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Pandey R, Kalra A (2003). Root knot disease of ashwagandha Withania somnifera and its ecofriendly cost effective management. J. Mycol. Pl. Pathol. 33(2): 240-245.

Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds) Biological Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International, pp. 181-190.

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Full Length Research Paper

Use of nutrient stock:balance (NSB) ratio for assessment of sustainability of agricultural system

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Nutrient stock:balance (NSB) ratio is a valuable tool for assessing the sustainability of agricultural land. The experiment design was split plot in randomised complete block design (RCBD) done in triplicate. The NSB ratio was monitored by the effect of applied fertilizer (N and K) and soybean nitrogen contribution in cassava/soybean intercrop system. With respect to nitrogen nutrition, sole soybean produced the highest NSB ratio (14.58) and highest nutrient balance of +1597.52 Kg ha^{-1} followed by intercrop with NSB ratio of 11.00 and nutrient balance of 3461.86 Kg ha^{-1}. Lowest NSB ratio (1.99) was obtained at sole cassava having a negative nutrient balance (-11.33 Kg ha^{-1}). Within the fertilizer rates, N_0K_{50} gave the highest NSB ratio hence sole soybean at N_0K_{50} fertilizer rate will be the most sustainable (15 years), followed by intercrop at N_0K_{50} fertilizer rate (11 years.) while sole cassava cropping system at all fertilizer rates will be the least sustainable (1 or 2 years). Nutrient stock:balance (NSB) ratio for potassium was also highest in sole soybean (12.02), followed by intercrop (8.74). Lowest NSB ratio was obtained at sole cassava (0.86). Within the fertilizer rates, N_0K_{50} gave the highest NSB ratio, hence sole soybean at N_0K_{50} fertilizer rate will be the most sustainable (12 years), followed by intercrop at N_0K_{50} fertilizer rate (10 years.) while sole cassava cropping system at N_0K_0 and $N_{45}K_0$ fertilizer rates will be the least sustainable (1 year).

Key words: Intercrop, nutrient balance, nutrient stock, sustainability.

INTRODUCTION

Nutrient stock (residue + fertilizer + biological nitrogen fixation): balance (total input – total output) ratio serves as an indicator for predicting sustainability of cropping systems (Defoer et al., 2000). Stock of nutrient in the soil is usually made up of the total input from crop residue, applied fertilizer and biological nitrogen fixation (BNF). These nutrients are stored in two forms: Soil dynamic nutrient reserve and soil inert nutrient reserve. Soil dynamic nutrient reserve is a fraction of soil organic matter with readily available nutrient stored in the relatively active form. Soil inert reserve is a fraction of organic matter which does not easily release its nutrient (Defoer et al., 2000). Accumulation of these nutrients occurs only when more nutrients are added to the soil than removed. When the nutrients extracted from the soil through crop yield and depleted through

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leaching or volatilization roughly equals the nutrient brought back through residue, fertilizer and BNF, we assume that the system is in equilibrium. A good knowledge of possible changes of the nutrient stock therefore involves balancing of nutrient input and output (Nutrient flow Analysis) (Smaling et al., 1996). A large negative or positive difference is cause for concern and will require some form of correct action. A negative balance means that the production system is being degraded as the store of available soil nutrient is depleted. Nutrient stock:balance ratio is therefore the ratio of quantified dynamic reserve:nutrient balance which gives an indication on how long farming can continue in the same way, given the available nutrients. For cassava-based cropping system, total nutrient losses due to cassava cultivation are known to be quite high, especially those of N and K when cassava yields are high, or when crop is grown on slopes (Howeler, 2001). This can result in losses of soil nutrient in eroded sediments which tend to be high also in N and K. Defoer et al. (2000), noted that only about 1 to 4% of the dynamic reserve is directly available for crop production, and this is subject to losses amounting to 15 to 90 kg ha^{-1} per unit weight of soil containing 0.1% N. To maintain a positive nutrient balance and high NSB ratio in such cropping system, it is important to maintain a high input diversification (Fertilizers and manures that are high in N and K) or other cultural practices as suggested by Umeh and Mbah (2010a). The objective of the present investigation was therefore, to assess the effect of applied fertilizer (N and K) and soybean nitrogen contribution on nutrient stock: balance ratio in cassava/soybean intercrop system.

MATERIALS AND METHODS

The cassava/soybean intercrop experiment was conducted at University of Nigeria, Nsukka farm located at latitude 06° 52''N and longitude 07° 24''E and at 447 m above the sea level, between August 2004 and July 2006. The experiment was laid out in a split plot design, having two factors; fertilizer rates and cropping systems. The four fertilizer rates, N_0K_0, N_0K_{50}, $N_{45}K_0$ and $N_{45}K_{50}$ kg ha^{-1}, were randomised in the main plot, while the twenty cropping systems comprising six sole soybean, two sole cassava and twelve cassava/soybean intercrop were randomised in the sub-plots. The nitrogen source was Urea and potassium source was muriate of potash. A uniform application of 39 kg ha^{-1} of P as single supper phosphate was applied to all plots.

Plot size was 4.0 m × 3.0 m, containing 4 ridges at 1.0 m spacing. Soybean was planted on both sides of the ridges at a plant distance of 10.0 cm showing a plant population of 200,000 plants ha^{-1} while cassava was planted at a plant distance of 0.75 m on the crest of the ridges, a plant population of 13,333.33 plants ha^{-1}. Soil samples at 0 to 30 cm. depths were taken at the beginning of the experiment, at harvest of soybean and at harvest of cassava, and analysed for soil minerals and organic matter. Planting was done in early August and weed was controlled manually. At maturity, four middle rows of soybean were harvested to determine the seed yield and the nutrient content (N and K) of both the grain and the crop residue. Cassava tuber yield and nutrient content (N and K) were determined at 12 months after planting.

Nutrient balance method

The quantities of nutrients (N and K) entering and leaving the field were estimated and the balances for N and K were calculated for the various treatments and cropping systems. This was achieved by aggregating input and output data for all the plots using the following equation:

$$Rn_m = \sum^{in} (AP_1 + AR_{\Delta t} - RM_{\Delta t} - L_{\Delta t})$$

Where, Rn_m, is the quantity of inorganic and organic nutrients remaining in the soil at time (in) AP_1 the soil inorganic and organic nutrients (dynamic reserve) present at time t; $AR_{\Delta t}$, is the inorganic (N_0K_0, N_0K_{50}, $N_{45}K_0$ or $N_{45}K_{50}$) and organic (crop residue) nutrients added or returned to the soil at the time interval Δt; $RM_{\Delta t}$, estimates the plant nutrients removed with the harvested product and residue management during the time interval Δt; $nL_{\Delta t}$, is the organic and inorganic nutrients lost during the time interval Δt; The value of t represents the beginning time period; m, represents the ending time period; Δt, is the time interval between t and m.

The production of crop outputs and residues is used to calculate total crop nutrient uptake from soil. Nutrient stock:balance ratio are assessed by calculating and using estimates of nutrient gain to the application of mineral (N_0K_0, N_0K_{50}, $N_{45}K_0$ or $N_{45}K_{50}$) fertilizers and to biophysical processes of deposition, sedimentation and fixation. Information on weather, soil constraints and soil characteristics is used to estimate soil nutrient losses resulting from erosion, leaching and volatilization (gaseous losses). Estimates of nutrient gains and losses are developed from assumed soil nutrient transfer functions and from estimation of empirical statistical models.

$$\text{Dynamic Reserve} = ((B_1P_N) + B_2)e1$$

Where: P_N = %N in dry matter; B_2 = Total stock including applied fertilizer $e1$ = % soil –N; N and K were nitrogen and potassium respectively, subscripts 0, 45 and 50 were levels of N and K kg ha^{-1} respectively.

Data analysis

Data collected were analysed using procedures outlined by Obi (2002) for split plot in randomised complete block design (RCBD). Differences among treatment means were determined by the use of Fisher's least significant difference (F-LSD) at 5% probability procedure outlined by Obi (1996). Combined analysis of variance (ANOVA) was done using the general linear model procedure (GLM) to determine differences and effects between cropping system, soil amendment effect, crop yield and system efficiency.

RESULTS AND DISCUSION

The result of the soil analysis of plots before the study is shown on Table 1. The textural class of the soil was a

Table 1. Some soil properties of the experimental site at the beginning of the experiment.

Soil dept (cm)	pH (H_2O)	OM (%)	C (%)	K (Meq/100 g)	$N0_3$ (%)	N (%)	Clay (%)	Silt (%)	Fine sand (%)	Coarse sand (%)
0-30	4.1	1.74	0.81	0.11	8.7	0.045	332.5	8.5	19.93	39.1
30-60	4.2	1.18	0.68	0.12	6.7	0.043	40.5	6.5	26.7	26.3
60-90	4.1	0.80	0.46	0.09	3.0	0.038	30.5	4.6	21.1	43.8

Table 2. Nitrogen stock: balance ratio for sole cassava cropping system at various fertilizer rates.

Fertilizer rate	Dynamic Reserve (kg. ha^{-1})	Stock (Kg ha^{-1})	Balance (Kg ha^{-1} $year^{-1}$)	Ratio	Year
N_0K_0	2.25	6.62	-27.33	0.88	1
N_0K_{50}	2.25	6.64	-11.33	1.99	2
$N_{45}K_0$	6.16	28.44	-4.12	1.50	2
$N_{45}K_{50}$	5.52	36.95	-2.88	1.93	2
F-$LSD_{0.05}$	0.52	0.73	0.21		

combination of sandy-clay and sandy-clay-loam. The pH of the soil at different soil depths (0-90 cm) was similar. The range was 4.0 to 4.2. Organic matter (OM) at the top soil (0-30 cm) were highest and ranged 1.43 to 1.74%, followed by 30 to 60 cm depth (1.04-1.18%) while 60 to 90-depth had the lowest organic matter (0.76-0.94%). Similarly the highest organic carbon (0.81-0.84%) was on the top soil (0-30 cm depth), followed by 30 to 60 cm depth (0.6-0.7%) while 60 to 90 cm depth had a range of 0.44 to 0.54%.

Potassium content was similar in 0 to 60 cm depth in all the plots with a range of 0.11 to 0.13 meq/100 kg while 60 to 90 cm depth had a range of 0.08 to 0.09 meq/100 gK. Soil-N at 0 to 30 and 30 to 60 cm depths were similar and had the range of 0.041 to 0.050%, while 60 to 90 cm depth had the range of 0.032 to 0.038%. Soil-$N0_3$ was highest at the 0 to 30 cm depth followed by 30 to 60 cm depths. Lowest soil NO_3 was obtained at 60 to 90 cm depth under sole cassava (Table 2) highest nutrient stock balance (NSB) ratio was 1.99 with a nitrogen balance of -11.33 Kg N ha^{-1} $year^{-1}$ obtained at N_0K_{50} fertilizer rate. It has a nutrient stock of 6.64 Kg ha^{-1}. This result did not differ significantly with the NSB ratio obtained at $N_{45}K_0$ and $N_{45}K_{50}$ fertilizer rates (1.50 and 1.93 respectively). The lowest NSB ratio (0.88) was at N_0K_0 fertilizer rate which had the lowest dynamic nutrient reserve (2.25 Kg N ha^{-1}). This finding revealed that cassava has a high requirement for nitrogen. The negative nitrogen balance of -11.33 Kg N ha^{-1} $year^{-1}$ showed that the production system degraded, the sustainability of the cropping system will be less than 2 years (NSB ratio 1.99). The result agreed with the result of experiments conducted within the ecological zone which led to the tentative recommendation of 56, 28 and 112 kg ha^{-1} of NPK fertilizer respectively for cassava production in pure stand (ARTS, 1994; Nweke et al., 1994; Ikeorgu and Iloka, 1994). They observed that after several years of planting cassava in monoculture, the soil was eroded and was confirmed by the negative nitrogen balance (-22.33 kg ha^{-1} $year^{-1}$) obtained at N_0K_0 fertilizer rate which will result in soil degradation and crop failure in less than 1 year (NSB ratio 0.88).

With potassium nutrition (Table 3), highest potassium NSB ratio (4.46) and nutrient balance (+10.75 kg K ha^{-1}) in sole cassava, were obtained at N_0K_{50} fertilizer rate which differed significantly with other fertilizer rates. The positive potassium balance of 12.23 and 10.75 kg K ha^{-1}at the application of 50 kg K showed that the production system could be sustained for about 5 years with potassium application (NSB ratio 4.46 and 3.88 respectively). Production system without potassium (N_0K_0 and $N_{45}K_0$) showed negative K balances thus NSB ratio less than 1.

In the sole soybean (Table 4), the highest NSB ratio (14.58) and nutrient stock (1597.52 Kg ha^{1}) were obtained at N_0K_{50} fertilizer rate, which was significantly higher than NSB ratios of 9.46 and 10.44, obtained at $N_{45}K_{50}$ and $N_{45}K_{50}$ fertilizer rates, respectively. Nutrient stock: balance ratio (8.82) obtained at $N_{45}K_{50}$ fertilizer rate was significantly lower than at all other fertilizer rates. This result showed that use of inorganic fertilizer reduces sustainability of a farming system. Inclusion of legumes in farming systems is a better method for improving soil-N. The role of legume as soil improver has long been recognised by farmers throughout the world. Leihner (1988) suggested that the amount of fertilizer recommended for cassava at sole

Table 3. Potassium Stock:balance ratio for sole cassava cropping system.

Fertilizer rate	Dynamic Reserve (Kg. ha^{-1})	Stock (Kg ha^{-1} year^{-1})	Balance	Ratio	Year
N_0K_0	10.69	11.75	-12.40	0.86	1
N_0K_{50}	47.95	100.70	+10.75	4.46	5
$N_{45}K_0$	6.37	14.01	-6.40	0.99	1
$N_{45}K_{50}$	103.10	226.82	+12.23	3.88	4
F-$LSD_{0.05}$	1.30	1.42	0.44		

Table 4. Nitrogen stock:balance ratio for sole soybean cropping system.

Fertilizer rate	Dynamic reserve (kg. ha^{-1}	Stock (kg ha^{-1} year^{-1})	Balance	Ratio	Year
N_0K_0	133.01	846.72	+12.62	10.44	11
N_0K_{50}	916.35	1597.52	+62.85	14.58	15
$N_{45}K_0$	405.52	1443.32	+42.45	9.46	10
$N_{45}K_{50}$	412.66	1279.70	+46.81	8.82	9
F-$LSD_{0.05}$	2.62	6.75	0.47		

Table 5. Potassium Stock:balance ratio for sole soybean cropping system.

Fertilizer rate	Dynamic reserve (kg. ha^{-1})	Stock (kg ha^{-1} year^{-1})	Balance	Ratio	Year
N_0K_0	79.60	159.21	+8.24	9.65	10
N_0K_{50}	1060.65	2862.00	+88.24	12.02	12
$N_{45}K_0$	50.95	152.85	+7.65	6.66	7
$N_{45}K_{50}$	718.74	2299.68	+85.26	8.43	8
F-$LSD_{0.05}$	6.99	8.27	3.33		

would be reduced if the cassava were planted in association with efficient nitrogen fixing legume. Ngo et al. (2005) reported that intercropping cassava with cowpea resulted in 20 to 100% greater land use efficiency than for either of the crops grown alone. The role drives mainly from ability of legumes to fix atmospheric nitrogen in symbiosis with *ryzobia.* At the application of potassium alone, nutrient balance was +62.85 kg ha^{-1} year^{-1} and nutrient stock of 1597.52 Kg ha^{-1} and sustainability of 15 years.

Sole soybean highest NSB ratio 12.02 (Table 5) was also obtained at N_0K_{50} fertilizer rate which has nutrient balance of +88.24 kg K ha^{-1} and was significantly higher than the second highest potassium NSB ratio (9.65) obtained at N_0K_0 fertilizer rate. Lowest NSB ratio (8.43) was obtained at $N_{45}K_{50}$ fertilizer rate. This finding agrees with the report of Howeler (2001) who observed that potassium taken from the solution phase of the soil would be replenished through ion exchange, by dissolution from solid mineral phase, or by mineralization of organic compounds. Defoer et al. (2000) noted that when plants take up potassium, the equilibrium between the dynamic and inert reserve is temporally disrupted, some of the exchangeable potassium must then be released into the soil solution to re- establish this equilibrium.

At intercrop (Table 6), N_0K_{50} fertilizer rate had the highest NSB ratio (11.0) which was significantly higher (about 4, 3, and 2 times) than NSB ratio at $N_{45}K_0$, N_0K_0 and $N_{45}K_{50}$, respectively. Lowest NSB ratio (3.2) was obtained at N_0K_0 fertilizer rate. This result showed that the benefit of including legumes in intercrop systems goes beyond sparing effect of nitrogen, competitive interaction between the crop components or reduced competition. The fertilizer rates $N_{45}K_0$ and $N_{45}K_{50}$ with higher nitrogen application produced NSB ratio of 4.0 and

Table 6. Nitrogen stock:balance ratio for cassava/soybean intercrop.

Fertilizer rate	Dynamic reserve (kg. ha^{-1})	Stock (kg ha^{-1} year^{-1})	Balance	Ratio	Year
N_0K_0	13.11	93.64	-4.37	3.15	3
N_0K_{50}	484.66	3461.86	+44.06	11.60	12
$N_{45}K_0$	100.00	357.14	+25.00	4.00	4
$N_{45}K_{50}$	129.65	540.21	+25.93	5.00	5
F-$LSD_{0.05}$	4.26	22.61	1.27		

Table 7. Potassium Stock:balance ratio for cassava/soybean intercrop.

Fertilizer rate	Dynamic reserve (kg. ha^{-1})	Stock (kg ha^{-1} year^{-1})	Balance	Ratio	Year
N_0K_0	16.04	72.90	+4.01	3.86	4
N_0K_{50}	654.30	2044.69	+72.70	8.74	9
$N_{45}K_0$	22.96	114.80	+5.74	3.98	4
$N_{45}K_{50}$	381.50	1467.31	+76.30	4.77	5
F-$LSD_{0.05}$	12.12	14.73	2.67		

5.0, respectively, indicating that high application rate of mineral fertilizer did not necessarily increase the cropping system nutrient balance or its NSB ratio. The result rather confirmed that there were substantial yield advantages obtained in intercropping systems involving legumes as were reported by many workers (Tijani and Akinnifesi, 1996; Unkovick and Pate, 2000; Umeh and Mbah, 2010b). These advantages are not commonly the sparing effects of inputs or biophysical compatibility, but can be attributed to better use of resources when crops are grown together than when in moncrop systems. Certainly, different crops may be complementary to each other and make better use of resources when grown together. Whereas in potassium nutrition (Table 7), highest NSB ratio (8.74) and highest nutrient stock (2044.69 kg K ha^{-1}) with nutrient balance of +72.70 kg K ha^{-1}) were again obtained at N_0K_{50} which was significantly higher than all other fertilizer rates. The result supports that soybean may have played a role on the metabolic processes of potassium in the cropping system by influencing the release of potassium from the soil inert potassium reserve. The second highest NSB ratio (4.77) was obtained at $N_{45}K_{50}$ fertilizer rate. While the lowest NSB ratio of 3.86 obtained at N_0K_0 did not differ significantly with the NSB ratio (3.98) obtained at $N_{45}K_0$ fertilizer rate.

Conclusion

1. With respect to nitrogen nutrition among the cropping systems, sole soybean produced the highest NSB ratio followed by intercropped system. Lowest NSB ratio was obtained at sole cassava. Within the fertilizer rates, N_0K_{50} gave the highest NSB ratio hence sole soybean at N_0K_{50} fertilizer rate will be the most sustainable (15 years), followed by intercrop at N_0K_{50} fertilizer rate (11 years.) While sole cassava cropping system at all fertilizer rates will be the least sustainable (1 or 2 years).

2. Nutrient stock:balance (NSB) ratio for potassium, was also highest in sole soybean, followed by intercropped system. Lowest NSB ratio was obtained at sole cassava. Within the fertilizer rates, N_0K_{50} gave the highest NSB ratio, hence sole soybean at N_0K_{50} fertilizer rate will be the most sustainable (12 years), followed by intercrop at N_0K_{50} fertilizer rate (10 years.) While sole cassava cropping system at N_0K_0 and $N_{45}K_{50}$ fertilizer rates will be the least sustainable (1 year).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Effects of stocking density on growth and feed utilization of grouper (*Epinephelus coioides*) reared in recirculation and flow-through water system

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Stocking density is well known as an important field in aquaculture. Although many studies on stocking density have been conducted, however effects of rearing grouper (*Epinephelus coioides*) with different densities in recirculation and flow-through system are not well known. In this study, grouper juveniles (14.22 ± 0.67 g) were reared into 100-L aquaria at 15, 20 and 25 fish/aquaria and cultivated for 70 days. Statistical analyses showed that the highest growth performances observed in group reared at high densities (25 fish/aquaria) in recirculation system (R25), with an average final body weight and length were 95.82 ± 4.24 g and 18.72 ± 1.40 cm, respectively. Significant increase in weight gain and specific growth rate and decrease in food conversion ratio were observed in R25 after 10 weeks. However, no statistically significant different was found in survival rate and condition factor in all treatments. This study found that the effects of stocking density on growth and feeding ratio were higher in recirculation system compared with flow-through system. Further analysis determined that high stocking density in recirculation system and medium density in flow-through could affect the growth, feeding and fish behavior of this species.

Key words: Feeding ratio, high stocking density, specific growth rate, water system, weight gain.

INTRODUCTION

Recently, research on stocking density is receiving a great attention (Turnbull et al., 2005) since this study is necessary to provide information on a better aquaculture management in relation to the fish welfare. Although some studies on stocking density have been published, it is still difficult to obtain information on better densities for each species, because the best densities are affected by different culture systems, fish species and fish age (Ellis et al., 2002; Jorgensen et al., 1993; Greaves and Tuene, 2001). In aquaculture system, mostly aquaculturists cultivate their fish in high stocking density in order to maximize productivity (Iguchi et al., 2003). Therefore, knowing appropriate stocking density is recognized as an essential aspect because it plays a big role in increasing the fish production to meet the continuous increase in fish demand and maintain the profitable and economic sustainable for aquaculturist (Rafatnezhad et al., 2008).

Generally, stocking density is well known as the weight of fish per unit volume (Ellis, 2001) or the number of fish stocked at the beginning of experiment (Ruane et al., 2002). Stocking density is identified and may affect fish growth performances, physiology and fish behavior (Holm

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et al., 1990; Wedemeyer, 1997; Schreck et al., 1997; Ellis et al., 2002), influence feeding activities, metabolism distortion and digestive utility (Vijayan et al., 1990; Holm et al., 1990; Adams et al., 1998; Dibattista et al., 2005), feed utilization (Jorgensen et al., 1993; Alanara and Brannas, 1996), hormonal alteration (Kebus et al., 1992); and immunological activities (Pottinger and Pickering, 1992; Yin et al., 1995; Tort et al., 1996). In high stocking density, usually fish exhibit aggressive behavior, especially when food availability is limited. This condition often leads to fish stress; particularly it may affect the fish health. Therefore, food availability is very important when it concerns fish density (Holm et al., 1990). Moreover, disproportionate density may cause poor fish welfare, hence affecting the profitableness of the commercial fish industry (Ellis et al., 2002; Conte, 2004; Turnbull et al., 2005; Huntingford et al., 2006; North et al., 2006). Stocking density not only reportedly affects the fish productivity, but also affects water availability, land requirements and production costs.Previous studies have also reported on positive and negative effects of stocking density; for example, in Arctic charr, *Salvelinus alpines* (Jorgensen et al., 1993) and halibut, *Hippoglossus hippoglossus* (Bjornsson, 1994). Those species showed positive impacts when stocked at high densities. In contrast, gilthead sea bream, *Sparus auratus* (Montero et al., 1999) and sea bass, *Dicentrarchus labrax* (Vazzana et al., 2002; Gornati et al., 2004) showed negative impact when reared at high densities, because high densities lead to increased stress level; consequently resulting in poor growth rate and feeding behavior.

Based on the attempts mentioned above, it is worthy to note that studies on fish density have been carried out on some species; however, to this day information on stocking density is still limited (Turnbull et al., 2005), especially in groupers. Thus, this study was designed to investigate the effects of stocking density of grouper, *Epinephelus coioides,* cultivated in different water systems: recirculation and flow-through system.

MATERIALS AND METHODS

Experimental fish preparation

Grouper (*E. coioides*) weighed 14.22±0.67 g body weight were obtained from Aquatic Animal Center and then acclimated in the hatchery of the Department of Aquaculture, National Taiwan Ocean University, for two weeks prior to experimentation. Fish were reared and fed twice a day by feeding commercial diet. Fish of each group were distributed into 100-L total water volume (60 cm length, 50 cm wide, 35 cm height) at 15, 20 and 25 fish/aquarium. Well aerated water was provided from a storage fiberglass. Water quality parameters were maintained at temperature 29.0±1°C; pH 8.0±1 and salinity 34±1 ppt. These ranges are considered within optimal values for grouper juveniles.

Experimental design

The experimental facility was composed of 18 aquaria (100-L each); whereas nine aquaria run- in recirculation water system, others run in flow-through system. Well-aerated water was provided from storage of fiberglass tank, filtered and supplied to the system. Those two water systems were equipped with mechanical filter (spongy), UV light, automatic heater and supplied with compressed air via air-stones from air pumps.

Water flows in experimental aquaria were measured and adjusted before the experiment in order to be proportional to the fish density and to ensure sufficient water circulation. The experimental site was maintained under 24-h photoperiods. The fish were stocked into aquaria based on densities treatments and randomly allocated to triplicate aquaria.

Fish growth measurements

Measurements on parameters such as growth performances, feeding activities, survival rate and water quality was carried on based on required data. Data on growth rate was recorded regularly every two weeks by weighed and measured individual fish from each aquarium. On each sampling day, each individual fish was caught from aquarium using a small net. Then the fish were quickly weighed and measured.

The body wet weight was measured using an analytical balance (Ohaus Navigator, no. 4120, Canada) and the total length using digital caliper (Mitutoyo, Absolute Digimatic, Japan). Immediately after measurements, the fish were carefully returned to its original aquaria. Growth performances were calculated as following:

Specific growth rate (SGR, %/day) = 100 x ($\ln W_2 - \ln W_1$) / T

where: W_1 and W_2 are initial weight and final weight, respectively and T is the number of days in the feeding periods.

Weight gain (WG, %) = 100 x [(final weight (g) – initial weight (g)] / initial weight (g)

and

Condition factor (K) = [(10^5 x weight of fish (g) / (length of fish)^3 (cm)]

In experiment on survival rate, all treatments were observed daily and the data was calculated by the following formula:

Survival rate (SR, %) = (Final no. of fish / initial no. of fish) x 100

Feed utilization measurements

Fish were handfed twice daily (at 08:00 and 17:00) at 3% of the biomass by feeding commercial diet. Consequently, the total number and feed size changed as fish grew and as a result of mouth gape. During the experiment, uneaten pellets were collected and measured after each feeding time. For feed utilization, the amount of food consumed was calculated as the difference between dry diet given and dry diet remained.

Feed intake (FI, g/fish/days) = [dry diet given (g) – dry diet remained (g)] / no. of fish

Feed conversion ratio (FCR) = dry feed intake (g) / [final body weight (g) – initial body weight (g)]

and

Feed efficiency ratio (FER) = [final body weight (g) – initial body weight (g)] / dry feed intake (g).

Table 1. Growth responses, condition factor and survival rate of grouper reared at different stocking density.

Variables		Length (cm)		Weight (g)		WG (%)	SGR (%)	K	SR (%)
		Initial	Final	Initial	Final				
					Recirculation system				
Density	R15	9.43 ± 0.58	15.87 ± 1.12^{c}	14.23 ± 0.67	64.17 ± 3.78^{c}	351 ± 20^{c}	2.15 ± 0.06^{c}	1.61 ± 0.10	100
	R20	9.42 ± 0.58	16.13 ± 0.90^{bc}	14.22 ± 0.67	73.53 ± 3.62^{b}	417 ± 25^{b}	2.35 ± 0.07^{b}	1.67 ± 0.15	100
	R25	9.43 ± 0.58	18.72 ± 1.40^{a}	14.24 ± 0.67	95.82 ± 4.24^{a}	574 ± 30^{a}	2.72 ± 0.06^{a}	1.46 ± 0.08	100
					Flow-water system				
Density	F15	9.43 ± 0.58	15.47 ± 1.0^{c}	14.24 ± 0.67	59.82 ± 3.05^{c}	321 ± 14^{c}	2.05 ± 0.05^{c}	1.56 ± 0.11	100
	F20	9.43 ± 0.58	16.72 ± 1.10^{b}	14.23 ± 0.67	75.99 ± 3.70^{b}	435 ± 26^{b}	2.39 ± 0.07^{b}	1.63 ± 0.08	100
	F25	9.42 ± 0.58	15.82 ± 0.90^{c}	14.22 ± 0.67	62.00 ± 3.46^{c}	336 ± 17^{c}	2.10 ± 0.06^{c}	1.57 ± 0.09	100

WG: weight gain; SGR: specific growth rate; K: condition factor; SR: survival rate; R: recirculation system; F: flow-through system. Values are means of triplicate groups' ± S.D. Within a column, means with different letters are significantly different ($P < 0.05$). Means with the same letters or absence of letters indicate not significantly different between treatments.

