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Full Length Research Paper

Evaluation of biocontrol agent and wheat straw mulch on yield and yield components of groundnut (*Arachis hypogaea* L.) genotypes in Central Tigray, Northern Ethiopia

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Groundnut (*Arachis hypogaea* L.) is the 13 and 4th most important food and oil seed crops in the world. Groundnut is a source of vitamin E. However, due to low soil fertility, drought stress, fungal diseases and lack of appropriate management, production has been constrained qualitatively and quantitatively in the study area. *Trichoderma harzianum* and wheat straw mulch could increase the growth performance of groundnut and soil moisture content at maturity and near harvest to overcome terminal drought stress. Therefore, a field experiment was conducted in Rama, Central Zone of Tigray, Northern Ethiopia, to evaluate the effects of wheat straw mulch and *T. harzianum* on yield and yield components of groundnut genotypes (ICGV00308, ICIAR19BT, Werer-961 and Rama local). The treatments were arranged in factorial randomized complete block design (RCBD) with three replications. Results showed that groundnut genotypes, *T. harzianum* and wheat straw mulch highly significantly ($p < 0.01$) affected plant height with the tallest plant height 25.40 cm was recorded from Rama local while the shortest 23.15 cm from ICGV00308. The highest (37.06) mean number of seeds per plant was obtained from ICGV00308 treated with wheat straw mulch, followed by 32.05 and 32.91 from ICIAR19BT treated with wheat straw mulch and Rama local treated with *T. harzianum* and wheat straw mulch, respectively. Higher 28.44 and 24.97 numbers of pods per plant were recorded on ICIAR19BT and ICGV00308 genotypes, respectively that were mulched with wheat straw. ICGV00308 genotype produced the highest seed yield per plant (14.69 g), hundred seed weight (51.00 g) and total seed yield (1.00 t ha⁻¹) as compared to the other genotypes.

Key words: Groundnut genotype, *Trichoderma harzianum*, wheat straw mulch.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an annual legume crop originated from South America, and gradually

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became the most popular and universal crop and it is currently cultivated over 107 countries in six continents of the world. According to Wiess (2000) groundnut covers an area of 25.34 million hectares in the world with a total production of 42.63 million metric tons with the highest production of 46.76% from Asia and 41.99% from Africa (USDA, 2017). The major groundnut growing countries of the world are India (20.92%), China (18.75%) and Nigeria (18.70%) and Sudan (7.10%) (USDA, 2017). Its cultivation is mostly confined to the tropical, sub-tropical and warm temperate regions of the world ranging from 40°N to 40°S (IOPEPC, 2017).

Italian explorers introduced groundnut from Eritrea to Ethiopia through Hararghe in 1920's and it is relatively a new crop to Ethiopia (Daniel, 2009). Its production accounted for about 75255.73 ha with a corresponding gross annual production of 115, 150.04 metric tons in 2015/2016 cropping season (CSA, 2017). However, its productivity is limited to about 1.53 t ha⁻¹, which is lower than the average global yield of 1.68 t ha⁻¹ but with good management practice, the potential of the crop can reach up to 3.0 t ha⁻¹ (CSA, 2009). Groundnut is widely growing in eastern part of the country, that is Hararghe (Daniel, 2009). The crop is also produced in Tanqua Abergelle and Mereb Leke in Central and some districts in northwestern zone of Tigray (Dereje et al., 2012; Gebreselassie et al., 2014; Brhane et al., 2016).

The crop is 13th most important food and the 4th most important oilseed crop of the world. Its kernel contains 40 to 50% oil, 20 to 50% protein and 10 to 20% carbohydrates and the seeds are also source of vitamin E, niacin, riboflavin, thiamine and rich source of some minerals, namely calcium, magnesium, phosphorus, potassium, iron and zinc (Surendranatha et al., 2011).

In spite of its multipurpose function crop, groundnut production is constrained both in quantity and quality by several biotic, abiotic and socio-economic factors. The biotic factors are associated with unique plant character of groundnut, where flowers are formed and fertilized above the soil surface, but the subsequent fruit development takes place under the soil. This unusual characteristic exposes the crop to different soil borne fungal pathogens. Due to this character, the pod is therefore in profound close contact with many soil microorganisms.

Rama is one of the lowland areas in Tigray, agro-ecologically ideal for groundnut production, but the productivity of the crop is far below its potential, which is about 0.7 t ha⁻¹ that is much lower than the national average of 1.53 t ha⁻¹ (CSA, 2017). Lower productivity is mainly due to biotic and abiotic factors, such as poor soil fertility, prevalence of soil borne fungal diseases, occurrence of frequent drought stress, lack of appropriate agronomic technologies, low awareness of the producers towards aflatoxigenic fungi and aflatoxin contaminations, limited access to resistant/tolerant varieties and low

integrated disease management practices (Kahsay and Mewael, 2014). Dereje et al. (2012) reported the presence of *Aspergillus flavus* and *Aspergillus niger* in the study area (Rama) with seed infection level of 41.5 and 12.3% by *A. flavus* and *A. niger*, respectively, and aflatoxin B₁ contamination ranged from 0.1 to 397.8 ppb. Thus, the detected aflatoxin B₁ value is much higher than the standard set for the European Union (5 ppb), FAO/WHO (15 ppb) and USA (20 ppb).

Therefore, the present research work focused on reduction of the inocula of soil borne fungi, specifically the pathogens *Aspergillus* species in the soil to reduce the yield loss and fungal infection level through integrated management using different groundnut genotypes, natural antagonistic fungal strain *Trichoderma harzianum* isolate BD-13 and wheat straw mulch to retain soil moisture and overcome terminal drought stress and stabilize soil temperature. Hence, the study was carried out with the specific objective of assessing the effects of application of biocontrol agent *T. harzianum* and wheat straw mulch singly or in integration on yield and yield components of groundnut genotypes.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Rama; Mereb-Leke District, Central zone of Tigray, northern Ethiopia, during the 2016 main cropping season. Rama is located at 14°22'25" N latitude and 038°47'32" E longitude at an elevation of 1390 meters above sea level (Figure 1). The area is 1041 km far from Addis Ababa towards the north. It lies in the low land agro-ecological zone and the soil type is sandy clay loam. The mean annual rainfall in the area ranges from 400 to 600 mm and the rainfall distribution is mono-modal with an erratic distribution beginning in late June and terminating in the last week of August. The mean maximum and minimum temperature of Rama during the 2016 growing season was 33.9 and 18.7°C, respectively, and the average temperature of the study area was 26.3°C in the season, while the total annual rainfall of Rama Experimental Site during 2016 main cropping season was 586.9 mm (NMA, 2017). Commonly grown crops are finger millet, maize, sorghum, tef and groundnut. According to Mereb-Leke District Office of Agriculture and Rural Development (DOARD), *Citrus* species and mango are also among the commonly grown fruit crops in the study area.

Experimental materials

Planting materials

Four groundnut genotypes, namely ICGV00308, ICIAR19BT (introduced from ICRISAT as drought tolerant), Werer-961 and Rama local as drought susceptible were used as planting materials in the study. Seeds of these cultivars were obtained from Mekelle University RUFORUM Groundnut Project. Werer-961 was released from Werer Agricultural Research Center in 2004 and the other two genotypes ICGV00308 and ICIAR19BT were introduced from ICRISAT.

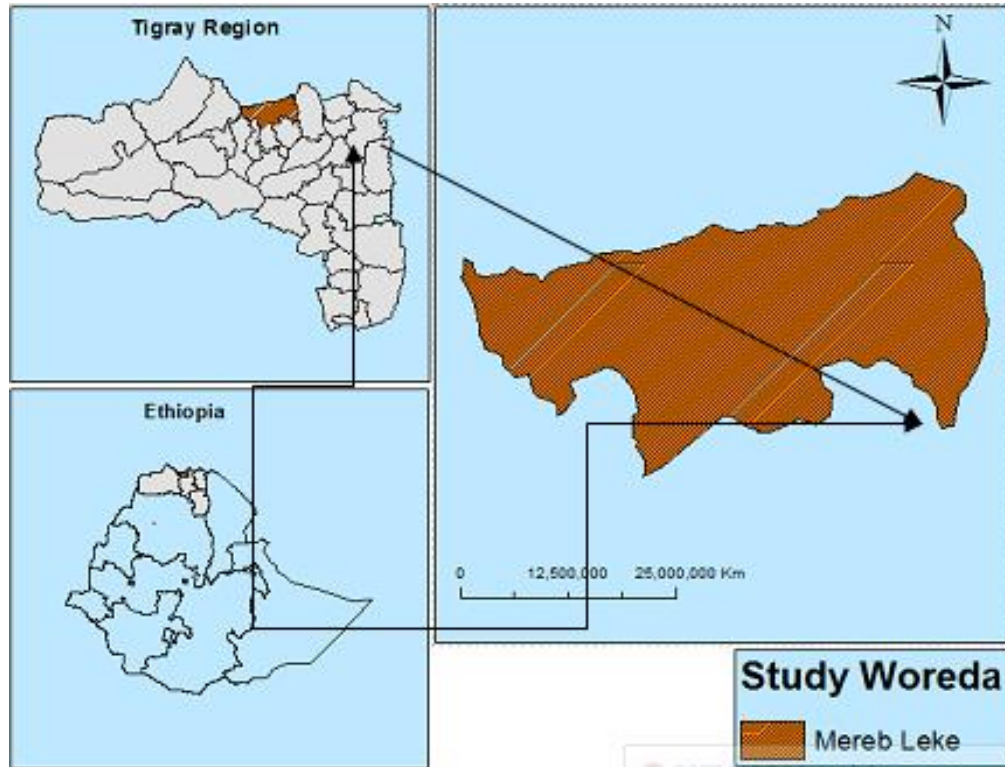


Figure 1. Map of the study area, Merib-Leke District in Central Zone of Tigray, Northern Ethiopia.

