 20.50 | 20.50 |
| Chlorine | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 |
| Lysine | 10.73 | 10.73 | 10.73 | 10.73 | 10.73 | 10.73 | 10.73 | 10.73 | 10.73 |
| Methionine + cystine | 77.30 | 77.30 | 77.30 | 77.30 | 77.30 | 77.30 | 77.30 | 77.30 | 77.30 |
| Threonine | 69.70 | 69.70 | 69.70 | 69.70 | 69.70 | 69.70 | 69.70 | 69.70 | 69.70 |
| D.E.B (mEq/kg)* | 231 | 231 | 231 | 231 | 231 | 231 | 231 | 231 | 231 |

†Semiurified glycerin from soybean oil (GENPA®, Granol Indústria, Comércio e Exportação S/A). ‡Supplied per kg of the diet: Zn, 55 mg; Se, 0.18 mg; I, 0.70 mg; Cu, 10 mg; Mn, 78 mg; Fe, 48 mg. ●Supplied per kg of the diet: folic acid, 0.48 mg; pantothenic acid, 8.7 mg; biotin, 0.018 mg; butylated hydroxytoluene (BHT), 1.5 mg; niacin, 11.1 mg; vitamin A, 6,000 IU; vitamin B1, 0.9 mg; vitamin E, 12.15 IU; vitamin B12, 8.1 µg; vitamin B2, 3.6 mg; vitamin B6, 1.8 mg; vitamin D3, 1,500 IU; vitamin K3, 1.44 mg. ►Glycerol from glycerin supplementation. *Dietary electrolyte balance (D.E.B.) calculated using the equation proposed by Mongin (1981), which correlates the calculated concentrations of sodium, potassium and chloride ($Na^+ + K^+ – Cl^-$). ME: metabolizable energy.

there is a production of glycerin above the market demand, which drives the researchers to seek new uses of this byproduct. Due to the lack of legislation to dispose the glycerin produced in excess, this byproduct can become in this way, an environmental problem.

As crude glycerin may be considered a good source of dietary energy for poultry and pigs (Cerrate et al., 2006; Dozier et al., 2008) it is possible to suggest its inclusion in animal diet, which is a viable and environmentally sustainable alternative to allocate part of this byproduct on the market, as it may prevent the over production from having inadequate destinations and contaminating the environment. Thus, this study aimed to evaluate the performance and carcass yield of broilers from 22 to 35 days old, receiving diets with different levels of crude glycerin derived from soybeans and semipurified glycerin.

## MATERIALS AND METHODS

The experiment was conducted at the Poultry Section of the Department of Animal Science, Federal University of Lavras, Lavras - MG, using 792 male broilers of the Cobb 500 strain, in a 4x2 +1 factorial arrangement, with a design in blocks randomized by the weight of the birds, being four levels of inclusion of two glycerins in the diets (17.5, 35.0, 52.5 and 70.0 g/kg diet) and a control treatment without any addition of glycerol. Crude soybean glycerin (SG), and semipurified glycerin - GENPA® (PURG) were used. Diets were formulated to meet nutritional requirements in accordance with recommendations of the Brazilian tables (Rostagno et al., 2005) (Table 1). Due to the high sodium concentration in the

**Table 2.** Chemical composition of the different glycerin types*.

| Parameter | Crude glycerin from soybean oil | Semipurified glycerin from soybean oil‡ |
|---|---|---|
| Moisture and volatiles (g/kg) | 167.5 | 110.8 |
| Karl Fischer moisture (g/kg) | 124.5 | 101.5 |
| Glycerol (g/kg) | 700.0 | 793.1 |
| Gross energy (MJ//kg) | 15.33 | 15.48 |
| Crude protein (g/kg) | 0.3 | 0.4 |
| Methanol● | 181.3 | 20.6 |
| Sodium (g/kg) | 23.8 | 21.6 |
| Total phosphorus (g/kg) | 0.0 | 0.3 |
| Potassium (g/kg) | 0.6 | 0.9 |
| pH in aqueous solution | 6.05 | 5.72 |

*Chemical analyses performed by CBO laboratory analyses, Campinas/São Paulo, Brazil. ‡Semipurified glycerin from soybean oil (GENPA®, Granol Indústria, Comércio e Exportação S/A). •Units are mg/L for the glycerin from crude soybean and semi-purified sources and g/kg for the mixed glycerin.

glycerins used, we adjusted the salt in the diet formula, with the necessary corrections for sodium and chlorine, the latter corrected by the addition of calcium chloride. In the formulating diets, we used an average value of corrected apparent metabolic energy for nitrogen for each glycerin (3279 and 3304 kcal/kg of natural matter, respectively), previously determined by Lima et al. (2012). The nutritional composition of the glycerins used was analyzed by CBO Laboratory Analysis (Table 2). We evaluated the performance and carcass yield and cuts of chicken from 22 to 35 days old.

In all, there were nine treatments with four replicates of 22 birds each. The birds were housed in brick shed, divided into boxes of 3 $m^2$. The floor of each box was covered with wood shavings and each box contained a tubular feeder and drinker a pendulum. The animals were exposed to continuous light, receiving water and ration *ad libitum*.

To evaluate the performance, birds and diets were weighed at the beginning and at the end of the experiment (at 22 and 35 days old) for weight gain, diet intake and feed conversion. The mortality of birds was recorded and considered for the correction of performance data.

At 35 days old, for carcass yield evaluation, two birds per experimental unit, were selected and slaughtered (eight birds per treatment in total) with a weight of approximately 5.0% of the respective box average. The carcass yield was calculated on the weight of the clean carcass and eviscerated without going through the chiller in relation to the fasting body living weight. The chilled carcasses were manually cut into pieces (breast, thigh + drumstick and abdominal fat).

Statistical analysis of the performance variables and carcass yield were performed by the statistical software SAEG (UFV, 2007). The ANOVA of performance data and carcass yield and cuts was conducted, and when significant, sources of glycerin were compared by the Student-Newman-Keuls test and the glycerin levels by regression analysis. The control treatment was compared to the average of treatments with glycerin by the Dunnett's test.

## RESULTS AND DISCUSSION

The diet intake was not influenced by the sources nor by the levels of glycerin inclusion (Table 3) and did not differ from the control diet (P>0.05) either. This result is according to Mclea et al. (2011), who at the moment of inclusion of two sources of glycerin (with apparent metabolizable energy values of 3093.11 and 3487.21 kcal/kg of natural matter) and three levels (33.63 and 100 g/kg) for broilers in the period from 7 to 28 days and did not observe any difference in the diet intake. Cerrate et al. (2006) did not observe differences in the diet intake in broilers from 0 to 35 days old fed with 0, 50 and 100 g of glycerin/kg of diet either. Probably, the non-difference in the diet intake observed in the present experiment was due to the fact that the experimental diets are isonutritious; therefore, it can be inferred that the concentration of glycerin in the diet does not interfere in the diet intake, as long as the poultry nutritious requirements are met.

A significant interaction was observed (P<0.05) between the sources and levels of glycerin tested for the weight gain (Table 3), where the quadratic effect of the evaluated levels was observed for the soybean glycerin, and for the semi purified glycerin the levels did not influence the weight gain. The effect of the levels of crude soybean glycerin on the weight gain in the broilers can be expressed by the $Y= -0.0002x^2+0.01423x+1.2119$ ($R^2= 0.95$) equation, suggesting the best level for dietary inclusion of the soybean glycerin as 35.58 g/kg.

The difference observed in the weight gain in the present experiment was not observed by Mclea et al. (2011), who studied the inclusion of glycerin sources and levels of 33.0, 67.0 and 100.0 g/kg for broilers in the period from 21 to 28 days old. Cerrate et al. (2006) verified that even the inclusion of 50 g/kg of crude glycerin in the diet of broilers in the period from 0 to 35 days old does not alter the weight gain of the birds. However, 100 g/kg of glycerin negatively affected breast weight gain (BWG). Jung and Batal (2011a) included up to 75.0 g/kg of glycerin in the diets of broilers in the period from 16 to 34 days old and did not observe difference in weight gain either.

The worsening in weight gain from 35.58 g/kg of inclusion of the soybean glycerin and the contradictory results to those found by the supracited authors can be

**Table 3.** Diet intake (kg), weight gain (kg) and feed conversion (kg/kg) of birds fed with diets containing glycerins in different levels in the period from 22 to 35 days old.

| Evaluation parameter | Diet intake (kg) | | | | |
|---|---|---|---|---|---|
| | Level (g/kg) | | | | |
| Glycerins | 17.5 | 35.0 | 52.5 | 70.0 | Average |
| Soybean $^{ns}$ | 2.210 | 2.252 | 2.260 | 2.251 | 2.243 |
| Semipurified $^{ns}$ | 2.223 | 2.241 | 2.230 | 2.228 | 2.231 |
| Probability | | | P>0.05 | | |
| Control Treatment | | | 2.192 | | |
| Variation Coeficient (%) | | | 3.806 | | |
| | **Weight gain (kg)** | | | | |
| | **Level (g/kg)** | | | | |
| Glycerins | 17.5 | 35.0 | 52.5 | 70.0 | Average |
| Soybean $^{Q}$ | 1.410$^{A}$ | 1.450$^{A}$ | 1.453$^{a}$ | 1.256$^{B}$* | 1.392 |
| Semipurified | 1.391$^{A}$ | 1.341$^{B}$ | 1.396$^{a}$ | 1.434$^{A}$ | 1.391 |
| Probability | | | P<0.05 | | |
| Control Treatment | | | 1.433 | | |
| Variation Coeficient (%) | | | 4.530 | | |
| | **Feed conversion (kg)** | | | | |
| | **Level (g/kg)** | | | | |
| Glycerins | 17.5 | 35.0 | 52.5 | 70.0 | Average |
| Soybean$^{Q}$ | 1.57$^{A}$ | 1.55$^{A}$ | 1.56$^{a}$ | 1.79$^{B}$* | 1.62 |
| Semipurified $^{Q}$ | 1.60$^{A}$* | 1.67$^{B}$* | 1.60$^{A}$* | 1.56$^{A}$ | 1.61 |
| Probability | | | P<0.05 | | |
| Control Treatment | | | 1.53 | | |
| Variation Coeficient (%) | | | 2.052 | | |

A,B differ through the Student-Newman-Keuls test; * Average differs from the control treatment through the Dunnett test; NS = non-significant; $^{Q}$ Quadratic effect.

partially explained by the composition of the evaluated glycerins. For example, the crude glycerin used by Mclea et al. (2011) contained 523 g of glycerol per kilo of crude glycerin; and the crude soybean glycerin used in the present experiment contained 700 g of glycerol per kilo. Possibly, the greater concentration of glycerol in the crude glycerin used in the present work, may have surmounted its metabolization capacity by the animal, promoting a worsening in the performance with higher levels of inclusion. Considering that the glycerol, when not metabolized, needs to be excreted, such process promotes energetic expenditure which can compromise the broilers performance.

In relation to the control treatment, the inclusion of 70 g of crude soybean glycerin/kg of diet was the first treatment which differed (P<0.05), promoting a worst weight gain to the birds (Table 3). This result may be due to the ingestion of a greater amount of glycerol than the metabolization capacity of this by the birds.

For the feed conversion, a significant interaction was observed (P<0.05) between the sources and levels of the tested glycerins (Table 3), where the quadratic effect was verified in both sources. The effect of the crude soybean glycerin on the feed conversion in the birds from 22 to 35 days old ($Y= 0.0002x^2 - 0.0142x + 1.765$; $R^2=0.94$), allowed a better feed conversion with the inclusion of 35.5 g of this glycerin per kilo of diet, similar to the result obtained for the weight gain. For the purified glycerin, the inclusion of 39.44 g/kg, was the one which promoted the worst conversion ($Y= -0.00009x^2 + 0.0071x + 1.5118$; $R^2= 0.77$). These results differ from those reported by Cerrate et al. (2006), who did not observe difference in the food conversion of broilers in the period from 0 to 35 days old when comparing the inclusion of 25.0 and 50.0 g/kg of glycerin. Simon et al. (1996) also found different results from the ones observed in this work, where the referred authors included up to 25.0 g/kg of pure glycerol in diets of broilers and did not verify difference in the feed conversion until the inclusion of 10.0 g/kg.

In relation to the control treatment, the level of 70 g/kg of crude soybean glycerin and 17.5; 35; 52.5 g/kg of semipurified glycerin showed the worse feed conversion (P<0.05) (Table 3). That is, when comparing the soybean glycerin with a diet without glycerin, the inclusion of 70.0 g/kg of this glycerin damages the performance of the birds may be suggested, possibly due to the excess of

**Table 4.** Carcass yield (%), Thigh + Drumstick yield (%), Breast yield (%) and Abdominal fat (%) of birds fed with diets containing glycerins in different levels in the period from 22 to 35 days old.

| Evaluation parameter | Carcass yield (%) | | | | |
|---|---|---|---|---|---|
| | Level (g/kg) | | | | |
| Glycerins | 17.5 | 35.0 | 52.5 | 70.0 | Average |
| Soybean | 73.48 | 72.44 | 73.22 | 71.85 | 72.75 |
| Semipurified | 72.44 | 72.44 | 72.69 | 73.09 | 72.67 |
| Probability | | | NS | | |
| Control Treatment | | | 72.80 | | |
| Variation coefficient (%) | | | 1.033 | | |
| | **Thigh and Drumstick yield (%)** | | | | |
| | **Level (g/kg)** | | | | |
| Glicerins | 17.5 | 35.0 | 52.5 | 70,0 | Average |
| Soybean $^{Q}$ | 30.46A | 28.96B | 29.12[a] | 31.81A* | 30.09 |
| Semipurified | 30.46A | 30.31A | 29.88[a] | 30.35B | 30.25 |
| Probability | | | P<0.05 | | |
| Control treatment | | | 29.19 | | |
| Variation coefficient (%) | | | 2.818 | | |
| | **Breast yield (%)** | | | | |
| | **Level (g/kg)** | | | | |
| Glycerins | 17.5 | 35.0 | 52.5 | 70.0 | Average |
| Soybean $^{Q}$ | 37.16A | 37.38A | 37.77[a] | 33.12B* | 36.36 |
| Semipurified $^{Q}$ | 37.33A | 37.51A | 37.99A* | 37.19[a] | 37.51 |
| Probability | | | P<0.05 | | |
| Control Treatment | | | 37.18 | | |
| Variation coefficient (%) | | | 1.124 | | |
| | **Abdominal fat (%)** | | | | |
| | **Level (g/kg)** | | | | |
| Glycerins | 17.5 | 35.0 | 52.5 | 70.0 | Average |
| Soybean | 1.97 | 1.85 | 1.60* | 1.78 | 1.80[a] |
| Semipurified | 1.64* | 1.64* | 1.54* | 1.59* | 1.60B |
| Probability | | | P<0.05 | | |
| Control treatment | | | 2.03 | | |
| Variation coefficient (%) | | | 11.890 | | |

A,B differ through Student-Newman-Keuls test; * Average differs from the control treatment through the Dunnett test; NS = non-significant; $^{Q}$ Quadratic effect.

glycerol ingested or due to the effect of contaminants present in this byproduct, which in higher levels may be toxic to the animal, as for example, the methanol which is present in the soybean glycerin in the concentration of 181.31 mg/L. An inverse effect was observed for the semipurified glycerin, where the levels below 70 g/kg showed greater feed conversion in relation to the control treatment (P<0.05). The improvement in the feed conversion with the inclusion of 70 g/kg of semipurified glycerin may be partially explained by the low concentration of methanol (20.62 mg/L), which would allow the supply of glycerin levels to be higher in the studied animal. However, the limitation to a higher inclusion of this semipurified glycerin would be the sodium concentration in it.

The inclusion of 35.0 and 70 g/kg of the soybean glycerins and semipurified, respectively, can be noticed by the performance results, allowing a sustainable and environmentally correct fate for the glycerins originating from the production of biodiesel.

For the carcass yield there was no interaction (P>0.05), nor influence of the sources and levels of glycerin (Table 4). No difference (P>0.05) in the yield of the birds fed with the different diets in relation to the control treatment was

observed either (Table 4). The same was observed by Cerrate et al. (2006), working with the inclusion of 0; 25.0 and 50.0 g/kg of glycerin. However, these same authors, including 0; 50.0 and 100.0 g/kg of glycerin in another experiment, verified the reduction in the carcass yield with the inclusion of 100.0 g/kg.

A significant interaction was observed (P<0.05) between sources and levels of glycerin for the thigh and drumstick yield (TDY) (Table 4). The levels of soy glycerin provided a quadratic effect of TDY ($Y = 0.0034 \times -0.2734 + 34.2520x^2$, $R^2 = 0.99$), where the lowest TDY was found with 40.21 g/kg inclusion of SG. Results contrary to those of Cerrate et al. (2006), who did not observe any influence of glycerin levels on the yield of the thigh and drumstick. The purified glycerin levels did not influence the TDY (P>0.05).

Only the birds fed with 70.0 g/kg of SG differed from the control treatment in relation to TDY (P<0.05), with poorer outcome. Such result can be justified by the results of weight gain and feed conversion found in this study. Probably, there was saturation of the kinase glycerol enzyme, limiting the use of glycerol by the body, and also the performance of the chickens may have been damaged by the ingestion of high amounts of methanol present in the crude soybean glycerin. This explanation would agree with Doppenberg and Van der Aar (2007), who suggested that there was over 50 g of glycerol per kilogram of feed, as there may be saturation of glycerol kinase.

According to Jung and Batal (2011b), there is variation in the composition of methanol in different glycerins produced from biodiesel, highlighting the importance of analyzing them before feeding the birds, since this alcohol, in higher concentrations, can be harmful to animals.

There was a significant interaction (P<0.05) between levels and sources of glycerin for breast yield, and quadratic effects were observed for both sources (Table 4). A higher breast yield was obtained with the inclusion of 35.15 g/kg of soybean glycerin ($Y = -0.004x^2 + 0.2812 + 33.19x$, $R^2 = 0.90$) and 43.69 g/kg of semi purified glycerin ($Y = -0.0008 + 0.0699x + 36.274x^2$, $R^2 = 0.66$). At levels of 25.0 and 50.0 g of glycerin inclusion per kilo of diet, Cerrate et al. (2006) did not observe difference in breast yield to the levels tested. However, in relation to the glycerin-free diet, the presence of this byproduct showed higher breast yield in broilers.

The addition of 70.0 g/kg of soybean glycerin provided breast yield lower than the control treatment, and including 52.5 g/kg of semi purified glycerin resulted in breast yield higher than the control treatment. These results do not confirm the theory that glycerol could save gluconeogenic amino acids (Cryer and Bartley, 1973) increasing protein retention, resulting in higher breast yield, since the other levels of tested glycerins did not differ from the control treatment, similar to the results of Cerrate et al. (2006) that included 0.0, 50.0 and 100.0 g glycerol/kg and found no difference in breast yield.

The percentage of abdominal fat was only influenced by sources of evaluated glycerins (P <0.05), where the lowest fat percentage was obtained with the use of semipurified glycerin. It could be deduced that the concentration of glycerol does not increase lipogenesis, since the glycerin with the highest percentage of glycerol was the source that provided thinner carcass. The inclusion of 17.5, 35.0 and 70.0 g/kg of soybean glycerin provided percentage of abdominal fat similar to the control treatment, and the level of 52.5 g/kg of soybean glycerin and all levels of the semi purified glycerin presented a lower percentage of abdominal fat in relation to the control treatment.

## Conclusion

The addition of 35.5 g/kg of crude soybean glycerin and 70.0 g/kg of purified glycerin gave the best performance results of broilers in the period from 22 to 35 days, within the evaluated levels. The use of glycerin from biodiesel can be applied to the nutrition of broilers achieving good results, since it is considered a contaminant concentration in the byproduct since, at high concentrations they can harm the animal metabolism, resulting in lower animal performance.

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## Conflict of Interest

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Fuel demand as a function of furrow opener and soil conditions in no-tillage system

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**In a no-tillage system, the timing of beginning to work with agricultural machines and tools is of great importance because it may be the key to the low cost of the operation. This study was conducted to evaluate the fuel consumption of a tractor and the effects of using different types of hoe-type openers on the soil disturbance at two soil moisture contents in a no-tillage system. The experiment was conducted in an area of the Department of Rural Engineering, UNESP/FCAV, Jaboticabal-SP-Brazil. The area was divided according to a randomized block design with a factorial scheme of 3 × 2 × 2 with four replications. The tractor used was a BH125i-model Valtra-AGCO with 91.9 kW of rated engine power and a pantograph planter with four rows. The treatments were three hoe-type furrow openers, two soil water content profiles (WCS1 and WCS2), and two working depths. The WCS2 profile consisted of a water content of 23.1% in the layer from 0.0 to 0.10 m deep and 23.8% in the layer from 0.11 to 0.20 m deep. The WCS1 profile consisted of a water content of 15.6% in the layer from 0.0 to 0.10 m deep and 21.3% in the layer from 0.10 to 0.20 m deep. The working depths were 0.10 and 0.15 m. Increasing the working depth provides greater tillage. The greater the working depth is and the lower the soil water content is, the better the operational fuel consumption. The combination of the rake angle and the thickness of the FO3 opener resulted in the lowest operational and hourly fuel consumption levels.**

**Key words:** Soil mobilization, consumption, direct seeding, tractor performance.

## INTRODUCTION

No-tillage seeding is one of the key operations of conservation agriculture (Baker et al., 2007). It is a system that minimizes soil disruption by leaving crop residue on fields after harvest, where it acts as a mulch to protect the soil from erosion and fosters soil productivity. To sow seeds, farmers use specially designed seeders that penetrate the residue and the undisturbed soil below,where the seeds can germinate and surface as new crops (Huggins and Reganold, 2008).

In Brazil, this technique has been used for approximately 30 years and is widely applied in the southern central region. Currently, more than 27 million ha in Brazil are cultivated under this system (Boddey et al., 2010), which makes Brazil the country with the

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second-highest no-tillage cultivated area in the world, behind only the United States of America.

In a no-tillage system, the soil may be opened by coulters, row cleaners, disc openers, in-row chisels, hoe-type, or rototillers prior to planting the seed. All of these terms related to conservation tillage describe operational aspects of conservation agriculture (Ling-Ling et al., 2011).

The main functions of a hoe-type furrow opener in a no-tillage system are decompression and disturbance of the subsoil in depth and extension (Cepik et al., 2005). This decompression and disturbance of the subsoil reduces the bulk density of the soil and its mechanical resistance to penetration (Mello et al., 2003).

An important aspect of this type of mechanism is the tractive effort required by the opener, which depends on its physical characteristics, such as the angle of attack, tip width, tip shape, thickness, and angle of inclination (Sánchez-Girón et al., 2005).

Information on the results of evaluations of furrow-opening mechanisms for no-tillage planters helps companies in the sizing of soil-opening tools to reduce energy requirements (Mion et al., 2009).

The possibility of rupturing compacted surface soil layers, even in a localized manner, has induced famers to utilize shanks that can reach greater depths-in some cases, as great as 0.20 m. However, shanks with narrow points, such as the ones used in the majority of seed drill-fertilizers suitable for no-tillage, are limited in their ability to increase the operation depth because of soil–shank interactions and soil structure rupturing behavior (Conte et al., 2011; Godwin and O'Dogherty, 2007; Hemmat and Adamachuk, 2008).

Germino and Benez (2006) evaluated two types of hoe-type furrow openers for a no-tillage planter, working at four depths (0.12, 0.23, 0.28, and 0.33 m) in a distroferric red ultisol, and concluded that there was no difference in the performance of the two furrow openers when working at the recommended depth (0.13 m) but that the differences between the two types of furrow openers were accentuated when the working depth exceeded the critical working depth.

In a series of tests carried out on a sandy clay loam and loamy sand; Damora and Pandey (1995) found that furrow openers with lower drafts had smaller widths and wedges and rake angles of 40º or less. Soil disturbance is also affected by furrow opener design. The cross-sectional area of a furrow increases with increasing rake angle and wedge angle (Siemens et al., 1965; Abernathy and Porterfield, 1969). The rake angle also affects the working depth of a furrow opener and the variation in depth due to changes in the forward speed. Abernathy and Porterfield (1969) observed the furrow depth to be greater for openers with large rake angles and wedge angles.

Altuntas et al. (2006) evaluated the effects of three types of furrower mechanisms and reported that the design of the shank involves factors that affect its performance and the quality of the operation. Montanha et al. (2011) commented that the intensification of activities in mechanized agriculture results in higher energy costs for farms, mainly in fuel consumption by agricultural tractors.

Two conditions must be fulfilled before the start of field operations in the spring or after a rainy period: "The surface layer must be friable down to the intended tillage depth, and the underlying soil must have a sufficient bearing capacity for the machinery" (Heinonen, 1985). Soil friability is related to the soil water content and reaches a maximum at water contents close to the plastic limit (Utomo and Dexter, 1981; Watts and Dexter, 1998). To avoid compaction, field operations should not be carried out when the soil water content exceeds the lower plastic limit (Rounsevell, 1993).

This study was conducted to evaluate the fuel consumption of a tractor and the effects on the soil of three types of hoe-type furrow openers at two working depths and two soil water contents in a no-tillage system.

## MATERIALS AND METHODS

The experiment was conducted in experimental area of the Department of Rural Engineering of São Paulo State University - UNESP/FCAV, Jaboticabal-SP-Brazil, in 2011 and 2012. The average slope of the area is 4%. The Köeppen classification of the climate is Aw climate (subtropical). The soil is classified as a eutroferric Red Latosol with 469 g $kg^{-1}$ of clay, 307 g $kg^{-1}$ of silt, and 224 g $kg^{-1}$ of sand, managed for nine years under a no-tillage system. The soil's mechanical resistance to penetration was 0.8 and 2.7 MPa for the layers from 0.0 to 0.10 m deep and 0.11 to 0.20 m deep, respectively.

The experiment was conducted according to a completely randomized design with a 3 × 2 × 2 factorial scheme with four replications: three hoe-type furrow openers (FO1, FO2, and FO3) (Figure 1), two soil water contents (WCS1 and WCS2), and two working depths of the furrow openers (WD1 and WD2). The tractor used had 91.9 kW of rated engine power at 2300 rpm to pull a pantographic planter along four rows of corn, with a spacing of 0.90 m between rows. The average planting speed was 5.9 km $h^{-1}$.

To analyze the water contents of the soil, soil samples were collected from the layers 0.0 to 0.10 m deep and 0.10 to 0.20 m deep. A total of 20 soil samples per layer were collected from each plot. To permit planting at two soil water content levels, the experimental area was irrigated for approximately 12 h (6 h on one day + 6 h on the next day) at an average precipitation rate of 10 mm $h^{-1}$. The first planting was begun 36 h after the irrigation was completed. At that time, the WCS2 water content profile consisted of a water content of 23.1% in the 0.0 to 0.10 m layer and 23.8% in the 0.11 to 0.20 m layer. After 72 h, the second planting was conducted. At that time, the WCS1 water content profile consisted of a water content of 15.6% in the 0.0 to 0.10 m layer and 21.3% in the 0.10 to 0.20 m layer. The working depths were 0.10 m (WD1) and 0.15 m (WD2).

The soil area disturbed and the width and depth of the furrow were evaluated. The furrow was opened manually to make it possible to model the furrow. The furrow width (FW) and working effective depth (WED) were analyzed using a profile meter with 45 rods that are 30 cm high and spaced 1 cm apart (Figure 2). A piece of cardstock paper with horizontal lines spaced 0.5 cm apart was nailed to the back of the profile meter for precision and ease of reading. The positions of the upper ends of the rods reproduce the shape of the furrow. A digital camera was used to capture the

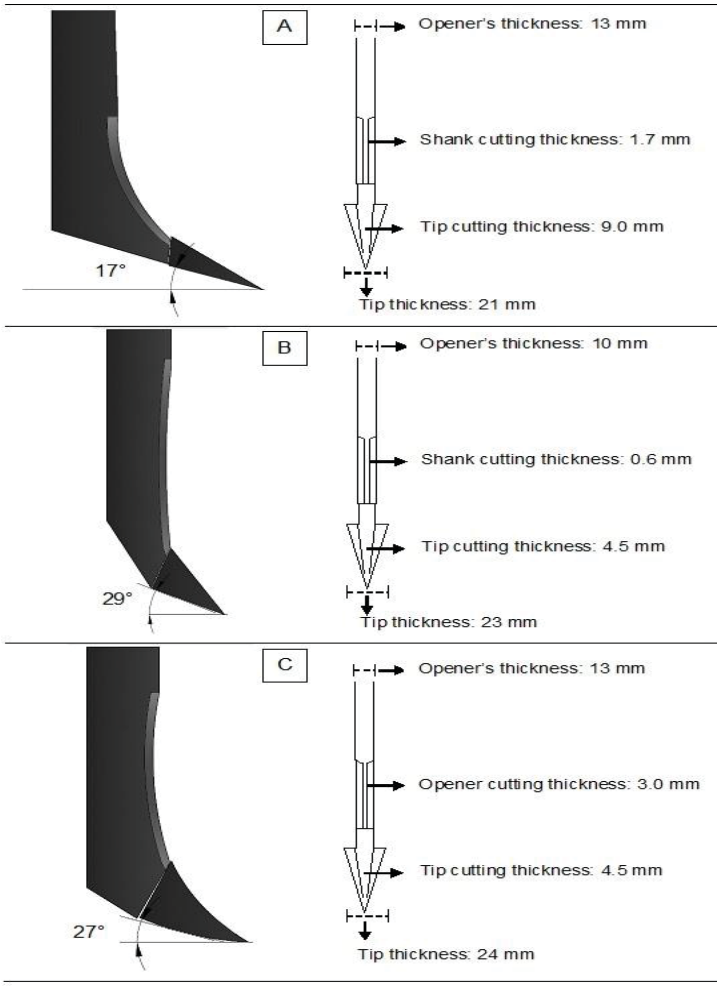


**Figure 1.** Characteristics of the furrow openers FO1 (a), FO2 (b), and FO3 (c).

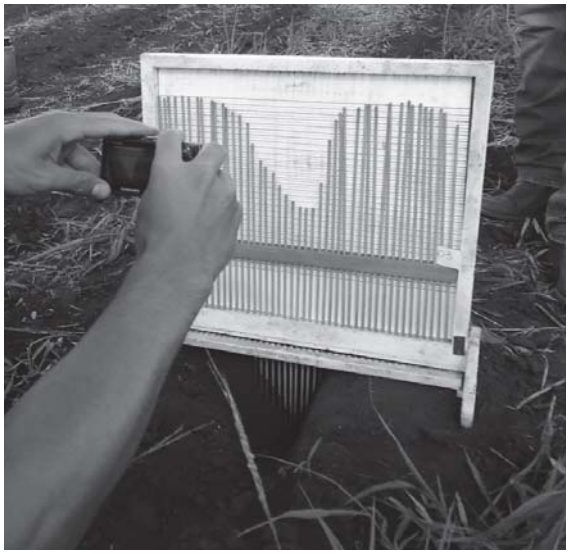

**Figure 2.** Profile meter used to analyze the furrow conditions after the passing of the opener.

**Table 1.** Analysis of variance for soil disturbance (SD), furrow width (FW), working effective depth (WED), hourly fuel consumption (FC - L $h^{-1}$), operational fuel consumption (FC - L $ha^{-1}$), and specific fuel consumption (SFC).

| Factor | SD | FW | WED | --------- FC ---------- | | SFC |
|---|---|---|---|---|---|---|
| | ($cm^2$) | ------- (cm) ------- | | ($L\ h^{-1}$) | ($L\ ha^{-1}$) | ($ml\ m^{-3}$) |
| **WCS** | | | | | | |
| WCS1 | 167.4 | 26.1 | 11.6 | 8.9 | $6.1^{a}$ | $37.2^{a}$ |
| WCS2 | 155.9 | 26.6 | 12.3 | 8.6 | $5.7^{b}$ | $33.0^{b}$ |
| **Furrow openers (FO)** | | | | | | |
| FO1 | 149.9 | 26.5 | 12.6 | $9.6^{a}$ | $6.8^{a}$ | $40.0^{a}$ |
| FO2 | 156.8 | 25.4 | 12.2 | $9.1^{b}$ | $6.0^{b}$ | $33.9^{ab}$ |
| FO3 | 178.2 | 27.1 | 11.1 | $7.8^{c}$ | $4.8^{c}$ | $31.4^{b}$ |
| **Working depth (WD)** | | | | | | |
| WD1 | $129.3^{b}$ | 24.8 | $9.8^{b}$ | $8.4^{b}$ | $5.6^{b}$ | $40.5^{a}$ |
| WD2 | $193.9^{a}$ | 27.9 | 14.1 a | $9.2^{a}$ | $6.1^{a}$ | $29.7^{b}$ |
| CV (%) | 4.6 | 8.9 | 5.2 | 2.9 | 3.6 | 20.4 |

Means followed by the different letters are significantly different according to Tukey test at 95% confidence level. CV: coefficient of variation.

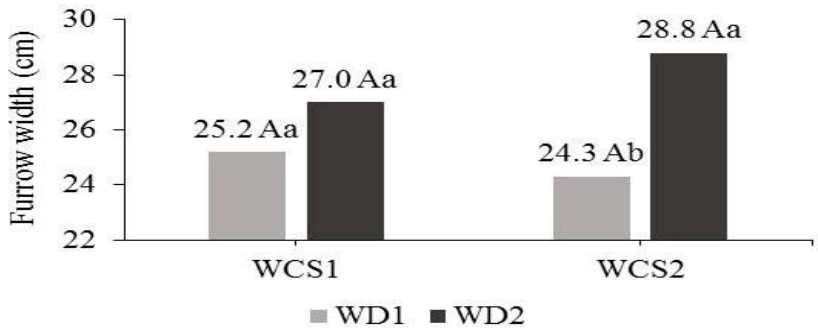


**Figure 3.** Interaction between water content of soil (WCS1 and WCS2) and working depth (WD1 and WD2) for furrow width. Means followed by the same letter (uppercase for WCS and lowercase for WD) are not significantly different according to Tukey test at 95% confidence level.

readings, which were analyzed using a computer. FW was defined with respect to the first rod that fell on the ground inside the furrow. WED was defined as the average of the heights of the two first rods with the highest values.

Soil disturbance was obtained by the transversal section of disturbed soil and the data were analyzed by the integral of the trapezoidal rule.

To determine the demand of fuel of the tractor, we obtained by the flow meter Oval-III model, with 0.01 ml of precision, installed in the tractor and collecting the difference between the measured amount of fuel in the input and return of the fuel injection pump. The values were stored in a "CR23X micrologger, Campbell Scientific Company".

Specific fuel consumption was evaluated. To calculate this variable, the area of disturbed soil data was transformed to volume of disturbed soil per hectare (m3 ha-1). Then, the data of fuel consumption was transformed from liters to milliliters and divided it per volume of disturbed soil (ml m-3).

The statistical programs used were the SISVAR (Ferreira, 2011) and ASSISTAT (Silva and Azevedo, 2006) to ANOVA, using F test of Snedecor and, when significant, applied the Tukey test ($p<0.05$). When the values presented asymmetric by Anderson-Darling test, applied the transformation [X=log(x)].

## RESULTS AND DISCUSSION

An analysis of variance of the measurement obtained was conducted. The results are presented in Table 1 and Figures 3, 4, and 5 (which illustrate the interactions between factors). The coefficient of variation was low for most of the variables, which can be attributed to the logarithmic transformation of the data, performed on the basis of the asymmetry detected using the Anderson–Darling test. The asymmetry that was observed can be attributed to the natural variability of soil in an experiment

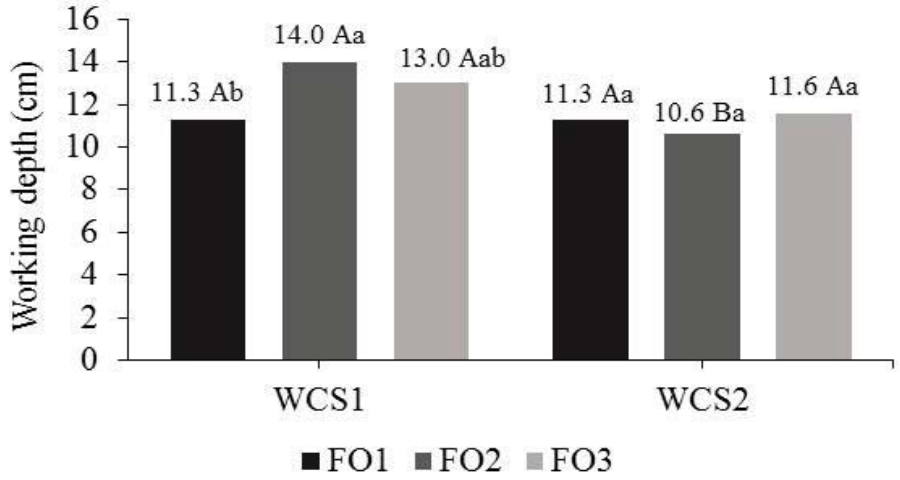


**Figure 4.** Interaction of water content of soil (WCS1 and WCS2) and furrow openers (FO1, FO2, and FO3) for working effective depth. Means followed by the same letter (uppercase for WCS and lowercase for FO) are not significantly different according to Tukey test at 95% confidence level.

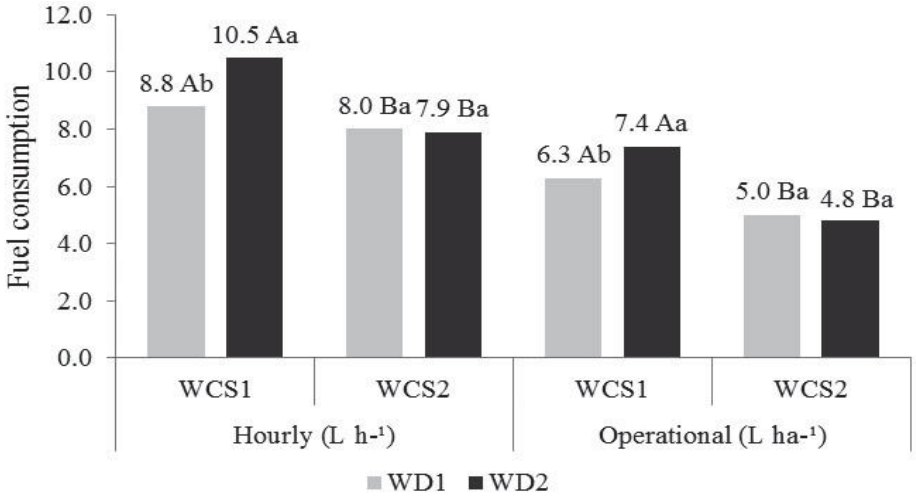


**Figure 5.** Interaction of the water content of soil (WCS1 and WCS2) and working depth (WD1 and WD2) for hourly (L $h^{-1}$) and operational fuel consumption (L $ha^{-1}$). Means followed by the same letter (uppercase for WCS and lowercase for WD) are not significantly different according to Tukey test at 95% confidence level.

of this type.

Despite the differences in the soil water content and the design characteristics (the thickness and tilt angle) of the openers, the degrees to which the soil was disturbed by the different openers were not significantly different (Table 1). The deeper the openers penetrated into the soil, the higher the disturbed area was. The soil disturbance increased by approximately 33% when the openers penetrated more deeply into the soil. According to Mion et al. (2009), the furrow opener achieves a greater depth due to the action of the opener's tip angle, which has a tendency to suck it down.

Use of the FO1 opener resulted in a higher fuel volume demand per hour and a higher consumption of fuel per hectare (Table 1) than the other openers. For example, use of the FO1 opener required 29% more fuel per hectare than the FO3 opener.

When the openers worked at a greater depth, the fuel consumption was higher because of the larger contact area of the openers with the soil and the greater resulting resistance of the soil to penetration.

A significant difference in the operational fuel consumption

at WCS1 and WCS2 was observed. At lower water content, the fuel consumption was greater, which is consistent with the findings reported by Toro and Arvidsson (2003), who found in working in a clayey soil at different soil moisture contents that greater resistance to penetration was encountered when the soil contained less water.

