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Journal of Horticulture and Forestry

Full Length Research Paper

Multiplication of a woody forest gallery species: *Pterocarpus santalinoides* L'Hér. ex DC.

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With climatic change, and despite their protection by the Malian forest code, species characterizing the natural forest galleries of rivers in southern Mali are threatened with extinction because of the development of agricultural parcels. A study of seeds germination and the growth of branch cuttings of *Pterocarpus santalinoides* L'Hér. ex DC. were carried out for multiplication of this typical gallery species. Seeds and branch cuttings were collected from the banks of the river Bagoé. The study revealed that scarification of seeds increases the germination rate (up to 5 times) for *P. santalinoides* L'Hér. ex DC. The branch budding rate and the total number of buds varied with cutting diameter size. The larger the diameter, the better were the results. Studies such as this one might be carried out on other typical forest gallery species for a better understanding of the functioning of these particular ecosystems with a view to identify how best they might be protected and conserved.

Key words: Southern Mali, woody vegetation, forest gallery, diversity, plant multiplication.

INTRODUCTION

In the Sahelian African countries, plant resources play an important role in the survival of populations. Ecosystems are rich with various plant species and constitute a heritage that deserves to be safeguarded. Unfortunately, they are subject to severe degradation due to the combined effects of climatic hazards and human pressure (MEATEU, 2000).

In southern Mali, socio-economic surveys reveal that a reservoir of biological resources is the forest gallery (Arbonnier, 2000), a part of the closed forest formation according to the classification of Yangambi (Letouzey, 1982).

Before the 1970s, forest galleries played an important role in the protection of the banks, in the fight against the evaporation of river waters and were considered as a reservoir of biological diversity in general and plant species in particular. Due to the favorable water conditions, some plant species are encountered exclusively in forest galleries (Yossi, 1996).

During the last decades, forest galleries in the Sudano - Sahelian zone become increasingly at risk because of agricultural development (Karembé, 1996). The forest

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galleries are threatened with extinction in favor of agricultural parcels despite their protection by the Malian forest code. This explains, partly, the denudation and filling of several rivers in Mali (Yossi, 1996).

Mali's vegetation has been the subject of several studies, (PIRT, 1983; Boudet, 1979; Penning de Vries and Djitèye, 1982; Breman and De Ridder, 1991; Yossi, 1996; Dembélé, 1996; Sakiliba, 2006). These studies have sought to characterize the country's soil and vegetation resources, namely pastoral resources, specifically woody, or fallow land. However, none of these studies, to our knowledge, has focused on the plant diversity of forest galleries and their eventual restoration. In order to re-introduce the gallery species for the restoration and conservation of the banks and thus participate in the search for an adequate solution toward limiting the threat of development, a study was undertaken on the biology of plant species in the forest galleries of rivers in southern Mali.

The results presented here focused on the multiplication of *Pterocarpus santalinoides* L'Hér. ex DC. which is of vital importance from both a food and medicinal point of view, (Arbonnier, 2000) and especially for the conservation of the forest galleries. *P. santalinoides* L'Hér. ex DC. is located in the West Tropical Africa (Senegal to Gabon); South America (Paraguay, Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela) and the Guyanas (Caribbean – Trinidad) according to (http://tropical.theferns.info/), accessed on May 29, 2018.

Germination was carried out using scarified and non-scarified seeds. No other pre-treatment was used as in (Amusa, 2011) for Afzelia africana Sm. and in (Pathak et al., 2013) for Psidium guajava L. All seeds were from the same location in comparison to the study reported in (Fredrick et al., 2015) for Faidherbia albida (Delile) A.Chev. The growth of branch was done using various cuttings diameter and height in comparison with the value-year-old tree used as parameter in (Ambebe et al., 2017) for the growth responses of Cordia Africana Lam. We recorded the germination score data in order to assess the effect of scarification on the germination rate of *P. santalinoides* L'Hér. ex DC. The influence of the branch cuttings diameter and height sizes were analyzed on the budding rate and the total number of buds. Seeds germination and growth of branch cuttings of *P. santalinoides* L'Hér. ex DC. were done in natural conditions in order to better understand the behavior of this species typical of galleries in common environments.

MATERIALS AND METHODS

This study was carried out at a site located on latitude 10°03'35''N, longitude 6°50'59''W and altitude 311 m above the sea, in the Guinea savannah with an average annual temperature of 27°C. The average rainfall recorded between 1984 and 2014 in the town Konlondiéba, near to the site, is 1090 mm. Seeds of *P. santalinoides* L'Hér. ex DC. were gathered on August 6, 2012, from the banks of the river Bagoé near the bridge of the village Tiendaga, (Figure 1), using polystyrene bags. The collection did not take into account either the types of formations or the characteristics of the river. Axes were also used to cut the branches of *P. santalinoides* L'Hér. ex DC. in the banks of the river Bagoé on August 6, 2012. Branch cuttings of different sizes were obtained with secateurs and packed in polystyrene bags. An objective of this study was to introduce the species under fairly natural conditions (Figure 1).

Germination experiment

Germination experiments were carried out with scarified and non-scarified seeds that we termed T1 and T2 treatments, respectively. For T1, a knife was used to make a crack on seed shell. For each type of seed, three parcels of land (repetitions) were used and in each parcel of land 10 pots (replicas) each filled with manure. The manure comprised of 6 wheelbarrows of clay soil from the river Sikoro (Bamako) and 4 wheelbarrows of sifted rubbish heap. Each pot contained a single seed and 60 pots were prepared: 30 for T1 and 30 for T2 seeds. The diameter and height of a pot were 8 cm and 20.5 cm, respectively, filled with 1030 cm3 of manure. After installation, the 60 pots were watered with 30 liters of water using a watering can at 8.00am and at 6.00pm during one year and a half. Observations were then made with each new sprout being noted. For each treatment (scarified and non-scarified seeds) the number of sprouts and their occurrence dates were recorded. Measurements were recorded in a Microsoft Excel spreadsheet.

Branch cuttings experiment

The branch cuttings were measured, sorted and stored on the ground under shade of trees two days before the installation. Two principal factors were studied: the cutting height (h=15, 25 and 35 cm) and the cutting diameter (d=1.5, 2.5 and 3.5 cm). We thus have 9 model treatments (permutations) and adopted the randomized complete block design with 4 repetitions leading to 36 plots. In each parcel of land, 10 pots (replicas) were used, leading to a total of 360 branch cuttings used in the experiment. Each branch of *P. Santalinoides* L'Hér. ex DC. is in its own bag filled with manure. The manure was made up as follows: 6 wheelbarrows of clay soil from the river Sikoro (Bamako) and 4 wheelbarrows of sifted rubbish heap. We used a soil designed to encourage the development of young plants derived from cuttings. Each bag had 17.5 cm and 35 cm as diameter and height, respectively, filled with 6020 cm3 of manure. The experiment was followed to observe and note new and dead buds. Figure 2 shows a partial image (shot on April 25, 2013) of the branch cuttings experiment with treatment T9 in the front. For each branch cuttings two values were recorded: presence of bud and the total number of buds. For each diameter 120 (4 repetitions x 3 height sizes x 10 replicas) branch cuttings were made. The data were analyzed to understand the budding of Pterocarpus santalinoides L'Hér. ex DC.

Statistical analysis

The data obtained were categorical count values. We used an exact binomial distribution test for the small size germination data (n=30). The proportion test was used to compare percentages of cuttings group's data. For the larger size cuttings data for which different diameters and/or heights were used (n>50), the normal distribution approximation applies. Hence, we used an analysis of variance (ANOVA) through the linear model framework, in the comparison of branch cuttings data. For the post-hoc analysis, we used the Newman-Keuls procedure with the Student t-test or the

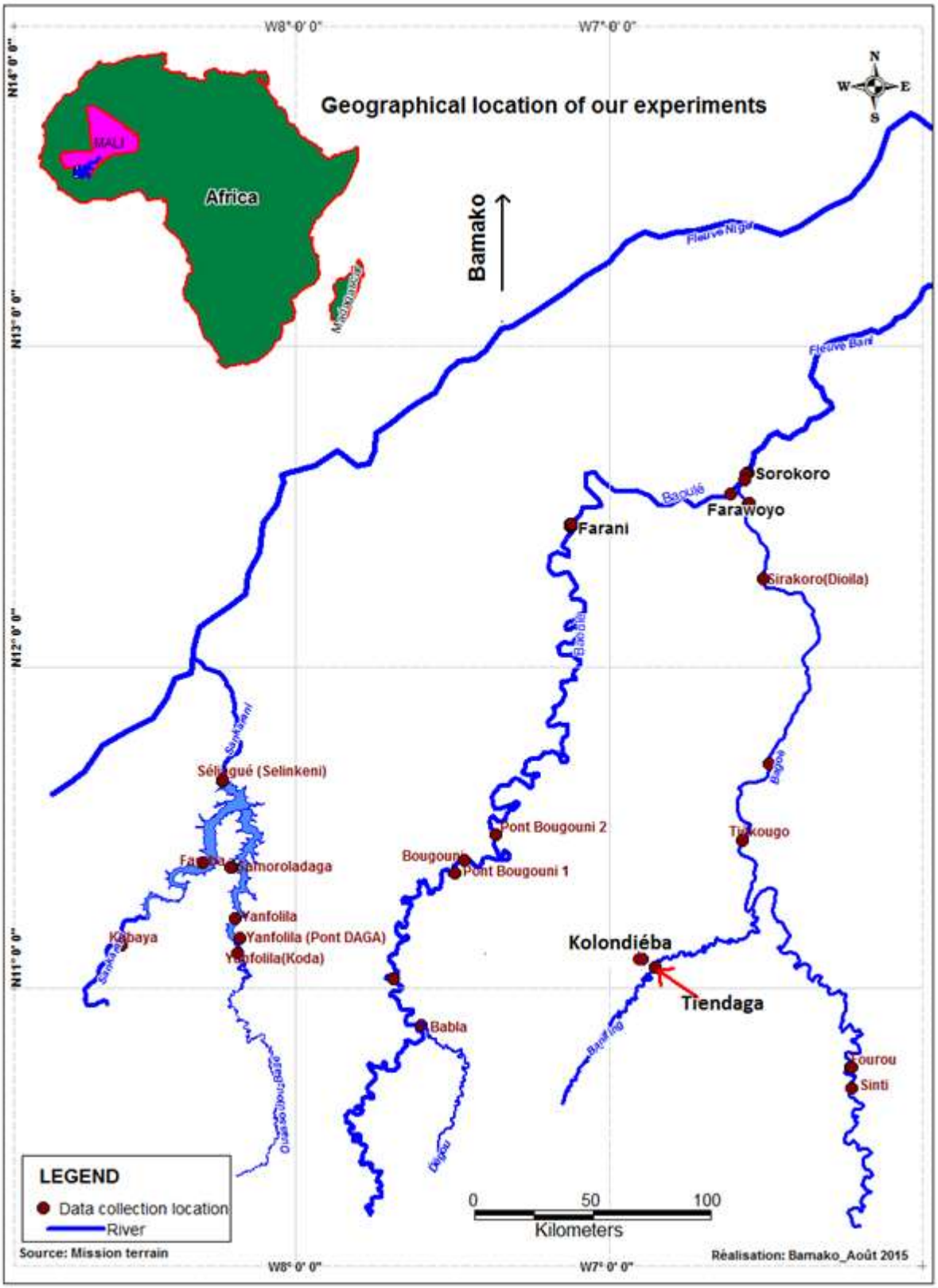

Figure 1. Geographical location of our experiments.

Figure 2. Partial image of the branch cuttings experiment.

proportion test at 5% threshold and the Bonferroni correction. The calculations were performed on the same computer (a laptop equipped with i7-2640M processor, 8GB of RAM, under Microsoft Windows Professional 7) and R version 3.3.3.