Water quality measurements

Water parameters including dissolved oxygen (DO), temperature, pH, salinity, ammonia and nitrite were sampled every five days. DO and temperature were measured *in situ* using DO meter (DO600: Waterproof ExStick, Extech Instument Corp. USA), pH with a pH meter (PH100: ExStick, Extech Instument Corp. USA), salinity using refractomoter (ATAGO S/Mill-E, ATAGO CO. LTD, Japan). Data collection was conducted by placing the detector (at the tip of equipments) into the water surface. All displayed number was recorded as water quality parameters. Total ammonia nitrogen (TAN) was examined using phenol-hypochlorite method. It was carried on by prepared 1000 µL of filtered water samples in 1.5 mL tube; and then 40 µL of phenol alcohol solution and 40 µL of sodium nitroprusside were added and mixed into the samples, followed by added 100 µL prepared oxidation solution. After mixing, all samples were stored at 22-27°C for 1 hour, and then samples were analyzed at 640 nm absorbance using spectrophotometer (Ultrospec 8000, Biochrom, Cambridge, UK). Nitrite (NO_2-N) was determined using Wood-Armstrong-Richard method. It was conducted by prepared 1000 µL of filtered water sample in 2 mL tube; 20 µL sulfanilamide was added followed by 20 µL of N-(1-naphthyl)-ethelene diamine solution and mixed. Then samples were stored at 22 to 27°C. After 15 min, all samples were analyzed at 543 nm absorbance using spectrophotometer. During the whole experiments, sea water was changed around 10% daily in recirculation system, whereas in flow-through system, sea water changed approximately 200% daily.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) with water system and fish densities as factors. When the differences were significant at $P < 0.05$ level, Tukey's test was used to compare the means between individual treatments. Statistical analysis was performed using the SAS software (SAS Inc. Cary, NC, USA).

RESULTS

Fish growth performances

Fish growth was significantly affected by water system and fish densities. A summary of growth responses, condition factor and survival rate in each trial is provided in Table 1. In recirculation system (R), the highest growth performances were observed in group reared with high density (R25). It showed a significantly different ($P < 0.05$) compared to R15 (low density) and R20 (medium density), with an average final body weight 95.82 ± 4.24 g and final length 18.72 ± 1.40 cm. In flow-through system (F), group F20 (juveniles reared in medium density of 20 fish/aquaria) showed the best growth rate. The mean body weight and final length were 75.99 ± 3.70 g and 16.72 ± 1.10 cm, respectively. Among treatment groups, the lowest fish growth was occurred at F15 (juveniles reared in low density of 15 fish/aquaria) in flow-through system. However, it found that there is no significant difference between groups F15 and F25 in their final weight and length. During the whole experimental periods, all groups increase the weight and length steadily every week. Both R-system and F-system showed the greatest weight increments, with fish cultivated in R-system tended to have a better growth performances compare with fish in F-system (Figure 1A, B). However, no statistical differences were observed on the survival rate (SR, %) and condition factor (K) during the 10-weeks rearing (Table 1).

The greatest improvements in weight gain (WG) and specific growth rate (SGR) were observed in group R25 ($P < 0.05$), maintaining a significantly higher WG and SGR than F20 and R20 (Figure 2A, B). The poorest fish growth was obtained in group F15, with the percentage of weight gain and specific growth rate being 321 ± 14 and 2.05 ± 0.05, respectively. There was a decrease of SGR in both cultivation systems. In R-system, at the first measurements (2 weeks) the mean SGR was 2.73%/day (ranging from 2.65 to 2.86%), then decrease steadily to 2.40%/day (ranging from 2.15 to 2.69%) after 10 weeks. Similarly, in F-system, the mean SGR value reduced from 2.54%/day (ranging from 2.38 to 2.67%) to 2.18%/day

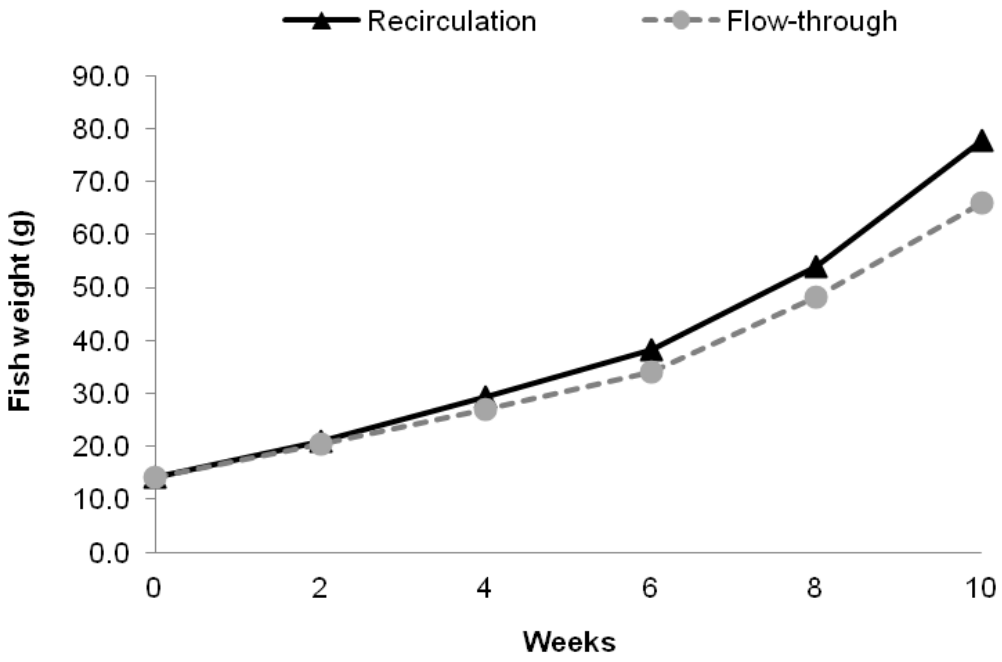

Figure 1A. Comparative of fish weight reared at different stocking density in recirculation and flow-through water system. Values are means of three treatments in each water system.

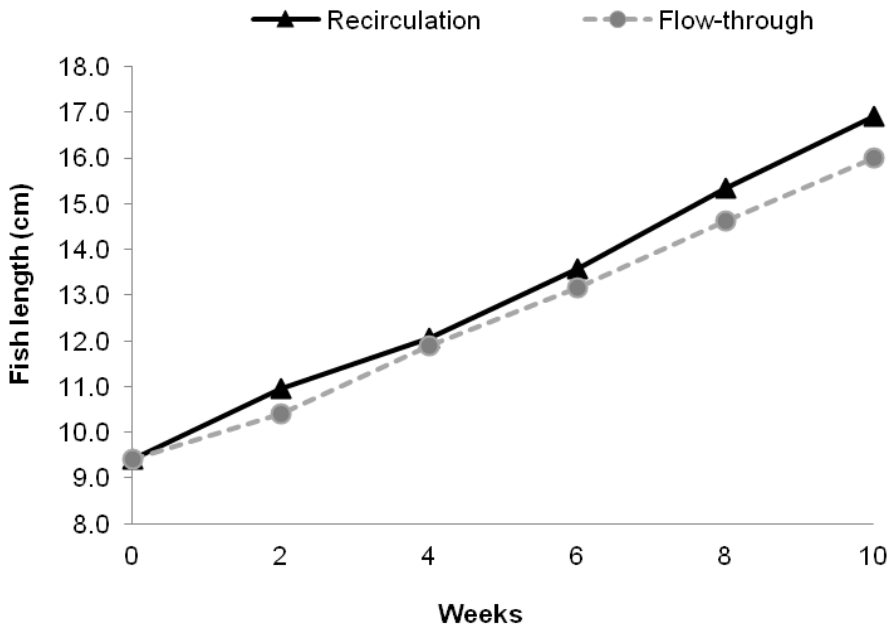

Figure 1B. Comparative of fish length reared at different stocking density in recirculation and flow-through water system. Values are means of three treatments in each water system.

(ranging from 2.05 to 2.39%) at the end of experiment. In contrast, the increased in WG was observed during the experimental time. Mean WG increased from 47% (ranging from 45 to 49%) to 448% (ranging from 351 to 574%) in R-system; and increased from 42% (ranging from 37 to 45%) to 364% (ranging from 321 to 435%) in F- system, respectively. Result on the WG_s and SGR_s indicated that fish cultivated in R-system could enhance overall relative

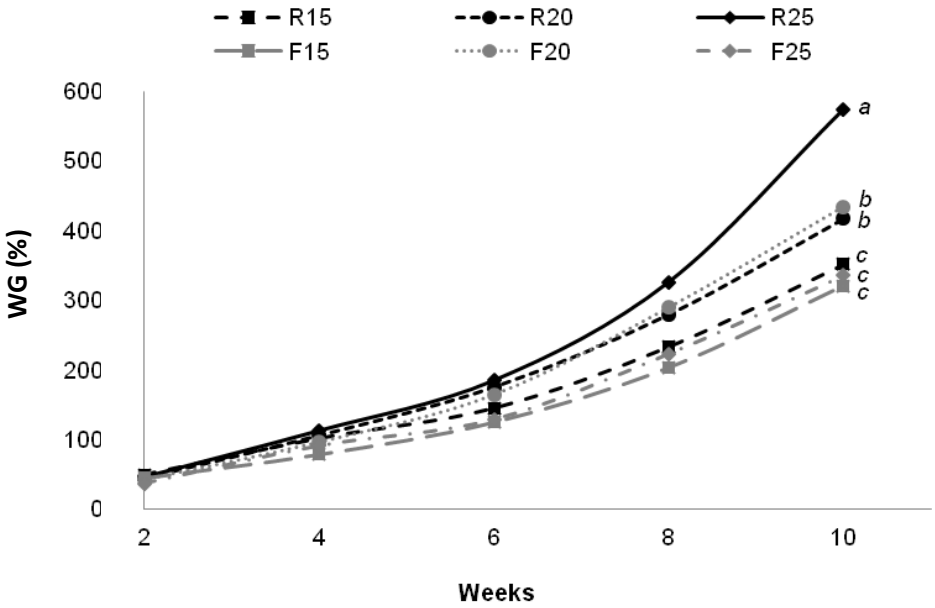

Figure 2A. Mean weight gain (WG) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of triplicate groups. Different superscripts at the end of each line chart indicate significant different (P < 0.05) among treatments.

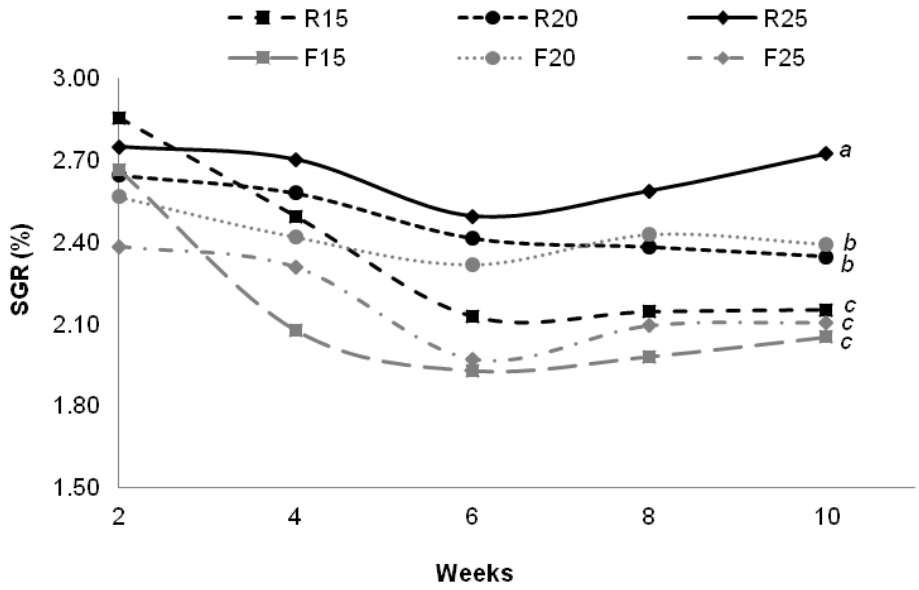

Figure 2B. Mean specific growth rate (SGR) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of triplicate groups. Different superscripts at the end of each line chart indicate significant different (P < 0.05) among treatments.

growth rate compare with fish cultured in F-system. However, statistical analyses showed that there were no significant differences between R20 with F20 and between R15 with F15 and F25.

Feeding performances

Feeding parameters such as feed intake (FI), feed conversion ratio (FCR) and feed efficiency ratio (FER)

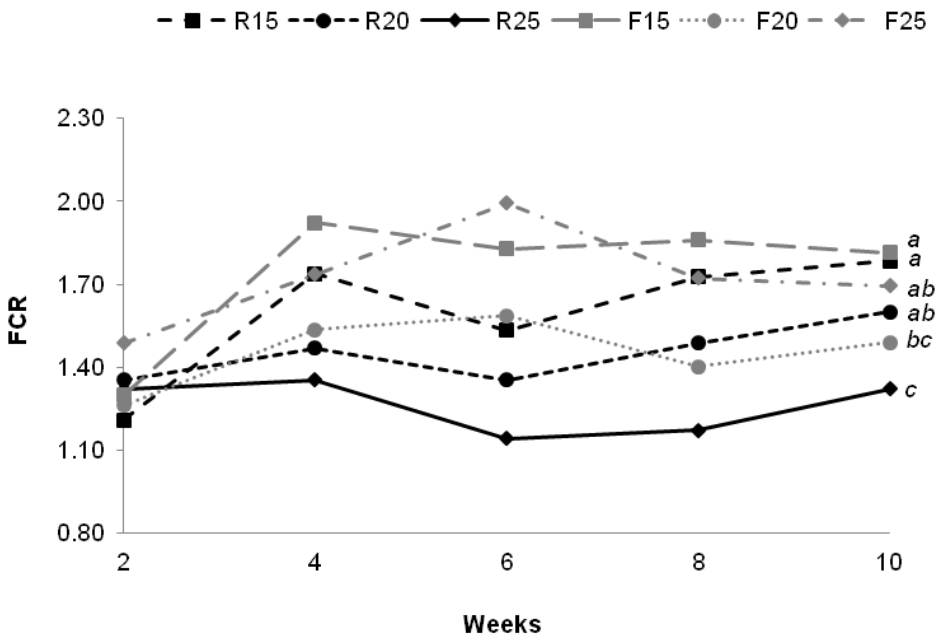

Figure 3. Mean feed conversion ratio (FCR) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of triplicate groups. Different superscripts at the end of each line chart indicate significant different (P < 0.05) among treatments.

Table 2. Feed utilization of grouper reared at different stocking density.

Variables		FI	FCR	FER
		Recirculation system		
Density	R15	89.05 ± 0.85^{c}	1.79 ± 0.18^{a}	0.56 ± 0.03^{c}
	R20	94.68 ± 1.07^{b}	1.60 ± 0.12^{ab}	0.63 ± 0.04^{bc}
	R25	107.8 ± 4.04^{a}	1.32 ± 0.05^{c}	0.76 ± 0.03^{a}
		Flow-water system		
Density	F15	82.69 ± 0.96^{d}	1.82 ± 0.18^{a}	0.55 ± 0.02^{c}
	F20	91.92 ± 0.63^{bc}	1.49 ± 0.12^{bc}	0.67 ± 0.04^{ab}
	F25	80.81 ± 0.77^{d}	1.69 ± 0.19^{ab}	0.59 ± 0.03^{bc}

FI: feed intake (g feed/fish/day); FCR: feed conversion ratio; FER: feed efficiency ratio. Values are means of triplicate groups' ± S.D. Within a column, means with different letters are significantly different (P < 0.05). Means with the same letters indicate not significantly different between treatments.

were significantly affected by water system and fish densities (P < 0.05). In R-system, the best feed utilization was obtained by R25, with FCR showing significant difference (P<0.05) compared to R15 and R20 (Figure 3). Similarly, in F-system, group, F20 showed the best feeding activity with the mean of FI and FCR being 91.92 ± 0.63 and 1.49 ± 0.12, respectively (Table 2). This study found that, fish cultured in R-system tended to consume more feed compared with fish in F-system (Figure 4). The highest FI was found at R25 (107.8 ± 4.04 g), while the lowest one was at F25 (80.81 ± 0.77 g). In R-system, the FCR decreased and FER ratio increased significantly in group R25 compared to other groups. Similar result showed in F-system; the FCR decreased and FER ratio increased significantly in group F20 compared to F15. In contrast, group R15 showed increasing FCR and decreasing FER ratio from 4 weeks until the end of experiment. In both culture systems, the total FCR values were lower in fish cultivated in R-system than fish cultivated in F-system. Furthermore, overall FER values were higher in fish cultured in R-system compare with F-system. Data analyses found that the best FCR and FER were obtained by R25 at 1.32 ± 0.05 and 0.76 ± 0.03, respectively; while the poorest FCR and FER were seen

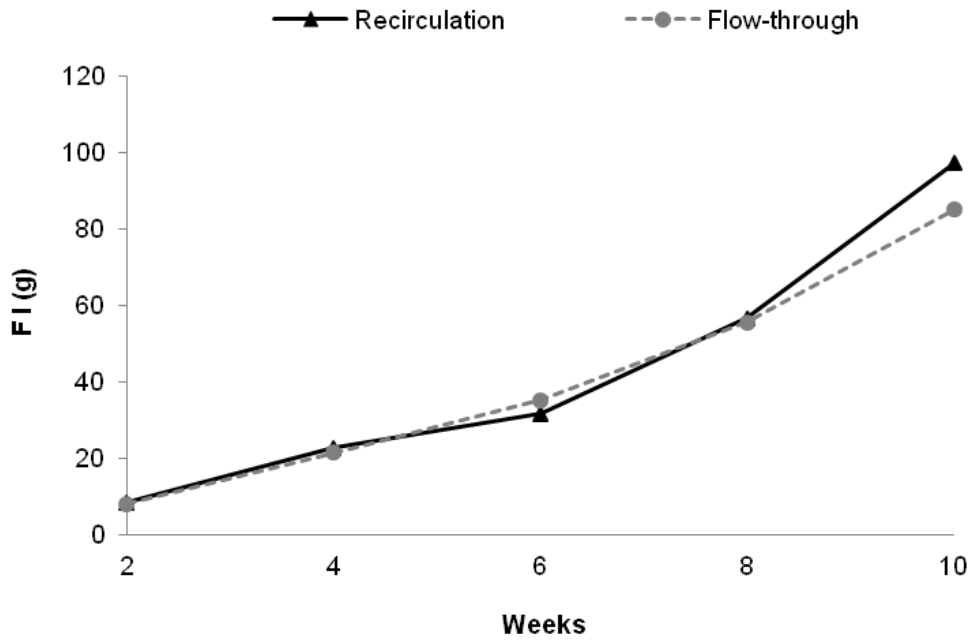

Figure 4. Comparative feed intake (FI) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of three treatments in each water system.

at F15 at 1.82 ± 0.18 and 0.55 ± 0.02, respectively.

Water quality parameters

A summary of water quality parameters in each trial is provided in Table 3. Water quality parameters such as: salinity, dissolved oxygen (DO), temperature and pH were checked regularly every five days until the end of experiment. During the experiment, temperature in R-system ranged between 30.69 to 30.76°C, with the highest temperature found in higher density compare with lower density. In F-system, the temperature remained stable within the range of 29.09 to 29.10°C. The data showed that temperature in F-system tended to be lower than R-system. This low temperature is estimatedly caused by regularly water changes up to 200% daily. In R-system, DO levels were between 5.81 to 5.96 ppm, while in F-system, it ranged from 6.37 to 6.44 ppm. Significant differences were observed in both water systems, whereas DO concentration seems to be high in F-system than R-system. The highest DO was obtained by F15, whereas the lowest one was occurred in R25. The data also indicated that the higher stocking density tended to be lower in DO concentrations.

pH was significantly different in each treatment, with the lowest pH occurring in R25. This low pH value was predicted due to high fish stocking density. Observation on water quality also found that there was no obvious effect of stocking densities on salinity in the treatment groups. Mean water salinity was dependent on the daily supply of seawater. During 70 days of experiment, the range of salinity in R-system was 34.15 to 34.28 ppt, while in F-system, salinity was around 32.87 to 33.08 ppt. These ranges are considered within optimal values for fish culture (Barnabe, 1990; Shepherd and Bromage, 1992; Boyd, 2000; Ismi et al., 2012).

On the first two sampling times, total ammonia nitrogen (TAN) was low in both groups. In R-system, mean of 3 treatments ranged at 0.11 to 0.12 ppm, whereas in F-system ranged from 0.09 to 0.10 ppm. The differences between groups increased with time (Figure 5). In R-system, TAN increased steadily and reached a peak at day 45, with the mean value of 3 treatments 1.20 ppm, however TAN slightly lower until the final sampling day. In contrast, TAN were continuously increasing in F-system with the highest concentration found at the end of sampling time; with the mean of 3 treatments being 1.04 ppm. During the experimental days, the highest TAN was found at R25 with the mean value being 1.39 ppm. In the present study, nitrite concentration was always lower than TAN concentration in both culture systems. It is found that during 5 times of samples measurements, nitrite was almost similar in each group (<0.14 ppm). In all groups, there was a steadily increasing nitrite with time (Figure 6). In R-system, nitrite reached a peak (mean of 3 treatments: 0.33 ppm) on the tenth sampling date, followed by a gradual decline. On the other hand, nitrite showed an increase in F-system until the end of experiment, with the highest mean value attained by

Table 3. Mean Salinity, dissolved oxygen, temperature and pH.

Parameters		Salinity (ppt)	Dissolved oxygen (ppm)	Temperature (°C)	pH
			Recirculation system		
Density	R15	34.21 ± 0.67^{a}	5.96 ± 0.35^{b}	30.69 ± 1.40^{a}	7.76 ± 0.29^{c}
	R20	34.28 ± 0.62^{a}	5.88 ± 0.37^{bc}	30.72 ± 1.42^{a}	7.75 ± 0.29^{c}
	R25	34.15 ± 0.59^{a}	5.81 ± 0.39^{c}	30.76 ± 1.47^{a}	7.70 ± 0.30^{d}
			Flow-water system		
Density	R15	33.05 ± 0.62^{b}	6.44 ± 0.48^{a}	29.09 ± 1.72^{b}	8.02 ± 0.19^{a}
	R20	33.08 ± 0.64^{b}	6.43 ± 0.48^{a}	29.10 ± 1.66^{b}	8.01 ± 0.18^{ab}
	R25	32.87 ± 0.58^{b}	6.37 ± 0.47^{a}	29.10 ± 1.62^{b}	7.99 ± 0.20^{b}

Values are means of triplicate groups' ± S.D. Within a column, means with different letters are significantly different ($P < 0.05$). Means with the same letters indicate not significantly different between treatments.

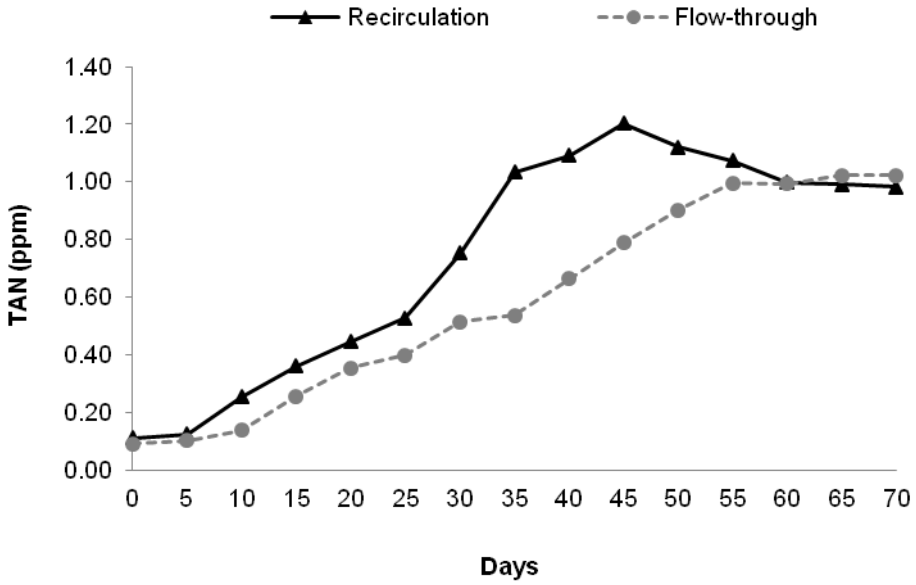

Figure 5. Comparative total ammonia nitrogen (TAN) between recirculation and flow-through water system of grouper reared at different stocking density. Values are means of three treatments in each water system.

F20 at 0.41 ppm.

DISCUSSION

This present study indicated that water system and stocking density had greater effects on fish growth performances, water quality and feed utilization. Growth rate and feed utilization were higher in R-system than in F-system. Presumably, this was caused by a more stable water quality in R-system compare to F-system due to the ability of this system to maintain a constant water quality (Roque d'Orbcastel et al., 2009), improve waste management and nutrient recycling (Piedrahita, 2003), and hygienic (Summerfelt et al., 2009; Tal et al., 2009).

The results showed the positive effect of high stocking density and medium stocking density of *E. coioides* reared in R-system and F-system, respectively. Significant differences in the value of growth were observed among different groups in all the growth parameters studied. The fish cultured at a higher density in R-system reached significantly higher weight and length value than those at lower densities. Further, it found that the greatest improvements in WG and SGR in group R25 were 574 and 2.72%, respectively. It is assumed that higher density caused less swimming due to limited space availability. This condition has been attributed to metabolic savings and low energy expenditure. As a result, consumed nutrients can be utilized for growth maximization. This finding was similar

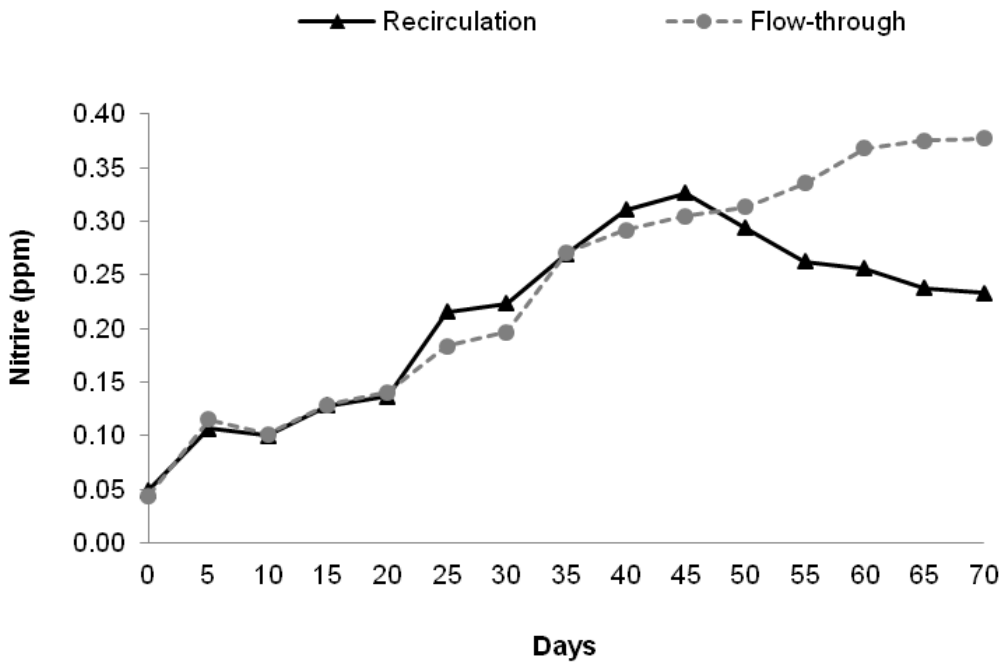

Figure 6. Comparative of nitrite between recirculation and flow-through water system of grouper reared at different stocking density. Values are means of three treatments in each water system.

to previous studies conducted by Jorgensen et al. (1993) on the Arctic charr *S. alpines*; North et al. (2006) on Rainbow trout *Oncorhynchus mykiss*; Papoutsoglou et al. (1998) on sea bass *D. labrax,* and Howell (1998) on turbot (*Scophthalamus maximus*), which described an increment on growth with the increasing of stocking density. However, some studies reported on negative effect when fish is cultivated in higher density, for example in Brook charr *Salvelinus fontinalis* (Vijayan et al., 1990), Rainbow trout and Brown trout (Sirakov and Ivancheva, 2008), and Rainbow trout juvenile (Procarione et al., 1999). On the other hand, fish reared in F-system at medium density (F20) showed the highest growth increment compared with higher (F25) and lower density (F15). It is suggested that medium density is the optimal stocking density of this species when reared in F-system. This may be due to individuals acquiring space, food and water fluctuation during the experiment. It has been demonstrated that appropriate stocking density depends on species, social interaction, water quality and environmental conditions, therefore studies on stocking densities need to be determined in each species for management efficiency in increasing productivity and profitability (Salari et al., 2012; Baldwin, 2010; Ellis et al., 2002).