Conte et al. (2011) commented that in evaluating the performance of furrow openers, it is very important to determine the soil mobilization index to make it possible to analyze the specific energy demand of the equipment used.

Although, there was no significant difference in fuel consumption with soil disturbance, when the amount of fuel consumed by the tractor was divided by the amount of soil disturbed, differences were observed in the real working conditions. This calculation makes it possible to assess the actual efficiency of the furrow opener mechanism.

When the tractor-seeder worked at WCS1, that is, under drier soil conditions, the fuel consumption per volume of disturbed soil was higher. Thus, WCS2 was more favorable because the amount of fuel consumed per volume of disturbed soil was smaller.

Although, the openers were not significantly different in terms of soil disturbance, use of the FO3 opener resulted in lower fuel consumption per volume of disturbed soil. The use of the FO1 resulted in the highest fuel consumption per volume of disturbed soil. This is an interesting finding because the geometries of these openers are completely different. The FO3 opener is larger than the FO1 opener, and the FO1 opener has a smaller angle of inclination.

The specific fuel consumption (SFC) for the WD2 was smaller than for the WD1 because the soil disturbance was higher and the fuel consumption was smaller. Kichler et al. (2011), working with a strip-tillage system, obtained values of 5.9 L $h^{-1}$ per opener for openers operating at a depth of 30 cm (Table 1).

There was a significant interaction between the factors WCS and WD for the furrow width, as shown in Figure 3. In the interaction between WCS and WD for furrow width (Figure 3), there was an increase in width when the WD2 was utilized in the WCS2 soil because of the higher furrow opening angle promoted by the openers.

In the interaction between WCS and FO for the working effective depth (Figure 4), the FO2 opener resulted in greater depths than the FO1 opener in the WCS1 soil, but no differences were detected between the other openers when worked in the WCS2 soil. The reason for the difference observed is that the FO2 is thinner than the other openers. Another interesting characteristic of the FO2 is that it was able to penetrate the WCS2 soil but was not able to keep the furrow open, most likely because of the high soil moisture.

For Conte et al. (2009), the increasing depth of the FO action suggests a viable strategy for increase grain yield under conditions of water stress. Kichler et al. (2011) found a difference of 2 to 5 cm in the working depth between two subsoiling shanks and attributed this difference to differences in the geometries of the shank.

The interaction between WCS and WD for the variable hourly and operational fuel consumption (Figure 5) indicates that the tractor–planter set was better when working in the WCS2 soil. These results indicated that the WCS2 soil moisture content can be recommended for the soil studied, which has been managed for nine years under a no-tillage system. When the tractor–planter set worked under the drier soil conditions, the fuel demand was higher because of the amount of clay dried and the length of time for which the soil had been managed under a no-tillage system. In evaluating two planters with hoe-type furrow openers in a Red–Yellow Podzolic soil at four soil moisture contents under a no-tillage system, Reis et al. (2002) did not detect any significant differences in the hourly fuel consumption of the tractor. It is very important to study the characteristics of each mechanism because the tractor performance will vary depending on the texture, moisture, and physical attributes of the soil. This is particularly true in conservation agriculture systems.

## Conclusions

Increasing the working depth achieves greater located tillage. A greater working depth together with a lower soil water content yields low operational fuel consumption. The combination of the rake angle and the thickness of the FO3 furrow opener resulted in the lowest operational and hourly fuel consumption.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Profitability analysis and determinants of fruit tree based agroforestry system in Wondo District, Ethiopia

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**Fruit-tree based agroforestry represents a more environmentally friendly system, the economic returns and adoption determinants of which have only been modestly studied to date. This study investigated the determinants of practicing fruit-tree based agroforestry and the associated costs incurred and returns earned by practitioners. It contrasted the economic performance of agroforestry based systems versus monocropping systems using economic performance indicators at the household level in Wondo District. Data were collected from 149 selected households through structured interviews, focus group discussions, key informant interviews, market assessments as well as field observation. Variables including nearness to the main road, farming experience, labor, landsize and income significantly affected the practice of fruit tree based agroforestry system. Attention is needed in the design of policies and strategies for promoting the fruit-tree based agroforestry system which is more attractive financially, in addition to being labor saving and less risky investment than the monocropping systems.**

**Key words:** Agroforestry, annual equivalent value, benefit cost ratio, cost benefit analysis, net present value.

## INTRODUCTION

In the past, monocropping systems were considered to be the most desirable agricultural development since high production under such systems was presumed to solve the problem of food shortages (Tesfaye, 2005). Recently, concerns have developed on the long-term sustainability and environmental consequences of the environmentally destructive practice of intensive monoculture. Because of its various positive contributions, emphasis has been given promoting agroforestry as a viable land use particularly in developing countries (Rasul and Thapa, 2002).

Agroforestry involves the cultivation and use of trees in farming systems and is a practical and low-cost means of implementing many forms of integrated land management, especially for small-scale producers (Leakey, 2010). Agroforestry is found to be one of the appropriate means of achieving sustainable production without causing any environmental destruction (Padmavathy and Poyyamoli, 2013). According to Dhyani et al. (2009) agroforestry is a key path to prosperity for millions of farm families, leading to extra income, employment generation, greater food and nutritional security and meeting other basic human needs in a sustainable manner. In addition to satisfying basic human

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needs, agroforestry has positive impacts on the conservation of the natural resource base and in the protection of the environment (Ajayi et al., 2005). Ultimately, agroforestry can significantly contribute for the achievement of the MDGs; Goal 1 (eradicating extreme poverty and hunger) and Goal 7 (ensuring environmental sustainability) on the same piece of land (Weidner et al., 2011).

Agroforestry is an age-old practice in Ethiopian farming systems. The practice differs in different agro-ecological zones mainly driven by farmers' strategy for maximizing production and maintaining livelihood options with a limited resource such as land. For instance, in Sidama, the main agroforestry practices include (i) tree–enset–coffee, (ii) tree–enset (iii) Eucalyptus woodlot (iv) scattered/parkland trees on maize (v) boundary planting, and (vi) scattered trees on grazing fields (Zebene and Agren, 2007). In Gedeo area, three basic agroforestry systems are practiced: trees agroforestry system, enset-coffee-trees agroforestry system and coffee-fruit crops-trees agroforestry system (SLUF, 2006). However, in addition to the aged traditional practices new agroforestry technologies are being introduced in different areas. Intercropping of fruit trees with other perennials in Wondo area is one such example.

While agroforestry has made tremendous strides in recent years, many challenges remain in terms of its wider application (SWF, 2005) and its success largely depends upon how it is accepted by farmers (Dwivedi et al., 2007). Simply, the wider acceptance of agroforestry practices depends on the potential economic benefits it generates to farmers (Neupane and Thapa, 2001). However, studies to assess the economic benefits of agroforestry are rather limited, although the extent to promote this technology critically depends on the size and nature of the benefits (Otsuki, 2010). Especially in Ethiopia, despite the tremendous economic and ecological benefits of multi-strata agroforestry land-uses, no systematic efforts have been made to document and improve the practice (SLUF, 2006). This was the basis for undertaking this study.

The objectives of this study were:

i. To estimate the production costs involved and the tangible benefits obtained by farm households,
ii. To analyze and compare the profitability of the fruit tree based agroforestry system and the monocropping systems, and
iii. To identify factors that influence the practice of fruit tree based agroforestry by farm households in the study area.

## MATERIALS AND METHODS

### Study area

The study was conducted in Wondo District which is located about 267 km away from Addis Ababa between $38^0$ 04' 04" - $39^0$ 46' 08" East longitude and $6^0$ 12' 29" - $7^0$ 42' 55" North latitude. The landscape of the study area varies with an altitude ranging between 1700 and 2300 m.a.s.l. The rainfall distribution of the study area is bi-modal where short rain falls during spring and the major rain comes in summer and stays for the first two months of autumn season. The annual temperature and rainfall range between 17 to 19°C and 700 to 1400 mm, respectively.

### Data collection

Wondo District was selected purposively based on the presence of fruit-tree based agroforestry systems. From the district, two Kebeles were selected based on the presence of fruit production and ease of accessibility. Several rules-of-thumbs were suggested for determining the minimum number of households required to conduct logistic regression analyses. In this study, the rule-of-thumb that $N \geq 50 + 8$ m was adopted, where N is the minimum required number of households and m is explanatory variables (Green, 1991) to limit the size of sampled households for the interview. The explanatory variables were ten. Thus, the minimum sample size was 130. For this study a total sample of 149 individuals was selected and interviewed. Stratified sampling was employed to identify farmers as practitioners of the fruit-tree based agroforestry system and non practitioners (those who practice the monocropping system).

Both primary and secondary data were collected for the purpose of this study. Primary data were collected through key informant interviews, structured questionnaire, focus group discussions, field observation and market assessment. Individuals who have lived in the area for a long time, active and knowledgeable of their localities were selected as key informants (six individuals in each kebele) by adapting the snow-ball method and one-on-one interviews were conducted with the selected key informants to know the history of land use practice and introduction of fruit-trees in the area. For focus group discussions, individuals who have good experience in fruit production were selected to discuss specific issues related to the purpose of the study by forming small groups (members of 5-6) with a homogenous composition and members sharing similar background and experience on the issues under study.

### Data analysis

To meet the objectives of the study, both cost benefit analysis and econometric analysis were employed. The data collected were analyzed using Stata 11 and Excel 2007. In the cost benefit analysis (CBA) economic performance indicators such as net present value (NPV), benefit cost ratio (BCR) and annual equivalent value (AEV) were calculated to address the first two objectives. The mathematical procedure for each of the economic performance evaluation parameters is displayed in Table 1.

Econometric model was used to analyze the factors that influence the adoption of the fruit-tree based agroforestry system. The logit model was used because it is computationally easier to use and leads itself to a meaningful interpretation than the other types such as probit and tobit (Gujarati, 1995). The logistic distribution function for practicing the fruit-tree based agroforestry system can be specified as (Hosmer and Lemeshew, 1989):

$$P_i = \frac{1}{1 + e^{-z_i}} = \frac{e^z}{1 + e^z} \quad (1)$$

Where $P_i$ is the probability of practicing the fruit-tree based agroforestry system for the $i^{th}$ farmer and it ranges from 0-1. P is the observed response of the $i^{th}$ farmer (that is, the binary variable, P =

**Table 1.** Equations of economic performance indicators with critical min values.

| Financial indicator | Computation | Critical min value |
|---|---|---|
| Net present value | $NPV = \sum_{i=1}^{t} \frac{Bt}{(1+r)^t} - \sum_{i=0}^{t} \frac{Ct}{(1+r)^t}$ | NPV>0 |
| Benefit cost ratio | $BCR = \sum_{i=1}^{t} \frac{Bt}{(1+r)^t} \Big/ \sum_{i=0}^{t} \frac{Ct}{(1+r)^t}$ | BCR>1 |
| Annual equivalent value | $AEV = NPV / \sum_{t=1}^{n} \frac{1}{(1+i)^t}$ | |

Where t = time in number of years (1, 2,… n), r = discounting rate, $B_t$ = total revenue earned from sale of the outputs in year t, Ct = total cost incurred from the different activities at the time of production in year t.

1 for a practitioner, P = 0 for a non practitioner) and $Z_i$ is a function of *m* explanatory variables ($X_i$) which is expressed as:

$$Z_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + - - - + \beta_m X_m \quad (2)$$

Where $\beta_0$ is the intercept and $\beta_i$ are the slope parameters in the model. The slope tells how the log-odds in favor of being practitioner of the fruit-tree based agroforestry system as independent variables change.
If $P_i$ is the probability of occurrence of an event, then (1 - $P_i$), the probability of non occurrence event will be (Gujarati, 2004):

$$1 - P_i = \frac{1}{1 + e^{Z_i}} \quad (3)$$

Then, $P_i$/ (1-$P_i$) are simply the odds ratio in favor of occurrence of the event - the ratio of probability of occurrence of an event to the probability of non-occurrence of an event will be specified as:

$$\frac{P_i}{1 - P_i} = \frac{1 + e^{Z_i}}{1 + e^{-Z_i}} = e^{Z_i} \quad (4)$$

And

$$\frac{P_i}{1 - P_i} = \frac{1 + e^{Z_i}}{1 + e^{-Z_i}} = e^{\beta_0 + \sum_{i=1}^{m} \beta_i X_i} \quad (5)$$

$Z_i$ - is a function of m- explanatory variables ($X_i$) which is also expressed by taking natural log in both sides of Equation (4):

$$Ln\left[\frac{P_i}{1 - P_i}\right] = Ln\left[e^{\beta_0 + \sum_{i=1}^{m} \beta_i X_i}\right] = e^{Z_i} \quad (6)$$

If the disturbance term Ui is taken into account, the logit model becomes:

$$Z_i = \beta_0 + \sum \beta_i X_i + U_i \quad (7)$$

## RESULTS

### Demographic and socio-economic characteristics of households

Among the total sampled respondents, 83.22% were male headed households and 16.78% were female headed households. The average family size of the respondents was 6.94. The mean age of the respondents was about 43.53 years. About 28.77% of the respondents were unable to read and write whereas 71.23% of the respondents had attended from primary school up to college at the time of survey. The average educational attainment of the respondents was 4.16 years.

On the average, total land holding in the area was 0.43 ha (Table 2) representing land shortage is a basic problem that resulted in small scale production. The average number of livestock in the area was small and it was about 2.18 tlu (tropical livestock unit). The main problems in the area were shortage of grazing lands and insufficient health care service. 70.33% of non practitioners used irrigated agriculture. However, only 47.27% of practitioners used irrigated agriculture. The major components of the agroforestry system were avocado, banana, papaya, coffee and enset.

### Economic performance evaluation of the two land use systems

The NPV measures the present value of the streams of net benefits from any land use system. In order for the land use system to be acceptable, the NPV must be greater than zero (that is, positive). Note that the life span of the whole practice is 25 years. So the costs and benefits are the sum of the whole life of each practice. Since the net present values of mutually exclusive projects with different lives cannot be compared, we

**Table 2.** Household characteristic and land holding (ha) of the sampled respondents.

| Household characteristic | Family size | Age (year) | Education (Year) | Total land holding (ha) |
|---|---|---|---|---|
| Minimum | 2 | 20 | 0 | 0.2 |
| Maximum | 11 | 75 | 12 | 2 |
| Mean | 6.94 | 43.53 | 4.16 | 0.43 |

replicated the projects till they have the same life (or) convert the net present values into annuities. A discount rate of 10% was used.

Results of the cost benefit analysis (Table 3) revealed that the NPV of fruit-tree based agroforestry system was found to be about one and half times higher than the NPV of sugarcane, more than two times higher than the NPV of the sequential monocrop of tomato with maize, and nearly four times higher than the NPV of the sequential monocrop of potato with maize, respectively. The BCR of fruit-tree based agroforestry system was also found to be 1.41 times higher than the BCR of sugarcane monocrop, 1.81 times higher than the BCR of the sequential monocrop of tomato with maize, and 2.38 times higher than the BCR of the sequential monocrop of potato with maize, respectively.

The annual equivalent value (AEV) for the fruit-tree based agroforestry system indicated that the expected annual income of the fruit-tree based agroforestry system was 80,600.28 ETB per annum, whereas the AEV for sugarcane monocrop was 52,089.97 ETB per annum, AEV for the sequential monocrop of tomato with maize was 36,445.68 ETB per annum, and AEV for the sequential monocrop of potato with maize was 20,625.17 ETB per annum.

The sensitivity of NPVs was examined in both benefits and costs structures of the model. At the best case scenario (increase in price/yield and simultaneous decline in discount rate and wage), the monocropping systems were more favored than the fruit-tree based agroforestry system. Whereas, at the worst case scenario (decline in price/yield and simultaneous rise in labor and discount rate), the agroforestry-based system was highly favored than the monocropping systems. The result of the sensitivity analysis is presented in Appendix 1.

### Determinants of fruit tree based agroforestry practice

Binary logistic regression was run to identify factors affecting practice of FTBAFS (Table 4). The Goodness of Fit test showed that the overall model fits reasonably well as indicated by a Wald Chi-square value of 39.72 with 10 degrees of freedom with a probability value of over 0.0000. Five variables were significant at the 10% probability level. These variables were size of land holding (landsize), farming experience (experie), nearness to the main road (road) , total income of the household (income), and family labor (famlabor).

Consistent with the prior expectation nearness to the main road was found to be negatively associated with agroforestry practice. The odd ratio of 0.02 indicated that, other factors held constant, the likelihood of a household in favor of practicing the fruit-tree based agroforestry system increases by a factor of 0.02 over those who are practicing monocropping land use system. Farming experience was found to have a positive effect at less than 1% significance level on the adoption of fruit-tree based agroforestry system. The experience of the farmers in practicing the modern agroforestry system is attributed to practicing the traditional agroforestry system. The odd ratio of 1.21 indicated that, other factors held constant, the likelihood of a household in favor practicing the fruit tree based agroforestry system increases by a factor of 1.21 over those who are practicing the monocrop land use. Contrary to the prior expectation, family labor was negatively associated with the practice of fruit-tree based agroforestry system at less than 5% significance level. The odds ratio showed that, other variables held constant, the probability of practicing the fruit-tree based agroforestry system will decrease by 0.573 than for those who practice the monocropping systems.

Total land size influenced the probability of adopting fruit-tree based agroforestry system at less than 10% significance level. The odds ratio for land size indicated that, other variables held constant, the odds of practicing the fruit based agroforestry system will increase by 3.24 when compared to those who do not practice it. In line with the prior expectation, income is positively associated with the adoption of fruit-tree based agroforestry system at less than 10% significance level. The odds ratio 1.05 indicated that, other variables held constant, the probability of the household to practice the fruit-tree based agroforestry system will increase by a factor of 1.05 when compared to those who practice monocropping systems.

## DISCUSSION

### Economic performance evaluation

The results from the three economic performance indicators showed that the fruit-tree based agroforestry system has the highest NPV, BCR and expected annual

**Table 3.** Results of NPV, BCR and AEV per ha (Ethiopian Birr (ETB)).

| Financial indicator | Agroforestry land use | Mono crop land use | | |
|---|---|---|---|---|
| | FTBAFS | Sugarcane | Tomato + maize | Potato + maize |
| NPV | 731,608.35 | 472,820.76 | 330,817.59 | 187,214.76 |
| BCR | 3.43 | 2.43 | 1.90 | 1.44 |
| AEV | 80,600.28 | 52,089.97 | 36,445.68 | 20,625.17 |

1USD = 17.80 ETB.

**Table 4.** The maximum likelihood estimates of the binary logit model for FTBAFS.

| E/variables | Coefficients | Std. Err. | Significance (p value) | Odds ratio |
|---|---|---|---|---|
| landsize | 1.177 | 0.697 | 0.092 | 3.243* |
| tlu | -0.070 | 0.140 | 0.617 | 0.932 |
| experie | 0.188 | 0.056 | 0.001 | 1.207*** |
| educ | 0.027 | 0.108 | 0.805 | 1.027 |
| road | -3.932 | 0.797 | 0.000 | 0.0196*** |
| market | 0.0206 | 0.063 | 0.745 | 1.021 |
| income | 0.049 | 0.027 | 0.069 | 1.051* |
| offfarm | 0.236 | 0.588 | 0.688 | 0.789 |
| famlabor | -0.557 | 0.226 | 0.014 | 0.573** |
| gender | -0.317 | 0.776 | 0.683 | 0.729 |
| _cons | 4.204 | 1.903 | 0.027 | |

Wald chi2(10) = 39.72; N = 149; Prob > chi2 = 0.0000; Pseudo R2 = 0.5955; Log pseudo likelihood = -38.72857; *, **, and *** represents statistical significance at 10%, 5% and 1%, respectively.

income (AEV) followed by monocrop of sugarcane, sequential monocrop of tomato with maize, and sequential monocrop of potato with maize, respectively. Therefore, this result revealed that the agroforestry land use is the best land use practice with the highest financial return than that of the monocrop land use.

In line with this, a study conducted in Vietnam by Kham and Thuy (1999) revealed that AFSI (forest trees + fruit trees + annual crops) is the most profitable system with a net present value (NPV) of $5,950 and an annualized income of $874. Another study conducted in Sri Lanka on economic feasibility and biological productivity of coconut-based agroforestry revealed that the NPV of agroforestry was about 4-5 times greater than that of the monocrop at different discount rates, indicating that investment in mixed farming is more beneficial than the adoption of monocropping (Peiris et al., 2003). In addition, a finding in Bangladesh in the case of multi-strata agroforestry and traditional monocropping agriculture revealed that agroforestry was substantially more profitable in terms of net present value (NPV), benefit-cost-ratio (B/C), internal return rate (IRR) and annual net cash rate (ANCR) than the traditional system (Rahman et al., 2007).

Accordingly, Muhammad et al. (2011) also confirmed that in agroforestry, the combination of trees with the annual crops increases the overall farm income of per unit land area of farmland and reduces the risks and broadens the sphere of alternatives. Similarly the finding of Neupane and Thapa (2001) who studied the impact of agroforestry intervention on farm income under the subsistence farming system in Nepal, revealed that the mean annual net return of farming 'with' agroforestry was estimated to be $1582/ha compared to $804/ha 'without' agroforestry intervention.

## Factors influencing the practice of FTBAFS

In this study, variables that showed significant differences for the practice of fruit tree based agroforestry practice were nearness to the main road, farming experience, family labor, total household income and total land holding.

Nearness to the main road was found to be negatively associated with agroforestry practice. Therefore, those residing far away from the main road have fewer tendencies to practice the agroforestry land use system as compared to those who reside nearby the main road. The nearer to the main road, the better would be the access to market and information so that the better would be the rate of adoption (practicing).

Farming experience was another variable found to be positively associated with agroforestry practice . In line

with this finding, a study conducted in western Sudan by Gibreel and Bauer (2007) found positive effect of farming experience on gum agro-forestry system adoption at 1% level of significance. Another finding by Nkamleu and Manyong (2005) on factors affecting the adoption of agroforestry practices by farmers in Cameroon revealed that farmers' experience positively and significantly influences the adoption of improved fallow, suggesting that the higher the level of experience, the greater the likelihood of farmers using improved fallow.

In this study, family labor negatively affected agroforestry adoption, suggesting that monocropping systems are labor intensive farming system than the agroforestry-based system. In line with this result, a study on factor affecting farmers land use options in gum-belt of western Sudan revealed that total working days is found to be negatively associated with adoption decision (Gibreel and Bauer, 2007). However, a contrasting finding by Thangata and Alavalapati (2003) who studied on agroforestry adoption in southern Malawi revealed that a positive relationship between agroforestry adoption and the number of people in the household who contribute to farm work. Another contrasting finding by Nkamleu and Manyong (2005) also revealed that household family size is positively and significantly related to farmers' adoption of agroforestry implying that larger families with increased labor supply are more likely to adopt the technologies than smaller households.

As an important variable, total land holding was positively associated with the practice of fruit tree based agroforestry system. This finding complies with the finding by Siddig (2008) who studied the adoption determinant factors in Sudan and in the finding it is revealed that the total area of owned land ranked as an important significant determinant of the respondents' innovativeness. In the same study, the positive relation between land holding and adoption is justified as since one of the main constraints of agroforestry adoption is the perception that trees compete with agricultural crops for land particularly when the size of holding is small, this perception will not hold true when the size of the farmers holding is large enough to accommodate both agricultural crops and trees (Siddig, 2008).

Income is another important variable that positively affected the practice of fruit tree based agroforestry system. This result complies with the finding of Kham and Thuy (1999) which revealed that income of the household is a very important factor that affects the adoption decision of farmers in Vietnam. Thus, the higher the income of the farmer, the greater is the probability of adoption.

## CONCLUSIONS AND POLICY IMPLICATIONS

Agroforestry land use, the combination of fruit trees with perennials like Enset and Coffee, generated the highest economic profit than the crops cultivated in the monocrop land use system. It is economically more attractively profitable, labor saving and less risky investment with a diversified income sources than the monocropping systems. However, the practice of fruit tree based agroforestry system was influenced by a number of factors. Total land holding, family labor, farming experience, nearness to main road, and gross annual income significantly affected the practice of fruit tree based agroforestry system. Therefore, differences in the above factors should be considered in promoting the fruit tree based agroforestry.

Based on the findings, the following recommendations are offered:

i. The economic performance evaluation has shown that the fruit-tree based agroforestry system is financially more attractive than that of the different crop components in the monocropping systems. Hence, the fruit-tree based agroforestry should be promoted by smallholder farmers in the study area and nearby localities,
ii. It was found that the agroforestry practice is more sensitive to high cost of capital (discount rate). So, it would be better to provide improved varieties of perennials with short maturity period to counteract the effect of higher discount rate,
iii. Since only the marketable benefits were evaluated in this study, further study is needed to estimate the total economic value of the fruit-tree based agroforestry system including the environmental functions served by the system.

## Conflict of Interest

The authors have not declared any conflict of interest.

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**Appendix 1.** Results of sensitivity analysis.

| Change of key variable | FTBAFS (%) | Sugarcane (%) | Tomato + maize (%) | Potato + maize (%) |
|---|---|---|---|---|
| Price increase (10%) | 114.11 | 116.61 | 120.75 | 132.62 |
| Price decrease (10%) | 85.89 | 82.59 | 78.51 | 67.46 |
| Wage increase (10%) | 98.70 | 98.47 | 96.56 | 94.82 |
| Discount rate increase (10%) | 89.37 | 91.67 | 92.71 | 92.7 |
| Best case scenario (p↑,w↓, i↓) | 129.29 | 129.21 | 134.48 | 149.22 |
| Worst case scenario (p↓, w↑, i↑) | 75.50 | 74.91 | 69.58 | 57.7 |

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*Full Length Research Paper*

# Assessment of plant biodiversity on and off mature stands of *Androstachys johnsonii* Prain and *Colophospermum mopane* (J.Kirk ex Benth.) J. Leonard

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**The species that were growing on the stands of *C. mopane* and on open space stands weighed more than those on the stands of *A. johnsonii* in both loam and sandy soil areas. This is probably an indication that there are some toxic chemicals that are excreted from various parts of *A. johnsonii* that caused the suffering and hence lesser weights of the understory species of *A. johnsonii*. The conditions were found to be unfavorable for the majority of the understory species to grow and flourish well under the canopies of *A. johnsonii*. This therefore, might imply that even though *A. johnsonii* possess allelochemical materials, some understory species may adapt to such conditions in the proximity of its vicinity. The decomposition of different parts of the plant on the ground surface under *A. johnsonii* canopies probably results in relatively highest concentration of allelochemical materials in the soil on *A. johnsonii* stands. Limiting factors like shading, temperature, water availability, and soil type may not necessarily be causes of the observed differences as the investigation was carried out from the same areas. The present investigation revealed that *A. johnsonii* in woodlands chokes its understory plants and such plants might eventually die out.**

**Key words:** Allelochemical materials, *Androstachys johnsonii, Colophospermum mopane,* biodiversity, impact.

## INTRODUCTION

Plant species grow in very complex situation wherein each one is in demand of space, nutrients, water as well as other resources needed for their survival. In this regard, plants begin to compete with each other due to short supply of resources. Plants then realize the danger of being outcompeted, as such, they develop some defensive mechanisms like emergence of superficial lateral roots which draw water before penetrating into the deeper horizons of the soil and dense canopies which will shade their understory species; ultimately such understories get outcompeted. The introduction of certain species in the area sometimes may result in the reduction of species that originate there. For example, an annual grass called cheatgrass (*Agropyron spicatum*) from

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Europe was accidentally introduced to the area. From the time of its introduction to the present, ranchers have noticed an enormous increase in the abundance of cheat grass and an equally notable decrease in the abundance of blue bunch wheatgrass (Mack, 1981). What caused the shift? Mack (1981) pointed out that the presence of cheat grass greatly reduced the growth and survival of blue bunch wheatgrass.

In other systems, plants interact with others mutualistically, commensualistically and amensualistically instead of competing with them. Amensualism is an interaction that depresses one organism while the other remains stable. One example of amensualism is allelochemical interaction, which involves the inhibition of one organism by another through a release of metabolic by-products into the environment. The by-products which are normally secondary metabolites are selectively toxic affecting some species but not others. One of the most famous examples of allelopathic plants is Black Walnut (*Juglans nigra*). The chemical responsible for the toxicity in Black Walnut is jug lone and is a respiratory inhibitor. Solanaceous plants such as tomato, pepper and eggplant are especially susceptible to jug lone (http: //ceirp.cornell.edu/projects/AR/Allelopathy.html.). These plants, when exposed to the allelotoxin, exhibit symptoms such as wilting, chlorosis and eventually death. Plants that have been observed to be tolerant to jug lone include lima bean, beets, carrot, corn, cherry, catalpa, violets and many others (http://ceirp.cornell.edu/projects/AR/Allelopathy.html.).

Allelochemical materials are viewed by some biologists as simply the mechanism for an aggressive form of competition. Mutualism is a mutually beneficial relationship between two or more organisms, especially one in which neither organism can survive without another. Common examples of mutualism are lichens (algae + fungi), mycorrhizae (fungi + higher plants), symbiotic nitrogen-fixation (bacteria or blue-green algae + higher plants), pollination (insects, birds or mammals + flowering plants), zoochory (animal dispersal of plant propagules), and myrmecophytes (ants + woody plants). Commensualism is a relationship between two organisms in which one lives on or in another organism that is not harmed (or benefitted) by its presence. Commensualism includes epiphytic orchids or bromeliads living on the branches of host trees.

To a large degree, decomposers in the soil and litter beneath communities are affected by the species shedding the litter and penetrating the soil with the roots. For example, soils beneath northern conifer forests are largely acidic, because conifer litter is acidic and its decomposition influences soil pH (Maguire and Forman, 1983). Although forest understory herbs are notably patchy, and over story tree seedlings may be positively or negatively associated with the herbs (Maguire and Forman, 1983), it is likely that amensualism is usually involved. Instead, herb pattern is often determined by the physical quantity and quality of litter, by uneven distribution of soil nutrients, and by micro environmental patterns of soil drainage (Rogers, 1985). Cater and Chapin (2000) studied the effects of competition and microenvironment on boreal tree seedlings. Allelopathy and allelochemicals in rice weed management have extensively been studied (Asaduzzaman et al., 2010).

Plant biodiversity will be less on *A. johnsonii* stands than on the stands of *C. mopane* and on open space stands, whilst plant biodiversity on *C. mopane* stands will be less than on open space stands. *Androstachys johnsonii* is known to be allelopathic to plant species that grow under it whilst *C. mopane* has not been proven as such. Open space stands or stands without huge canopies are generally also not known to be allelopathic. Accordingly it should not be uncommon to observe plant biodiversity more on open space stands than on *A. johnsonii* stands which are both allelopathic and canopied. It should also not be puzzling to further on find more plant species on open space stands than on *C. mopane* stands because of shading from *C. mopane* canopies. What could be the long-term impact of *A. johnsonii* and *C. mopane* on both species diversity and abundance in woodlands and hence in the broader management of these ecosystems?

The aim of this current study is to assess plant biodiversity on and off *A. johnsonii* and *C. mopane* stands. The presence of one species may limit the distribution of others through competitive interaction, such as allelopathy. Allelopathy is a type of competition that occurs between any two or more species that use the same types of resources and live in the same place. For instance, solanaceous plants, such as tomato, pepper, and eggplant are especially susceptible to juglone (a toxic chemical released by walnut tree) (Coder 1999). The objective of this study is to acquire more knowledge on woodland dynamics and hence add such knowledge to the already existing know-hows in the management of woodlands where *A. johnsonii* and *C. mopane* prevail.

The study was conducted at Makuya Nature Reserve in the far northeastern part of the Limpopo Province, South Africa. Makuya Nature Reserve is about 104 kilometers from Thohoyandou town; Thohoyandou town is the former capital city of the former Venda homeland. The study area lies between 30° 50′E and 31° 05′E and 22° 25′S and 22° 35′S. The soil type of the study area varies from loamy sand to clayey in the undulating granitic landscape of the northern Kruger National Park. Annual summer rainfall is from 250 to over 500 mm.

The vegetation is classified as Mopane Bushveld (Low and Rebelo, 1996), and is characterized by a fairly dense growth of Mopane (*Colophospermum mopane*) and mixtures of *Combretum apiculutum*, associated with *Acacia nigrescens*, *Adansonia digitata*, *Commiphora spp*, *and Terminalia pruniodes* and on rocky outcrops, *Androstachys johnsonii*. The sandy-loam soils, low

**Figure 1.** A photograph illustrating a 4 m × 10 m quadrat under the canopies of *Androstachys johnsonii* (*A. johnsonii*) plants that were growing on loam soil area at Makuya Nature Reserve.

**Figure 2.** A photograph showing a 4 m x 10 m quadrat under the canopies of *Colophospermum mopane* (*C. mopane*) plants that were growing on loam soil area at Makuya Nature Reserve.

rainfall, high temperatures and lack of frost influence the distribution of this vegetation type (Low and Rebelo, 1996).

## MATERIALS AND METHODS

4 m x 10 m quadrats were constructed using a 50 m measuring tape. The figures referred to here are representative samples of how the forty quadrats were constructed and sampled. The plant species that were found in each quadrat were counted and then collected. All collected plant species were put in separate envelopes for biomass determination in one of the biology laboratories of the University of Venda. In the process of collection secateurs were used to cut the plants that could not be uprooted. A digital camera was used to take the photographs of sites in the reserve where quadrats were constructed (Figures 1 to 6).

After measuring their fresh weights/biomasses, the species were then oven dried at 60°C for 48 h. The dry weights were then measured through the use of mettler balances after the 48 h drying period. The dried materials were again put back into the oven to re-dry them until constant weights were achieved. Such constant weights were the ones considered for final recordings.

## RESULTS

Generally there were very few or no plant species seen growing in the stands of *A. johnsonii*. In cases were species occurred, they were found not surviving well as compared to the plant species that grew in both *C. mopane* and open space stands on both loam and sandy soil areas. Species biomass and number of species that were understories in the stands of *A. johnsonii* were less as contrasted to those that were understories in both *C. mopane* and open space stands on both loam and sandy soil areas (Figures 1, 4 and 2, 5).

Both the biomass and number of species of almost all the individual species that were growing on all the three categories of stands, that is, *A. johnsonii*, *C. mopane* and open space stands on both loam and sandy soils were found to differ significantly (Table 1a); the biomass and numbers of species that were understories of *A. johnsonii* were less than the understories of *C. mopane* and the species that were growing on open space stands (Figures 1, 4 and 2, 5). It is only between *A. johnsonii* versus *C. mopane* stands on loam soil areas where no significant difference between the numbers of species was found (Table 1a). Significant difference was also found between the biomass and numbers of the species that were growing on *C. mopane* stands and on open space stands (Table 1a); the species biomass and numbers of the species that were growing on open space

**Figure 3.** A photograph showing a 4 m x 10 m quadrat on a loam soil area without overstory plants (open space stand) growing thereon at Makuya Nature Reserve.

**Figure 4.** A photograph illustrating a 4 m x 10 m quadrat under the canopies of *A. johnsonii* plants that were growing on sandy soil area at Makuya Nature Reserve.

**Figure 5.** A photograph showing a 4 m × 10 m quadrat under the canopies of *C. mopane* plants that were growing on sandy soil area at Makuya Nature Reserve.

stands were greater than those of the species that were growing on *C. mopane* stands (Figures 1, 4 and 2, 5).

Generally the number of individuals of the different species that were found occurring on both loam and sandy soil areas were found to differ (Table 1b); with the highest number of individuals appearing on open space stands followed by those that occurred on *C. mopane* stands and then the ones that were growing on *A. johnsonii* stands (Table 1b). Significant difference was noticed between the numbers of individuals that were

**Figure 6.** A photograph illustrating part of a 4 m x 10 m quadrat on a sandy soil area without overstory plants (open space stand) growing thereon at Makuya Nature Reserve.

**Table 1(a).** One-way analysis of variance of biomass of species collected from loam soil areas, biomass of species collected from sandy soil areas, number of species collected from loam soil areas, number of species collected from sandy soil areas, number of individuals of different species collected from loam soil areas and number of individuals of different species collected from sandy soil areas.

| Parameter | SS | df | MS | F-values | P |
|---|---|---|---|---|---|
| Biomass of species collected from loam soil areas | 1816.91 | 2 | 908.45 | 19.30 | 0.000 |
| Biomass of species collected from sandy soil areas | 417.79 | 2 | 208.89 | 12.81 | 0.00 |
| Number of species collected from loam soil areas | 1799.62 | 2 | 899.81 | 185.88 | 0.00 |
| Number of species collected from sandy soil areas | 992.27 | 2 | 496.13 | 435.8 | 0.00 |
| Number of individuals of different species collected from loam soil areas | 9643805.0 | 2 | 4821903 | 3.75 | 0.03 |
| Number of individuals of different species collected from sandy soil areas | 2460144 | 2 | 1230072 | 3.98 | 0.03 |

SS refers to sum of squares, df refers to degree of freedom and MS refers to mean of squares. $P < 0.05$.

**Table 1(b).** Duncan multiple range test analysis of the number of species and species biomass collected from *A. johnsonii* stands, *C. mopane* stands and open space stands. A, B, C and D refer to No. of species that were growing on loam soil areas, biomass of species that were growing on loam soil areas, No. of species that were growing on sandy soil areas and biomass of species that were growing on sandy soil areas respectively.

| Treatments | Groups | | | | Replicates |
|---|---|---|---|---|---|
| | A | B | C | D | (n) |
| *A. johnsonii vs C. mopane* | ns | * | ** | * | 40 |
| *A. johnsonii* vs open space | ** | ** | ** | ** | 40 |
| *C. mopane* vs open space | ** | ** | ** | ** | 40 |

ns, * and ** refer to not significant, significantly different and highly significantly different respectively. $P < 0.05$.

collected from *A. johnsonii* stands versus those that were collected from open space stands and also from those that were collected from *C. mopane* versus those that were collected from open space stands.

## DISCUSSION

Although *A. johnsonii* and *C. mopane* shared the same kind of environmental conditions, it was indeed intriguing to note that the wellbeing of both common and non-common factor understory plant species of *A. johnsonii* and *C. mopane* were not matching; the understories of *C. mopane* were more flourishing than those of *A. johnsonii*. Comparing the numbers of plant species that were found in all plots under the canopies of *A. johnsonii* versus those under *C. mopane* canopies, it was somehow visually tempting to conclude that *A. johnsonii* is somehow hindering the growth of its understories. Puzzling was nevertheless the observation where the number of the understory species on *A. johnsonii* stands on loam soil areas was found not to differ significantly from the number of species on *C. mopane* stands also on loam soil areas, the number of species on *A. johnsonii*

stands and *C. mopane* stands on sandy soil areas however differed significantly. The probable cause of this manifestation might be because loam soils are rather nutrimentally rich and could therefore confound the true outcome of the expected events in ecosystems, in this instance the real effects of allelopathic materials on understory plants. Plants growing on sandy soils (that is, soils with little or no nutrients at all) infested with allelochemicals are inflicted with allelochemicals only; hence a cogent significant difference between the number of species which are understories of *A. johnsonii* versus those which are understories of *C. mopane* on sandy soil areas.

The species that were growing on the stands of *C. mopane* and on open space stands weighed more than those on the stands of *A. johnsonii* in both loam and sandy soil areas. This is probably an indication that there are some toxic chemicals that are excreted from various parts of *A. johnsonii* that caused the suffering and hence lesser weights of the understory species of *A. johnsonii*. These differences might therefore persuade one to allege that there is better life off *A. johnsonii* stands than in their stands. Even though conditions were found not to be favorable for the majority of the understory species to grow and flourish well under the canopies of *A. johnsonii*, there was at least an exception of about ten percent. Rice (1995) pointed out that the response of plants to allelochemical materials differs from one species to the other due to the genetic make-up of any particular species. The presence of genetic variation in response to environmental cues is necessary for the ability of populations to evolve adaptations to the local environment (Catrine and Ehlers, 2010). Similarly, Ehlers and Thompson (2004) conducted studies where they checked the germination and growth rates of the grass *Bromus erectus* which was planted on the soil collected from thyme chemotype. The results showed *Bromus erectus* adapting well by germinating and growing on the soil collected from thyme chemotype. The above mentioned studies demonstrate that plant allelochemicals can act as a strong selective force driving evolution in plant-plant interaction. Variation in performance and sensitivity among individual plants to a plant allelochemical has been documented (Callaway et al., 2005a). This therefore might imply that even though *A. johnsonii* possess allelochemical materials, some understory species may adapt to such conditions in the proximity of its vicinity.