RESULTS

Germination

The germination of the seed of *P. santalinoides* L'Hér. ex DC. is of epigean type. The scarified and non-scarified seeds were sown on the same date. Two scarified seeds germinated 13 days after the sowing date, while one non-scarified seed germinated 30 days after sowing. The data obtained are shown in Figure 3. The measurements from the three parcels of land have been grouped for each treatment. A total of 25 scarified seeds germinated compared to 15 for the non-scarified seeds during the observation period of the experiment leading to a germination rate difference of 33.3%. To show the significance of this difference, we modeled the germination of the scarified seeds using a binomial distribution B(n, p) where n = 30 and p = 25/30. Our null hypothesis is: the germination rate for seeds T1 and T2 are the same. The p-value obtained, p-value=2.62E-5, shows a highly significant difference between the germination of the seeds T1 and T2. The odds ratio or cross-product-ratio was computed (Agresti, 2002): 25*15/15*5 = 5. This value means that the odds of success with scarified seeds are 5 times higher than non-scarified seeds. Figure 3 presents the number of sprouts obtained for scarified and non-scarified seeds. This Figure shows a peak on September 8 for the number of sprouts associated with the scarified seeds. The peak for the number of sprouts associated with the non-scarified seeds appears 22 days later. For comparison purpose, the data reported in (Diop et al., 2012) for the germination of shelled seed of the *Jatropha curcas* L. were re-analyzed, the p-value obtained was 0.0078. An odds ratio was also calculated and the value obtained was 3.27.

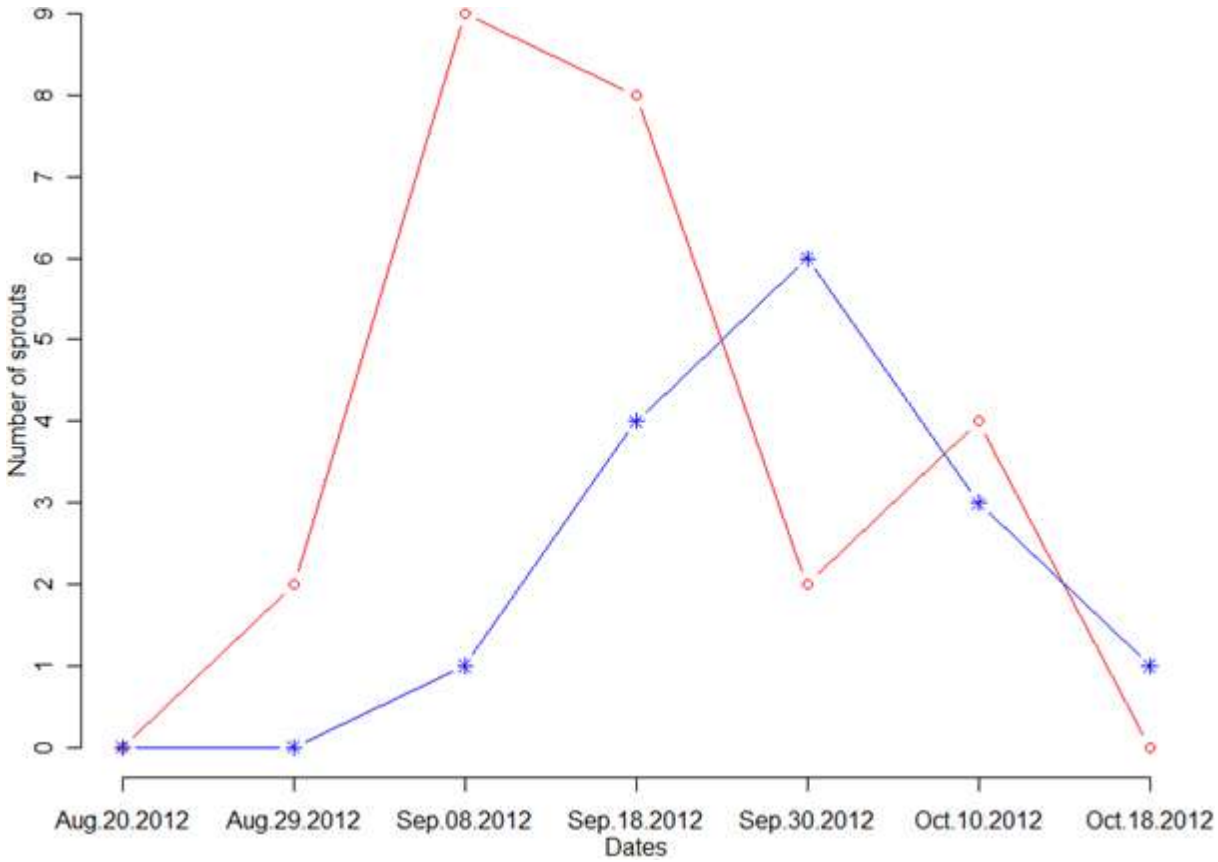

Figure 3. Number of sprouts for scarified seeds (red circle line) and for non-scarified seeds (blue star line) during the experiment observation time.

Branch cuttings

For each branch cuttings, two cases were considered to record data:

(a) The presence of bud and
(b) The total number of buds.

The counting data obtained are recorded in Table 1. In this table, values in column "buds presence" are the total number of branch cuttings having at least one bud. The values in column "buds total" are the total number of buds. The 10 replicas of each treatment were grouped for each repetition. The data in Table 1 were used to test the influence of cutting diameter and height on the budding rate (bud's presence) and the total number of buds observed. An ANOVA was used to test equality of the average presence of buds for the 9 treatments. The p-value obtained, 0.00494, is significant and shows a difference between treatment results. Similarly, an ANOVA was used with the total number of buds. The p-value obtained, 0.000409, is significant and shows a difference between treatment results. We tested separately then together the influence of the diameter and the height on the budding rate on the one hand and on the total number of buds on the other hand.

Influence of the cutting diameter on the budding rate

An ANOVA was used with data in columns "buds presence" and "diameter" of Table 1. The p-value obtained, 0.0105 is significant meaning the budding rate varies with the diameter of the branch cuttings. Using a post-hoc analysis, we obtained two groups: the diameters 1.5 and 2.5 cm forming one and the diameter 3.5 cm the other. The 120 branch cuttings associated with each diameter combined with the values in Table 1 makes it possible to calculate the budding rate per diameter, that is, 30.0, 42.5 and 53.3% respectively for the diameters 1.5, 2.5 and 3.5 cm, respectively. Using the proportion test, we observed a significant difference (p-value=0.0012) between the budding rate for the three branch cuttings diameters. A post-hoc analysis (using proportion test) shows no significant difference (p-value = 0.105) between the budding rates of diameters d1 and d2, while a significant difference (p-value = 0.003) is observed between budding rates of diameters d1 and d3.

Table 1. Number of branch cuttings with buds (Buds presence) and total number of buds (Buds total) for a branch cuttings when the cutting diameter and height vary: d1=1.5 cm, d2=2.5 cm, d3=3.5 cm, h1=15 cm, h2=25 cm and h3=35 cm.

Treatment	Repetition	Diameter	Height	Buds presence	Buds total
T1	R1	d1	h1	5	6
T1	R2	d1	h1	3	5
T1	R3	d1	h1	4	7
T1	R4	d1	h1	4	11
T2	R1	d1	h2	2	4
T2	R2	d1	h2	6	13
T2	R3	d1	h2	3	4
T2	R4	d1	h2	4	10
T3	R1	d1	h3	2	3
T3	R2	d1	h3	0	0
T3	R3	d1	h3	0	0
T3	R4	d1	h3	3	9
T4	R1	d2	h1	2	2
T4	R2	d2	h1	6	8
T4	R3	d2	h1	5	7
T4	R4	d2	h1	4	4
T5	R1	d2	h2	3	4
T5	R2	d2	h2	8	18
T5	R3	d2	h2	5	11
T5	R4	d2	h2	6	10
T6	R1	d2	h3	4	7
T6	R2	d2	h3	4	9
T6	R3	d2	h3	2	6
T6	R4	d2	h3	2	6
T7	R1	d3	h1	5	7
T7	R2	d3	h1	5	7
T7	R3	d3	h1	8	14
T7	R4	d3	h1	6	15
T8	R1	d3	h2	3	7
T8	R2	d3	h2	7	33
T8	R3	d3	h2	6	31
T8	R4	d3	h2	7	23
T9	R1	d3	h3	4	25
T9	R2	d3	h3	3	10
T9	R3	d3	h3	6	22
T9	R4	d3	h3	4	17

From these results, we conclude that cuttings of larger diameter (3.5 cm) have the highest budding rate compared with those of smaller diameter.

Influence of the cutting height on the budding rate

An ANOVA was used with data in columns “buds presence” and “height” of Table 1. The p-value obtained was 0.00895, which means the rate of budding also varies according to the height of the cuttings. Using a post-hoc analysis (t-test), we obtained 2 groups: heights of 15 and 25 cm in one group and height 35 cm forming the other.

The budding rates obtained were 47.5%, 50.0% and 28.3% respectively for the heights 15, 25 and 35 cm. Using the proportion test, significant difference (p-value = 0.00098) was observed between the budding rates associated with cutting heights. The budding rate associated with the cutting height h3 (35cm) is significantly different (proportion test, p-value < 0.0017) to those associated to h1 and d2.

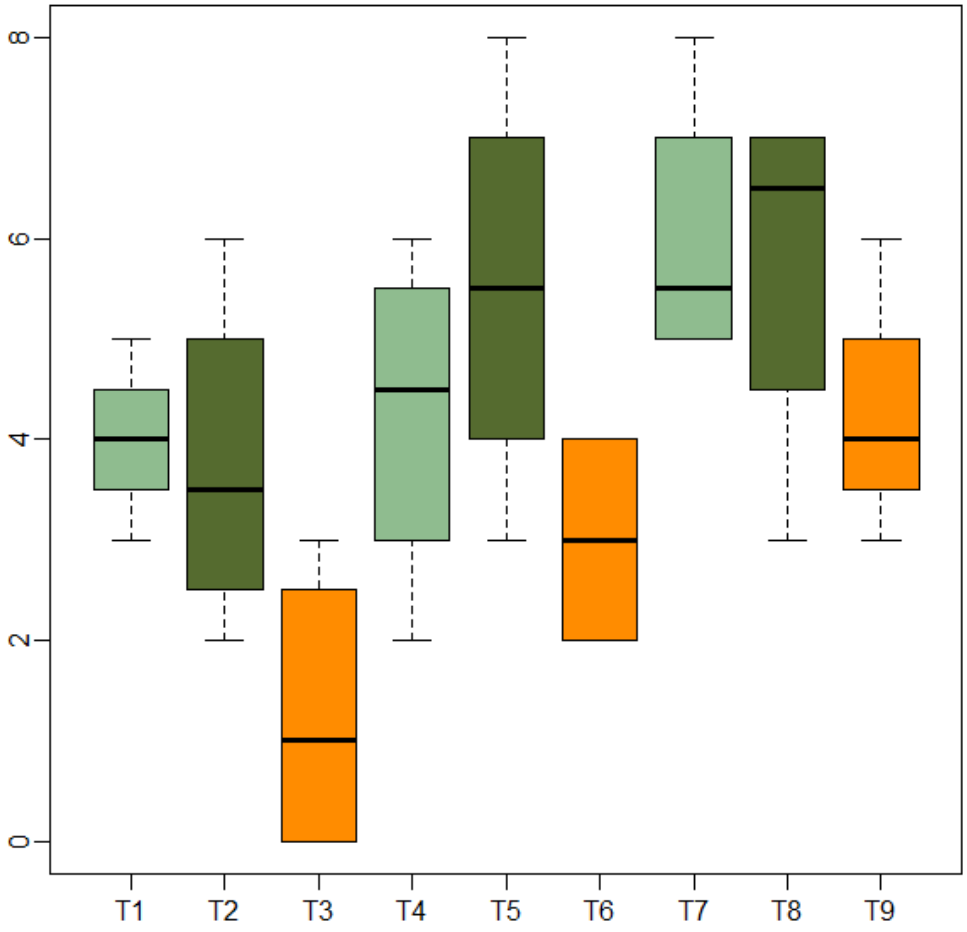

Figure 4. Boxplot of the number of "buds present" for each treatment.