T. harzianum

T. harzianum isolate BD-13 was used as biocontrol agent against groundnut seed infection of *Aspergillus* species that mainly cause seed infection and aflatoxin contamination at pre-harvest and post-harvest conditions.

Preparation of *T. harzianum* inoculums and inoculation techniques

Culture of *T. harzianum* isolate BD-13 was prepared on potato dextrose agar (PDA) plates that were incubated under fluorescent light at 25°C until sporulation was visible. The conidial inoculum was harvested by washing plates with 10 ml distilled sterile water and the suspension was filtered through nylon mesh into a tube. The concentration of the spore suspension was standardized via haemocytometer count and the spore suspension was adjusted with distilled sterile water to produce a spore suspension of 10^6 spores per milliliter. Finally, a drop of Tween 20 was added to the adjusted spore suspension at a rate of 0.5% to disperse the spores and to increase the effectiveness of inoculation by attaching the spores with the seeds. 70 seeds of groundnut per plot were inoculated in 50 ml spores suspension by seed priming techniques (soaking seeds in the spores suspension) for 3 h just before planting.

The inoculated seeds (Tr_1) were air dried under shade for 40 min and, similarly, the remaining non-inoculated seeds (Tr_0) were soaked with sterile distilled water for similar duration and air dried in the same manner to that of the *T. harzianum* inoculated seeds. Additionally, spore suspension of the *T. harzianum* isolate BD-13

was prepared in a similar procedure with the aforementioned paragraph was sprayed to soils of groundnut seedling plots that were first planted with inoculated seeds. Similarly, the non-inoculated seedlings were sprayed with sterile distilled water only. One time spraying was done using hand sprayer (atomizer) around the root zone (rhizosphere) of the seedlings 59 days after planting, when the groundnut growth reached the first pegging stage at a rate of 50 milliliter per plot.

Wheat straw mulching material

Wheat straw was used as a source of mulching to increase the soil moisture holding capacity and to reduce the terminal drought stress by decreasing evaporation rate. It is well known that pre-harvest contamination is higher under conditions of drought stress and higher soil temperature (25-32°C) during pod-filling period. Mulching could create conducive microclimatic conditions for the atoxigenic soil microorganisms and this helps them to suppress the *Aspergillus* species living in the soil. Wheat straw mulch was first chopped into pieces of size 5 to 10 cm to cover the plots properly. Wheat straw mulch was applied at a rate of 12 t ha^{-1} in split application of first half 27 days after planting and the second half 49 days after planting or 22 days after the first mulching based on the treatments randomizations and un-mulched (ML0) plots were used as control.

Treatments and experimental design

The treatments consisted of factorial combinations of three factors:

four groundnut genotypes (ICGV00308, ICIAR19BT, Werer-961 and Rama local), two *T. harzianum* with and without inoculation (Tr₁ and Tr₀) and two wheat straw mulching with (ML₁) and without mulching (ML₀) (Table 2). The experimental plot size was 2 m long and 3.6 m wide with a plot size of 7.2 m² with six rows per plot. The net harvested area was 4.8 m (2.4 m × 2.0 m) leaving one outermost row in both sides as borders. Groundnut genotypes were sown in row maintaining 0.60 and 0.20 m spacing between rows and plants, respectively. Each treatment and treatment combination was assigned to the respective plot randomly and the treatments were laid out in a randomized complete block design (RCBD) with three replications. Distances (1 and 1.5 m) were maintained among plots and between adjacent replications, respectively.

Management of experimental field

Groundnut seeds were sown in rows on well-prepared land at Rama, Central Tigray, Northern Ethiopia on 13/07/2016. Each plot was divided into six planting rows and data were collected only from the harvested four central rows in the plot, which was considered as net plot. Diammonium phosphate (DAP) fertilizer was uniformly applied at planting at a rate of 100 kg ha⁻¹ (18 kg ha⁻¹ N and 46 kg ha⁻¹ P₂O₅) for all plots at the time of sowing of the groundnut seeds. Similarly, all other management practices (hand weeding and hoeing) were done manually to all plots uniformly.

Data collection

Data on soil moisture content

Soil moisture content was measured at harvest by taking undisturbed soil samples using the core sampler at a depth of 0 to 40 cm from each plot to see the effect of wheat straw mulch on soil moisture conservation capability and its indirect effect on the management of aflatoxin contamination level. Comparison among different treatments was performed to determine the capacity of mulching for soil moisture retention, management of *Aspergillus* species and its effect on groundnut yield improvement as well as suppression of the percentage seed infection by *Aspergillus* species.

Phonological and growth parameters' data

Days to 50% emergence is the number of days from sowing to the date on which 50% seedlings in each plot emerged was recorded and days to 50% flowering refers to the number of days from emergence to the date on which 50% of the plants in each plot had at least shown the first flowers. Similarly, Days to 90% physiological maturity is the number of days from emergence to the date at which 90% of the plants in each plot reached physiological maturity; pods were considered as matured when the kernels were fully developed, testa assuming the varietal color, the inside wall of pods become dark to brown and the pods were strong when pulling by fingers or when squeezed between the thumb and the index finger. Plant height measurement was done by measuring the height (in centimeters) of twelve randomly taken plants from the middle four harvestable rows from the ground level to the uppermost point and measurements were taken with a ruler. The measurements were averaged to get the plant height.

Yield and yield components

Number of pods per plant, number of seeds per pod and number of

seeds per plant were computed from counts carried out on twelve randomly taken plants from the middle four harvested rows of each plot at harvest and average number of seeds per plant was calculated. Similarly, seed yield per plant (g plant⁻¹) was computed from twelve randomly taken plants from the central harvested four rows of each plot and average per plant was calculated. Seed yield (ton ha⁻¹) and hundred seed weight was computed from data collected in plot wise. These parameters were measured after harvesting, threshing and weighing.

Data analyses

All data collected during the study were subjected to analysis of variance (ANOVA) using SAS version 9.1.3 computer software (SAS Institute Inc., 2004). The least significant difference (LSD) test was used to compare the treatment means at 5% probability level.

RESULTS AND DISCUSSION

Effect of wheat straw mulch on residual soil moisture content at harvest

Wheat straw mulch highly and significantly ($p \leq 0.01$) influenced the soil moisture content at harvest. However, the main effect of groundnut genotypes, *T. harzianum* and their interaction effect did not show significant difference on the soil moisture content at harvest.

Significantly higher soil moisture content (24.64%) was recorded on wheat straw mulched plots as compared to 11.85% in the non-mulched plots. Application of wheat straw mulching on the groundnut plots increased soil moisture content at harvest by 12.79% as compared to the bare soil. Mulching protects the soil from direct sunshine, wind blowing, runoff and decreases the rate of evaporation (Bhardwaj, 2013). Similarly, wheat straw mulch increases the infiltration rate as straw holds and retains soil moisture for a longer period.

Straw mulch significantly affected yield and yield related groundnut parameters, like plant height, days to physiological maturity, number of pods per plant, number of seeds per plant, seed yield per plant and total seed yield, whereas parameters, like the number of seeds per pod and hundred seed weight, were not significantly influenced by the wheat straw mulching.

Ghosh et al. (2003) reported that soils covered with wheat straw mulch and black polythene retained higher amount of available soil moisture at 0 to 10 cm soil depth continuously through the crop growth period than the non-mulched plots. Khan and Chatta (2005) also reported that straw conserved higher soil moisture up to 55% more than non-mulched plots. Application of wheat straw mulch at a rate of 6730 kg ha⁻¹ increased the available soil moisture significantly up to 1.5 m soil depth as compared to the bare soil (Khan and Chatta, 2005). The present result is in agreement with the observation of Bhatt et al. (2004) who reported that mulching significantly enhanced soil moisture content with maximum (17.0%) soil moisture

content at harvest in mulched plot as compared to non-mulched soil (14.0%).

Effect of *T. harzianum* inoculation and wheat straw mulch on phenology and growth characters of groundnut genotypes

Days to 50% emergence and 50% flowering

There was no significant ($p > 0.05$) difference due to the main effects and their interaction effects on days to 50% emergence and days to 50% flowering. The absence of significant difference to 50% emergence and 50% flowering might be attributed due to the presence of sufficient soil moisture at earlier growth stages of the crop until flowering period for all treatment combinations. Thus, there might not be critical soil moisture difference as a result of the application of wheat straw mulch until flowering period. Genotypes did not also show significant difference on days to 50% seed emergence and flowering.

Plant height

Three-way interaction effect of genotypes, *T. harzianum* and straw mulch showed significant ($p \leq 0.05$) difference on plant height. The tallest plant heights of 28.78 and 28.19 cm were recorded in IRTTr1ML1 and RLTr1ML1 treatment combinations, while the shortest 19.25, 22.17 and 22.39 cm were observed on IVTr0ML0, WTr0ML0 and IRTTr0ML0 treatment combinations, respectively. The significant increase in plant height in the straw mulched and *Trichoderma* treated plots might be due to improved soil physical conditions, such as total N, available P, CEC and O.M and more soil water conservation available to the crop treated with wheat straw mulch than in the control plots. In line with this result, Pervez et al. (2009) obtained the tallest plant height and highest seed yields of from wheat straw mulched plots as compared to plants from the non-mulched plots.

Soils mulched with straw can decrease the rate of evaporation, run-off, soil erosion and can increase the rate of water infiltration because straw mulch holds or retains soil moisture available around the root zone for a longer period than in soil without mulching. Mulches may create sustainable and available soil moisture at the root zone throughout the whole growth period and this may lead to better growth performance and taller plants on mulched plots than on the non-mulched plots. In agreement with this result, Oberson et al. (2006) reported that rice straw mulch boosts soil biological activities, preserves soil organic matter and improves soil nutrients that, in turn, contribute to plant growth.