One would naturally and perhaps normally expect to see vigorous growth and flourishing of understory species occurring on *A. johnsonii* stands because of the amount of litter that is available in such stands. Surprisingly, the opposite was found to be the case under the canopies of *A. johnsonii*. Allelochemical materials possessed by *A. johnsonii* are probably contained in different parts of the tree including leaves, barks, fruits and roots. Decomposition of such different parts in the ground surface under *A. johnsonii* canopies probably results in relatively highest concentration of allelochemical materials in the soil on *A. johnsonii* stands.

The fact that the number of species and species biomass of plant species that were growing on *C. mopane* stands were lesser than those of species that were growing on open space stands on both loam and sandy soils might be an indication that *C. mopane* also releases some allelochemicals that have negative impact on its understories. Such allelochemicals are probably not as deleterious as those possessed by *A. johnsonii* because throughout the current research we found that the understory species of *A. johnsonii* were both less in numbers and species biomass to those of *C. mopane.* On the other hand the lesser number of species on *C. mopane* stands versus those that were on open space stands might be because of the shading of understories by *C. mopane.*

Limiting factors like shading, temperature, water availability, soil type and others may not necessarily be causes of the observed differences in this study because of the fact that both *A. johnsonii* and *C. mopane* stands of this current study were sampled from the same areas. This current study survey revealed that the prevalence of *A. johnsonii* in woodlands chokes its understory plants and such understories might eventually die out.

## Conclusion

Since phytochemical materials appear to be fatal to the growth of some plant species in association with those that secrete such allelochemicals, it would therefore be wise for managers of Makuya Nature Reserve and other reserves in South Africa and other countries outside South Africa to take precautionary measures. They may have to reduce the number of species or stands of species such as *A. johnsonii* in the reserves. The understory species are generally grasses, herbs and even shrubs which serve as food for the game like water buck, impalas, nyalas and others. It is therefore, necessary and economical to promote conditions that are conducive to an increase in the number of plants such as grasses, herbs and shrubs for these are food to some animals in the nature reserves.

## Conflict of Interest

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Farmers' perception on climate change in Sokoto State, Nigeria

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**The study examined farmer's perception on changes in climate variables in Sokoto State. Eight local government areas in Sokoto East senatorial district were purposively selected due to their vulnerability to climatic changes. Proportionate sampling was employed to select the eight villages. A total of two hundred and twenty three (223) questionnaires were administered. Descriptive statistics was used to analyze socio-economic characteristics of the farmers. ANOVA was used to test the significant differences between climatic variables in ten years. The results indicated that majority of the respondents (67.9%) agreed that rain normally starts by April-May and that the month of August had the highest amount of rainfall; and 2010 recorded the highest amount of rainfall in ten years from 2000-2010. It was evident from the results that the highest dry spell was recorded in 2011. 56.1% of the respondents perceived that August was the period of lowest temperature (31.97°C) and 20.6% reported that the year 2008 had the highest temperature (36.91°C) in ten years, from 2000-2010. Farmers were aware of the increased change in the climatic indices. It is recommended that farmers need to be sensitized on the importance of afforestation programme to mitigate climate change.**

**Key words:** Perception, climate change, climate variables.

## INTRODUCTION

Climate change is a change in the statistical distribution of weather over long periods of time that ranges from decades to millions of years (Cramer et al., 2001). This usually refers to changes in the climatic variables such as temperature, rainfall, wind and humidity. The continuous increase in atmospheric concentration of carbon dioxide due to the release of gasses from the continuous burning of fossil fuel by human activities is predicted to lead to significant change in climate (Cox et al., 2000) more generally known as "global warming". Developed countries are responsible for most of the causes of this phenomenon that affects the developing countries of the world. For example Africa, with about 25% of the world's arable land, contributes only 10% to the global agricultural output (Jayaram et al., 2010). The increased frequency, intensity and magnitude of drought and floods have adversely impacted food and water security, water quality, energy and sustainable livelihoods of rural communities in the study area (AAI, 2006).

People have perceived changes in rainfall and

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temperature patterns over the years on evidences of climate change, the area south of the Sahara are worst hit. Mendelsohn et al. (2006) found that farmers' perception on climate change as affected by an increase in temperature; reduced intensity and distribution of rainfall in many African countries has improved.

Adger et al.(2007) perception on climate change showed that a significant number of farmers believe that temperature has already increased and that rainfall pattern has declined for African countries leading to low yield of agricultural crops, less vegetation for livestock and water for irrigation. Due to the limited water resources agricultural policies play a vital role in agricultural water management. Africa has the most population growth in the world while, the actual yield as percentage of potential yield is 40% for North Africa, and 30% for Sub-Saharan Africa (FAO 2012). Africa needs specific attention; Namara, et al. 2010, mentioned the role of agricultural water management as a panacea to reduce poverty in the world.

Valipour (2012) working in North Africa mentioned the status of irrigation and rain-fed agriculture in the world and summarized the advantages and disadvantages of irrigation system, and attempt to update irrigation information. The result shows that 46% of cultivated areas in the world are not suitable for rain-fed agriculture because of climate changes and other metrological conditions. The value of irrigation equipped areas as share of cultivated areas was 5.8% and value of water management areas as share of cultivated areas was 6.7% for Africa. Burney et al. (2013) argued favorably on the impact of investment in agricultural water management for green revolution in Africa, claiming that poverty was significantly reduced in irrigation equipped area than in rain fed areas. Frank et al. (2008) examined developing capacity for agricultural water management in current practices and future directions, and suggested increased attention to monitoring and evaluation of capacity development and closer link to emerging work on water governance. Wheater and Evans (2009) studies relationship between land use, water management and future flood risk, their study mentioned that apart from irrigation issue, water related implications of climate change for future land use remain relatively unexplored. To conserve usable water resources, land uses which increase evapotranspiration or rapid run off should be discouraged, particularly in the south and east, there is need for continuous efforts to maintain good chemical water gravity in rivers, and ground water resources constrains will limit opportunities to use irrigation as a counter to climate change, which in turn will influence where irrigation production can be located (Weatherhead and Howden 2009).

Ozon and Aisharif (2013) showed home owners irrigated more to meet the water need of their farmlands despite the restrictions imposed by their local government. Characteristics of land tenure and use policy during 30 years of small irrigation system operations in Niger have enhanced beneficiary incentives and project sustainability. Tilman et al. (2012) studied agricultural sustainability and intensive production practices. The use of new incentives and policies for ensuring the sustainability of agriculture and ecosystem services would be crucial to meet the demands of improving yields without compromising environmental integrity. Viata (2008) assessed water management in agriculture successfully using FAO database.

A close look at the historic weather records of Maiduguri (1986-1996) showed that rural people though not literate, have good knowledge of the changes in the climatic variables (Mendelsohn et al., 2005). The mean atmospheric temperature of the area has been on the increase since 1986 with low humidity. They also observed that the little rainfall received has been associated with flooding. Darkoh (1998) also reported climate change and variability in the Sahel region, and on the causes of desertification in the dry land of Africa. Similar observation had been reported by Kandji et al. (2006) on the climate change variability in the Sahel region and on the causes of desertification in dry land of Africa respectively. These observations also corroborate the scientific studies in general (IPCC, 2007). Thus climate change is already visible in the study area. While many factors continue to influence climate, human activities like overgrazing, coupled with bush burning and other forms of degradation of natural vegetation have become dominant forces (Darkoh, 1998.)

## MATERIALS AND METHODS

The study purposively considered eight local government areas of Gada, Wurno, Goronyo, Rabah, Illela, Gwadabawa, Isa, and Sabon Birni in Sokoto East. These areas are more prone to the effects of climate change. The number of villages and households in each local government are not the same; therefore thirty percent of the villages in each local Government were proportionately selected. A total of 223 questionnaires were administered.

Primary and Secondary data were collected: the primary data were collected using structured and open ended questionnaires on the socio- economic characteristics of the farmers and the level of farmer's perception on climate change. Secondary data on rainfall, temperature, wind and humidity, were obtained from the Metrological Centre, Sokoto. The data collected were subjected to descriptive statistical analysis (frequency and percentages) to analyze socio- economic characteristics of the farmers. Descriptive statistics was also used to measure the perception and awareness of the farmers. ANOVA was used to test the significant difference between climate variables over 10 years. Statistical package for social science (SPSS) was used for the analysis.

## RESULTS AND DISCUSSION

### Socio-economic characteristics of the respondents

Table 1 showed that 26.0% were within the age range of 22-32 years, 36.7% of the respondents were within the

**Table 1.** Socio-economic characteristics of the respondents (223).

| Variables | Frequency | Proportion (%) | Variables | Frequency | Proportion (%) |
|---|---|---|---|---|---|
| Age (years) | | | Occupation | | |
| 22-32 | 58 | 26.0 | Crop Production | 142 | 63.6 |
| 33-42 | 82 | 36.7 | Animal Production | 2 | 0.8 |
| 43-52 | 38 | 17.0 | Trading | 0 | 0.0 |
| 53-62 | 33 | 14.7 | Craft | 0 | 0.0 |
| 63-72 | 12 | 5.3 | Both | 79 | 36.1 |
| Total | 223 | 100 | Total | 223 | 100 |
| Gender | | | Cropping Sys. Pattern | | |
| Male | 221 | 99.1 | Commercial | 11 | 8.4 |
| Female | 2 | 0.9 | Subsistence | 212 | 91.6 |
| Total | 223 | 100 | Total | 100 | 100 |
| Marital Status | | | Farm Size (ha) | | |
| Married | 220 | 98.6 | 1-4 | 86 | 38.5 |
| Single | 3 | 1.4 | 5-8 | 78 | 34.9 |
| Divorce | 0 | 0.0 | 9-12 | 49 | 21.9 |
| Total | 223 | 100 | 13-16 | 8 | 3.5 |
| Family Size | | | 17-20 | 2 | 0.8 |
| 1-6 | 103 | 46.2 | Total | 223 | 100 |
| 7-12 | 72 | 32.3 | Seed (kg / hectare) | | |
| 13-18 | 31 | 13.9 | 1-40 | 125 | 56.9 |
| 19-25 | 17 | 6.7 | 41-80 | 37 | 17.1 |
| Total | 223 | 100 | 81-120 | 28 | 12.1 |
| Education | | | 122-160 | 17 | 7.5 |
| Primary | 71 | 30.8 | 161-200 | 8 | 3.2 |
| Secondary | 30 | 13.4 | 201-225 | 6 | 3.0 |
| Tertiary | 17 | 8.5 | Total | 223 | 100 |
| Quranic | 88 | 39.4 | Yield (kg / hectare) | | |
| Adult Education | 15 | 7.5 | 200-4000 | 138 | 61.8 |
| Total | 223 | 100 | 4001-8000 | 29 | 13.2 |
| | | | 8001-12000 | 18 | 7.5 |
| | | | 12001-16000 | 25 | 11.2 |
| | | | 16001-20000 | 7 | 2.9 |
| | | | 20001-23000 | 6 | 3.4 |

Source: Field Survey 2011.

age of 33-42 years; 17.3% of the farmers have attained the age range of 43-52, 14.7% of the farmers were 53-62 years of age, while 63-72 years of age range constituted 5.3% of the total respondents. Males formed the majority of the respondents with 99.1% and female the minority with 0.9%. This indicated that males dominate agricultural work force in the study area. It agrees with Adedoyin et al. (2005) who reported that male folks dominated the agricultural workforce in Nigeria. The high proportion of males to females may be because religion and custom play crucial roles in the livelihoods of the study area. For instance, males who are mostly the household heads, have more access to land and participate more in out door activities than females. Majority of the respondents (98.6%) in the area reported that they were married, while (1.4%) were single. This indicated that majority of the respondents have family responsibilities to cater for which affects their farming activities.

The result showed that 46.2% of the respondents had family sizes in the range of 1-6, (46.2%) 7-12 (32.3%) in the range 13-18, (3.9%) and those with 19-25 members per household were 6.7%. On the educational level, it was reported that 30.8% of the respondents in the study area had primary education, 13.4% had secondary education, 8.5% had tertiary education, 39.4% had quranic and 7.5% had adult education. The study showed that 63.6% of the farmers engaged in crop production, animal production 0.8% while other livelihood engagements including crafts, trading and animal rearing was 35.4%. Most of the farmers (91.6%) were engaged in

subsistence farming, while only few (8.3%) engaged in commercial agriculture. Farm size varied from 1 to 20 ha, with majority (38.6%) having between 1 and 4 ha, while 35.5% had between 5 and 8 ha. About 56.9% of the farmers planted 1 to 40 kg of seeds, 17.1% of the farmers had planted between 41 to 80 kg per hectare.

Yield obtained by the farmers ranged between 233-230000 kg of seed per ha. About 61.8% of the farmers harvested 200 to 4000 kg, 13.2% harvested 4001 to 8000 kg, 11.2% had 8001 to 12000, while others recorded 20,001 to 23000 kg of seed yield per hectare. Low levels of education, small farm sizes and low income in the selected communities had contributed to their vulnerability to climate change.

**Farmers' perception on climatic change**

Table 2, showed that 67.9% of the respondents agreed that rainfall starts by May, 32.2% believed that rain starts by June, while 0.8% maintained that rainfall starts by July. This indicated that the farmers are aware of the onset of rainfall. Information from the respondents revealed that drought causes stress to forest trees by affecting their life, most especially young trees, the higher the temperature, the higher the evapotranspiration and the lower the availability of water to the plant which in turn affects young trees, *Mangifera indica,* and *Psidium guajava* experience low production during drought. According to AAI (2006) "from July to August every year, there were heavy rains, the dry season starts in October and last until May. Rainy season starts late, sometimes as late as June; December and January were extremely cold months with frequent fogs. Water collects in rivers and ponds take longer time to dry up. Now they frequently dry up as early as November. "Majority of the respondents (87.0%) in the area reported that August had the highest amount of rainfall, 9.4%observed that September had the highest amount while 3.6% were undecided.

The result showed that 98.2% of the respondents were of the view that 2010 had the highest amount of rainfall in ten years, and 1.8% were undecided. 67.2% of the respondents believed that 2011 was the year of highest dry spell within ten years. 21.6% observed 2008 as the year of highest dry spell. While only 11.2% were undecided, 63.2% of the respondents opined that May-September had the highest duration of rainfall, 35.8% pointed June –Septembet, and 0.9% July-October as the highest duration of rainfall.

The study showed that 17.9% of the respondents said that 2010 had prolong harmattan, while 82.1% were undecided. 43.9% of the respondents viewed January as the period of low temperature, while 56.1% observed low temperature between December to January. 34.6% of the respondents opined that April was the period of highest temperature, while 64.4% observed highest temperature in April-May. 20.6% of the respondents agreed that 2008 had the highest temperature, 7.6% pointed to 2006, while 71.8% were undecided. The climate record of Sokoto state from April- May 2000-2010 indicated highest temperatures of 35.15-36.91°C, August 2000-2010 has the lowest rainfall of 42.88-95.55 mm. This implies that an increase in the average global temperature is very likely to cause death of livestock, agricultural and forest products. It can also lead to changes in precipitation and atmospheric moisture because of changes in atmospheric circulation and increases in evaporation. According to AAI (2006) rainfall and climate are affected by the mountain forest, and also partly by the Chiperoni Mountain in adjacent Mozambique. The climate is warm, hot and humid throughout most of the year, with annual temperature averaging 21-23°C and maximum temperatures around 32-35°C in the months of November and December.

During the dry season (June to mid-August), as a result of wind coming from the Chiperoni mountains, the phalombe plains, south of the Mulanje experience cooler weather. During this period , temperature on mount Mulanje occasionally drops to freezing point. Tea estates located within several kilometres of the southern foot of mount Mulanje experience dry season, rainfall and occasional mists and fogs. At the Mimoso Tea Research station (5 km from the mountain and 650 m above the sea level ), the average annual rainfall is 1,626 mm, with 16% falling during the dry season (that is May to October). 41.1% of the respondents believed that changes in weather condition most especially temperature affects people's health as evidenced by widespread diseases such as malaria and high blood pressure,as well as stress to trees. 52.5% of the respondents agreed that November- February had the highest dust storms, 37.2% mentioned December-February, only 10.3% pointed to November –December, as the highest dust storm period.

From the perspective of dust, many people experience eye problems and asthmatic attacks as one of the serious effect of dust storms. 55.2% of the respondents showed that November-January had the highest deposition of sand dune, 23.8% observed November-Febuary 2008 as the year with highest deposition of sand dunes, while only 21.0% believed it to be the period between December- Febuary. 53.4% of the respondents agreed that October had the lowest amount of wind, 35.9% agrued for August, 8.0% indicated May as the period of lowest wind speed, while 3.6% were undecided. 65.9% of the respondents agreed that May had the highest amount of wind, 22.9% argued for June, while only 11.2% argued for July. Wind affect trees during flowering period and also destroy tree branches. 80.3% of the respondents agreed that August had the highest humidity, 8.5% believed it to be July, 5.8% argued for June, and only 5.4% proved to be undecided. According to some respondents (59.2%) the lowest humidity was

**Table 2.** Farmers' perception on climate change.

| Variables | Frequency | Proportion% | Variables | Frequency | Proportion % |
|---|---|---|---|---|---|
| Onset of Rainfall | | | Persistent Dust Storms | | |
| May | 149 | 67.9 | Nov –Dec | 22 | 10.3 |
| June | 72 | 32.2 | Dec- Feb | 83 | 37.2 |
| July | 2 | 0.8 | Nov-Feb | 117 | 52.5 |
| Highest Amount of Rain | | | Deposition of Sand Dune | | |
| Aug | 196 | 87.0 | Nov- Feb | 53 | 23.8 |
| Sept | 21 | 9.4 | Dec-Feb | 47 | 21.0 |
| Undecided | 8 | 3.6 | Nov-Jan | 123 | 55.2 |
| Years with Highest Rainfal in 10 years | | | Period of Low Wind | | |
| 210 | 219 | 98.2 | Oct | 119 | 53.4 |
| Undecided | 4 | 1.8 | Aug | 80 | 35.9 |
| When was the highest Dry spell in 10 years | | | May | 18 | 8.0 |
| 2011 | 150 | 67.2 | Undecided | 8 | 3.6 |
| 2008 | 48 | 21.6 | Period of Highest Wind | | |
| Undecided | 25 | 11.2 | May | 147 | 65.9 |
| Duration of Rainfall in a year | | | June | 51 | 22.9 |
| May-Sept | 141 | 63.2 | July | 25 | 11.2 |
| June- Sept | 80 | 35.8 | Period of Highest Humidity | | |
| July –Oct | 2 | 0.9 | Aug | 179 | 80.3 |
| Prolong Harmattan within 10 years | | | July | 19 | 8.3 |
| 2010 | 40 | 17.9 | Sept | 12 | 5.4 |
| Undecided | 183 | 82.1 | June | 13 | 5.8 |
| Period of low Temperature | | | Period of Low Humidity | | |
| Dec- Feb | 98 | 43.9 | Feb | 75 | 33.7 |
| AUG | 125 | 56.1 | March | 132 | 59.2 |
| Period of Highest Temperature | | | April | 10 | 4.4 |
| April | 77 | 34.6 | May | 6 | 2.7 |
| April-May | 146 | 64.4 | | | |
| Years of Highest Temperature | | | | | |
| 2008 | 46 | 20.6 | | | |
| 2006 | 17 | 7.6 | | | |
| Undecided | 160 | 71.8 | | | |

Source: Field Survey 2011.

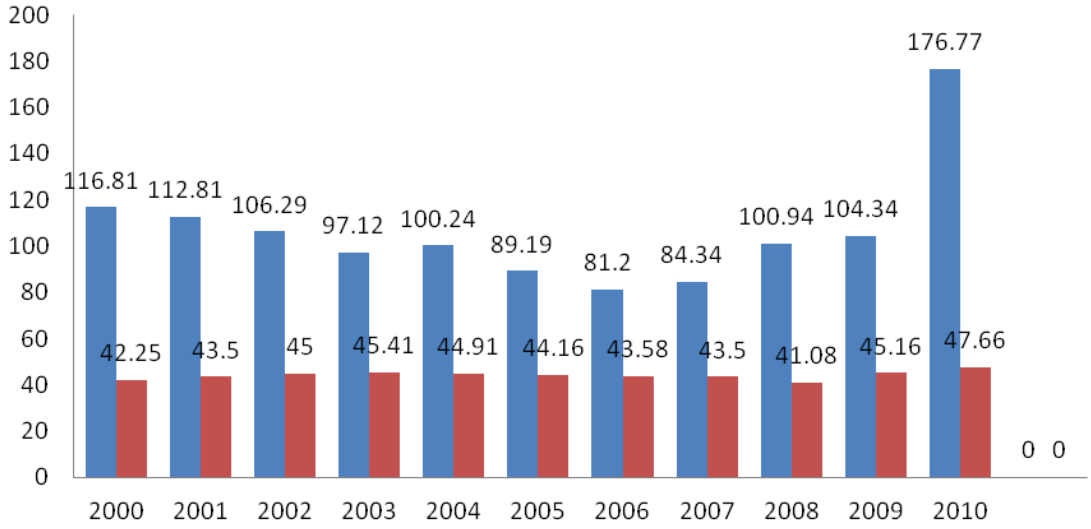


**Figure 1.** Distribution of wind and humidity over ten years. Source: Metrological Station, 2011 Sokoto.

recorded in March 33.7%, in February, 4.4% in April and only 2.7% discovered May as the period of lowest humidity. This indicated that there were significant differences between climatic indices and perception of farmers in Sokoto state.

The data of the mean annual rainfall and temperature for the period of 10 years (Figure 1) revealed that August 2001 and 2010 had the highest amount of rainfall within ten years. 2010 had highest rainfall within a ten- year period. That climatic record of Sokoto state showed that 2010 had the highest amount of rainfall within eleven years. 2011 had the highest dry spell within ten years, majority of the farmers were aware of the year with prolong harrmattan. From the perspective of temperature majority of the farmers believed that December-January had the lowest from 2001-2010, and April-May had the highest temperature.

The data of the mean annual wind and humidity for the period of 10 years (Figure 1) reveal that August had the highest humidity while March had the lowest humidity. October had the lowest wind speed while May had the highest wind. This indicates that the higher the velocity of wind the higher its impact on livelihood (UNFCC, 2007). The wind speed are particularly critical to the success of agricultural resources, which most negatively affects sustainbility. These results showed that both the perception of farmers and the climate records are in agreement.

According to AAI (2006) temperature data from the Mimosa Tea Research Foundation showed a steady increase in maximum and minimum temperatures over the past twenty years. From 1963-1986, the average maximum temperature howered around 28.5°C. The period between 1986 and 2006 saw an increase of over 1°C, with an average maximum temperature of 30.0°C. The minimum temperatures have shifted to a similar degree over the same period.

## Conclusion

The results indicated that farmers were aware that the area is getting warmer and drier with change in the time of rains. The implication is that farmers need to adjust their management practices to ensure that they make efficient use of the limited rainfall and water resources for food production and other needs.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Effects of some insecticidal chemicals under laboratory condition on honeybees [*Apis mellifera* L. (Hymenoptera: Apidae)] that forage on onion flowers

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**In Ethiopia, *Apis mellifera* L. are the most important honeybees to produce diverse bee products. In addition, bees also improve crop yields through their active and efficient pollination. However, they have been killed due to the misuse of various insecticides. For this reason this study was proposed to determine in the laboratory the level of toxicity of some insecticides used widely on honeybees foraging on onion flowers. Adult *A. mellifera* bandasii honeybees 21 to 24 days old were collected at the Adami Tullu Agricultural Research Center (ATARC) apiary. Easy to clean and well-ventilated 15 x 10 x 15 cm cages were used for feeding, contact and fumigation tests. Six insecticides: deltamethrin 2.5 EC, diazinon 60 EC, endosulfan 35 EC, lambda-cyhalothrin 5 EC, malathion 50 EC and profenofos 750 EC, were evaluated in the laboratory at the rates suggested for onion field spraying. Each treatment was replicated three times and arranged in a completely randomized design. All of the insecticides in the contact and feeding tests were toxic to honeybees and caused mortality within a few hours after exposure. In the fumigation test, profenofos did not cause honeybee mortality until the third hour, but the remaining insecticides caused varying mortality within 24 h.**

**Key words:** Insecticides, toxicity, honeybee, bee products and crop yields.

## INTRODUCTION

In Ethiopia, *Apis mellifera* races are the most economically important honeybees (Amssalu et al., 2004) which are used to produce diverse bee products, of which only honey and beeswax benefit small producers and the country as a whole. However, honeybees have been killed due to the misuse of various insecticides (MOWR, 2007). Reduction of honeybee colonies, which later result in reduction of bee products and crop yields are the major constraints of beekeeping in the country. This study was undertaken to determine the level of toxicity in the laboratory of some insecticides used widely, on honeybees that forage onion flowers.

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**Table 1.** Insecticides evaluated in laboratory toxicity tests on honeybees in the Adami Tullu District in 2012/2013.

| Common name | Trade name | Rate (L/ha) | Spray volume (L) |
|---|---|---|---|
| Deltamethrin | Ethiodemethrin 2.5% EC | 0.11 | 917 |
| Diazinon | Ethiozinon 60% EC | 0.50 | 1000 |
| Endosulfan | Ethiosulfan 35% EC | 0.20 | 1000 |
| Lambda-cyhalothrin | Karate 5% EC | 0.32 | 1000 |
| Malathion | Ethiolathione 50% EC | 0.20 | 1000 |
| Profenofos | Selecron 750% EC | 0.60 | 1200 |

## MATERIALS AND METHODS

Adult *A. mellifera* bandasii bees 21 to 24 days old were collected in the evening from hives without brood at the Adami Tullu Agricultural Research Center (ATARC) apiary and set 5 min at 0°C in a refrigerator to reduce their mobility and aggressiveness. Wooden well-ventilated and easy to clean 15 x 10 x 15 cm cages were used to determine acute toxicity tests in feeding, fumigation and contact tests for deltamethrin, diazinon, endosulfan, lambda-cyhalothrin, malathion, and profenofos. Handling procedures, treatment and observations were conducted during day time at the commercial rates recommended for controlling thrips in onion plants (Table 1).

### Feeding test

Twenty bees were placed in each of the test cages, which were placed randomly on a working table in the laboratory and after 2 h without food to homogenize their gut content they were exposed to the treatments and concentrations recommended. Only healthy bees were used. They were provided with 75 ml 50% honey solution in Petri dishes containing each of the aforementioned insecticide treatments. Insecticide toxicity was compared with that of 0.3 μg reference standard of the highly toxic dimethoate (Rogor) insecticide, and a separate control (bees fed on 50% honey solution only), with 3 replicates and a completely randomized design. Since bees did not survive 24 h after treatment, death losses were recorded at 0.5, 1, 2, 3, 4, 6, and 24 h after spraying (Amssalu, 2010).

### Contact test (residual exposure method)

The experiment and normal feeding (that is, 50% honey) were set as that for the feeding test above. 9 cm diameter filter papers were immersed in 100 ml of solutions containing the recommended concentrations of the insecticides and later they were set to dry, and then they were attached to a wall of the cages, where 20 worker honeybees were introduced for exposure. Contact actions of each insecticide treatment were compared with the standard (0.15 μg dimethoate) and the control (paper immersed in distilled water only). Mortality losses of honeybees were recorded separately at 0.5, 1, 2, 3, 4, 6, and 24 h after exposure (Amssalu, 2010).

### Fumigation test

The experiment set-up and normal feeding to bees (that is, 50% honey solution) were the same as for the feeding and contact tests. Glass tubes were filled with 10 ml of each treatment and were tapped with cotton wicks for slow diffusion, and placed into the test cages. The death rate was compared with 0.0009% of the standard toxic chemical dimethoate (Rogor) and the control (the tube filled with water). Fumigation action of each treatment was recorded through honeybee mortality at 0.5, 1, 2, 3, 4, 6, and 24 h after exposure, as in the other tests (Amssalu, 2010).

### Data collection

#### *Mortality (%)*

Mortality of bees at each observation time was recorded for each treatment and compared with the standard toxic chemical and the control, and they were corrected by Abbott (1925):

$$\text{-\% of mortality: Correct mortality} = \frac{\text{\% mortality in treatment - \% mortality in control}}{\text{100- Mortality in control}} \times 100$$

Average insecticide mixed food consumption per g in the feeding test was recorded at the end.

### Data analysis

Results were analyzed using SAS software version 9.1. The insecticide treatments were compared with the controls and the standard, and the 5% LSD test was used for mean separation whenever significant differences occurred. Before data analysis, data were revised for normality and when they were not normally distributed, they were transformed by $\sqrt{(x + 0.5)}$.

## RESULTS AND DISCUSSION

### Feeding test

Insecticides differed significantly ($p \leq 0.001$) in causing mortality to honeybees in the feeding test (Table 2). All of them caused significant bee mortality 0.5 h after exposure when compared with the control, that is, water. On the other hand, all the insecticides, except lambda-cyhalothrin, were not statistically different in bee mortality from the toxic standard dimethoate at 0.3 μg. Maximum honeybee mortality was recorded from bees fed on lambda-cyhalothrin (14.71%), followed by diazinon (11.54%). Also, all insecticides were toxic to honeybees 1 h after feeding exposure, although endosulfan (6.46%) was relatively less toxic.

Toxicity levels of all insecticides were not significantly different from the standard 2, 3, 4, and 6 h after feeding exposure, but they were significantly different from the

**Table 2.** Toxicity of insecticides to honeybees in a laboratory feeding test in 2012/2013.

| Treatments | Corrected mortality of honeybees in percent (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.5 h | 1 h | 2 h | 3 h | 4 h | 6 h | 24 h |
| Deltamethrin | 8.14 (2.94)$^{b}$ | 9.55 (3.17)$^{c}$d | 21.59 (4.70)$^{a}$ | 21.59 (4.70)$^{ab}$ | 19.75 (4.50)$^{a}$ | 15.02 (3.94)$^{bc}$ | 0.00 (0.71)d |
| Diazinon | 11.54 (3.47)$^{ab}$ | 18.25 (4.33)$^{a}$ | 21.59 (4.70)$^{a}$ | 23.22 (4.87)$^{a}$ | 11.54 (3.47)$^{bc}$ | 11.54 (3.47)$^{c}$ | 0.00 (0.71)d |
| Dimethoate | 5.70 (2.49)$^{b}$ | 13.26 (3.71)$^{abc}$ | 21.59 (4.70)$^{a}$ | 21.59 (4.70)$^{ab}$ | 16.56 (4.13)$^{ab}$ | 15.02 (3.94)$^{bc}$ | 0.00 (0.71)d |
| Endosulfan | 5.70 (2.49)$^{b}$ | 6.47 (2.64)d | 16.56 (4.13)$^{b}$ | 16.15 (4.08)$^{ab}$ | 14.71 (3.90)$^{abc}$ | 15.02 (3.94)$^{bc}$ | 13.86 (3.79)$^{a}$ |
| Lambda-cyhalothrin | 14.71 (3.90)$^{a}$ | 16.56 (4.13)$^{ab}$ | 20.02 (4.53)$^{a}$ | 14.71 (3.90)$^{b}$ | 9.99 (3.24)$^{c}$ | 18.25 (4.33)$^{ab}$ | 0.00 (0.71)d |
| Malathion | 8.14 (2.94)$^{b}$ | 8.14 (2.94)$^{c}$d | 20.02 (4.53)$^{a}$ | 19.93 (4.52)$^{ab}$ | 15.02 (3.94)$^{ab}$ | 19.75 (4.50)$^{a}$ | 5.26 (2.40)$^{c}$ |
| Profenofos | 8.14 (2.94)$^{b}$ | 11.53 (3.47)$^{bc}$ | 20.02 (4.53)$^{a}$ | 18.25 (4.33)$^{ab}$ | 11.54 (3.47)$^{bc}$ | 15.02 (3.94)$^{bc}$ | 12.17 (3.56)$^{ab}$ |
| Control | 0.00 (0.71)$^{c}$ | 0.00(0.71)e | 0.00 (0.71)$^{c}$ | 0.00 (0.71)$^{c}$ | 0.00 (0.71)d | 0.00 (0.71)d | 7.79 (2.88)$^{bc}$ |
| **LSD (5%)** | **0.88** | **0.81** | **0.37** | **0.82** | **0.69** | **0.54** | **0.71** |
| **CV (%)** | **17.6** | **14.7** | **5.4** | **11.7** | **11.5** | **8.6** | **21.1** |

Means in parenthesis are data transformed by square root; those in the column followed by the same letter are not significantly different at the 5% probability level.

water-treated control. However, at 2 and 4 h after exposure, deltamethrin caused maximum mortality, while at the 3 and 6 h time period diazinon (23.22%) and malathion (19.75%) were markedly toxic to honeybees. In the water-treated control no mortality occurred throughout the test. However, after 24 h feeding exposure, higher mortality was recorded in endosulfan (13.86%), profenofos (12.17%), malathion (7.79%) and water fed (5.26%) honeybees. In the other treatments, the bees died before 24 h.

All the tested insecticides were toxic when fed to honeybees. Also, they exhibited some unusual behaviors, such as lack of coordination, trembling and tumbling. Our results agree with those of Atkins et al. (1981), who also found that ingestion of deltamethrin resulted in mortality and impaired learning ability in honeybees. Similarly, diazinon mixed in the food of the bees was extremely toxic when ingested. Gary and Mussen (1984) also reported that malathion caused significant mortality of honeybees in the laboratory. Kidd and James (1991) found that endosulfan is toxic to honeybees and profenofos remained highly toxic to them when tested orally. According to the USEPA (1988), lambda-cyhalothrin is highly toxic to bees when they are fed orally.

Exposure of honeybees to insecticides may result in acute mortality and sublethal effects. Data in Table 3 indicate that all insecticides mixed with honey were not readily accepted by honeybees. When they ingested insecticide poisoned food, they exhibited unusual behavior, like paralysis, and abnormal jerky and spinning movements. In contrast, honeybees fed normal food consumed more than those provided with insecticide tainted food. Bees fed with diazinon laced food consumed the lowest.

## Contact test (residual exposure method)

All the insecticide treatments killed significantly ($p \leq 0.01$) more honeybees than the control (Table 4). Moreover, 0.5 h after exposure, all insecticides were not statistically different from the highly toxic standard dimethoate at 0.15 μg. The honeybees died at the same level (9.99%) when exposed to profenofos, endosulfan, lambda-cyhalothrin, and diazinon, whereas low mortality occurred equally (6.47%) in bees exposed to malathion and deltamethrin. However, 1, 2, 3 and 4 h after exposure, there were no significant differences ($p \geq 0.05$) between the toxic standard and all the insecticides tested.

An hour after exposure, minimum and maximum mortality occurred with profenofos (8.14%) and diazinon (13.26%), respectively. However, 6 h later, profenofos, endosulfan, diazinon, malathion, and deltamethrin caused significantly more honeybee deaths than the toxic standard dimethoate. At the 24 h exposure there were more dead bees with the water control than in those exposed to the insecticides. Bee mortality in the water control might be attributed to their exhaustion when they were trying to leave the cage. There were also some dead bees among those exposed to lambda-cyhalothrin, malathion, and deltamethrin. In the contact test all insecticides

**Table 3.** Effect of insecticide treatments on honeybee food consumption in the Adami Tullu Jido Kombolcha District in 2012/2013.

| Insecticide treatments | Food ingested by honeybees (g) |
|---|---|
| Deltamethrin | 1.48[c] |
| Diazinon | 1.32[d] |
| Dimethoate | 1.58[c] |
| Endosulfan | 1.73[b] |
| Lambda-cyhalothrin | 1.84[b] |
| Malathion | 1.56[c] |
| Profenofos | 1.74[b] |
| Control | 5.33[a] |
| **LSD (5%)** | **0.15** |
| **CV (%)** | **4** |

Means in the column with same letter are not significantly different at the 5% probability level.

**Table 4.** Residual effects of insecticides on honeybees in the laboratory in the Adami Tullu Jido Kombolcha District in 2012/2013.

| Treatments | Corrected mortality of honeybees (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.5 h | 1 h | 2 h | 3 h | 4 h | 6 h | 24 h |
| Deltamethrin | 6.47 (2.64)[b] | 8.14 (2.94)[b] | 13.41(3.73)[a] | 15.02 (3.94)[ab] | 11.40 (3.45)[a] | 18.51 (4.36)[ab] | 7.40 (2.81)[b] |
| Diazinon | 9.99 (3.24)[a] | 13.26 (3.71)[a] | 14.94 (3.93)[a] | 15.02 (3.94)[ab] | 9.80 (3.21)[a] | 18.51 (4.36)[ab] | 0.00 (0.71)[b] |
| Dimethoate | 9.99 (3.24)[a] | 8.14 (2.94)[b] | 13.41 (3.73)[a] | 16.56 (4.13)[a] | 12.75 (3.64)[a] | 13.41 (3.73)[c] | 0.00 (0.71)[b] |
| Endosulfan | 9.99 (3.24)[a] | 8.14 (2.94)[b] | 13.41 (3.73)[a] | 15.02 (3.94)[ab] | 9.80 (3.21)[a] | 20.38 (4.57)[a] | 0.00 (0.71)[b] |
| Lambda-cyhalothrin | 9.99 (3.24)[a] | 11.54 (3.47)[ab] | 15.26 (3.97)[a] | 13.26 (3.71)[b] | 11.40 (3.45)[a] | 13.41 (3.73)[c] | 5.8 (2.51)[b] |
| Malathion | 6.47 (2.64)[b] | 8.14 (2.94)[b] | 11.75 (3.50)[a] | 15.02 (3.94)[ab] | 12.75 (3.64)[a] | 16.89 (4.17)[b] | 8.14 (2.94)[b] |
| Profenofos 750 | 9.99 (3.24)[a] | 8.14 (2.94)[b] | 13.41 (3.73)[a] | 15.02 (3.94)[ab] | 12.75 (3.64)[a] | 16.89 (4.17)[b] | 0.00 (0.71)[b] |
| Control | 0.00 (0.71)[c] | 0.00 (0.71)[c] | 1 .06 (1.25)[b] | 0.00 (0.71)[c] | 0.00 (0.71)[b] | 0.00 (0.71)d | 16.56 (4.13)[a] |
| **LSD (5%)** | **0.47** | **0.76** | **0.88** | **0.31** | **0.76** | **0.37** | **1.54** |
| **CV (%)** | **9.6** | **15.3** | **14.6** | **5.1** | **13.9** | **5.7** | **17.0** |

Means in parenthesis are data transformed by square root; those in the column followed by the same letter are not significantly different at the 5% probability level.

were toxic to honeybees throughout the test hour. This result was the same in other studies (Hagler et al., 1989; Pilling, 1992), where diazinon and lambda-cyhalothrin were highly toxic to honeybees within 24 h on an acute contact basis. Endosulfan causes also acute contact toxicity to bees (WHO, 1998).

Tomlin (2006) found also malathion, deltamethrin, and profenofos highly toxic to honeybees in contact toxicity tests.