In these trials where different stocking density had shown effects on juveniles' growth rate, the feed utilization was also observed. The results exposed that FI, FCR and FER were better in R-system compared with F-system. It is assumed that more constant water condition and long-day photoperiods during this experiment affected the fish appetites and feed intake. Similar studies have been reported on the effects of water circumstances and photoperiods in increasing appetite (Taylor et al., 2006; Saunders et al., 1994; Trippel and Neil, 2003), feed intake (Imsland et al., 1995), feeding ratio (Nordgarden et al., 2003; Boeuf and Le Bail, 1999; Boujard et al., 1995), and reducing food costs in commercial farming (Trippel and Neil, 2003). Based on the results, it is alleged that continuously light and schooling behavior seems playing a big role in increasing fish appetence, therefore feed intake can be improved in R25 and F20 in order to gain a maximum growth increment. This finding was in agreement with studies conducted by Webster et al. (2001) and Nordgarden et al. (2003); both suggested that growth may be enhanced by increasing feed consumption to meet the energy demand in maintaining continually growth. In this aspect, we recommend that increase in feeding percentage should be considered in recently studied species; however, well feeding administration is acquired to avoid FCR and FER values deterioration.

Studies on stocking density involve many interrelated parameters including water quality and food availability (Hastein et al., 2005; Ellis et al., 2002). During the experiment, measurements on water quality on both R-system and F-system were examined in the morning. All water samples including TAN and nitrite were taken before the feeding activity, while diurnal water changes were not measured. This practice was similar with the

experiment on juvenile cod, *Gadus morhua* (Bjornsson and Olafsdottir, 2006) in agreement with an assumption that all water quality measurements must be checked at minimum values (Burel et al., 1996). The present experiments found that, water quality parameters were significantly different in both R-system and F-system. Deterioration seemed to be affected by different stocking density in R-system, with higher density which showed poorer in DO, pH and ammonia concentration. However, R-system was provided with good controlling water facilities including aeration and filters supported by relatively low water exchange (around 10% daily), which caused more constant and stable water in this system. Boyd (2000) and Agarwal (1999) mentioned that in high stocking density, the problems such as oxygen deficiency, ammonia-nitrogen and carbon dioxide accumulation and other organics pollution are frequently occurring; by then aeration is considered the best way to solve this matter. On the other hand, although some water parameters such as salinity, DO and temperature were not significantly affected by different densities in F-system, it is however observed that pH, TAN and nitrite were influenced by stocking density where each value was slightly decreased with higher density. It is estimably caused by fish excretion in the culture media as a result of metabolic activities.

In the present study, both TAN and nitrite showed a variation in different culture systems. In R-system, TAN increased steadily at the beginning of experiment followed by slightly decreasing until the final sampling day. In contrast, TAN were continuously increasing in F-system with the highest concentration found at the end of sampling time. It is presumably caused by bacteria and other microorganisms' activities. Those microorganisms usually form a complex community interactions commonly known as biofilms. Biofilms seemed to be formed earlier in R-system rather than in F-system; it is estimably caused by lower water exchange compare to F-system which undergoes high water exchange. Although the true function of biofilms is still not yet understood (Flemming, 2008) especially in fish culture system, it has long been recognized that biofilms is essential in water considering its ability in providing protection against environmental stressor (Kokare et al., 2009). In general, biofilms also believed to be involved in biogeochemical cycles of nitrogen, hydrogen, oxygen, carbon, sulphur and phosphorus (Ehrlich, 2002); mineral weathering processes, oxygen production, ammonia and nitrite oxidation (Flemming, 2008); enhancing nutrient availability and removal toxic metabolites (Decho, 1990). These studies imply that bacteria and other microorganisms may affect water quality. In this study, even though no specific experiment was carried out to determine the effect of microorganisms in both R-system and F-system, observation found that microorganisms play a big role for maintaining good water quality in culture system.

Conclusion

The present study indicates that growth performances, feed utilization and water quality were affected by culture systems and stocking densities. Thus, for grouper juveniles, it is suggested to be reared in high stocking density in recirculation system to attain a maximum growth. However, maintaining acceptable water quality and providing enough food is required to sustain fish health and to prevent aggressive behavior.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Occurrence and antimicrobial resistance of *Salmonella* serotypes isolates recovered from poultry of Western Paraná, Brazil

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This study was conducted to determine the occurrence and antimicrobial resistance of *Salmonella* serotypes isolates from feces, feed and broiler litter in poultry broilers in western Paraná between 2006 and 2010. The antigenic characterization and identification of serotypes confirmed 118 samples of *Salmonella* spp., belonging to 38 different serotypes. The most common serotypes identified were *S. enteritidis* (16.1%), *S. heidelberg* (5.9%), *S. typhimurium* (5.9%), *S. hadar* (5.0%), *S. albany* (4.2%), *S. enterica* (4.2) and *S. saintpaul* (4.2%). The serotypes of *Salmonella* spp. isolates were examined for resistance against 13 antibiotics using the disc diffusion method. Thirty-six samples (30.5%) were susceptible to all antimicrobials, and among the 82 resistant strains, there were 25 different resistance patterns. Serotypes' multi-resistant to streptomycin, nalidixic acid and tetracycline (20.7%), nalidixic acid (13.4%) and nalidixic acid and tetracycline (11.0%) were found at higher frequency. The results revealed high prevalence of *Salmonella* spp. mainly *S. enteritidis* (16.1%) in aviary environment and percentage of strains' resistant to conventional antibiotics.

Key words: *Salmonella* enteritidis, broilers, antimicrobial susceptibility, poultry.

INTRODUCTION

The poultry industry is currently one of the major sectors of the Brazilian agro-industrial complex that is expanding constantly and highlighted in the international market. Paraná is the state that mostly produces chickens in Brazil; quality, sanitation and price are the main factors contributing to increased productivity (UBA, 2012). However, the chicken meat is the main vehicle for transmission of *Salmonella*, responsible for huge losses in the poultry industry (Téo and Oliveira, 2005).Due to the ability to colonize the digestive tract of animals and to present various reservoirs involved in fecal excretion and environmental pollution, researchers around the world agree that it is virtually impossible to eradicate *Salmonella* spp. from poultry and, therefore, there must be a constant control and monitoring of all serotypes, including those involved in the health of poultry and

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consumers (Andreatti Filho et al., 2009).

The use of synthetic antimicrobial helps to improve poultry health and reduces the rearing time. But, the indiscriminate use of subtherapeutic concentrations for various purposes, such as growth promoters, therapeutics and prophylactics provides the selection and prevalence of different serotypes of *Salmonella* and other microorganisms resistant to antibiotics. This reduces their effectiveness in clinical cases, besides contributing to the presence of residues in meat and meat products (Bila and Dezotti, 2003; Sakaridis et al., 2011).

It is well known that an increase is occurring in the isolation of *Salmonella* serotypes prevalent and resistant to synthetic antimicrobials all over the world. For example, a few studies were found reporting the isolation of salmonella serotypes with high-level resistance in Brazil (Silva and Duarte, 2002; Cardoso et al., 2006; Ribeiro et al., 2007; Souza et al., 2011), Spain (Carramiñana et al., 2004), Lithuania (Ruzauskas et al., 2005), Nigeria (Okolli et al., 2006), United States (Alali et al., 2010) and Greece (Sakaridis et al., 2011). These studies reported that *Salmonella* from poultry source are resistant to a wide range of antimicrobials and confirmed that poultry are important reservoirs of multidrug-resistant *Salmonella*. Between 1999 and 2008, there were 254 outbreaks of salmonellosis in Paraná, and eggs, meat and their derivative products are the main food connected to these outbreaks (Kottwitz et al., 2010). Although salmonellosis is one of the foodborne diseases more common in Paraná, there is little information of the occurrence and frequency of different *Salmonella* serotypes involved with outbreaks of this disease, emphasizing the importance of this work.

The aim of this study was to evaluate the occurrence and antimicrobial resistance profile of serotypes of *Salmonella* spp. in samples isolated from batches of broilers in Western Paraná.

MATERIALS AND METHODS

Sampling and serotype identification

Between 2006 and 2010 about 396 samples isolated from feces (cloacal swabs), broiler litter (drag swab) and feed (flour/feed inputs) were analyzed; 118 strains of the genus *Salmonella* were confirmed from two different aviaries with capacity of 5000 chickens, both located in the western region of Paraná. The samples were provided by the Veterinary Laboratory MercoLab (Cascavel / PR, Brazil) and the complete antigenic characterization and identification of serotypes were performed by the Adolfo Lutz Institute (São Paulo, Brazil). The occurrence was determined by observing the most frequent serotypes among the numbers of samples, arranging them in descending order.

Antimicrobial susceptibility test

The samples were recovered on Brain-Heart Infusion broth (BHI) (HIMEDIA ®) at 37°C for 18 h before testing. The antimicrobial susceptibility was evaluated according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2007). The antimicrobial agents tested included (LABORCLIN®): nalidixic acid (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), enrofloxacin (10 µg), streptomycin (10 µg), imipenem (10 µg), gentamicin (10 µg), norfloxacin (10 µg) sulfazotrin (25 µg), tetracycline (30 µg), and tobramycin (10 µg). All samples that showed intermediate resistance were considered sensitive to avoid overestimation of resistance. Reference strains of *Salmonella* Typhimurium ATCC 14028 (American Type Culture Collection) and *Escherichia coli* ATCC 25922 were used. The results obtained were compared with the data in Table 2A of the document M100-S17 (CLSI, 2007). The strains with resistance to three or more antibiotics were considered as multi-resistant.

RESULTS

38 different serotypes were found of a total of 118 isolates distributed. The main serotypes isolated from broiler litter, diet and feces in poultry were *S. enteritidis* (16.1%), followed by *S. heidelberg* (5.9%), *S. typhimurium* (5.9%), *S. hadar* (5.0%), *S. albany* (4.2%), *S. enterica* (4.2) and *S. saintpaul* (4.2%) (Table 1).

Regarding the source of *Salmonella* spp. samples, 73 strains were from broiler litter (61.9%), 23 from poultry feed (19.5%) and 22 from poultry feces (18.6%) (Table1).

In strains isolated from broiler litter, 15 different serotypes were identified, with *S. enteritidis* being the most frequent serotype, followed by *S. typhimurium*, *S. hadar*, *S. heidelberg*, *S. enterica*, *S. derby*, *S. mbandaka*, *S. infants*, *S. livingstone* and *S. kentucky*. About the diet, among the 13 different serotypes isolated, the most frequent were *S. enteritidis*, *S. heidelberg*, *S. panama*, *S. corvallis*, *S. senftenberg* and *S. gafsa*. In relation to the feces, among the 22 serotypes, *S. enteritidis* was the most common serotype, followed by *S. heidelberg*, *S. albani*, *S. enterica* and *S. saintpaul* (Table 1).

About the antimicrobial susceptibility and resistance of samples, it was observed that 36 samples (30.5%) were susceptible to all antimicrobials tested, while 82 (69.6%) were resistant to at least one antimicrobial agent, namely: 15 (12.7%) were resistant to one antimicrobial agent, 22 (18.6%), resistant to two, 30 (25.4%), to three, 14 (11.9%), to four and 1 (0.8%) was resistant to five antibiotics (Table 2). 45 of samples showed multidrug resistance (that is, resistance to three or more antimicrobials) (Table 2).

As for the 82 samples that showed resistance to at least one antimicrobial, 55 were reported to nalidixic acid, 54 to streptomycin, 53 to tetracycline, 14 to ampicillin, 8 to chloramphenicol, 7 to gentamicin, 5 to enrofloxacin, 4 to cephalothin, 3 to norfloxacin, 3 to sulfazotrin, 2 for ciprofloxacin and 1 to imipenem. No serotype was resistant to tobramycin and none of the samples tested showed 100% resistance to all antimicrobials used (Table 3). 25 different antimicrobial resistance patterns were found: 7 was associated with 1 or 2 antimicrobials and 18 was associated with three or more antimicrobials

Table 1. Distribution of 118 samples of *Salmonella* spp. isolated from broiler farms, according to the occurrence and source of isolation in the period 2006-2010 of Brazil.

Isolation sources	Serotypes	N° (%)	Feces	Litter	Feed
All sources	Enteritidis	19 (16.1)	6	9	4
	Heidelberg	7 (5.9)	2	3	2
	Typhimurium	7 (5.9)	1	5	1
	Hadar	6 (5.0)	1	4	1
	Albany	5 (4.2)	2	2	1
	Saintpaul	5 (4.2)	2	2	1
Feces and litter	Enterica	5 (4.2)	2	3	0
	Mbandaka	4 (3.4)	1	3	0
	Newport	3 (2.5)	1	2	0
	Cerro	2 (1.7)	1	1	0
	Tennesse	3 (2.5)	1	2	0
	Cubana	2 (1.7)	1	1	0
	Lexigton	2 (1.7)	1	1	0
Litter and feed	Give	2 (1.7)	0	1	1
	Derby	4 (3.4)	0	3	1
	Braenderup	2 (1.7)	0	1	1
	Gallinarium	2 (1.7)	0	1	1
Litter	Infantis	3 (2.5)	0	3	0
	Livingstone	3 (2.5)	0	3	0
	Kentucky	3 (2.5)	0	3	0
	Schwarzengrund	2 (1.7)	0	2	0
	Brandenburg	2 (1.7)	0	2	0
	Anatum	2 (1.7)	0	2	0
	Montevideo	2 (1.7)	0	2	0
	Ohio	2 (1.7)	0	2	0
	Agona	2 (1.7)	0	2	0
	Minnesota	2 (1.7)	0	2	0
	Pullorum	1 (0.8)	0	1	0
	Muenchen	1 (0.8)	0	1	0
	Morehead	1 (0.8)	0	1	0
	Worthington	1 (0.8)	0	1	0
	Grumpensis	1 (0.8)	0	1	0
	Orion	1 (0.8)	0	1	0
Feed	Gafsa	2 (1.7)	0	0	2
	Corvallis	2 (1.7)	0	0	2
	Panamá	2 (1.7)	0	0	2
	Senftenberg	2 (1.7)	0	0	2
	Rissen	1 (0.8)	0	0	1
Total		118 (100)	22 (18.6)	73 (61.9)	23 (19.5)

(multidrug resistance patterns) (Table 3). More frequent were those resistant to tetracycline (17 patterns), streptomycin (16 patterns) and nalidixic acid (13 patterns). Also, more frequent were those resistant to nalidixic acid-streptomycin-tetracycline (17 samples), those resistant only to nalidixic acid (11 samples) and those resistant to nalidixic acid-streptomycin (9 samples) (Table 3). Among the 16 samples of *S. enteritidis* isolated, 100% were resistant to nalidixic acid, 8 (50%) to tetracycline, 7 (44%) to streptomycin and 2 (12.5%) to

Table 2. Number of antibiotic resistance of 118 *Salmonella* isolates in different sources in broiler farms in Brazil in the period 2006-2010.

Number of antimicrobials	Number of isolates (or strains)			
	Feces	Litter	Feed	Total (%)
Susceptible	3	20	13	36 (30.5)
1	3	8	4	15 (12.7)
2	3	14	5	22 (18.6)
3	6	23	1	30 (25.4)
4	5	9	0	14 (11.9)
5	1	0	0	1 (0.8)

enrofloxacin (Table 3). The 6 samples of *S.* Hadar showed resistance to three different patterns of resistance: tetracycline-streptomycin, nalidixic acid-tetracycline, and nalidixic acid-tetracycline-streptomycin. As *S*. *hadar*, all isolates of *S*. *typhimurium* showed three different patterns of resistance: Gentamicin-nalidixic acid, streptomycin-tetracycline-nalidixic acid and nalidixic acid-streptomycin-tetracycline-gentamicin (Table 3).

About the multidrug resistance patterns, the serotypes Mbandaka and Saintpaul showed the highest amount of patterns (3): Norfloxacin-tetracycline-streptomycin, streptomycin-tetracycline-chloramphenicol, ciprofloxacin-ampicillin-streptomycin-norfloxacin-chloramphenicol to Mbandaka, and streptomycin-nalidixic acid-chloramphenicol, tetracycline-streptomycin-nalidixic acid-chloramphenicol and sulfazotrin-tetracycline-streptomycin-nalidixic acid to Saintpaul. After these, the serotypes, S. *typhimurium,* S. *enteritidis,* S. *enterica,* S. *albany,* S. *newport* and S. *anatum* showed 2 multidrug resistance patterns and the serotypes, S. *livingstone,* S. *give,* S. *hadar,* S. *kentucky,* S. *infantis,* S. *tenesee,* S. *heidelberg,* S. *schwarzengrund* and S. *montevideo* showed only 1 multidrug resistance pattern (Table 3).

DISCUSSION

The occurrence of antimicrobial resistance in zoonotic bacteria such as *Salmonella* has major public health implications, and drug resistance is almost an inevitable consequence of the use of antimicrobial drugs in food-producing animals. The monitoring of occurrence of multi-resistant *Salmonella* serotypes in poultry is critical to the protection of human and veterinary health (Therelfall, 2002).

The presence of the main serotype, *S*. *enteritidis* in poultry was also confirmed by other researchers in various regions of Brazil, such as Paraná (Souza et al., 2011), São Paulo (Hofer et al., 1998; Lima et al., 2009), Bahia, Ceará, Goiás, Paraná, Mato Grosso, Mato Grosso do Sul e Santa Catarina (Kanashiro et al., 2005), Rio Grande do Sul (Ribeiro et al., 2007) and the other states in the Northeast (Duarte et al., 2009). Similarly, in other countries, the occurrence of *S*. *enteritidis* isolated from broilers in Portugal (Antunes et al., 2003), Spain (Fernandez -Rubio et al., 2009) Lithuania (Ruzauskas et al., 2005) and United States (Burkholder et al., 2008) was observed.

Regarding the source of *Salmonella* spp. samples in broiler litter, the high incidence of *Salmonella* spp. may be due to the repeated use of the same litter for several flocks of broiler, enhancing the dissemination of *Salmonella* spp. (Chernaki-Leffer et al., 2002). In addition, the humidity content of the broiler litter and consequently temperature, are factors that contribute to the proliferation of microorganisms (Mcward and Taylor, 2000).

The serotypes isolated from broiler litter were found by Andreatti Filho et al. (2009) who reported *S*. *enteritidis*, *S*. *infants* and *S*. *kentuky* present in broiler litter in the state of São Paulo, Brazil, and also Souza et al. (2011) who researched the resistance profile of quinolones against strains of *Salmonella* isolated from poultry farms in Paraná and found out that out of the 123 isolates, 90 belonged to *S. enteritidis*.

Contamination of broiler litter becomes a problem for poultry chain, that if infected by *Salmonella*, there is the propensity to transmit it to the poultry and then to humans through consumption of meat and meat products. So the control of dissemination of *Salmonella* is dependent on the control of transmission sources (Frederick and Huda, 2011). One reason that may explain the presence of *S. enteritidis* in poultry refers to the high incidence of mealworm (*Alphitobius diaperinus*) considered a pest and transmission vector of *Salmonella* (Crippen et al., 2012). Therefore, the mealworm elimination in broilers is considered an important measure for controlling the salmonellosis in poultry (Leffer et al., 2010).

About the diet, Hofer et al. (1998) found variation in serotypes isolated from raw materials and feed in seven different regions of Brazil; the most common being *S*. *montevideo*, *S*. *senftenberg*, *S*. *havana*, *S*. *mbandaka*, *S*. *tennessee*, *S*. *infants* and *S*. *agona*. The authors mention that probably the large number of serotypes recognized results from the mixture of a large number of inputs of same rating but from different sources; this probably

Table 3. Antibiotic resistance of 82 *Salmonella* isolates from broiler farm.

No. resistance	Resistance to *												Samples (%)	Serovars
	Amp	Cip	Clo	Cef	Enr	Gen	Imi	Nal	Sul	Str	Tet	Nor		
1								+					11 (13.4)	Enteritidis (7), Heidelberg (1), Kentucky (1), Minnesota (2)
											+		4 (4.9)	Enterica (1), Mbandaka (1), Cubana (1), Derby (1)
	+			+									1 (1.2)	Lexington (1)
						+		+					4 (4.9)	Typhimurium (3), Infantis (1)
2	+										+		1 (1.2)	Agona (1)
										+	+		7 (8.5)	Hadar (1), Derby (3), Corvalis (1), Enterica (1), Panama (1)
								+		+			9 (11.0)	Enteritidis (1), Hadar (3), Muenchen (1), Gallinarium (1), Pullorum (1), Infantis (1), Cubana (1)
	+			+							+		2 (2.4)	Livingstone (2)
				+			+				+		1 (1.2)	Give (1)
								+		+	+		17 (20.7)	Typhimurium (3), Enteritidis (6), Hadar (2), Kentucky (1), Enterica (2), Infantis (1), Tennnesee (2)
3														
					+			+			+		2 (2.4)	Enteritidis (2)
			+					+		+			1 (1.2)	Saintpaul (1)
	+									+	+		5 (6.1)	Heidelberg (5)
	+							+			+		2 (2.4)	Albany (2)
	+									+	+	+	1 (1.2)	Mbandaka (1)
	+		+							+	+		1 (1.2)	Mbandaka (1)
					+			+		+		+	1 (1.2)	Newport (1)
						+		+		+	+		1 (1.2)	Typhimurium (1)
4			+					+		+	+		3 (3.6)	Saintpaul (1), Schwarzengrund (1), Enterica (1)
					+			+		+	+		1 (1.2)	Anatum (1)
		+			+			+		+			1 (1.2)	Newport (1)
								+	+	+	+		2 (2.4)	Saintpaul (1), Anatum (1)
			+			+				+	+		2 (2.4)	Albany (2)
			+						+	+	+		1 (1.2)	Montevideo (1)
5	+	+	+							+		+	1 (1.2)	Mbandaka (1)
Total samples	**14**	**2**	**8**	**4**	**5**	**7**	**1**	**55**	**3**	**54**	**53**	**3**	**82**	

* 36 isolates were susceptible to antimicrobials: Amp: Ampicillin; Cef: Cefalotin; Cip: Ciprofloxacin; Clo: Chloramphenicol; Enr: Enrofloxacin; Imi: Imipenem; Gen: Gentamicin; Nal: Nalidixic acid; Nor: Norfloxacin; Str: Streptomycin; Sul: Sulfazotrin; Tet: Tetracycline; Tob: Tobramycin.

being the same reason for the difference between the serotypes isolated from this study. In relation to feces, the data of the present study disagree with Oliveira et al. (2006), who analyzing samples of feces and carcasses of broilers in the state of Ceará, Brazil observed contamination by *Salmonella* spp. only in the carcasses. This suggests that the cages might be considered free of *Salmonella* contamination. This study also disagrees with Kottwitz et al. (2008), searching the incidence of *Salmonella* spp. in poultry production chain in isolates from feces, cloacal swabs and eggs in Paraná, found prevalence of *S. enterica* (67.0% of isolates) and absence of *S. enteritidis*.

The differences between studies of the occurrence, prevalence and serotypes resistance profiles of *Salmonella* in poultry can be explained by the fluctuation of dominant serotypes that occurs between geographical regions; and additionally, the amplitude of serotypes inside the same region may be related to propagation of *Salmonella* by feed, derived from supplies from different locations, collaborating to increase the diversity of serotypes (Hofer et al., 1997). Also, the differences between the data of prevalence of *Salmonella* serotypes can be associated to the age of the samples, differences in origin, variation in sampleolation, sampling procedures, poultry contamination, differences in sample size, among others (Parveen et al., 2007).

About the susceptibility and resistance of samples against antimicrobials, Castagna et al. (2001), researching the resistance of *Salmonella* isolated from swine, found that the highest rates of resistance found were to tetracycline and ampicillin; agreeing in the sense that these were among the main antimicrobials that *Salmonella* were resistant to. It is suggested that the highest levels of antimicrobial resistance occur with the antimicrobials available and used on the market for longer period, since it increases the possibility of the selection of resistant microorganisms.

About the resistance of the serotypes to enrofloxacin and ciprofloxacin, these data agree with Sakaridis et al. (2011), who analyzed the susceptibility of *Salmonella* samples isolated from chicken carcasses in Northern Greece against synthetic antimicrobials, and found low resistance to these antimicrobials. Considering the multi-resistance to the antimicrobials, Sakaridis et al. (2011) reported high rates of resistance to tetracycline, streptomycin and nalidixic acid, corroborating with this study. The authors attributed the increased resistance of some antimicrobial agents compared to others to the constant and intensive use of these agents as therapeutic and preventive in flocks.

In the study of Lima et al. (2009) for antimicrobial susceptibility of isolates from poultry products, *S. gallinarum* and *S. pullorum* were resistant to nalidixic acid, but sensitive to tetracycline, partially corroborating with this study. It is known that serotypes that can produce large losses to poultry industry include *S. gallinarum*, which causes clinical disease known as typhoid fever, and *S.* pullorum that causes pulorosis; both show resistance to tetracycline and nalidixic acid, constantly used in poultry.

About the resistance of *S.* enteritidis to antimicrobials, the results agree with Vaz et al. (2010) who isolated *S. enteritidis* from outbreaks of salmonellosis and products related to poultry and reported lower rates of resistance to nalidixic acid, streptomycin and tetracycline. Also, the result of Cardoso et al. (2006) mentions, besides ciprofloxacin, the susceptibility to gentamicin, norfloxacin and sulfazotrin of strains isolated from chicken carcasses in the state of Rio Grande do Sul is similar to our results. Medeiros et al. (2011), researching the resistance of 18 *Salmonella* serotypes isolated from frozen broiler carcasses in 15 Brazilian cities against antimicrobials, found that strains of *S. enteritidis* were resistant to all antibiotics tested, at various levels, including antimicrobials used in this study. Also, the highest levels of bacterial resistance to antimicrobial agents were assigned to Streptomycin, florfenicol, sulfonamide, nalidixic acid, ampicillin, ceftiofur, aztreonam, cefoxitine, cephalotin, gentamicin and tetracycline, partially corroborating with our results. This is because these are among the antibiotics that are more resistant (*Salmonella)*, but none were resistant to all antimicrobial agents tested.

It is noteworthy that *S. enteritidis* has emerged as a major problem in poultry and public health in Brazil from the 1990s, probably introduced by the importation of hatching eggs and a-day-old chicks infected. Moreover, the expansion of the Brazilian poultry industry concomitant with the indiscriminate use of antimicrobials in poultry has created favorable conditions for the maintenance and proliferation of *S. enteritidis* as well as induced the maintenance of positive batches (Silva and Duarte, 2002).

The resistance shown by *S. hadar* to three different patterns of multidrug resistance corroborates with the values previously reported by Antunes et al. (2003) and Ribeiro et al. (2007), who although having performed works on carcasses and other poultry products, found that all isolates of *S. hadar* were resistant to antimicrobials, with high rates of resistance to tetracycline, nalidixic acid, enrofloxacin and streptomycin.

About the resistance patterns shown by *S. typhimurium*, Bauer-Garland et al. (2006) reported the transmission of *S. typhimurium* multidrug-resistant and sensitive, noting that multi-resistant *Salmonella* are more virulent under selection pressure, which allows them to diffuse more quickly from animal to animal and also not responding well to antimicrobial therapy.About *S. mbandaka*, in a study on the characterization of class 1 integrons and antibiotic resistance genes in multidrug resistant *S. enterica* from foodstuff was reported genes resistant to ampicillin, chloramphenicol, nalidixic acid, sulfamethoxazole/trimethoprim, streptomycin and tetra-

cycline (Ribeiro et al., 2011). This corroborates the results of this study. No reports of susceptibility and resistance of this serotype to antimicrobials were found. This is the first study on resistance and susceptibility of S. mbandaka; and, since this serotype 3 showed patterns of multidrug resistance, it suggests a greater attention needs to be given to it to control it. About the serotype *S. saintpaul*, there are no reports of its occurrence in poultry environment or their antimicrobial resistance profile, which is similar to the first report.

The results from this study highlight the need for continuous monitoring in the poultry industry regarding the presence of *Salmonella* spp., mainly *S*. enteritidis, which is more prevalent and already recognized as a major problem in the poultry sector. It is also important to make good use of antimicrobials (since *Salmonella* serotypes exhibit different patterns of resistance and variation in sensitivity to antimicrobials tested) to replac the commonly used antibiotics such as streptomycin, nalidixic acid, tetracycline and chloramphenicol, once a high number of strains showed resistance to these antimicrobials.

We also suggest a plan of actions to prevent outbreaks of *Salmonella* and the adoption of strict hygienic and sanitary measures throughout the production chain. They include exchange of poultry litter broiler frequently and tighter control of quality of feed inputs, besides the control of the mealworm which represents a critical factor for the reduction of *Salmonella* spp. in flocks.

It is concluded from this study that *S*. enteritidis was the most prevalent serotype of *Salmonella* in poultry of Western Paraná, Brazil. For resistance, most isolates were resistant to two or more antibiotics, especially when considering the ones mostly used in the poultry sector.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effect of silicon on real time nitrogen management in a rice ecosystem

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Field experiments were conducted during *Kharif* season of 2009 on sandy loam soil at Doddajala, Bangalore, India of eastern dry zone soils of Karnataka to investigate the effect of silicon (Si) and nitrogen application on the growth and yield of rice, using Cv. BI-34, a medium duration genotype. The results revealed that higher grain and straw yield was noticed with application of calcium silicate at 2 t ha^{-1} along with application of N at 100 kg ha^{-1} recommended dose of fertilizer (RDF) followed by silica gel at 500 kg ha^{-1} over RDF under both aerobic and wetland rice conditions. The combined application of Si sources along with Leaf Colour Chart (LCC) based N application of 75 kg N ha^{-1} (Basal 30 kg N ha^{-1}+LCC) under aerobic and wetland rice recorded on par grain and straw yield compared to RDF alone. Combined application of silicon and nitrogen significantly increased the effective number of tillers, number of grains per panicle, 1000-grain weight. Higher agronomical efficiency (AE_N), recovery efficiency (RE_N), Partial factor productivity (PFP_N) values were noticed with LCC based application along with calcium silicate at 2t ha^{-1} under both aerobic and wetland rice.