It was found that the biocontrol agent *T. harzianum*

resulted in a highly significant ($p \leq 0.01$) difference on the plant height of groundnut. *T. harzianum* inoculated plants were 11.04% taller than non-inoculated plants. Thus, the difference in plant height between *T. harzianum* inoculated and non-inoculated might have resulted due to the bio-fertilization and growth stimulating nature of *T. harzianum*. Harman (2004) reviewed that some strains of *T. harzianum* are usually associated with plant roots, root ecosystems and, thereby, can enhance the growth performance of crops when inoculated with *Trichoderma* and described *Trichoderma* strains as plant symbiosis and opportunistic virulent organisms that are able to colonize plant roots by mechanisms similar to rhizobial bacteria.

Days to 90% physiological maturity

The main effect of wheat straw mulching had highly significant ($p \leq 0.01$) influence on days to 90% physiological maturity of groundnut. A significant ($p \leq 0.05$) difference was also observed in days to 90% physiological maturity among the tested groundnut genotypes. The earliness in days to 90% physiological maturity was observed in ICIAR19BT, Rama local and ICGV00308 with 96.92, 97.00 and 98.25 days, respectively, required to reach 90% physiological maturity after planting. Prolonged physiological maturity of 101 days was recorded in Werer-961 to reach 90% physiological maturity. Among the tested four groundnut genotypes, Werer-961 was found to be a late maturing variety with a mean value of 101 days as compared to the other tested genotypes and Rama local. However, there was no significant difference in days to 90% physiological maturity between *T. harzianum* inoculated and non-inoculated plots and interaction effect of the main effects.

The main effect of wheat straw mulch significantly ($p \leq 0.05$) affected days to 90% physiological maturity and prolonged maturity (101 days) was observed on straw-mulched plots as compared to the non-mulched plots that required shorter (95.58 days) to reach 90% physiological maturity. Application of wheat straw mulch significantly extended the number of days required to reach physiological maturity. Days to 90% physiological maturity of groundnut plants grown on mulched plots were exceeded by 5.42 days as compared to non-mulched plots (Table 1). This prolonged physiological maturity period (days) in the mulched plots might be attributed to the availability of enough soil moisture content. This indicated that application of straw mulch on groundnut planted plots at a rate of 12 t ha⁻¹ avoided forced maturity and terminal moisture stress. Moisture stress was one of the most frequently occurring problems of groundnut production in the study area. Mulching retained soil moisture, protected the soil from direct

Table 1. Main effect of groundnut genotypes, *T. harzianum* and wheat straw mulch on groundnut plant height, days to 90% physiological maturity and number of pods per plant at Rama during 2016 main cropping season.

Factor	Plant height (cm)	Days to 90% physiological maturity (days)	Number of pods per plant
Groundnut genotypes			
Werer-961	24.86 ^a	101 ^a	19.66 ^{ab}
ICGV00308	23.15 ^b	98.25 ^b	20.97 ^a
ICIAR19BT	24.85 ^a	96.92 ^b	22.44 ^a
Rama local	25.40 ^a	97.00 ^b	16.63 ^b
LSD (0.05)	1.42	1.80	3.42
<i>T. harzianum</i>			
Inoculated	25.96 ^a	98.50 ^a	19.95 ^a
Non inoculated	23.38 ^b	98.08 ^a	19.90 ^a
LSD (0.05)	1.00	Ns	Ns
Wheat straw mulching			
Mulched	26.21 ^a	101.00 ^a	21.50 ^a
Non mulched	23.13 ^b	95.58 ^b	18.35 ^b
LSD (0.05)	1.00	1.27	2.41
CV (%)	8.91	2.20	20.59

LSD: Least significant difference, CV: coefficient of variation. Means followed by the same small letter(s) in the same column are not significantly different from each other at 5% probability level.

Table 2. Interaction effects among groundnut genotypes (G), *T. harzianum* (Tr) and wheat straw mulch (ML) on number of seeds per plant at Rama in 2016 main cropping season.

Treatment interactions	Mean number of seeds per plant
ICGV00308*Tr1*ML1	26.95 ^{abcd}
ICGV00308*Tr1*ML0	28.19 ^{abc}
ICGV00308*Tr0*ML1	37.06 ^a
ICGV00308*Tr0*ML0	22.47 ^{bcd}
ICIAR19BT*Tr1*ML1	29.89 ^{abc}
ICIAR19BT*Tr1*ML0	21.89 ^{bcd}
ICIAR19BT*Tr0*ML1	32.05 ^{ab}
ICIAR19BT*Tr0*ML0	19.14 ^{cd}
Werer961*Tr1*ML1	25.72 ^{abcd}
Werer961*Tr1*ML0	18.33 ^{cd}
Werer961*Tr0*ML1	25.61 ^{abcd}
Werer961*Tr0*ML0	18.53 ^{cd}
Rama local*Tr1*ML1	32.91 ^{ab}
Rama local*Tr1*ML0	23.58 ^{bcd}
Rama local*Tr0*ML1	15.47 ^d
Rama local*Tr0*ML0	24.47 ^{bcd}
LSD (0.05)	11.58
CV (%)	27.67

*= interaction, LSD: Least significant difference, CV: coefficient of variation, Tr1: with *T. harzianum*; Tr0: without *T. harzianum*, ML1: with wheat straw mulching, and ML0: without wheat straw mulching. Means followed by the same letter(s) in the same column are not significantly different from each other at 5% probability level.

sunlight and consequently decreased the rate of evaporation. On the other hand, mulching might decrease

immediate deep percolation of rainwater and increased soil moisture retention capacity. Due to this reason,

plants grown on mulched plots had high probability of obtaining the required available amount of soil moisture during their late maturity stage and could mature slowly. This research result is in accordance with the finding of another researcher (Anonymous, 2007) who reported that straw mulches with a thickness of 3.81 cm reduced the rate of evaporation by 35% as compared to bare soil.

Effect of *T. harzianum* and wheat straw mulching on yield components and yield of groundnut genotypes

Number of pods per plant

Number of pods per plant was significantly ($p \leq 0.05$) influenced by the main effects of genotypes and wheat straw mulching. However, the main effect *T. harzianum* and the interaction effect of the three-factors (groundnut genotypes, bio-inoculant *T. harzianum* and straw mulches) were not significant on the number of pods per plant of groundnut. The highest number of pods per plant (22.44) was recorded by the genotype ICIAR19BT while the lowest number (16.63) was recorded by Rama local (Table 1). Here, gives the possible reasons for the result and compare and contrast with similar studies. Straw mulched plots gave significantly higher number of pods per plant (21.50 g) than the non-mulching. The application of wheat straw mulch increased the number of pods per plant by 17.17% over the bare soil plots (Table 1). The increase in number of pods per plant with the application of wheat straw mulch might be due to the improvement of available soil moisture, plant nutrients and favourable soil temperature created throughout the crop growth period. The current result corroborates with the observation of Ghosh et al. (2006) who reported that straw mulch produced more pods, that is, 17 to 24% higher pod which crop than polyethylene mulch and no mulch due to the favourable soil moisture and soil temperature from straw mulch.

Similarly, Awal et al. (2016) stated that the important pea (give scientific name) yield contributing parameters, like number of pods per plant, number of seeds per pod, seed weight per plant and hundred seed weight, were significantly increased in mulched-plots as compared to non-mulched plots and attributed this to the availability of soil moisture. Other researchers also reported that mulches retained higher amount of soil water with efficient utilization of soil nutrients that might enhance plant growth and might maximize the yield attributes (Awal and Ikeda 2003; Awal et al., 2008). This current result also agrees with the findings of Ghosh et al. (2003) who reported that data collected for over eight years (1990-1997) in India revealed that application of wheat straw as a mulch on the soil increased pod yield by 19.4% over non-mulched plots. Similarly, Zayton et al. (2014) reported that mulching highly and significantly ($p \leq$

0.01) increased the mean number of pods per plant of groundnut as compared to the non-mulched plots. Number of pods per plant recorded from mulched plots was 37.56, while 33.25 was recorded from non-mulched plots.

Number of seeds per pod

The analysis of variance (ANOVA) showed that the main effect genotype highly significantly ($p \leq 0.01$) influenced the number of seeds per pod, whereas the other two main effects, that is, *T. harzianum* inoculation, wheat straw mulching and their interaction effects were not significant. Rama local produced the highest number of seeds per pod (2.58) than all the other tested genotypes. Thus, the highest number of seeds per pod recorded from Rama local might be due to the genetic potential of the crop to produce higher number of seeds per pod and the adaptability of the genotype to the area for a long period.

Number of seeds per plant

The main effect of mulching and the interaction effect of the three factors, that is, variety \times *T. harzianum* \times wheat straw mulch showed significant ($p \leq 0.05$) difference on number of seeds per plant of groundnut genotypes. Seed yield per plant of ICGV00308 under straw mulch gave the highest also include about *T. harzianum* mean number of seeds per plant (37.06), followed by the genotype ICIAR19BT (32.05) under no-mulch and no-*T. harzianum* treatment, and Rama local (32.91) under wheat straw mulch and *T. harzianum* application. On the other hand, the lowest number of seeds per plant was from Rama local under wheat straw mulch but *T. harzianum* untreated (Table 2). Similarly, wheat straw mulching significantly ($p \leq 0.05$) increased the number of seeds per plant from 22.11 in without mulching to 28.21 seeds per plant on straw mulched plots. This increased number of seeds per plant might have resulted due to the application of straw mulches increased soil moisture content and plant nutrition that might have been mineralized from the straw mulches.

In line with this result, Bhardwaj (2013) reported that mulching provides an environment conducive for the growth and development of crops; as a result, plants become vigorous and healthier than plants on non-mulched plots. Similarly, Albuquerque et al. (2001) have shown that in maize production higher number of seeds per cob was obtained from wheat straw mulched plots. This higher number of seeds per cob in maize might have resulted due to the change in soil physical, chemical and biological characteristics after the soils were mulched with wheat straw.