**Fumigation test**

The fumigation toxicity test yielded also highly significant ($p \leq 0.001$) mortality on honeybees (Table 5). Mortality due to deltamethrin (11.13%),

**Table 5.** Fumigation effects of insecticides on honeybee in the laboratory in the Adami Tullu Jido Kombolcha District in 2012/2013.

| Treatments | Corrected mortality of honeybees in percent | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.5 h | 1 h | 2 h | 3 h | 4 h | 6h | 24h |
| Deltamethrin | 11.13 (3.41)$^{ab}$ | 8.62 (3.02)$^{ab}$ | 17.45 (4.24)$^{a}$ | 17.65 (4.26)$^{a}$ | 17.65 (4.260)$^{a}$ | 25.00 (5.05)$^{ab}$ | 0.00 (0.71)$^{c}$ |
| Diazinon | 8.92 (3.07)$^{b}$ | 8.92 (3.07)$^{ab}$ | 17.65 (4.26)$^{a}$ | 19.93 (4.52)$^{a}$ | 19.93 (4.52)$^{a}$ | 25.00 (5.05)$^{ab}$ | 0.00 (0.71)$^{c}$ |
| Dimethoate | 5.34 (3.98)$^{a}$ | 11.13 (3.41)$^{a}$ | 19.93 (4.52)$^{a}$ | 17.65 (4.26)$^{a}$ | 15.34 (3.98)$^{ab}$ | 22.44 (4.79)$^{b}$ | 0.00 (0.71)$^{c}$ |
| Endosulfan | 0.00 (0.71)d | 5.26 (2.40)$^{b}$ | 15.34 (3.98)$^{ab}$ | 19.93 (4.52)$^{a}$ | 15.34 (3.98)$^{ab}$ | 26.44 (5.19)$^{ab}$ | 0.00 (0.71)$^{c}$ |
| Lambda-cyhalothrin | 7.00 (2.74)$^{b}$ | 5.26 (2.40)$^{b}$ | 15.34 (3.98)$^{ab}$ | 15.34 (3.98)$^{a}$ | 19.75 (4.50)$^{a}$ | 25.00 (5.05)$^{ab}$ | 4.65 (2.27)$^{b}$ |
| Malathion | 2.89 (1.84)$^{c}$ | 6.90 (2.72)$^{ab}$ | 10.79 (3.36)$^{b}$ | 15.34 (3.98)$^{a}$ | 17.65 (4.26)$^{a}$ | 27.69 (5.31)$^{a}$ | 6.26 (2.60)$^{a}$ |
| Profenofos | 0.00 (0.71)d | 0.00 (0.71)$^{c}$ | 0.00 (0.71)$^{c}$ | 5.26 (2.40)$^{b}$ | 11.13 (3.41)$^{b}$ | 13.34 (3.98)$^{c}$ | 5.26 (2.40)$^{ab}$ |
| Control | 0.00 (0.71)d | 0.00 (0.71)$^{c}$ | 0.00 (0.71)$^{c}$ | 0.00 (0.71)$^{c}$ | 0.00 (0.71)$^{c}$ | 0.00 (0.71)d | 23.40 (4.89)$^{a}$ |
| **LSD (5%)** | **0.88** | **0.86** | **0.85** | **0.59** | **0.85** | **0.46** | **0.33** |
| **CV (%)** | **23.6** | **21.4** | **15.1** | **9.5** | **13.1** | **6.0** | **10.1** |

Means in parenthesis are data transformed by square root; those in the column followed by the same letter are not significantly different at the 5% probability level.

diazinon (8.92%), and lambda-cyhalothrin (7.00%) 0.5 h after fumigation were significantly greater than the toxic standard, which suggests that these insecticides were applied under the 0.0009% concentration level. Similarly, 1 h after fumigation diazinon (8.92%), deltamethrin (8.62%), and malathion were not significantly different from the toxic standard. However, 2 and 3 h after fumigation, all the insecticides except profenofos, caused similar degrees of mortality as the toxic standard. However, profenofos did not cause mortality until 3 h after the beginning of the

test. Similarly, at the 4 and 6 h fumigation all the insecticides caused significant mortality, although profenofos caused relatively less mortality than the toxic standard. After 24 h fumigation, there were more dead honeybees in the water control than in the insecticide fumigated bees. As in the honeybee consumption test, bee mortality in the water control might be attributed to their suffocation when they were trying to leave the cage.

The insecticides evaluated did not knock down honeybees but nearly all of them caused significantly more mortality than the control. This could be due to their fume properties. According to U.S. Department of Health and Human Services (2006), diazinon volatilizes in the air at room temperature and is transformed to diazoxon (an even more potent ChE inhibitor than diazinon), where it

remains active. This property of diazinon makes it more toxic to honeybees. The same Agency (2009) also reported that deltamethrin has a higher potential to volatilize from water compared with other pyrethroids. The USEPA (1988) also indicates that lambda-cyhalothrin and endosulfan are moderately toxic to invertebrate animals, while malathion is very low in toxicity to animals when inhaled (USEPA, 2006).

## Conclusion

All of the insecticides evaluated were toxic to honeybees and caused mortality within a few hours after exposure to laced food. In the fumigation test, profenofos did not cause honeybee mortality until the third hour, but the other insecticides caused varying mortality within 24 h. The government ought to exclude products that are toxic to honeybees and the environment in general as criteria for registration and marketing of imported as well locally formulated insecticides. Further research is needed on these insecticides to determine their $LD_{50}$ on Ethiopian bee races.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Efficacy of fungicides for the control of leaf spot disease of ginger under the field conditions of Chhattisgarh (India)

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**Leaf spot of ginger caused by *Phyllosticta zingiberi* is a very serious phytopathological constraint in the cultivation of ginger in India. Among the five fungicides (Copper-Oxy-Chloride, Mancozeb, Thiophanate Methyl, Metalaxyl, and Carbendazim), tested for the control of *Phyllosticta* leaf spot of ginger, Carbendazim (0.1%) three sprays, starting with the first symptoms appearance in the field followed by two more sprays at monthly interval, was found more effective in reducing the severity of the diseases (21.3%) that consequently increased the fresh rhizome yield (173.0 q/ha) significantly, followed by Metalaxyl (118.0 q/ha) and Thiophanate Methyl (116.0 q/ha), respectively. Mean apparent rate of infection (ARI) was also the lowest in Carbendazim (0.1%) spray.**

**Key words:** *Gingiber officinales*, *Phyllosticta zingiberi*, leaf spot, fungicidal control.

## INTRODUCTION

Ginger (*Gingiber officinales*) is an important spice crop commercially grown in Kerala, Karnataka Tamil Nadu, West Bengal, Bihar, Orissa, Madhya Pradesh, Uttar Pradesh, Himanchal Pradesh, Meghalaya and Sikkim (Dake, 1995; Spices Board, 2009) with an area of 155000 ha having production of 756000 t, with average productivity of 4900 kg $ha^{-1}$ (NHB, 2012). India stands at second position next to China regarding ginger production and productivity (Abubacker, 2011).

Ginger is affected by many diseases but rhizome rot (*Pythium* spp.) and leaf spot (*Phyllosticta zingiberi*) are the main constraints which cause economic loss to this crop (Iyer, 1988). It has been reported in Himanchal Pradesh (Sohi et al., 1973), Maharashtra (Kanware, 1947), Kerala (Anonymous, 1974) and also a serious problem in Chhattisgarh (Singh, 1998). Symptoms are observed on leaves as oval to elongated spots that later turn to whitish spots surrounded by dark-brown margin with yellowish halo (Brahma and Nambiar, 1982; 1984). The pathogen *P. zingiberi*, survives through matured pycnidia even up to 14 months through the lesions on the leaves fallen in the soil as plant debris (Brahma and Nambiar, 1982). The spores ooze out into water droplets on the leaves and get dispersed through rain splashes (Brahma and Nambiar, 1984). Continuous cultivation of ginger in the same field(s) helps in the buildup of higher concentrations of inoculum and early infection of the plant fails the vigor leading to drastic reduction in the rhizome

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yield (personal observation).

Till date, not even a single variety/cultivar is reported as a source of resistance but few moderate resistant sources has been identified (Kanware, 1974; Nybe and Nair, 1979; Premnathan et al., 1980; Dohroo et al., 1986 and Rao et al., 1995). Partial shade (30-40%), up to only initial three months, significantly reduces the disease without affecting yield potential (Nizam and Jayachandran, 1997; Singh et al., 2004). Thus, only fungicides remain an open option towards managing this disease. Partial management of this disease has been reported with the two spray of Bordeaux mixture (1%) (Ramakrishnan, 1942; Sohi et al., 1973) and Mancozeb (0.3%) (NRCS, 1989); Prochloraz, Tebuconazole, Chlorothalonil, Mancozeb, Captan and Chlorothalonil + Copper (Nazareno, 1995) and Captan (0.3%) (Das and Senapati, 1998). However, very scanty information is available on the control of leaf spot through fungicidal sprays. Hence, an attempt was made to control this disease with other fungicides which were not evaluated against this disease.

## MATERIALS AND METHODS

A field experiment was conducted at Regional Agricultural Research Station, Indira Gandhi Krishi Vishwavidyalaya, Raigarh (Chhattisgarh), in Randomized Block Design with five different fungicides alongwith one check as treatments and four replications, to control the leaf spot disease with fungicidal sprays. Fungicides were Copper-Oxy-Chloride (local action), Mancozeb (local action), Thiophanate Methyl (systemic action), Metalaxyl (systemic action) and Carbendazim (systemic action). Planting of rhizome was done at 20 X 30 cm in each plot and fertilizers, at the rate of 150:100:100 kg nitrogen, phosphorus and potash per ha, respectively along with all other recommended package of practices, were applied to raise good crops. First spray, as per the treatment of each fungicide, was started as the first symptoms appeared in the experimental field followed by two more sprays at monthly intervals. Disease severity was recorded, on 20 randomly selected leaves in each plot, just one day before each spraying and one month after the last spraying, on 1-9 point disease rating scale (Singh et al., 2000). Disease index, in each replication, was worked out by using the following formula (Ayyangar, 1928):

$$\text{Disease Index} = \frac{\text{Sum of all ratings}}{\text{Total number of rating X Maximum disease grade}} \times 100$$

Fresh rhizome per plot yield was recorded at the time of digging. Disease severity and yield data were analyzed through analysis of variance (ANOVA). Apparent rate of infection (r) was worked out by the following formula (Vanderplank, 1963):

1. When the disease severity was less than 5% (logarithmic infection rate)

$$r = \frac{2.3}{t_2 - t_1} \log 10 \frac{X_2}{X_1}$$

2. When the disease severity was more than 5% (non-logarithmic infection rate)

$$r = \frac{2.3}{t_2 - t_1} \log 10 \frac{X_2 (1-X_1)}{X_1 (1-X_2)}$$

Where r = apparent rate of infection, $X_1$ = disease severity at the first observation ($t_1$), $X_2$= disease severity at the second observation ($t_2$), $t_1$= time of first observation and $t_2$= time of second observation.

## RESULTS AND DISCUSSION

Data depicted through Figure 1, reveals all the fungicides, tested in the present investigation, reduced the disease severity significantly. The lowest disease severity was achieved in spraying of Carbendazim (21.3%) followed by Mancozeb (22.9%), Thiophanate Methyl (24.2%) and Metalaxyl (24.6%), respectively. Maximum disease severity was recorded in Copper-Oxy-Chloride spraying. Among all the tested fungicides, none was found statistically significantly effective in comparison to the other. Effectiveness of Copper-Oxy-Chloride (Copper fungicide) and Mancozeb (Dithane M-45), for the reduction of the disease severity, confirms the results of Sohi et al. (1973) and NRCS (1989), that *Phyllosticta* leaf spot can be managed by one and two sprays of Bordeaux mixture (Copper fungicide) and Dithane M-45, respectively. But in the present investigations, Carbendazim followed by Thiophante Methyl showed better performance in comparison to the above reported effective fungicides.

Effective fungicide(s) increase(s) crop yield through the reduction in disease severity in cases of fungal diseases. In this study, higher fresh rhizome yield was recorded in the case of all fungicidal spray but significantly higher, and with maximum yield in Carbendazim (0.1%) followed by Metalaxyl (0.05%) and Thiophanate Methyl (0.1%), respectively. Here, fresh rhizome yield of ginger remained unaffected statistically in Mancozeb (0.3%) and Copper-Oxy-Chloride (0.3%), in comparison to control, though the disease severity was significantly reduced in these fungicides. This may be attributed to local but not systemic, action of the fungicides. This study on the effectiveness of Mancozeb (0.3%) for the reduction of leaf spot of ginger is in affirmation with the findings of NRCS (1989), but differs regarding the significant increase in the yield of fresh rhizome of ginger (Figures 2 and 3).

Study on the effect of different fungicidal spray on the apparent rate of infection (ARI), data depicted through Table 1 and Figure 4, reveals that mean apparent rate of infection was significantly reduced in all the fungicidal, spray over control, with lowest in Carbendazim (0.1%) followed by Mancozeb (0.3%) and Thiophanate Methyl (0.1%), respectively. Though, this is the first study in ginger to record the ARI in a case of leaf spot disease, this may be attributed to the higher yield of fresh rhizome of ginger because of low apparent rate of infection that delayed the spread of the pathogen, consequently

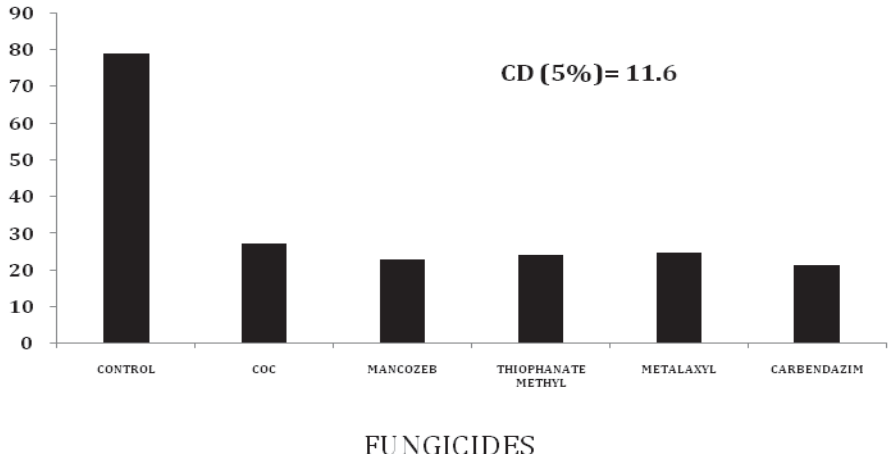


**Figure 1.** Effect of fungicidal spray on the disease severity of leaf spot on ginger.

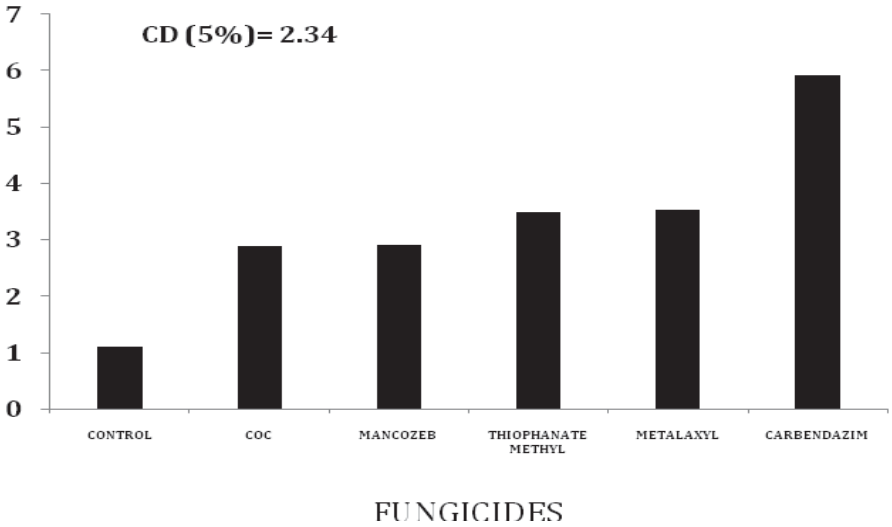


**Figure 2.** Effect of fungicidal spray on the ginger fresh rhizome yield kg/3 $m^2$.

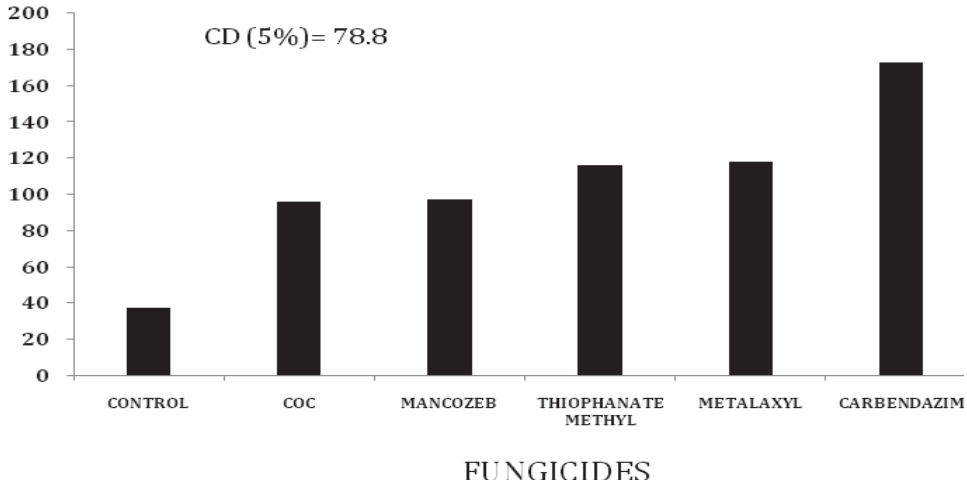


**Figure 3.** Effect of rhizome yield (Q/Ha) as affected by different fungicidal spray.

**Table 1.** Effect of different fungicides on the apparent rate of infection at different interval of different fungicidal spraying under field conditions.

| Serial Number | Treatment | Apparent rate of infection (ARI) during different spraying interval | | | Mean ARI |
|---|---|---|---|---|---|
| | | 70-105 DAS | 106-136 DAS | 137-167 DAS | |
| 1 | Control (No spray) | 0.079 | 0.030 | 0.019 | 0.043 |
| 2 | Copper-Oxy-Chloride (0.3%) | 0.059 | 0.011 | 0.005 | 0.025 |
| 3 | Mancozeb (0.3%) | 0.054 | 0.005 | 0.003 | 0.021 |
| 4 | Thiophanate Methyl (0.1%) | 0.055 | 0.011 | 0.005 | 0.023 |
| 5 | Metalaxyl (0.05%) | 0.056 | 0.011 | 0.066 | 0.024 |
| 6 | Carbendazim (0.1%) | 0.053 | 0.054 | 0.002 | 0.019 |
| CD (5%) | | - | - | - | 0.012 |

DAS= Days after sowing, ARI= Apparent rate of infection.

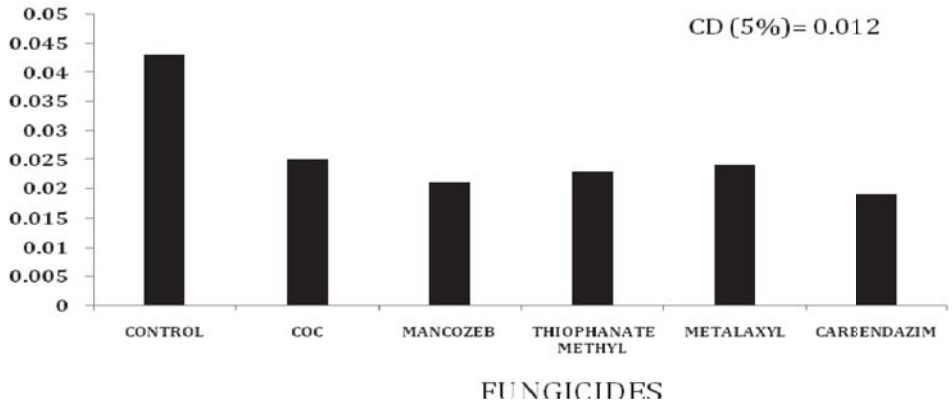


**Figure 4.** Effect of different fungicidal spray on the mean ARI of leaf spot on ginger.

resulting in the development of lowest disease severity in the case of Carbendazim (0.1%) spraying.

Thus, considering all the parameters studied and already proven facts by other researchers (Brahma and Nambiar, 1982), three spraying of Carbendazim (0.1%), at monthly interval, starting with the appearance of first symptoms in the field, may be integrated with crop rotation, collection and destruction of plant debris, partial shade (Singh et al, 2004) and use of moderately resistant cultivar(s) (Kanware, 1974; Nybe and Nair, 1979; Premnathan et al., 1980; Dohroo et al., 1986 and Rao et al., 1995) for the harvest of higher yield of ginger in endemic areas of its cultivation.

## Conflict of Interest

The author(s) have not declared any conflict of interest.

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# Profile distribution and degradation of soil properties of an ultisol in Nsukka semi-humid area of Nigeria

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**Better understanding of how soils respond to land use is needed to enable science-based land management interventions. The present study investigated the relative changes in properties of ultisol under conventional tillage for arable crops and compared with fallowed plot (greater than 10 years of continuous no-till fallow) in the semi-humid Nsukka of southeastern Nigeria. Soil samples were collected from designated profile horizons for determination of soil properties. In cultivated plot relative to fallowed plot, soil erodibility increased by 2.5% (Ei + 0.11), total porosity decreased by 1.1%, whereas macro- and microporosity increased by 3.4 and 7.1%, respectively. Soil saturated hydraulic conductivity increased by 4.9%. The degree of topsoil saturation with water was similar in both the cultivated and the fallowed plots. Soil pH increased (7%) when exchangeable acidity increased (21.3%, +0.46 cmol (+) $kg^{-1}$). Losses of organic carbon (28%, -2.58 $gkg^{-1}$), total N (26%, -0.17 $gkg^{-1}$), available P (47%, -2.63 $mgkg^{-1}$), Ca (55%, -1.75 cmol (+) $kg^{-1}$), CEC (17%, -1.11 cmol (+) $kg^{-1}$), and base saturation (11.3 %) due to cultivation were observed. Since the fallowed plot that was previously under cultivation was able to show more favourable values of the measured soil properties than the cultivated plot without human activity, the study deduces that an ultisol can be resilient.**

**Key words:** Ultisol, land use system, soil property, semi-humid, Nigeria.

## INTRODUCTION

Environmental degradation caused by unsuitable land use is a worldwide problem. Soil's capacity to carry out its functions of biological production, environmental protection and human health sustenance is impaired due to climate effects and anthropogenic disturbances (Hertmink et al., 2008).

Land use profoundly influences the functionality of the three facets of soil properties: Physical, chemical and biological in terms of relative changes at multiple levels of the agro-ecosystem. Soil properties that can be changed in a short time by land use are dynamic soil quality indicators (Sanchez- Maranon et al., 2002), while an assessment of dynamic soil quality indicators influenced by land use requires four approaches to avoid misuse of soil quality paradigm and include: (i) Separation of soil environments with narrow climate and soil ranges; (ii) Identification of native and current soil properties within an environment; (iii) Description of the relative quantified

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changes of individual soil properties, and (iv) Inference of the state of individual soil functions.

Spatial variability in soils exists at many scales with different dominant controlling factors. An understanding of the variability and distribution of soil nutrients as influenced by site characteristics (controlling factors) including climate, landscape features, and land use is critical for assessing the future of land use change in soil nutrients (Kosmas et al., 2000; ). Also, the direction and degree of soil quality changes in managed ecosystems depend on these factors.

Soil-landscape relationships result from short and long-term pedogeomorphic processes. Parent material, climate, and geological history are major factors affecting the distribution of soil properties at continental scale (Akamigbo and Asadu, 1983), whereas land use, land use history, and topography are the dominant controls at smaller scales such as catchment scale (Fu et al., 2000). Furthermore, land use influences soil property variations since land use and soil management practices influence such processes as erosion, oxidation, mineralization and soil nutrient leaching.

In field experiments, Wei et al. (2010 found that land use conversions resulted in significant soil degradation through loss of fine soil particles, soil organic carbon (SOC), and nutrients. However, after 28 years of afforestation of grassland, SOC and STN increased. This study suggests that land use changes affect soil properties and soil quality indicators.

A key feature of soils is profound variation in their properties and may differ among soil profile horizons due to the transport and storage of water and nutrients across and within the soil profile. Both horizontal and vertical variability of soil properties have been studied in temperate soils (Schilling et al., 2009) when compared to little work and literature availability on the variability of Nigerian soils (Oku et al., 2010).

Nutrient and water uptake are not always the same at different soil depths because of soil-forming factors that affect different properties differently at different depths (Cassel, 1983). Limited studies on vertical soil variability (Oku et al., 2010) show soil pH to increase with depth and least variable, irrespective of depth. They also found variability of soil organic matter, total N, available phosphorus and CEC to increase with depth and ranged between moderate and high.

An important question is to what extent are soil properties different along profile depths in the different land uses of the study sites. Understanding the vertical distribution of soil properties along profile depths could help to predict the proneness of such soils to erosive influence. Knowledge of their variability is essential in applying location-specific land management interventions. The objective of this study was to quantify and compare soil properties of 4-year conventionally and continuously tilled arable cropland with those of an adjacent native site of greater than 10 years of continuous no-till fallowed sites in the semi-humid zone of Nsukka, Nigeria.

## MATERIALS AND METHODS

### Features of the study location

The study was conducted on an ultisol of Nsukka (06° 52' N; 07° 24' E), Nigeria on an altitude of 400 m above sea level (m asl). Its climate is characteristically sub-humid tropical, with mean annual total rainfall of about 1600 mm; of which distribution is bimodal, with peaks during July and October in the first and second phases, respectively. Mean minimum temperature is 21.8°C and relative humidity ranges between 70 and 80% (Oko-Ibom and Asiegbu, 2006). The soil of the study location is an utisol characterized as low activity clays (Ezeaku, 2006). The soil is well drained, prone to erosion and leaching losses of nutrients, hence of low fertility status. The vegetation is characteristically derived savanna (Savanna-mosaic) agroecology, which represents different land uses such as forest, secondary forest, cultivated areas, and grazed grass pasture in a soil-landscape system.

Two profile pits were dug on a 4-year conventionally and continuously tilled arable cropland system and another two sited on a plot under 10-year natural fallow system. The two land uses were 500 m apart, while the distance between the two profiles was 530 m apart. Profile samples were collected at different soil horizons (0-20, 20-45, 45-70, 70-100 m) for the first pit on a 4-year arable cropland) using cores of 0.050 m in diameter and 0.051 m in height. The method of sampling was discrete, which involved collecting samples at designated soil profile horizons (Muller and McBratney, 2001). Horizon depths for soil sampling among the different profile pits vary. However, soil horizon samples on each system were bulked and air-dried, crumbled and sieved through a 2-mm screen.

The analytical characteristics of the soil horizon samples were determined in the following manner. Particle-size distribution was determined by the pipette method (Gee and Bauder, 1986), soil bulk density as described by Blake and Hartge (1986), while porosity was calculated as a function of the total volume not occupied by soil solids, assuming a particle density of 2.65 $gcm^{-3}$ and mathematically expressed as follows:

$$f = 1- Bd/Pd \times 100\ \% \quad (1)$$

Where f = total porosity (%), Bd = bulk density ($gcm^{-3}$), Pd = particle density assumed (2.65 $gcm^{-3}$)

Erodibility index of the soil was calculated as a ratio of sand + silt to clay (Hudson and Vooorees, 1995). Soil saturated hydraulic conductivity (Ks) was determined based on Klute and Dirksen (1986) method and calculated by using the transposed Darcy's equation for vertical flows of liquids:

$$Ks = (Q/At)/(L/DH \quad (2)$$

Where, Ks = saturated hydraulic conductivity (cm $h^{-1}$), Q = steady-state volume of water outflow from the entire soil column ($cm^3$), A = the cross-sectional area ($cm^2$), t = the time interval (h), L = length of the sample (cm), and DH = change in the hydraulic head (cm).

Soil pH was determined using 1:2.5 soil water suspension (adequate to wet the glass electrode) and read off using pH meter (McLean, 1982). Organic carbon was obtained by the wet dichromate acid oxidation method (Nelson and Sommers, 1982). Total nitrogen was determined using the Micro-kjeldhal method (Bremmer and Mulvaney, 1982), while available phosphorus was assayed by Bray P-2 bicarbonate extraction method (Olson and Sommers, 1982). Exchangeable bases ($Ca^{2+}$, $Mg^{2+}$, $K^+$ and $Na^+$) were extracted in 1 N $NH_4OAc$ buffered at pH 7.0. Exchangeable

**Table 1.** Variation in soil physical properties in relation to soil depth (cm) of cultivated land.

| Hz | Depth (cm) | Sand | Silt | Clay | Tx | Si/Clay | Ei | Bd $Mgm^{-3}$ | Tp | Ma.p | Me.p | Mi.p | Ks $cm\ h^{-1}$ | Sa $cm^3/cm^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (%) | | | | | | | (%) | | | | | |
| Ap | 0-32 | 74.2 | 7.4 | 18.4 | Sl | 0.40 | 4.43 | 1.35 | 49.1 | 23.7 | 13.3 | 12.1 | 0.82 | 0.44 |
| AB | 33-65 | 70.3 | 9.9 | 19.8 | Sl | 0.50 | 4.03 | 1.37 | 48.3 | 21.4 | 10.2 | 16.7 | 0.70 | 0.45 |
| $Bt_1$ | 66-99 | 55.7 | 12.5 | 31.8 | Scl | 0.39 | 2.14 | 1.47 | 44.5 | 17.8 | 6.4 | 20.3 | 0.24 | 0.48 |
| $Bt_2$ | $100^+$ | 54.2 | 10.9 | 34.9 | Scl | 0.31 | 1.87 | 1.49 | 43.7 | 14.0 | 6.2 | 23.5 | 0.19 | 0.49 |
| **Mean** | | 63.6 | 10.2 | 26.23 | | 0.40 | 3.12 | 1.42 | 46.4 | 19.2 | 9.0 | 18.2 | 0.46 | 0.46 |
| **SD** | | 10.1328 | 2.1376 | 8.3436 | | 0.0778 | 1.2996 | 0.0702 | 2.6957 | 4.2461 | 3.3925 | 4.8973 | 0.3191 | 0.0238 |
| **CV (%)** | | 16 | 21 | 32 | | 20 | 42 | 5 | 6 | 22 | 38 | 27 | 66 | 5 |

Tx= texture, Si= silt, Ei= erosion index, Bd= bulk density, Tp= total porosity, Ma.p= macro porosity, Me.p= meso porosity, Mi.p= micro porosity, Ks= saturated hydraulic conductivity, Sa= degree of saturation. CV= coefficient of variability.

**Table 2.** Variation in soil physical properties in relation to soil depth (cm) of fallow land.

| Hz | Depth (cm) | Sand | Silt | Clay | Tx | Si/Clay | Ei | Bd $Mgm^{-3}$ | Tp | Ma.p | Me.p | Mi.p | Ks $cm\ h^{-1}$ | Sa $cm^3/cm^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (%) | | | | | | | (%) | | | | | |
| Ap | 0-30 | 72.6 | 8.6 | 18.8 | Sl | 0.45 | 4.32 | 1.37 | 48.3 | 24.2 | 7.8 | 16.3 | 0.78 | 0.44 |
| AB | 31-82 | 70.0 | 10.1 | 18.9 | Sl | 0.54 | 4.23 | 1.40 | 45.3 | 20.3 | 7.9 | 17.1 | 0.78 | 0.44 |
| $Bt_1$ | 83-135 | 66.4 | 12.5 | 29.6 | Scl | 0.42 | 2.66 | 1.47 | 44.5 | 12.6 | 11.7 | 20.2 | 0.25 | 0.47 |
| $Bt_2$ | $135^+$ | 54.8 | 10.9 | 32.1 | Scl | 0.34 | 2.05 | 1.48 | 44.2 | 13.0 | 8.5 | 22.7 | 0.23 | 0.48 |
| **Mean** | | 65.9 | 10.5 | 24.9 | | 0.44 | 3.32 | 1.43 | 45.6 | 17.5 | 8.98 | 19.0 | 0.51 | 0.46 |
| **SD** | | 7.856 | 1.626 | 7.003 | | 0.083 | 1.137 | 0.054 | 1.875 | 5.686 | 1.843 | 2.944 | 0.312 | 0.021 |
| **CV (%)** | | 12 | 15 | 28 | | 19 | 34 | 3 | 4 | 32 | 21 | 15 | 61 | 5 |

Tx= texture, Si= silt, Ei= erosion index, Bd= bulk density, Tp= total porosity, Ma.p= macro porosity, Me.p= meso porosity, Mi.p= micro porosity, Ks= saturated hydraulic conductivity, Sa= degree of saturation. CV= coefficient of variability.

acidity (EA) was determined by titration with 0.05 N NaOH, while CEC was determined titrimetically using 0.01 N NaOH.

### Statistical analysis

All data were statistically analyzed using Genstat 9.2 Edition. A *t*-test was used to verify whether there were statistically significant differences. Changes in soil properties can be used to determine whether soil quality, from environmental viewpoint, is improving, stable, or declining with changes in land use (Brejda et al., 2000). Soil variability was estimated using mean and coefficient of variation (CV). Soil properties with larger CV values are more variable than those with smaller CV values. Ranking of variability was done using the classification scheme by Wilding (1985) as follows:

Little variation (CV = < 0-15%)
Moderate variation (CV = 16-35%
High variation (CV = > 36%)

## RESULTS AND DISCUSSION

### Variability in soil properties

Representative soil profiles showing some properties of the fallowed and the cultivated land uses were studied. The physical and chemical properties of the soils are shown in a horizon sequence A-$Bt_2$ (Tables 1 to 4). Tables 1 and 2 present the physical properties of the soil profiles.

**Table 3.** Variation in soil chemical properties in relation to soil depth (cm) of cultivated land.

| Hz | Depth (cm) | pH | OC | TN | Av. P | Ca | Mg | Na | K | EA | CEC | BS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $H_2O$ | $gkg^{-1}$ | | $Mgkg^{-1}$ | $cmol\ kg^{-1}$ | | | | | | % |
| Ap | 0-30 | 5.11 | 9.12 | 0.65 | 5.63 | 3.18 | 1.30 | 0.03 | 0.07 | 2.16 | 6.40 | 43.22 |
| AB | 31-82 | 5.04 | 10.80 | 0.71 | 6.40 | 2.60 | 1.80 | 0.02 | 0.13 | 3.28 | 7.24 | 52.01 |
| $Bt_1$ | 83-135 | 4.71 | 14.10 | 0.87 | 7.12 | 2.30 | 1.85 | 0.06 | 0.18 | 3.42 | 8.10 | 55.86 |
| $Bt_2$ | $135^+$ | 4.68 | 14.92 | 0.93 | 8.36 | 2.14 | 2.24 | 0.08 | 0.24 | 4.06 | 9.27 | 60.11 |
| **Mean** | | 4.89 | 12.24 | 0.79 | 6.88 | 2.56 | 1.79 | 0.05 | 0.16 | 3.23 | 7.75 | 52.80 |
| **SD** | | 0.222 | 2.739 | 0.1317 | 1.161 | 0.458 | 0.3856 | 0.0275 | 0.0723 | 0.7900 | 1.2268 | 7.193 |
| **CV(%)** | | 5 | 22 | 17 | 17 | 18 | 22 | 58 | 47 | 25 | 16 | 14 |

OC= organic carbon, TN= total nitrogen, Av.P= available phosphorus, Ca= calcium, Mg= magnesium, Na= sodium, K= potassium, EA= exchangeable acidity, ECEC= effective cation exchange capacity, BS= base saturation percentage. CV= coefficient of variability.

Soil texture ranged between sandy loam and sandy clay loam. Sand fraction was predominant in relation to the other size fractions in the study site. The predominance of sandy loam is an indication of the uniformity of the site in lithological origin, being false-bedded sandstone of the cretaceous sediment (Akamigbo and Asadu, 1983).

In both land uses studied, the trend in sand and clay movement with depth was irregular. In the topsoil (0-32 cm) of the cultivated soil relative to reference (native) soil, sand had the least variation, while silt and clay varied moderately (CV >16,<35%). Percentage clay content (18.4 %) was less in the upper surface layer as compared with 34.9% obtained in the lower ($100^+$ cm) soil depths, indicating that clay increased with depth, perhaps due to the pedogenetic process of illuviation. Silt/clay ratio varied between 0.31 and 0.50 (mean = 0.40; CV = 19.5 %) in the cultivated soil and from 0.34 to 0.54 (CV = 18.9 %) in the fallowed soil (Tables 1 and 2). Mean silt/clay ratio was less than unity, signifying low weatherability of the soil and pedogenesis under the land uses.

In terms of soil degradation, soil erodibility at the soil surface increased by 2.5% (Ei + 0.11) in cultivated soil (Table 1) relative to native soil (Table 2). However, the variability of degradation index for both soils is generally high (> 36 %) (Wilding, 1985).

Other properties of the soils such as bulk density, porosity, saturated hydraulic conductivity (Tables 1 and 2) varied. Bulk density increased (1.63%) with depth. In the cultivated site minimum bulk density (1.35 $Mgm^{-3}$) occurred at the 0-30 cm depth (which is more or less the plough depth), suggesting pulverization of the surface soils during tillage. Urioste et al. (2006) and Hertmink et al. (2008) also associated low soil bulk density to cultivation; which destroys organo-mineral complexes and the release of soil organic matter and nitrogen, when exposed to oxidation. Channeling and loosing effect of roots could have caused lower bulk density values obtained in the topsoil of the two land uses. The values were all below the critical minimum value (1.5 Mg $m^{-3}$) (Aune and Lal, 1997). Situations of soil bulk density values above this critical value are capable of impeding crop root growth and development, thereby reducing crop yields.

The distributions of bulk density, Ks and porosity with depth in the two sites are shown in Tables 1 and 2. Some workers have indicated that Ks is affected more by the proportion of the water-conducting pores (macro- and mesopores) relative to that of the micropores (Mbagwu, 1995). The report further showed that high bulk density reduced *Ks,* by decreasing drainable porosity through compaction at the soil surface and consolidation in the subsoil.

In the cultivated and native sites, the distribution of the macro, meso + microporosity varied with soil depth. While saturated hydraulic conductivity and macroporosity decreased, microporosity increased with depth. When compared to the fallow site, the value of microporosity decreased by 34.7% in cultivated land use (Table 1). This observation accords earlier reports (Mbagwu et al., 1983; Oku et al 2010) for some soils in southern Nigeria.

Macroporosity and bulk density are the two important physical properties influencing the Ks of soils. As bulk densities increased to 1.49 Mg $m^{-3}$; Ks decreased to 0.19 cm $hr^{-1}$ (Table 1). High Ks values found on surface soils could be associated to abundant biopores, textures coarser than loamy fine sand and strong, fine to medium blocky structures in the surface soil (Fu et al., 2000; Mbagwu, 1995).

On the other hand, low Ks on the sub surface suggests low water transmission rate due to clay accumulation and siltation of the pedogenic horizons. Thus, micro-aggregates could have blocked and reduced the number of available pores open for hydraulic flow. This corresponds to increases in clay contents and microporosity as well as higher degree of water saturation as observed in the subsoils of the study sites.

For these test sites, variation was observed with the hydraulic property (Ks) than with porosity and bulk density (Tables 1 and 2). Other investigators, for example Ahuja et al. (1989) and Franzmeier (1991), also reported more variability in Ks than in the total porosity.

The results in Table 3 show that soil acidity (pH)

**Table 4.** Variation of soil chemical properties in relation to soil depth (cm) of fallow land.

| Hz | Depth (cm) | pH | OC | TN | Av.P | Ca | Mg | Na | K | EA | CEC | BS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $H_2O$ | $gkg^{-1}$ | | $Mgkg^{-1}$ | cmol $kg^{-1}$ | | | | | | % |
| Ap | 0-30 | 5.47 | 11.70 | 0.82 | 8.26 | 4.93 | 1.81 | 0.06 | 0.09 | 1.70 | 7.51 | 48.12 |
| AB | 31-82 | 5.22 | 13.54 | 0.86 | 10.80 | 4.20 | 2.16 | 0.09 | 0.16 | 2.16 | 9.51 | 58.31 |
| $Bt_1$ | 83-135 | 5.00 | 17.20 | 0.94 | 16.00 | 3.40 | 3.03 | 0.10 | 0.26 | 3.20 | 12.14. | 61.50 |
| $Bt_2$ | $135^+$ | 4.93 | 18.05 | 0.98 | 18.30 | 3.22 | 2.70 | 0.12 | 0.25 | 3.30 | 12.30 | 64.20 |
| Mean | | 5.16 | 15.12 | 0.90 | 13.34 | 3.94 | 2.43 | 0.09 | 0.19 | 2.59 | 9.77 | 58.03 |
| SD | | 0.244 | 3.006 | 0.0730 | 4.616 | 0.787 | 0.5447 | 0.0250 | 0.8042 | 0.7859 | 2.406 | 7.033 |
| CV(%) | | 5 | 20 | 8 | 35 | 20 | 23 | 27 | 42 | 30 | 25 | 12 |

OC= organic carbon, TN= total nitrogen, Av.P= available phosphorus, Ca= calcium, Mg= magnesium, Na= sodium, K= potassium, EA= exchangeable acidity, ECEC= effective cation exchange capacity, BS= base saturation percentage. CV= coefficient of variability.

increased (7 %) when exchange acidity increased (21.3%, + 0.46 $cmolkg^{-1}$) in cultivated soil. Spark (1995) made similar observation.