Key words: Rice, silicon, nitrogen use efficiency, leaf colour chart, real time N management.

INTRODUCTION

Rice is the staple food of about 3 billion people and demand is expected to continue to grow as population increases (Carriger and Vallee, 2007). Globally rice is grown over an area of about 149 million ha with an annual production of 600 million tons (Bernier et al., 2008). In India, rice is cultivated round the year in one or the other part of the country, in diverse ecologies spread over 44.6 M ha with a production of 132 MT of rice and average productivity of 2.96 t ha^{-1} (Rai, 2006). The 79 million ha of irrigated lowlands provide 75% of the world's rice production (Maclean et al., 2002).

Asia's food security depends largely on the irrigated rice fields, which produces three quarters of all rice harvested. But rice is a profligate user of water, consuming half of all developed fresh water resources. The increasing scarcity of water threatens the sustainability of the irrigated rice production system and hence the food security and livelihood of rice producers

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and consumers. Aerobic rice is a rice production system for water-short environments where adapted rice varieties are grown under aerobic soil conditions (Atlin et al., 2006; Bouman et al., 2006; Wang et al., 2002). Although the system of aerobic rice can produce high yields (Bouman et al., 2006; Wang et al., 2002, Peng et al., 2006).

Silicon (Si) is the second most abundant element in the earth's crust, with soils containing approximately 32% Si by weight (Lindsay, 1979). Si is a major constituent of the Earth's crust, forming the silicate minerals. In soils, these minerals undergo chemical and physical weathering, resulting in the release of Si in solution, which is either combined with other elements to form clay minerals, or released toward the streams and the oceans or absorbed by the vegetation. In agronomy, Si is generally not considered an essential element. The major reason is that there is no evidence to show that Si is involved in the metabolism of plant, which is one of the three criteria required for essentiality established by Arnon and Stout (1939). Although agricultural soils are largely composed of silicate minerals, many soils contain an inadequate supply or are naturally low in plant available Si. Most likely, the Si content in some regions might be limiting to sustainable rice production (Savant et al., 1999). In addition, Si depletion can occur in traditional rice soils from the continuous monoculture of high-yielding cultivars with intensive cultivation practices (Miyake, 1993).

Rice is considered to be a Si accumulator plant and tends to actively accumulate Si to tissue concentrations of 5% or higher. Recently Si has been regarded as quasi-essential element (Epstein, 2002). Some of the studies suggest that Si, enhances disease resistance in plants, imparts turgidity to the cell walls and has a putative role in mitigating the metal toxicities (Datnoff et al., 1997). It is suggested that the Si plays a crucial role in preventing or minimizing the lodging in the cereal crops (Munir et al., 2003), a matter of great importance in terms of agricultural productivity.

Nitrogen fertilizer has played an important role in increasing rice yields, and total consumption of N for rice production has increased gradually worldwide (Singh et al., 2012). However, fertilizer N use efficiency of rice is generally low for rice grown in a transplanted culture ranging from 25 to 45%, and average about 35% (Dobermann and Cassman, 2002).

A deficiency of N that is directly involved in synthesis of protein or chloroplast pigments or electron transfer, however, lowers the photosynthetic efficiency (Takahashi 1990). Nitrogen is one of the most important plant nutrients and plays a vital role in plant photosynthesis and biomass production. Several studies showed that when N is slightly deficit within plants, the demand for NO_3, free amino acid, and free amino N increases quickly, without necessarily bringing a simultaneous marked change in total nitrogen (Wang et al., 2005). More than half of the N fertilizer applied is lost and results not only in an environmental hazard but also a substantial economic loss (Li et al., 2009). About one-third of applied N is lost by different processes (Abrol et al., 2007). Blanket or packages of fertilizer recommendations over large areas are not efficient because indigenous nitrogen supply (INS) varies widely among rice field. Rice crops require different amount of nutrients, depending on native nutrient supply and demand. Leaf colour chart (LCC) and chlorophyll meters are the promising tools developed in recent years for need-based N management in rice crops. Application of N with the LCC usage helps in reducing the leaching loss and enhances the nutrients uptake of crop. The LCC is simple and alternative method for monitoring the relative greenness of a rice leaf as an indicator of leaf N status (Shukla et al., 2004). Fertilizer application for wetland rice with the usage of LCC helps in saving N to an extent of 27 to 56 kg ha^{-1} in Punjab, 19 to 39 kg ha^{-1} in Haryana, 30 to 40 kg ha^{-1} in Bihar and 42 to 50 kg ha^{-1} in West Bengal as compared to fixed-time blanket N recommendation or farmers practice (Bijay and Yadvinder, 2003). However, there is no information regarding N management in aerobic rice by adopting LCC method.

Application of nitrogenous fertilizers is an important practice for increasing rice yields. However, when applied in excess may limit yield because of lodging, promote shading and susceptibility to insects and diseases. These effects could be minimized by the use of Si (Ma et al., 1989; Munir et al., 2003). Due to a synergistic effect, the application of Si has the potential to raise the optimum N rate, thus enhancing productivity of existing lowland rice fields (Ho et al., 1980). Silicon has been reported to raise the optimum level of N in rice. However, information on Si and N interaction in aerobic/upland/rainfed rice is very limited. In this context the present study was undertaken to evaluate the effect of Si and N on yield, yield components and NUE of aerobic and wetland rice.

MATERIALS AND METHODS

Experimental site

The experimental site belongs to eastern dry zone of Karnataka, situated at Doddajala, Bangalore North with latitude of 12°10' N and longitude of 76°35' E 650 m above mean sea level (MSL) and receives annual rainfall of 766 mm during the cropping period. The meteorological data of the location indicating rainfall, temperature and humidity recorded during the experimental period is mentioned in Table 1. The initial pysico-chemical soil analysis of the experimental is given in Table 2.

Experimental design

Two field experiments were conducted during *Kharif 2009*, under aerobic and wetland conditions simultaneously, the experiments consisted of seven treatments with three replications laid out in randomized block design (same set of treatments were followed for

Table 1. Normal and actual monthly weather data recorded at Doddajala, Bengaluru (North) during 2009.

Month	Rainfall (mm)			Mean temperature (°C)						Mean relative humidity (%)			No. of rainy days
				Maximum			Minimum						
	N	A	D	N	A	D	N	A	D	N	A	D	
July	102.0	55.8	-46.8	28.2	28.7	0.5	19.0	19.5	0.5	87	69.5	-17.5	2
Aug	129.0	106.8	-22.2	27.2	28.3	1.1	18.8	19.2	0.4	88	75.0	-13.0	7
Sep	203.2	231.5	28.3	28.1	28.0	-0.1	18.8	19.2	0.4	88	84.0	-4.0	12
Oct	173.9	29.6	-144.3	27.7	28.1	0.4	18.3	17.5	-0.8	87	71.5	-15.5	4
Nov	53.9	49.4	-4.5	26.6	27.0	0.4	16.6	17.7	1.1	86	74.5	-11.5	5
Dec	13.3	11.0	-2.3	26.1	26.8	0.7	14.3	16.3	2.0	86	73.0	-13.0	1

N, Normal meteorological data (Mean of 1972-2008); A, actual meteorological data (Year 2009-2010); D, deviation from the normal (A-N).

Table 2. Pre-sowing physico-chemical soil analysis.

Parameter	Aerobic rice	Wetland rice
pH (1:2.5)	6.29	6.30
EC (d Sm^{-1})	0.23	0.20
OC (g kg^{-1})	6.40	6.90
Particle size distribution		
Sand (%)	65.39	61.80
Silt (%)	15.48	17.60
Clay (%)	18.57	20.30
Textural class	**Sandy loam**	
CEC (Cmol $P^{(+)}$ kg^{-1})	17.25	19.57
Available N (kg ha^{-1})	378.00	389.00
Available P_2O_5 (kg ha^{-1})	39.30	54.10
Available K_2O (kg ha^{-1})	144.50	156.6
Available S (ppm)	18.30	17.50
Exchangeable Ca (c mol $P^{(+)}$ kg^{-1})	1.40	2.20
Exchangeable Mg (c mol $P^{(+)}$ kg^{-1})	1.00	0.90
Available Si (ppm) (0.5 M Acetic acid extractable)	44.00	45.93

both aerobic and wetland rice).

Field experiment

Aerobic rice

Rice seeds of BI 34 were sown at 2 seeds $hill^{-1}$ with spacing of 30 × 10 cm and the crop was irrigated once in 4 to 5 days.

Wetland rice

25th day old seedlings of BI 34 were transplanted at two seedlings per hill with spacing of 20 × 10 cm and irrigation was given to maintain the submergence condition throughout the crop growth period. The calculated quantity of silicon sources, viz. calcium silicate and silica gel were applied to soil two weeks prior to sowing/transplanting. All the treatments received a common recommended dose of 50 kg ha^{-1} of P_2O_5 and K_2O. For RDF (Recommended dose of fertilizer) treatment, 100 kg N ha^{-1} was applied at three splits (50 kg N ha^{-1} at the time of sowing / transplanting and remaining 50 kg as two equal splits during maximum tillering and panicle initiation stage). The LCC treatments receives, 30 kg N ha^{-1} at the time of sowing and remaining amount of N supplied based on LCC critical values. During the growth periods, the LCC readings were taken at ten days intervals starting from 14 days after transplanting in wetland rice and 21 days after sowing in case of aerobic rice. Based on critical value (LCC-3 for aerobic rice and LCC-4 for wetland rice) assessed in the respective treatment, N was applied at at 15 kg ha^{-1} at each time when LCC value fell below the critical value. Grain and straw yield and yield components were recorded in each treatment at harvest and grain yields were adjusted to 14% of moisture level. Grain and straw

samples were analyzed by using CHNS (LECO - 900, USA) analyzer for total N content. Nitrogen use efficiency (NUE) in rice was calculated by using different efficiency formulae (Cassman et al., 1998).

Source of silicon and composition

Calcium silicate was used as a source of silicon which was procured from Excell Minerals, USA (www.excellminerals.com) which consists of Si 12%, CaO 30%, Mg 7%, S 0.2%, Fe 4%, Mn 1%, Al 3% Cr 0.2%, Ti 0.5% and Ni 0.04% and another source was silica gel which was procured from Shijo, Japan.

Nitrogen use efficiency parameters

$$AE_N \text{ (kg grain kg}^{-1} \text{ N applied)} = \frac{\text{Grain yield (kg ha}^{-1}\text{) in N fertilized plot - grain yield (kg ha}^{-1}\text{) in no N plot}}{\text{Quantity of fertilizer N applied (kg ha}^{-1}\text{) in N fertilizer plot}}$$

$$RE_N (\%) = \frac{\text{Total N uptake (kg ha}^{-1}\text{) in N fertilized plot - Total N uptake (kg ha}^{-1}\text{) in no N plot}}{\text{Quantity of fertilized N applied (Kg ha}^{-1}\text{) in N fertilizer plot}} \times 100$$

$$PFP_N \text{ (kg grain kg}^{-1} \text{ N applied)} = \frac{\text{Grain yield (Kg ha}^{-1}\text{) in N fertilized plot}}{\text{Quantity of fertilized N applied (Kg ha}^{-1}\text{)}}$$

Where, AE_N = Agronomic efficiency; RE_N = Apparent recovery efficiency, and PFP_N = Partial factor productivity.

Method of application of N based on LCC

Leaf colour chart procured from Nitrogen parameter, Adambakkam, Chennai - 600088. India, (e-mail:lccenquiry@gmail.com) was used in the present investigation. LCC is a simple, cheap, and easy-to-use tool that can help farmers to manage N judiciously. The critical value of LCC-3 was used for aerobic rice and LCC-4 for wetland rice. The critical or threshold value of the LCC is defined as the intensity of green colour that must be maintained in the uppermost fully opened leaf of the rice plant and fertilizer N needs to be applied whenever leaf greenness is below the critical LCC value. Leaf greenness or leaf N content is closely related to photosynthesis rate and biomass production and is a sensitive indicator of changes in crop N demand during the growing season. Thus, maintaining the leaf greenness just above the LCC critical value ensures high yields with need-based N application thereby leading to high fertilizer N use efficiency.

Estimation of total N in plant samples

The total nitrogen was determined using CHN analyzer (CHNS, LECO). The powdered samples were weighed (5-10 mg) and mixed with an oxidizer [vanadium pentoxide (V_2O_5)] in a tin capsule, which is then combusted in a reactor at 1000°C. The sample and container melt, and the tin promote a violent reaction (flash combustion) in a temporarily enriched oxygen atmosphere. The combustion products CO_2 and NO_2 are carried by a constant flow of carrier gas (helium) that passes through a glass column packed with an oxidation catalyst of tungsten trioxide (WO_3) and a copper reducer, both kept at 1000°C. At this temperature, the nitrogen oxide is reduced to N_2. The CO_2 and N_2 are then transported by the helium and separated by a 2-m-long packed column (Poropak Q/S 50/80 mesh) and quantified with a thermal conductivity detector (**TCD)** (set at 290°C.).

Statistical analysis and interpretation of data

The analysis and interpretation of the data were done using the Fisher's method of analysis and variance technique as given by Panse and Sukhatme (1967). The level of significance used in 'F' and't' test was 5% probability and wherever 'F' test was found significant, the 't' test was performed to estimate critical differences among various treatments.

RESULTS AND DISCUSSION

Growth parameters

The plant height was significantly affected by the combined application of Si and N under aerobic rice, but not in wetland rice (Table 3). There was a significant increase in the plant height with the application of calcium silicate at 2 t ha^{-1} along with RDF (100 kg N ha^{-1}) (87 cm) over control (78 cm). Application of Si was effective in preventing lodging in rice by increasing the thickness of the culm and size of the vascular bundles thereby enhancing the strength of the culm (Shimoyama, 1958). Application of calcium silicate at 2 t ha^{-1} along with basal 30 kg N ha^{-1} with LCC recorded higher number of productive tillers in both aerobic and wetland rice. Higher number of productive tillers was recorded in aerobic rice compared to wetland rice mainly due to rice plants develop relatively more tillers at wider spacing because of advantage of space, nutrition and sunlight. The plant spacing significantly influenced tillering capacity of rice.

Yield parameters

Number of grains per panicle was significantly increased by application of silicon and N under aerobic and wetland condition (Table 3). In the present study LCC based N was applied at different crop growth stages (basal, tillering stage and before flowering stage, Table 5). Increase in the number of grains per panicle was mainly attributed increased application of N from 0 to 100 kg ha^{-1} along with the application of calcium silicate or silica gel, which might have enhanced the accumulation of photosynthates under both aerobic and wetland condition. However, there was no significant difference in the number of grains per panicle with application of calcium silicate at 2 t ha^{-1} along with RDF (124 and 137) and LCC (121 and 129) based N application under both conditions, respectively. Fageria et al. (2001) reported that number of panicles along with number of grains per

Table 3. Effect of silicon and nitrogen on growth and yield parameters of aerobic and wetland rice.

Treatments		Plant Height (cm)		No. of productive tillers		No. of grains per panicle	
Fertilizer	**Silicon source**	**A**	**W**	**A**	**W**	**A**	**W**
RDF P and K (Control -N)		78	82	11	7	97	120
RDF P and K + Basal 30 kg N ha^{-1} + LCC	Without silicon	81	88	15	8	120	128
	$CaSiO_3$ at 2 t ha^{-1}	86	88	17	10	121	129
	Silica gel at 500 kg ha^{-1}	84	87	16	9	121	129
RDF NPK	Without silicon	84	90	14	9	118	125
	$CaSiO_3$ at 2 t ha^{-1}	87	93	16	9	124	137
	Silica gel at 500 kg ha^{-1}	80	91	16	10	122	132
LSD (0.05)		**8**	**10**	**3**	**4**	**5**	**9**

RDF, Recommended dose of fertilizer (100:50:50 kg ha^{-1}); A, Aerobic. W, Wetland; $CaSiO_3$, Calcium Silicate; LCC, Leaf Colour Chart; N, Nitrogen; P, Phosphorus; K, Potassium.

Table 4. Effect of silicon and nitrogen on test weight (g), grain and straw yield of aerobic and wetland rice

Treatments		Test weight (g)		Yield (kg ha^{-1})			
				Grain		Straw	
Fertilizer	**Silicon source**	**A**	**W**	**A**	**W**	**A**	**W**
RDF P and K (Control -N)		18.8	20.1	2712	3723	3828	4705
RDF P and K + Basal 30 kg N ha^{-1} + LCC	Without silicon	22.8	24.7	4045	4409	5397	5265
	$CaSiO_3$ at 2 t ha^{-1}	24.0	25.1	4544	4692	5502	5649
	Silica gel at 500 kg ha^{-1}	23.1	24.5	4405	4622	5440	5370
RDF NPK	Without silicon	23.4	24.8	3807	4805	4999	6041
	$CaSiO_3$ at 2 t ha^{-1}	23.7	25.4	4640	5425	5539	6842
	Silica gel at 500 kg ha^{-1}	23.1	24.9	4588	5077	5523	6173
LSD (0.05)		**1.4**	**1.4**	**626**	**567**	**763**	**316**

RDF, Recommended dose of fertilizer (100:50:50 kg ha^{-1}); A, Aerobic. W, Wetland; $CaSiO_3$, Calcium Silicate; LCC, Leaf Colour Chart; N, Nitrogen; P, Phosphorus; K, Potassium.

panicle and paddy yield was significantly affected by N application in splits at different growth stages.Application of Si sources along with RDF and LCC based N application significantly increased the test weight under both aerobic and wetland situations over control. It may be due to higher N rates, which primarily increased the chlorophyll concentration in leaves and thereby higher photosynthetic rate and ultimately plenty of photosynthates available during grain development (Mahzoor et al., 2006). Increase in test weight could also be due to greater deposition of Si on paleae and lemma (Balastra et al., 1989). Application of silicon sources along with N significantly increased grain yield of rice when applied along with 100 kg N ha^{-1} as compared to RDF alone and control (Table 4). However, there was numerically increased in yield of LCC based N management in aerobic rice compared to wetland rice. The grain yield response to Si application may be due to increased leaf erectness, decreased mutual shading caused by dense planting and high N application. N increases susceptibility to various disease in rice but application of Si decreases the occurrence of pest and disease in rice (Yoshida et al., 1969). Increase in yields of flooded rice with Si fertilization has been already reported in India. Prakash et al. **(**2002) reported that application of calcium silicate at 3 to 4 t ha^{-1} as Si source significantly increased grain yield over control and other treatments; Prakash et al. (2010) also reported that there was response of rice for the application of calcium silicate in coastal and hilly zone soils of Karnataka, South India. Takahashi et al. (1990) reported particularly striking rice yield responses to Si application especially when application rates of other conventional fertilizers were rather high. Snyder et al. (1986) showed that calcium silicate application increased rice yield on Histosols mainly due to the supply of plant available Si and not due

Table 5. LCC values recorded at different growth stages of Aerobic and Wetland rice.

Treatments		Aerobic rice					
Fertilizer	**Silicon source**	**40 DAS**	**50 DAS**	**60 DAS**	**70 DAS**	**80 DAS**	**90 DAS**
RDF P and K (Control -N)		1.9	2.1	2.6	2.3	2.6	2.6
RDF P and K + Basal 30 kg N ha^{-1} + LCC	Without silicon	2.6*	2.8*	3.1	2.9*	3.1	3.2
	$CaSiO_3$ at 2 t ha^{-1}	2.5*	2.7*	3.1	2.9*	3.1	2.8
	Silica gel at 500 kg ha^{-1}	2.6*	2.9*	3.3	2.8*	3.2	2.9
RDF NPK	Without silicon	3.2	3.4	3.3	3.6	3.4	3.4
	$CaSiO_3$ at 2 t ha^{-1}	2.8	3.0	2.9	3.1	3.0	3.1
	Silica gel at 500 kg ha^{-1}	2.9	3.2	3.3	3.1	3.0	3.1
Treatments		**Wetland rice**					
		20 DAT	**30 DAT**	**40 DAT**	**50 DAT**	**60 DAT**	**-**
Fertilizer	**Silicon source**						
RDF P and K (Control -N)		3.5	3.3	3.4	3.5	3.5	-
RDF P and K + Basal 30 kg N ha^{-1} + LCC	Without silicon	3.7**	3.6**	3.7**	4.1	4.0	-
	$CaSiO_3$ at 2 t ha^{-1}	3.9**	3.6**	3.7**	4.1	3.9	-
	Silica gel at 500 kg ha^{-1}	3.8**	3.6**	3.9**	4.2	4.0	-
RDF NPK	Without Silicon	4.2	4.2	4.0	4.2	4.2	-
	$CaSiO_3$ 2 t ha^{-1}	4.0	3.8	4.0	4.2	4.0	-
	Silica gel at 500 kg ha^{-1}	4.2	4.1	4.1	4.1	4.2	-

DAS, Days after sowing; DAT, days after transplanting; *, **, 15 kg N was applied based on the LCC reading for aerobic and wetland rice respectively.

to supply of other nutrients. The results of field trials on rice soils with different levels of available Si in South China suggested a synergistic effect of added N on performance of Si fertilizer (Ho et al., 1980). Higher grain yield levels at 75 kg N ha^{-1} as compared to RDF under rice was mainly due to efficient utilization of applied N at spilt doses, which matches the crop N requirement. Adequate N supply is needed throughout the active growing period of rice. Thus proper N management is very crucial for successful rice production. Excessive and moderate application lead to an inefficient N acquisition by the crop and results in reduced yield.

Among the different sources, application of calcium silicate and silica gel found to be on par with each other in their yield levels under both aerobic and wetland conditions respectively. The lower yield with the silica gel compared to calcium silicate application may be due to leaching and fixation loss of silicon in submerged conditions. It may also be due to less supply of Si and may be inadequate in attaining the Si requirement by the crop for producing higher grain yield.

In the present investigation, the LCC critical value three (aerobic) or four (wetland) based N (30 kg N ha^{-1} as basal and three splits of 15 kg N ha^{-1} each time) matched the crop demand at different physiological stages and might have reduced the losses through nitrification, leaching and volatilization and resulted in the highest grain yield. It was also on par with 100 kg N ha^{-1} (Table 5). Tran et al. (2002) reported that the N application method based on the leaf colour diagnosis helped in saving on the N fertilizers applied and increased grain yield. The N rate of 60 to 80 kg ha^{-1} for the dry season and 40 to 60 kg ha^{-1} for the wet season was recommended. N split application at early tillering and at panicle initiation or booting stage was optimum for early and late maturing cultivars.

Increase in straw yield was mainly attributed to higher tiller numbers, biomass observed in the treatment with calcium silicate at 2 t ha^{-1} under both aerobic and wetland situations (Table 4). The enhanced straw yield with calcium silicate at higher N levels may be attributed to leaf erectness which facilitated better penetration of sunlight leading to higher photosynthetic activity of plant and higher production of carbohydrates (Ma et al., 1989; Korndorfer et al., 2001). Agarie et al. (1998) reported that maintenance of photosynthetic activity due to Si fertilization could be one of the reasons for increased dry matter production in rice crop. Savant et al. (1997) noted beneficial effects of Si on plant growth in terms of increased number of leaves. Ma et al. (1989) observed that addition of 100 ppm SiO_2 as silicic acid during the reproductive stage markedly increased straw yield of rice.

N Uptake (kg ha^{-1})

N uptake of both grain and straw was higher when silicon

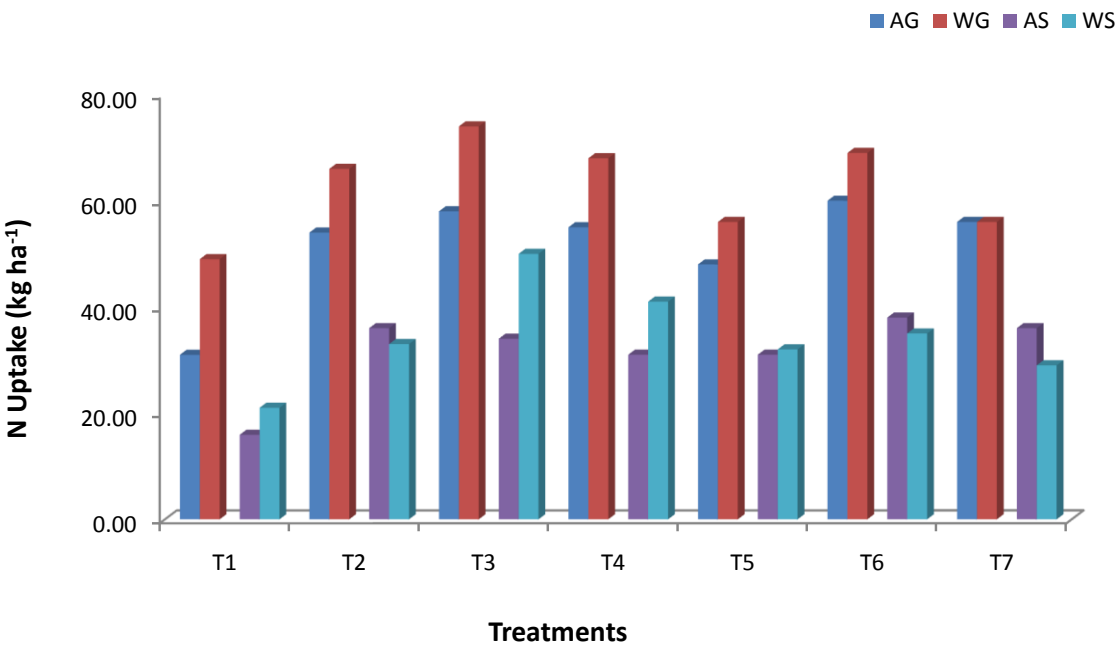

Figure 1. Effect of Si and N on N uptake (kg ha^{-1}) of grain and straw in aerobic and wetland rice. AG, Aerobic rice grain; WG, Wetland rice grain; AS, aerobic rice straw; WS, wetland rice straw. AEN, Agronomic efficiency; REN, apparent recovery efficiency; PFPN, partial factor productivity

applied along with RDF of N (100 kg ha^{-1}) compared with LCC based N application (75 kg ha^{-1}) (Figure 1). However, N application along with silicon recorded higher N content than RDF of N alone. This might be due to the possibility of dilution effect when Si fertilized with less application of N. Due to a synergistic effect, the application of Si has the potential to raise the optimum N rate, thus enhancing productivity of existing lowland rice fields (Ho et al., 1980). Silicon has been reported to raise the optimum level of N in rice. Snyder et al. (1986) reported that a decline in N concentration in Histosol grown rice and attributed it to the possibility of dilution in the larger Si fertilized plants. In greater biomass where N is limiting, the plants will show lower N concentration due to dilution. The mean total N content in the Si fertilized treatments were statistically non significant when silicon applied along with LCC based N application (75 kg ha^{-1}) than recommended N (100 kg ha^{-1}). However, Beyrouty et al. (1994) recorded no difference in total plant N content between alternately submerged, non submerged and flooded rice.

Nitrogen use efficiency (NUE)

Application of N based on LCC in combination with silicon sources significantly affected the NUE of aerobic rice. The AE_N, RE_N and PFP_N values were higher for LCC based N application along with calcium silicate at 2 t ha^{-1} and silica gel at 500 kg ha^{-1} (Figure 2). Generally AE_N, RE_N and PFP_N are greater when less N fertilizer was used, but this was achieved with the use of the LCC without sacrificing yield. The AE_N for LCC based N management treatments was the same or higher as in fixed schedule recommended N treatment. The AE_N, RE_N, PFP_N values ranged from 10.95 to 24.43 and 42.81 to 68.15, 38.07 to 60.59 in aerobic rice and 9.10 to 17.0, 18.0 to 72.0, 48.5 to 62.56 in wet land rice respectively (Figures 1 to 3). The AE_N is a function of both physiological efficiency and RE_N of applied N. Application of N using LCC resulted in increased leaf N concentration. The AE_N was greater when less N fertilizer was applied, but this was achieved with LCC without sacrificing the yield under both aerobic and wetland conditions. Basal application of 30 kg N ha^{-1} compared to 50 kg N ha^{-1} (RDF) efficiently utilized the applied N, whereas at later stages of the crop growth N was applied based on the crop requirement which was measured through LCC based on critical values. Spilt application of N at 15 kg N ha^{-1} was applied at each time against the recommended fixed dose of 25 kg N ha^{-1}. Cassman and Pingali (1985) reported AE_N values of 24 to 30 in rice by improved timing and further revealed that crop demand of applied N could improve the AE_N to some extent.