Seed yield per plant

Seed yield per plant was significantly ($p \leq 0.05$) influenced by the main effect of genotypes and wheat straw mulching but the main effect *T. harzianum* and the interaction effect of the three factors did not show significant effect on seed yield per plant. The highest mean seed yield per plant (14.69 g) was obtained from ICGV00308 whereas the lowest seed yield per plant (10.18 g) was recorded from Were-961. The genotype ICGV00308 produced 20.21, 29.66 and 44.30% higher seed yield per plant than ICIAR19BT, Rama local and Werer-961, respectively. This variation in mean seed yield per plant might be due to the inherent genetic potential of the genotypes. Likewise, wheat straw mulching had significant effect on seed yield per plant, where it enhanced seed yield per plant by 20.69% over the non-mulched plots. Mulched plots recorded higher seed yield per plant (13.24 g) than the non-mulched plots with a mean (10.97 g) seed yield per plant of 10.97 g.

Wheat straw mulching reduces soil deterioration through preventing the runoff and soil loss, minimizes the weed infestation and checks the water evaporation. Thus, it facilitates more soil moisture retention and helps in the control of soil temperature fluctuations, improves the soil physical, chemical and biological properties. In the present study, the effect of wheat straw mulch and *T. harzianum* treatment on available N, P and K was significantly higher than in soil conditions in non-treated plots before harvest. Straw mulch supplies plant nutrients to the soil and ultimately enhances the growth performance and yield of crops (Patil Shirish et al., 2013). Fang et al. (2011) also reported that straw mulching improved soil nitrogen availability, increased plant growth and influenced the physical and chemical properties of the soil. Awal et al. (2016) also stated that the important yield contributing parameters of pea, like number of pods per plant, number of seeds per pod, seed weight per plant and hundred seed weight, significantly increased in mulched-plots as compared to non-mulched plots. It was also recognized that the availability of soil moisture significantly improved the various yield parameters in field pea too (Awal et al., 2016).

Hundred seed weight (HSW)

Analyses of variance showed that groundnut genotypes and bio-inoculant *T. harzianum* significantly ($p \leq 0.05$) influenced hundred seed weight. However, the main effect of wheat straw mulch and the interaction effect of the three main effects did not show significant difference on hundred seed weight. There was significant difference among the tested four genotypes. The genotypes ICGV00308 and Werer-961 gave significantly higher hundred seed weight of 51.00 and 48.08 g, respectively.

Hundred seed weight ranged from 51.00 for ICGV00308 to 43.08 g for ICIAR19BT. Thus, ICGV00308 resulted in 18.38 and 17.7% higher hundred-seed weight than the lowest ICIAR19BT and Rama local, which is currently the most popular variety under production in the study area. Therefore, this genotype could be considered as a better optional genotype for farmers of the study area.

Studies have confirmed that *T. harzianum* and *Trichoderma viridi* are capable of enhancing seed germination, and root and shoot length (Dubey et al., 2007) as well as increasing the frequency of healthy plants, and boosting yield (Rojoa et al., 2007). Similarly, Chaur-Tsuen and Chien-Yih (2002) screened *T. harzianum* strains for their effects on plant growth and root growth of bitter melon, loofah and cucumber and noted that *Trichoderma* strains significantly increased the seedling height by 26 to 61%, root exploration by 85 to 209%, leaf area by 27 to 38% and root dry weight by 38 to 62% 15 days after sowing. Methanol extract of *T. harzianum* and *T. viridi* significantly improved various growth parameters of okra (Prasad and Anes, 2008). Vigour index (VI) was also significantly affected by the application of different *Trichoderma* strains both in the laboratory and under field conditions. This current result conforms to the findings of Harman (2006) who reported that *T. harzianum* strains frequently increased plant growth and productivity.

Seed yield

Seed yield was obtained as a function of different yield components like number of pods per plant, number of seeds per pod, number of seeds per plant, seed yield per plant and hundred seed weight. These all parameters had their own contribution to the final seed yield. The main effect of the tested groundnut genotypes and wheat straw mulch was significant to the seed yield ($t \text{ ha}^{-1}$). On the other hand, the main effect of *T. harzianum* and the interaction effects among the three factors did not show significant variation in the seed yield. The highest (1.0 t ha^{-1}) seed yield was recorded from the genotype ICGV00308 and it was significantly higher than the other three genotypes tested in this study (Table 3). The mean value of seed yield ranged from 1.00 ha^{-1} for ICGV00308 to 0.63 t ha^{-1} for Werer-961. This indicated that the genotype ICGV00308 produced 58.7, 29.87 and 28.21% higher seed yield than Werer-961, Rama local and ICIAR19BT, respectively. Rama local is the only genotype currently under production in the study area. Therefore, ICGV00308 genotype can be considered as an important and superior genotype to the other tested groundnut genotypes to improve productivity in the study area.

Wheat straw mulch showed significant ($p \leq 0.05$) difference in seed yield as compared to the non-mulched

Table 3. Main effect of genotypes (G), *T. harzianum* (Tr) and wheat straw mulching (ML) on number of seeds per pod, seed yield per plant, hundred seed weight and seed yield at Rama in 2016 cropping season.

Factor/Treatment	Number of seeds per pod	Seed yield per plant (g)	Hundred seed weight (g)	Seed yield (t ha ⁻¹)
Genotypes				
Werer961	1.84 ^b	10.18 ^b	48.08 ^a	0.63 ^b
ICGV00308	1.89 ^b	14.69 ^a	51.00 ^a	1.00 ^a
ICIAR19BT	1.88 ^b	12.22 ^b	43.08 ^b	0.78 ^b
Rama Local	2.58 ^a	11.33 ^b	43.33 ^b	0.77 ^b
LSD(0.05)	0.11	2.44	3.58	0.16
<i>T. harzianum</i>				
<i>T. harzianum</i> inoculated	2.02 ^a	12.91 ^a	0.80 ^a	0.80 ^a
<i>T. harzianum</i> non inoculated	2.08 ^a	11.30 ^a	0.78 ^a	0.79 ^a
LSD(0.05)	NS	NS	NS	NS
Wheat straw mulching				
With mulching	2.06 ^a	13.24 ^a	46.42 ^a	0.86a
Without mulching	2.04 ^a	10.97 ^b	46.33 ^a	0.73b
LSD (0.05)	NS	1.73	NS	0.11
CV (%)	6.64	24.24	9.28	24.75

NS: Non significant, LSD: least significant difference, CV: coefficient of variation. Means followed by the same letter in the same column are not significantly different from each other at 5% probability level

plots. Mulched plots produced significantly higher (0.86 t ha⁻¹) seed yield (0.86 t ha⁻¹) than the non-mulched plots (0.73 t ha⁻¹). Seed yield from mulched plot was 17.80% higher than the non-mulched plots. Application of wheat straw mulch showed significant increase in seed yield which might be due to the soil moisture retaining ability of straws. Uwah and Iwo (2011) stated that plant height, seed yield per cob and the seed yields per hectare were significantly increased as the rate of straw mulch increased; hence, 61% yield increase was obtained as the mulch increased from 0 to 2 t ha⁻¹ and further increase in the mulch from 2 to 4 t ha⁻¹, resulted in 87.5% yield increase, and finally 155.5% yield increase was recorded when the plot was mulched with 6 t ha⁻¹. In agreement with this resulted. Zamir et al. (2013) also reported that highest number of seeds per cob was recorded from wheat straw mulched plots. This result is in agreement with the findings of Zayton et al. (2014) who reported that mulching highly and significantly increased the mean total kernel yield of groundnut as compared to the yield from the non-mulched plots. The total kernel yield recorded from mulched plots was 1.14 t ha⁻¹, while 0.918ha⁻¹ was recorded in non-mulched plots.

Conclusions

The analysis of variance (ANOVA) showed that plant height and number of seeds per plant were significantly influenced by the interaction effect of groundnut

genotypes, *T. harzianum* and wheat straw mulching. However, soil moisture content at harvest, days to 90% physiological maturity, number of pods per plant, number of seeds per pod, seed yield per plant, hundred seed weight and total seed yield were significantly affected by the main effect of genotypes, *T. harzianum* and wheat straw mulch separately.

The highest soil moisture was conserved on wheat straw mulched-plots. The tallest plant height was observed in Rama local, werer-961 and ICIAR19BT with values of 25.40, 24.86 and 24.85 cm, respectively. The highest (22.44) number of pods per plant was recorded on ICIAR19BT, followed by ICGV00308 (20.97), while the least (16.63) number of pods per plant was recorded on Rama local. Rama local held the highest (2.58) number of seeds per pod as compared to the other genotypes. Genotype ICGV00308 was superior in number of seeds per plant, seed yield per plant, hundred seed weight and total seed yield compared to the other genotypes. The maximum number of seeds per plant was obtained from ICGV00308 treated with both *T. harzianum* and wheat straw mulching. Significantly, higher seed yield per plant as well as total seed yield was recorded in ICGV00308 genotype treated with wheat straw mulch. Likewise, higher hundred seed weight was obtained from the genotype ICGV00308 than the other genotypes when treated with *T. harzianum* biocontrol agent.