Amounts of soil organic carbon (SOC) found in both soils showed an increase with depth. The decrease on the surface (0-32 cm) of cultivated soil was 28% (-2.58 $gkg^{-1}$) and accumulated by 63.0% at 100+ cm soil depth (Table 3). Frequent erosion phenomena and crop harvests could be the cause of low organic carbon in arable (cultivated) soils. Similarly, inversion and pulverization of the soil during tillage makes for accelerated mineralization of exposed organic matter (Connoly, 1998).

Mean SOC was higher by 28.3% under fallow soil in relation to cultivated land use (Table 4). The high SOC could be associated to the ability of the fallow soil to sequester higher quantity of carbon due to their long-term existence with very minimal disturbance, decomposition of plant (litter falls) and animal tissue in the soil thereby releasing soil organic matter. Wei et al (2010) reported that environmental and climatic factors also favor higher level of carbon sequestration in the fallow soils as they are covered by trees and as they are under cover vegetation that reduces extreme climatic elements and thus preserves soil moisture and reduces high thermal fluctuations.

The mean values of most of the chemical properties showed increases with soil depth. However, compared to the control (native) soil, there were decreases in the mean values of total N (26%, -0.17 $gkg^{-1}$), available P (47%, -2.63 $mgkg^{-1}$), $Ca^{2+}$ (55%, -1.75 cmol (+) $kg^{-1}$), CEC (17%, - 1.11 $cmolkg^{-1}$), and base saturation (11.3%) observed in cultivated surface soil (0-32 cm) (Table 3). These results are in line with the findings by Urioste et al. (2006) that cultivation affected the distribution of organic carbon, total nitrogen and phosphorus in soils of the semiarid region of Argentinian Pampas.

Soil CEC has been classified as low (< 6 cmol. $kg^{-1}$), medium (6-12 cmol. $kg^{-1}$) and high (> 12 cmol. $kg^{-1}$) for some Nigerian soils (Ezeaku et al., 2012). On the basis of this classification, mean CEC of cultivated and fallow soils was low (6.4 $cmolkg^{-1}$) and medium (7.51 $cmolkg^{-1}$), respectively. Decrease in CEC suggests decrease in buffering capacity, and is a cause for concern as both land use types with low to medium CEC can be catalogued as unsustainable land use. Low CEC value of tropical soils is due to dominance of kaolinitic clays in the fine earth fraction (Spark, 1995). The CEC generally increases with soil pH, an indication that low CEC obtained under cultivated soils could be accounted for by the low soil pH.

## Conclusion

The results revealed that there were variations in some of the measured soil properties in cultivated and fallowed fields. Soil properties under fallow have the highest contents of chemical properties and hence an improved soil fertility. The lower content of some of the measured soil properties observed in the cultivated soil was associated to land use modification when compared to the soil properties under 10 year fallow land use. Restoring vegetative cover is critical to remediate degraded land as in the cultivated land and to achieve its sustainable use. The combination of tillage-mulch practices with cropping systems may have synergistic effects on the soil properties. Therefore, location specific adoption of tillage-mulch-crop combination for the soil is recommended for higher SOC and to decrease risk of soil erosion and productivity decline.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Effects of season and species on *in sacco* degradability of forages in the sub-humid subtropical savannah

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**Effect of season and plant species on *in sacco* dry matter (DM) and crude protein (CP) degradability of five plant species were investigated. Plant species were *Acacia natalitia*, *Acacia nilotica*, *Dichrostachys cinerea*, *Scutia myrtina* and *Chromolaena odorata*. Leaves were harvested during dry, early wet and late wet seasons, subjected to degradation in cows' rumen using nylon bags technique. Season affected potential DM degradability and effective degradation of DM and CP. Species affected all parameters except slowly degradable fraction of CP. Interaction between season and species affected the parameters except potential and slowly degradable fraction of CP. *Chromolaena odorata* had highest estimated parameters of degradation among seasons compared to others. Based on potential and effective degradation, plants followed this decreasing order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. These plants have a potential as feed supplements. *C. odorata* has the highest potential as feed protein source in ruminants. It concluded that season and species affected *in sacco* degradability of DM and CP of browse species.**

**Key words:** Dry matter, crude protein, *Chromolaena odorata*, ruminants, nutritive value.

## INTRODUCTION

Smallholder farmers in subtropical savannah of Africa keep different ruminant species, most of which survive on natural pastures (Ugwu, 2007). The productivity of these ruminant species depends on quantity and quality of feeds (forage), which is affected by seasonal fluctuations (Abusuwar and Ahmed, 2010). The lowest quantity of forage occurs during dry season and may limit feeding and production of livestock. One strategy to increase value is the use of trees and shrubs as a sufficient source of food for ruminants. Some of forages are legumes, and legumes offer important sources of protein to maintain ruminant production in tropical savannah (Belachew et al., 2013; Gusha et al., 2013). Browse and shrub fodders are essential because they reduce seasonal limitation in ruminant feed (Balgees et al., 2013; Belachew et al., 2013). However, the distribution of tannins and other phenolic compounds in shrubs and tree leaves limits their utilization as animal feed (Belachew et al., 2013). Hence,

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**Table 1.** Chemical compositions (g $kg^{-1}$ DM) of five main browse species selected by goats sampled in three seasons at Zululand Thornveld.

| Season | Species | Parameters | | | | |
|---|---|---|---|---|---|---|
| | | CP | NDF | ADF | ADL | CT |
| Dry | *A natalitia*[a] | 121.9 | 405.8 | 275.9 | 200.0 | 117.7 |
| | *A. nilotica* | 115.7 | 228.1 | 130.6 | 79.5 | 3.8 |
| | *D. cinerea* | 109.1 | 452.7 | 330.5 | 207.0 | 45.8 |
| | *S. myrtina* | 105.0 | 429.4 | 291.6 | 199.7 | 32.2 |
| | *C. odorata*[b] | 185.8 | 360.0 | 218.6 | 97.5 | 0.5 |
| Early wet | *A. natalitia* | 140.7 | 380.6 | 259.7 | 203.6 | 211.7 |
| | *A. nilotica* | 136.1 | 242.9 | 120.5 | 90.2 | 16.5 |
| | *D. cinerea* | 172.8 | 427.3 | 243.3 | 161.2 | 59.0 |
| | *S. myrtina* | 122.8 | 452.0 | 247.8 | 181.7 | 207.6 |
| | *C. odorata* | 215.7 | 212.9 | 130.2 | 56.4 | 0.5 |
| Late wet | *A. natalitia* | 132.7 | 497.6 | 401.3 | 324.0 | 97.0 |
| | *A. nilotica* | 137.7 | 272.4 | 180.6 | 119.5 | 2.6 |
| | *D. cinerea* | 169.9 | 585.6 | 511.6 | 337.0 | 33.8 |
| | *S. myrtina* | 128.9 | 471.2 | 400.0 | 277.9 | 133.4 |
| | *C. odorata* | 226.4 | 336.6 | 297.9 | 204.7 | 0.5 |
| | RMSE | 2.0 | 8.4 | 32.2 | 18.1 | 5.1 |
| **Sources of variation effects** | | | | | | |
| Season | | *** | *** | *** | *** | *** |
| Species | | *** | *** | *** | *** | *** |
| Season × Species | | *** | *** | * | *** | *** |

[a]Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive non-native species; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; CT, condensed tannin; RMSE = root mean square error; ns ($P > 0.05$); * ($P < 0.05$); *** ($P<0.0001$).

evaluation of nutritive value of browse trees becomes important only when browse species is used as ruminant feeds.

*In sacco* degradability is a main evaluation technique of nutritive value of forages (Ørskov and McDonald, 1979). It is a useful method for ranking browse trees in terms of quality (Mehrez and Ørskov, 1977), and for evaluating the digestive abilities of ruminant species (Migongo-Bake, 1992). *In sacco* estimation has a benefit of estimating the degradation of particular constituent of feed such as dry matter (DM), crude protein (CP). *In sacco* technique also does not only determine the extent of degradation, but the part that degrades fast and its rate (Ørskov and McDonald, 1979). Moreover, estimation of soluble and slowly degradable fractions is necessary for dietary protein, thus estimating dietary protein used by rumen microbes, and that which bypass the rumen and become available for digestion in the small intestine.

The objective of this study was to determine the effect of season and plant species on *in sacco* degradation characteristics of dry matter and nitrogen on edible forage of browse species in sub-humid subtropical savannah.

## MATERIALS AND METHODS

### Plant samples and their collecting area

Leaves of five plant species (main in the field and goat's diet) selected by goats were sampled during dry (June/July 2008), early (November/December 2008) and late wet (February/March 2009) seasons at the Owen Sitole College of Agriculture (OSCA), Empangeni, South Africa (Basha et al., 2012). The mean annual rainfall of OSCA is 1022 mm and temperature is 26°C, and the type of soil is Mayo/Tambankulu. The early and late wet seasons are each part of the wet (rainy) season. January is the middle of the wet season. Plant species were *Acacia natalitia* (Mely), *Acacia nilotica* (L. Willd ex Del), *Dichrostachys cinerea* (L.Wight Arn), *Scutia myrtina* (Burm. f.) and *Chromolaena odorata* (King and Robinson). Browse samples were randomly sampled by collecting leaves 1.0 to 1.5 m above ground from three non-browsed trees per species per season. Once collected, leaf samples were kept in paper bags and air dried prior to oven drying at 60°C for 48 h. Part of dried samples was ground through 1-mm mesh sieve (Retsch GmbH & Co. KG 5657 HANN 1, West-Germany) for chemical analysis (Table 1). Other part of dried samples was milled through a 2-mm mesh sieve for *in sacco* purpose. All ground samples were stored in sealed plastic bottles until used.

Samples were analysed for chemical composition on dry matter basis using pseudo replicates (3 replicates). Nitrogen (N)

concentration was determined based on AOAC (Wendt, 2003) using a LECO, FP2000, nitrogen analyser. Crude protein (CP) was calculated as 6.25 × N concentration. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991) using ANKOM Technology Technique. The NDF was determined with α-amylase. The acid-butanol proanthocyanidin assay (Porter et al., 1985) was used to determine condensed tannin (CT) (Makkar, 1995). Cellulose was calculated as the difference between ADF and ADL, while hemicellulose was derived from the difference between NDF and ADF.

### *In sacco* degradability

The experiment was conducted at the Livestock Section of the University of KwaZulu-Natal Research Farm (Ukulinga), South Africa. Three rumen-fistulated cows (average weight: 350± 45 kg LW) were used. Cows were each fed 2 kg of Lucerne hay per day, with *ad libitum* access to hay, water and a mineral lick. Cows were adapted to the diet for one week before being used in the experiment. The experiment followed the nylon bag technique described by Mehrez and Ørskov (1977).

Three grams of each dry sample per incubation period replicated thrice (three nylon bags) were incubated for 0, 3, 6, 9, 12, 24, 48 and 72 h in three fistulated cows (one bag/cow). The whole bag size was 18×8 cm with pore size of 40 to 60 µm. Samples were incubated in four batches; all samples within a batch were withdrawn simultaneously. Withdrawn samples were cleaned with water and kept in a refrigerator till washing date, when all samples were washed together including zero hour ones. Washing occurred in a semi–automatic washing machine (Hoovermatic model T4350, South Africa) in 6 cycles of 5 min each. The washed bags were dried in an oven (LABCO, model 5SOE1B, P.O. Box 155, Maraisburg 1700) at 60˚C for 48 h, cooled in a desiccator and weighed. Residues were analysed for nitrogen using a LECO, FP2000, nitrogen analyzer. The DM and CP degradation data were fitted to the exponential equation (McDonald, 1981):

$$Y = a + b\,(1 - e^{-c(t-lt)})$$

where, $Y$ is the degradability of DM and CP at time ($t$), $a$ is the soluble fraction which is rapidly washed out the bags, $b$ is the insoluble fraction which is potentially degradable by micro-organisms, $c$ is the degradation rate of fraction b per hour and $lt$ is the lag time.

The effective degradability (ED) of DM and CP were calculated at a rumen out flow rate ($r$) of 0.03 $h^{-1}$ using the following equation:

$$ED = a + b*c/(c+r).$$

### Statistical analysis

All degradation variables are followed by subscript 'dm' or 'n' to indicate the nutrient being degraded (dry matter or nitrogen). Data were subjected to analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS (2002) in a 3 seasons ×5 feeds factorial design with three replicates. The model used was:

$$Y_{ijk} = \mu + s_i + p_j + (sp)_{ij} + \varepsilon_{ijk};$$

where, $Y_{ijk}$ is the observation, µ is the population mean, $s_i$ is the season effect ($i$ = 1–3), $p_j$ is the plant species effect ($j$ = 1–5), $(sp)_{ij}$ is the interaction between season and plant pecies and $\varepsilon ijk$ is the residual error. Statistical significance was declared at P<0.05. Means were compared by least significant difference (LSD). Correlation was used to test the relationships between *in sacco* degradability and chemical variables of browse species.

## RESULTS

### Effects of season and plant species on *in sacco* dry matter degradation

Table 2 shows the effect of season and plant species on *in sacco* dry matter degradation. Season affected (P<0.001) the soluble fraction ($a_{dm}$), potential degradability ($PD_{dm}$), effective dry matter degradability ($ED_{dm}$) and lag time ($lt_{dm}$). Browse species and its interaction with season affected (P<0.001) all variables ($a_{dm}$, $b_{dm}$, $c_{dm}$, $PD_{dm}$, $ED_{dm}$ and $lt_{dm}$). Among the three seasons, the soluble fraction was higher in the dry season than in the early wet and the late wet seasons. The $PD_{dm}$ and $ED_{dm}$ were higher in the dry season than in the early wet and late wet seasons. The $lt_{dm}$ was longest in the late wet season and shortest in the dry season.

Among five plant species, *A. natalitia* had the highest soluble fraction ($a_{dm}$) and *C. odorata* had moderate $a_{dm}$, while *A. nilotica.*, *D. cinerea* and *S. myrtina* had similar $a_{dm}$. *C. odorata* had highest insoluble degradability ($b_{dm}$), while *A. natalitia* and *S. myrtina* had similar and moderate $b_{dm}$, and *A. nilotica* and *D. cinerea* had lowest $b_{dm}$. *C. odorata* had the fastest degradation rate, followed by *A. natalitia*, *A. nilotica*, *D. cinerea* and *S. myrtina* in this order. The $PD_{dm}$ and $ED_{dm}$ showed similar trend among the species, *C. odorata* had highest values followed by *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea* in this order. *Acacia natalitia* had the longest $lt_{dm}$, whilst *S. myrtina* had the shortest $lt_{dm}$.

Interaction between season and browse species showed different trends for these variables. For the five species, except *D. cinerea*, soluble fraction decreased from the dry to the early wet seasons then decreased in the late wet season (*A. natalitia*, *A. nilotica* and *C. odorata*) or increased in the late wet season (*S. myrtina*), while the soluble fraction of *D. cinerea* increased from the dry to the early wet seasons from where it decreased in the late wet season. The degradation rate ($c_{dm}$) of all five species except *C. odorata* were low and did not change throughout the three seasons, while degradation rates of *C. odorata* decreased from the dry to the early wet and late wet seasons in this order.

The $PD_{dm}$ for *A. natalitia* decreased from the dry season to the early wet and late wet seasons in this order. For *A. nilotica*, the $PD_{dm}$ was similar between the dry and the early wet seasons but decreased in the late wet season. The $PD_{dm}$ of *D. cinerea* did not change during the three seasons. For *S. myrtina* and *C. odorata*, the $PD_{dm}$ decreased (*S. myrtina*) or increased (*C. odorata*) from the dry to the early wet and late wet seasons which were similar.

The $ED_{dm}$ for *A. natalitia*, *A. nilotica* and *S. myrtina* decreased from the dry to the early wet and late wet seasons in this order. For *D. cinerea* and *C. odorata* the

**Table 2.** *In sacco* dry matter degradation constants of plant species harvested at different three seasons from sub-humid subtropical savanna.

| Season | Species | $a_{dm}$ | $b_{dm}$ | $c_{dm}$ | $PD_{dm}$ | $ED_{dm}$ | $lt_{dm}$ |
|---|---|---|---|---|---|---|---|
| Dry | *A natalitia*[a] | 308.5 | 520.6 | 0.032 | 829.0 | 576.1 | -1.256 |
| | *A. nilotica* | 527.6 | 359.6 | 0.066 | 887.2 | 773.7 | 0.847 |
| | *D. cinerea* | 307.2 | 289.3 | 0.026 | 596.5 | 429.5 | -0.377 |
| | *S. myrtina* | 292.3 | 519.4 | 0.022 | 811.7 | 492.8 | -2.102 |
| | *C. odorata*[b] | 352.7 | 484.3 | 0.341 | 837.1 | 795.9 | -0.064 |
| Early wet | *A. natalitia* | 267.8 | 451.0 | 0.035 | 718.8 | 510.7 | -0.241 |
| | *A. nilotica* | 500.1 | 357.4 | 0.066 | 857.5 | 745.1 | 1.001 |
| | *D. cinerea* | 331.3 | 227.3 | 0.040 | 558.6 | 458.4 | 1.052 |
| | *S. myrtina* | 241.7 | 448.8 | 0.020 | 690.5 | 422.6 | -0.568 |
| | *C. odorata* | 328.9 | 609.4 | 0.280 | 938.2 | 879.1 | 0.056 |
| Late wet | *A. natalitia* | 207.8 | 390.5 | 0.042 | 598.3 | 433.5 | 0.235 |
| | *A. nilotica* | 327.4 | 443.2 | 0.088 | 770.7 | 657.8 | -0.262 |
| | *D. cinerea* | 221.5 | 324.4 | 0.039 | 545.9 | 394.0 | 0.545 |
| | *S. myrtina* | 255.2 | 398.7 | 0.021 | 654.0 | 408.8 | 0.833 |
| | *C. odorata* | 316.8 | 632.2 | 0.229 | 949.0 | 875.7 | -0.030 |
| | RMSE | 7.1 | 50.9 | 0.029 | 50.2 | 10.6 | 0.648 |
| **Sources of variation effects** | | | | | | | |
| Season | | *** | ns | ns | *** | *** | ** |
| Species | | *** | *** | *** | *** | *** | ** |
| Season x Species | | *** | *** | * | *** | *** | ** |

[a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive none-native species; $a_{dm}$, the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable; $b_{dm}$, the proportion of insoluble nutrient which is potentially degradable by micro-organisms; $c_{dm}$, the degradation rate of fraction $b_{dm}$ per hour; $PD_{dm}$, the potential degradability; $ED_{dm}$, effective dry matter degradability; $lt_{dm}$, lag time; RMSE, root mean square error; ns (P>0.05); **(P<0.01); * (P<05); *** (P<0.001).

$ED_{dm}$ increased from the dry to the early wet seasons then decreased in the late wet season (*D. cinerea*) or remained similar between the early wet and the late wet season (*C. odorata*).

The lag time ($lt_{dm}$) for *A. natalitia* and *S. myrtina* increased from the dry to the early wet and late wet seasons in this order. For *A. natalitia*, *D. cinerea* and *C. odorata* the $lt_{dm}$ increased from the dry to the early wet seasons from where it decreased in the late wet season.

## Effects of season and plant species on *in sacco* nitrogen degradation

Table 3 shows the effect of season and plant species on *in sacco* nitrogen degradation. Season affected (P<0.001) only the soluble fraction ($a_n$) and $ED_n$. Species strongly affected (P<0.001) $a_n$, $c_n$, $PD_n$ and $ED_n$, weakly affected (P<0.05) the $lt_n$. Interaction between season and species affected (P<0.001) $a_n$, $c_n$ and $ED_n$. Among these three seasons, $a_n$ and $ED_n$ were higher in the early wet season than in the dry and the late wet seasons.

Among the five plant species, *C. odorata* had the highest soluble fraction ($a_n$), while *A. nilotica* and *S. myrtina* had moderate ($a_n$), and *A. natalitia* and *D. cinerea* had similar and low $a_n$. *C. odorata* had the fastest degradation rate ($c_n$) followed by *A. nilotica*, *A. natalitia*, *D. cinerea* and *S. myrtina* in this order. *C. odorata* had the highest $PD_n$ and $ED_n$ followed by *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea* in this order. *A. nilotica* had the longest $lt_n$, whilst *S. myrtina* had the shortest $lt_n$.

According to interaction between season and browse species, the $a_n$ fraction in *A. natalitia*, *S. myrtina* and *C. odorata* decreased from the dry to the early wet seasons which was either similar to the late wet season (*A. natalitia* and *S. myrtina*) or lower than in the late wet season (*C. odorata*). For *A. natalitia* and *D. cinerea,* $a_n$ increased from the dry to the early wet seasons then either decreased in the late wet season (*A. natalitia)* or remained similar to the late wet seasons (*D. cinerea*).

During the dry season, the degradation rate ($c_n$) was slowest with *D. cinerea*, *A. natalitia* and *S. myrtina;* intermediate with *A. nilotica* and fastest with *C. odorata.* During the early wet season, the degradation rate was slowest for *A. natalitia* and *S. myrtina*; intermediate for *A. nilotica* and *D. cinerea*; and fastest for *C. odorata.* For the late wet season, the degradation rate was fastest for *C. odorata followed by A. nilotica*, *A. natalitia, D. cinerea*, and *S. myrtina* in this order. Among seasons, *A. nilotica*

**Table 3.** *In sacco* nitrogen degradation constants of plant species harvested at three seasons harvested at different three seasons from sub-humid subtropical savanna.

| Season | Species | $a_n$ | $b_n$ | $c_n$ | $PD_n$ | $ED_n$ | $lt_n$ |
|---|---|---|---|---|---|---|---|
| Dry | *A. natalitia*[a] | 267.8 | 646.2 | 0.030 | 914.1 | 569.5 | 0.534 |
| | *A. nilotica* | 351.2 | 522.0 | 0.057 | 873.2 | 692.3 | 1.649 |
| | *D. cinerea* | 171.7 | 543.2 | 0.014 | 714.9 | 337.7 | 0.712 |
| | *S. myrtina* | 336.3 | 449.9 | 0.033 | 786.2 | 535.7 | -0.088 |
| | *C. odorata*[b] | 433.7 | 480.6 | 0.282 | 914.3 | 867.9 | -0.069 |
| Early wet | *A. natalitia* | 231.5 | 545.0 | 0.036 | 776.5 | 526.1 | -0.747 |
| | *A. nilotica* | 432.3 | 452.2 | 0.054 | 884.5 | 722.4 | 1.694 |
| | *D. cinerea* | 276.2 | 2558 | 0.056 | 532.1 | 442.7 | -0.893 |
| | *S. myrtina* | 286.8 | 432.1 | 0.026 | 718.9 | 464.3 | -0.619 |
| | *C. odorata* | 407.3 | 566.6 | 0.265 | 973.9 | 916.0 | 0.108 |
| Late wet | *A. natalitia* | 242.0 | 404.1 | 0.037 | 646.1 | 464.1 | 0.298 |
| | *A. nilotica* | 298.6 | 466.6 | 0.077 | 765.2 | 631.9 | 0.836 |
| | *D. cinerea* | 261.0 | 433.8 | 0.037 | 694.8 | 453.9 | -0.756 |
| | *S. myrtina* | 279.1 | 507.3 | 0.024 | 786.5 | 461.9 | -1.001 |
| | *C. odorata* | 388.1 | 580.5 | 0.209 | 968.6 | 895.7 | 0.036 |
| | RMSE | 20.0 | 106.2 | 0.014 | 103.7 | 15.6 | 1.199 |
| **Sources of variation effects** | | | | | | | |
| Season | | *** | ns | ns | ns | *** | ns |
| Species | | *** | ns | *** | *** | *** | * |
| Season x Species | | *** | ns | *** | ns | *** | ns |

[a] Formerly part of *Acacia karroo* (Coates Palgrave, 2002); [b] invasive none-native species; $a_n$, the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable; $b_n$, the proportion of insoluble nutrient which is potentially degradable by micro-organisms; $c_n$, the degradation rate of fraction $b_n$ per hour; $PD_n$, potential degradable; $ED_n$, effective nitrogen degradability; $lt_n$, lag time; RMSE, root mean square error; ns (P>0.05); * (P<05); *** (P<0.001).

for which the degradation rates were similar between the dry and the early wet seasons but higher during the late wet season. The degradation rate of *D. cinerea* was fastest during the early wet season and slowest during the dry season. For *C. odorata,* the degradation rate was fastest, moderate and low during the dry, early wet and late wet seasons, respectively.

The effective nitrogen degradability ($ED_n$) for *A. natalitia* and *S. myrtina* decreased from the dry to the early wet seasons from where it remained similar (*A. natalitia*) or decreased in the late wet season (*S. myrtina*). For *A. nilotica*, *D. cinerea* and *C. odorata* the $ED_n$ increased from the dry to the early wet seasons then decreased in the late wet season (*A. nilotica* and *C. odorata*) or remained similar between the early wet and the late wet seasons (*D. cinerea*).

## Correlation between chemical composition and *in sacco* degradability

Table 4 presents the correlations between the CP, NDF, ADF, ADL, CT, cellulose (Cell) and hemicellulose (Hcell), and DM and nitrogen degradation parameters. Crude protein was strongly and positively correlated (P<0.001) with degradation rate of $b_{dm}$ and $b_n$, and negatively correlated (P<0.05) to CT. Crude protein was strongly and positively correlated (P<0.001) with $ED_n$ and moderately and positively correlated (P<0.01) with $ED_{dm}$ and $a_n$. Crude protein was weakly and positively correlated (P<0.05) with $b_{dm}$, $PD_{dm}$ and $PD_n$. The soluble fraction ($a_{dm}$) was strongly and negatively correlated (P<0.001) to fibre fractions (NDF, ADF and ADL) while $a_n$ was moderately and negatively correlated (P<0.01) to NDF and ADL as well as $a_n$ was weakly correlated (P<0.05) to ADF. Neutral detergent fibre was strongly and negatively correlated (P<0.001) with $PD_{dm}$ and $ED_{dm}$ which were moderately correlated (P<0.01) with ADF and ADL. Effective N degradation ($ED_n$) and $lt_n$ had negative correlations (P<0.01) with NDF; and weak negative correlations (P<0.05) with ADF, ADL and CT. The $PD_n$ was negative correlated (P<0.05) to NDF. Condensed tannins had negative correlation (P<0.05) with $a_{dm}$, $a_n$ and $ED_{dm}$. Cellulose negatively correlated (P<0.05) with $a_{dm}$ and $lt_n$.

## DISCUSSION

Seasonality was hypothesized to affect rumen

**Table 4.** Correlation coefficient between chemical composition, and *in sacco* dry matter and nitrogen degradation and estimated parameters.

| Parameters | Chemical constituents | | | | | | |
|---|---|---|---|---|---|---|---|
| | CP | NDF | ADF | ADL | CT | Cell | Hcell |
| $a_{dm}$ | 0.13$^{ns}$ | -0.78*** | -0.77*** | -0.77*** | -0.52* | -0.55* | -0.02$^{ns}$ |
| $b_{dm}$ | 0.57* | -0.36$^{ns}$ | -0.22$^{ns}$ | -0.21$^{ns}$ | -0.08$^{ns}$ | -0.18$^{ns}$ | -0.33$^{ns}$ |
| $c_{dm}$ | 0.83*** | -0.47$^{ns}$ | -0.35$^{ns}$ | -0.48$^{ns}$ | -0.53* | 0.06$^{ns}$ | -0.30$^{ns}$ |
| $PD_{dm}$ | 0.55* | -0.81*** | -0.69** | -0.68** | -0.41$^{ns}$ | -0.51$^{ns}$ | -0.29$^{ns}$ |
| $ED_{dm}$ | 0.76** | -0.86*** | -0.70** | -0.74** | -0.62* | -0.41$^{ns}$ | -0.38$^{ns}$ |
| $lt_{dm}$ | 0.22$^{ns}$ | -0.14$^{ns}$ | -0.05$^{ns}$ | -0.08$^{ns}$ | -0.17$^{ns}$ | 0.04$^{ns}$ | -0.22$^{ns}$ |
| $a_n$ | 0.65** | -0.66** | -0.59* | -0.65** | -0.56* | -0.27$^{ns}$ | -0.17$^{ns}$ |
| $b_n$ | 0.22$^{ns}$ | -0.31$^{ns}$ | -0.12$^{ns}$ | -0.13$^{ns}$ | 0.01$^{ns}$ | -0.07$^{ns}$ | -0.46$^{ns}$ |
| $c_n$ | 0.84*** | -0.48$^{ns}$ | -0.36$^{ns}$ | -0.49$^{ns}$ | -0.52* | 0.04$^{ns}$ | -0.29$^{ns}$ |
| $PD_n$ | 0.56* | -0.63* | -0.45$^{ns}$ | -0.50$^{ns}$ | -0.34$^{ns}$ | -0.22$^{ns}$ | -0.44$^{ns}$ |
| $ED_n$ | 0.82*** | -0.74** | -0.58$^{*}$ | -0.64* | -0.55$^{*}$ | -0.28$^{ns}$ | -0.39$^{ns}$ |
| $lt_n$ | 0.05$^{ns}$ | -0.65** | -0.58* | -0.52* | -0.51* | -0.57* | -0.17$^{ns}$ |

CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CT, condensed tannin; Cell, cellulose; Hcell, hemicellulose; $a_{dm}$, the soluble fraction of dry matter which is rapidly washed out of the bags and is assumed to be completely degradable; $b_{dm}$, the proportion of insoluble nutrient which is potentially degradable by micro-organisms; $c_{dm}$, the degradation rate of fraction $b_{dm}$ per hour; $PD_{dm}$, the potential degradability; $ED_{dm}$, effective dry matter degradability; $lt_{dm}$, lag time of dry matter; $a_n$, the soluble nitrogen fraction which is rapidly washed out of the bags and is assumed to be completely degradable; $b_n$, the proportion of insoluble nitrogen which is potentially degradable by micro-organisms; $c_n$, the degradation rate of $b_n$ per hour; $PD_n$, potential degradable; $ED_n$, effective nitrogen degradability; $lt_n$ , lag time of nitrogen; $^{ns}$ (P>0.05); * (P<0.05); ** (P<0.01); *** (P<0.001)

degradability due to variation in forage quality among seasons. Results supported the hypothesis. The *in sacco* degradability ($a_{dm}$, $PD_{dm}$ and $ED_{dm}$) of browse species was lower during the wet season than the dry season in agreement with a previous finding (Camacho et al., 2010). This decrease in DM degradability can be attributed to the effects of CT on accessible N, which can decrease ammonia concentrations and microbial growth in the rumen (Salem et al., 2007). Van Soest (1994) suggested that lignin and its cross-linkage to hemicellulose, polysaccharides and proteins could also depress digestibility. High $a_n$ and $ED_n$ of browse species during the early wet season is partly in agreement with Ramírez-Orduña (2003) who reported high $ED_n$ of browse plants during autumn and winter at Baja California Sur, Mexico. There may be variation in climate factors between the locations of two studies. Ramírez et al. (2000b) suggest that plants may response to produce new foliage with highly soluble CP due to warm temperatures and wet climate that arise sometime at the end of winter at Northeastern Mexico.

Rumen degradability was hypothesized to vary among plant species due to their variation in chemical composition and results supported the hypothesis. Consistent with our results, Melaku et al. (2003), Anele et al. (2009) and Balgees et al. (2013) reported significant variations in DM and CP degradation parameters of multipurpose trees. The DM and CP potential degradability in the current study overlapped the range of 720 - 914 and 546 – 949 g/kg, respectively, reported by Melaku et al. (2003). The current study had values that are higher than the range of 362 - 673 for the $PD_{dm}$ reported by Anele et al. (2009). Based on potential and effective degradation of both DM and CP, the plant species followed this order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. These differences in degradation may be associated to the structural and non-structural protein and carbohydrate fractions (Belachew et al., 2013). Previous reports suggested that the variation in the degradation parameters of the browse species may be due to the variation in chemical composition (Kamalak, 2006; Belachew et al., 2013; Gusha et al., 2013). Furthermore, the variations in chemical composition between seasons or among plant species have been reported (Gusha et al., 2013) which indicate the variation in degradation material of browse species. These variations in $PD_{dm}$ and $PD_n$ in the rumen have been reported as a result of variations in fibre content and tannins levels (Gusha et al., 2013) or due to other factors such as ash (Benjamin et al., 1995) or maturity (Kamalak, 2006; Gusha et al., 2013) . Moreover, $PD_{dm}$ and $PD_n$ were negatively correlated with NDF, ADF and CT (Kamalak, 2006). Acid detergent fibre (ADF) and tannins were negatively correlated with $PD_{dm}$ (Vadiveloo and Fadel, 1992). With regards to *C. odorata*, there has not been any previous report on the $PD_{dm}$ and $PD_n$.

The soluble fraction of DM ($a_{dm}$) and CP ($a_n$) varied within and among plant species, the highest $a_{dm}$ in *A. nilotica* during the three seasons while the highest $a_n$ values recorded in *C. odorata* in the dry and the late wet

seasons and *A. nilotica* in the early wet season. Comparable to the others, differences among these species may be because of variation in the type of carbohydrates (in term of structure and content). The $a_{dm}$ and $a_n$ were negative correlated with ADF, NDL and CT of browse species (Ramírez et al., 2000a).

Melaku et al. (2003) also reported that $a_{dm}$ and NDF were negatively correlated, and agree with the negative correlation in this study between $a_{dm}$ and $a_n$, and fibre fractions and CT. In addition, these results are consistent with the lowest values of $a_{dm}$ and $a_n$ in *A. natalitia* in the late wet season and in the two wet seasons, respectively, and *D. cinerea* in the late wet season and in the dry season. These species had higher fibre fractions in these seasons.

The greatest value of the slowly degradable fraction of DM ($b_{dm}$) and CP ($b_n$) in *A. natalitia* in the dry season and *C. odorata* during the two wet seasons and the lowest values of $b_{dm}$ and $b_n$ recorded in *D. cinerea* in the three seasons and in the early wet season, respectively. These parameters were not related to any measured chemicals in this study except $b_{dm}$ was positive correlated to CP. On the other hand, Ramírez et al. (2000a) reported that the slowly degradable fraction of plant cell wall was limited by ADL and tannins and by other factors not measured in the current study such as organic matter, ash and insoluble ash. Many studies reported that the extent of degradation of DM or CP was negatively correlated with NDF, ADF, ADL and CT (Melaku et al., 2003).

Markedly higher degradation rate of DM ($c_{dm}$) and protein ($c_n$) in *C. odorata* and the slowest rates ($c_{dm}$ and $c_n$) observed with *S. mytrina* in the three seasons; reflect differences in chemical composition between the plant species. For instance, *C. odorata* had higher CP and lower CT contents in the three seasons whilst *S. mytrina* had lower CP and higher CT. Results showed $c_{dm}$ and $c_n$ are positively correlated with CP but negatively correlated with CT and is consistent with findings of Kamalak (2006). Balgees et al. (2013) reported that the rate of degradation of protein ($c_n$) was negatively correlated to NDF and ADF concentrations. Melaku et al. (2003) found negative relationship between $c_n$ and CT, and between $c_{dm}$ and neutral detergent fibre bound nitrogen (NDF-N) and ADL, and positive relationship between $c_n$ and NDF-N.

The effective degradability of DM ($ED_{dm}$) and CP ($ED_n$) were positive correlated with CP, but negatively related with fibre fractions and CT. This is in agreement with results of previous studies (Kamalak, 2006; Gusha et al., 2013) reporting that $ED_{dm}$ and $ED_n$ were negatively correlated with NDF and ADF concentrations and $ED_{dm}$ was positive correlation to CP concentration. Melaku et al. (2003) found negative relationship between $ED_n$ and ADL. The differences in $ED_{dm}$ and $ED_n$ may be attributed to structural and non-structural CP and carbohydrate fractions, which affect protein solubility and bio-availability (Belachew et al., 2013).

## Conclusion

A significant variation in *in sacco* degradability parameters were reported among seasons and different browse species harvested from sub-humid subtropical savannah of South Africa, during dry, early, and late wet seasons. These variations were more related with fibre fractions than with tannins content. Fibre concentration appears to be the main factor limiting *in sacco* degradability. It is suggested that the dilution rate and other factors in the rumen may limit tannin effects on degradability. Based on potential and effective degradability, the plant species can be placed in the following decreasing order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*. Consequently, *C. odorata* is the best supplementary protein source like high-quality leguminous forages.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Fertigation studies in Japanese mint (*Mentha arvensis* L.) under humid climate in Odisha, India

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**The study was conducted to optimize the use of water and nutrients by Japanese mint (*Mentha arvensis* L.) with three moisture regimes [$I_1$ drip irrigation at 100%, $I_2$ at 80% and $I_3$ at 60% pan evaporation (PE)] and three fertility levels ($F_1$ 100%, $F_2$ 75% and $F_3$ 50% recommended dose of NPK) with an extra (control) treatment having surface irrigation and soil application of fertilizer. The experiment was laid out in Factorial Randomized Block Design with three replications at the Experimental Farm of the Directorate of Water Management, Bhubaneswar India (20° 30' N lat., 87° 48' E long, 45 m above mean sea level) during winter (dry) seasons of 2005-2006 and 2006-2007. Drip irrigation increased the herbage and oil yield by 15.9 and 15.2%, respectively as compared to surface irrigation. It saved 29% water as compared to the latter (925 mm). Soil moisture regimes maintained at 100% PE significantly enhanced crop growth, herbage yield (34,798 kg ha$^{-1}$), essential oil yield (254 kg ha$^{-1}$) and N uptake (120 kg ha$^{-1}$) compared to 60% PE. Application of 100% recommended dose of fertilizer significantly produced maximum herbage (32,572 kg ha$^{-1}$) and oil yield (246 kg ha$^{-1}$). Combination of irrigation at 100% PE with 100% RD of fertilizer produced maximum quantity of oil (260 kg ha$^{-1}$) with improvement in its quality as compared to other levels tested.**

**Key words:** Drip irrigation, fertilizer, Japanese mint, pan evaporation, recommended dose.

## INTRODUCTION

Japanese mint, also known as corn mint or menthol mint (*Mentha arvensis* L.), is one of the commercially cultivated and important essential oil bearing industrial crops in northern semi-arid and sub tropical region of India. It is a potential source of natural menthol and other ingredients viz., mint terpenes, menthone, isomenthone, menthyl acetate etc., which are extensively used in pharmaceutical, cosmetic, food and flavour industries. India is currently producing more than 18,000 tonnes of mint oil per year and has emerged as a major world supplier of mint oil and menthol (Patra, 2008). Mint being a leafy herb, responds to frequent irrigation during dry season's months to obtain good growth and high yields as reported by Shormin et al. (2009). It absorbs

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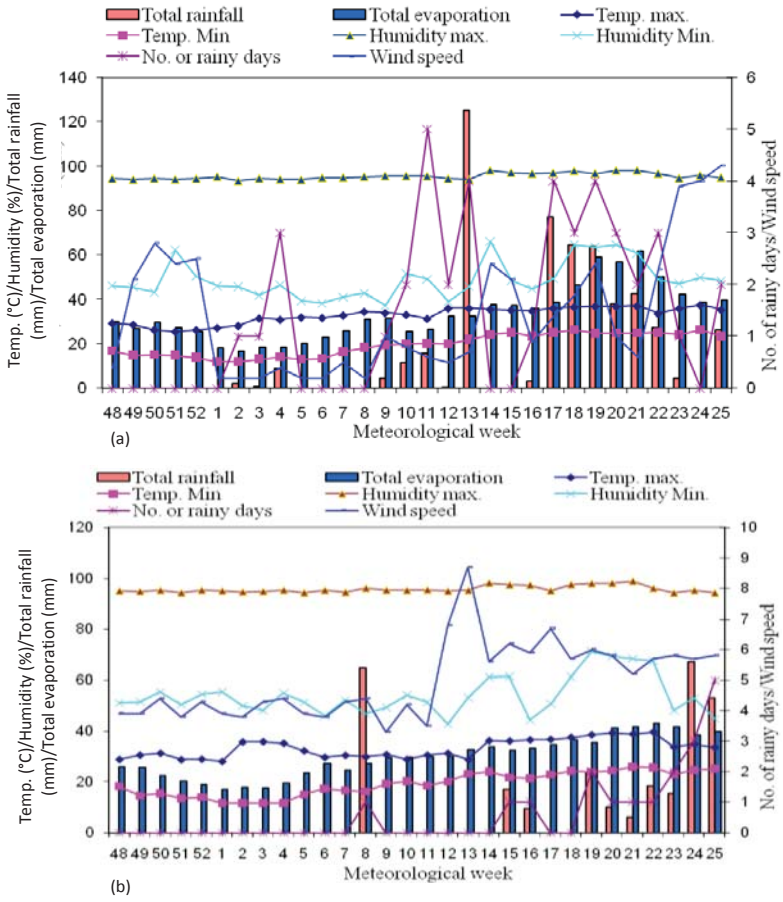


**Figure 1.** General climatic condition of experimental site during (a) 2005-2006, (b) 2006-2007.

substantial quantities of N, P and K as compared to other mint species. It responds well to high levels of nitrogen fertilizer, between 150 and 250 kg $ha^{-1}$, depending upon different agro-climatic conditions (Shormin et al., 2009). The irrigation requirement of mint differs from location to location depending on soil type, soil fertility status and climatic conditions. As little information is available on interaction effects of irrigation and fertilizer in a sandy loam acid soil under humid climate, the present investigation was undertaken to assess the irrigation and fertilizer in relation to its growth, yield and quality of the oil.