Application of N based on LCC achieved higher PFP_N values against RDF of fixed N spilt application under both

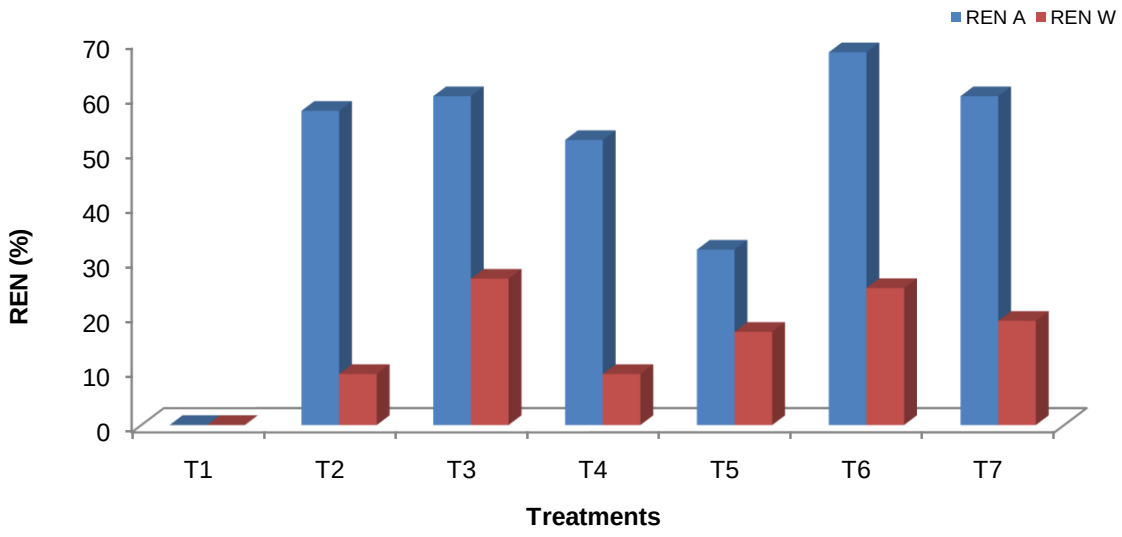

Figure 2. Effect of SI and N on RE_N in aerobic rice.

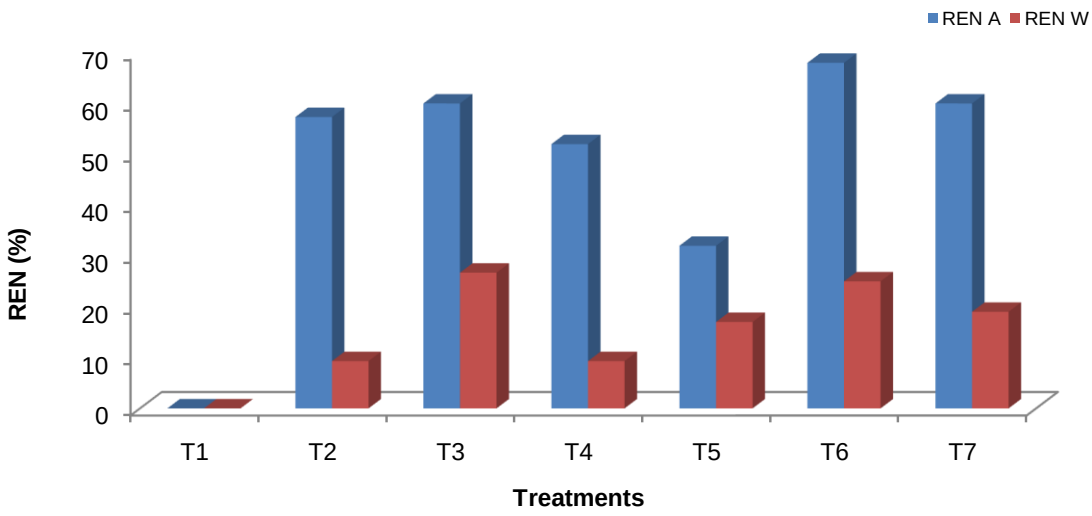

Figure 3. Effect of silicon and nitrogen on AE_N and PFP_N in aerobic and wetland rice.

aerobic and wetland rice. Half of the 100 kg N ha^{-1} (RDF) was recommended as basal application. As rice seeds take about 5 to 7 days to germinate and 2 to 3 leaf seedlings in fields, it is very likely that most of N applied as basal immediately after sowing is not used by plants and is subjected to lose by many ways. Rice seedlings need about 7 to 10 days to recover from transplanting shock and hence, N uptake within two weeks of transplanting could be very small. The usefulness of applying a lower dose of N is sufficient or at later stages of crop, that is, 30 days after sowing in aerobic rice, and 14 days after transplanting in wetland rice need to be examined. Shulka et al. (2004) and Alam et al. (2004) observed not only higher NUE, but also higher yields through LCC based management. Yogendra et al. (2011) reported that basal application of low dosage of N fertilizer (30 kg ha^{-1}) along with calcium silicate as a source of Si was effective for aerobic rice. Application of calcium silicate along with LCC based N application has achieved high N and Si use efficiency in aerobic rice. Yogendra et al. (2013) reported a significant increase in the grain yield of wetland rice and nitrogen use efficiency was noticed with the application of calcium silicate at 2 t ha-1 in eastern dry zone soils of Karnataka. This result indicate that the current recommendation of fixed time split N applications at specified growth time is not

adequate to synchronize N supply with actual crop N demand due to poorly designed N splitting and variations in crop N demand (Bijay et al., 2002; Nachimuthu et al., 2007). Furthermore, N application in recommended splits are not based on the indigenous N supply (INS) (Shukla et al., 2004). The INS is defined as total plant N uptake at physiological maturity in zero N plots, which represents all sources of N (soil, organic materials, rhizosphere N fixation, crop residues, rainfall, irrigation water, etc.) available to crops during the growing season (Dobermann et al., 2003). This varies with crop, soil and cropping season (Stalin et al., 1996).

Conclusion

It is evident from our results that the use of LCC with reduced the basal N application (30 kg N ha^{-1}) along with calcium silicate at 2 t ha^{-1} as a source of silicon resulted on par grain yield as compared to the recommended N treatments under both aerobic and wetland rice. Monitoring rice plant N status and N requirement is an important subject with improving the balance between crop N demand and N supply from soil and applied fertilizer. In many field situations in Karnataka, more than 50% (50% of total N is supplied as basal application) of applied N is lost due in part to the lack of synchrony of plant N demand with N supply. The LCC is simple and easy-to-use tool that can help farmers avoid over application of N in rice plant. The LCC based management in rice suggests that N application can be saved with no yield lose by appropriately revising the fertilizer recommendation. Thus, there is considerable opportunity to increase farmers yield and N recovery efficiency levels through improved N management with the LCC. In the situation of using fixed-time split N recommendations, refining fixed time split N recommendations periodically will be needed with the real-time N management to tackle high spatial and temporal variability in INS.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Efficacy of some spices as sorghum grain protectants against *Sitophilus zeamais* Motschulsky [Coleoptera: Curculionidae]

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Studies were carried out to determine the efficacy of three spices (*Allium sativum* L.*, Capsicum frutescens* L., and *Zingiber officinale* Rosc.) against *S. zeamais* reared on sorghum grains. Doses of 0.5, 1.0 and 1.5 g of each of *A. sativum, C. frutescens* and *Z. officinale* and 0.12 g of permethrin were applied to 20 g of sorghum grains infested with *S. zeamais* under constant conditions of 30 ± 2°C and 65 ± 5% R.H. In all the treatments, 100% mortality among adult of *S. zeamais* reared on sorghu treated with all the powders was obtained. The effect of the spices on adult mortality was significant ($p<0.05$) between the powdered spices and the control. The effect of the different spices applied at varying amounts on grain damage caused by *S. zeamais* was significantly ($p<0.05$) different with the highest (33.30%) in petri dishes with 1.5 g of *C. frutescens* and the least (3.30%) from treatments of 0.5, 1.0 and 1.5 g of *Z. officinale* and *C. frutescens,* respectively. The findings of this study indicated that the selected spices showed their potentiality in reducing sorghum grain damage caused by *S. zeamais*.

Key words: *Allium sativum, Capsicum frutescens,* Protectants, *Sitophilus zeamais,* Sorghum grain, *Zingiber officinale.*

INTRODUCTION

Sorghum is the primary food crop in virtually all parts of northern Nigeria (USDA, 2010). The whole grain may be ground into flour or decorticated before grinding to produce either a fine particle product or flour, which is then used in various traditional foods (Leder, 2004). Sorghum is also used extensively in brewing, and industrial demand for sorghum by beer manufacturers is rising steadily, in step with rising demand for their products (USDA, 2010).

Storage is an important activity, which enhances marketing efficiency by providing utility (Adejumo and Raji, 2007). The loss of food grain during storage due to various insect pests is a very serious problem. Climate and storage conditions, especially in the tropic countries like Nigeria, are often highly favourable for insect growth and development, which leads to their damages to the stored grains that ranges from 5 to 30% of the world's total agricultural production (Pugazhvendan et al., 2009). In sorghum, the losses incurred through insect damage in store, is estimated to be in the region of 35% of total production (NAERLS, 2002).

The grain weevils (*Sitophilus* spp.) and the grain moth (*S. cerealella*) were the major pests of sorghum (NAERLS, 2002). The species of the genus *Sitophilus* (grain weevils) feed on cereals (Abate et al., 2000), causing serious management problem facing agriculture

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in developing countries.

The use of botanical products is more prevalent in the control of insect pests in storage systems, farmers can grow them and then can also be cheaper and easier to use than the synthetic insecticides (Govindan et al., 2010). Plant materials with insecticidal properties are one of the most important locally available, biodegradable and inexpensive methods for the control of pests of stored products (Jayakumar, 2010). Resource poor farmers in developing countries use different plant materials to protect stored grains against pest infestation by mixing grains with protectants made up of plant products (Udo, 2005). Many plant powders were evaluated and found effective in the management of *S. zeamais,* attacking maize grains in the stores (Danjumma et al., 2009; Suleiman et al., 2011). Spices are one of the important plant powders tested and found efficacious against insect pests of stored products (Rajapakse and Ratnasekera, 2008; Ukeh et al., 2008; Danjumma et al., 2009). This study describes laboratory bioassays to evaluate the efficacies of three local spices; *Allium sativum* (L.), *Capsicum frutescens* (L.) and *Zingiber officinale* (Rosc.) as possible stored sorghum grains protectants against *S. zeamais* in the tropics.

MATERIALS AND METHODS

Rearing of *S. zeamais*

Adults of *S. zeamais* were cultured in the laboratory at 30 ± 2°C in the Biology Laboratory 2, Umaru Musa Yar'adua University, Katsina. The food media used was whole maize grains. Fifty pairs of *S. zeamais* were introduced into 1 L glass jar containing 400 g weevils susceptible sorghum grains. The jars were then covered with muslin cloth held in place with rubber bands, newly emerged adults of *S. zeamais* were then used for the experiment.

Collection and preparation of spices

Spice materials namely; Garlic (*Allium sativum* L.), Chilli pepper (*Capsicum frutescens* L.) and Ginger (*Zingiber officinale* Rosc.) used in this experiment were purchased from Katsina central market. The spices were dried in a well ventilated area in the Laboratory for seven days before grinding into fine powder. The spices were ground using laboratory blender and made into fine powder. The powders were separately kept in polythene bags under room temperature for use during the experiment.

Adult mortality test

To study the adult survival of *S zeamais*, methods of Dawit and Bekelle (2010), was employed. Twenty gram of clean disinfested sorghum grains was weighed into sterilized petri dishes. Quantities (0.5, 1.0 and 1.5 g) of each of the spices were added to first three petri dishes separately, 0.12 g of permethrin as chemical check to the fourth and zero spices was added to the fifth (control). The spices were thoroughly mixed with the disinfested sorghum grains with the aid of glass rod to ensure thorough admixture. The treated sorghum was left undisturbed for an hour. Thereafter five pairs of newly emerged adult weevils were introduced into each of the treated and untreated sorghum in the petri dishes. Each of the petri dishes was covered with muslin cloth and tied with rubber band. All the petri dishes were then kept in the incubator. Each treatment was replicated four times. The petri dishes were arranged in Completely Randomized Design (CRD). Observations were made on adult mortality daily for 14 days during which dead adults were removed. The petri dishes were kept in the incubator at 32 ± 2°C and 65 ± 5% R.H for another 14 days for damage assessment.

Damage assessment

Damage caused by the weevils to the sorghum grains was assessed using the methods of Adedire and Ajayi (1996) and Asawalam et al. (2007). Ten grains were sampled randomly from each petri dish. Grains with characteristics hole were separated from healthy ones and counted then percentage grain damage was calculated using the formula (Fatope et al., 1995):

$$\% \text{ Damage} = \frac{\text{Number of grains perforated}}{\text{Number of grains sampled}} \times 100$$

The weevil perforation index (WPI) is defined as follows:

$$\text{WPI} = \frac{\% \text{ Treated grains perforated}}{\% \text{ Control grains perforated} + \% \text{ Treated grains perforated}} \times 100$$

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using a general linear model procedure (SAS, 2000) at 5% level of significance. Significantly different means were separated by using least significant difference (LSD).

RESULTS

The effect of the spicy powders on mortality of adults of *S. zeamais* is shown in Table 1. The Table shows that all the spices applied at varying amounts (0.5, 1.0 and 1.5 g/20 g) resulted in 100% adult mortality 14 days after application, while 13.3% adult mortality was observed in the control at the same period. The spices were observed to show similar effects to the conventional insecticide (Permethrin). Adult mortality was observed to be significantly ($p<0.05$) different between the spices and control.

The effect of the different spices applied at varying amounts on sorghum grain damage caused by *S. zeamais* is presented in Table 2. The Table indicates that the least damage (3.30%) was observed from sorghum grains treated with 1.0 g of *C. Frutescens* and 0.5 g and 1.5 g of *Z. officinale* respectively, while the highest grain damage (33.3%) was obtained from sorghum grains treated with 1.5 g of *C. frutescens*. There grain damage caused by *S. Zeamais* was significantly ($p<0.05$) different between the treatments and the control.

Table 1. Effect of the spices on mortality of adults of *S. zeamais* on sorghum grains.

Test powders	Amount applied (g/20g)	No of weevils introduced	Adult mortality (percent)
	0.5	10	100.00
A. sativum	1.0	10	100.00
	1.5	10	100.00
	0.5	10	100.00
C. frutescens	1.0	10	100.00
	1.5	10	100.00
	0.5	10	100.00
Z. officinale	1.0	10	100.00
	1.5	10	100.00
Permethrin	0.12	10	100.00
Control	0.00	10	13.30
S. E.	-	-	14.33

Table 2. Effect of the spices applied at varying amounts on sorghum grain perforation caused by *S. Zeamais.*

Test powder	Amount applied (g/20 g)	Mean No. of grain sampled	Mean no. of perforated grain	Grain damage (Percent)
	0.5	10	1.00	10.00
A. sativum	1.0	10	1.67	16.70
	1.5	10	1.00	10.00
	0.5	10	0.67	6.70
C. frutescens	1.0	10	0.33	3.30
	1.5	10	3.33	33.30
	0.5	10	0.33	3.30
Z. officinale	1.0	10	0.67	6.70
	1.5	10	0.33	3.30
Permethrin	0.12	10	0.00	0.00
Control	0.00	10	5.33	53.30
S. E.	-	-	1.15	11.51

Table 3 shows the weevil perforation index (WPI) obtained from the study conducted. The least (0.00) WPI value was obtained in petri dishes treated with 1.5 g of *C. frutescenc*e and 0.12 g of permethrin respectively, while the highest (23.86) WPI value was observed in 1.0 g of *A. sativum*. Statistical analysis showed that there was a significant ($p<0.05$) difference between the WPI values from the different spices applied and the control.

DISCUSSION

From the results obtained it shows that all the plant powders used during the investigation have significant ($p<0.05$) effect on the mortality of adult *S. zeamais* in which all the test powders resulted in 100% mortality. Arannilewa et al. (2006) reported that 1.5 g of *A. sativum* applied to 25 g of maize grains caused mortality of 85% in adult *S. Zeamais,* 14 days after application. Similarly, Danjumma et al. (2009) reported that *A. sativum* gave 90% mortality at 1.5 g per 50 g of maize grain followed by *Z. officinale* with 86% after 7 days of application. *A. sativum* may have been very potent because of its strong odours which may have exerted a toxic effect by disrupting normal respiratory acting of the weevils as

Table 3. Effect of the spices on weevil perforation index (WPI) of sorghum grains.

Test powder	Amount applied (g/20 g)	No of sampled sorghum grains	Mean WPI
	0.5	10	15.80
A. sativum	1.0	10	23.86
	1.5	10	15.80
	0.5	10	11.17
C. frutescens	1.0	10	5.83
	1.5	10	0.00
	0.5	10	5.83
Z. officinale	1.0	10	11.17
	1.5	10	5.83
Permethrin	0.12	10	0.00
Control	0.00	10	50.00
S. E.	-	-	9.57

suggested by Adedire and Ajayi (1996). The present findings agree with that of Al-Moajel (2004) who reported 100% mortality of *S. zeamais* 14 days after application of *C. frutescens*. Asawalam et al. (2007) also reported that application of 0.4 g of *C. frustecens* caused 75% mortality on adult *S. zeamais* in 20 g maize grains. The ability of these plants to cause mortality of *S. zeamais* adult on maize grains might be attributed to the contact toxicity of powder on the weevil. The findings of this study also revealed that the selected spices applied at varying amounts were effective in reducing sorghum grain damage caused by *S. zeamais*. Among the spices applied *Z. officinale* was found to be the most effective spices in reducing grain damage which was in agreement with the findings of Asawalam et al. (2007) who recorded 7% grain damage on maize grains treated with *C. frutescens* at the rate of 0.4 g/20 g. *A. sativum* was also found promising in reducing grain damage. This agrees with the findings of Arannilewa et al. (2006) who reported 2.81% grain damage of maize when 1.5% of *A. sativum* was applied. This is due to the strong aroma of the powder which might have served as feeding deterrent to the weevils. The reduction in grain damage was observed to be directly proportional to the amount of the spices applied.

The results obtained revealed that all the spices applied had positive protectant ability of sorghum grains against *S. zeamais* by resulting in WPI value of <50 as suggested by Asawalam et al. (2007). *C. frutescens* was found to be the most effective spice in protecting sorghum grains against *S. zeamais.* This agrees with the findings of Adedire and Ajayi (1996) who reported 0.00 WPI value when 1.0 cm^3 of *C. frutescens* extract was applied to 10 g of maize grains against *S. zeamais.*

The findings of this research have shown that the selected spices were effective in reducing sorghum grains damage caused by *S. zeamais* and had positive protectant ability against the weevil. In addition, the spices used are edible since they are used either as ingredients for soup or medicinal preparations.

Conflict of Interests

The author has not declared any conflict of interests.

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Full Length Research Paper

Chemical composition and antimicrobial and antioxidant activity of essential oil and various plant extracts from *Prunus myrtifolia* (L.) Urb

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In this study focused on research on plants as a source of alternative and natural antimicrobial substances, the chemical composition of the essential oil from *Prunus myrtifolia* (L.) Urb. was assessed through gas chromatography coupled to mass spectrometry (GC/MS) and phytochemical screening of different extracts (aqueous, ethanolic, ethyl acetate, and hexanic) from the same plant, as well as the antimicrobial effect against the following microorganisms: *Pseudomonas aeruginosa, Salmonella* Typhimurium, *Proteus mirabilis, Klebsiella pneumoni, Escherichia coli, Enterococcus faecalis*, *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis* and *Candida albicans*, through determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, using the micro-dilution broth method. Finally, the goal was to assess the antioxidant activity of essential oil and plant extracts using the DPPH free radical method (2.2-diphenyl-1-picrylhydrazyl). The largest class of volatile compounds identified in *P. myrtifolia* oil belongs to aldehydes represented by benzaldehyde compounds. With respect to antimicrobial activity, all extracts and essential oil showed activity against the microorganisms assessed, with exception of hexanic extract. Among the extracts assessed, aqueous and ethanolic extracts were the most effective. Antioxidant activity of aqueous, ethanolic and ethyl acetate extracts was confirmed; however, antioxidant activity of essential oil and hexanic extract was not observed.

Key words: Antimicrobial activity, gas chromatography–mass spectrometry (GC/MS), native plants, chemical composition, antioxidant activity, essential oil, plant extracts.

INTRODUCTION

Brazil has the largest equatorial and humid tropical forest on the planet and, consequently, little explored extensive plant genetic diversity. With respect to the medicinal potential, only approximately 17% of plants have been studied (Pinto et al., 2002). Exploration of these plants is required, because potentially useful compounds can be lost due to the extinction of some species (Patinõ and Cuca, 2011). Due to this diversity, Brazil came to prominence in the search for potential bioactive compounds that can be used for various purposes, such

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as alternative antimicrobial products for controlling pathogens (Pupo et al., 2007) used in the pharmaceutical and food industries (Cehyan et al., 2012).The family Rosaceae comprises around 100 genera and 3000 species. Concentrated in the northern hemisphere, it is one of the leading families from an economic point of view, showing a few native species in Brazil (Souza and Lorenzi, 2005). Some species have great pharmacological and nutrition potential and are used in popular medicine for the treatment of various diseases and for the maintenance of good health. The genus *Prunus* is composed of approximately 130 species that occur in the northern, southern and southeastern regions of Brazil. Various fruits introduced and consumed in Brazil belong to this genus, such as peaches (*P. persica*), nectarines (*P. persica* var. nucipersica), plums (*P. domestica*), almonds (*P. dulcis*), and cherries (*P. avium*, *P. cerasus*) (Souza and Lorenzi, 2005).

Regarding Brazilian native species of the genus *Prunus*, the species *Prunus myrtifolia* (L.) Urban deserves attention. For being a species of wide geographic distribution, it is synonymous with *P. sphaerocarpa* Hook and *P. sellowii* Koehne (Souza and Lorenzi, 2005).

In recent years, the chemical compositions as well as the antioxidant and antimicrobial properties of plants have gained interest in the search for alternative products. Essential oils can contain from 20 to 60 (or more) diverse compounds and in the most varied concentrations (Bakkali et al., 2008). The analysis requires the application of current analytical methods and adapted instrumentation, which allows assessing the quality of essential oils and ensure the identification of their constituents. Plant extracts are targets of great interest due to the presence of secondary metabolites in their composition, which are substances used against pathogenic microorganisms, insects and herbivorous animals. In addition, they have a varied chemical composition with the presence of terpenoids, alkaloids and coumarins, which often feature antimicrobial activity (Reschke et al., 2007).With the progressive development of synthetic antimicrobial resistance, the biological properties of plant products have been studied in search of alternative products with antimicrobial action (Arya et al., 2010). In this context, essential oils and plant extracts stand out as efficient antimicrobials (Bona et al., 2010).

The search for new natural antioxidants has increased and led food, cosmetics and pharmaceutical industries to focus their searches on materials of plant origin. Plant antioxidants are very varied, but the phenolic compounds have been considered responsible for greater antioxidant capacity, being represented by flavonoids and isoflavones, tannins, lignans, and xanthones, among others (Razavi et al., 2008).The goal of this study was to determine the chemical composition of the essential oil and various plant extracts from *P. myrtifolia*, as well as their antimicrobial effect against different microorganisms, such as: *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* typhimurium (ATCC 14028), *Proteus mirabilis* (ATCC 25933), *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 19433), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (CCD-04) and *Candida albicans* (ATCC 10231). Finally, we aimed to assess the antioxidant activity of the essential oil and plant extracts.

MATERIALS AND METHODS

Plant material

The leaves of *P. myrtifolia* were collected in the western region of the State of Parana, Brazil (24°57' S - 53°28' W), in January and February 2013. The material was identified and incorporated into the Herbarium of the West of Parana State University (UNOP) under number 25 J. Silva, J. P. B.

The leaves collected were dried in an oven with air circulation at 40°C for 48 h and subsequently ground using a cutting mill with less than 0.42 mm granulometry. The plant material ground was stored protected from the light until its use for the production of extracts.

Obtaining aqueous extract (W)

We added 20 g of the ground plant material to a container with distilled water that was kept in a rotary shaker at 220 × g for 24 h. Subsequently, the material was filtered in filter paper (Whatman Nº 1) and centrifuged at 5000 x g for 15 min. The supernatant material was collected and the final concentration was 200 mg/ml. The extract was stored at 4°C until use.

Obtaining of organic extracts

The organic extracts were obtained according to the methodology described by Ceyhan et al. (2012) with modifications. Ethanol (95%), ethyl acetate and hexane were used as organic solvents. Starting with 10 g, the ground plant material was added to 100 ml organic solvent and placed in a rotary shaker at 220 x g for 24 h. Subsequently, it was filtered in filter paper (Whatman Nº 1) and centrifuged at 5000 × g for 15 min. The supernatant material was collected and submitted to roto-evaporation in order to remove the solvent. The extract obtained was diluted at a concentration of 150 mg/ml for ethanolic extracts (ET) and ethyl acetate (EA) and at a concentration of 6 mg/ml for hexanic extract (H) with 10% dimethyl sulfoxide (DMSO), following the proportion of its weight and volume. The extracts obtained were stored at 4°C until use.

Phytochemical screening

The main secondary metabolites were detected in accordance with the methodology developed by Matos (1997).

Essential oil extraction (EO)

Nearly 70 g of fresh leaves of *P. myrtifolia* in 600 ml distilled water were submitted to standard water steam dragging methodology for three hours using Clevenger-type equipment. The oil was collected directly with no addition of solvent and stored at 4°C.

Chemical composition analysis

The constituents of the essential oil were identified through gas chromatography coupled to mass spectrometry (GC-MS) and the determination of their Kovats retention index (KI).

GC-MS

Analysis of oil from *P. myrtifolia* was carried out using a Thermo-Finnigan GC-MS system, composed of a FOCUS GC gas chromatograph (Thermo Electron), coupled to a DSQ II mass spectrometer (Thermo Electron) and a TriPlus AS automatic injector (Thermo Electron). Chromatographic separation was performed with an HP-5ms fused silica capillary column (30 m long, 0.25 ID and 0.25 µm film; composition of 5% phenyl-95% dimethylpolysiloxane).

The temperature of the injector was 250°C. Samples and patterns of alkanes were injected using the split mode with a split ratio of 1:25. The programming of the temperature used was: 50°C maintained for 2 min; temperature rise to 180°C at a ratio of 2°C min-1; followed by an increase to 290°C at a ratio of 5°C min^{-1}. The interface between the GC and MS was maintained at 270°C and the temperature of the ionization source of the mass spectrometer was 250°C. The identification of the components was performed by comparing their retention times with those obtained in the literature (Adams, 2007) for the same compounds analyzed by means of Kovats retention index.

Microorganisms used

To perform the antimicrobial activity test of the essential oil and plant extracts from *P. myrtifolia*, we used 5 gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853; *Salmonella* Typhimurium ATCC 14028; *Proteus mirabilis* ATCC 25933; *Klebsiella pneumoniae* ATCC 13883; and *Escherichia coli* ATCC 25922), 4 Gram-positive bacteria (*Enterococcus faecalis* ATCC 19433; *Staphylococcus epidermidis* ATCC 12228; *Staphylococcus aureus* ATCC 25923; and *Bacillus subtilis* CCCD-B005) and *Candida albicans* ATCC 1023 as yeast.

Microorganisms previously kept at -20°C were recovered in enriched medium (Brain Heart Infusion) and incubated at 36°C for 24 h. After this period, they were re-suspended in 0.9% sterile saline solution to obtain the standard inoculum at a concentration of 1×10^8 UFC/ml on the MacFarland scale. Subsequently, dilutions were performed in 0.9% sterile saline solution in order to obtain a final inoculum at a concentration of 1×10^5 UFC/ml, with the exception of *C. albicans* that was used at the final concentration of 1×10^6 UFC/ml.

Determination of minimum inhibitory concentration (MIC)

Essential oil

The MIC of the essential oil was determined using the broth microdilution method. We used 96-well plates, according to the CLSI document M31-A317 with modifications. We added 200 µl of EO from *P. myrtifolia*, at a concentration of 7000 µg/ml with Mueller-Hinton broth (MH) for bacteria and RPMI for yeast in the first well and, after homogenization, successive dilutions were held, obtaining final concentrations from 7000 to 13.67 µg/ml. Aliquots (10 µl) of microorganisms' dilution were distributed in each well containing the EO in its final dilutions. The plates were incubated at 36°C for 24 h. After turbidity was observed, each well received an aliquot of 10 µl of 0.5% triphenyl tetrazolium chloride (TTC). After three more hours of incubation at 36°C, the MIC was defined as the lowest concentration of oil in µg/mL able to prevent microbial growth (Sartoratto et al., 2004).

Plant extracts

The MIC of extracts was determined using the broth microdilution method proposed by Ayres et al. (2008) with modifications. Aliquots (10 µl) of dilution were distributed in 96-well microtitre plates, containing 150 µl of MH broth (double concentration) for bacteria and RPMI for yeast, with the previous addition of extracts. The extracts were diluted in concentrations between 100 and 0.04 mg/mL (W), between 75 and 0.035 mg/mL (ET and EA), and between 3 and 0.0012 mg/mL (H). The plates were incubated at 36°C for 24 h. After turbidity was observed, we followed the same assessment standards used for the essential oil.

Determination of the minimum bactericidal concentration (MBC)

The MBC was determined based on the methodology described by Santurio et al. (2007). From the wells in which there was no visible bacterial growth in the MIC test, prior to the addition of TTC, we withdrew an aliquot of 10 µl and inoculated it on the Mueller-Hinton agar surface. The plates were incubated for 24 h at 36°C and, after this procedure; the MBC was defined as the lowest concentration of the extract/oil able to cause the death of the inoculum. The tests of MIC and MBC were carried out in triplicate.