Application of wheat straw mulching at a rate of 12 t ha⁻¹ as split application half at 27 DAP after the establishment of seedlings and the remaining half at 49

DAP showed significant effect on soil moisture conservation and agronomic parameters. From this observation, wheat straw mulch exerts significant effects on yield related parameters and yield of the crop. The newly introduced groundnut genotype ICGV00308 was highly superior in quantitative and qualitative yield related parameters and yield compared to other tested genotypes. The current research findings imply that field demonstration need to be conducted and seeds should be accessible to farmers through the responsible bodies, like Rural Development and Agricultural Offices, research centers, universities and other governmental and non-governmental organizations (NGO). Wheat straw mulched soils were also found to retain higher soil moisture than non-mulched plots at harvest and the maturity period of crops on mulched plot extended by five days more than that of the non-mulched plots. It is recommended that farmers have to use locally available mulching materials at a rate of 12 t ha⁻¹ to solve late drought stress that forces early physiological maturity of groundnut. You need to say something about *T. harzianum* and its effects.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

REFERENCES

- Albuquerque A, Sangoi L, Ender M (2001). Modification in the soil physical properties and maize parameters, including by cropping and grazing under two tillage systems. *Revista- Brasileira- de- Cienciado- Solo* 25(3):717-723.
- Anonymous A (2007). Impact of mulching on landscape plants and the environment -a review Washington State University, Research and Extension Center. *Journal of Environmental Horticulture* 25(4):1-10.
- Awal M, Dhar P, Sultan M (2016). Effect of Mulching on Microclimatic Manipulation, Weed Suppression, and Growth and Yield of Pea (*Pisum sativum* L.). *Journal of Agriculture and Ecology Research International* 8(2):1-12.
- Awal MA, Ikeda T (2003). Controlling canopy formation, flowering, and yield in field grown stands of peanut (*Arachis hypogaea* L.) with ambient and regulated soil temperature. *Field Crops Research* 81:121-132.
- Awal MA, Das GK, Haque MS, Karim MA (2008). Growth and yield response of maize to intercropping with peanut and straw mulching. *Bangladesh Journal of Program on Science and Technology* 6(1):81-84.
- Bhardwaj RL (2013). Effect of mulching on crop production under rainfed condition; A review. *Agricultural Research Communication Center* 34(3):188-197.
- Bhatt R, Khera KL, Arora S (2004). Effect of tillage and mulching on yield of corn in the submontaneorainfed region of Punjab, India. *International Journal of Agricultural Biology* 6:126-128.
- Brhane S, Guesh T, Melesse H (2016). Response of groundnut (*Arachis hypogaea* L.) to different rates of phosphorus fertilizer at Tanqua-Abergelle District, northern Ethiopia. *Basic Research Journal of Agricultural Science and Review* 5(1):1-4.
- Chaur-Tsuen L, Chien-Yih L (2002). Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. *Plant Pathology Bulletin* 11:215-220.
- Central Statistics Agency (CSA) (2009). Agricultural sample survey of area and production of crops of 2008/2009 in Ethiopia. Government annual report on area and production of crops, Addis Ababa, Ethiopia pp. 1-128.
- Central Statistics Agency (CSA) (2017). Agricultural sample survey 2015/2016 report on area production and farm management practice of belg season crops for private peasant holding, Addis Ababa, Ethiopia.
- Daniel E (2009). Groundnut research. *In: Presentation for Workshop, Werer Agricultural Research Center, Ethiopia* pp. 1-3.
- Dereje A, Muez T, Skinnies H (2012). Natural occurrence of toxigenic fungi species and aflatoxin in freshly harvested groundnut kernels in Tigray, northern Ethiopia. *Journal of the Dry Lands* 5(1):377-384.
- Dubey SC, Suresha M, Singha B (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* sp. *ciceris* for integrated management of chickpea wilt. *Biological Control* 40:118-127.
- Fang SZ, Xie BD, Liu D, Liu JJ (2011). Effects of mulching materials on nitrogen mineralization, nitrogen availability and poplar growth on degraded agricultural soil. *New Forests* 41:147-162.
- Food and Agriculture Organization (FAO) (2006). *Production Yearbook*. Rome, Italy P 60.
- Gebreselassie R, Dereje A, Solomon H (2014). On Farm Pre-harvest Agronomic Management Practices of *Aspergillus* Infection on Groundnut in Abergelle, Tigray. *Journal of Plant Pathology and Microbiology* 5:2-7.
- Ghosh PK, Dayal D, Bandyopadhyay KK, Mohanty M (2006). Evaluation of straw and polythene mulch for enhancing productivity of irrigated summer groundnut. *Field Crops Research* 99:76-86.
- Ghosh PK, Singh DV, Naik PR (1997). Improvement of yield of summer groundnut through mulching and criss-cross sowing in Gujarat, India. *International Arachis News Letter* 17:61-62.
- Harman GE (2006). Overview of mechanisms and uses of *Trichoderma* species. *Phytopathology* 96:190-194.
- Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004). *Trichoderma* species-opportunistic, a virulent plant symbionts. *National Review Microbiology* 2:43-56.
- Indian Oilseed and Produce Export Promotion Council (IOPEPC) Kharf. (2017). Survey of Groundnut crop. <http://www.iopepc.org/Groundnut%20Survey%20Report-Kharif%202017.pdf>
- Kahsay T, Mewael K (2014). *Aspergillus* species groundnut seed invasion as influenced by soil solarization and time of planting. *International Journal of Advanced Research in Biological Sciences* 1(8):121-129.
- Khan M, Chatta TH (2005). Growth and yield response of tomato (*Lycopersicon esculentum* L.) to organic and inorganic mulches. *Asian Journal of Plant Science* 2:128-131.
- National Metrological Agency Mekelle branch (NMA) (2017). <https://www.preventionweb.net/organizations/10322>
- Oberson AB, Else KF, Dennis KR, Idupulapati MS, Poul CT, Benjamin LF, Emmanuel F (2006). Improving phosphorus fertility in tropical soils through biological intervention. *In: Norman, T. (eds.) Biological approaches to sustainable soil system*. CRC Press, Taylor and Francis Group P 531.
- Patil Shirish S, KelkarTushar S, BhaleraoSatish A (2013). Mulching: A Soil and Water Conservation Practice Research. *Journal of Agriculture and Forestry Science* 1(3):26-29.

- Pervez MA, Iqbal M, Shahzad K, Hassan A (2009). Effect of mulch on soil physical properties and NPK concentration in maize (*Zea mays* L.) shoots under two tillage systems. *International Journal of Agricultural Biology* 11(2):119-124.
- Prasad D, Anes KM (2008). Effect of metabolites of *Trichoderma harzianum* and *Trichoderma viride* on plant growth and *Meloidogyne incognita* on okra. *Annual Plant Protection Science* 16:461-465.
- Rojas FG, Reynoso MM, Fereza M, Chulze SN, Torres AM (2007). Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Protection* 26:549-555.
- Statistical Analysis System (SAS) (2004). SAS 9.1.3. Qualification Tools User's Guide, Cary, NC: SAS Institute Inc. https://support.sas.com/documentation/installcenter/the_sas_system/9.1.3_TS1M3/qualification_tools_guide.pdf
- Surendranatha EC, Sudhakar C, Eswara NP (2011). Aflatoxin contamination in groundnut induced by *Aspergillus flavus* type fungi: a critical review. *International Journal of Applied Biology and Pharmaceutical Technology* 2:2-9.
- United States Department of Agriculture (USDA) (2017). World Agricultural Production. <https://www.fas.usda.gov/data/world-agricultural-production>
- Uwah DF, Iwo GA (2011). Effectiveness of organic mulching on the productivity of maize and weed growth. *Journal of Animal and Plant Science* 21(3):525-530.
- Wiess EA (2000). *Oil Seed Crops* 2nd edition. Blackwell Science private company limited, Oxford, London, Berlin Carlton, Paris pp. 31-36.
- Zamir MSI, Javeed HMR, Ahmed W, Sarwar N, Shehzad M, Sarwar MA, Iqbal S (2013). Effect of Tillage and Organic Mulches on Growth, Yield and Quality of Autumn Planted Maize (*Zea mays* L.) and Soil Physical Properties. *Cercetări Agronomice în Moldova* 46(2):17-26.
- Zayton AM, Guirguis AE, Allam KHA (2014). Effect of Sprinkler Irrigation Management and Straw Mulch on Yield, Water Consumption and Crop Coefficient of Peanut in Sandy soil. *Egyptian Journal of Agricultural Research Institute* 92(2):657-673.

Full Length Research Paper

Performance of CIP and Dutch potato varieties under Rwanda climate conditions

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In attempt to develop new potato varieties with the current market lead traits, nine Dutch potato varieties namely Fabula, Panamela, Sagitta, Challenger, Sifra, Rosi, Memphis, Taurus and Derby, and International Potato Center (CIP) varieties Kinigi and Kirundo were evaluated in three Rwandan sites for two growing seasons in a randomized complete block design (RCBD) with tree replications. The data collected consists of plant vigor, late blight incidence and severity, yields and processing qualities. The collected data were submitted to analysis of variance. The results showed that variety, site, season, and all interactions of main variation factors have significant effects on total yields. The average total yields of CIP varieties were extremely high and significantly different compared to tested Dutch varieties. These yields were 34.2 and 30.7 tons ha⁻¹ for Kirundo and Kinigi, respectively. The best performing among tested Dutch varieties were Panamela with 20.2 tons ha⁻¹, Rosi with 16.4 ton ha⁻¹, Sagita with 15.8 tons ha⁻¹, and Taurus with 14.4 tons ha⁻¹. The CIP varieties Kinigi (7.2 score) and Kirundo (7.2 score) revealed a big plant vigour compared to tested Dutch varieties. Among Dutch varieties, Taurus (6.9 score) and Panamela (6.2 score) showed the highest plant vogour. CIP varieties Kinigi and Kirundo, and Dutch varieties Sifra and Taurus revealed the same trends for late blight (less than 30% of plants revealed late blight symptoms. Taurus, Challenger, and panamela revealed the highest dry matter content of 22.4, 21.7, and 20.0%, respectively. This dry matter content is high compared to CIP varieties Kinigi (19.20%), commonly used for French fries and crisps in Rwanda. Dutch varieties Rosi, Challenger and Taurus revealed crisps with yellow color without rolling. Panamela and Kinigi showed crisps with similar characteristics. The results from this study suggested that any private company planning to invest in potato processing for French fries and crisps, the Dutch varieties such as Rosi, Challenger, Taurus and Panamela are good candidates for raw materials.