Influence of the cutting diameter and height together on the budding rate

An ANOVA was used to test interaction of cuttings diameter and height on the budding rate. Significant results were obtained for diameter (p=0.004) and for height (p=0.0035) but a non-significant result was obtained (p=0.76) for diameter*height. Positive estimates for model parameters were obtained for diameter (2.0 for d3) and negative estimates for height (-2.75 for h3). Hence, a larger cuttings diameter size increases budding rate while a larger cuttings height penalizes the budding rate. Figure 4 shows the boxplot of the number of the "bud's presence" for each treatment. Notice that height h3 is used in the treatments T3, T6 and T12. Figure 4 shows clearly a less good result for cutting height h3 compared to those obtained using h1 and h2.

Influence of the cutting diameter on the total number of buds

The total number of buds on each cutting was analyzed. We observed a significant difference (ANOVA, p = 0.000136) between the average of the total number of buds when the diameter varies. A post-hoc analysis produced 2 groups: the diameters 1.5 and 2.5 cm in one with average values for the total number of buds equal to 6 and 7.7 respectively; and the diameter 3.5 cm forming the other with a average value for the total number of buds equal to 17.6. We can then state that the total number of buds depends strongly on the diameter of the cuttings.

Influence of the cutting height on the total number of buds

The results obtained with ANOVA reveal no significant difference (p = 0.1447) between the average value of the total number of buds when we vary the height from 15 to 35 cm.

Influence of the cutting diameter and height on the total number of buds

An ANOVA was used to test interaction of diameter and

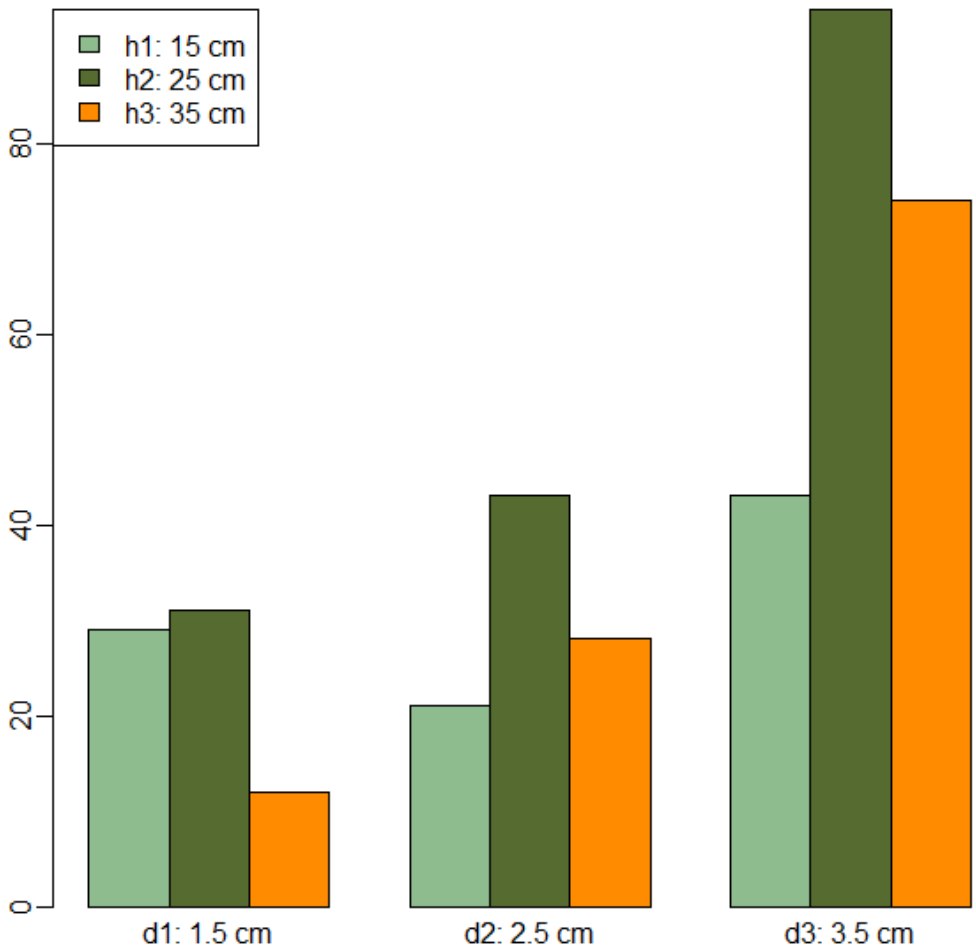

Figure 5. Bar-plot of the total number of buds recorded for diameter-height parameters.

height on the total number of buds. Significant results were obtained for diameter (p=4.93E-5) and for height (p=0.033) but a non-significant result was obtained (p=0.218) for diameter*height. Positive estimate for model parameter was obtained for diameter (3.5 for d3) and negative estimate for height (-4.25 for h3). Hence, a larger cuttings diameter size increases the number of buds and a larger cuttings height acts in opposite direction. The highest model parameter estimate (12.25) was associated with d3*h2. This again revealed the importance of the cutting diameter on the success of the total number of buds. Figure 5 shows the bar plots of the total number of buds for diameter and height sizes. The combination of d3 and h2 seems to be the good choice for multiplication of *P. Santalinoides* L'Hér. ex DC. when branch cuttings are used.

DISCUSSION

Experimentation on the multiplication of *P. santalinoides* L'Hér. ex DC. concerning germination and branch cuttings concludes with the following 3 results. First, scarification has a positive effect on germination rate and emergence rate. This result is comparable with that of Hessou et al. (2009), on the germination of seeds of *Caesalpinia bonduc* (L.) Roxb. in Benin. These authors reported that untreated seeds take longer to germinate (16.4 days), than those removed from their pericarp (13.1 days). It is reported in (Diop et al., 2012), that no significant difference was observed regarding the germination of shelled seeds (87%) and unshelled seeds (73.3%) of the *J. curcas* L. However, a re-analysis of their data show a concordance with present result.

Second, the budding rate is proportional to the diameter of the *P. santalinoides* L'Hér. ex DC. branch cuttings. This result is comparable with that of (Ky-Dembélé et al, 2010) on *Detarium microcarpum Guill* & Perr. where it is reported that in dry environments, budding rates are higher for larger diameter sizes. This results is also comparable with that of (Ky-Dembélé et al., 2016) on the growth of the rooted cuttings of *P. santaniloides* L'Hér. ex DC. This result is also comparable with those of the in situ cuttings of *Lawsonia*

inermis L. (henna) in which different lengths (15 and 40 cm) were used and where the basal cuttings of height 15 cm gave the best recovery rates (Sanogo et al., 2008).

The present result is also comparable with that for Vitex doniana Sweet (Mapongmetsem et al., 2012), where it is shown that whatever the length of the root segment in question, the budding rate of root cuttings of height 10 cm is higher than that of cuttings of height 15 cm. However, the present results contrast with those of (Stenvall et al., 2003), on the Populus hybrid in which budding decreases with increasing diameter.

Third, budding varies with the diameter of *P. santalinoides* L'Hér. ex DC. branch cuttings. This result is consistent with that reported by Detrez (1994), where it is shown that large-diameter cuttings yield larger-sized rejects than small-diameter cuttings. The present results are also comparable with that of (Mapongmetsem et al., 2012), for cuttings of the root segments of Vitex doniana Sweet and which maintain that in 24 months after cultivation, cuttings of 1.1 to 2.5 cm diameter (86.0±7.8 %), performed better than those of 0.5 to 1.0 cm (21±1.8%). It is also consistent with the morphological and growth characteristics of *J. curcas* L. seedlings (Diop et al., 2012). Branch segments close to the stem have a better yield (80%) than the apical segments (50%).

Conclusions

For *P. santalinoides* L'Hér. ex DC. scarification has a positive effect on the germination and emergence rates of seeds. The non-scarified seeds takes longer (30 days), to germinate than those scarified (13 days). Branch cuttings of the species is possible under very experimental conditions and the recovery rate is proportional to the diameter of the cuttings. Budding varies with the diameter of the cuttings. The control of the multiplication of woody species, characteristic of gallery, will permit a better protection of the riverbanks against degradation. The multiplication of woody species characteristic of a gallery should be undertaken so that the emerging results will permit the maintenance, protection and restoration of the galleries within the framework of a sustainable management of the natural resources linked to the rivers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of inorganic NP fertilizers and vermicompost on growth, seed yield and yield components of onion (*Allium cepa* L.) at Maitsebri, northern Ethiopia

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The NP fertilizer or organic resources alone may not provide sufficient amounts or may be unsuitable for alleviating specific constraints to crop production. Therefore, a field experiment was conducted at Maitsebri Research Station of Shire-Maitsebri Agricultural Research Center (SMARC) to study the effect of inorganic NP fertilizers and vermicompost on growth, seed yield and yield components of red onion (*Allium cepa* L.) variety during 2016/17 dry season under irrigation. The treatments consisted of five NP fertilizer rates {0, 25, 50, 75 and 100% of recommended NP rates (69 kg N and 92 kg P_2O_5 ha^{-1})} and four rates of vermicompost (0, 2.5, 5.0, 7.5 t ha^{-1}). The experiment was laid out in a factorial arrangement using randomized complete block design (RCBD) with three replications. Results of the analysis revealed that the interaction effects on inorganic NP fertilizer and vermicompost significantly (P<0.05) affected plant height. Days to bolting, days to 50% flowering, flower stalk diameter and days to maturity were significantly (P<0.05) affected by the main effect of NP fertilizer rates and vermicompost. Similarly, numbers of umbels per plant, umbel diameter, number of seeds per umbel and seed weight per umbel were significantly affected by the main effect of NP fertilizer rates and vermicompost. The highest seed yield per hectare (1462.5 kg ha^{-1}) was obtained from the plants grown at 75% of RDF with vermicompost at 2.5 t ha^{-1} which was about 263% higher than seed yield from unfertilized control plot. It can, thus, be concluded that the combined application of 75% of RDF with vermicompost at 2.5 t ha^{-1}can improve growth, seed yield and yield components of Bombay red onion variety in the study area.

Key words: Growth, onion, NP fertilizers, parameters, seed yield, vermicompost.

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important vegetable crops commercially grown in the world. Seed production is a vital part in onion growing and is highly specialized business. About 9,745.36 tons of onion seed was produced in the world (FAO, 2013). In Asian Vegetable Research and Development Center (AVRDC)

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Africa Regional Program a yield of onion seed 0.5 to 0.6 t ha^{-1} is considered as average. However under favorable conditions, yields up to 2.0 t ha^{-1} are achieved (Chadha et al., 1997). In Ethiopia onion seed yield of average 1.0 to 1.3 t ha^{-1} (Lemma et al., 2006) and also 1.3 - 2.0 t ha^{-1} has been reported (Nikus and Fikre, 2010).

The nutrient management paradigm acknowledges the need for both inorganic NP and organic inputs to sustain soil health and crop production due to positive interactions and complementarities between them (Sanchez and Jama, 2001; Vanlauwe et al*.,* 2002). It is a strategy that incorporates both inorganic NP and organic inputs plant nutrients to attain higher crop productivity, prevent soil degradation, and thereby helps meet future food supply needs (Vanlauwe et al., 2002; Place et al., 2003). This is due to practical reasons as fertilizer or organic resources alone may not provide sufficient amounts or may be unsuitable for alleviating specific constraints to crop production (Sanchez and Jama, 2001). Especially, vermicompost with inorganic NP fertilizers is one of the promising techniques for improving soil fertility and increasing vegetables production (Kachapur et al., 2001; Linus and Irungu, 2004).