Distilled water, ethanol and ethyl acetate were used as negative control; gentamicin was used as positive control for bacteria; and nystatin was used for *C. albicans* (Table 1). Synthetic antimicrobials were tested at concentrations of 100 to 0.78 mg/ml.

Antioxidant activity

The measurement of the activity of free radicals scavenging (2.2-diphenyl-1-picrylhydrazyl, DPPH) was assessed as described by Scherer and Godoy (2009) and Rufino et al. (2007) with modifications. For the analysis, 0.1 ml of each dilution of samples or patterns were placed in test tubes containing 3.9 ml DPPH radical (0.2 mM) diluted with methanol and homogenized in a test tube agitator. For the negative control, we used 0.1 ml control solution (methyl alcohol, acetone and water) with 3.9 ml DPPH radical, which were homogenized. We used the commercial synthetic antioxidant butylated hydroxytoluene (BHT) following the same procedure used for the negative control. Methyl alcohol was used as whitening agent in order to calibrate the spectrophotometer (UV mini-1240, Shimadzu Co., Japan). The mixtures were incubated in the absence of light at room temperature until measurement. Subsequently, the absorbance at 515 nm was measured using a spectrophotometer and monitored every 30 min until stabilization. The tests were carried out in triplicate.

The DPPH index was calculated using the antioxidant activity equation (%) = [(Abs0 -Abs1) /Abs0] × 100, where Abs0 is the absorbance of the whitening agent and Abs1 the absorbance of the sample.

The concentrations of the samples (extracts and EO) responsible for 50% decrease in the initial activity of DPPH free radical (IC_{50}) were calculated through linear regression of the antioxidant activity.

Statistical analysis

The data obtained by calculating the DPPH index and IC_{50} were analyzed through Tukey test at 5% significance using the Sisvar software (Ferreira, 2007).

Table 1. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of distilled water, organic solvents and reference antibiotics on pathogenic microorganisms.

Microorganisms	MIC/MBC (mg/ml)				
	Distilled water	Ethanol	Ethyl acetate	Gentamycin	Nystatin
P. aeruginosa ATCC 27853	Na	Na	Na	6.25/6.25	Nt
S. TyphimuriumATCC 14028	Na	Na	Na	3.125/6.25	Nt
P. mirabilis ATCC 25933	Na	Na	Na	6.25/6.25	Nt
K. pneumoniae ATCC 13883	Na	Na	Na	6.25/6.25	Nt
E. coli ATCC 25922	Na	Na	Na	6.25/6.25	Nt
E. faecalis ATCC 19433	Na	Na	Na	3.125/6.25	Nt
S. epidermidis ATCC 12228	Na	Na	Na	6.25/6.25	Nt
S. aureus ATCC 25923	Na	Na	Na	6.25/6.25	Nt
B. subtillis CCD-04	Na	Na	Na	6.25/6.25	Nt
C. albicans ATCC 10231	Na	Na	Na	Nt	6.25/6.25

Na, No activity (100<); Nt, not tested.

Table 2. Classes of secondary metabolites identified in different extracts from *Prunus myrtifolia*.

Classes of metabolites	Extracts			
	W	ET	EA	H
Tannins	+	+	-	-
Alkaloids	-	-	-	-
Coumarins	-	-	-	-
Saponins	-	+	-	-
Anthocyanins	-	-	-	-
Anthocyanidins	-	-	-	-
Flavonoids	+	+	+	-
Triterpenoids	-	+	-	-
Steroids	-	-	-	-

-, Absent; +, present; W, aqueous extract; ET, ethanolic extract; EA, ethyl acetate extract; H, hexane extract.

RESULTS AND DISCUSSION

The tests conducted for phytochemical screening (Table 2) showed that the aqueous extract had only the classes tannins and flavonoids. The ethanolic extract showed the greatest number of classes of substances: Tannins, saponins, flavonoids and terpenes. The extract with ethyl acetate solvent only showed flavonoids and the hexanic extract did not show positive results for the classes of substances tested.

It is known that the chemical constitution of Rosaceae includes especially tannins (Okuda et al., 1992), flavonoids (Harbone, 1998), triterpenes and steroids (Wallaart, 1980). The data obtained in our research agree with studies of these authors, except for the class of steroids, which was not found in any of the extracts tested.

Three compounds were found in the volatile composition of essential oil from *P. myrtifolia*, and the largest class of compounds identified belonged to aldehydes, represented by benzaldehyde, which constituted approximately 97% of the total area of the chromatogram peaks. It was followed by lower percentages of alcohol classes (3-hexen-1-ol) and esters (benzyl benzoate), with 0.07 and 0.09% total peak area, respectively (Table 3).

These data agree with those found by Ibarra-Alvarado et al. (2009), when they identified the volatile compounds of oil from *P. Serotina*, they also detected benzaldehyde as majoritary compound. It is known that benzaldehyde is one of the main components responsible for the characteristic odor of essential oils (Kerdogan-Orhan and Kartal, 2011) and it is related to various biological activities, such as antimicrobial and antifungal (Fujii et al., 2005).

The results summarized in Table 4 indicate that all

Table 3. Volatile composition of *P. myrtifolia* through GC-MS.

RT	Compound name	KI	Area (%)
5.74	3-Hexen-1-ol	852	0.07
10.22	Benzaldehyde	964	96.96
57.22	Benzyl benzoate	1759	0.09

RT, Retention time; KI, Kováts retention index calculate.

Table 4. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of essential oil and different extracts of *P. myrtifolia* on pathogenic microorganisms.

Microorganisms	MIC/MBC			
	EO (µg/ml)	W (mg/ml)	ET (mg/ml)	EA (mg/ml)
P. aeruginosa ATCC 27853	3500/3500	12.5/12.5	9.38/18.75	37.5/75
S. TyphimuriumATCC 14028	1750/3500	12.5/25	18.75/37.5	150/150
P. mirabilis ATCC 25933	3500/7000	12.5/12.5	18.75/18.75	37.5/75
K. pneumoniae ATCC 13883	3500/7000	12.5/12.5	18.75/37.5	37.5/37.5
E. coli ATCC 25922	1750/7000	12.5/25	9.38/37.5	37.5/75
E. faecalis ATCC 19433	1750/7000	12.5/25	9.38/18.75	9.38/18.75
S. epidermidis ATCC 12228	3500/7000	1.56/1.56	1.18/2.35	4.69/9.38
S. aureus ATCC 25923	3500/7000	0.04/0.09	0.07/0.15	2.34/4.68
B. subtillis CCD-04	3500/7000	3.13/6.25	4.69/4.69	4.69/9.38
C. albicans ATCC 10231	3500/7000	6.25/6.25	4.69/9.37	9.38/9.38

EO, Essential oil; W, aqueous extract; ET, ethanolic extract; EA, ethyl acetate extract; Hexane extract, no activity.

extracts and the essential oil tested showed antimicrobial activity against the microorganisms assessed, with exception of the hexanic extract that showed no activity.

The essential oil had MIC values ranging from 3500 to 1750 µg/ml over the microorganisms tested. For the majority of microorganisms, the MBC was 7000 and 3500 µg/ml only for *P. aeruginosa* and *S. Typhimurium*. The activity found in the oil can be due to the presence of benzaldehyde in its composition. This compound is environmentally safe when used as an antimicrobial, considering its wide spectrum of inhibitory effect. It is also used as a bactericide and fungicide. Benzaldehyde activity has similarities to the antimicrobial activity of phenols, because it interacts with the surface of the cell and leads to cell death by disintegration of the cell membrane and release of intracellular components (Alamri et al., 2012).

Aqueous, ethanolic and ethyl acetate extracts had MIC values ranging from 0.04 to 150 mg/ml, comparable with standard antimicrobials, which ranged from 3.125 to 6.25 mg/ml. Thus, the extracts were as potent antimicrobials inhibiting the growth of microorganisms' strains as synthetic antimicrobials. With respect to gram-positive microorganisms, the same extracts had smaller MIC (0.04 to 4.69 mg/ml) compared with gentamicin (6.25 mg/ml). Regarding ethanolic extracts, *C. albicans* also had lower MIC value (4.69 mg/ml) compared to nystatin (6.25 mg/ml). When the different plant extracts (aqueous, ethanolic and ethyl acetate), were assessed regarding the gram-negative microorganisms, they had MIC ranging from 9.38 to 150 mg/ml, which were higher concentrations when compared to gentamicin concentrations (3.125 to 6.25 mg/ml). The same ratio found in the MIC was observed with respect to MBC, with values ranging from 0.09 to 150 mg/ml.

A growing number of mechanisms with inhibitory action-such as the secondary metabolites-have been assigned to active compounds present in plant extracts. Thus, the antimicrobial activity observed in aqueous, ethanolic and ethyl acetate extracts can be related to the presence of flavonoids (W, ET, and EA), tannins (ET and W), triterpenoids (ET), and saponins (ET) (Table 2), which have already proved active in different studies described in the literature (Recio et al., 1989).

It is known that the presence of flavonoids is related to most antimicrobial activities of extracts, including antibacterial (Gibbons, 2008) and antifungal potential (Cao et al., 2008). In this study, we observed greater activity against Gram-positive bacteria. This fact can result from the presence of flavonoids, agreeing with the results found by Taleb-Contini et al. (2003). The compounds commonly related to antimicrobial activity,

Table 5. DPPH average and standard deviation (% sequestration) and IC_{50} values of essential oil and different extracts from *Prunus myrtifolia* in the different concentrations tested.

Extracts/oil	Antioxidant activity (%)	IC_{50} (mg/ml)
BHT	95.85±0.07[a]	11.52±0.96[a]
W	91.27±0.67[a]	20.12±0.05[a]
ET	94.12±0.64[a]	15.43±0.0[a]
EA	78.49±0.98[a]	14.58±0.28[a]
H	2.81±0.039[b]	186.26±0.01[b]
EO	8.69±0.97[b]	175.17±0.99[b]

Standard error followed by the same letter in the column do not differ through Tukey test ($p<0.05$); EO, Essential oil; W, aqueous extract; ET, ethanolic extract; EA, ethyl acetate extract; H, hexane extract.

such as flavonoids, tannins, saponins, and triterpenes, generally act in the microorganism's membrane or cell wall. Flavonoids act in the bacterial cell through complexes between proteins and the cell wall causing its breakage (Taguri et al., 2004). Tannins act in microorganisms by preventing their growth through the inhibition of nutrients transport to the cell caused by the formation of complexes between the organism and the cell wall (McSweeney et al., 2001). The action mechanism of triterpenes in microorganisms is related to the breakage of lipophilic compounds of microbial membranes (Bagamboula et al., 2004). Lastly, with respect to the saponins, they act actively in the membrane sterols (Sparg et al., 2004).

The difference between the activity found in the extracts can be attributed to the fact that the components extracted from aromatic plants with antimicrobial activity have greater solubility in solvents like ethanol, compared to hexane, for example (Cowan, 1999). Similarly, the results obtained agree with those found by Rojas et al. (2006) in which the ethanolic extract has antimicrobial activity in comparison with hexane extract, confirming the fact that the latter did not have activity at the concentration tested.

In general, aqueous and ethanolic extracts demonstrated inhibitory activity regarding all strains tested in smaller concentrations when compared to ethyl acetate extract, agreeing with Yiğit et al. (2009), who reported antimicrobial activity for ethanolic and aqueous extracts from *P. armeniaca* against Gram-negative and gram-positive bacteria and yeast as *C. albicans*.

With respect to antioxidant activity, it should be noted that the IC_{50} values are inversely related to the percentage of DPPH sequestration, since the higher the rate of sequestration, the lower IC_{50}, establishing a relationship between the values (Table 5).

The results of the antioxidant activity, expressed as IC_{50}, showed no significant difference between the synthetic antioxidant (BHT) and aqueous, ethanolic and ethyl acetate extracts; thus, they can be considered excellent antioxidants. On the other hand, there was significant difference ($p<0.5$) when compared to BHT, essential oil and hexanic extract, and no antioxidant activity was detected in these compounds. The same correlation can be observed in relation to the DPPH sequestration percentage. It is worth mentioning that the IC_{50} determines the minimum sample amount needed to reduce the DPPH free radical absorbance by 50%. However, the analysis of antioxidant activity expressed in percentages can underestimate the real potential of the samples.

According to Gao et al. (1999) phenolic compounds such as flavonoids, triterpenes and tannins are excellent antioxidants. These compounds were found in the phytochemical screening of the extracts tested (Table 2). Ethno-pharmacological data have been reported in studies conducted on the genus Prunus regarding the relationship of antioxidant activity and the presence of flavonoids (Nakatani et al., 2000). The values obtained for the DPPH sequestration index-which is similar to those obtained for BHT, aqueous and ethanolic extracts-agree with the data found by Yiğit et al. (2009).

The non-detection of antioxidant activity with respect to the essential oil may be due to the presence of its majoritary compound, that is, benzaldehyde, which features moderate to low antioxidant activity (Thanh and Hoai, 2012).

The genus *Prunus* has economic importance for the food and phytopharmaceutical industries. The literature reports more than 100 patents involving different *Prunus* species in their formulation for multiple purposes: Skin whitening (Pieroni et al., 2004); sunscreens and anti-aging skin care (Sachdeva and Katyal, 2011); essential oils used in the chemical industry (Bachheti et al., 2012); livestock food (Khanal and Subba, 2001); antimalarial treatment (Muñoz et al., 2000); asthma treatment (Karani et al., 2013); and cardiovascular disease prevention (Negishi et al., 2007).

The increased growth of antimicrobial-resistant microorganisms commonly used is one of the most serious threats to the successful treatment of microbial diseases. Thus, the search for products that replace

synthetic antimicrobials, such as essential oils and plant extracts, is increasing primarily because they are associated with the treatment of infectious diseases (Bharathi et al., 2010). Therefore, testing new natural compounds with antimicrobial action is of great value.

Within this context, it is worth mentioning the importance of phytochemical studies, since they confirm the biological activities found. It is also worth noting the importance of preliminary studies to determine the activity of these compounds so that they can serve as the basis for subsequent studies in order to isolate different compounds with antimicrobial activity. The antioxidant activity has to be determined, since the compound has to be both antimicrobial and antioxidant.

In conclusion, the presence of flavonoids and terpenoids, among other metabolites, was detected in aqueous, ethanolic and ethyl acetate extracts. With respect to the essential oil, benzaldehyde was found as the majoritary compound. Regarding antimicrobial activity, microorganisms proved susceptible to aqueous, ethanolic and ethyl acetate extracts, and essential oil, demonstrating the antimicrobial potential of *P. myrtifolia*. With respect to antioxidant activity, the ethanolic, aqueous and ethyl acetate extracts had significant values comparable to those of synthetic antioxidant.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Growth, thermal tolerance and oxygen consumption in rohu, *Labeo rohita* early fry acclimated to four temperatures

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Determination of early life stages of fish for thermal tolerance, optimum temperature for growth, rate of oxygen consumption is becoming essential for aquaculture industry in the climate change scenario due to long-term and short term (seasonal and diurnal) variability in temperature. Emphasis was made to understand the response of early of rohu (0.09 to 0.1 g) to thermal acclimation. Three hundred early fry stage rohu, *Labeo rohita* (initial weight 0.097±0.01 g) were equally distributed at four different temperatures (28, 30, 32 and 34°C) each with three replicates for a period of 40 days. Highest body weight gain was between 30 and 32°C and lowest feed conversion ratio (FCR) was at 30°C. The percentage weight gain and specific growth rate at 30°C were 382±8.01 and 0.88±0.03% respectively, significantly higher than other acclimation temperatures. Thermal tolerance and oxygen consumption rate were analyzed to determine the temperature tolerance limits and metabolic activity at four acclimation temperatures. Critical thermal maxima (CTmax) was 42.86±0.04, 43.3±0.02, 44.45±0.02 and 45.42±0.03; critical thermal minima (CTmin) was 13.07±0.04, 14.35±0.02, 14.92±0.04 and 15.64±0.03 and oxygen consumption rate was 110.75±0.44, 126.57±0.60, 146.22±0.68, 166.47±0.86 mgO_2 $kg^{-1}h^{-1}$ at 28, 30, 32 and 34°C respectively and increased with increasing acclimation temperatures. Oxygen consumption rate for four acclimatization temperatures increased significantly, 110.75±0.44, 126.57±0.60, 146.22±0.68, 166.47±0.86 mgO_2 $kg^{-1}h^{-1}$ at 28, 30, 32 and 34°C respectively. Temperature preference of the early fry of rohu derived from relationship between acclimation temperatures and Q_{10} values for 28 to 30°C, 30 to 32°C, 32 to 34°C were 1.94, 2.05, and 1.91 respectively. The optimum temperature range for growth was 30 to 32°C and Q_{10} value was 32 to 34°C. Survival at different acclimation temperatures was between 98.7±2.31, 96.0±4.0, 93.3±2.31 and 94.7±4.62%, from lower to higher acclimation temperatures.

Key words: Acclimation temperature, critical temperature, critical thermal maxima (CTmax), critical thermal minima (CTmin), *Labeo rohita.*

INTRODUCTION

Fish inhabiting freshwaters are exotherms and cannot regulate body temperatures through physiological means (Moyle and Cech, 2004) as their body temperatures are very identical to the environment they inhabit. Temperature affects fish physiology in terms of thermal tolerance, growth, metabolism, food consumption,

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reproductive success and ability to maintain internal homeostasis due to variability in external environment (Fry, 1971). Temperature tolerance differs with species, acclimation temperature, acclimation duration and salinity (Ficke et al., 2007; Das et al., 2004; Das et al., 2005; Diaz et al., 2007; Manush et al., 2004). Laboratory quantification of lower and upper temperatures tolerance of aquatic animals is referred as critical thermal methodology (CTM) (Cowles and Bogert, 1944) in which fish is subjected to a continuous, constant increase or decrease in temperature till near-lethal or lethal endpoint is reached. CTM is the arithmetic mean of the thermal points at which locomotion of fish becomes disorganized and it loses the ability to escape the conditions that would result in death (Cox, 1974; Lowe and Vance, 1955). CTM is an ecologically relevant lethal index because fishes in nature encounter such temperatures either temporally or spatially as acute fluctuations outside of their limits (Brett, 1956; Hutchison, 1976). Acclimation response ratio (ARR) is an index of the magnitude of the thermal acclimation of the organism (Claussen, 1977; Re et al., 2005). In tropical freshwaters diurnal water temperature fluctuations approach their incipient upper thermal limits (United Nations Economic Commission for Asia and the Far East (1972); Irion and Junk (1997). Tropical fishes endure these temperatures (Milstein et al., 2000) but a small increase in temperature (1-2°C) in the region may cause the daily temperature maxima to exceed these limits, particularly for fish that currently exist in thermally marginal habitats (Roessig et al., 2004). In India freshwater aquaculture grew ten-fold from 0.37 million tonnes in the year 1980 to 4.04 million tonnes in 2010, with mean annual growth rate of over 6% (Handbook of fisheries and aquaculture). To sustain the aquaculture production in the climate change scenarios, information on fish physiological responses to various abiotic stress factors would help in better management of fishery and aquaculture resource. CTMax and CTMin are essential parameters of fish exposed to the cold winter and severe summer or drought months.

In the present study *Labeo rohita* early fry stage fish acclimated at 28, 30, 32 and 34°C under laboratory conditions were studied for their critical temperature tolerance limits, growth, feed conversion ratio and oxygen consumption rate.

MATERIALS AND METHODS

Experimental design

L. rohita spawn were procured from Khopoli Fish Farm, Government of Maharashtra and were hatched in CIFA portable hatchery/circular tanks at an ambient temperature of 28°C in the aquaculture laboratory, National Institute of Abiotic Stress Management, Baramati and acclimated for 20 days to recover from transportation stress. Before initiating the experiment 300 uniform sized fry were equally distributed between four treatments (28, 30, 32 and 34°C) with each replicated three times following a completely randomized design, with a stocking density of 25 fry/75 L water. Rearing conditions were kept uniform in the four experimental groups except water temperatures at 28, 30, 32 and 34°C.

Rearing for growth study

The temperatures were maintained at 28°C initially and were gradually increased by 1°C/day to 30, 32 and 34°C and were maintained for 40 days. Fish were fed for 40 day growth study. Photoperoid of 12 h light and 12 h dark was maintained with light exposure from morning 6 to evening 18 h. Dissolved oxygen level were maintained by aeration in all experiments. Ammonia and pH were monitored at regular interval (APHA, 1998) and maintained.

Feed and feeding

During the feeding trail fish were fed with pelleted feed containing 35% crude protein as recommended for *L. rohita* (Renukardhyay and Varghese, 1986). Initially rohu fry were fed twice a day (8 and 20 h) at 10% of body weight, which was determined periodically at ten day interval up to 40 days. Feed waste and fish excreta were removed daily before feeding. Every day, 50% water was exchanged with fresh chlorine free water. Fish were starved for a day prior to the assessment of growth, thermal tolerance and oxygen consumption.

Growth measurements

Growth rate of fish was measured in terms of percentage weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) as given below:

Percentage weight gain = final weight × initial weight/ initial weight*100
Specific growth rate = final body weight-initial body weight/duration of experiment (days) × 100
Feed conversion ratio (FCR) = Feed given (dry weight)/Weight gain (wet weight)
Survival = Number of fish harvested/Number of fish stocked × 100

Oxygen consumption

Rate of oxygen consumption was measured under identical conditions at 28, 30, 32 and 34°C acclimation temperature to estimate significant change in oxygen consumption at acclimation temperatures. Three replicates of six fish from each acclimation temperature were kept individually in sealed 5 L glass chambers. The glass chamber was made airtight after insertion of dissolved oxygen probe. The chamber was placed inside the temperature controlled aquaria at acclimation temperatures and the water was continuously circulated. The aquaria were covered with opaque screen to reduce stress due to visual treatment. The initial and final oxygen content was measured using Eutech cyberscan 600. Oxygen consumption was calculated as:

Oxygen consumption = Final oxygen concentration-Initial oxygen concentration /weight of fish (kg) × Time (H)

Critical thermal tolerance

To estimate thermal tolerance, CTmax and CTmin of rohu fry, randomly selected six fish from each acclimation temperature were transferred to 52 L tank and maintained at acclimation temperatures

of 28, 30, 32 and 34°C. Fish were exposed to a constant increase or decrease of temperature (0.3°C/min) till the loss of equilibrium (LOE), the designated end point for critical thermal maxima (CTMax) and critical thermal minima (CTMin) respectively (Paladino et al., 1980; Beitinger et al., 2000) was observed. The fishes were rescued and recovered from the CTM experiments of the four acclimation temperatures. Beitinger and McCauley (1990) have used the CTM method for analyzing the physiology of stress and adaptation in fish. The acclimation response ratio was calculated as stated by Claussen (1997) by dividing the tolerance change by the total change in acclimation temperature.

Statistical analysis

One way ANOVA was performed using the mean values of all parameters (SPSS, version 16.0). Duncan's multiple range test (DMRT) was carried out for post hoc mean comparisons. Regression analysis was carried out to know the relationship between acclimation temperatures with growth, CTmax, CTmin and oxygen consumption.

RESULTS

Water quality parameters of rearing tanks of four acclimation temperatures were maintained for dissolved oxygen (DO), pH, and ammonia (mg L^{-1}). The DO levels decreased significantly with increasing water temperatures. Hydrogen ion concentration increased with increase in temperatures. Ammonia was monitored for accumulation of toxic nitrogenous waste products from fish metabolism in all acclimation tanks. In these experiments all parameters were maintained at optimum level with only variable of acclimation temperature.

Growth of *Labeo rohita* fry raised at four acclimation temperatures is presented in Table 2. It was observed that at 30°C acclimated rohu fry gained highest body weight (%) along with the highest specific growth rate, followed by 32°C and lowest by 34°C. FCR was significantly different at 30°C than 28, 32 and 34°C. Fry survival at all acclimation temperatures was similar and was not lethal to the rohu fry at the experimental acclimation temperatures. Preferred temperature was estimated using Q_{10} relationship with acclimation temperature which is considered to coincide with optimum temperature for growth (Brett, 1971; Kellog and Gift, 1983). Preferred temperature is the point at which Q_{10} value starts to decrease with increase acclimation temperature (Kita et al., 1996). The final preference temperature for *L. rohita* fry was found to be 30°C based on the Q_{10} value and growth data indicated that optimum temperature range for rohu fry was 30°C. The estimation of Q_{10} suggests the optimal temperature requirements for fish. Highest body weight gain (%) and lowest FCR at 30ºC indicates that temperature of 30°C is optimum for growth in early fry of *L. rohita*.

CTMax and CTMin increased significantly ($p<0.05$) with increasing acclimation temperatures (Table 1). The fish recovered the CTM temperatures completely. The fry at higher acclimation temperature exhibited higher CTMax and CTMin values. At 0.3°C min^{-1} heating and cooling rate, CTMax ranged from 42.86±0.03 to 45.42±0.03 and CTMin ranged from 13.07±0.04 to 15.64±0.03 in 28-34°C acclimation temperatures. Both CTMax and CTMin regression analysis showed a positive relationship to acclimation temperature (CTmax = 30.89 + 0.42 × Acclimation temperature; $P = 0.001$, $r^2 = 0.96$ and CTMin = 1.63 + 0.41 × Acclimation temperature, $P = 0.001$, $r^2 = 0.96$). The average ARR for CTMax and CTMin was 0.43 for early fry of *L. rohita* at the range of 6 degree differential in acclimation temperature. Thermal tolerance polygon for early fry of *L. rohita* was 178.74°C^2 at 28 to 34°C acclimation temperatures used in the experiments (Figure 1).

The increase in oxygen consumption rate was significant to the increase in acclimation temperature ($p<0.05$) (Table 1). Mean oxygen consumption at 28, 30, 32 and 34°C were 110.75±0.44, 126.57±0.60, 146.22±0.68, 166.47±0.86 mg O_2 Kg^{-1} h^{-1} respectively. Q_{10} values were estimated and extrapolated as 1.94 (between 28 and 30°C), 2.05 (between 30 and 32°C) and 1.91 (between 32 and 34°C) (Table 1). The temperature and oxygen consumption regression model established was oxygen consumption = -152.06+9.34 × Acclimation temperature, $P = 0.001$, $r^2 = 0.99$.

DISCUSSION

In the present study early fry stage fish of initial weight 0.09 to 0.11 g were studied at acclimation temperatures of 28, 30, 32 and 34°C. The CTMax and CTMin values get influenced by rate of temperature change, size of fish, condition factor (K) of the fish and water toxicity (Baker and Heidinger, 1996; Beitinger et al., 2000). Similarly, Das et al. (2005) studied thermal tolerance, growth and oxygen consumption at 26, 31, 33 and 36°C acclimated *L.rohita* fry of initial weight 9.8 to 10.36 g which represent advance fry stage or early fingerling stage of *L. rohita*. The CTMax and CTMin (Das et al., 2005) values ranged from 42.33 to 45.60°C and CTMin ranged from 12.0 to 14.43°C which are comparable to CTMax 42.86 to 45.42°C; CTMin values of 13.07 to 15.64°C in 28-34°C acclimation temperatures indicate lesser tolerance to cold temperatures by 1.07 to 1.21°C in the early fry stage. Rohu early fry have less tolerance at cold temperatures than the advanced fry as reported, confirming that thermal exposure affects thermal tolerance of early life stage of fish (Das et al., 2004). A strong relation was observed between acclimation temperature and thermal tolerance (CTM) level.

Oxygen consumption rate (OCR) was between 110.75 to 166.47 mg O_2 kg^{-1} h^{-1} which was almost twofold higher than 58.02 to 93.27 mg O_2 kg^{-1} h^{-1} as observed by Das et al. (2005) suggesting that rohu fry at the early fry stage consumes more oxygen. According to Tom (1998), oxygen requirements per unit weight of fish significantly decline with increasing individual body weight. It has

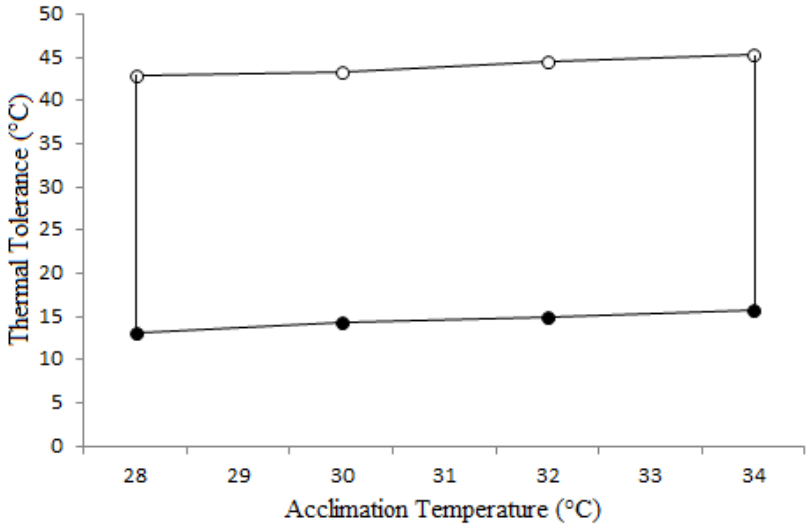

Figure 1. Thermal tolerance polygon of early fry of *Labeo rohita* over four acclimation temperatures (28, 30, 32 and 34°C). The area of thermal tolerance polygon was calculated as 178.74°C^2.