Key words: Crisps, Dutch, yield, blight symptoms.

INTRODUCTION

Potatoes (*Solanum tuberosum*) is the fourth among the world's food crops after wheat, rice and maize, grown in more than 100 countries at an area estimated at 18.4

million hectares of land, with a productivity of 18.4 ton ha⁻¹ and annual production of 347 million tons (FAOSTAT, 2015). Globally, it is the third consumed food commodity

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after rice and wheat and has therefore been recommended as a food security crop by the Food and Agriculture Organization of the United Nations. Potato is viewed as an important food crop to face the world uncertainties in food supply, and steady hunger rates of the current growing population leading to an increasing demand for food (FAO, 2009; Devaux et al., 2014). Potato provides more food with high nutrient content in a short period (FAO, 2008; Lutaladio and Castaldi, 2009). Currently, the cultivation of potato is becoming an integral part of the global food system based on nutritional value, and social and economic importance of this plant (Paul, 1985).

Rwanda is one of the sixth potato producing countries in Africa after Egypt, Malawi, Kenya, Algeria, South Africa, and the third in sub-Saharan Africa, and the largest producer of potatoes in East Africa (CIP, 2011; FAOSTAT, 2015). Potato is one of the six priority crops (potato, maize, rice, wheat, cassava and beans) supported in the Crop Intensification Program established by the Ministry of Agriculture and Animal Recourses in Rwanda (Kathiresan, 2011). Due to the growing market demand in Rwanda and neighbouring countries (USAID, 2016), potato production has increased substantially, particularly in the Northern and Western provinces of Rwanda where agricultural conditions are favourable for potato production (MINAGRI, 2014).

It covers 4% of total cultivated land in each growing season, and provides 10% of total production of main food crops in Rwanda (NISR, 2015). Most potato cultivation consists of small family farms that intercrop potato with beans and maize, and the yield average is still low (around 10 tons ha⁻¹) compared to other countries that can reach up to 35 tons ha⁻¹ (FAOSTAT, 2015). This low productivity is mainly due to low soil fertility, unavailability of clean seeds, poor market accessibility and lack of financial investment, a range of diseases, and lack of access to high yielding varieties (Muhinyuza et al., 2012). Most of the current famous grown potato varieties in Rwanda have been developed 20 years ago, based on needs of that time (Monares, 1984; Muhinyuza et al., 2012). Nowadays, end users' needs have changed. Current established potato processing plants require potato with specific characteristics such as high dry matter, low reducing sugar content, good shape and shallow eyes. In attempt to develop other potato varieties meeting end user's preferences, the RAB Potato Sub-Programme has introduced and evaluated nine new potato varieties, in The Netherlands in comparison with CIP varieties. This publication reports the results.

MATERIALS AND METHODS

Planting

Nine Dutch potato varieties (Fabula, Panamera, Sagitta, Challenger, Sifra, Rosi, Memphis, Taurus and Derby) belonging to The

Netherlands were introduced in the form of minitubers by BRAMIN Ltd., in March 2015. After seeds increase, trials were established with Dutch varieties and two CIP varieties Kinigi (CIP 378699.2) and Kirundo (CIP 8212.5). The description of tested materials is shown in Table 1.

Trial establishment

The National Performance Trials (NPTs) were established at Cyanika sector in Burera district (2,300 m), Cyuve sector in Musanze district (1,860 m), and Mudende sector in Rubavu district (2,300 m) for two growing seasons. Cyanika site has an annual rainfall and temperature of 1200 mm and 20°C, respectively, while Mudende receives an annual average rainfall and temperature estimated at more than 1450 mm, and 15°C, respectively. The soils of both sites were derived from volcanic parental materials. Cyuve has 20% organic volcanic soils and bimodal rainfall with the short and long rains being received in October to December and March to June, respectively. The annual mean temperature and rainfall received is 18.5°C and 1400 mm, respectively (RAB, 2014). The first season was carried out from March to July 2017 and the second season was performed from September 2017 to February 2018. Trials were established in a randomized complete block design with three replications of 40 hills per plot. The experimental plot consisted of four rows with ten plants in each row. The spacing was 80 and 30 cm between rows and plants, respectively. Trials were surrounded with three guard rows planted with Kinigi potato variety. Fertilizers N₁₇P₁₇K₁₇ and Urea (46-0-0) were applied at planting with a rate of 300 and 200 kg/ha, respectively. Aphids (virus vectors) and other insects were controlled by applying Rocket 44 EC (Profenofos 40% + cypermethrin 4% EC) at a dilution of 30 ml in 15 L of water. Late blight was controlled by using Dithane M45, a contact fungicide, wettable powder 80% Mancozeb at a rate of 2.5 g/L sprayed before the appearance of symptoms of late blight infection and by Ridomil gold, a systemic fungicide, wettable powder 64% Mancozeb, 4% Metalaxil at a rate of 2.5 g/L when symptoms appear. The trials were treated with Ridomil gold once and Dithane M45 eight times with 7 days of intervals. The weeding was carried out manually during the growth of the crop, when it was needed. Soil ridging up was also done manually and no irrigation was applied. Trials were managed by farmers who carried out all crop husbandry activities, and dehauling at fully maturity (120 days after planting). Harvesting was carried out at 135 days after planting.

Data collection

Disease and pest data

Late blight damage was assessed visually using the scale of 1-9, where no symptom, 5: medium (20-50%) damage and 9: very severe (75-100%) damage at 60 days after planting. Virus disease and leaf infection were assessed using a score of 0 to 5, where 0: (0-5%), no serious symptom, 1: (6-15%) maximum foliage infected is below 20%, 2: (16-35%) about 25% of foliage is affected, 3: (36-65%) about 50% of foliage area is affected, 4: (66-85%) about 75% forage area is affected, 5: (86-100%) all leave are affected or dead (Bonierbale et al., 2006).

Yield data

The yield data including total yield, marketable and unmarketable yields, number and weight of tubers per plot, and dry matter content of tubers were collected at harvesting following the approach

Table 1. Characteristics of tested materials.

Variety	Maturity	Skin colour	Flesh colour	Shape
Challenger	Medium late	Yellow	Light yellow	Oval / Long oval
Derby	Early	Yellow	Light yellow	Oval
Fabula	Medium late	Yellow	Light yellow	Oval
Memphis	Medium early	Red	Light yellow	Oval / Long oval
Panamera	Late	Yellow	Light yellow	Oval
Rosi	Late	Red	Light yellow	Oval
Sagitta	Medium early	Yellow	Light yellow	Oval
Sifra	Late	Light yellow	Cream	Round oval
Taurus	Medium late	Yellow	Light yellow	Round / Round oval
Kinigi	Medium late	Red	Light yellow	Round
Kirundo	Medium early	white	Light yellow	Round oval

described by De Haan et al. (2014). To determine the marketable and unmarketable yields, tubers were sorted and classified into three groups: Tubers weighing 200 to 300 g or tubers with a diameter equal or bigger than 60 mm, Tubers weighing 80 to 200 g or tubers with a diameter ranging between 30 and 60 mm, and tubers weighing less of 80 g or tubers with a diameter that is less than 30 mm. The number and weight of tubers of each category and for each variety were determined. The category one and two were classified in marketable yields while the last category was classified as unmarketable yields.

Processing test

This test consists of identifying the phenotypic characteristics of processing qualities such as shape, type and number of eyes using visual inspection. The dry matter was determined using under-water weight methods as detailed by Haase (2004). The Ashton Mayer's tool was set to zero. Potato sample was put in bags, determined the weight in air, and record the results. Eighty liters of water were filled in an open tank and float the weighed potato in air. The potato samples in bag were dropped until down of tank and reset tools to zero. Bags of samples were kept suspended in water and read the number in ashton Mayer's tool. Weight of samples in water was recorded using the following Formula 1 and 2:

$$\text{Sp.gr} = [\text{weigh in air} / (\text{weigh in air} - \text{weigh in water})] \quad (1)$$

$$\text{Dry matter (\%)} = [24.182 + 211.04 (\text{sp.gr} - 1.0988)] \quad (2)$$

Other processing characteristics such as frying time, color (sugar content), taste, crunch, waste, conversion, and others were tested by WINNAZ, a processing factory. The sugar content was assessed using color chart after frying (Bonierbale et al., 2006).

Data analysis

Data collected were processed by analysis of variance (ANOVA) using GenStat 15th edition. When the significance of differences among genotypes, sites, and seasons were detected, the mean separation was performed with the Least Significant Difference (LSD) test ($P = 0.01$ and $P = 0.05$) (Payne et al., 2011). The qualitative data (such as disease and pests score, and plant vigor) were processed by determining their averages using SPSS (PASW statistics 18.0) computer package (SPSS, 2006), and Microsoft Excel (Windows Office 2012; Microsoft Inc., Redmond, WA).

RESULTS

Yield results

Effects of variety, site, season, and interactions of variety \times season, site \times season, and variety \times site \times season on marketable yields of potato were significant. However, the interaction of varieties \times site was not significant (Table 2).

The CIP varieties showed the highest marketable yields compared to Dutch varieties (Table 2). The yield of these varieties was 28 and 22.8 tons ha^{-1} for Kirundo and Kinigi, respectively. The highest yielding of marketable tubers among tested Dutch varieties were Panamela with 17.5 tons ha^{-1} , Rosi with 13.6 tons ha^{-1} , Taurus with 13 tons ha^{-1} , Sagita with 12.9 tons ha^{-1} , and Deby with 12.5 tons ha^{-1} . The lowest yielding among tested Dutch varieties were Challenger with 9 tons ha^{-1} and Fabula with 8.4 tons ha^{-1} (Table 3).