Despite increase of area of coverage, the productivity of onion in Ethiopia is much lower than the expected production level. The bottleneck problem for high production and productivity is lack of adaptable high yielding varieties, lack of proper soil fertility management practice and other agronomic practices, diseases and insects etc. The problem of farmers throughout the country is that they have little knowledge on the optimum amount of NP fertilization and advantage of incorporating organic fertilizer, especially vermicompost, with inorganic fertilizer for the production of bulb, as well as seed of onion (Zende et al*.,* 1998; Nikus and Fikre, 2010). Farmers in Tigray, particularly in Northwestern Zone of Tigray also faced problems similar to other farmers in the country. Therefore, the aim of this research was to study the effect of inorganic NP fertilizers and vermicompost on growth, seed yield and yield components of onion under irrigation at Maitsebri, Northern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The field experiment was conducted at the Research Station of Shire-Maitsebri Agricultural Research Center during the dry season of 2016/2017. The experimental site is located 85 km from Shire along the way Shire to Gondar and lies at 13°05'N and 38°08'E and at an elevation of 1,304 m above sea level. The mean annual temperature ranges between a minimum of 15.7°C (November-January) and a maximum of 36.6°C (February-May). It is a low altitude area with an average 5 years annual rainfall of 1,296.5 mm.

Description of the experimental materials

'Bombay Red' onion variety was used for the study. This variety can grow at an altitude of 700-2000 m.a.s.l. and it takes 110-120 days for seed production by bulb-to-seed method (Rahim et al., 1982; EIAR, 2012). Bombay Red is one of the most commonly and widely used variety in Tigray region. The sources of the NP fertilizers were urea (46% N) and Triple Super Phosphate (TSP) (46% P_2O_5) for supplying nitrogen and phosphorus, respectively. The vermicompost was obtained from Shire Soil Research Center.

The treatments comprised of 4 × 5 factorial combinations of vermicompost (0, 2.5, 5.0 and 7.5 t ha^{-1}) and NP fertilizers {0, 25, 50, 75 and 100% of recommended NP rates (69 kg N and 92 kg P_2O_5 ha^{-1})}. The treatments were replicated three times and laid out in a randomized complete block design. Onion bulbs were planted in double rows; the spacing between furrows was kept at 50 cm, between the double rows 30 cm and between plants 20 cm (EARO, 2004).

Experimental procedure and field management

The planting materials collected from Kobo-Alamata were medium sized bulbs of uniform diameter (4-5 cm), which were free from insect, disease and mechanical injuries, twins or split and very large bulbs were discarded. Before planting, the biological material was kept in storage house on wooden shelves for 15 days. For final planting, only the bulbs which were free from diseases, non-early sprouts and non-splitter were selected during the 15 days storage period. Also, prior to planting, one fourth of the bulb tops were sliced off to promote sprouting (Sukprakarn et al., 2005).

The selected bulbs were planted on 10 October 2016, planting time for bulb to seed method most commonly used in Ethiopia. All of the phosphorus fertilizer rates in the form of triple superphosphate (TSP) and vermicompost were applied once along the rows at planting. Urea as nitrogen source was applied in split application where half rate was applied at planting and the other half a month after emergence placed in rows along the onion plants at five centimeters away from the plants and covering with a five centimeters thick soil.

A 2.5 m × 2.8 m (7.0 m^2) gross plot size was used for each experimental unit. There were five double rows per plot and 28 plants in single double row. The blocks were separated by 1.5 m width whereas the space between each plot within a block was 1.0 m. The plots were irrigated as per the recommendation for the area, that is, at the interval of three days during the first phase of active growth of the plant. Later, the irrigation gap was increased to seven days interval (Lemma and Shimelis, 2003). Hoeing was done manually and the field was kept free of weeds throughout the growing period. For the control of diseases, such as purple blotch (*Alternaria porri*) and downy mildew (*Perenospora destractor*), Ridomil Gold 68WP at 2.5 kg ha^{-1} and Thilt 250 EC at 400 ml ha^{-1} were applied. For control of thrips (*Thrips tabaci*), Dimethoate spray at 20 ml 15 L^{-1} water was used (Nikus and Fikre, 2010).

Harvesting was started when over 50% of black seeds were exposed on an umbel and was done by hand three times, as all umbels per plant do not mature at one time due to difference in the stalks flowering. The umbels were dried on canvas and threshed by hand. The seeds were separated from stalks and other debris by winnowing and the chaff seeds were separated from well filled seeds by soaking seeds in water filled in buckets. The floating seeds were discarded as chaffy seeds because they were hollow and unable to sink in water while the sinking ones were considered as well filled and viable. The sinking seeds were dried under shade to 8% moisture content, weighted and recorded as seed weight per plot (Nikus and Fikre, 2010).

Data collection

Data were collected from the entire four double rows excluding the two extreme single rows on both sides. From each double row, two

stalks and per plot also eight stalks were randomly taken for data collection. Growth, seed yield and yield components of onion seed were collected from 8 randomly selected and pre-tagged plants from the four central double rows of each plot:

Days to bolting: This was recorded as the number of days from date of planting up to when 50% of the plants in a plot produced flower stalk.

Days to 50% flowering: This was recorded as the number of days from date of planting up to when 50% of the flower stalks in each plot produced flowers.

Days to maturity: This was recorded as the number of days from date of planting up to when 50% of the plants in each plot matured or ready for harvest (when the seed colour changed to black or the capsule turned brown and started splitting).

Plant height (cm): This refers to the mean height of 8 randomly selected plants from the central rows from each plot. It was measured from the soil surface to the tip of the plant after development of umbels of the plant.

Flower stalk diameter (cm): This was measured for 8 randomly selected plants from the central rows from each plot at flowering stage and the average was calculated to record the parameter.

Number of flower stalks per plant: Numbers of flower stalks of the 8 randomly selected plants per plot from 4 double central rows was counted and the average calculated and recorded as the number of flower stalks per plant.

Number of umbels per plant: Numbers of umbels from the 8 randomly sampled plants was counted and the average calculated and expressed as the number of umbels per plant.

Umbel diameter (cm): This refers to the mean umbel diameter of the 8 randomly sampled plants in each plot. The diameter was measured using a ruler or a caliper two times measuring in two opposite direction (north-south and east to west).

Number of seeds per umbel: Five umbels were randomly taken from the 8 randomly sampled plants in each plot, dried, threshed and then counted to obtain number of seeds per umbel.

Seed weight per umbel (g): Five randomly sample umbels were harvested, dried, threshed to determine seeds weight per umbel and adjusted to a moisture content of 8%; the average weight of seed per umbel was calculated by dividing the total weight of seeds to number of the umbels.

Seed yield (kg ha^{-1}): The yield was estimated from seed yield per plot.

Statistical analysis: All collected data in this study were subjected to analysis of variance (ANOVA) following a procedure appropriate to a randomized complete block design using Gen Stat 14th edition statistical software according to Gomez and Gomez (1984). Mean comparison was done using least significant difference (LSD) at 5% probability level.
The statistical model used for analysis of the data collected from the experimental field is given by:

$$Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}$$

Where:

Y_{ijk} = the response variable
μ = Overall mean
A_i = Effect of factor A (NP fertilizer),
B_j= Effect of factor B (Vermicompost),
ε_{ijk} = Treatment error of factor A (NP fertilizer) and factor B (Vermicompost) and replication as block K.

RESULTS AND DISCUSSION

Days to bolting

Days to bolting was significantly (P<0.05) affected by the main effect of NP fertilizer rates and by vermicompost. Bombay Red onion variety bolted early (about 5 to 8 days earlier) when it was grown at NP fertilizer rate of 50% of RDF compared to the control plots that did not receive NP fertilizer rate (70 days). There was no significant difference among plants treated with 50, 70 and 100% RDF NP fertilizer rates (Table 1).

Similarly, increasing the rate of vermicompost from nil to 5.0 t ha^{-1} was decreasing days to bolt three days (Table 1). Vermicompost can be described as a complex mixture of earthworm feces, which when added to the soil or plant growing media accelerates the development of vegetable crops (Lazcanoa and Dominguez, 2011). It has a positive effect on vegetative growth, stimulating shoot and root development of potato and okra (Edwards et al., 2004; Yourtchi et al., 2013; Mal et al., 2013).

Days to 50% flowering

Days to 50% flowering were significantly (P<0.05) affected by the main effects of NP fertilizers and by vermicompost. The earlier days to 50% of flowering were attained in plants grown at 50, 75, 100 and 25% of RDF NP fertilizer rates with no significant difference among them while the delayed 50% days of flowering was in plants grown with no NP fertilizer application (Table 1).

In agreement with present result, Ali et al. (2007) and Law-Ogbomo and Egharevba (2009) reported that nitrogen and phosphorous fertilizers have enhanced days to 50% flowering. This might be due to metabolic and physiological activities that increase respiration and growth of the plant (Suthar, 2012).
Increasing the rate of vermicompost (VC) from nil to 7.5 t ha^{-1} decreased days to 50% flowering by three days compared to the control with no significant difference among the VC levels (Table 1).

Days to maturity

Days to maturity were other growth parameters of seed onion. The obtained results were shown to be highly and significantly (P<0.01) influenced by the main effects of NP fertilizer. Bombay Red onion variety seed maturity

Table 1. Phenology and growth parameters of onion seed as affected by NP fertilizers and vermicompost.

Inorganic NP fertilizer (% of RDF)	Days to Bolting	Days to 50% Flowering	Days to 50% maturity	Flower stalk diameter (cm)
0	70.42^{c}	90.08^{b}	127.7^{a}	1.233^{c}
25% of RDF	65.17^{b}	86.17^{a}	127.7^{a}	1.348^{b}
50% of RDF	62.50^{a}	83.58^{a}	127.2^{a}	1.463^{a}
75% of RDF	62.83^{a}	84.67^{a}	128.8^{a}	1.503^{a}
100% of RDF	62.75^{a}	84.67^{a}	133.3^{b}	1.473^{a}
LSD (5%)	1.847	2.498	1.970	0.0720
VC (t ha^{-1})				
0	66.07^{b}	87.93^{b}	128.9^{a}	1.290^{b}
2.5	64.60^{ab}	84.93^{a}	128.3^{a}	1.440^{a}
5.0	63.60^{a}	84.87^{a}	128.7^{a}	1.423^{a}
7.5	64.67^{ab}	85.60^{a}	130.1^{a}	1.463^{a}
LSD (5%)	1.652	2.234	NS	0.0644
CV (%)	3.5	3.5	1.8	6.2

Means followed by the same letter in the same columns are not significantly different at 5% probability level according to least significant difference (LSD (5%)). RDF = recommended dose of fertilizer, N = Nitrogen, P = Phosphorus, VC = Vermicompost, NS = non-significant.

Table 2. Plant height (cm) of onion seed as affected by NP fertilizers and vermicompost.

VC (t ha^{-1})	Inorganic NP fertilizer (% RDF)				
	0	25% RDF	50% RDF	75% RDF	100% RDF
0	68.27^{e}	75.53^{d}	74.27^{d}	75.43^{d}	75.40^{d}
2.5	75.73^{cd}	79.67^{bcd}	87.57^{a}	79.37^{bcd}	82.63^{ab}
5.0	74.30^{d}	81.40^{bc}	86.80^{a}	78.23^{bcd}	77.53^{bcd}
7.5	76.77^{cd}	75.50^{d}	77.43^{bcd}	78.40^{bcd}	80.00^{bcd}

LSD (5%) = 4.837; CV (%) = 3.8. Means with the same letter(s) in the table were not significantly different at 5% probability level according to least significant difference. LSD (5%) = least significant difference at P = 0.05, CV (%) = Coefficient of variation in percent, VC = vermicompost, N = Nitrogen, P = Phosphorus, RDF= Recommended Dose of Fertilizer.

was significantly delayed when grown at 100% of RDF NP fertilizer (133.3 days), a delay of 4 to 6 days compared to lower NP fertilizer rates and the control which did not show significant differences among them (Table 1). The delay in maturity at high rates of NP fertilizer application could be possibly due to the fact that those elements affect the supply of carbohydrate during the critical period of reproductive phase through reduction of sugar concentration in the leaves during the early ripening stage and inhibition of the translocation of assimilated products to the seed (Marschner, 1995).