## MATERIALS AND METHODS

### Location of the experiment

The field experiment was conducted during winter (dry) seasons of 2005-2006 and 2006-2007 at the research farm of the Directorate of Water Management, Bhubaneswar, India, located at 20° 30' N latitude and 87° 48' E longitude at an elevation of 45 m above mean sea level. It is about 52 km away towards the west from the Bay of Bengal, representing warm, moist with hot and humid summer and mild winter.

### Soil

The soil of the experimental site was a sandy loam with pH 5.7. The bulk density ranged from 1.44 to 1.52 g $cm^{-3}$, field capacity 19.47% to 26.10% (w/w %) and permanent wilting point 8.56 to 12.49% (w/w %). The available soil moisture was 111 mm per 0 to 60 cm soil depth with organic carbon content of 0.46% (Jackson, 1967). The available N was 159 kg $ha^{-1}$ (Subbiah and Asija, 1956), P 21 and K 183 kg $ha^{-1}$ (Jackson, 1973) in 0 to 15 cm soil profile.

### Climate

The climate is warm, moist with hot and humid summer and mild winter. The mean annual rainfall of the place was 1439 mm (1995-2004). The total amount of rainfall received during the cropping seasons of mint was 464 mm in 2005-2006 and 359 mm in 2006-2007 in 42 and 23 rainy days respectively. The total evaporation from open pan evaporimeter was 959 and 850 mm, maximum temperature ranged from 25.5 to 37.5°C and 28.1 to 39.6°C, whereas minimum temperature ranged from 12.0 to 26.1°C and 11.5 to 25.9°C in 2005-2006 and 2006-07, respectively (Figure 1a and b). The former season (2005-2006) was hotter than the latter (2006-2007). The relative humidity varied from 93.3 to 98.1%; 94.0 to 98.9% in the morning and 37.2 to 66.0% and 44.4 to 71.3% in the afternoon hours. The weekly total radiation varied from 4306 to 7405 $Wm^{-2}$ in 2005-2006 and 4305 to 7346 $Wm^{-2}$ in 2006-2007. The

**Table 1.** Number of irrigations applied to Japanese mint.

| Irrigation regimes based on pan evaporation | Number of irrigations per month | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **2006** | Dec | Jan | Feb | March | April | May | June | Total |
| 100% PE | 15 | 16 | 11 | 12 | 9 | 4 | 7 | 74 |
| 80% PE | 15 | 16 | 11 | 12 | 9 | 4 | 7 | 74 |
| 60% PE | 15 | 16 | 11 | 12 | 9 | 4 | 7 | 74 |
| Surface irrigation | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 11 |
| **2007** | | | | | | | | |
| 100% PE | 15 | 15 | 10 | 16 | 12 | 11 | 5 | 84 |
| 80% PE | 15 | 15 | 10 | 16 | 12 | 11 | 5 | 84 |
| 60% PE | 15 | 15 | 10 | 16 | 12 | 11 | 5 | 84 |
| Surface irrigation | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 11 |

One common pre-planting irrigation of 60 mm depth was given uniformly to all the treatments.

average wind speed ranged from 0.2 to 4.3 km $hr^{-1}$ in 2005-2006 and 3.3 to 8.7 km $hr^{-1}$ in 2006-2007.

### Treatment details

The treatments were consisting of three irrigation regimes based on pan evaporation ($I_1$ drip irrigation at 100% PE, $I_2$ at 80% PE and $I_3$ at 60% PE) and three levels of fertilizer [($F_1$100%, $F_2$ 75% and $F_3$ 50% of the recommended dose of NPK that is, 150-60-60 kg N-$P_2O_5$-$K_2O$ $ha^{-1}$) were tested in a Factorial Randomized Block Design with three replications. For comparison of experimental results between drip fertigation (DF) and surface irrigation, one treatment of surface irrigation and soil application of fertilizer was maintained as control.

Healthy and disease free suckers of variety “Koshi ” were used for planting at the rate of 0.5 t $ha^{-1}$. The suckers were dipped in 0.5% benelate solution for 10 min before planting to safeguard against root rot disease. About 10 cm long pieces of suckers were placed at 5 cm soil depth in furrows spaced at 60 cm. It was covered with thin layer of soil followed by a light irrigation to ensure good sprouting. Suckers were planted in the second week of December during both the years. The recommended dose (RD) of fertilizer consisting of 150-60-60 kg N, $P_2O_5$ and $K_2O$, $ha^{-1}$ was applied to the crop. Full dose of phosphorus was applied basally at the time of planting. It was placed in open furrows about 2.5 cm below the suckers and mixed well with the soil. Fertigation was given in equal splits at fortnightly interval from 15 days after planting (DAP) up to 30 days before harvest as per the treatment. Required amount of urea (46% N) and potash (60% K) were dissolved in water and fed to the drip system through a ventury. Fertigation was made by regulating the taps of the laterals by allowing the solution to the specified plots as per the treatments.

### Irrigation scheduling

Differential amount of water was supplied as per treatment, on the basis of two days cumulative pan evaporation (CPE) through meteorological approach (Pruitt, 1966; Jenson et al., 1961) Cumulative pan evaporation for different treatments was computed using data from a standard US Weather Bureau Class A open pan evaporimeter. The depth of water during each irrigation was maintained at 6 cm in case of surface irrigation. The water was drawn from the secondary reservoir. First irrigation was given one day prior to planting. Subsequent irrigations were given at two days interval in drip irrigation and at 60 mm CPE value in case of surface irrigation method. Irrigation was applied after deducting the rainfall if rainfall event occurred between irrigation cycles. Computation of irrigation water through drip system was made according to the following equation.

$$\text{Amount of irrigation water in litre} = \frac{\begin{array}{c}(\text{Lateral spacing in mm} \times \text{dripper spacing in mm}) \\ \times \text{Wetted area (60\%)} \times \text{crop coeficient at diferent} \\ \text{crop growth period} \times \text{two days} \\ \text{pan evaporation (mm)}\end{array}}{\text{Uniformity coefficient}}$$

The crop coefficient values of 0.60, 1.15 and 1.10 were used during vegetative, full growth and later part of the growth stages, respectively. In this experiment, the observed uniformity coefficient (UC) values varied from 92 to 94% for different treatments as discharge rate of drippers was measured frequently. Depending upon the discharge rate and UC, the time of operation of drip system was adjusted, and treatment wise irrigation water was applied. Time of operation of drip irrigation was calculated for 100% PE as follows as total number of drippers were 96 (32 in each plot ).

$$T = \frac{IW}{Oem \times Nem} \times 60$$

Where, T = Time in minutes,IW = Irrigation water (litre) = depth of irrigation (100% PE in mm) x plot area ($m^2$), $O_{em}$ = Output of emitter (litre $h^{-1}$), $N_{em}$ = Number of emitters per plot.

The number of irrigations given per month at two days interval under drip irrigation was worked out to be 74 in 2005-2006 and 84 in 2006-2007 (Table 1). The number of irrigation applied in the second year was more than the first due to dry spells prevailed during the month of March. Ground water contribution was considered zero as the depth of ground water table during the study period in the experimental field was beyond 8 m. Effective rainfall was taken in to account for computing consumptive use of water. Water use under different irrigation treatments was calculated by adding different components of moisture use (irrigation water applied ± change in soil profile moisture + effective rainfall). Water use efficiency (WUE) was expressed as the ratio of oil yield to that of the water used in kg ha-$mm^{-1}$.

The soil moisture content (v/v %) was monitored with the help of TDR moisture meter (model TRIME FM) in all irrigation levels (in drip and surface irrigation method) after installing 1 m length

**Table 2.** Growth characters of menthol mint as influenced by different levels of irrigation regime and fertilizer during 2006.

| Treatment | Plant height (cm) | | Leaf area index | | Leaf-stem ratio | | Dry matter ( g $m^{-2}$) | |
|---|---|---|---|---|---|---|---|---|
| | 35 days after first harvest | 75 days after first harvest | Before first harvest | Before second harvest | 35 days after first harvest | 75 days after first harvest | 35 days after first harvest | 75 days after first harvest |
| Method of irrigation | | | | | | | | |
| Control | 17.0 | 45.1 | 5.22 | 3.09 | 1.08 | 0.87 | 127.5 | 234.9 |
| DF | 24.0 | 51.5 | 6.66 | 3.51 | 1.11 | 0.94 | 143.6 | 270.7 |
| SE (m)± | 0.10 | 0.28 | 0.023 | 0.005 | 0.005 | 0.004 | 0.22 | 0.29 |
| CD (0.05) | 0.290 | 0.82 | 0.071 | 0.015 | 0.015 | 0.014 | 0.65 | 0.87 |
| Irrigation (I) | | | | | | | | |
| $I_1$ = 100% PE | 25.6 | 53.7 | 7.11 | 3.67 | 1.13 | 0.90 | 148.1 | 281.2 |
| $I_2$ = 80% PE | 24.0 | 51.8 | 6.95 | 3.57 | 1.10 | 0.86 | 146.3 | 276.8 |
| $I_3$ = 60% PE | 22.4 | 49.0 | 5.91 | 3.30 | 1.09 | 0.86 | 136.3 | 254.1 |
| **SE (m) ±** | 0.17 | 0.48 | 0.041 | 0.008 | 0.009 | 0.008 | 0.38 | 0.51 |
| CD (0.05) | 0.50 | 1.42 | 0.123 | 0.026 | 0.028 | 0.025 | 1.13 | 1.51 |
| Fertility (F) | | | | | | | | |
| $F_1$ = 100% RD | 24.6 | 52.5 | 6.85 | 3.66 | 1.12 | 0.88 | 151.3 | 286.4 |
| $F_2$ = 75% RD | 24.2 | 51.7 | 6.67 | 3.55 | 1.11 | 0.87 | 144.8 | 273.2 |
| $F_3$ = 50% RD | 23.3 | 50.3 | 6.45 | 3.32 | 1.09 | 0.87 | 134.7 | 252.4 |
| **SE (m) ±** | 0.17 | 0.48 | 0.041 | 0.008 | 0.009 | 0.008 | 0.38 | 0.51 |
| CD (0.05) | 0.50 | 1.42 | 0.123 | 0.026 | 0.028 | NS | 1.13 | 1.51 |

access tube near the emitter in drip irrigation. In surface irrigation it was placed between two crop rows. The depth interval for soil moisture measurement was fixed at 20 cm, which was continued up to 80 cm soil depth. Total available soil moisture in 80 cm depth was 9.38 cm. The depletion of soil moisture at different depth was computed based on the observations recorded frequently to assess degree of moisture stress in plant under different irrigation treatment in drip and surface method.

#### Plant analysis

The crop was harvested by taking the first cut at 115 days after planting and the second at 75 days after the first cut during both the years. The essential oil was extracted from the fresh herbage through steam distillation method using Clevenger`s type extracting apparatus made of glass (British Pharma Copoeia, 1958). The volume of oil was recorded and oil percent was computed by the following formula.

$$\text{Oil content (\%, w/w) on fresh weight basis} = \frac{\text{Weight of oil}}{\text{Weight of fresh herb}} \times 100$$

The oil percentage was multiplied with corresponding fresh herbage yield of each treatment to get the oil yield. The oil was analysed at the Central Institute for Medicinal and Aromatic Plants, Lucknow, India by gas liquid chromatography (Hewlett Packard 5890, column AT 1000, temperature from 100 to 170°C raised to 5°C per minute, carrier gas-nitrogen at 1 ml $min^{-1}$) for principal chemical constituents such as limonene, menthone, isomenthone, methyl acetate, neomenthol and menthol. Nitrogen content in the plant sample was estimated by the micro-Kjeldahl method. Leaf area was determined using a LICOR Leaf Area Meter model 3100.

## RESULTS AND DISCUSSION

### Crop growth

Drip fertigation significantly increased the growth attributes such as plant height (41.4 and 14.0%), leaf stem ratio (2.8 and 8.0%) and dry matter accumulation (12.6 and 15.2%) of mint crop after 35 and 75 days of first harvest during both years as compared to surface method (Table 2). Increasing the level of irrigation from 60% PE to 100% PE significantly enhanced the growth attributes such as plant height, leaf stem ratio and dry matter accumulation after 35 and 75 days of first harvest. Irrigating the crop at 100% PE had maximum leaf-stem ratio, which decreased marginally with 80 and 60% PE by 0.8 to 4.4% in 2005-2006 and 2.5 to 8.4% in 2006-2007. Similarly, application of irrigation at 100% PE ($I_1$) had maximum LAI (3.76 to 7.27) followed by 80% and 60% PE (Table 2). It decreased from 2.3 to 13.3% during both the seasons by reducing the quantity of irrigation water from 100% PE to 60% PE. Maximum amount of dry matter was produced by application of irrigation water at 100% PE and minimum with 60% PE.

Frequent irrigation enhanced the growth parameters due to quick development of extensive root system, which created a conducive environment to absorb more water and nutrient. It is well known that proper supply of moisture and nutrients helps in maintaining high photosynthetic rate, which increases the cell elongation and its multiplication at a much faster rate. It is further

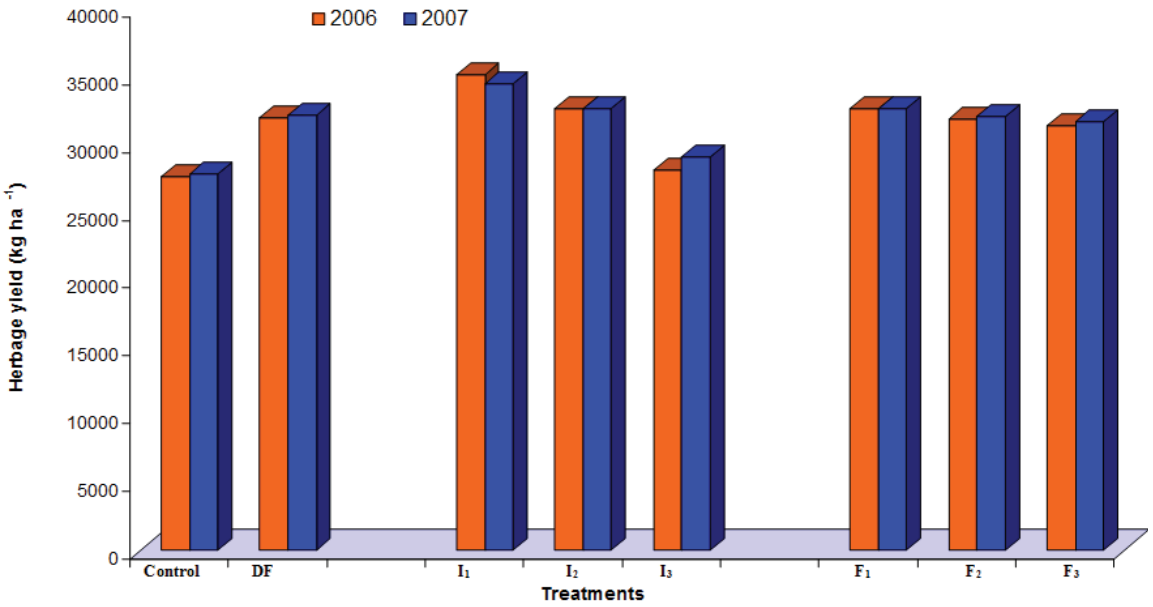


**Figure 2.** Effect of drip fertigation on herbage yield.

confirmed by the fact that higher Relative Growth Rate (RGR) and Crop Growth Rate (CGR) might be due to high rate of photosynthesis, which resulted in more accumulation of dry matter. Higher leaf temperature under low frequency irrigation might have increased respiration and decreased net assimilation rate resulting in low accumulation of dry matter. In the present study, all these factors cumulatively affected the plant growth from the initial stage to harvest under different irrigation regimes. Ram et al. (2006) and Shormin et al. (2009) obtained similar results. Plant height, leaf area index and dry matter accumulation were significantly influenced by application of 100% recommended dose of fertilizer. This may be attributed to more proliferation of root biomass resulting in more absorption of nutrients and water from the soil leading to production of higher vegetative biomass. At 35 days after first harvest, 100% RD increased LAI by 2.4 and 5% as compared to 75 and 50% RD, respectively. The dry matter was increased by 4.5 and 12.3% in 2005-2006 and 4.5 and 11.8% in 2006-2007, respectively in comparison with 75 and 50% RD at 35 days after first harvest. Adequate nutrition plays an important role on plant growth and development. It promotes vegetative growth through cell enlargement, multiplication and increase in the rate of photosynthesis (Patra et al., 2003; Rahman et al., 2003).

## Herbage and essential oil yield

### *Herbage yield*

Maximum herbage yield of 31, 925 kg ha$^{-1}$ in 2005-2006 and 32, 142 kg ha$^{-1}$ in 2006-2007 with mean yield of 32,034 kg ha$^{-1}$ were recorded with drip fertigation (Figure 2). Drip fertigation increased the yield by 16% compared to surface irrigation. The herbage yield was affected by different irrigation levels during both the seasons. Maximum herbage yield of 34,463 to 35,132 kg ha$^{-1}$ was obtained at 100% PE. The minimum yield of 28,079 to 29,010 kg ha$^{-1}$ was recorded at 60% PE ($I_3$). Application of irrigation at 100% PE increased the total yield from 7.7 to 25.1% in 2005-2006 and 5.8 to 18.8% in 2006-2007. The mean yield increased from 6.7 to 21.9% due to favorable soil moisture conditions maintained throughout the crop growth period. The favorable effect of irrigation in enhancing herb yield of various mint species have also been reported by Singh et al. ( 2002), Ram et al. ( 2006) and Shormin et al. (2009).

Application of 100% RD ($F_1$) produced maximum yield (32,558 to 32,586 kg ha$^{-1}$) with mean yield of 32,572 kg ha$^{-1}$. These results are in close conformity with the findings of Fasina et al. (2008). Reduction of 25% ($F_2$) and 50% fertilizer ($F_3$) from the recommended dose ($F_1$) decreased the total herbage yield by 1.6 to 2.1% and 3.0 to 3.8%, respectively. The yield was reduced more in first harvest than in the second one. High yield of menthol mint with high rate of NPK has been reported on soils with low N content (Table 3).

### *Oil yield*

Maximum oil yield was obtained with drip fertigation (232 to 240 kg ha$^{-1}$). Drip fertigation increased it from 17.0 to 20.3% at first harvest and 13 to 16.5% at the second

**Table 3.** Growth characters of menthol mint as influenced by different levels of irrigation regime and fertilizer during 2007.

| Treatments | Plant height (cm) | | Leaf area index | | Leaf-stem ratio | | Dry matter ( g $m^{-2}$) | |
|---|---|---|---|---|---|---|---|---|
| | 35 days after first harvest | 75 days after first harvest | Before first harvest | Before second harvest | 35 days after first harvest | 75 days after first harvest | 35 days after first harvest | 75 days after first harvest |
| Method of irrigation | | | | | | | | |
| Control | 19.9 | 52.2 | 6.18 | 3.20 | 1.06 | 1.08 | 137.0 | 233.8 |
| DF | 27.4 | 55.7 | 6.89 | 3.59 | 1.13 | 1.16 | 146.4 | 274.2 |
| SE (m)± | 0.25 | 0.53 | 0.020 | 0.005 | 0.004 | 0.004 | 3.39 | 0.37 |
| CD (0.05) | 0.743 | 1.57 | 0.060 | 0.015 | 0.014 | 0.012 | NS | 1.10 |
| Irrigation (I) | | | | | | | | |
| $I_1$ = 100% PE | 30.0 | 57.7 | 7.27 | 3.76 | 1.19 | 1.12 | 153.0 | 290.2 |
| $I_2$ = 80% PE | 27.4 | 56.5 | 7.09 | 3.60 | 1.11 | 1.06 | 153.0 | 278.8 |
| $I_3$ = 60% PE | 24.9 | 53.0 | 6.30 | 3.41 | 1.09 | 1.06 | 133.2 | 253.6 |
| **SE (m) ±** | 0.43 | 0.92 | 0.036 | 0.009 | 0.008 | 0.007 | 5.87 | 0.64 |
| CD (0.05) | 1.29 | 2.72 | 0.107 | 0.028 | 0.024 | 0.021 | 17.60 | 1.90 |
| Fertility (F) | | | | | | | | |
| $F_1$ = 100% RD | 28.3 | 57.7 | 7.02 | 3.66 | 1.14 | 1.10 | 154.0 | 288.8 |
| $F_2$ = 75% RD | 27.4 | 55.2 | 6.88 | 3.59 | 1.13 | 1.08 | 147.4 | 276.6 |
| $F_3$ = 50% RD | 26.6 | 54.2 | 6.76 | 3.52 | 1.11 | 1.06 | 137.8 | 257.1 |
| **SE (m) ±** | 0.43 | 0.92 | 0.036 | 0.009 | 0.008 | 0.007 | 5.87 | 0.64 |
| CD (0.05) | 1.29 | 2.72 | 0.107 | 0.028 | 0.024 | 0.021 | NS | 1.90 |

(Figure 3). The mean oil yield increased by 16.7%. Maximum yield was recorded with application of irrigation at 100% PE (250 and 257 kg $ha^{-1}$) followed by 80 and 60% PE. The total oil yield increased by 5.4 to 18.1% in 2005-2006 and 6.2 to 16.9% in 2006-2007 in case of $I_1$ as compared to $I_2$ and $I_3$. The mean oil yield in $I_2$ and $I_3$ decreased by 5.5 to 14.9% in comparison with $I_1$. Application of irrigation at 80% PE also increased the mean oil yield by 11% above that of 60% PE. The increase in yield in the above two treatments were due to favorable soil moisture conditions maintained throughout the crop growth period. Mentha is a succulent, multi-cut crop that has high water requirement during its growth period especially in dry months when the evaporation demand is relatively high. The favorable effect of irrigation in enhancing herb and oil yields of various mint species have been reported by Ram et al. (2006). It is evident from the results that the plant height, number of branches, number of leaves, crop growth rate and dry matter accumulation were significantly higher under high frequency irrigation than the low frequency ones, which contributed to higher herbage and oil yield.

Application of 100% RD ($F_1$) produced maximum oil (246 kg $ha^{-1}$) followed by $F_2$ (236 kg $ha^{-1}$) and $F_3$ (226 kg $ha^{-1}$). It increased the total oil yield from 4.0 to 7.9% in 2005-2006 and 3.9 to 9.0% in 2006-2007 and the mean yield by 4.0 to 8.5% as compared to $F_2$ and $F_3$ (Figure 3). The yield also increased from 3.7 to 4.9% by 75% RD as compared to 50% RD. Anwar et al. (2010) reported favorable effect of graded levels of NPK fertilizers on oil yield of mint.

## Interaction effect of irrigation and fertility levels

Maximum quantity of oil (260 kg $ha^{-1}$) was harvested from the crop (Figure 3) at 100% PE irrigation level with 100% recommended dose of fertilizer ($I_1F_1$). It was significantly superior to other treatment combinations (Table 4). The oil yield has been maximized due to adequate availability of moisture, which enhanced the uptake of nutrients resulting in high herbage yield (Ram et al., 2006).

## Nitrogen uptake

Uptake of N by the crop, in general, was higher in the first cutting than the second. The uptake was more in 2006-2007 than 2005-2006 (Figure 4). Drip fertigation increased the nitrogen uptake by 21.0% as compared to surface irrigation (95 kg $ha^{-1}$). Application of irrigation water at high frequency and application of fertilizers in the effective crop root zone through fertigation increased the uptake. Maximum amount of 120 kg N $ha^{-1}$ was taken up by the plants through irrigation at 100% PE followed by 80% PE (119 kg $ha^{-1}$) and 60% PE (107 kg $ha^{-1}$). There was no significant difference in uptake between 100% PE and 80% PE. Application of irrigation at 80% PE

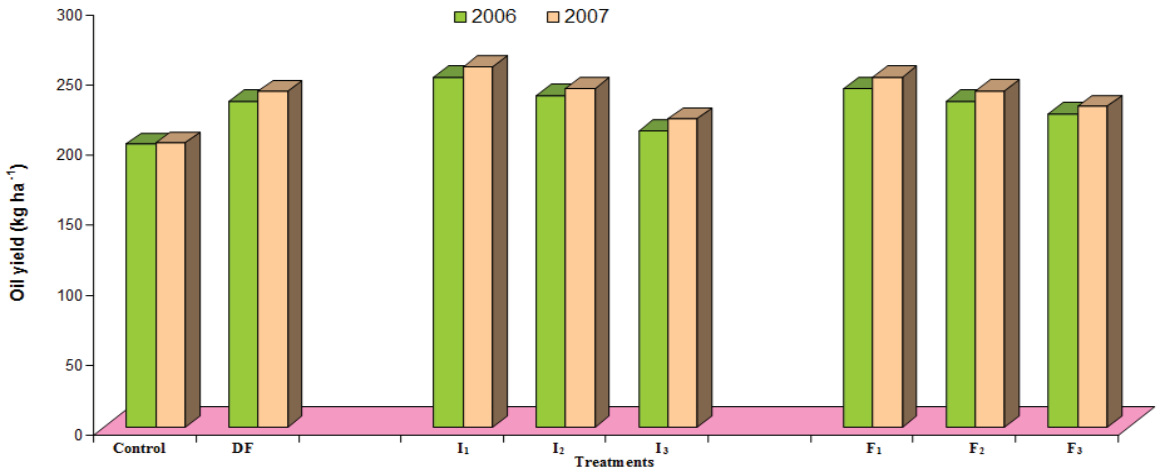


**Figure 3.** Effect of drip fertigation on oil yield.

**Table 4.** Interaction effect of irrigation and fertility on mean oil yield (2006 and 2007) of mint (kg ha$^{-1}$).

| Irrigation | Fertilizer | | | |
|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | I Mean |
| $I_1$ | 260 | 254 | 246 | 253 |
| $I_2$ | 248 | 240 | 231 | 239 |
| $I_3$ | 229 | 215 | 202 | 215 |
| F mean | 246 | 236 | 226 | |
| CD (0.05)= 5.10 | | | | |

increased N uptake by 11.3% than that of 60% PE.

Maximum nitrogen (120 kg ha$^{-1}$) was taken up by plant that received 100% RD. Reduction of 25% fertilizer from 100% RD decreased the uptake by 2.6% and that of 50 by 7.7%. Application of 75% RD ($F_2$) increased the nitrogen uptake by 5.6% than 50% RD ($F_3$). High dose of N increased the total fresh herbage yield, which ultimately led to an increase in uptake of N. Saxena and Singh (1996) and Ram et al. (2006) reported more uptake of N under different water and N levels due to more vegetative growth.

## Quality of essential oil

Surface irrigation with 100% RD (control) increased the limonene (4.53%) and menthyl acetate (6.56%) content (Table 5). Fertigation at 100% PE with 100% RD allowed the crop plants to synthesize more menthol (71.53%) than control. It also increased the terpinoids such as menthone (9.33%), isomenthone (3.41%) and neomenthol (2.14%) contents as compared to control. Anwar et al. (2010) reported that menthol content was not significantly affected due to NPK application but neo-menthol, menthyl acetate, isomenthone and menthone were considerably affected by fertilizer levels.

## Soil moisture status

To assess soil moisture stress in plant through both drip and surface irrigation method, soil moisture content at 20, 40, 60 and 80 cm soil depth was monitored in drip fertigation at weekly interval, started from 2.2.2006 in first year and from 5.2.2007 in second year. The observations were continued up to 8.6.2006 and 10.6. 2007. In case of surface irrigation method, it was monitored before each irrigation.

The soil moisture status in surface layer was more as compared to deeper layer. Hence, the depletion of available soil moisture (ASM) in surface layer was less than deeper layer (Tables 6 and 7). The soil moisture in each soil depth under 100% PE was higher than 80% PE and 60% PE. Due to good amount of soil moisture, depletion of available soil moisture in 100% PE was minimum and ranged from 1.6 to 19.0%. Less amount of moisture was depleted from 0 to 20 cm soil depth when rainfall was received in the month of March, 2006. In case of 80% PE, the depletion of soil moisture ranged 4.35 to 20.8% in 0 to 20 cm soil depth. The depletion was

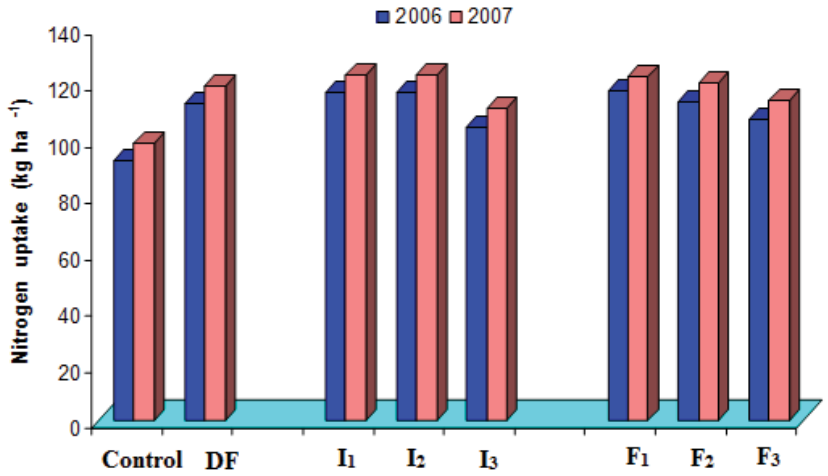


**Figure 4.** Effect of drip fertigation on nitrogen uptake.

**Table 5.** Effect of fertigation on quality of oil.

| S/ No | Constituents (%) | Control | $I_1F_1$ |
|---|---|---|---|
| 1 | Limonene | 4.53 | 2.92 |
| 2 | Menthone | 5.10 | 9.33 |
| 3 | Isomenthone | 3.54 | 3.91 |
| 4 | Menthyl acetate | 6.56 | 1.41 |
| 5 | Neomenthol | 1.97 | 2.19 |
| 6 | Menthol | 67.15 | 71.53 |

higher in 20 to 40 cm soil depth than surface layer. In 40 to 60 and 60 to 80 cm soil layers, the depletion of ASM was quite low as crop water demand was fulfilled from 0 to 40 cm soil depth. The root density of this crop was also high in the surface layer. In case of 60% PE irrigation schedule actual soil moisture content in the surface layer was comparatively low than 80% PE and 100% PE as very low amount of irrigation water was provided each time to this treatment. .

In case of surface irrigation method, total 11 irrigations were given at 60 mm CPE in both the years. The actual soil moisture content determined at 20, 40, 60 and 80 cm soil depth before each irrigation ranged 13.7 to 15.8%, 19.1 to 20.3%, 21.0 to 22.9% and 22.8 to 24.2% (v/v) in respective depth during 2005-2006. The depletion of soil moisture in surface layer was 33.6 to 52.9%. The rate of soil moisture depletion decreased with soil depth and it was found that moisture depletion varied from 28.8 to 38.4% in 40 cm, 11.0 to 27.1% in 60 cm and 8.1 to 20.2% in 80 cm soil depth. Similar trend was observed during 2006-2007. The soil moisture content before each irrigation in 20 cm soil depth was 13.3 to 14.6% in 40 cm 18.2 to 19.8% in 60 cm 20.4 to 22.4% and in 80 cm 21.9 to 23.7%. The depletion of available soil moisture ranged from 44.6 to 56.6% in 20 cm, 32.8 to 44.0% in 40 cm, 15.2 to 32.2% in 60 cm and 12.4 to 27.9% in 80 cm soil depth. The total amount of rainfall received during the growth period in 2005-2006 was 196.0 mm and in 2007, 22.31 mm, which helped in reducing irrigation requirement of crop.

## Consumptive use of water

The crop consumed more water in 2006-2007 than 2005-2006 (Figure 5). Application of water through furrow irrigation used more water than drip irrigation. The drip irrigation method saved 34.5 and 24% water in first and second year, respectively as compared to surface irrigation. It consumed on an average 654 mm of water as against 924 mm in surface irrigation method.

Drip irrigation at 100% PE required more water than the lower values of PE. Maximum amount of water (730 to 812 mm) was used at 100% PE followed by 80% PE (623 to 681 mm) and 60% PE (521 to 556 mm) during 2005-2006 and 2006-2007. The former ($I_1$) consumed 18.3 to 43.0% more water than $I_2$ and $I_3$. Irrigating the crop at 80% PE used 19.6 and 22.5% more water than that of 60% PE during 2005-2006 and 2006-2007, respectively. Saxena and Singh (1996) reported that mint required 300 to 400 mm of water at IW/CPE ratio of 0.5 and 250 mm at IW/CPE 0.3 under shallow water table conditions (62 to 119 cm). Variation in fertility level affected the

**Table 6.** Effect of irrigation regimes on soil moisture depletion pattern (%) in mint (2006).

| Date of observation | 100% PE | | | | 80% PE | | | | 60% PE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil depth (cm) | | | | Soil depth (cm) | | | | Soil depth (cm) | | | |
| | 20 | 40 | 60 | 80 | 20 | 40 | 60 | 80 | 20 | 40 | 60 | 80 |
| 2.2.2006 | 8.9 | 1.5 | 3.4 | 16.7 | 10.7 | 14.4 | 0.8 | 14.2 | 15.3 | 16.0 | 0.8 | 10.7 |
| 9.2.2006 | 12.6 | 0.2 | 1.7 | 15.9 | 14.4 | 16.8 | 0.8 | 13.3 | 14.4 | 18.4 | 3.4 | 10.7 |
| 16.3.2006 | 19.0 | 10.4 | 5.1 | 14.4 | 20.8 | 12.8 | 7.6 | 11.6 | 20.8 | 15.2 | 9.3 | 9.9 |
| 23.3.2006 | 16.2 | 4.4 | 14.4 | 10.7 | 19.9 | 24.8 | 16.9 | 8.1 | 17.1 | 27.2 | 19.5 | 6.4 |
| 28.3.2006 | 3.0 | 1.2 | 5.9 | 13.3 | 1.2 | 19.2 | 9.3 | 10.7 | 1.6 | 21.6 | 11.9 | 8.1 |
| 1.4.2006 | 9.8 | 1.2 | 1.7 | 11.6 | 12.6 | 19.2 | 3.4 | 9.0 | 10.7 | 20.8 | 5.9 | 6.4 |
| 12.4.2006 | 19.0 | 5.3 | 5.1 | 7.3 | 21.7 | 26.4 | 7.6 | 4.7 | 19.9 | 29.6 | 9.3 | 2.1 |
| 19.4.2006 | 8.9 | 1.7 | 8.5 | 10.7 | 11.6 | 20.8 | 11.0 | 8.1 | 9.8 | 22.4 | 13.5 | 5.6 |
| 26.4.2006 | 14.4 | 11.2 | 9.3 | 1.3 | 19.0 | 36.8 | 11.0 | 1.3 | 15.3 | 38.4 | 13.5 | 3.0 |
| 3.5.2006 | 1.6 | 16.8 | 11.9 | 0.4 | 4.3 | 19.2 | 13.5 | 2.1 | 8.0 | 21.6 | 15.2 | 4.7 |
| 11.5.2006 | 2.5 | 12.8 | 11.0 | 4.7 | 5.2 | 15.2 | 13.5 | 1.3 | 7.1 | 16.8 | 15.2 | 1.3 |
| 18.5.2006 | 4.3 | 8.8 | 9.3 | 3.9 | 7.1 | 11.2 | 11.9 | 6.4 | 12.6 | 13.6 | 14.4 | 1.3 |
| 25.5.2006 | 6.1 | 10.4 | 4.2 | 5.6 | 8.9 | 12.8 | 6.8 | 3.0 | 11.6 | 15.2 | 8.5 | 0.4 |
| 1.6.2006 | 8.0 | 10.4 | 5.9 | 2.1 | 10.7 | 12.8 | 8.5 | 4.7 | 13.5 | 14.4 | 10.2 | 7.3 |
| 8.6.2006 | 8.9 | 12.0 | 6.8 | 3.9 | 11.6 | 14.4 | 9.3 | 6.4 | 15.3 | 16.8 | 11.9 | 9.0 |

**Table 7.** Effect of irrigation regimes on soil moisture depletion pattern (%) in mint (2007).

| Date of observation | 100% PE | | | | 80% PE | | | | 60% PE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil depth (cm) | | | | Soil depth (cm) | | | | Soil depth | | | |
| | 20 | 40 | 60 | 80 | 20 | 40 | 60 | 80 | 20 | 40 | 60 | 80 |
| 5.2. 2007 | 7.1 | 4.8 | -5.1 | -13.3 | 10.7 | 8.0 | -0.8 | -14.2 | 12.6 | 10.4 | 0.8 | 10.7 |
| 11.2.2007 | 8.9 | 4.0 | 0.8 | -9.9 | 14.4 | 10.4 | 0.8 | -13.3 | 14.4 | 13.6 | 3.4 | 10.7 |
| 18.3.2007 | 9.8 | 5.6 | 5.1 | -9.9 | 15.3 | 12.8 | 7.6 | -11.6 | 18.1 | 15.2 | 9.3 | -9.9 |
| 25..3.2007 | 5.2 | 10.4 | 6.8 | -8.1 | 16.2 | 16.8 | 11.0 | -8.1 | 17.1 | 19.2 | 13.5 | -6.4 |
| 30.3.2007 | 13.5 | 12.0 | 5.9 | -13.3 | 16.2 | 19.2 | 9.3 | -10.7 | 19.9 | 20.0 | 11.9 | -8.1 |
| 3.4. 2007 | 9.8 | 17.6 | 8.5 | -11.6 | 19.0 | 16.0 | 3.4 | -9.0 | 16.2 | 20.8 | 5.9 | -6.4 |
| 15.4.2007 | 19.0 | 16.8 | 5.1 | -7.3 | 21.7 | 18.4 | 2.5 | -4.7 | 19.9 | 21.6 | 9.3 | -2.1 |
| 21.4.2007 | 8.9 | 18.4 | 8.5 | -10.7 | 18.1 | 20.8 | 11.0 | -8.1 | 17.1 | 22.4 | 13.5 | 1.3 |
| 28.4. 2007 | 14.4 | 19.2 | 9.3 | -1.3 | 19.0 | 14.4 | 11.0 | 1.3 | 15.3 | 19.2 | 13.5 | 3.0 |
| 5.5.2007 | 10.7 | 16.8 | 11.9 | -0.4 | 14.4 | 19.2 | 13.5 | 2.1 | 20.8 | 21.6 | 15.2 | 4.7 |
| 13.5.2007 | 11.6 | 12.8 | 11.0 | -4.7 | 16.2 | 15.2 | 13.5 | -1.3 | 21.7 | 16.8 | 13.5 | 1.3 |
| 20.5.2007 | 13.5 | 8.8 | 9.3 | -3.9 | 16.2 | 11.2 | 11.9 | 6.4 | 12.6 | 13.6 | 11.9 | 1.3 |

**Table 7.** Contd.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27.5.07 | 11.6 | 10.4 | 4.2 | 0.4 | 18.1 | 12.8 | 6.8 | -3.0 | 20.8 | 15.2 | 8.5 | 2.1 |
| 3.5.07 | 12.6 | 10.4 | 5.9 | 2.1 | 19.9 | 11.2 | 8.5 | 4.7 | 22.6 | 14.4 | 10.2 | 7.3 |
| 10..6.07 | 14.4 | 12.0 | 6.8 | 3.9 | 20.8 | 14.4 | 9.3 | 6.4 | 24.5 | 16.8 | 11.9 | 9.0 |

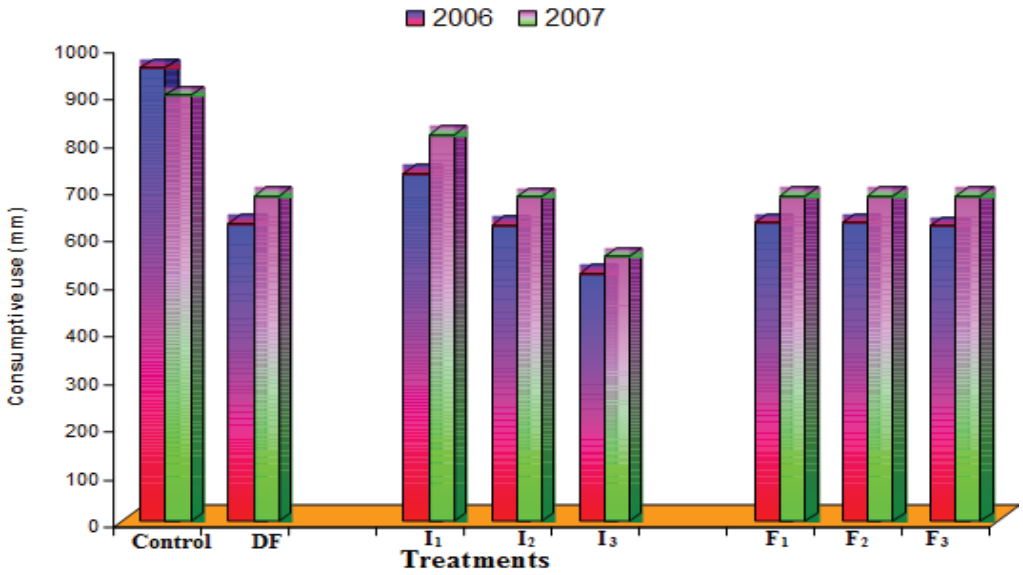


**Figure 5.** Effect of drip fertigation on consumptive use.

consumptive use only in 2006. However, maximum amount of 655 mm was used by the plants receiving 100% RD, which was equal to that of 75% RD and at par with 50% RD (652 mm). The interaction effect was not significant.