Table 1. Thermal tolerance (CTmax and CTmin), oxygen consumption and Q_{10} value of *Labeo rohita* fry acclimated at four different temperatures (28, 30, 32 and 36°C)

Parameter	Acclimation temperatures (°C)			
	28	30	32	34
CT_{max}	42.86±0.03^a	43.35±0.02^b	44.45±0.02^c	45.42±0.03^d
CT_{min}	13.07±0.04^a	14.35±0.02^b	14.92±0.04^c	15.64±0.03^d
Oxygen consumption (mg O_2 kg^{-1} h^{-1})	110.75±0.44^a	126.57±0.60^b	146.22±0.68^c	166.47±0.86^d
Q_{10} value	1.94 (between 28 and 30°C), 2.05 (between 30 and 32°C), 1.91 (between 32 and 34°C)			

Different superscripts (a,b,c,d) in the same row indicate significant difference ($p<0.05$) amongst different acclimation temperature.

Table 2. Growth parameters and survival of *Labeo rohita* fry reared at four temperatures (28, 30, 32 and 34°C).

Parameter	Acclimation temperature (°C)			
	28	30	32	34
Initial weight (g)	0.10±0.01	0.09±0.01	0.09±0.01	0.11±0.02
Final weight (g)	0.37±0.03^a	0.44±0.02^b	0.37±0.01^a	0.38±0.01^a
Weight gain (%)	286.6±45.6^a	382.0±8.0^b	290.8±13.0^a	270.3±46.4^a
Specific growth rate (%/day)	0.69±0.08^a	0.88±0.03^b	0.68±0.01^a	0.70±0.03^a
Feed conversion ratio	1.32±0.51^a	1.03±0.06^b	1.22±0.09^a	1.76±0.33^a
Survival (%)	98.7±2.31	96.0±4.00	93.3±2.31	94.7±4.62

Superscripts indicate significant differences ($p<0.005$).

been observed that the oxygen-consumption rate of silver carp fry was 5 to 10 times greater than those of summer fingerlings and 15 to 20 times greater than those of 2-year-old fingerlings (NACA, 1989). Regression models though showing positive relation were different due to lower initial weight of rohu early fry for, CTMax (CTMax = 30.89 + 0.42 × Acclimation temperature: CTMax = 41.94 + 1.03 × Acclimation temperature) and CTMin (CTMin = 1.63 + 0.41 × Acclimation temperature: CTMin = 11.01 + 0.86 × Acclimation temperature). Regression model for oxygen consumption (Oxygen consumption = -152.0 + 9.340 × Acclimation temperature; oxygen consumption = 44.40 + 11.59 × Acclimation temperature) differ which may be due to weight dependent oxygen consumption rates of fish. Highest body weight gain and lowest FCR observed at 30°C correspond to the highest body weight

gain and lowest FCR at 31°C as observed by Das et al. (2005). Acclimation response ratio, an index of the magnitude of thermal acclimation of organism (Claussen, 1997) is dependent on geographical temperature gradient (Herrera et al., 1998). It has been observed that tropical species have higher ARR values than temperate regions (Herrera et al., 1998; Re et al., 2005; Rodriguez et al., 1996) which is due to adaptation of a species to fluctuating temperature seasonally and over long terms. ARR differed from that reported by Chatterjee et al. (2004) which may be due to early life stage of fish. Thermal tolerance polygons provide important insights into fish ecology and distribution and are used to identify temperature related survival tactics (Bennett and Beitinger, 1997) and is also a useful comparative index of eurythermicity between species (Eme and Bennett, 2009).

These findings present the impact of rearing temperatures on the early fry stage of *L. rohita*. The early fry stage fish are very susceptible to change in environmental conditions and determination of their thermal tolerances, growth and oxygen consumption may help aquaculture industry to effectively manage fisheries and aquaculture of *L. rohita* for its growth trait.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Characteristics and suitability of some arid soils in Southeastern Iran for wheat cultivation

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A study was conducted to characterize the calcareous soils of the Askara plain in Southeastern Iran and evaluate their potential for wheat production. Sixteen soil profiles within the colluvial fan in different physiographic units were studied. Four phases of soils were identified namely the Ashkara, Dareabad, Khalandi, and Sheikhabad soils. All soils were classified as Entisols, namely Aridic Ustortents and Aridic Ustifluvents. The Ashkara soils were marginally suitable while the Dareabad and Khalandi soils were moderatelly suitable for wheat growing. The Sheikhabad soil units, however, were evaluated as S1, S2, S3 and N1 classes. The results suggested that most important limiting are high $CaCO_3$, pH, texture and salinity as the major constraints to wheat productivity for soils of the Ashkara plain. There are about 60% of the plain that are suitable for wheat growing and only 1.15% that is not suitable due to poor moisture availability. The rest 38.85% of the plains are marginal soils.

Key words: Calcareous soils, wheat cultivation, land suitability evaluation.

INTRODUCTION

The arid and semi-arid regions cover more than 60% of the country. In this agro-pastoral transition region, the rains are highly variable in time, space, amount and duration, and water is the most important limiting factor for biological and agricultural activities. Seasonal changes in rainfall pattern may alter the hydrological cycle and environmental processes (Delitalia et al., 2000) as well as the vegetation and the entire ecosystem (Lazaro et al., 2001; Ni and Zhang, 2000). These areas have low production potential due to the restrictical rainfall (Zeynaddini and Banaei, 2001).

Iran's population increased dramatically during the later half of the 20th century, reaching about 72 million by 2008. Proper land resource ultilization for agriculture is therefore crucial. Part of the solution to the land-use problem can be solved through land evaluation in support of rational land use planning and sustainable use of natural and human resources (Rossiter, 1996). Several land evaluation studies for some important crops in Iran had been reported by Moghimi (2002), Garkani Negad et al. (2009. These authors agreed that for arid and semi-arid lands of Iran, the soil aridity, salinity, acidity and high carbonate content in soils as among the serious limiting factors. Calcareous soils covered vast areas of Iran. The soils in which a high amount of calcium carbonate dominates the problems related to agricultural land

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Figure 1. Location of studied area.

use. They are characterized by the presence of calcium carbonate in the parent material and by a calcic horizon, a layer of secondary accumulation of carbonates (usually Ca or Mg) in excess of 15% calcium carbonate equivalent and at least 5% more carbonate than an underlying layer. In some soils the calcium carbonate deposits are concentrated into layers that may be very hard and impermeable to water. These soils are generally very fertile, alkaline and saline. Due to alkalinity, they are proned to Zn, Fe, Mn and Cu deficiency (Cakmak et al., 1996) and experienced phosphorus (P) deficiency and low P-use efficiency (Korkmaz et al., 2009). Salinity affects plant growth by weakening the plant's ability to absorb water from the soil. Wheat, like many other crops show intraspecific variation in response to salinity (Kingsbury and Epstein, 1986; Parida and Das, 2004) and physical properties of soil (Bagherzadeh, 2013). The present study was to characterize some calcerous soils of the Ashkara plain and evaluate them for their potential in wheat production.

MATERIALS AND METHODS

Site description

The Ashkara plain occupied about 17600 ha of land and is located about 150 km north of Bandarabbas city in the north part of Hormozgan province of southeast Iran, from 28° 8′ to 28° 11′ North longtitude and 56° 7′ to 56° 11′ East latitudes (Figure 1). The climate is arid with an average annual precipitation and evaporation of about 162 (Figure 2) and 4243 mm, respectively. The moisture and temperature regimes are aridic ustic and hyperthermic (Banaei, 1998). The altitude of the region is 850 m a.s.l.

Sixteen soil profiles within the colluvial fan in different physiographic units were dug and studied. The morphological properties of the profiles were described in the field using the field book of USDA (2003). The four representative profiles were internationally classified using the criteria of soil taxonomy (Soil Taxonomy, 2010) and their approximate classes in the IUSS working group WRB (2007) world reference base for soil resources. Two famillies and four phases of soils were identified. Soil samples were taken from pedogenic horizons or layers of the profiles for various laboratory analysis.

Laboratory analysis and soil classification

Soil samples were air-dried in the laboratory ground and sieved through a 2 mm sieve. The percentage gravel content was calculated on the basis of subsamples (500 g each) of whole samples. Particle-size distribution was determined after the removal of $CaCO_3$ with 2N HCl and organic matter with 30% H_2O_2 by the pipette method (Day, 1965). Organic carbon was measured by Walkly and Black (1947) procedure and total N by the micro-kejeldal technique (Bremner and Mulvaney, 1982). The soil pH was determined in a saturated paste by a glass electrode (McLean, 1982). The electrical conductivity (EC) was measured in the saturated extract (Salinity Laboratory Staff, 1954). The calcium carbonate equivalent (lime) was measured by acid neutralization (Allison and Moodi, 1965). Cation exchange capacity (CEC) was determined using sodium acetate (NaOAc) at pH 8.2 (Chapman, 1965). The complexometric titration method described by Chapman (1965) was used for the determination of calcium and magnesium. Potassium and sodium were determined from ammonium acetate leachate using the flame photometer. The sodium adsorption ratio (SAR) was calculated from soil solution data (Na^+, Ca^{2+}, Mg^{2+} given in $cmol_c kg^{-1}$): $SAR = Na^+[(Ca^{2+}+Mg^{2+})/2]^{-0.5}$. Available P was

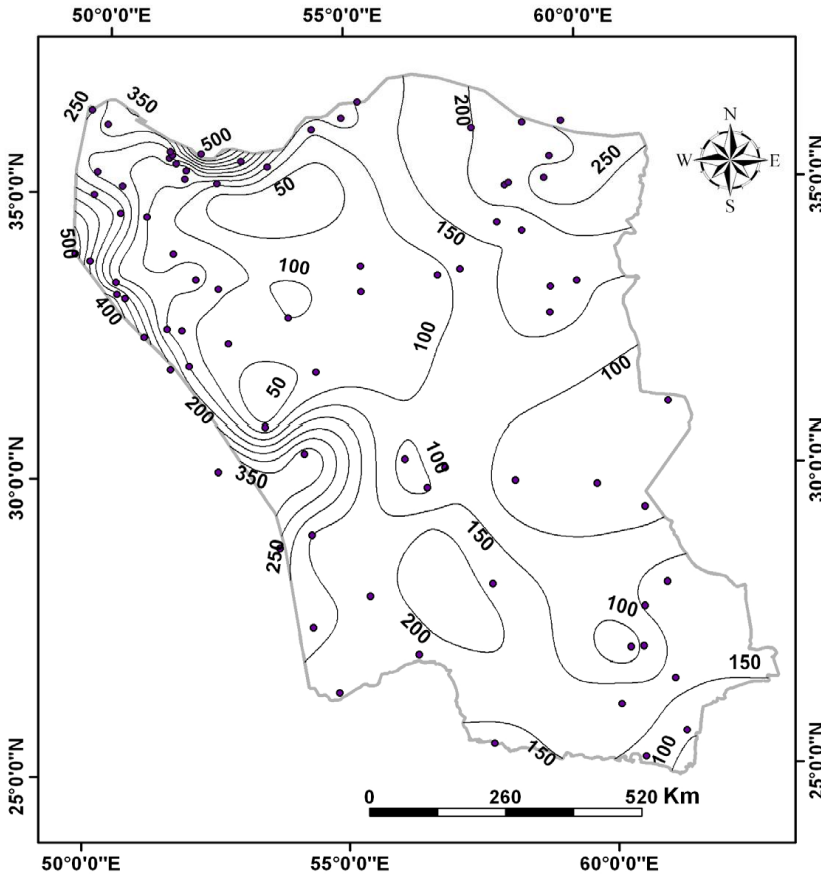

Figure 2. Mean annual precipitation over the study area during 1965-2000.

extracted by the Bray method and determined colorimetrically (Watanabe and Olsen, 1965). For the land evaluation suitability study, the soils were evaluated for wheat following the method of Sys et al. (1991a, b, 1993). Soils were placed in the suitability classes by matching their characteristics with the requirement of wheat (Table 1) using the simple limitation and parametric methods (square root method). In this analysis, the classes S1, S2, S3, N1 and N2 represent highly, moderately, marginally, actually notsuitable but potentially suitable and actually and potentially not suitable, respectively.

RESULTS AND DISCUSSION

Soil morphological properties

Four soil phases were identified, namely the Ashkara, Dareabad, Khalandi and the Sheikhabad soil. The soils were deep but the soil texture changes throughout the profile quite obvious in almost all soils. Since the plain is part of the colluvial fan, the deposition of materials from the alluvial wash vary from time to time creating different textural layers. This is observed in all soils studied where the soils textural changes were quite drastic, particularly for the silt and sand content. There were also variation in their structures and this is also associated to the amount of sand and silt in the soil. The soil tends to be massive when the silt content is high otherwise the structure would be singular when the sand content is high. Gravel was observed in the Ashkara soil but not in other soil types. The rounded nature of these gravels suggested that it was deposited along part of the plain. All soils were weakly developed with only weak profile horizon formation because of the slow chemical weathering in these normally dry and hot soils.

The Ashkara soil had brown (10YR4/3) loamy sand to sandy texture and were gravelly throughout the profile (Table 2). The gravel were rounded and increased down the profile from 10% at topsoil to 60% at 85 cm depth. Similar trend was observed for the sand content (Table 3). The soil structure was somewhat massive at the top 30 cm but became singular down the profile as sand content increases. The soil was observed to be well drained. The Dareabad soil was characterized by dark brown (7.5YR4/4) loamy topsoil to more sandy subsoil. The sand content was 90% at 120 cm as compared to only 45% at the surface. The Khalandi soil had brown (10YR5/3) sand on top coming down to pale brown(10YR6/3) at the subsoil. Their textures were somewhat irregular changing from sandy to sandy loam,

Table 1. Climate, soil and land requirements for wheat production (Sys et al., 1993).

Land, soil and climate characteristics	S1	S2	S3	N1	N2
Climate					
Mean tem. of the growing cycle (°C)	12-23	23-10	8-25	-	>30, <8
Mean tem. of the vegetation stage (°C)	6-18	6-4,18-24	4-2,24-28	-	<2, >28
Mean tem. of the flowering stage (°C)	18-12,18-26	12-10,26-32	10-8,32-36	-	<8, >36
Mean tem. of the ripening stage (°C)	20-16,20-24	14-12,30-36	12-10,36-24	-	<10, >42
Average daily min. tem. Coldest month (°C)	<8	>8	8-19	-	-
Average daily max. tem. Coldest month (°C)	<21	<21	>21	-	-
Physical soil characteristics (s)					
Texture	Si,SiC,SiL,CL,L	SCL	SL,LfS	-	Cm,fS,LcS
$CaCo_3$ (%)	3-30	30-40	40-60	-	>60
Soil fertility characteristics (f)					
Apparent CEC (cmol(+)/kg clay)	16-24, >24	<16 (-)	<16 (+)	-	-
pH H_2O	6-8.2	5.6-6,8.2-8.3	5.2-5.6,8.3-8.5	<5.2	>8.5
Salinity and alkalinity (n)					
ECe (ds/m)	0-3	3-5	5-6	6-10	>10
ESP (%)	0-20	20-35	35-45	-	>45

SL = Sandy loam, LS = loamy sand, L = loam, S = sand, SiL = silty loam, Si = silty, CL = clay loam, LfS = loamy fine sand, SCL = sandy clay loam, LcS = loamy coarse sand, SiC = silty clay, S1 = very suitable, S2 = suitable, S3 = marginally suitable, N1 = currently not suitable, N2 = permanently not suitable; tem., temperature.

Table 2. Field morphological description of representative pedons.

Groups of soils	Depth (cm)	Color	Clay	Silt	Sand	Texture	Structure	Consistence	Boundry	Gravels
			%			++	*	+	**	(%)
Ashkara	0-12	10YR4/3	4.3	13.7	82	LS	Ma	VFi	-	10
	12-30	10YR4/3	4.2	11.8	84	LS	Ma	Fi	Cs	30
	30-60	10YR4/3	3.9	6.1	90	S	Ma-Sin	VFi	Cs	45
	60-85	10YR4/3	3.8	6.2	90	S	Sin	Lo	Cs	35
	85-150	10YR4/3	3.5	6.5	90	S	Sin	Lo	Cs	60
Dareabad	0-30	7.5YR4/4	12	40	48	L	Ma	Fi	-	-
	30-50	7.5YR4/4	14	36	50	L	Ma	Fi	Cs	-
	50-120	10YR4/4	8	22	70	SL	Ma	VFi	Cs	-
	120-150	10YR4/3	6	4	90	S	Ma-Sin	Lo	Cs	-
Khalandi	0-25	10YR5/3	4	8	88	S	Ma	VFi	-	-
	25-55	10YR5/3	6	44	50	SL	Ma	VFi	Cs	-
	55-85	10YR6/3	4	20	76	LS	Ma-Sin	VFi	Cs	-
	85-150	10YR6/3	4	8	88	S	Sin	Lo	Cs	-
Sheikhabad	0-12	10YR5/3	10	60	30	SiL	Ma	Fi	-	-
	12-35	10YR5/3	14	45	32	SiL	Ma	Fi	Cs	-
	35-60	7.5YR3/4	10	56	34	SiL	Pla	Fi	Cs	-
	60-100	7.5YR3/4	10	20	70	SL	Pla	VFi	Cs	-
	100-150	10YR5/3	2	2	96	S	Sin	Lo	Cs	-

Texture++: SL = sandy loam, LS = loamy sand, L = loam, S = sand, SiL = silty loam, Structure* :Ma = massive, Sin = singular, Pla = platy, consistence +: VFi = very firm, Fi = firm, Lo = loose, boundry **: Cs = clear smooth.

Table 3. Chemical properties of representative pedons.

Depth (cm)		Ave.P (ppm)	O.C (%)	SAR	EC (dSm^{-1})	pH H_2O	$CaCO_3$ (%)	HCO^{3-}	$SO_4^{=}$	Cl^-	CEC	K^+	$Ca^{2+}+Mg^{2+}$	Na^+
								$cmol_c kg^{-1}$						
A	0-12	6.7	0.39	1.3	1.3	8.25	25.8	2.2	1.2	2.0	6.5	0.15	3.8	1.9
	12-30	6.5	0.38	1.4	1.2	8.15	32.5	1.6	0.5	2.2	5.2	0.11	2.8	1.6
	30-60	6.3	0.21	2.8	0.9	8.30	25.3	2.6	0.0	1.6	5.0	0.10	1.6	2.6
	60-85	5.9	0.06	3.5	1.1	8.35	25.5	4.2	0.0	3.0	8.2	0.08	3.2	4.5
	85-150	5.5	0.02	4.5	1.1	8.4	27.5	2.2	1.9	2.0	6.9	0.07	1.8	4.3
B	0-30	10.2	0.52	5.9	3.6	7.7	24	6.4	10.5	21.2	42	0.45	17	21.5
	30-50	0.13	0.15	10.3	4.3	8.15	23.8	1.8	20	22.5	48	0.47	15.6	29
	50-120	0.05	0.06	9.4	1.9	8.17	25.8	2.0	8.9	8.8	24	0.24	5.0	15
	120-150	0.02	0.02	5.1	0.8	8.28	25.8	3.4	2.2	3.0	13	0.20	2.8	6.2
C	0-25	6.3	0.10	1.2	1.5	8.0	28	3.7	0.0	1.8	9.1	0.44	3.0	2.5
	25-55	4.6	0.13	2.5	1.2	8.4	30.3	2.3	2.4	1.6	10.4	0.25	3.4	3.3
	55-85	0.05	0.07	2.6	1.6	8.4	25	2.0	0.0	5.5	10.8	0.15	3.6	3.4
	85-150	0.04	0.11	0.8	1.4	8.3	25.3	0.6	0.8	5.4	11.4	0.16	6.6	1.5
D	0-12	13.1	0.4	10	9.4	8.4	24.0	3.2	24	26.4	64	2.52	21	32
	12-35	3.2	0.1	8.1	5.0	8.05	24.1	2.1	18	28.1	53	1.26	21	26
	35-60	0.04	0.17	2.4	3.6	8.1	24.2	1.8	9.5	12.6	30	1.22	8.8	16
	60-100	0.03	0.17	6.6	3.6	8.1	28.7	1.8	24	15.4	44	1.20	14.2	25
	100-150	0.01	0.07	6.4	1.7	8.0	33.0	1.2	2	9.0	13	1.05	2.4	7

A = Ashkara soil, B = Dareabad soil, C = Khalandi soil, D = Sheikhabad soil.

loamy sand and finally became sandy at deeper depth.

These textures changes were due to the irregular changes of the silt and sand content. The Sheikhabad soil was characterised by grayish brown (10YR5/3) silty loam topsoil coming to dark yellowish brown (7.5YR3/4) sandy loam subsoil.

All the four soils were well drained. The Ashkara, Dareabad and Khalandi soils had massive to singular structure while the Sheikhabad soil was more platy down the profile (Table 2). In all soils, the sand content increases with depth resulting a more loose single structure. The consistency of all soils were also quite similar in their trend where soils were firm at the top but loose at the bottom layers. Soil structures were, however, very weak and unstable.

Soil chemical properties

All soils of the Ashkara plain exhibit some common properties with some variation (Table 3). They were all calcareous in nature containing 23 to 33% of $CaCO_3$ throughout the profile. The average electrical conductivity (EC) values were somewhat variable among the soils studied, placing these soils from slightly saline to saline level. The Ashkara and Dareabad soils were slightly saline with EC values less than 5.0 Ds/m, while some soil units of the Khalandi and Sheikhabad soils were slightly saline to saline with EC values ranging from 3 to 20 Ds/m. The sodium content showed slight variability among soils studied. The Ashkara and Khalandi soils contain less than 4.5 $cmol_c kg^{-1}$ of sodium as compared to

Table 4. Soil classification and land suitability evaluation of representative pedons.

Soil classification												
Soil No.	**Soil unit**	**USDA**	**FAO**	**Flooding**	**Texture**	**$CaCO_3$ (%)**	**pH H_2O**	**ECe dSm^{-1}**	**SAR**	**Suitability SLM**	**Suitability PSR**	**Area (%)**
A	1.1	Aridic Ustortents	Calcaric Regosols	F1	LS	27.6	8.3	0.3	2.2	S3s	S3s	7.8
B	2.1	Aridic Ustortents	Calcaric Regosols	F0	SL	24.3	7.7	3.3	7.7	S2s	S2s	12.15
	2.2	Aridic Ustortents	Calcaric Regosols	F0	SL	26.7	8.2	2.3	10	S2s	S2sf	2.55
C	3.1	Aridic Ustifluvent	Calcaric Arenosols	F0	SiL	29.1	8.0	9.4	15	S2n	S2n	3.0
	3.2	Aridic Ustifluvent	Calcaric Arenosols	F0	SL	28.5	8.6	1.2	4.6	S2sf	S3f	5.7
	3.3	Aridic Ustifluvent	Calcaric Arenosols	F0	SL	28.0	8.0	0.7	1.8	S2s	S2s	4.85
	3.4	Aridic Ustifluvent	Calcaric Arenosols	F0	SL	28.8	8.1	8.9	14.2	S2s	S3n	9.4
	3.5	Aridic Ustifluvent	Calcaric Arenosols	F0	L	27.4	8.0	8.8	14.7	S2n	S2n	6.8
D	4.1	Aridic Ustifluvent	Calcaric Fluvisols	F0	SiL	28.4	8.2	3.9	10.7	S1	S2f	3.95
	4.2	Aridic Ustifluvent	Calcaric Fluvisols	F0	L	24.8	8.1	5.2	9.4	S1	S1	4.35
	4.3	Aridic Ustifluvent	Calcaric Fluvisols	F0	SiL	29.1	7.6	12	14.9	S2n	S2n	9.65
	4.4	Aridic Ustifluvent	Calcaric Fluvisols	F0	L	28.6	8.2	3.5	4.4	S1	S1	5.7
	4.5	Aridic Ustifluvent	Calcaric Fluvisols	F0	L	27.6	8.0	17.3	18.9	S3n	S3n	11.75
	4.6	Aridic Ustifluvent	Calcaric Fluvisols	F0	L	27.4	8.3	3.1	4.1	S1	S1	7.5
	4.7	Aridic Ustifluvent	Calcaric Fluvisols	F0	SiL	27.1	8.4	20	19.3	N1n	N2n	1.15
	4.8	Aridic Ustifluvent	Calcaric Fluvisols	F0	L	30.6	8.2	18.2	9.9	S3n	S3n	3.7

SLM, Simple Limitation Method, PSR: Parametric Square Root Method, A = Ashkara soil, B = Dareabad soil, C = Khalandi soil, D = Sheikhabad soil.

Dareabab and Sheikhabad soils containing up to 6.2 cmolckg-1. The exchange capacity (CEC) content represented high variability among the soils. The CEC in The Ashkara and Khalandi soils less than 11.4 cmolckg-1 compared to Dareabab and Sheikhabad soils up to 13 cmolckg-1. All soils have pH values above 8.0 suggesting that they are alkaline and may induce deficiency problems for P and some micronutrients. Soil organic carbon were low in all soils, which is common for soils of these regions where vegetations are strongly influenced by the hask climatic condition.

Based on the soil morphological characteristics and chemical data analyzed, and following the Soil Taxonomy (2010), all soils were classified in the order of Entisol. The Ashkara and Dareabad soils were group as Aridic Ustortent while the Khalandi and Sheikhabad soils were classified as Aridic Ustifluvent. The soils of the Ashkara plain were basically young soils with little pedological development due to the sandy soil materials and little water available to promote weathering.

Suitability evaluation for wheat production

The morphological description and chemical date available suggested that soils of the Ashkara plain were rather sandy, calcerous, alkaline, slightly saline and received very little rainfall throughout the year. Based on these characteristics, these soils were evaluated of their potential for wheat production. Using the conversion table of Sys system (Table 1), the results of the land evaluation for wheat production is presented in Table 4.

The mean annual temperatures of the study site is within 23.7°C, hence they all fall within S1 (Highly suitable) class with reference to tempreture requirement. All soils were not flooded and were well drained and therefore qualified for the S1 class when drainage and flooding were considered. Looking at the textural class as the evaluation criteria, Sheikhabad soils qualified into the S1 class, while the Ashkara, Dareabad and

Khalandi soils into the S3 class. This is because of their loamy sand and sandy loam texture throughout the soil profiles that reduce the soil moisture availability when compared to the silty loam of the Sheikhabad soils. Considering the soil pH and the electerical conductivity values, the Ashkara soils were finally evaluated as S3 class while the Dareabad and Khalandi soils as S2 class. The Sheikhabad soil units, however, were very variable, finally evaluated as S1 (unit 4.1, 4.2, 4.4, 4.6), S2 (unit 4.3), S3 (unit 4.5, 4.8) and N1 (unit 4.7) classes. The evaluation suggested that aridity, alkalinity, high $CaCO_3$ and certain level of salinity as the major constraints to wheat productivity for the soils of the Ashkara plain. The Ashkara soils contain 10 to 30% gravels at top 30 cm depth and this would seriously affect the farm mechanization in the future.

Conclusions

The Ashkara plain soils were sandy, calcerous, high soil pH, saline and received little rainfall annually. These soils, however, were still potentially viable for wheat cultivation. The results of this study indicated that the largest parts of the study area were classified as suitable for wheat cultivation. These soils were evaluated as marginally (S3), moderately (S2) and highly suitable (S1) for wheat cultivation.The results of various methods demonstrated that the most important limiting factors are high $CaCO_3$ and sodium content, high pH and sandy texture. They were also low in organic matter and subsequently little nitrogen available. The high pH level results in unavailability of phosphate and sometimes reduced micronutrient availability such as zinc and iron. There may be also problems of potassium and magnesium nutrition as a result of the nutritional imbalance between these elements and calcium.

The salts or exchangeable sodium hinder crop growth. For efficient crop production salts must be therefore leached from the root zone which is in itself problematic because irrigation water is scarce in most regions where these soils occur. Plant growth is directly affected, as sodium in high concentrations is toxic for most crops, while the dense subsoil and unfavourable physical properties of these soils hinder downward water percolation and the growth of roots. Nutrients contents and nutrient retention are normally low, thus causing a low inherent fertility status for agricultural production. Nutrients are easily leached out of the solum. The poor soil structure also makes the soils very susceptible to wind erosion.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Performance of wheat (*Triticum aestivum* L.) crop under different spacings of trees and fertility levels

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A field experiment was laid out at Pantnagar (Uttarakhand, India) in Mollisols during rabi season (2009-10) to study effect of spacing and fertility levels on growth and yield of wheat (*Triticum aestivum* L.) under different tree species in Terai region. The experiment was conducted in split-split plot design comprising four tree species in main plots, four spacing treatments in sub plots and four fertility levels in sub-sub plot with three replications. The wheat crop var. PBW-502 was sown on November 29, 2009 and harvested on April 21, 2010. Significant higher grain yield was recorded under Poplar (44.60 qha^{-1}); however, it was statistically at par with Melia (42.60 qha^{-1}) inter-phases at 180-60-40 kg NPK ha^{-1} fertility level. At 3 × 2.5 m, the wheat growth, yield attributes and yield (grain, straw and biological yield) under Poplar was significantly higher than closer spacing. Application of 180-60-40 kg NPK ha^{-1} had significant effect on crop growth and grain yield than other levels of fertility. The correlation coefficient (r) studies exhibited that wheat growth and yield attributing characteristics are significantly ($p<0.05$) and positively correlated with each other.