Plant height (cm)

The interaction effect of NP fertilizer and vermicompost had significantly (P<0.05) influenced plant height. Application of 50% of RDF NP fertilizer with 2.5 and 5.0 t ha^{-1}vermicompost had recorded maximum plant height (87.57 and 86.80 cm), which were 27.1 and 28.3% respectively taller than plants that did not receive the NP fertilizer and vermicompost (Table 2). Gonzalez et al. (2001) reported that inorganic fertilizer and organic manure supplied all the essential nutrients at growth stage resulting in increase of measured variables like the plant height. Suthar (2012) concluded that the combination of vermicompost with chemical fertilizer increased the budget of essential soil micronutrients and promotes microbial population, which ultimately promotes the plant growth and production at sustainable basis.

Flower stalk diameter (cm)

Flower stalk diameter was highly and significantly (P<0.01) influenced by the main effect of NP fertilizers as well as vermicompost. Bombay Red onion variety flower stalk diameter was highest (1.503 cm) when grown at

Table 3. Yield components of onion seed as affected by NP fertilizers and vermicompost.

Inorganic NP fertilizer (% of RDF)	Number of flower stalks per plant	Number of umbels per plant	Umbel diameter (cm)
0	5.04[c]	5.04[c]	5.04[b]
25% of RDF	5.45[b]	5.45[b]	5.38[a]
50% of RDF	5.93[a]	5.93[a]	5.68[a]
75% of RDF	5.73[ab]	5.73[ab]	5.69[a]
100% of RDF	5.62[ab]	5.62[ab]	5.58[a]
LSD (5%)	0.349	0.349	0.321
VC (t ha^{-1})			
0	5.22[b]	5.22[b]	5.21[b]
2.5	5.63[a]	5.63[a]	5.48[ab]
5.0	5.73[a]	5.73[a]	5.63[a]
7.5	5.64[a]	5.64[a]	5.59[a]
LSD (5%)	0.313	0.313	0.288
CV (%)	7.6	7.6	7.1

Means followed by the same letter in the same columns are not significantly different at 5% probability level according to least significant difference (LSD (5%). N = Nitrogen, P = Phosphorus, RDF = Recommended Dose of Fertilizers, VC = Vermicompost.

75% of RDF NP fertilizer followed by plants grown at 50 and 100% of RDF NP fertilizer (1.463 and 1.473 cm) respectively, with no statistical difference among them. Plants in the control treatment recorded the least (1.233 cm), but the 25% of RDF NP fertilizer had 9.3% larger flower stalk diameter compared to the control (Table 1). Similar results is also reported by Getachew (2014) who found significantly more flower stalk diameter from NP fertilizer treated plants compared to control plants that did not receive NP fertilizer.

Similarly, vermicompost application at 2.5 to 7.5 t ha^{-1} resulted in 10.3 to 13.4% increment in flower stalk diameter compared to the lowest (1.290 cm) recorded from nil application of vermicompost (Table 1). Vermicompost applications 2.5, 5.0 and 7.5 t ha^{-1} had no significant difference among them on flower stalk diameter. According to Arancon et al. (2003), vermicomposts applied at very low rates 2.5 or 5.0 t ha^{-1} can significantly increase growth parameter of highly valuable vegetable crops such as plant height, flower stock diameter, yield etc due to attributed quality mineral nutrients.

Number of flower stalks and umbels per plant

Significant ($P<0.05$) differences were calculated in both number of flower stalks and number of umbels per plant due to the effect of NP fertilizers and vermicompost application rates. Increasing the application rates of NP fertilizer from 0 to 50% RDF NP fertilizer increased number of flower stack by 17.66%. Increasing the rate of NP fertilizer beyond 50% RDF NP fertilizer did not show any increase in number of flower stalk (Table 3).

Similarly, VC application at the rate of 5.0 t ha^{-1} gave significantly highest number of flower stalk (5.73) per plant compared with plots which did not receive VC, but gave the least number of flower stalks per plant (5.22). No significant differences were established in the number of flower stalk per plant among the VC application rates of 2.5, 5.0 and 7.5 t ha^{-1} (Table 3).

Results on the number of umbels per plant also followed similar trend to the number of flower stalk per plant. The application of NP fertilizer at 50, 75 and 100% RDF had significantly higher number of umbels per plant compared with the no NP application and 25% RDF. Increasing application rates of NP fertilizer from 0 to 50% RDF increased the number of umbels per plant by 17.66% (Table 3). In agreement with the present result, Tamrat (2006) and Rashid and Singh (2000) reported that increase in NP fertilization, increases number of umbels and flower stalks per plant. The finding of Nwadukwe and Chude (1995) show the interaction effects of applied NP fertilizer rates 50-100 N and 50 P_2O_5 kg ha^{-1}; also, an increased number of umbels and flower stalks per plant was noticed.

Similarly, increasing VC application rates from 0 to 5.0 t ha^{-1} gave 9.77% increase in the numbers of umbels per plant. No significant differences have been emphasized in the number of umbels per plant, among the various rates of VC except the nil application (Table 3).

Similar results were also reported that vermicompost stimulate plant flowering, increase the number and biomass of the flowers produced (Atiyeh et al., 2002; Arancon et al., 2008) as well as increase fruit yield (Atiyeh et al., 2000; Singh et al., 2008).

Table 4. Yield components of onion seed as affected by NP fertilizers and vermicompost.

Inorganic NP fertilizer (% of RDF)	Number of seeds per umbel	Seed weight per umbel (g)
0	675.0[b]	2.28[c]
25% of RDF	714.2[b]	2.63[b]
50% of RDF	825.3[a]	2.80[ab]
75% of RDF	897.0[a]	3.02[a]
100% of RDF	860.0[a]	2.97[a]
LSD (5%)	83.0	0.284
VC (t ha^{-1})		
0	724.7[b]	2.51[b]
2.5	792.4[ab]	2.73[ab]
5.0	831.2[a]	2.86[a]
7.5	829.0[a]	2.85[a]
LSD (5%)	74.3	0.249
CV (%)	12.6	12.3

Means followed by the same letter in the same column are not significantly different at 5% probability level according to LSD. N = Nitrogen, P = Phosphorus, RDF = Recommended Dose of Fertilizers, VC = Vermicompost.

Umbel diameter (cm)

Umbel diameter was significantly (P<0.05) affected by the main effect of NP fertilizer and by the vermicompost application rates. The highest umbel diameter (5.69 cm) was obtained with plots that received NP fertilizer at the rate of 75% RDF, which was significantly higher over the control (5.04 cm) but statistically at par with other rates of RDF NP fertilizer application rates. Applying NP fertilizer rates from nil to 75% RDF increased umbel diameter by 12.90% (Table 3).

Vermicompost at 5.0 t ha^{-1}gave significantly highest umbel diameter of 5.63 cm compared with nil application of VC (5.21 cm) and followed by VC rates at 7.5 t ha^{-1} (5.59 cm) and 2.5 t ha^{-1} (5.48 cm) which did not statistically vary among them. Application of 5.0 t ha^{-1} VC gave 8.06% increase in umbel diameter compared with the control (Table 3). This result is in line with that of Rashtbari et al. (2012) who reported that vermicompost significantly increased all the growth attributes such as plant height, umbel diameter, number of leaves, and leaf area index in response to applied municipal solid waste and vermicompost under well-watered, moderate and severe stress conditions.

Number of seeds per umbel

Main effect of NP fertilizer and vermicompost showed significant (P<0.05) differences on the number of seeds per umbel. The highest numbers of seeds per umbel were recorded in plots which received 50, 75 and 100% RDF NP fertilizer rates (825.3, 897 and 860 respectively) compared with unfertilized (675) and 25% RDF NP fertilized plots (714.2). There were no significant differences in the number of seeds per umbel among plots which received 50, 75 and 100% RDF NP fertilizer. The seeds per umbel obtained from 75% RDF NP fertilizer was 32.89% more compared with nil application of NP fertilizers, which was statistically at par with 25% RDF NP fertilizer application rates (Table 4).

This result was similar with the reports of Getachew (2014) who found that the highest number of seeds per umbel (914.6) was recorded from 115 N kg ha^{-1} and 114 P_2O_5 kg ha^{-1} and lowest from control with no significant difference from plants that received 85.5 N and 86.25 P_2O_5 kg ha^{-1}.

Vermicompost fertilized plots at the rate of 2.5, 5.0 and 7.5 t ha^{-1} gave significantly more number of seeds per umbel (792.4, 831.2 and 829 respectively) than VC unfertilized plots (724.7) (Table 4). No statistical differences in the number of seeds per umbel among the VC fertilized plots. The increment in seed per umbel was about 9.3 and 14.70% as VC application rate increased from nil to 2.5 and 5.0 t ha^{-1}, respectively. In agreement with the present result, vermicompost have been shown to increase the number and biomass of the flowers produced (Atiyeh et al., 2002; Arancon et al., 2008) and increase the number of fruits of vegetable crops such as tomato and strawberries (Atiyehn et al., 2000; Singh et al., 2008).

Seed weight per umbel (g)

Seed weight per umbel was significantly (P<0.05) affected by the main effect of NP fertilizer and vermicompost application. The highest significant seed

Table 5. Seed yield (kg) per hectare of onion as affected by NP fertilizer and vermicompost.

VC (t ha^{-1})	Inorganic NP fertilizer (% RDF)				
	0	25% RDF	50% RDF	75% RDF	100% RDF
0	402.8^{h}	454.7^{h}	527.8^{gh}	597.2^{gh}	743.1^{fg}
2.5	553.5^{gh}	865.2^{ef}	1192.3^{bcd}	1462.5^{a}	1189.7^{bcd}
5.0	948.1^{def}	869.3^{ef}	1448.6^{a}	1356.9^{abc}	1145.8^{bcd}
7.5	1050.9^{de}	958.8^{def}	1300.8^{abc}	1385.2^{ab}	1128.5^{cd}

LSD (5%) = 217.4; CV (%) = 13.4. Means in rows and columns with the same letter(s) in each treatment are not significantly different. LSD (5%) = least significant difference at P=0.05, CV (%) = Coefficient of variation in percent, VC = Vermicompost, N = Nitrogen, P = Phosphorus, RDF = Recommended Dose of Fertilizers.

weight per umbel was obtained from plants grown at 75% of RDF NP fertilizer (3.02 g) which was statistically at par with the application of 50% RDF (2.8 g) and 100% RDF (2.97 g) NP fertilizer rates. Significantly small seed weight (2.28 g) per umbel was recorded from nil application of NP and followed by 25% RDF NP fertilizer (2.63 g). The increase in the seed weight per umbel at 75% RDF NP fertilizer applied plots compared with unfertilized plots was about 32.46% (Table 4). The result are in agreement with the study of Getachew (2014) and Ali et al. (2008) who reported that seed weight per umbel was significantly increased by NP fertilizer 115 N kg ha^{-1} and 114 P_2O_5 kg ha^{-1} and 150 N and 80 P_2O_5 kg ha^{-1} application, respectively.

In the same way, plots which received vermicompost at 5.0 and 7.5 t ha^{-1} gave significantly highest seed weight of 2.86 and 2.85 g per umbel respectively, compared with nil vermicompost application (2.51 g) (Table 4).

The increase in seed weight from nil to 5.0 t ha^{-1}vermicompost application was by about 13.94%. No statistical differences have been recorded in seed weight per umbel, between nil and 2.5 t ha^{-1} vermicompost applications and 2.5 t ha^{-1}vermicompost with 5.0 and 7.5 t ha^{-1} vermicompost application rates. The increased seed weight per umbel could be due to the role of vermicompost which is known to contain micronutrients, apart from major nutrients and to contain several plant growth promoters, enzymes, beneficial bacteria and mycorrhizae (Gupta, 2005).

Seed yield per hectare

The interaction effect of 75% RDF NP fertilizer and 2.5 t ha^{-1}vermicompost showed the highest significant seed yield (1462.5 kg ha^{-1}), followed by 50% RDF NP × 5.0 t ha^{-1} and 75% RDF NP × 7.5 t ha^{-1} VC (1448.6 and 1385.2 kg ha^{-1} respectively) with no significant difference among them. The lowest seed yield per hectare was obtained from the plots with no NP fertilizer × no VC (402.8 kg ha^{-1}) followed by 25 to 75% RDF NP fertilizer without VC and no NP fertilizer with 2.5 t ha^{-1} VC, which did not vary significantly (Table 5). Application of 75% RDF NP × 2.5 t ha^{-1} gave 263% seed yield ha^{-1} increment compared with no NP fertilizers and no VC application rates.