## Water use efficiency

Irrigation method affected the water use efficiency (WUE) in both the years (Figure 6). Irrigating the crop through drip system increased WUE by 65.3% as compared to surface irrigation (control). The average WUE was 0.362 kg oil ha-mm$^{-1}$ in case of the former and 0.219 kg ha-mm$^{-1}$ in case of the latter. Kannan (2006) reported increased water use efficiency (20 to 50%) due to drip irrigation compared to surface irrigation in case of medicinal coleus crop. Similar findings were reported by Imtiyaz et al. (2000). Water use efficiency decreased with an increase in irrigation water application. Maximum WUE of 0.40 kg oil ha-mm$^{-1}$ water was recorded with 60% PE ($I_3$) followed by 80% PE (0.368 kg ha-mm$^{-1}$) and 100 % PE (0.329 kg ha-mm$^{-1}$). Application of more water decreased WUE. Irrigating the crop at 80% PE increased the WUE by 11.9% as compared to 100% PE.

Maximum WUE (0.375 kg ha- mm$^{-1}$) was recorded with 100% RD ($F_1$) which decreased with

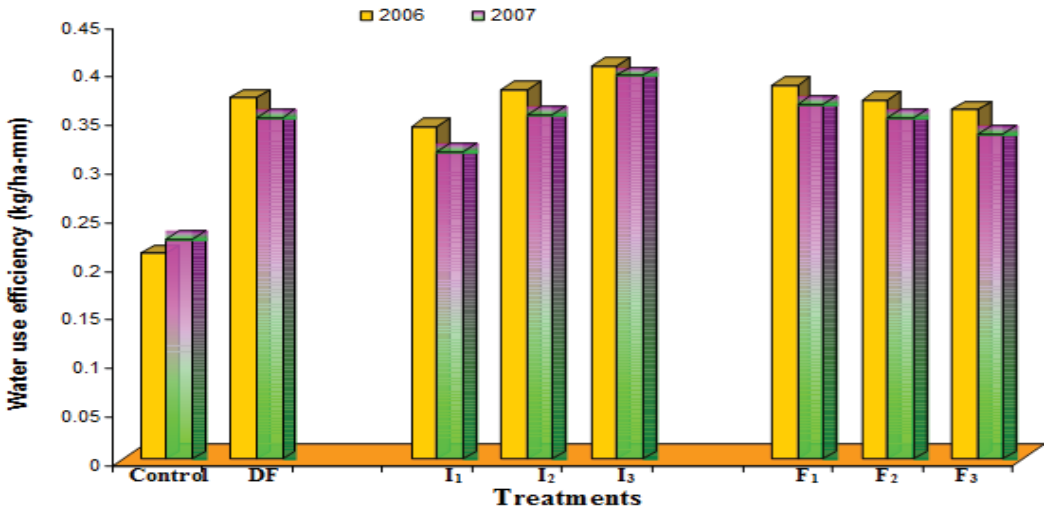


**Figure 6.** Effect of drip fertigation on water use efficiency.

reduction of fertilizer dose. It decreased by 3.7 and 7.2% due to reduction of 25 and 50% fertilizer from 100% RD, respectively. Drip irrigation increased the water productivity by 69%. The productivity decreased with increased in irrigation level but it increased with increased in fertility level. Kumar and Sood (2011) reported similar findings. Application of 75% RD had higher WUE (3.7%) than 50% RD. It was due to high herbage and oil yield that required more water for absorption of nutrients and trapping the $CO_2$ for photosynthesis.

## Conclusion

Japanese mint could be grown with drip irrigation at 100% PE with 100% recommended dose of fertilizer to give the highest oil yield of 260 kg $ha^{-1}$. It required 777 mm of water and saved 29% of water. It absorbed 120 kg N $ha^{-1}$ and produced high quality oil.

## Conflict of Interest

The authors have not declared any conflicts of interest.

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# Comparison of clustering methods for study of genetic dissimilarity in soybean genotypes

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**This study aimed to compare clustering methods based on the dissimilarity measures and get information on genetic diversity in twelve soybean genotypes. The experiment was conducted at the State University of Mato Grosso do Sul - Unit Aquidauna. The genotypes were grown in a randomized complete block design with four replications. The following quantitative traits were measured: Plant height, first pod insertion height, number of branches and pods, mass of hundred grains and grain yield. It were evaluated the clustering methods Ward, complete linkage, median, mean linkage within group and mean linkage between groups using as dissimilarity measures the mean standardized Euclidean distance (D) and the Mahalanobis's generalized distance ($D^2$). The diagnosis of multicollinearity revealed adequacy of data to the proposed study. Clustering based on standardized mean Euclidean distance is distinct from those formed based on the Mahalanobis's generalized distance, being this measure most recommended to quantify the genetic diversity in soybean genotypes based on morphological traits because it presents higher values cophenetic correlation coefficient (CCC) for all clustering methods. The mean linkage between groups's hierarchical method formed concordant groups for D and $D^2$, being recommended for these dissimilarity measures. According to both methods, the cross between the genotypes CD238 with SYN3358 and CD238 with Potência can generate hybrids with high heterotic effect due to different numbers of loci in which the dominance effects are evident.**

**Key words:** Dissimilarity measures, genetic divergence, *Glycine max*, quantitative descriptors.

## INTRODUCTION

Among the major oilseeds grown in the world, the soybean crop (*Glycine max* (L.) Merrill) excels with production of 253 million tons of grain (harvest 2012), with Brazil accounting for 25% of production total, characterizing it as the second largest crop producer (Fao, 2013). The average increase of 36 kg $ha^{-1}$ $year^{-1}$ in the yield, between 1976/77 up to 2012/13 (Conab, 2013), was provided mainly by genetic improvement that has obtained highly productive genotypes and adapted diverse edaphoclimatic conditions of the country.

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The establishment of homogeneous classes with heterogeneity among groups is a starting point in plant breeding programs. Cultivars in more distant groups provides the indicative of being dissimilar, may be considered more suitable to artificial crosses (Abreu et al., 1999), and once these materials are already on the market, these dissimilarities can be used in hybridization and/or selection schemes, aiming to incorporate the favorable traits and generate superior soybean genotypes (Cruz et al., 2004). For these measures, the clustering analysis stands out by having the purpose to gather, by some classification criterion, the parents in groups so that there is homogeneity within the group and heterogeneity between groups, being adequate to identify divergent genotypes and with most probability to succeed in the crosses (Bussab et al., 1990; Barroso and Artes, 2003).

The standardized mean Euclidean and Mahalanobis's generalized distances between pairs of genotypes are widely used as a measure of dissimilarity. The first, by not requiring repetitions, is widely used in germplasm collections, where the large number of genotypes compromises their utilization of experimental design. On the other hand the Mahalanobis distance offers the advantage of taking into account the correlations between the traits analyzed through the residual variances and covariance matrices, however, requires experiments with repetitions (Cruz et al., 2004).

The dissimilarity measure and the method used should ensure security in selection of parents for breeding. In cases of concordance of clustering, the choice of method should fall those simple to implement and easy to interpret. However, Mingoti (2005) reports that if there is disagreement between the methods, the choice of parents becomes dependent on the method used, with the need to choose the most efficient method.

Currently, research with genotypes of crops such as beans (Cargnelutti Filho et al., 2008), wheat (Bertan et al., 2006) and tomato (Karasawa et al., 2005) has been conducted aiming at establishing the most efficient dissimilarity measure and, consequently, the clustering method. However, there is heterogeneity between the results and lack of information for the soybean crop.

Given the above, the aim with this study was to compare clustering methods based on dissimilarity measures (standardized mean Euclidean and Mahalanobis's generalized), and get information about genetic divergence in twelve soybean cultivars based on six quantitative descriptors.

## MATERIALS AND METHODS

The experiment was installed in the experimental area of the State University of Mato Grosso do Sul, Unit of Aquidauana (UEMS/UUA), in the city of Aquidauana-MS, whose coordinates comprise 20°27'S and 55°40'W, with an average elevation of 170 m. The soil was classified as Ultisol sandy loam texture, with the following chemical features at layer 0 - 0.20 m: pH ($H_2O$) = 6.2; Al exchangeable ($cmol_c\ dm^{-3}$) = 0.0; Ca+Mg ($cmol_c\ dm^{-3}$) = 4.31; P ($mg\ dm^{-3}$) = 41.3; K ($cmol_c\ dm^{-3}$) = 0.2; Organic matter ($g\ dm^{-3}$) = 19.7; V (%) = 45.0; m (%) = 0.0; Sum of bases ($cmol_c\ dm^{-3}$) = 2.3; cation exchange capacity (or CEC) ($cmol_c\ dm^{-3}$) = 5.1. The climate of the region, according to the classification described by Köppen-Geiger, is Aw (Savanna Tropical) with a cumulative rainfall during the experiment of 450 mm. The minimum and maximum temperatures over experiment were 19 and 33°C, respectively.

Twelve commercial soybean genotypes were grown (P98Y70, CD238, CD241, BRS255, VMAX, NK7059, Magna, BRS245, Potência, SY3358, MS7909 and SY5909) in a randomized blocks with four replications. *Brachiaria decumbens* was grown as predecessor plant and desiccated with the active ingredient glyphosate, at dose of 1 kg $ha^{-1}$. Soybean seeds were inoculated with *Bradyrhizobium japonicum* at dose of 240 g 100 $kg^{-1}$ of seeds. The sowing was done manually on December 08th, 2011, under spacing of 0.45 m, with 16 seeds per meter. The base fertilization corresponded to 400 kg $ha^{-1}$ in formulation 4-20-20.

At 80 days after emergence (DAE) were measured plant height (PH) and first pod insertion height (PIH). At complete maturity (115 DAE) the number of branches (NB) and pods per plan (NPP) were assessed. All variables above mentioned were analyzed in ten plants per plot, while in two central lines the mass of hundred grains (MHG) and grain yield (GY) were evaluated, with humidity corrected to 13%.

For evaluating the variability among genotypes based on these quantitative traits was used analysis of variance, with the F-test at 1% probability. Subsequently, was determined the coefficient matrix of Pearson correlation among traits (phenotypic matrix) and realized the diagnosis of multicollinearity, as recommended by Montgomery and Peck (1982).

From these traits were determined matrices standardized mean Euclidean distance (D) and Mahalanobis's distance ($D^2$) among genotypes. These distance matrix, in relative scale, were used as dissimilarity measure for clustering analysis in cultivars by Ward's, hierarchical method of the single linkage (nearest neighbor), complete linkage (farthest neighbor), median (WPGMA), mean linkage within group and mean linkage between group (UPGMA) (Cruz and Carneiro, 2003).

The two matrices (D and $D^2$) were compared using the Pearson's correlation coefficient. To validate the clustering, it was verified the ability of the dendrogram to reproduce the dissimilarity matrices (D and $D^2$), and the cophenetic correlation coefficient (CCC) was calculated, on which values close to unity indicate the better representation (Barroso and Artes, 2003; Cruz and Carneiro, 2003). The concordance among hierarchical methods and measures of dissimilarity was verified by CCC (Bussab et al., 1990; Mingoti, 2005). Data were analyzed using the statistical software Genes (Cruz, 2013).

## RESULTS AND DISCUSSION

The F-test revealed the existence of genetic variability among the cultivars evaluated, which indicates that the population under study is promising for breeding (Table 1). Furthermore, all the traits presented heritability above 80%, value considered high by Cruz et al. (2004) which denotes low environmental influence on these traits. Similar results were also observed in other works with soybean genotypes (Sihag et al., 2004; Chettri et al., 2005; Malik et al., 2007; Rigon et al., 2012).

The diagnosis of multicollinearity, in Pearson's linear correlation coefficient among the traits revealed that the condition number (CN) was 42, featuring weak

**Table 1.** Values of average square and coefficient of variation of the variables plant height (PH) pod insertion height (PIH), number of branches (NB), number of pods per plant (NPP), mass of hundred grains (MHG) and grain yield (GY), evaluated in twelve soybean genotypes.

| Sources of variation | PH | PIH | NB | NPP | MGH | GY |
|---|---|---|---|---|---|---|
| Blocks | 4.37$^{ns}$ | 2.98$^{ns}$ | 8.41$^{ns}$ | 12.13$^{ns}$ | 23.44$^{ns}$ | 56.05$^{ns}$ |
| Genotypes | 44.09** | 75.01** | 91.14** | 101.20** | 134.05** | 201.07** |
| Mean | 0.80$^{m}$ | 0.12$^{m}$ | 15.13 | 84.07 | 22.32 g | 3230.12 kg ha$^{-1}$ |
| Heritability (%) | 81.32 | 80.04 | 83.06 | 91.14 | 90.87 | 86.17 |
| Coefficient of variation (%) | 3.75 | 6.99 | 4.77 | 8.99 | 6.01 | 9.97 |

$^{ns}$ and **: not significant and significant, respectively, by F-test at 1% probability.

**Table 2.** Mahalanobis's generalized distance ($D^2$ – upper diagonal) and standardized mean Euclidean distance (D – lower diagonal) among twelve soybean genotypes based in six quantitative descriptors.

| | P98Y70 | CD238 | CD241 | BRS255 | VMAX | NK7059 | Magna | BRS245 | Potência | SY3358 | MS7909 | SY5909 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P98Y70** | | 47.21 | 44.71 | 67.99 | 60.91 | 54.25 | 209.94 | 314.42 | 352.27 | 343.81 | 175.76 | 242.50 |
| **CD238** | 1.77 | | 18.91 | 57.58 | 17.85 | 58.52 | 223.15 | 340.74 | 499.72 | 485.85 | 190.17 | 263.79 |
| **CD241** | 1.38 | 1.25 | | 25.46 | 20.41 | 50.60 | 167.63 | 277.62 | 332.02 | 320.64 | 129.45 | 199.51 |
| **BRS255** | 1.32 | 1.39 | 0.64 | | 82.91 | 27.00 | 85.32 | 166.38 | 212.15 | 205.13 | 67.34 | 110.00 |
| **VMAX** | 1.58 | 0.89 | 0.49 | 0.92 | | 112.12 | 298.29 | 437.92 | 401.11 | 484.60 | 242.97 | 339.88 |
| **NK7059** | 0.97 | 1.06 | 1.18 | 1.12 | 1.20 | | 92.76 | 167.43 | 215.94 | 213.51 | 96.04 | 123.46 |
| **Magna** | 1.41 | 1.72 | 1.10 | 0.82 | 1.43 | 1.37 | | 14.23 | 33.84 | 32.46 | 10.12 | 2.75 |
| **BRS245** | 1.60 | 1.94 | 1.50 | 1.17 | 1.78 | 1.59 | 0.41 | | 8.04 | 8.69 | 36.77 | 8.41 |
| **Potência** | 1.76 | 2.42 | 1.89 | 1.56 | 2.21 | 2.01 | 0.87 | 0.64 | | 0.84 | 52.83 | 20.34 |
| **SY3358** | 1.84 | 2.36 | 1.90 | 1.57 | 2.19 | 2.03 | 0.87 | 0.58 | 0.21 | | 47.54 | 19.27 |
| **MS7909** | 1.65 | 2.07 | 1.23 | 1.03 | 1.61 | 1.80 | 0.52 | 0.70 | 0.84 | 0.85 | | 1.29 |
| **SN5909** | 1.55 | 2.06 | 1.30 | 1.04 | 1.68 | 1.69 | 0.38 | 0.52 | 0.66 | 0.70 | 0.28 | |

collinearity (CN <100), according Cruz et al. (2004), showing adequacy of the data the proposed study. In the presence of multicollinearity, the use of all traits in cluster analysis is not an appropriate procedure, because the multicolinear traits are implicitly weighted with the highest weight (Cruz and Carneiro, 2003).

Dissimilarity measures estimated from the standardized mean Euclidean distance (lower diagonal) and Mahalanobis's generalized distance (upper diagonal) are presented in Table 2. Similar groups were formed between SY3358 and Potência (D=0.21 and $D^2$=0.84), as well between SY5909 and MS7909 (D=0.28 and $D^2$=1.29). Such pairs, for having the same similarity standards, are not recommended for use in breeding programs by hybridization, avoiding restriction in the genetic variability, in order to derail the gains to be obtained by selection. More distances were observed between CD238 and Potência (D=2.42 and $D^2$=499.72) and between CD238 and SYN3358 (D=2.36 and $D^2$=485.85). This high divergence, in principle, allows recommend the crossing among these pairs in order to maximize the heterosis in progenies and increase the possibility of segregants in advanced generations (Cruz et al., 2004).

The matrix D and $D^2$ showed significant (P <0.05) and medium magnitude (r = 0.79) linear correlation. Correlations in higher magnitude were observed by Cargnelutti Filho et al. (2008) (r=0.92) and lower by Benin et al. (2003) (r=0.529). Cruz et al. (2004) highlight that D should be used in experiments that do not include repetition, because it is difficult to quantify the environmental act influences on genetic constitutions. These techniques are recommended for the evaluation of genotypes in germplasm collections, where the large number of genotypes compromises the experimental design utilization. However, the $D^2$ can be estimated only when the experimental design includes repetition, allowing the environmental effects quantification on genetic constitutions.

It is important mention that the quantification of genetic similarity of soybean genotypes based on molecular information is more accurate compared to morphological traits, because there is no influence of the environment (Cruz et al., 2004). However, the acquisition of molecular information demands a high financial cost for breeding programs and is not always possible in these institutions. Thus, it is necessary to seek alternatives to classify the most divergent genotypes, as the dissimilarity based on

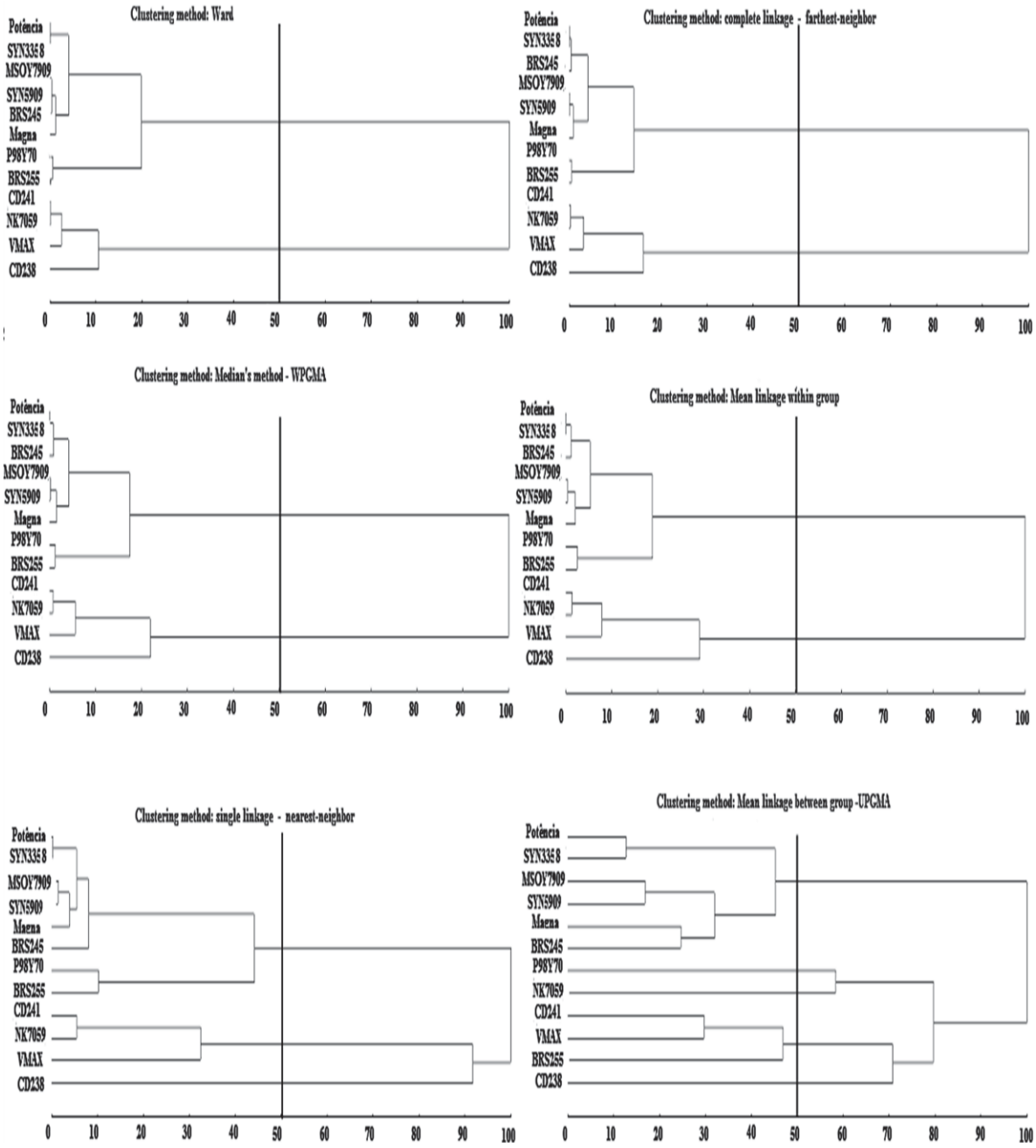


**Figure 1.** Dendrograms of hierarchical clustering method from the Mahalanobis's generalized distance ($D^2$) for twelve soybean cultivars based on six quantitative traits.

morphological traits.

In a dendrogram, large level change indicates heterogeneous cultivars union (Bonett et al., 2006). Thus, using 50% similarity as criterion for defining the groups (Cruz and Carneiro, 2003; Cruz et al., 2004) based on Mahalanobis's generalized distance ($D^2$), there was the formation of two identical groups by methods: hierarchical Ward's method, complete linkage, median and mean linkage within group (Figure 1), indicating a good correlation among them. These results are similar to those obtained by Cargnelutti Filho et al. (2008), Cargnelutti Filho et al. (2011) and Araújo et al. (2014), who verified a good correlation among the clustering methods evaluated, based on Mahalanobis's generalized distance ($D^2$).

Group 1 was formed by cultivars P98Y70, BRS255, Magna, BRS245, Potência, SYN3358, MS7909 and SYN5909, and the group 2 was formed by others

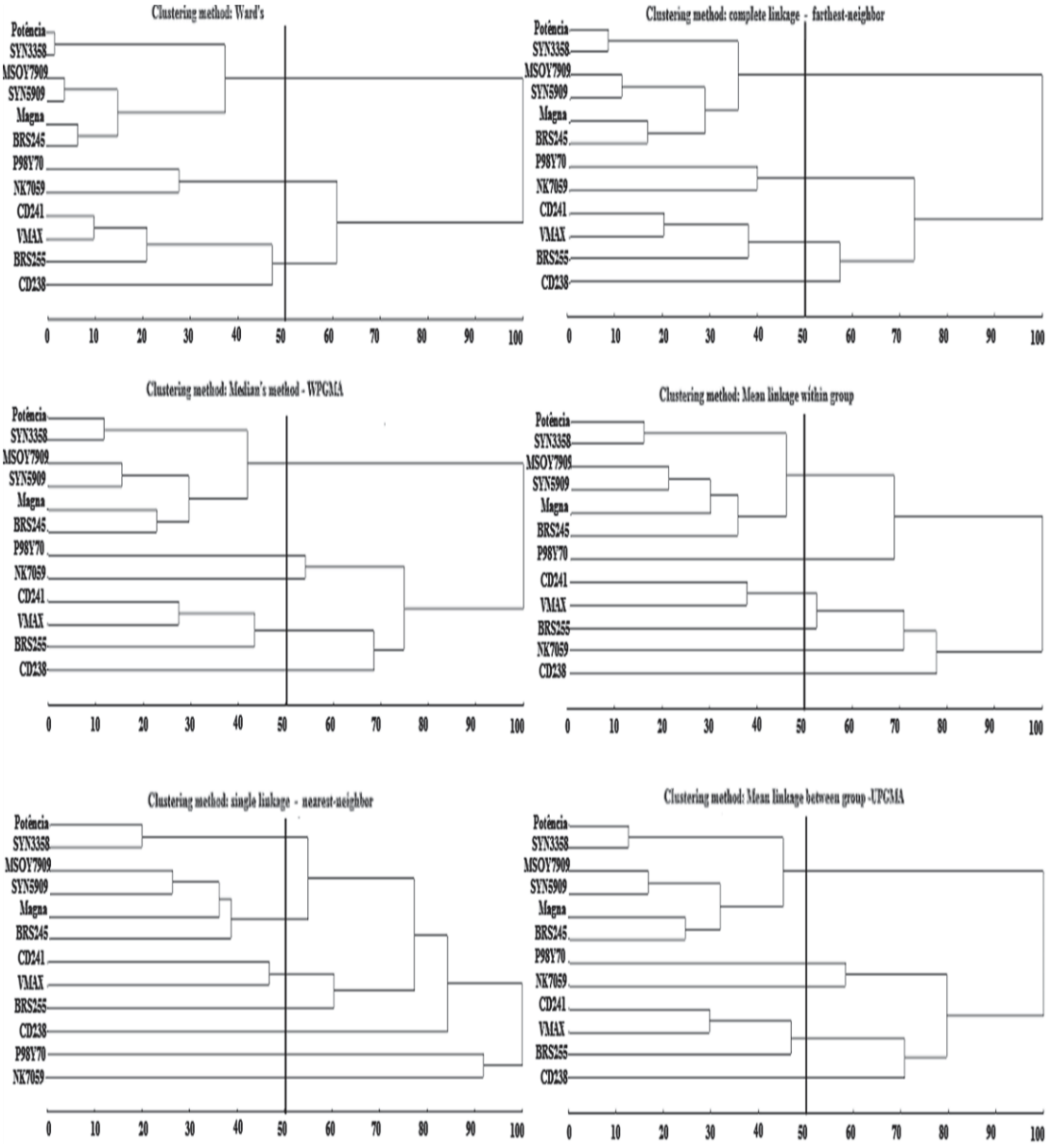


**Figure 2**. Dendrograms of hierarchical clustering method from standardized mean Euclidean distance (D) for twelve soybean cultivars based on six quantitative traits.

cultivars (CD241, CD238, VMAX and NK7059). There was, respectively, the formation of three and five groups using the hierarchical single linkage and mean linkage between groups, and in both cases the cultivar CD238 was part of an isolated group, so it can be used for obtaining of segregating populations with higher variability. The improvement with inbred lines extracted from improved cultivars is more favorable because such materials already possess high portion of favorable alleles and, concurrently, being evaluated in multiple environments (Amorim and Souza, 2005).

In contrast, the groups formed based on mean Euclidean distance (D) at 50% similarity were highly discordant among hierarchical methods (Figure 2). There was formation of seven, three, four, five and six groups of cultivars by the hierarchical methods single linkage, Ward, complete linkage, median, mean linkage within group and mean linkage between groups, respectively. These results are explained by the low magnitude ($0.6748 < r < 0.7183$) of the cophenetic correlation coefficient (CCC) (Table 3).

The CCC among the matrix of Mahalanobis's generalized distance ($D^2$) and C were significant with high magnitude, ranging from 0.7113 (single linkage) and 0.8384 (mean linkage between group). These results reinforce the hypothesis of greater consistency of groups

**Table 3.** Cophenetic correlation coefficient among matrix of standardized mean Euclidean (D - upper diagonal), Mahalanobis's generalized distance ($D^2$ - lower diagonal) and hierarchical clustering methods.

| | $D^2$ | SL | WA | CL | ME | MLWG | MLBG |
|---|---|---|---|---|---|---|---|
| **$D^2$** | 0.6748** | 0.7162** | 0.7170** | 0.7180** | 0.7181** | 0.7183** | 0.6748** |
| **SL** | 0.7113** | | 0.8897** | 0.6755** | 0.6800** | 0.6164** | 0.6784** |
| **WA** | 0.8215** | 0.9155** | | 0.9892** | 0.9858** | 0.8432** | 0.9800** |
| **CL** | 0.8332** | 0.9177** | 0.9982** | | 0.9963** | 0.8726 | 0.9938** |
| **ME** | 0.8373** | 0.9293** | 0.9981** | 0.9995** | | 0.8894** | 0.9987** |
| **MLWG** | 0.7797** | 0.9388** | 0.9961** | 0.9984** | 0.9996** | | 0.8973** |
| **MLBG** | 0.8384** | 0.9392** | 0.9970** | 0.9982** | 0.9996** | 0.9998** | |

** Significant at 1% probability by t-test with 64 degrees of freedom; SL, single linkage; WA, Ward's method; CL, complete linkage; ME, median; MLWG, mean linkage within group; MLBG, mean linkage between group.

formed based on Mahalanobis's generalized distance ($D^2$) regarding mean Euclidean distance (D).

Correlation coefficient among the clustering methods, obtained based on matrix D, ranged from 0.6164 (mean linkage within group and single linkage) and 0.9987 (mean linkage between group and median). While based on the matrix Mahalanobis's generalized distance ($D^2$), ranged from 0.9155 (single linkage and Ward) to 0.9998 (mean linkage between group and mean linkage within group). Mean linkage between group's method showed greater CCC in both dissimilarity measures.

In the conception of Sokal and Rohlf (1962), CCC values higher than 0.8 indicates good adjustment between the distance original matrix and the graphical distances derived. This proves the greater reliability of the clustering of mean linkage between groups, both for mean Euclidean distance (D) as well as for Mahalanobis's generalized distance ($D^2$), similar to results obtained by Cargnelutti Filho et al. (2008), Cargnelutti Filho et al. (2011) and Araújo et al. (2014). The best fit of the data when using this method can be explained by the fact that this method is based on arithmetic means of dissimilarity measures (Rocha et al., 2010).

From the plant breeder's perspective, the data processing by clustering methods and based on various dissimilarity measures and the consideration of particularities each one is suitable for better decision about crossings (Cargnelutti Filho et al., 2008). The clustering based on Mahalanobis's generalized distance ($D^2$) was consistent and all methods can be used. However, additional comparisons are needed before these considerations be generalized and also comparison with other methods based on further dissimilarity measures.

## Conclusion

Clustering based on standardized mean Euclidean distance is distinct from those formed based on the Mahalanobis's generalized distance, being this measure most recommended to quantify the genetic diversity in soybean genotypes based on morphological traits because it presents higher values cophenetic correlation coefficient (CCC) for all clustering methods. The mean linkage between groups's hierarchical method formed concordant groups for D and $D^2$, being recommended for these dissimilarity measures. According to both methods, the cross between the genotypes CD238 with SYN3358 and CD238 with Potência can to generate hybrids with high heterotic effect due to different numbers of loci in which the dominance effects are evident.

## Conflict of Interest

The author(s) have not declared any conflict of interest.

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# Quality evaluation of sunflower and groundnut oil produced by two cooperatives under the one village one product programme in central Malawi

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**Physicochemical properties of cooking oil as quality parameters are very important in predicting the appropriate uses of cooking oil. These properties have been known to be affected by several factors such as processing and storage conditions. In this study, quality evaluation was carried out on sunflower and groundnut cooking oils obtained from two cooperatives under the Malawi government initiated programme called One Village One Product. Furthermore, an evaluation was also carried on the cooperative's knowledge on food processing related standards as well as the extent of compliance of the processing premises to the stipulated standards. Results showed with exception of moisture content that values for peroxide value, saponification value and iodine value were within the ranges specified by the local and codex based standards. The cooperatives were also found to have adequate knowledge on food processing related standards and met most of the requirements with respect to the processing premises. However, little non compliance was also identified with respect to quality and processing premises compliance. The study concludes that the quality of sunflower and groundnut oil was of acceptable quality and safe to consumers.**

**Key words:** Sunflower oil, groundnut oil, peroxide value, saponification value, iodine value.

## INTRODUCTION

Edible vegetable oils are foodstuffs which are composed primarily of glycerides of fatty acids being obtained only from vegetable sources which may also contain small amounts of other lipids such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the fat or oil (FAO/WHO, 2001). Vegetable oils are important in human nutrition, providing energy and essential fatty acids and facilitating absorption of fat-soluble vitamins (PROTA Foundation, 2008).

The quality of the oil is very important and the extent of the oil quality can determine its desirable use. Oil quality is defined as physical and chemical properties of fats or oils that are necessary for any specific purpose as stated in a product specification or certificate of analysis. A number of factors have been reported to affect oil quality

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and include pre-processed factors such as growing season, soil fertility, post harvest storage conditions such as temperature and post process factors such as heat-thermal degradation and air contact (Turner, 2010). A number of authors have similarly highlighted other factors affecting quality of oil. The quality of vegetable oil has also been reported to be dictated by several physical and chemical parameters that are dependent on the source of oil, processing and storage conditions (Shahidi, 2005). Furthermore, some of the parameters used to evaluate the quality of the oils according to Chabiri et al. (2009) has been outlined and include moisture content, smoke point, saponification value, acid value, iodine value and peroxide value among other parameters.

Over the years in most developing countries, communities have realized the importance of adding value to their locally produced food resources. This realization has further been enhanced by a number of programmes involving communities implemented by various stakeholders including government initiated programmes. In Malawi, since the launch of a government initiated programme called One Village One Product in 2003, there have been a number of cooperatives under the programme who are involved in value addition. The One Village One Product (OVOP) programme is a community centered and demand driven regional economic development approach initiated by Oita prefecture in Japan in 1970s. The programme which was officially launched in Malawi in 2003 aims at generating incomes and wealth for the Malawian society by community mobilization to produce value added goods and services that are marketable in order to reduce wealth disparities(OVOP national secretariat, 2013). Although the emergence of small scale processors in any developing country such as Malawi is a welcome development, it is common knowledge that small scale food processing is usually constrained by a number of problems such as poor understanding of quality requirements, inadequate hygiene, inadequate processing skills, limited knowledge in both local and international food standards and many more others. These food processing related constraints affecting the small scale processors ultimately in most instances results in products with low quality whose safety cannot also be guaranteed. These problems are not confined to small scale processors only and in a study involving quality evaluation of 35 commercially available cooking oil brands sold in Pakistan market, it was found out that quality parameters such as free fatty acids, peroxide value, smell, weight and rancidity value significantly deviated from standards set by the Pakistan Standard Quality Control and Authority (Mehmood et al., 2012). It is clearly evident from a number of reported findings that most problems affecting small scale food processors are multi-faceted. Constraints faced by small scale oil palm fruit processors in Ghana were found to be multi-faceted and multi-scale and it was concluded that a cross-disciplinary research approach was needed to effectively address these complex issues and search for integrative solutions that are well embedded in the current local processing practices (Amponsah et al., 2012). Oxidation of oils is a major contributing factor to the reduction of quality and a number of authors have previously reported factors such as processing procedures, temperature, light and oxygen as the main causes of oxidation in edible oils (Jung et al., 1989; Shahidi and Spurvey, 1996; Yen and Shyu, 1989). With respect to edible oils, the type of raw oil, its colour, free fatty acid content, taste and other physical and chemical properties are such other parameters that need specific attention in order to obtain the much needed quality of the finished product (Egbuna et al., 2013). However, considering that a majority of food products produced by various small scale processors are rarely tested and coupled with limited knowledge in quality assurance and food safety, the likelihood of the food products deviating from standards set by food regulating agencies is assumed to be high and therefore every effort needs to be put in place to ensure that products from small scale processors are regularly evaluated to ensure consumer's health and safety. Information on the composition and quality characteristics of locally sourced lipids has been reported to be scarce (Babalola and Apata, 2011). The objective therefore in this current study was to evaluate the quality of sunflower and groundnut cooking oils based on selected physicochemical properties produced by two cooperatives under the One Village One Product Programme in two districts in central Malawi. The choice of the selected physicochemical properties of the oils was based on the relevance in the determination of oil quality. Furthermore, an evaluation was also carried out to determine the extent of the cooperatives knowledge in domestic food standards and compliance to relevant country's based mandatory food standards as well as some aspects in the Codex alimentarius code of hygiene practice.

## MATERIALS AND METHODS

### Identification of the cooperatives

The two cooperatives which were involved in this study were identified through consultation with management of the One Village One Product programme which is under the Malawi Government Ministry of Trade and Industry. Two cooperatives involved in the processing of sunflower and groundnut cooking oils from Lilongwe and Mchinji districts in central Malawi were purposefully chosen. Considerations on the choice were based on cooperatives performance with respect to product quality and marketing competence, products produced and proximity to the main study area which is Lilongwe University of Agriculture and Natural Resources where the analysis of the samples were carried out. Verbal consent to use the information and data obtained from the two cooperatives in this study was given by both the One Village One Product Programme secretariat and the two cooperatives. However, consent to use the names of the cooperatives was not

given and in this respect, the cooperative from Lilongwe district would be denoted as cooperative A and the cooperative from Mchinji district would be denoted as cooperative B in this study.