Key words: Tree species, spacing, fertility levels, wheat, growth, yield attributes, yield.

INTRODUCTION

The pressure from increasing population and urbanization, coupled with land degradation and climate change are the major causes for food insufficiency in developing world. Among different approaches to combat this problem, woody perennial based production systems has the great potential. Historically, agroforestry in India involved two distinct pathways, viz., growing food crops in the forests and establishing tree-crop production systems on arable lands. Agroforestry systems not only arrest land degradation but also improve site productivity through interactions among trees, soil, crops, and livestock (Kumar, 2006). This is the most important way to practice agriculture without deteriorating agro-diseases and environmental degradation is highly appreciable (Garrity, 2004).

Wheat (*Triticum aestivum* L.) is the most important food crop under agroforestry system in North Indian states, which accounted 88.31 million tones production in 2011-

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12. In India, it is widely intercropped cereal crop during *rabi* season (November-April) with Poplar, Eucalyptus and other fast growing short rotation tree species in Uttarakhand, Punjab, Haryana, U.P and Bihar states in north-and-parts of central and eastern states of M.P, Chhatisgarh and W.B. The micro-climate under agroforestry is modified by trees, under such conditions;ecosystems. Its role in the light of combating hunger, the growth response of under story wheat crop may be different from sole cropping system. The wheat production technology in Indo-Gangetic plains is well established but it may require some refinement in technology in mixed land-use systems, like agroforestry particularly nutrient management aspect, where wheat is grown in association with trees. Agroforestry systems have more than two components, which makes it productive and complex in nature as well. The fundamental challenge is therefore to develop a farming system that will be adopted by the farmers. The dynamic nature of nutrient cycling is one of the obstacles in nutrient management in agroforestry systems. It dictates that soil nutrient capital useful for supplying nutrients for plant growth must be equated with short to medium-term, rolling capital (the monthly or annual salary), rather than long-term reserves (gold in the bank). The role of organics is varied and complex, the challenge is to use organics of differing quality in combination with inorganic fertilizers to optimize nutrient availability to plants. A systematic framework for investigating the use of inorganic nutrient sources includes assessment of the fertilizer equivalency value for determining optimal use of nutrient sources. The desired outcome is tools that can be used by researchers and farmers for assessing options of using scarce resource for maintaining soil fertility and improving crop yields.

Usually farmers grow multipurpose tree species on their farmlands to meet their requirements. But the selection of tree species, spacing and the fertility levels are very important to reduce negative tree-crop interactions. Reduction in the yield of annuals arises either due to selection of non-compatible agricultural crop or improper tree spacing. Since no proper spacing as yet has been standardized in different agroforestry systems to avoid the adverse effect of trees on growth and yield of intercrops, therefore, there is a need to determine proper tree spacing for intercropping in agroforestry systems.

MATERIALS AND METHODS

The field experiment was conducted at Agroforestry research center, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (29°N Latitude, 79° 30' E longitude and at an altitude of 243.84 masl) during 2009-2010. The plot comprised silty-clay-loam soil with 1.2% of organic carbon, 227, 23 and 230 kg ha^{-1} available nitrogen, phosphorus and potassium, respectively. The site is characterized by a humid sub-tropical, cold and hot dry summers with 1400 mm mean annual rainfall, of which 80 to 90% is received between June and September. The remaining 10 to 20% rainfall is received during wheat-growing season (November to April). Soybean-wheat crop rotation was followed under short rotation fast growing tree species.

Four tree species of T_1: Poplar, S7C20 (*Populus deltoides*), T_2: Eucalyptus, K23 (*Eucalyptus camaeldulensis*), T_3: Leucaena, K636 (*Leucaena leucocephala* and T_4: Melia, Local (*Melia azedarach*) were planted in 2007 with four spacing treatments of S_1: 3 × 1.0 m, S_2: 3 × 1.5 m, S_3: 3 × 2 m and S_4: 3 × 2.5 m. The wheat (PBW-502) was sown with a uniform row-to-row distance of 20 cm. At the time of sowing half dose of N (N doses are varied), 60 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1} were applied. The remaining half nitrogen was applied before first irrigation (crown root initiation stage). Three irrigations were applied to the crop coinciding with crown root initiation (21 days after sowing), late jointing (65 DAS) and milking stage (105 DAS). The crop was fertilized with four treatments of F_0: No fertilizer, F_1: 120-60-40 kg ha^{-1} NPK, F_2: 150-60-40 kg ha^{-1} NPK and F_3: 180-60-40 kg ha^{-1} NPK through urea, diammonium phosphate (DAP) and murate of potash, respectively. The experiment was designed as split-split-plot with tree species in main plots, spacing in sub-plots and fertility levels treatments in sub-sub-plots and treatments were replicated thrice. The area of the net plot was 3 m^{-2} (3 × 1 m) for the wheat crop.

Leaf area was recorded at anthesis (90 DAS) by an area meter and converted to leaf area index (LAI) with dividing total leaf area by land area of the sample. The plant height and the dry matter accumulation were recorded at maturity stage (142 DAS). The number of spikes per meter row length, ear length, spikelets $spike^{-1}$, number of grains $spike^{-1}$ and 1000-seed weight was recorded at physiological maturity. The net plots were harvested to obtain grain/ seed, straw and biological yield. Harvest index was calculated as the ratio of grain to total biological yield.

Data obtained during the course of this investigation, was analyzed by using standard statistical procedure for split-split plot design with the help of computer for analysis of variance (ANOVA) technique (Snedecor and Cochran, 1967). Standard error of mean (SEm±) were computed in each case. The differences among treatments were compared by applying "F" test of significance at 5% probability. Correlation studies (Panse and Sukhatme, 1978) were also performed to study the inter-relationship between various parameters.

RESULTS AND DISCUSSION

Wheat growth

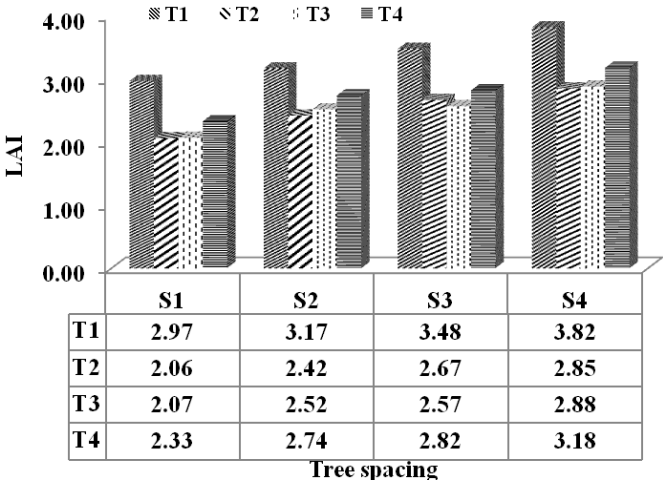
The wheat growth was significantly varied with respect to different tree species at different spacing and fertility levels (Table 1). The leaf area index (LAI) was significantly higher (3.36) under Poplar inter-phases. The LAI increased significantly with widening spacing of all tree species. It was significantly higher (3.18) at 3 × 2.5 m spacing. LAI was significantly increased from 2.30 to 3. 38 and 4.45 as nitrogen dose increases from 120 to 150 and 180 kg ha^{-1}, respectively. The tallest crop individuals were recorded under Poplar (81.6 cm); however, it was statistically at par with Melia (80.4 cm) inter-phases. The wider spacing (3 × 2.5 m) contributed to produce taller (81.9 cm) crop plants. The crop height was significantly higher at plots fertilized with 180-60-40 kg NPK ha^{-1}, whereas, it was statistically at par with 150-60-40 kg NPK ha^{-1} fertilized plots. Dry matter accumulation followed same pattern of the plant height and LAI. The interaction between tree species and fertility level for LAI was also found to be significant, whereas, tree species and their spacing interaction were

Table 1. Effect of tree species, spacing and fertility levels on wheat growth and yield.

Treatments	Growth			Yield attributes					Yield (q ha^{-1})			Harvest Index
	LAI	Plant height (cm)	Dry matter accumulation (g)	Ear bearing shoots m^{-2}	Spike length (cm)	Spikelets spike^{-1}	Grain number spike^{-1}	1000-grain weight (g)	Grain	Straw	Biological	
T1	3.36	81.6	739.4	406.5	8.2	16.9	43.7	36.4	25.6	45.6	71.2	0.34
T2	2.50	72.8	697.8	380.0	5.6	13.4	29.7	33.7	18.7	38.1	56.8	0.31
T3	2.55	77.8	713.9	383.4	5.9	14.7	34.8	34.6	21.1	40.7	61.8	0.32
T4	2.77	80.4	722.9	392.3	6.1	15.2	36.9	35.6	23.4	43.2	66.6	0.33
SEm±	0.030	0.73	4.2	5.0	0.04	0.30	0.78	0.16	0.48	0.38	0.83	0.004
CD $_{0.05}$	0.10	2.5	14.5	17.4	0.2	1.0	2.7	0.55	1.66	1.30	2.86	0.015
CV (%)	7.4	6.5	4.1	8.9	5.0	13.5	14.8	3.1	14.9	6.2	8.9	9.4
S1	2.35	76.1	675.4	377.4	5.7	13.8	29.4	32.7	18.0	37.7	55.7	0.30
S2	2.71	76.2	718.9	383.3	6.3	14.7	35.5	34.1	20.2	39.9	60.2	0.32
S3	2.93	78.3	726.9	392.1	6.7	15.1	37.4	35.6	23.3	43.0	66.2	0.33
S4	3.18	81.9	753.0	409.4	7.1	16.8	42.8	37.9	27.3	47.0	74.4	0.35
SEm±	0.029	0.69	4.9	3.6	0.04	0.27	0.73	0.089	0.42	0.42	0.83	0.003
CD $_{0.05}$	0.08	1.9	13.9	10.2	0.12	0.75	2.1	0.25	1.17	1.17	2.34	0.008
CV (%)	7.1	6.1	4.8	6.4	4.7	12.3	14.0	1.8	13.0	6.9	9.0	6.2
F0	1.04	55.9	545.5	350.3	4.1	9.4	13.4	27.4	9.2	28.9	38.0	0.23
F1	2.30	83.5	719.6	391.5	5.4	15.6	37.9	35.0	17.4	37.1	54.4	0.31
F2	3.38	85.8	773.5	401.8	7.2	17.0	42.5	37.6	24.0	43.7	67.6	0.35
F3	4.45	87.3	835.5	418.6	9.0	18.4	51.2	40.3	38.4	58.1	96.4	0.39
SEm±	0.03	0.60	4.3	3.5	0.04	0.30	0.86	0.14	0.45	0.45	0.90	0.004
CD $_{0.05}$	0.09	1.7	12.6	10.0	0.13	0.88	2.5	0.42	1.32	1.32	2.64	0.012
CV (%)	7.7	5.3	4.2	6.1	4.9	13.9	16.4	2.8	14.1	7.5	9.8	8.9

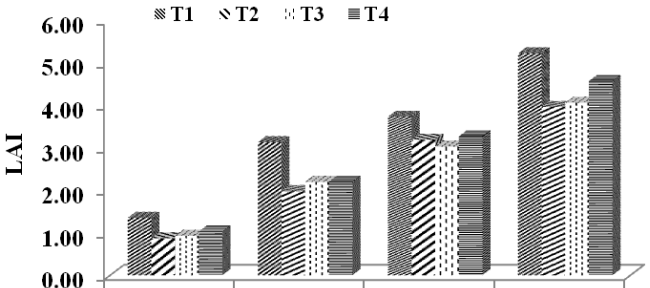
found to be non-significant. The LAI was significantly higher (5.22) under Poplar with 180-60-40 kg NPK ha^{-1} fertility level (Figure 1). The interaction among factors was non-significant for plant height and dry matter production.

The crop growth is mainly affected by light and nutrient availability. Leaf litter inputs from agroforestry trees could provide sufficient nutrients and organic matter to sustain crop growth (Lehmann et al., 2002; Bhardwaj et al., 2005). However, concentrations of foliar nutrients and organic constituents show considerable variation from different tree species. The leucaena added valuable nutritive leaf litter and improved soil properties, which help to improve crop growth. As poplar tree species shed their leaves before sowing of wheat crop, improves soil physic-chemical properties and nullified the negative tree-crop interaction for light. Corroborative findings have also been reported by Bhardwaj et al. (2005), Teklay (2004), Patil et al. (2002); Pant (1993) and Sidhu and Hans (1988). The poor wheat growth showed under eucalyptus due to inhibitory effects of allelochemicals. Similar findings were recorded by Fikreyesus et al. (2011) in the case of tomato; Ahmed et al. (2008); Kaushik and Singh (2001) for agricultural rabi crops. Negative tree-crop interactions for light, moisture and nutrients at closer spacing reduces crop growth rate. At closer spacing tree species



	S1	S2	S3	S4
T1	2.97	3.17	3.48	3.82
T2	2.06	2.42	2.67	2.85
T3	2.07	2.52	2.57	2.88
T4	2.33	2.74	2.82	3.18

Tree spacing



	F0	F1	F2	F3
T1	1.35	3.13	3.73	5.22
T2	0.87	1.98	3.19	3.96
T3	0.93	2.20	3.03	4.06
T4	1.03	2.20	3.27	4.57

Fertility levels

Figure 1. Wheat growth influenced by tree species, spacing and fertility levels.

utilize more resources for their growth and development. Sharma et al. (2000) also reported that close spacing increased amount of leaf litter, which inhibited the crop growth. Corroborative results were reported by Khan and Ehrenerich (1994). Additional supply of inorganic nutrients reduces tree-crop competition for nutrients resulting increase in crop growth. Corroborative results were reported by Ahmed et al. (2002) and Smithson and Giller (2002).

Yield attributes

Ear bearing shoots m^{-2}, spike length, spikelets $spike^{-1}$, grain number $spike^{-1}$ and 1000-grain weight (test weight) was significantly affected by tree species, spacing and fertility levels (Table 1). The ear bearing shoots m^{-2} were highest under Poplar (406.5) followed by Melia (392.3), Leucaena (383.4) and Eucalyptus (380.0) inter-phases. Wider spacing (3 m×2.5 m) and 180-60-40 kg NPK ha^{-1} fertility level recorded higher ear bearing shoots m^{-2}. The spike length was significantly higher under Poplar (8.2 cm) inter-phases at wider spacing (7.1 cm) with 180-60-40 kg NPK ha^{-1} fertility levels (9.0 cm). The spikelets $spike^{-1}$ was followed the same pattern of spike length. The Poplar (43.7) inter-phases at wider spacing (42.8) with 180-60-40 kg NPK ha^{-1} fertility levels (51.2) recorded significantly higher grain numbers $spike^{-1}$. Test weight was significantly higher under Poplar (36.4 g) at wider (37.9 g) spacing with 180-60-40 kg NPK ha^{-1} fertility levels (40.3 g). Poplar with 3 × 2.5 m spacing recorded highest test weight (39.0 g), whereas, statistically at par with Melia with 3 × 2.5 m spacing (38.9g). Leucaena inter-phases recorded highest (40.9 g) test weight, whereas; statistically at par with Melia (40.3 g) and Poplar (40.1 g) at 180-60-40 kg NPK ha^{-1} fertility level (Figure 2).

The yield attributes are mainly depends on the crop growth and significantly affected by tree species, spacing and fertility levels as they affect wheat growth. Similar results were reported by Kaushik and Singh (2001), Sharma

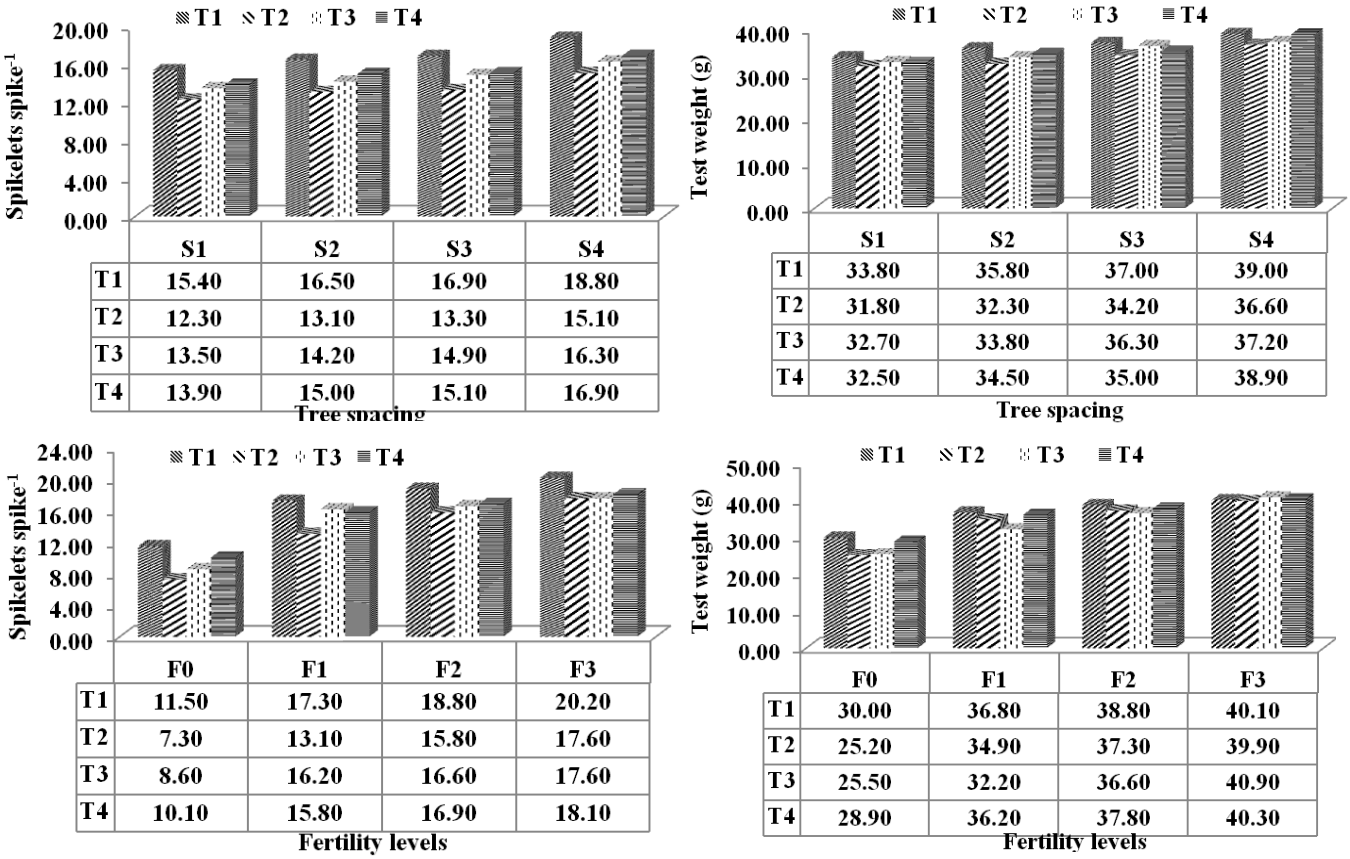


Figure 2. Wheat yield attributes influenced by tree species, spacing and fertility levels.

et al. (2000), Ahmed et al. (2002), Kumar and Rajput (2003) and Smithson and Giller (2002).

Yield

The reduction in grain, straw and biological yield was significantly higher under Eucalyptus inter-phases at closer (3 × 1 m) spacing and without fertilizer applications (Table 1). The grain (25.6 qha^{-1}), straw (45.6 qha^{-1}) and biological (71.2 qha^{-1}) yield were significantly higher under poplar inter-phases. The wider (3 × 2.5 m) tree species recorded higher grain (27.3 qha^{-1}), straw (47.0 qha^{-1}) and biological (74.4 qha^{-1}) yield as compared to closer spacing (3 × 1 m). The response crop to the fertility levels significantly affected grain, straw and biological yield. The crop grain (38.4 qha^{-1}), straw (58.1 qha^{-1}) and biological (96.4 qha^{-1}) yield at 180-60-40 kg NPK ha^{-1} were highest as compared to other fertility levels. Harvest index was significantly higher under poplar (0.34), however it was significantly lower under Eucalyptus (0.31) inter-phases. Wider spacing (3 × 2.5 m) had significantly effect on harvest index (0.35) as compared to 3 × 1 m spacing (0.30). Significant highest (0.39) harvest index was recorded under the plot fertilized by 180-60-40 kg NPK ha^{-1}. Interaction among the factors was significant for crop grain yield. It was significantly higher under Poplar at 3 × 2.5 m (31.10 qha^{-1}) followed by Melia at 3 × 2.5 m (29.40 qha^{-1}) spacing. At Poplar with 180-60-40 kg NPK ha^{-1} fertility levels, grain yield (44.60 qha^{-1}) was significantly higher, followed by Melia with fertilized by 180-60-40 kg NPK ha^{-1} (42.60 qha^{-1}). Highest HI was recorded under poplar with 3 × 2.5 m spacing (0.36) and Poplar inter-phases fertilized with 180-60-40 kg NPK ha^{-1} (0.41) fertility treatment (Figure 3).

The soil physico-chemical properties improvement and lower down the tree-crop competition for resources resulted that an increases in crop growth and yield. Similar findings were reported by Singh and Sharma (2007). During wheat crop maturity (March–April) poplar start sprouting and shade the crop which decreases light intensity and it becomes one of the limiting factors for reduction in wheat grain yield under poplar interspaces as compared to sole crop (Gill et al., 2009). Apart from nutrient and moisture, light is a major limiting factor for crop growth and yield under tree species. Being an evergreen tree species, eucalyptus reduces light availability and decreased crop yield. Corroborative results were reported by Tripathi et al. (2006), Kumar and Rajput (2003), Kaushik and Singh (2001), Fikreyesus et al. (2011), Willey and Holliday (1971) and Ahmed et al. (2008). Sharma et al. (2001) and Khan and Ehrenerich

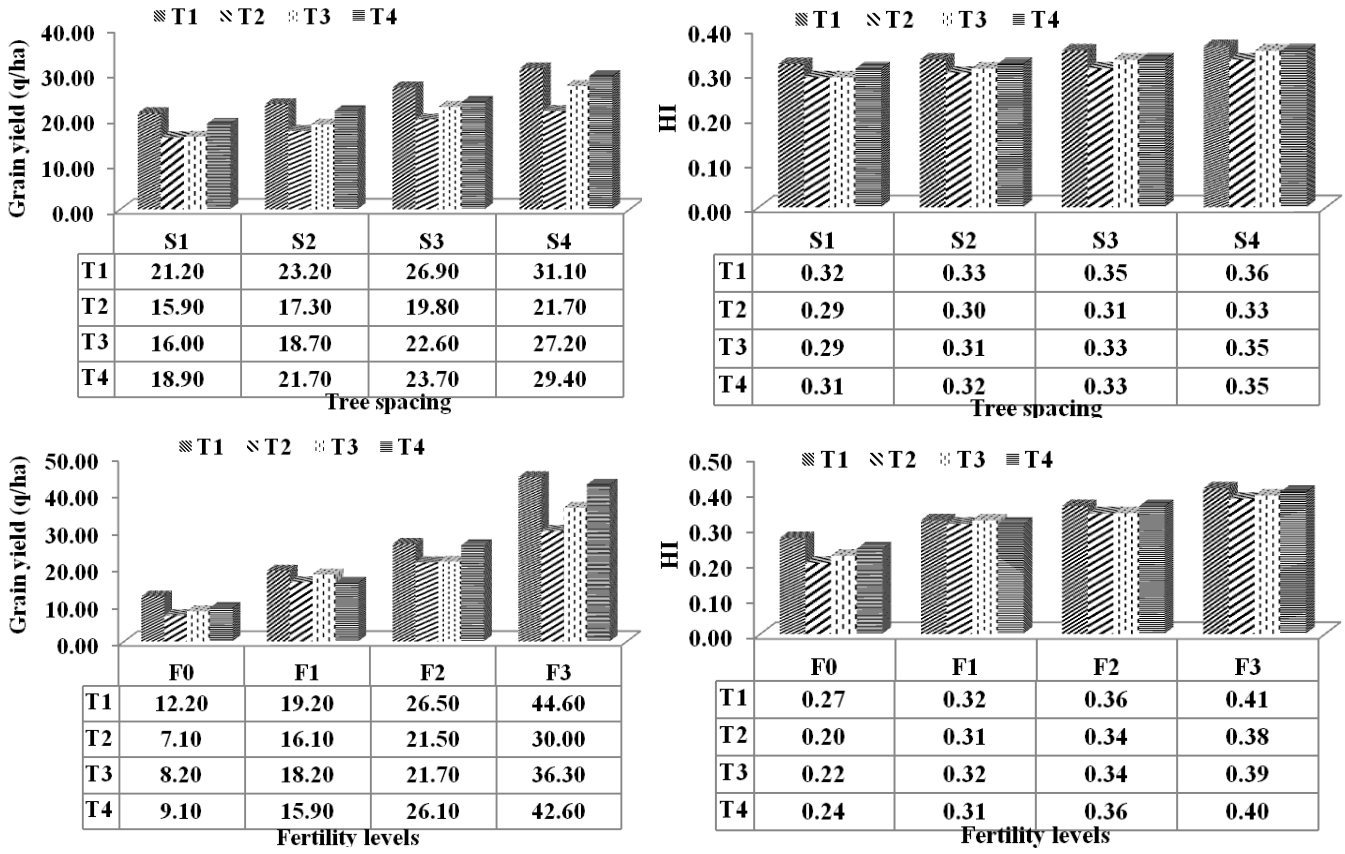


Figure 3. Wheat yield influenced by tree species, spacing and fertility levels.

Table 2. Correlation growth, yields attributes and yield of wheat under agroforestry system.

	2	3	4	5	6	7	8	9	10	11	12
1	0.856** (0.000)	0.960** (0.000)	0.943** (0.000)	0.964** (0.000)	0.937** (0.000)	0.949** (0.000)	0.956** (0.000)	0.972** (0.000)	0.972** (0.000)	0.972** (0.000)	0.851** (0.000)
2		0.945** (0.000)	0.914** (0.000)	0.763** (0.004)	0.966** (0.000)	0.949** (0.000)	0.937** (0.000)	0.785** (0.002)	0.786** (0.002)	0.786** (0.002)	0.826** (0.001)
3			0.947** (0.000)	0.874** (0.000)	0.970** (0.000)	0.976** (0.000)	0.985** (0.000)	0.915** (0.000)	0.914** (0.000)	0.915** (0.000)	0.880** (0.000)
4				0.916** (0.000)	0.981** (0.000)	0.983** (0.000)	0.981** (0.000)	0.919** (0.000)	0.921** (0.000)	0.920** (0.000)	0.864** (0.000)
5					0.891** (0.000)	0.908** (0.000)	0.887** (0.000)	0.939** (0.000)	0.943** (0.000)	0.941** (0.000)	0.757** (0.004)
6						0.995** (0.000)	0.980** (0.000)	0.886** (0.000)	0.888** (0.000)	0.887** (0.000)	0.864** (0.000)
7							0.983** (0.000)	0.911** (0.000)	0.913** (0.000)	0.912** (0.000)	0.844** (0.001)
8								0.930** (0.000)	0.929** (0.000)	0.929** (0.000)	0.902** (0.000)

Table 2. Contd.

9		1.000** (0.000)	1.000** (0.000)	0.816** (0.001)
10			1.000** (0.000)	0.812** (0.001)
11				0.814** (0.001)

** Correlation is significant at the 0.01% level (2-tailed); parenthesis values: Sig. (2-tailed); 1, LAI; 2, plant height; 3, dry matter accumulation; 4, ear bearing shoots per m-2; 5, spike length; 6, spikelets per spike; 7, grains per spike; 8, test weight; 9, grain yield; 10, straw yield; 11, biological yield; 12, HI.

(1994) reported that water use of the system increased up to a distance of 6 m from tree line, causes moisture stress to the crop. The tree and crop components compete for nutrients in simultaneous systems, but competition for growth resources is absent or minimal in sequential systems. Inorganic nutrients application may reduce tree-crop competition for nutrients. Corroborative results were reported by Yajun et al. (2009), Johannes et al. (2002), Pant (1993) and Steiner et al. (2007).

Correlation study

Correlations between growth, yield components and grain yield under four tree species, spacing and fertility treatments were evaluated for the present study (Table 2). The grain yield was positively significant correlated with crop growth parameters and yield attributes.

Straw, biological yield and HI were positively significant correlated with LAI, plant height, dry matter accumulation, ear bearing shoots m^{-2}, spike length, spikelets per spike and grains per spike. Subhani and Chowdhry (2000) and Attarbashi et al. (2002) reported similar data on these lines. Singh et al. (1995), Rana and Sharma (1997) and Deswal et al. (1996) in wheat also supported these findings.

It may be concluded that the nitrogen fixing deciduous tree species with wider spacing improves growth, yield attributes and yield of wheat. Poplar tree species reduces competition for light with crop by shedding their leaves during crop sowing period which may helps to improve soil properties.

The application of inorganic fertilizers also helps to minimize tree-crop competition for nutrients as added nutrients also utilized by fast growing tree species.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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