The combination of vermicompost with chemical fertilizer increases the budget of essential soil micronutrients and promotes microbial population, which ultimately promotes the plant growth and production at sustainable basis (Suthar, 2012). The current result are in agreement with the work of Alemu et al. (2016) who found that application of N at a rate of 46 kg N ha^{-1}, 92 kg P_2O_5 ha^{-1} and vermicompost from 0 to 5.0 t ha^{-1} increased the total bulb yields of garlic by about 14.29, 20.61 and 9.57% as compared to the untreated control, respectively. In onion bulb production highest yield was obtained with 50% of nitrogen fertilizer with 5.0 t ha^{-1} vermicompost applications (Yohannes, 2015). Similarly, Daniel (2006) showed that highest total yield of potato tuber 29.59 t ha^{-1} were obtained with combination of 75% of RDF NP fertilizer combined with 8.0 t ha^{-1}vermicompost.

Conclusion

The field experiment was conducted to determine the effect of inorganic NP fertilizer and vermicompost on growth, seed yield and yield component of onion (*Allium cepa* L.) under irrigation at Maitsebri, northern Ethiopia. The statistical results revealed that most of the parameters considered were significantly (P<0.05) affected by the main effect of NP fertilizer rates and vermicompost. Besides, the interaction effect of NP fertilizer and vermicompost was significant (P<0.05) on plant height and seed yield per hectare. Thus, the maximum seed yield (1462.5 kg ha^{-1}) at 75% of RDF NP fertilizer with vermicompost at 2.5 t ha^{-1} produced 263% more seed yield. This might be due to the positive and synergetic effect of inorganic NP fertilizers and vermicompost owing to the balanced nutrition, increased yield attributes of onion seed. So, to get better yield and higher economic benefit from onion seed productions, farmers are suggested to use the combination rates of NP fertilizers at 75% RDF × VC at 2.5 t ha^{-1}.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of volumetric soil water content and fertilizer rate on growth and baicalin accumulation in two species of *Scutellaria*

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***Scutellaria* or skullcap is a genus containing approximately 350 species of flowering plants, many of which are sold and marketed for their medicinal value. Flavonoids found in *Scutellaria* spp. have demonstrated anti-inflammatory, antiviral, sedative, antithrombotic and antioxidant affects. Baicalin, a flavonoid produced by *Scutellaria* spp., is an important compound used to treat anxiety. *Scutellaria* spp. have potential as commercially valuable specialty crops based on their visual and medicinal properties; however, a lack of commercial production techniques for successful cultivation of this genus precludes adoption by most growers. The influence of plant production techniques on flowering and baicalin synthesis is undocumented; thus, empirical research is needed for development of commercial production protocol. Objectives of this research were to investigate the effect of nutrient application rate and plant available water on growth and baicalin synthesis in *Scutellaria arenicola* and *Scutellaria integrifolia*, two common species of *Scutellaria* found in eastern United States. To accomplish these objectives, *S. arenicola* and *S. integrifolia* were cultivated in a greenhouse and subjected to one out of four nutrient application rates and one out of two volumetric water content rates. Results demonstrated that synthesis of baicalin, the main flavonoid of the *Scutellaria* genus that contributes to its reported medicinal benefits, occurred in both species. Fertilization rate and volumetric water content were found to influence both plant growth and baicalin concentration in *S. integrifolia*. In contrast to results observed for *S. integrifolia*, volumetric soil water and nutrient application rate did not influence plant growth in *S. arenicola*. *Scutellaria* spp. cultivated in the greenhouse had similar concentrations of baicalin to those harvested from the wild, undisturbed natural habitats. Results from this investigation will assist in development of commercial production protocol for these species and provides the first foundational research that has reported the presence of baicalin, a high value medicinal compound in *S. arenicola*.**

Key words: Skullcap, medicinal, flavonoid, cultivation, production.

INTRODUCTION

Plants of the *Scutellaria* genus are found around the globe and are prized for their medicinal qualities. In many Asian countries, *S. baicalensis* or Baikal skullcap, is prescribed to patients as an anti-inflammatory, antiviral and antithrombotic medicine (Shang et al., 2010). In the United States, *S. lateriflora* or American skullcap, is sold for its reported anxiolytic properties. Commercial sales and marketing for medicinal applications of *S. lateriflora* exist despite the lack of research documenting the presence of medicinally active compounds within the plant (Shang et al., 2010). Medicinal benefits of *Scutellaria* spp. are attributed to flavonoids present in vegetative and root tissue of most species. Flavonoids found in many species of *Scutellaria* include baicalin, baicalein and wogonin (Similien et al., 2016). Baicalin, one of the main flavonoids found in many species of *Scutellaria*, is valued for its anxiolytic effects without any sedative or myorelaxant effects (Liao et al., 2003; Xu et al., 2006). In the United States, skullcap has not been evaluated by the Food and Drug Administration as a medicine and thus has been designated as a herb of "undefined safety" (Awad et al., 2003). Nonetheless, dried skullcap shoots are commonly found in the market as an ingredient in tea, as a vitamin, and as liquid extract. Two species of *Scutellaria*, native to United States, Florida, *S. integrifolia* and *S. arenicola*, possess potential for commercial production; however, neither of them have been evaluated for growth and performance or baicalin content when cultivated within a greenhouse production environment.

Scutellaria spp. containing high concentrations of baicalin had high market value (Similien et al., 2016). Production of plant secondary metabolic compounds, such as baicalin, are believed to be produced by plants to protect plant cellular tissue, especially when plant stress is experienced (Kumar and Pandey, 2013). Plant stress experienced in natural, undisturbed environments are believed to result in greater secondary compound synthesis (Shippmann et al., 2002). When cultivated in protected commercial environments, medicinal plants are thought to produce decreased concentrations of secondary compounds. Relationships between nutrient availability and synthesis of baicalin may be explained by the Carbon-Nutrient Balance Hypothesis (CNBH) (Hamilton et al., 2001). The hypothesis predicts production of secondary metabolic compounds when deficiencies in carbon and nitrogen exist (Hamilton et al., 2001). Flavonoid development within *Scutellaria* spp. has been reported to occur in response to water and nutrient availability. Cao et al. (2012) found that baicalin concentrations in *S. baicalensis* were negatively correlated with nitrogen fertilizer application. Similarly, Similien et al. (2016) observed negative correlations between baicalin concentration and nutrient availability in *S. lateriflora.* Yuan et al. (2012) observed increased flavonoid concentrations in root and leaf tissue of *S. baicalensis* when subject to mild water stress (12% soil water content) as compared to a control that received sufficient water (16% soil water content). These results are in contrast to findings by Similien et al. (2016), who observed increased baicalin concentrations in *S. lateriflora* when irrigation was applied to plants cultivated on open field. When cultivated under 40% shaded conditions, however, field-grown *S. lateriflora* flavonoid concentrations were not significantly influenced by irrigation applications.

Rising popularity and interest in herbal medicines, coupled with the unknown influence of greenhouse production practices on plant growth and synthesis of the flavonoid baicalin within two *Scutellaria* spp., supports the need for research on this specialty crop. The objective of this study was to investigate the influence of nutrient application and plant available water on growth and baicalin synthesis of two native North American skullcap species, *S. integrifolia* and *S. arenicola*, within a protected greenhouse production environment. Results from this experiment can be used to develop cultivation practices for these potentially valuable medicinal plant species.

MATERIALS AND METHODS

Cultivation and preparation

On 6th June 2017, 48 cuttings of *S. integrifolia* were taken from a single stock plant, dipped in indole-3-butyric acid rooting hormone (Hormidin 1; OHP Inc., Mainland, PA, United States) and transplanted into 4 cm^3 rockwool cubes (Grodan, Milton, ON, Canada). Cuttings were placed in propagation chambers under continuous fluorescent lighting (T5; Sunblaster, Langley, BC, Canada), and kept at 25.5 °C and above 50% humidity for three weeks until rooted. On 27 June 2017, individual cuttings were transplanted into 2 L containers filled with a soilless substrate with a composition of 30% pine bark, 48% peat, 10% perlite and 12% vermiculite (Fafard 4P, Sun Gro Horticulture Canada, Ltd.,

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Agawam, MA, United States) and placed on a bench in a 30.5 x 14.6 m gutter-connected greenhouse with 30% light reducing polycarbonate paneling located in Apopka, Florida, United States (latitude 28°38' N, longitude 81°33' W). Environmental conditions within the greenhouse were measured and recorded every 15 min by a data logger (WatchDog 2475; Spectrum Technologies, Inc., Aurora, IL, United States). Forty-eight cuttings of *S. arenicola* were taken on 5 July 2017 and subject to the same propagation methods previously described for *S. integrifolia*. Due to poor rooting of this species, only 32 cuttings of *S. arenicola* were transplanted on 28th July 2017.

To quantify baicalin concentrations from plants found growing in a natural, undisturbed habitat (Lake County, FL, United States), field-harvested samples of *S. integrifolia* and *S. arenicola* were collected in August 2017. Given the commercial popularity of *S. latiflora* as an anxiolytic herbal medicine, samples of *S. latiflora* leaves were purchased from a local herbal medicine market (Leaves & Roots; Orlando, FL, United States).

Fertilization treatment

One week after transplant (WAT) on 5th July 2017, Osmocote 15-9-12 slow release fertilizer (Everris NA Inc., Dublin, OH, United States) was applied to *S. integrifolia* replicates (n=12) at one of the four rates: 0 (none), 3.8 (low), 9.2 (medium) or 13.8 g (high). Fertilizer formula includes 8.4% ammoniacal and 6.6% nitrate nitrogen, 9% phosphate and 12% soluble potash. Fertilizer application rate was selected based on manufacturer's recommendations. Upon transplant into 2 L containers, each plant received 112.5 mL of water daily using a pressure-compensating drip irrigation emitter (Woodpecker Pressure Compensating drippers; Netafim, Fresno, CA, United States). This treatment was repeated for *S. arenicola* on 3rd August 2017.

Volumetric water content treatment

Volumetric water content (VWC) was measured and recorded every 60 min throughout the experiment utilizing soil moisture sensors connected to a data logger (Em50; Decagon Devices, Pullman, WA, United States). Throughout WAT 1 to 3, substrate moisture was maintained among all plants at 56.2% VWC. Three weeks after transplant, half of the plants for each fertilizer treatment (n=6) for *S. integrifolia* were selected at random and subjected to reduced VWC of 9.9% to induce water stress. For *S. arenicola*, half of the plants for each fertilizer treatment (n=4) were selected at random and subjected to reduced VWC of 9.9% 4 WAT on 21st August 2017. An additional week of growth prior to implementation of reduced VWC for *S. arenicola* was provided to allow plants to reach sufficient size.

Measurements and data collection

Beginning from 1 WAT, plant growth indices (PGI = height × $width_1$ × $width_2$) were recorded weekly and continued until termination of the experiment 5 WAT. *S. integrifolia* were terminated 2nd August 2017 (5 WAT) when plants began flowering. Plants were destructively harvested by cutting the plant 2.5 cm above the crown and mass was recorded using a laboratory balance (Adventurer Analytical; Ohaus, Parsippany, NJ, United States). Plants were dried at room temperature until ≥ 70% moisture was lost and dry mass yield (DMY) was recorded. Dried plant material from the same treatment group were combined and vacuum-sealed for storage until ready for baicalin analysis using high performance liquid chromatography (HPLC). *S. arenicola* were also cultivated for five weeks and destructively harvested on 1 September 2017. Plant DMY was recorded and all replicates of each treatment were combined and vacuum-sealed for storage until HPLC analysis could be completed.