Variety, site, season, and interactions of variety \times season, site \times season, and variety \times site \times season showed significant effects on total yields of potato. The interaction effects of variety \times site on total tuber yields were not significant (Table 4).

The average total yields of CIP varieties were extremely high and significantly different compared to tested Dutch varieties. These yields were 34.2 and 30.7 tons ha^{-1} for Kirundo and Kinigi, respectively. The best performing among tested Dutch varieties were Panamela with 20.2 tons ha^{-1} , Rosi 16.4 tons ha^{-1} , Sagita with 15.8 tons ha^{-1} , and Taurus with 14.4 tons ha^{-1} . The lowest yielding among Dutch tested varieties were Fabula with 11.6 tons ha^{-1} and Challenger 13.5 tons ha^{-1} (Table 5).

Plant vigour, diseases and pest results

The CIP varieties Kinigi and Kirundo (both with a score of 7.2) revealed a big plant vigour compared to tested Dutch varieties. Among the Dutch varieties, Taurus and Panamela with scores of 6.9 and 6.2, respectively,

Table 2. Analysis of variance for marketable yields of CIP and Dutch potato varieties tested at three sites for two growing seasons in Rwanda.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	142.47	71.23	2.86	-
Varieties	10	6348.28	634.83	25.48	<0.001
Site	2	4322.15	2161.07	86.72	<0.001
Season	1	2631.59	2631.59	105.6	<0.001
Variety × Site	20	649.14	32.46	1.3	0.189
Variety × Season	10	779.34	77.93	3.13	0.001
Site × Season	2	3257.37	1628.68	65.36	<0.001
Variety × Site × Season	20	1107.98	55.4	2.22	0.004
Residual	130	3239.53	24.92	-	-
Total	197	22477.85	-	-	-

Table 3. Average of marketable yields (tons ha⁻¹) of potato varieties tested for two growing seasons at three sites in Rwanda.

Variety	Cyanika		Cyuve		Mudende		Average
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	
Challenger	4.0	4.4	16.0	4.4	20.9	4.4	9.0 ^d
Derby	6.3	6.6	23.7	6.3	28.0	4.4	12.5 ^{cd}
Fabula	3.3	4.3	8.9	4.5	19.2	10.2	8.4 ^d
Kinigi	13.2	19.0	24.3	22.9	29.7	27.8	22.8 ^{ab}
Kirundo	11.3	22.9	38.3	21.5	47.2	26.6	28.0 ^a
Memphis	5.9	7.1	12.2	8.7	27.0	10.7	11.9 ^d
Panamela	6.9	15.4	21.5	13.1	35.4	12.7	17.5 ^{bc}
Rosi	5.3	8.0	17.5	8.8	34.4	7.3	13.6 ^{cd}
Sagitta	5.3	8.5	26.0	5.0	25.0	7.8	12.9 ^{cd}
Sifra	5.6	10.3	13.9	6.2	20.1	13.0	11.5 ^d
Taurus	6.9	10.4	23.6	7.0	16.9	13.0	13.0 ^{cd}
% CV	-	-	-	-	-	-	15.7
LSD (0.05)	-	-	-	-	-	-	8.1

Table 4. Analysis of variance of total yields of CP and Dutch potato varieties tested at three sites for two growing seasons in Rwanda.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	16.26	8.13	0.3	-
Varieties	10	10085.99	1008.6	37.24	<0.001
Site	2	1308.99	654.5	24.16	<0.001
Season	1	6466.97	6466.97	238.75	<0.001
Variety × Site	20	773.21	38.66	1.43	0.121
Variety × Season	10	631.22	63.12	2.33	0.015
Site × Season	2	304.64	152.32	5.62	0.005
Variety × Site × Season	20	1012.34	50.62	1.87	0.02
Residual	130	3521.3	27.09	0	0
Total	197	24120.93	0	0	0

showed the highest plant vigour. The Dutch variety showing the lowest plant vigour was Fabula with a score

of 3.9 (Table 6). According to the late blight score, the CIP varieties Kinigi and Kirundo, and Dutch varieties Sifra

Table 5. Average total yields (tons ha⁻¹) of CIP and Dutch potato varieties tested for two growing seasons at three sites in Rwanda.

Variety	Cyanika		Cyuve		Mudende		Average
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	
Challenger	12.2	6.8	21.7	6.6	26.3	7.3	13.5 ^c
Derby	16.6	8.2	18.3	7.8	27.0	6.2	14.0 ^c
Fabula	16.4	5.7	12.1	4.4	21.6	9.3	11.6 ^c
Kinigi	43.0	21.2	27.5	21.6	39.7	31.4	30.7 ^a
Kirundo	37.1	23.9	39.8	25.1	51.4	27.8	34.2 ^a
Memphis	16.7	8.0	15.2	8.6	25.5	10.5	14.1 ^c
Panamela	22.2	15.2	22.7	11.9	30.7	18.4	20.2 ^b
Rosi	14.2	10.8	22.5	8.1	33.2	9.8	16.4 ^{bc}
Sagitta	19.6	9.5	30.9	6.6	19.7	8.7	15.8 ^{bc}
Sifra	11.6	10.7	12.3	8.1	23.3	16.2	13.7 ^c
Taurus	12.8	11.2	21.3	8.0	19.2	14.0	14.4 ^c
% CV	-	-	-	-	-	-	12.7
LSD (0.05)	-	-	-	-	-	-	8.5

Table 6. Plant vigor, late blight, virus, and leaf miner score of CIP and Dutch potato varieties tested for two growing seasons at three sites in Rwanda.

Variety	Vigour	Late blight score	Virus score	Leaf miner
Challenger	4.6	7.2	2.9	5.7
Derby	4.6	7.0	2.2	4.8
Fabula	3.9	7.0	2.2	4.1
Kinigi	7.2	3.0	3.1	3.9
Kirundo	7.2	2.3	1.2	2.8
Memphis	5.9	6.6	2.8	5.7
Panamela	6.2	4.2	1.4	2.9
Rosi	5.2	7.0	2.4	5.0
Sagitta	4.8	5.2	2.0	3.4
Sifra	5.7	2.9	2.6	3.2
Taurus	6.9	3.1	2.6	3.3

and Taurus revealed the same trends. Their late blight score was below 3 (less than 30% of plants revealed late blight symptoms) (Table 6). For virus diseases of both varieties, the score was not exceeding 3, meaning that less than 50% of foliage area of plants revealed viral symptoms (Table 6).

Processing results

The potato with high number of deep eyes was only a CIP variety Kinigi. The other varieties revealed a low number of shallow eyes (Table 7). In terms of dry matter content, Taurus, Challenger, and Panamela revealed the highest dry matter content of 22.4, 21.7%, and 20.0%, respectively. This dry matter content is high compared to CIP variety Kinigi with a dry matter content of 19.20%.

Results obtained after frying (Conversion, time for frying, color, taste, and crunch) are presented in Table 6.

Based on colour and rolling of processed chips, the Dutch varieties Rosi, Challenger and Taurus revealed crisps with clear yellow color without rolling. Panamela and Kinigi showed crisps with similar characteristics (Figure 1).

DISCUSSION

Yields

Adaptability of crop varieties can vary from location to location depending on the agro-ecology of a particular area. The development and dissemination of best suited varieties with specific adaptability to the environment was

Table 7. Results of processing test of nine Dutch potato varieties and one local variety, Kinigi.

No.	ID Clone	Shape (ovale)	Eyes type	Eyes number	sp.gr	DMC (%)	Conv	Frying time (s)	Colour	Taste	Crunch	General observations
1	Sifra	long	shallow	few	1.05	13.88	4.50	208	6.00	bad	bad	bad
2	Memphis	long	shallow	few	1.08	20.21	10.00	230	2.50	bad	bad	bad
3	Sagitta	long	shallow	few	1.06	15.99	5.00	251	3.50	bitter	bad	bad
4	Panamela	long	shallow	few	1.08	20.21	53.60	194	4.50	good	bad	bad
5	Derby	long	shallow	few	1.06	15.99	5.00	235	3.50	bad	good	bad
6	Rosi	round	shallow	more	1.07	18.10	3.90	270	2.00	good	good	good
7	Challenger	round	shallow	more	1.09	22.32	6.70	190	2.00	good	good	good
8	Taurus	round	shallow	few	1.09	22.32	6.70	236	2.00	bad	good	good
9	Fabula	round	shallow	more	1.06	15.99	4.30	235	3.00	bad	bad	bad
10	Kinigi	round	deep	more	1.08	20.21	4.10	270	2.50	good	good	good
11	Kirundo	-	-	-	-	-	-	-	-	-	-	-

Conv: Conversion, DMC: dry matter content, s: second.



Figure 1. Crisps processed by WINNAZ from Kinigi (a CIP potato variety) and nine Dutch potato varieties.

a sustainable strategy to increase the productivity and production, and to overcome economic and food security constraints. Therefore, it is essential to carry out specific adaptation trials to identify suitable varieties. In the evaluation of potato varieties at Bule Hora District, in Ethiopia, Addis et al. (2017), found a significant variation among potato varieties for tuber yields, number of tubers per hill, and tuber weight per hill. Statistical analysis of the dry matter content, and starch yields of various tested potato varieties revealed significant effects ($P < 0.01$) due

to genotype, location variation and genotype \times environment interaction. The dry matter content and starch yields ranged from 17.82 to 26.70% and from 2.21 to 6.91 %, respectively (Tesfaye et al., 2012). Habtamu et al. (2016) reported statistically significant variations in most of evaluated traits. The highest total yields of 56.52 tons ha^{-1} and marketable tuber yield of 53.97 tons ha^{-1} were observed. The results from this study agreed with these previous findings. Effects of variety, site, season, and interactions of variety \times season, site \times season, and

variety \times site \times season on marketable and total yields of potato were significant. The interaction effects of variety \times site on total tuber yields were not significant (Tables 2, 3 and 4).