**Focus group discussions and auditing of the processing premises**

A comprehensive checklist covering various aspects from the Codex Alimentarius General Principles of Food Hygiene (CAC/RCP 1-1969) and Malawi Standard 21: Food and Food Processing units-Code of hygienic conditions was compiled and used for both focus group discussions and auditing of the processing premises to evaluate the cooperative's knowledge and compliance to the stipulated mandatory standards. Furthermore, the checklist also included some questions on good manufacturing practices. Some of the leading questions in the checklist covered areas such as hygienic requirements in production areas, design and facilities in the establishment, personal hygiene and health requirements, sanitation, raw materials transportation and storage and hygienic processing requirements. A group of 10 to 15 members of the two cooperatives involved in the processing of the cooking oils participated in the focus group discussions.

**Sample collection**

Samples of recently produced sunflower and groundnut cooking oils were randomly selected from the two cooperatives. A total of 3 to 4 bottles each 250 ml from the two cooking oils were collected. The Lilongwe based cooperative provided the two types of the cooking oils while the Mchinji based cooperative provided only the sunflower cooking since it was only producing sunflower cooking oil.

**Physicochemical analysis**

The physicochemical analysis of the samples was carried out within a week after the samples were collected from the two cooperatives. Moisture content, smoke point, iodine value, peroxide value and saponification number were all determined by the procedure prescribed in AOAC (2002).

**Statistical analysis**

Data obtained from the physicochemical analysis of the sunflower and groundnut cooking oils was analysed using Gen-Stat (version 14.0). Responses obtained from the focus group discussions and observations from auditing of the processing premises were manually summarized and compared with the stipulated requirements in local mandatory food standards and Codex Alimentarius General Principles of Food Hygiene (CAC/RCP 1-1969).

## RESULTS AND DISCUSSION

### Cooperative's knowledge on food processing standards and processing premise's extent of compliance to local and codex alimentarius codes of hygiene practices

Responses obtained from the focus group discussions were manually summarized and compared with the specifications stipulated in both the local food standards (MS 21) and the Codex Alimentarius General Principles of Food Hygiene (CAC/RCP 1-1969) in order to draw meaningful conclusions on the cooperative's extent of knowledge. Similarly, observations made from the inspection or auditing of the processing premises were also accordingly compared to the stipulated specifications of the standards. A summary of the responses showed that both cooperative A and B had considerable knowledge of the local food standards regarding food processing requirements. This was evidenced by for example the cooperative's ability to keep records of different activities or operations at the factory including guidelines for choice of raw materials and transportation, availability of information about the processes which were well defined and controlled and availability of health records for the workers. Furthermore, there were written down procedures for oil processing and instructions for visitors. The members from the two cooperatives were also able to correctly respond to the questions pertaining to reasons behind undertaking some steps in the processing of oils and other issues which were general in nature. However, there other noted knowledge gaps as evidenced by the lack of HACCP system in place which is now a mandatory requirement, inability to explain the significance of the batch numbers on their products and having no end-product specifications. The food processing sector especially for the small scale processors is known to be constrained by inadequate processing methods, lack of access to equipment and packaging, weak linkages with producers and poor marketing skills (Byanyima, 2004). It was revealed during the focus group discussions that these two cooperatives are under the Malawi Bureau of Standards certification scheme where they are regularly monitored and this explained why they had a better understanding of the local mandatory food standards. Other authors have reported that small holders as compared to large scale farmers face difficulties in complying with standards due to a range of constraints such as access to information, capital, services and availability of labour (Asfaw, 2008). A summary of the observations from inspection of the processing premises of the two cooperatives showed that they have satisfied the majority of the requirements stipulated in the local food standards (MS 21) as well as those in Codex Alimentarius General Principles of Food Hygiene. The audit found out that the two cooperatives had met satisfactorily the following requirements: Buildings and facilities of permanent nature, availability of toilets, availability of changing rooms and hand washing facilities, availability of uniforms for the workers, insect proof window screens, waste disposal facilities, hygiene control programme, water-proof and washable walls and the floors were clean and walls smooth. It was noted that most of the specifications in the food standards were not applicable for a cooking oil processing facility and therefore those aspects were accordingly skipped during the inspection of the facilities. Despite satisfying a majority of the requirements, it was noted that there were some few notable non compliances. The non compliances included

**Table 1.** Physicochemical properties of sunflower cooking oil.

| Property | Cooperative A | Cooperative B |
|---|---|---|
| Peroxide value (meq/Kg) | 3.32 ± 1.15[a] | 3.30 ± 1.15[a] |
| Smoke Point ($^0$C) | 196.60 ± 0.17[a] | 196.50 ± 0.57[a] |
| Saponification_value (mg/KOH/g) | 188.90 ± 2.85[a] | 189.00 ± 1.91[a] |
| Moisture content (%) | 0.20 ± 0.10[a] | 0.27 ± 0.12[a] |
| Iodine value (g/100g) | 124.80 ± 3.45[a] | 125.70 ± 2.81[a] |

Means in the same row with the same superscripts are not significantly different (P<0.05).

non availability of pest control systems, non availability of a system for recalling batch of products and lack of product information and consumer awareness program. Considering the small scale status of the two cooperatives, it was concluded that these cooperatives are doing well with respect to their knowledge of food processing requirements and compliance to the relevant local standards and with the ongoing certification scheme, there is hope that they would further improve for the better and address the identified non compliances.

## Physicochemical properties of sunflower cooking oil

The results for physicochemical properties of sunflower cooking for cooperative A and cooperative B are presented in Table 1. Results from the analysis showed that all the physicochemical properties namely peroxide value, iodine number, smoke point, saponification number and moisture content were not significantly different in the two sets of the sunflower oil obtained from the two cooperatives. The value ranges for the physicochemical properties of the two sets of sunflower cooking oil from the cooperative A and B were as follows: Peroxide value ranged from 3.30-3.32 meq/kg, smoke point ranged from 196.5 to 196.6°C, saponification number ranged from 188.9 to 189.0 mg/KOH/g, iodine number ranged from 124.8 to 125.7g/100 g and moisture content ranged from 0.20 to 0.27%. When comparison was made with the values for peroxide value, saponification number and iodine number stipulated in the Codex Standard for named vegetable oils (CODEX STAN 210-1999), it was found out that the values were within the specified ranges. However, saponification and iodine number values were found to be within the specified ranges in the local standard (Table 3) while peroxide value and moisture content values were not within the stipulated ranges. The maximum permitted value of 2.5 was for refined sunflower oil and an evaluation of the refining process by two cooperatives which uses a white clean cloth meant this cannot qualify to be refined sunflower oil rather it should be categorized under cold pressed oils which the local standard did not specify. In view of the absence of the specifications in local standards for cold pressed oils for peroxide value, it can be assumed that the peroxide value was within the acceptable ranges based on the codex based specifications. Moisture content was found to be above the maximum permitted level (Table 3) and smoke point was not included in both the local and codex based standards but higher smoke points values may indicate suitability of oil for different purposes such as cooking and it has been reported that oils that have smoke points higher than 190 $^0$C are good for frying because they can be reused several times before they completely decompose (Culinary - Yours Consulting, 2011). Our results on the physicochemical properties of sunflower oil were found to differ from previous reported findings from other authors and this was not surprising as other researchers have reported that oil quality is dictated by several physical and chemical parameters that are dependent on source of oil, processing and storage conditions (Shahidi, 2005). Peroxide value, iodine value and saponification number which had value ranges of 3.30 to 3.32 meq/kg, 124.8 to 125.7 g/100 g and 188.9 to 189.0 mg/KOH/g respectively were different from values of 2.04meq/kg, 125.17g/100g and 151.33mg/KOH/g as reported by Babalola and Apata (2011). Furthermore, values for iodine and peroxide values were also different from those reported by Shastry et al. (2011) who found out that fresh sunflower oil had values of 132.0 g/100 g and 6.6 meq/kg for iodine value and peroxide value respectively and further reported that the values in reused sunflower oil increased to 145.5 g/100 g for iodine value and 17.3 meq/kg for peroxide value. The fact that the values obtained for all the physicochemical properties with the exception of peroxide value from sunflower oil from cooperative A and B were all within the ranges as stipulated in the local and codex alimentarius based standards suggest that sunflower oil produced by the two cooperatives was of acceptable standard and quality and therefore safe for the consumers. However, it is suggested that the two cooperatives should identify the reasons contributing to the higher moisture content so that all the physicochemical properties are within the acceptable ranges.

## Physicochemical properties of groundnut oil

Results on physicochemical properties of groundnut cooking oil for cooperative A which is based in Lilongwe

**Table 3.** Physicochemical properties values as specified in Codex standard for named vegetable oils (CODEX STAN 210-1999), MBS 77:1988, groundnut oil-specification and MBS 78: 1988, refined sunflower oil specification.

| Standard | Pv | Iv | Sn | Mc | Sp |
|---|---|---|---|---|---|
| **Codex Stan 210-1999** | | | | | |
| Sunflower oil | up to 10 | 118-141 | 188-194 | - | - |
| Groundnut oil | up to 10 | 86-107 | 187-196 | - | - |
| **MBS 77-Groundnut oil-specification** | | | | | |
| | up to 10 | 80-106 | 187-196 | 0.1 | - |
| **MBS 78- Refined sunflower oil specification** | | | | | |
| | up to 2.5 | 125-136 | 188-195 | 0.1 | - |

Pv=peroxide value (meq/kg), Iv=iodine value (g/100 g), Sn=saponification number (mgKOH/g), Mc=moisture content (%, m/m), Sp=smoke point ($^{o}$C), - = value not given.

**Table 2.** Physicochemical properties of groundnut oil for cooperative A.

| Property | Mean value |
|---|---|
| Peroxide value (Meq/kg) | 9.89 ± 0.16 |
| Smoke point (°C) | 226.10 ± 0.40 |
| Saponification_Value (mg/KOH/g) | 189.40 ± 0.93 |
| Moisture content (%) | 0.20 ± 0.10 |
| Iodine value (g/100g) | 91.46 ± 0.67 |

district in central Malawi are presented in Table 2. The Mchinji district based cooperative was not producing groundnut cooking oil and therefore the presented results are for cooperative A only. The obtained values for the different physicochemical properties just like in sunflower oil were different with those reported by other authors which could be attributed to a number of factors such as storage conditions and furthermore it has been reported that cooking oils can be spoiled by air and light and it is recommended that the packaging of any such products should exclude light and air (Fellows and Axtell, 2002). The peroxide value, iodine and saponification values obtained in this study for the groundnut oil (Table 2) were different to those reported by Babalola and Apata (2011) who reported 1.54 meq/kg, 13.27 g/g and 209.0 mgKOH/g for peroxide value, iodine value and saponification value, respectively. It was further observed that peroxide value (Table 2) was close to the maximum permitted levels as specified in both Codex alimentarius based and local standards (Table 3) which implied that there might be other post processing related factors contributing to the higher values as revealed in findings of Manral et al. (2008) who reported that peroxide value of sunflower oil used in frying of fish evaluated at 2 h interval for 14 h increased from 0.1 to 24.88 meq/kg while the iodine value decreased from 126.44 to 117.42 g/100 g. However, with the exception of moisture content, the values obtained for peroxide value, saponification number and iodine value were within the ranges as stipulated in the local standard covering groundnut oil (Table 3). Differences in the physicochemical properties of the groundnut oil has also been reported to be due to differences in cultivar type and our results which reported iodine value of 91.46 g/100 g was found to fall in the iodine value ranges of 85.77 to 98.43% for 20 groundnut varieties grown in Ghana (Asibuo et al., 2008). The moisture content was found to be outside the specified range suggesting that there is for the cooperative to work on the manufacturing process to address this non compliance as well as the peroxide value which needs to be significantly reduced.

## Conclusions

In this study, the physicochemical properties of sunflower and groundnut oil obtained from the two cooperatives under the Malawi government initiated One Village One Product Programme were investigated. Furthermore, an evaluation of the cooperative's knowledge on food processing related standards as well as extent of processing premises compliance to stipulated standards requirements was also carried. Findings revealed that the peroxide value, saponification and iodine values were within the ranges as specified in the local standard covering edible oils and Codex standard for named vegetable oils. This demonstrated that the cooking oils produced by the two cooperatives satisfactorily met the required quality standards and therefore safe to consumers. The study findings further revealed that the two cooperatives have considerable knowledge in food processing standards and the processing premises met a majority of the requirements stipulated in the standards. However, the moisture content for the sunflower and groundnut oil from the two cooperatives was higher than the maximum permitted levels and some noted non compliances with respect to the processing premises included the availability of pest control system, HACCP

system and a recall mechanism. Considering their small scale status, it can be concluded that quality of the sunflower and groundnut cooking oil produced by the two cooperatives is of acceptable standard and safe to consumers and that the cooperatives had better understanding of appropriate food standards and needs to be encouraged to continue complying to the requirements as stipulated in the standards with respect to oil quality and processing premises requirements.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Development and yield of maize (*Zea mays*) under plant densities using single and twin-row spacing

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**Among the main factors that influence higher maize yield are the use of more productive materials, plant arrangement more suitable to the cultivar, reduced spacing between rows and/or higher population density. In this context, the objective of this work was to evaluate the development and yield of maize under different plant densities using single and twin-row spacing configurations. The work was developed in the 2012/2013 harvest year, using a randomized block design, with four replications in a 5 x 5 factorial design, featuring five inter-row spacing arrangements (twin-rows: 0.4 x 0.2, 0.5 x 0.2, 0.6 x 0.2, 0.7 x 0.2 m; and conventional spacing between rows as control: 0.7 m) and five sowing densities (50,000; 65,000; 80,000; 95,000 and 110,000 plants ha$^{-1}$). The study evaluated plant height and first ear insertion height, stem diameter, number of row per ear, 1,000-kernel weight and yield. The t-test (p = 0.05) was used to evaluate the effects of twin-row spacing arrangements and the contrast between twin-rows and control (single-row). Whenever the interaction between twin-row spacing arrangements and plant population was significant, the data were submitted to Response-Surface Methodology. As population density increased, there were reductions in stem diameter, number of rows per ear, 1,000-kernel weight and yield. The greatest plant heights, first ear insertion heights and yields were obtained in conventional spacing. Kernel yield responded negatively to plant density increase.**

**Key words:** Crowding, plant arrangement, production system.

## INTRODUCTION

In recent years, the status of maize (*Zea mays* L.) has risen among farmers, going from a rotation crop to becoming an agricultural commodity. With growing worldwide demand for both human and animal

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consumption as well as to meet energy needs, there is growing pressure to increase the grain yield of this crop. According to data from National Food Supply Company (CONAB, 2014), the estimated 2013/2014 maize harvest will in total approximately be 78.2 million tons, with average yield of 4,996 kg $ha^{-1}$. The choice of row spacing and the proper number of plants in a given area are of utmost importance among the different management practices, as they determine the best possible use of abiotic factors such as water, light and nutrients so that the crop can express its full physiological potential (Penariol et al., 2003).

Considering the built of modern hybrids-featuring shorter plant and ear height, slighter leaf angle, higher yield capacity, reduced number of leaves, more erect leaves and smaller leaf area, minimizing competition for light- decreasing spacing may be an adequate practice (Argenta et al., 2001). The use of reduced spacing brings several advantages, one of which is the increased distance between each plant in the same row, resulting in a more equidistant arrangement of individuals in the crop area, reducing plant competition for water, light and nutrients (Porter et al., 1997).

Higher plant density is one of the easiest and most efficient ways to increase the interception of an incident solar radiation by maize plants. However, very high densities can reduce photosynthesis and the efficiency of photoassimilate conversion in grain production (Marchão et al., 2006). As a result, female sterility increases, and the number of kernels per ear and grain yield are compromised (Marchão et al., 2006; Pereira et al., 2008).

Another option for plant arrangement is the twin-row system, which is used to improve the configuration between plants, allowing for higher population density without compromising kernel yield. In this type of configuration, plants are arranged equidistant from one another, allowing for better land use, as well as lower plant competition for water, light and nutrients, both in the rows and between them (Balem et al., 2014).

Studies on twin-row systems in maize crops are recent, and the results are still inconsistent. While some results indicate higher kernel yield using twin-row systems (Gozubenli et al., 2004; Cox et al., 2006; Balem et al., 2014), others did not show any yield advantages when compared to single-row spacing (Robles et al., 2012; Novacek et al., 2013; Haegele et al., 2014).

Since this is a new form of plant arrangement, there is no sowing density or ideal spacing recommendation, considering that the ideal arrangement usually varies and is closely linked to differences in region, sowing season, crop system, edaphoclimatic conditions and choice of genotype. Thus, this study aimed to evaluate the effects of single and twin-row spacing under different population densities for maize crops.

## MATERIALS AND METHODS

The experiment was carried out during the 2012/2013 harvest on Oxisol, in São João, Paraná State - Brazil, (Soil Survey Staff, 2014), with loamy texture (76.5% clay, 8.0% sand and 15.5% silt), featuring the chemical profile shown in Table 1. The experimental area is located at 25°52'32" S and 52°47'58" W, with mean elevation of 620 m. The predominant climate in the region according to the Köppen classification is Cfa (temperate humid), with average temperatures below 18°C in the coldest month of the year and above 22°C in the warmest, featuring relatively hot summers, frequent frosts and well-distributed rainfall throughout the year. During the experiment, accumulated precipitation was 1,060 mm and mean temperature ranged from 18 to 23°C (SIMEPAR, 2013).

The experiment consisted of a 5 x 5 factorial arrangement in a randomized block design with four replications. The treatments resulted from the combination of five inter-row spacing configurations (four in twin-rows: 0.4 x 0.2, 0.5 x 0.2, 0.6 x 0.2, 0.7 x 0.2 m; one single spacing between rows: 0.7 m) and five sowing densities (50,000, 65,000, 80,000, 95,000, 110,000 plants $ha^{-1}$). Experimental units consisted of four single-rows in the conventional spacing configuration and four twin-rows in twin-row spacing, 5.0 m long. Evaluations and data collection were carried out over a useful plot of 3.0 m long and consisting of two single or twin central rows, according to the treatment.

Hybrid SUPERIS® was chosen for the study, characterized by early growth, high-yield potential, excellent leaf health, good rooting and stem quality, high kernel quality and stability (SYNGENTA, 2013). This material is considered a high investment hybrid, recommended for regular and off-season sowing. The hybrid features VIPTERA® technology, offered control of *Elasmopalpus lignosellus*, *Spodoptera frugiperda*, *Diatraea saccharalis*, *Helicoverpa zea* and *Agrotis ipsilon*. Sowing took place on September 27, 2012, done manually using jab planters, to an average depth of 5.0 cm. An extra 15% of seeds were sown, and when plants reached four expanded leaves, they were thinned to achieve the final stand for each treatment.

Basic fertilization consisted of 450 kg $ha^{-1}$ of 12-32-18 (N-P-K) formula, according to the chemical profile of the soil and maximum expected yield of 11,650 kg $ha^{-1}$ (SBCS, 2004). Nitrogen fertilizer (27% N) was used for nitrogen side dressing, applying 350 kg $ha^{-1}$ divided into two applications – one at V4 and the other at the V6 stage (Vn: vegetative phase with n developed leaves).

Weed control consisted of atrazine at a dose of 4.2 L $ha^{-1}$, when the crop was at stage V4. Pest control consisted of 0.4 L $ha^{-1}$ of beta-cyfluthrin at stage V4. A second application was carried out at stage V8, using the same insecticide and dose as before.

The following evaluations were carried out according to methodology proposed by Balem et al. (2014):

plant height - distance between the soil surface and the tip of the male inflorescence; height of the first ear insertion-distance between the soil surface and the first ear insertion; stem diameter-determined at the first internode above the plant collar; number of rows per ear; 1,000-kernel weight; and kernel yield. Plant height, height of first ear insertion and stem diameter were determined at phenological stage R5 (dent stage), based on a sample of 10 plants collected in each useful plot. For other evaluations, 10 ears were collected from each plot. The evaluations involving kernel weight were corrected for 13% moisture with manual harvesting.

Data were subjected to analysis of variance using Genes software to evaluate the effects of inter-row spacing and plant density factors, as well as the interaction between them (Cruz, 2006). To evaluate the effects of twin-row spacing and the contrast between twin-rows and control (single-row), means were compared through t-test ($p = 0.05$). Whenever the interaction between twin-rows and plant population was significant, the data were submitted to Response-Surface Methodology. Where the interaction between the contrast of twin-rows and control (single-row) and plant population was significant, polynomial regression was carried out. Models were chosen based on the significance of the coefficients of

**Table 1.** Chemical profile of the soil at the 0 to 0.2 m deep layer, sample before the experiment was established.

| pH | MO | P | H+Al | K | Ca | Mg | V |
|---|---|---|---|---|---|---|---|
| $CaCl_2$ | g $dm^{-3}$ | mg $dm^{-3}$ | | $cmol_c$ $dm^{-3}$ | | | (%) |
| 4.9 | 40.21 | 1.32 | 5.35 | 109.48 | 3.82 | 2.84 | 56.47 |

**Table 2.** Sources of variation, degrees of freedom (DF) and mean square of characters plant height (PH), height of first ear insertion (FEI), stem diameter (SD), number of rows per ear (NRE), 1,000-kernel weight (KW) and yield (Y) according to inter-row spacing configurations and plant populations.

| Sources of variation | DF | PH (m) | EIH (m) | SD (mm) | NRE | KW (g) | Y (t ha-1) |
|---|---|---|---|---|---|---|---|
| Block | 3 | 0.0263 | 0.0130 | 1.94 | 2.45 | 267.66 | 0.905 |
| Spacing (S) | 4 | 0.0295** | 0.0144** | 0.28 $^{ns}$ | 0.35 $^{ns}$ | 91.24 $^{ns}$ | 0.929** |
| Twin-Row (TR) | 3 | 0.0192** | 0.0121** | 0.37 $^{ns}$ | 0.34 $^{ns}$ | 114.45 $^{ns}$ | 0.978** |
| Crtl (C) vs | 1 | 0.0605** | 0.0213** | 0.02 $^{ns}$ | 0.39 $^{ns}$ | 21.62 $^{ns}$ | 0.781** |
| Population (P) | 4 | 0.0025 $^{ns}$ | 0.0119** | 58.29** | 1.35** | 2189.48** | 0.591** |
| TR x P | 12 | 0.0093** | 0.0076** | 0.48 $^{ns}$ | 1.06** | 132.06 $^{ns}$ | 0.397 $^{ns}$ |
| (C vs TR) x P | 4 | 0.0030 $^{ns}$ | 0.0021 $^{ns}$ | 0.35 $^{ns}$ | 0.22 $^{ns}$ | 144.43 $^{ns}$ | 0.257 $^{ns}$ |
| Error | 72 | 0.0080 | 0.0050 | 0.78 | 0.55 | 331.71 | 0.552 |
| Overall mean | --- | 2.24 | 1.09 | 20.01 | 15.42 | 357.40 | 7.25 |
| Twin-rows | --- | 2.23 b | 1.30 b | 20.00 a | 18.70 a | 357.63 a | 7.21 b |
| Single-rows | --- | 2.29 a | 1.33 a | 20.04 a | 18.54 a | 356.47 a | 7.43 a |
| CV (%) | --- | 3.99 | 10.35 | 4.42 | 5.91 | 5.09 | 10.25 |

Means followed by the same lower-case letter are not significantly different by the t-test (p = 0.05). ** and * significant (p <0.01) and significant (p <0.05), respectively, by F test; $^{ns}$: not significant (p>0.05); CV: coefficient of variation.

the fitted regression equation, tested by F test (p = 0.05), as well as the values of the coefficient of determination ($R^2$).

## RESULTS AND DISCUSSION

Mean values and the synthesis of the analysis of variance in the parameters of initial development of maize crops are presented in Table 2, demonstrating that the tallest plant heights were obtained in single spacing (control). This result may be attributed to how the plants are arranged in both spacing configurations. In single-row sowing, plants area distributed in a non-equidistant manner when compared to twin-row spacing arrangements, thus increasing intraspecific competition in the row, inducing plants to grow in search of light (Sangoi et al., 2010).

According to Argenta et al. (2001) and Alvarez et al. (2006), there is a natural tendency for greater plant heights in situations of intense competition for light. Demétrio et al. (2008), while evaluating two hybrids subjected to three inter-row spacing configurations (0.4, 0.6 and 0.8 m) and four population densities (30,000; 50,000; 70,000 and 90,000 plants $ha^{-1}$), observed that plant height was not influenced by the reduction in the spacing between rows; this result differs from the data found in this research.

The reduction in inter-row and plant population caused shorter plant heights, and the tallest heights were obtained at 0.7 m inter-row spacing and at populations between 65,000 and 85,000 plants $ha^{-1}$ (Figure 1). This result is justified, because whenever the space between rows is increased, the number of plants in the row necessarily increases along with it, in order to maintain a constant plant population. This increase naturally causes etiolation in plants in search of light. According to Sangoi et al. (2002), plant height will increase at the same rate as the population, due to the combined effect of intraspecific competition for light, resulting in stimulus of the apical dominance of plants.

Dourado Neto et al. (2003), evaluating the performance of three maize hybrids at three densities (30,000; 60,000 and 90,000 plants $ha^{-1}$) and two inter-row spacing (0.40 and 0.80 m), observed an increase in plant height for all three hybrids, with greater plant density, regardless of spacing. Yet, Farinelli et al. (2012), evaluating the performance of two maize hybrids in three sowing densities (40,000, 60,000 and 80,000 plants $ha^{-1}$) and three inter-row spacing (0.4, 0.6 and 0.8 m), observed finer plant heights at 0.6 and 0.8 m associated with the two greater sowing densities. According to those authors, this result was due to the natural tendency of increased heights for plants in situations of high population *densities.*

The greatest heights of first ear insertion were obtained

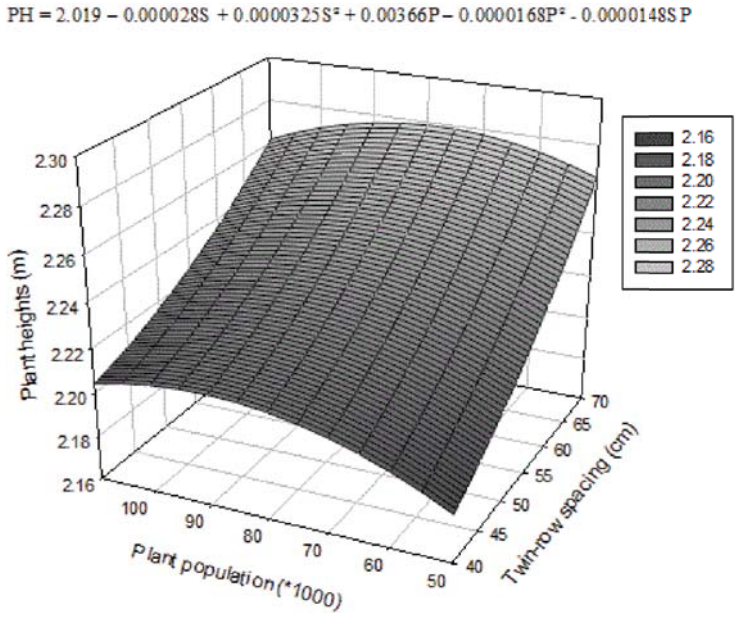


**Figure 1.** Plant heights (m) of maize crop according to plant population (plants ha$^{-1}$) and twin-row spacing (cm).

at single-row spacing. This result is attributed to the superior plant heights obtained in this spacing, as taller plants result in greater first ear insertion heights.

The tallest height of first ear insertion observed was 1.34 m, found at inter-row spacing of 70 cm and at a population density of 105,000 plants ha$^{-1}$ (Figure 2). This result may be due to the fact that the number of plant in the row increases whenever inter-row spacing is increased, causing natural blanching of plants in search of light.

The results found herein corroborate those observed by Kappes et al. (2011), which analyzing five maize hybrids, observed that the tallest height of first ear insertion occurred when density was increased from 50,000 to 90,000 plants ha$^{-1}$. However, the results differ from those obtained by Balem et al. (2014), while evaluating the maize yield in conventional and (0.70 m) and twin-row spacing (0.20 x 0.70 m) and using five plant populations (50,000; 65,000; 80,000; 95,000 and 110,000 plants ha$^{-1}$), did not observe any increase within plant population increases.

In regard to the number of rows per ear, the highest values were observed at the lowest plant population densities and greatest spaces between rows (Figure 3). Similar data were obtained by Brachtvogel et al. (2009) and Kappes et al. (2011), who observed a reduction in the number of rows per ear as population density increased. However, the decrease in 110,000 to 50,000 plants ha$^{-1}$, observed by Balem et al. (2014), showed no difference in NRE. These results also differ from those obtained by Marchão et al. (2005), evaluating six maize hybrids at two different locations during the same crop year, an increase of 40,000 to 100,000 plants ha$^{-1}$ showed no influence in NRE.

A population increase up to 64,600 plants ha$^{-1}$ caused a significant increase in 1,000-kernel weight (Figure 4). Similar results were obtained by Farinelli et al. (2012), studying three plant densities (40,000; 60,000 and 80,000 plants ha$^{-1}$), observed that kernel weight was lower at plant densities above 60,000 plants ha$^{-1}$. The use of high densities can reduce photosynthesis activity in the crop, as well as photoassimilate conversion efficiency in grain production (Marchão et al., 2006).

Inter-row spacing arrangement significantly influenced maize yield, with conventional spacing showing the highest yield (7.4 t ha$^{-1}$), approximately 3.0% greater than the yield obtained from twin-row spacing. This result went against expectations, considering that plants are better distributed in twin-row spacing, which would theoretically reduce competition among them, causing yield improvement.

Since this is a new form of plant arrangement, the effects on maize kernel yield are quite varied. Balkcom et al. (2011), while evaluating different hybrids, Pioneer 31N27, Pioneer 31N26, Dekalb DK697 and Dekalb DKC 69-72, at low density (40,000 to 44,000 plants ha$^{-1}$), average density (59,000 to 64,000 plants ha$^{-1}$) and high density (79,000 to 84,000 plants ha$^{-1}$) in twin-row (0.19 x 0.76 m) and conventional spacing (0.76 m), detected that twin-row spacing yielded 16% more than single spacing at the highest plant densities and 10% higher in medium densities, compared to conventional density.

Balem et al. (2014), while working with twin-row (0.2 x 0.7 m) and single spacing (0.7 m) using hybrid 30F53H,

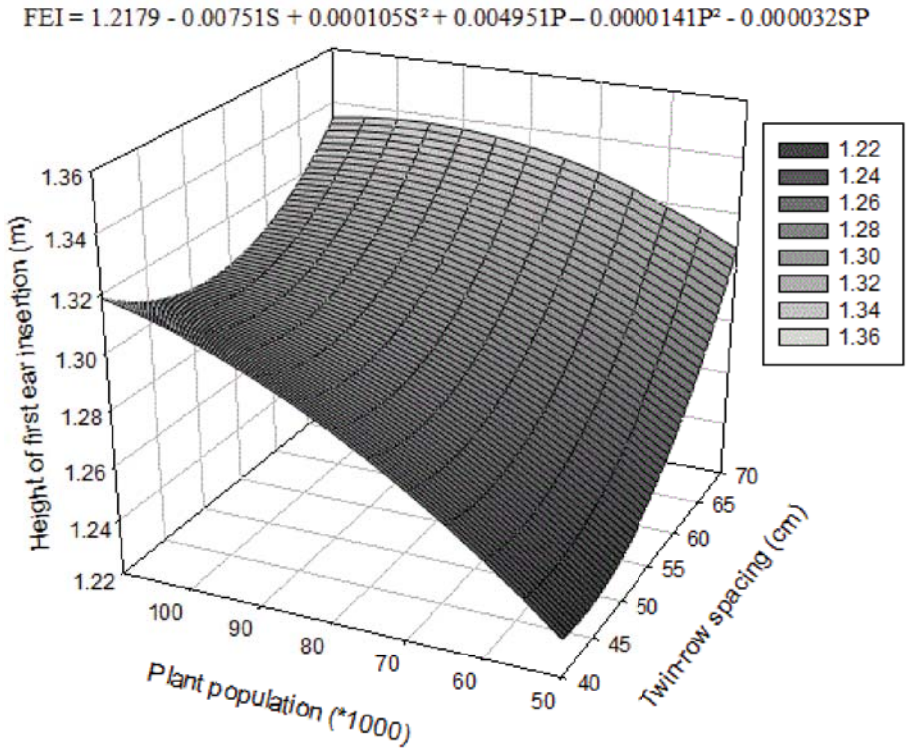


**Figure 2.** Height of first ear insertion (m) according to plant population (plants $ha^{-1}$) and twin-row spacing.

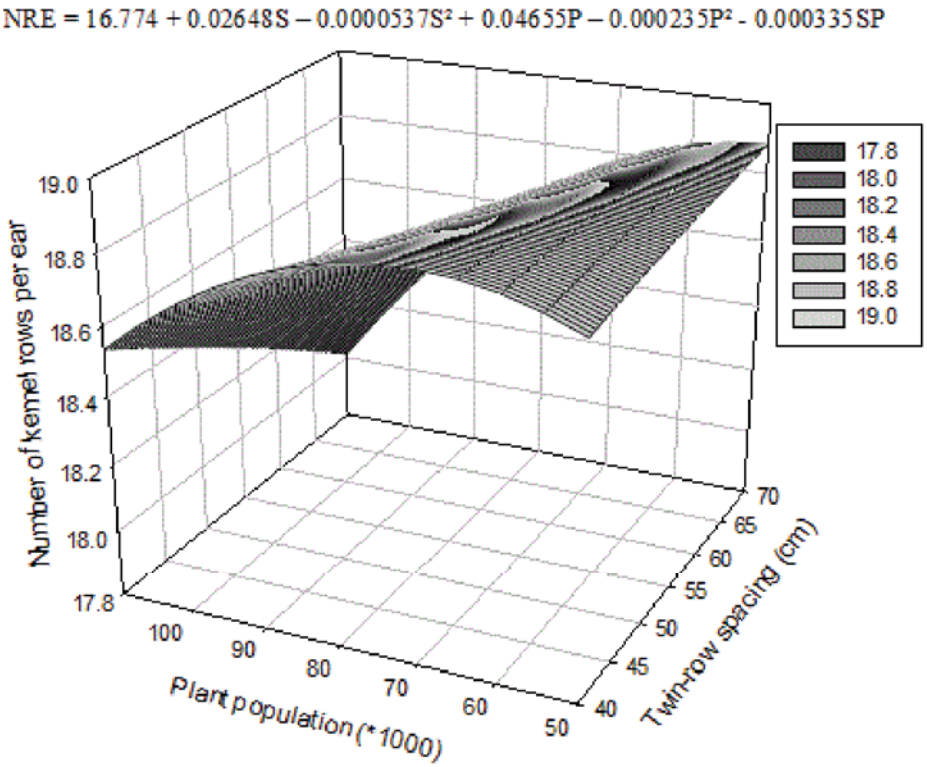


**Figure 3.** Number of rows per ear of maize according to plant population (plants $ha^{-1}$) and twin-row spacing (cm).

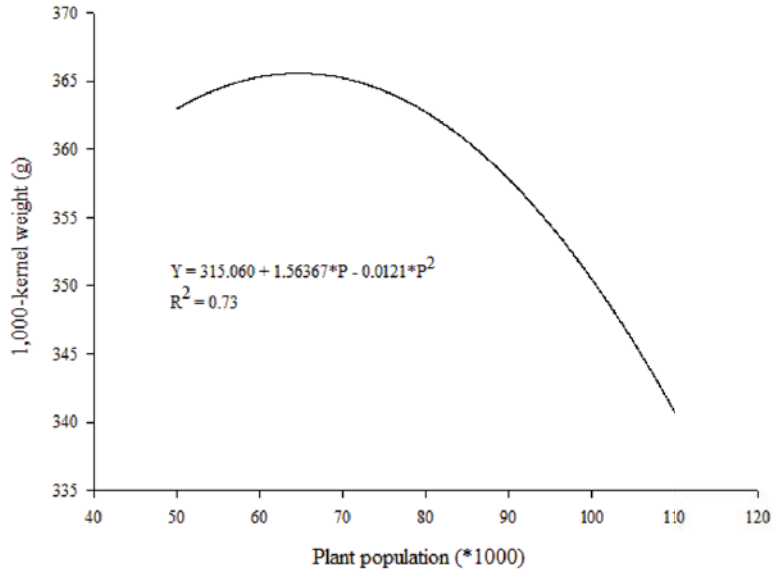


**Figure 4.** Thousand-kernel weight (g), according to plant population (plants $ha^{-1}$).

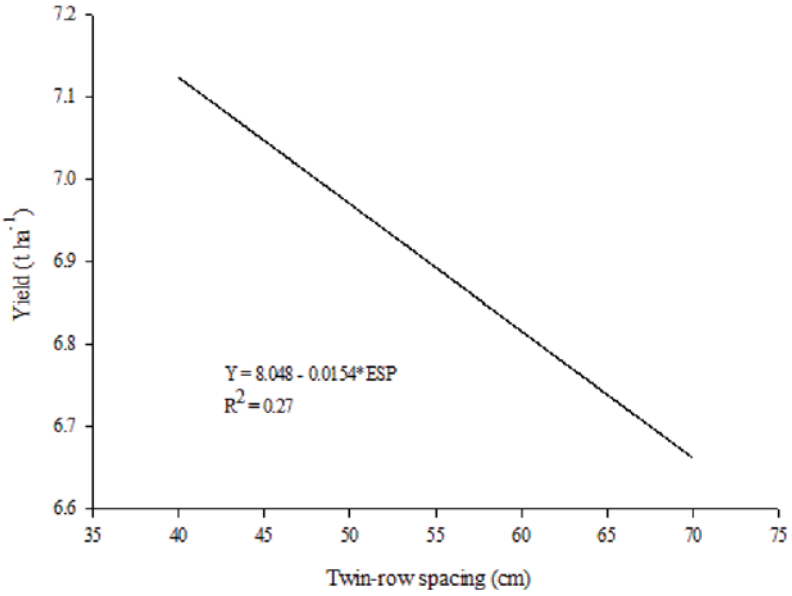


**Figure 5.** Maize yield (t $ha^{-1}$), according to twin-row spacing (cm).

also found higher yield in twin-row spacing. However, Robles et al. (2012), while evaluating three maize hybrids for three years, using twin-row (0.20 x 0.76 m) and conventional spacing (0.76 m), at four population densities (69,000; 81,000; 93,000 and 105,000 plants $ha^{-1}$), detected that maize kernel yield at twin-row spacing was not significantly superior to comparable yields in all hybrids and plant density levels.

Figure 5 shows a linear decrease in maize yield due to the increase of row spacing. This fact is due to the larger number of plants in the row in wider spacing configurations than in closer arrangements; in the latter, the plants are better distributed in the row, favoring root growth and nutrient absorption. Alvarez et al. (2006), studying two maize hybrids, observed a yield increase of 500 kg $ha^{-1}$ when spacing was reduced from 0.9 m to 0.7 m.

Farinelli et al. (2012) observed that decreasing spacing from 0.8 m to 0.4 m increased productivity of tested hybrids, regardless of plant density used (40,000; 60,000

and 80,000 plants $ha^{-1}$). Modolo et al. (2010) also found an increase in maize yield when spacing was reduced from 0.9 m to 0.45 m.

## CONCLUSIONS

With the increase in population density, plant and first insertion heights increased, while stem diameter, number of rows per ear, 1,000-kernel weight and crop yield decreased. Inter-row spacing influenced plant and first insertion heights as well as yield, with the best results found in single spacing. The use of twin-row spacing did not result in any advantages to conventional spacing for the different plant densities.

### Conflict of Interest

The author(s) have not declared any conflict of interest.

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