Preparation of leaf and stem extracts

Aliquots were prepared by randomly selecting a 0.1 g sample from the previously vacuum-sealed containers. Samples were placed in a test tube (13 × 100 mm Beaded Rim, Pyrex, Corning, NY, United States) and chemical extraction was performed using a prepared 0.005% acetic acid in a methanol : water extraction solution (80:20). 2 mL of extraction solution was added to each test tube and homogenized (Homogenizer 850; Thermo Fisher Scientific Inc., Waltham, MA, United States) at 21,000 rpm for a duration of 1 min. Extracts were placed into a 1.5 mL Eppendorf tube (1.5 mL Graduated Natural; Thermo Fisher Scientific Inc., Waltham, MA, United States) and centrifuged (Eppendorf Centrifuge 5418; Hauppauge, NY, United States) at 10,000 rpm for 10 min. Samples were then filtered through a 0.2 µm filter (25 mm Nylon Syringe Filter; Thermo Fisher Scientific Inc., Waltham, MA, United States) and placed in 2 mL HPLC ready vials (Clear Glass Sure Stop Vial; Thermo Fisher Scientific Inc., Waltham, MA, United States). Four extracts of each treatment combination for *S. integrifolia* were prepared and analyzed. Due to poor growth and lack of plant material, only two samples of each treatment combination for *S. arenicola* were prepared and analyzed. Four samples of field-harvested *S. integrifolia* and *S. arenicola*, and four samples of locally purchased *S. lateriflora* were extracted using the previously described extraction technique.

HPLC analysis

Baicalin concentration analysis was performed using a Dionex UltiMate 3000 HPLC (Thermo Fisher Scientific Inc., Waltham, MA, United States) with a C18 3 µm 3 × 150 mm column (Thermo Fisher Scientific Inc., Waltham, MA, United States). Analysis was performed utilizing the method described by Li et al. (2015). Mobile phase solutions were compromised of 0.01% phosphoric acid aqueous solution (A) and acetonitrile (B). The gradient elution was as follows: 0 to 3.5 min, 27% v/v B; 3.5-6 min, 27-60% B; 6-7 min, 60-40% B; 7-10 min, 40-27%; and then returned to initial conditions for 2 min for a total run time of 12 min. Column temperature was maintained at 30°C, scan wavelength ranged from 220-400 nm, flow rate was maintained at 0.8 mL min^{-1}, and sample injection volume was 1 µL. The wavelength for baicalin absorption, as determined by Li et al. (2015), was 278 nm. Peak detection occurred 1.89 min after sample injection (Figure 1).

Standard calibration

Baicalin standard was purchased from Indofine Chemical Company (Hillsborough, NJ, United States). Solid baicalin standard was dissolved in 100% methanol to achieve a standard concentration of 250 µg mL^{-1}. Standard calibration was achieved by doing serial injection at rates of 1, 5, 10, and 15 µL and resulted in a calibration

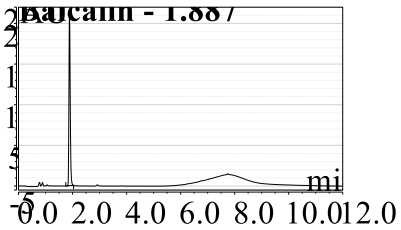

Figure 1. High performance liquid chromatography of baicalin illustrating retention time of 1.887 min.

correlation of r^2=0.99.

Statistical analysis

The experiment was arranged as a complete randomized design with irrigation and fertilizer application rate assigned as independent variables. Six replicates were cultivated for each treatment combination for a total of 48 experimental units for *S. integrifolia.* Four replicates were cultivated for each treatment combination for *S. arenicola*. Statistical analysis of PGI, DMY and baicalin was analyzed using mixed model analysis in JMP® Pro 13 (SAS; Cary, NC) with post-hoc mean separation tests performed using Tukey's honest significant difference test by WAT with variance within treatment combination replicates defined as the random error term. Statistical tests were considered significant if $P \leq 0.05$.

RESULTS

Greenhouse environmental conditions

Mean daily greenhouse temperature ranged from 24.0 to 33.6°C throughout the duration of the experiment. Mean relative humidity was 79.8% and photosynthetic active radiation varied from 59.6 to 311.6 µmol m^{-2} s^{-1}.

Plant growth index

No significant effects were observed for PGI among fertilizer or water stress treatments for *S. integrifolia* between 1 and 4 WAT. Plant growth was minimal between 1-2 WAT; however, beginning from 3 WAT, growth was quadratic (Figure 2). Plants subjected to the medium fertilizer application rate had the highest PGI, between 2 and 5 WAT. At termination of the experiment, 5 WAT, a water stress effect was observed (Figure 3). Least square means of PGI for plants subjected to high VWC were significantly greater (5.8 e^{-3} m^{-3}) for plants that were maintained at a high VWC than those subjected to low VWC (2.6 e^{-3} m^{-3}).

No significant effects were observed for PGI among fertilizer or water stress treatments for *S. arenicola* throughout the duration of the experiment. Moreover, PGI did not increase between between 1 and 5 WAT (data not shown). Plants that received the high fertilizer rates, regardless of water stress treatment, had the highest PGI at termination of the experiment with a least square mean of 4.1 e^{-5} m^3. Plants that received medium rate applications of fertilizer possessed the lowest overall PGI 5 WAT with a least square mean of 2.8 e^{-5} m^3.

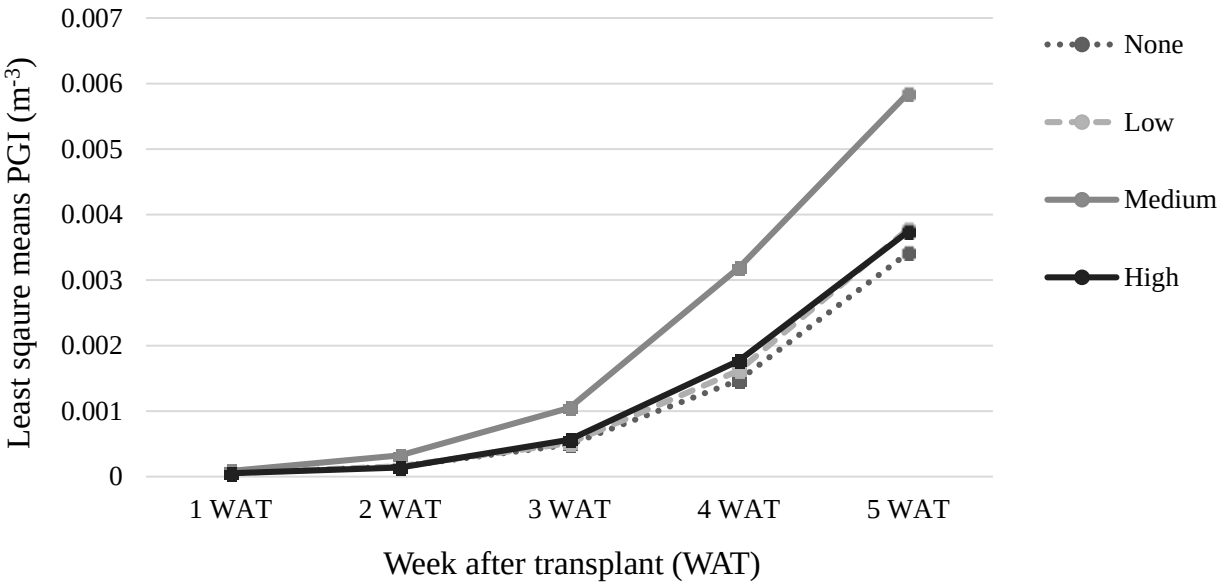

Figure 2. Least square means of plant growth indices (PGI) of *S. integrifolia* (n=12) in response to application of Osmocote 15-9-12 slow release fertilizer (Everris NA Inc., Dublin, OH, United States) applied at one out of four rates: 0 g (none), 3.8 g (low), 9.2 g (medium) or 13.8 g (high).

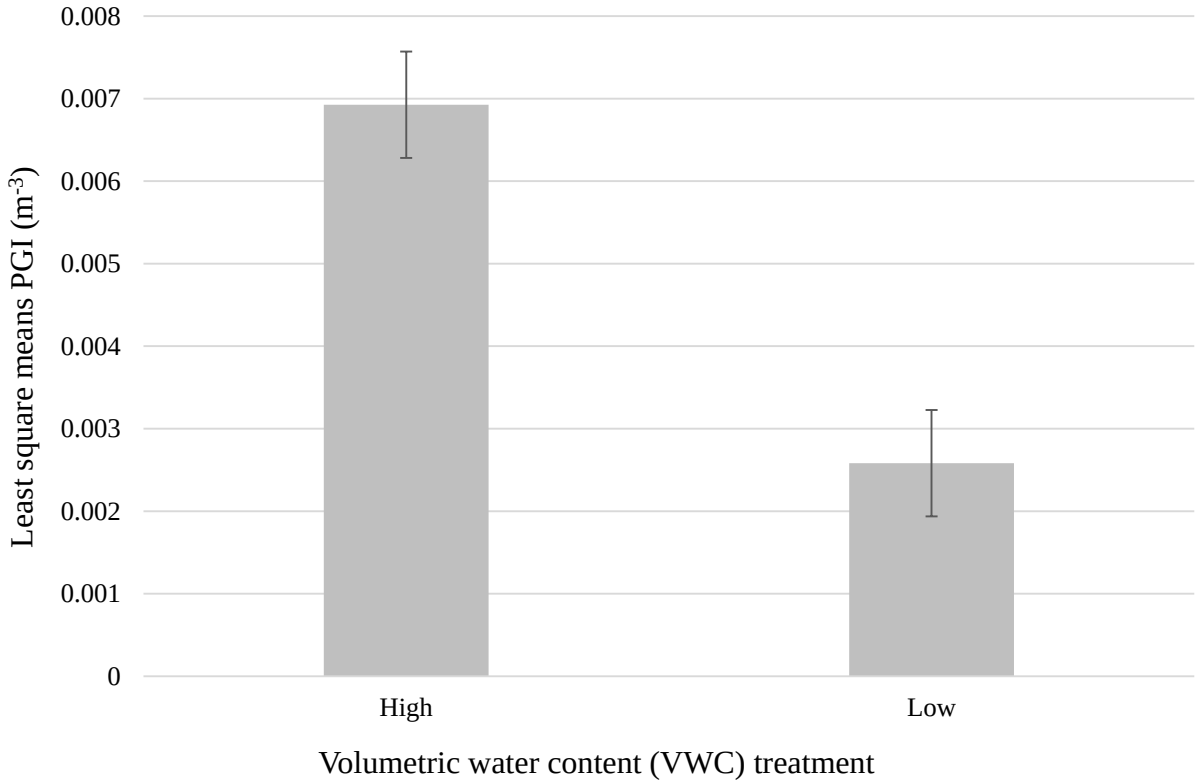

Figure 3. Least square means of plant growth indices (PGI) of *S. integrifolia* (n=6) in response to volumetric soil water content maintained at either high or low volumetric water content (VWC) of 56.2 and 9.9%, respectively.

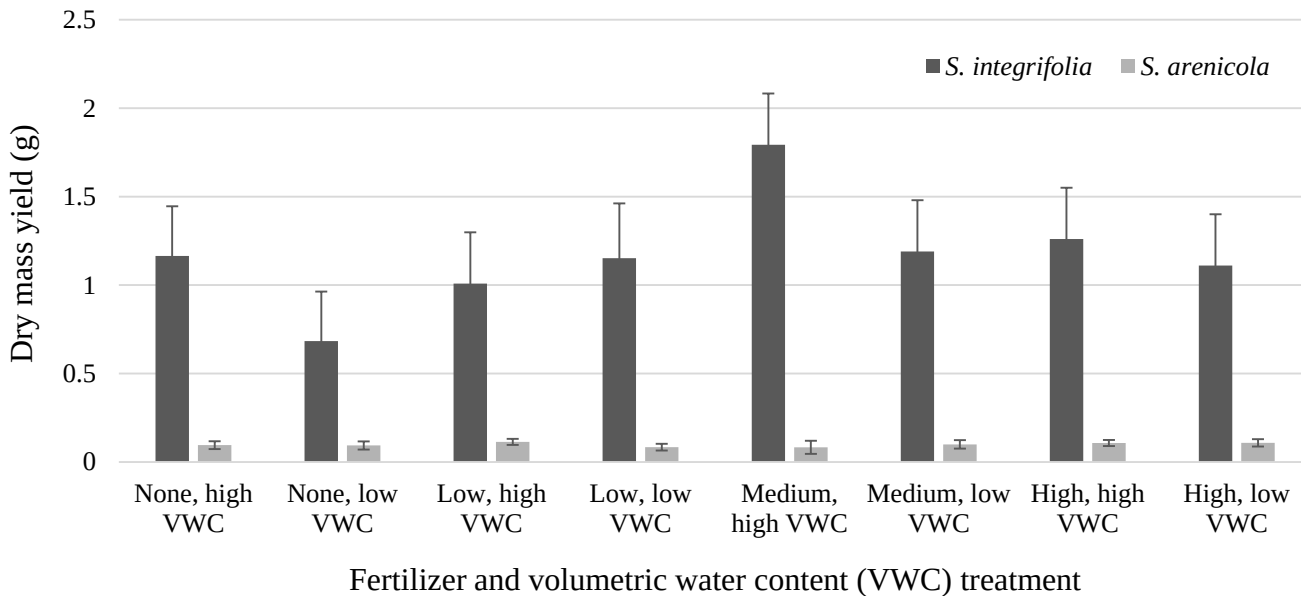

Figure 4. Means of shoot dry mass yield of *S. integrifolia* (n=6) and *S. arenicola* in response to application of Osmocote 15-9-12 slow release fertilizer (Everris NA Inc., Dublin, OH, United States) applied at one of four rates [0 g (none), 3.8 g (low), 9.2 g (medium), or 13.8 g (high)] and maintained at either high or low volumetric water content (VWC) of 56.2 and 9.9%, respectively.

Plant dry mass

No significant differences among treatment combinations were observed for DMY of *S. integrifolia* (Figure 4). Plants that received medium fertilizer application rates and subjected to high VWC had highest dry mass (1.8 g). Lowest DMY (0.7 g) was recorded for *S. integrifolia* that received no fertilizer and subjected to low VWC. Similar to results observed for *S. integrifolia,* no significant differences among treatment combinations were observed for *S. arenicola* (Figure 4). *S. arenicola* that received low fertilizer application rates and subjected to high VWC had the highest DMY of 0.11 g. Contrary to observations for *S. integrifolia*, *S. arenicola* that received medium fertilizer application rates and subjected to high VWC had the lowest DMY at 0.08 g.

Baicalin concentration

Baicalin concentrations ranged from 0.801 to 1.383 mg g plant shoot tissue^{-1} (Table 1). A fertilizer and water stress interaction was observed for baicalin concentrations of *S. integrifolia*. High concentrations of baicalin were observed in plants that received low and medium application rates of fertilizer, regardless of water stress treatment. *S. integrifolia* that received no fertilizer and subjected to low VWC possessed a high mean baicalin concentration (1.383 mg baicalin g plant shoot tissue^{-1}); however, plants that received the same fertilizer treatment (none) but were maintained at high VWC, possessed significantly less mean baicalin (0.870 mg g plant shoot tissue^{-1}). Based on DMY, greatest baicalin yield was observed when *S. integrifolia* received medium rate applications of fertilizer. When high rates of fertilizer were used during cultivation of *S. integrifolia*, low concentrations of baicalin were observed within the plant shoot tissue regardless of water stress treatment.

Baicalin concentrations in *S. arenicola* ranged from 1.861 to 2.899 mg g plant shoot tissue^{-1} (Table 1). Baicalin concentrations resulting from each treatment combination were approximately 2-fold greater than those for similar treatment combinations for *S. integrifolia*; however, limited plant mass resulted in insufficient replication to perform statistical mean separation tests among imposed treatments. Greatest baicalin concentrations were observed for *S. arenicola* that received high fertilizer application rates and subjected to low VWC. Given poor growth, baicalin yield of *S. arenicola* was low (0.2 to 0.3 mg).

Field harvested *S. integrifolia* and *S. arenicola* had mean baicalin concentrations of 1.457 and 2.348 mg g plant shoot tissue^{-1}, respectively (Table 1). Baicalin concentrations were similar to those cultivated within the

Table 1. Baicalin concentrations of *S. integrifolia*, *S. arenicola* and *S. lateriflora*. Baicalin yield based on mean dry mass yield (DMY) of harvested plants.

Species	Available water[z]	Fertilizer rate[y]	mg baicalin/g dried plant material	Baicalin yield (mg) based on DMY
S. integrifolia	High VWC	None	0.870bc[x]	1.0
		Low	1.294ab	1.3
		Medium	1.211ab	2.2
		High	0.273c	0.3
	Low VWC	None	1.383ab	0.9
		Low	1.093ab	1.3
		Medium	1.522a	1.8
		High	0.801bc	0.9
	Field harvested	--	1.457[w]	--
S. arenicola	High VWC	None	1.996	0.2
		Low	1.877	0.2
		Medium	2.159	0.2
		High	1.470	0.2
	Low VWC	None	1.861	0.2
		Low	2.065	0.2
		Medium	2.281	0.2
		High	2.899	0.3
	Field harvested	--	2.348[w]	--
S. lateriflora	Store-bought	--	0.274[v]	--

[z] Volumetric water content (VWC) maintained at high (56.2%) or low (9.9%) rates for weeks after transplanting of 3 to 5 and 4 to 5 for *S. integrifolia* and *S. arenicola*, respectively. [y] Osmocote 15-9-12 slow release fertilizer (Everris NA Inc., Dublin, OH, United States) applied at one out of the four rates (none = 0 g, low = 3.8 g, medium = 9.2 g, or high = 13.8 g). [x] Means (n=6) within column with the same letter are not significantly different ($P \leq 0.05$; Tukey's honest significant difference test). [w]Means (n=4) of baicalin concentrations from plants collected from a natural, undisturbed habitat (Lake County, FL, United States) in August 2017. [v] Means (n=4) of baicalin concentrations from dried plant shoot material purchased from a commercial store (Shoots & Roots; Orlando, FL, United States).

greenhouse and subjected to experimental treatments. *S. lateriflora*, purchased at a local medicinal herbal store, had low concentrations of baicalin 0.274 mg g plant shoot tissue^{-1} and were similar to concentrations observed for *S. integrifolia* that received high rates of fertilizer and high VWC (0.273 mg g plant shoot tissue^{-1}).

DISCUSSION

S. arenicola responded poorly to clonal propagation. Unlike *S. integrifolia,* which displayed logarithmic growth during the five-week study, *S. arenicola* did not increase in plant mass. Poor growth and performance of *S. arenicola* suggests that commercial cultivation techniques imposed in this study were not ideal for this species. Despite poor growth, however, baicalin concentrations in this species were approximately 2-fold greater than those observed in *S. integrifolia.* Detection of baicalin in *S. arenicola* has not been reported before. Relatively high concentrations of baicalin observed in *S. arenicola* warrant additional investigations to better understand relationships between plant growth and baicalin synthesis.

Increased fertilizer application rate, from none (0 g) to low (3.8 g) and medium (9.2 g), resulted in increased concentration of baicalin in both *S. integrifolia* and *S. arenicola*, regardless of VWC treatment. In contradiction to this trend, high fertilizer application rates (13.8 g) resulted in low baicalin concentrations in both *S. integrifolia* and *S. arenicola*, with exception of *S. arenicola* subjected to the low VWC treatment. Decreased baicalin concentration in response to increased application of fertilizer rates has been observed in both *S. baicalensis* and *S. lateriflora* (Cao et al., 2012; Shiwakoti et al., 2016; Similien et al., 2016). Results observed in the current investigation largely contradict these findings, thus suggest relationships

between nutrient and water availability and baicalin concentration are likely species dependent, and cannot be predicted simply by application of the CNBH. Empirical studies are therefore necessary to establish these relationships and assist in development of recommended commercial production practices.

Both *S. integrifolia* and *S. arenicola* cultivated in the current study were found to possess similar concentrations of baicalin to plants found growing in local, undisturbed natural environments. Results, therefore, do not support the assertion that medicinal plants produced within protected commercial environments will unremittingly possess lower concentrations of secondary metabolic compounds, such as baicalin, due to decreased exposure to environmental stressors. Baicalin concentrations within all cultivated plants in this study were found to be greater than concentrations observed within the limited, medicinal commercial samples of *S. lateriflora* analyzed in this investigation. Although, not an objective of this investigation, a more diverse sampling of commercially available *Scutellaria* spp. medicinal products would help define variability that exists in the marketplace. Given its relatively rapid growth rate coupled with high baicalin concentration, *S. integrifolia* likely possess qualities that would allow it to be successfully produced commercially for medicinal application. More specifically, production practices implemented in this investigation (medium application rates of fertilizer coupled with sufficient volumetric water content) provide a foundation for successful production of high yielding plant material that possess relatively high concentrations of baicalin.

Conclusion

Production of secondary metabolic compounds such as baicalin are believed to decrease in response to applications of fertilizer and when plants are cultivated within protected commercial greenhouse environments. Results of this investigation, however, showed that greenhouse cultivation of *S. integrifolia* and *S. arenicola* did not result in decreased production of baicalin as compared to plants obtained from local, undisturbed natural environments. Moreover, applications of fertilizer increased synthesis of baicalin in majority of the imposed treatments. *S. integrifolia* exhibited fast growth, reaching reproductive stage by five weeks, and responded well to commercial clonal propagation techniques. Medium fertilizer application rates and high VWC produced *S. integrifolia* with high baicalin yield. Although, *S. arenicola* exhibited poor growth trends throughout this study, high concentrations of baicalin present within this species support future studies. Empirical studies that examine the relationships between horticultural production techniques and plant growth and response, such as those presented here, are necessary for the establishment of commercial production practices for medicinally important plant species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

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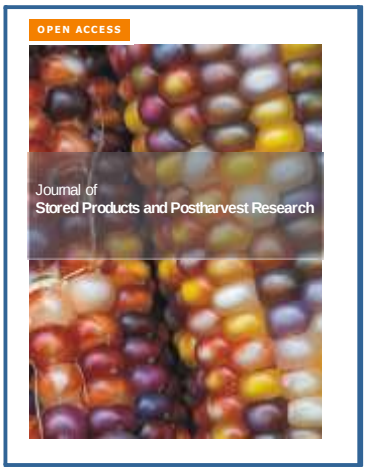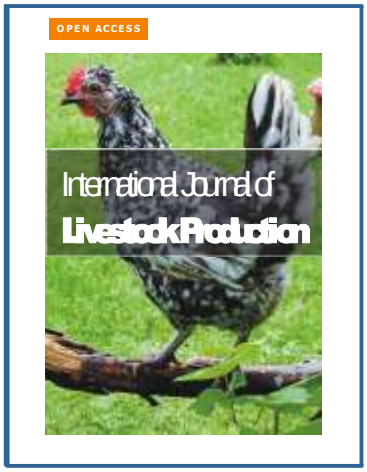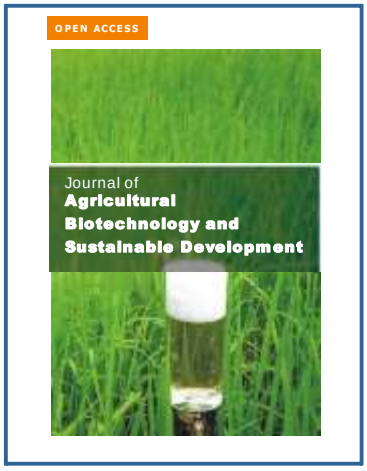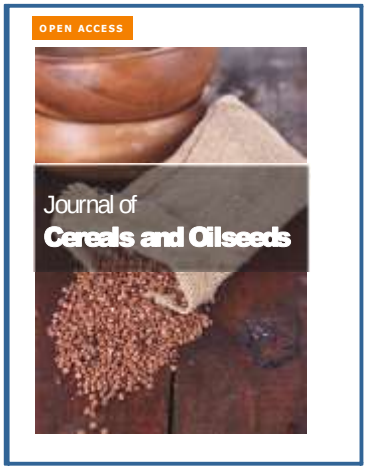
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