Marketable yields are an important trait for root and tuber crops because it is sold on market to get money while no marketable yield is mainly used by farmers for home consumption. This yields category is affected by different factors. For example, at the wider intra row spacing of 30 cm, and at the closer intra row spacing of 10 cm, the marketable tuber yields were 23.54 and 18.27 tons ha⁻¹, respectively. The highest marketable tuber yields of 27.48 tons ha⁻¹ were obtained at the 15 days earthing up whereas the lowest yield of 17.03 tons ha⁻¹ was obtained at the 45 days earthing up (Getachew et al., 2012). Through this study, the check varieties are adapted to local environmental conditions. Therefore, their average total yields were extremely high and significantly different compared to Dutch varieties under evaluation. These yields were 34.2 and 30.7 tons ha⁻¹ for Kirundo and Kinigi, respectively. The best performing among tested Dutch varieties were Panamela with 20.0 tons ha⁻¹, Rosi with 16.0 tons ha⁻¹, Sagita with 15.0 tons ha⁻¹, and Taurus with 14.4 tons ha⁻¹. The lowest yielding among Dutch tested varieties were Fabula with 11.6 tons ha⁻¹ and Challenger with 13.5 tons ha⁻¹. The same varieties have showed the same trends of marketable yields (Tables 4 and 5). These findings highlights that the tested varieties, the two growing seasons and the sites where potato varieties were tested are different. The tested varieties have a different genetic makeup. The sites where the trials were conducted have different soils characteristics, micro-climates. Also, the two growing seasons in which potatoes were tested are different. For example, the season A (September to December) is characterised by a heavy and long lasting rainfall, while the growing season B (March to June) is characterised by light and short lasting rainfall. These variations are the main causes of variations in marketable and total yields observed among tested potato varieties. Even though two seasons are recommended for National Performance Trials to evaluate new potato varieties released in COMESA region, the carried out trials did not reveal the trends of stability and adaptability of tested Dutch varieties. Therefore, the conclusion made is only based on yield across all seasons and sites.

The Dutch varieties are new in the Rwandese environmental conditions. This is the main reason their yields were low compared to local check varieties. This was also confirmed by diseases results (Table 6), where, the Dutch varieties revealed a high susceptibility to late blight. All tested plant materials (Dutch varieties and local check varieties) were treated in the same conditions but only two Dutch varieties Sifra and Taurus revealed the lowest disease score as Kinigi and Kirundo, the local checks. Others Dutch varieties were extremely susceptible to late blight (Table 6). Even though, an

extreme susceptibility was observed, the results revealed that by using special approaches for diseases control, the Dutch varieties such as Panamela, Sagita, Taurus and Rosi can produce appreciable yields as local varieties. However, these approaches have implications on the cost of potato production. Consequently, the selling price of the yields should be high compared to local varieties, because the local varieties can produce enough yield without special disease control approaches.

Plant vigour, diseases and pest results

The CIP varieties Kinigi and Kirundo (both with a score of 7.2) revealed a big plant vigour than Dutch varieties under evaluation. Among the Dutch varieties, Taurus and Panamela with scores of 6.9 and 6.2, respectively, showed the highest plant vigour. The Dutch variety showing the lowest plant vigour was Fabula with a score of 3.9 (Table 6). The Dutch varieties were developed in long day environments while the CIP varieties were developed at Lima, Peru within a short day environment. Trials were established in Rwanda where there is a short day environment. Therefore, the low plant vigor observed on Dutch varieties compared to CIP varieties should be associated with the day length. This has effects on variation of yield between these categories of potato varieties (Dutch and CIP varieties). It is known that the leaf canopy is the center of photosynthesis which produces plant assimilates for tuber formation. Canopy structure and vigor, reduce weeds and soil water evaporation. Farmers perceive that the crop varieties with high plant vigor produce high yield. However, this yield is associated with the genotype photosynthesis capacity. The same observation was reported by Pereira et al. (2017), in their study to identify the performance of advanced potato clones, plant vigor, tuber yield and specific gravity of Brazilian and European potato varieties. They found that under Brazilian soil and climate conditions, yields of European varieties are lower in comparison with the countries of origin, because these cultivars were selected under long photoperiod and low pressure conditions of some biotic factors affecting the crop in Brazil. Silva et al. (2014), reported that when best European varieties are cultivated under subtropical and tropical conditions, they show a shorter vegetative period, and, therefore, have a lower photoassimilate production. They suggested that it is necessary to use a large quantity of inputs to achieve higher yields, but this may lead to lower crop sustainability.

Processing qualities

Potato processing industries require continuous supply of tubers as raw materials for fries or crisps preparation (Adnan et al., 2016). Currently the most important

characteristics of potato production is processing quality. This is because farmers want a high price of their produce and consumers want to facilitate food preparation, by buying processed products. Potato supply as a raw material for processing industry must fulfil a number of qualities including low sugar contents, high dry matter, more specific gravity, high antioxidants, low weight loss, good color, and shallow eyes (Connor et al., 2001). Therefore, there is a need to develop and evaluate the processing characteristics of different commercial potato cultivars for the benefit of chips and crisps industries (Adnan et al., 2016). The results from the present study revealed that the potato with high number of deep eyes was a CIP variety Kinigi. The other varieties revealed a low number of shallow eyes (Table 7). In terms of dry matter content, Taurus, Challenger, and Panamela revealed the highest dry matter content of 22.4, 21.7, and 20.0%, respectively. This dry matter content is high compared to CIP variety Kinigi with a dry matter content of 19.20% (Table 7). Based on colour and rolling of processed chips, Dutch varieties Rosi, Challenger and Taurus revealed crisps with yellow color without rolling. Panamela and Kinigi showed crisps with similar characteristics (Figure 1). All tested Dutch varieties revealed the shape, type and number of eyes appropriate for processing varieties. These varieties have low number of shallow eyes (Table 7). At this point, the CIP variety Kinigi does not have these characteristics; its eyes are extremely deep. The dry matter content is very important characteristic for roots and tubers varieties for processing. However, this trait is significantly affected by the environment (Elfnech et al., 2011; Abebe et al., 2012). This study revealed that Dutch varieties such as Taurus with a dry matter content of 22.4% Challenger with a dry matter content of 21.7%, and Panamela with a dry matter content of 20.0% revealed the highest dry matter content, compared to CIP variety Kinigi with a dry matter content of 19.20%, commonly used for French fries and crisps (Table 6). This indicates that Dutch varieties can produce tubers with enough dry matter content meeting the processor needs. Based on taste, conversion, and crunch (Table 7) and on rolling and colour of processed crisps (Figure 1), the Dutch varieties such as Rosi, Challenger and Taurus were the best to give quality crisps. Panamela and Kinigi showed crisps with similar characteristics. Therefore, any private company planning to invest in potato processing, the aforementioned varieties are good candidates for crisps and chips.

Conclusions

Two growing seasons of National Performance Trials of a new varieties released in one country of COMESA region, are recommended if a breeder wants to release these varieties in another country of the same region.

The results from the established National Performance Trials did not reveal the trends of stability and adaptability of tested varieties. The Dutch potato varieties like other European grown varieties were selected in conditions of long photoperiod and low biotic and abiotic stresses. When these varieties are grown under tropical conditions, with a short photoperiod and high biotic and abiotic stresses, they showed a low photo assimilate production, highly susceptibility to the late blight, a low plant vigour, and yields compared to other potato varieties like CIP varieties developed in these conditions. Therefore, the cultivation of these Dutch varieties requires to use a special input to achieve higher yields, but this may lead to lower crop sustainability and negative implications on the cost of production. The Dutch varieties namely Panamela, Sagita, Taurus and Rosi can produce appreciable yields as CIP varieties if special measures are applied. Among tested varieties, the Dutch varieties of Rosi, Challenger and Taurus are the best to produce crisps, while Panamela produces crisps with similar characteristics as CIP variety Kinigi, commonly used for French fries and crisps. Therefore, any private company planning to invest in potato processing for French fries and crisps in Rwanda, the Dutch varieties of Rosi, Challenger, Taurus, and Panamela are good candidates to produce raw materials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Abebe T, Wongchaochant S, Taychasinpitak T, Leelapon O (2012). Dry matter content, starch content and starch yield variability and stability of potato varieties in Amhara region of Ethiopia. *Kasetsart Journal (Natural Sciences)* 46:671-683.
- Addis S, Regassa D, Tigre W (2017). Irish potato (*Solanum Tuberosum*) variety evaluation at Bule Hora District of Borena zone. *Global Journal of Science Frontier Research* 17:2.
- Adnan A, Randhawa MA, Butt MS, Asghar M (2016). Assessing the processing quality of different potato cultivars during storage at various temperatures. *Journal of Chemistry Society of Pakistan* 38(05):1005-1023.
- Bonierbale M, Stef de Haan, Forbes A (2006). Procedures for standard evaluation trials of advanced potato clones. An International Cooperators' Guide. International Potato Center, Apartado 1558, Lima 12, Peru.
- CIP (2011). Manual on quality seed potato production using aeroponics. International Potato Center, Lima, Peru.
- Connor CJ, Fisk KJ, Smith BJ, Melton LD (2001). Fat uptake in French fries as affected by different potato varieties and processing. *Journal of Food Sciences* 66:903-925.
- De Haan S, Forbes A, Amoros W, Gastelo M, Salas E, Hualla V, De Mendiburu F, Bonierbale M (2014). Procedures for standard evaluation and data management of advanced potato clones. Module 2: Healthy tuber yield trials. International Co-operators Guide. Lima (Peru). International Potato Center 44 p.
- Devaux A, Kromann P, Ortiz O (2014). Potatoes for Sustainable Global Food Security. *Potato Research* 57(3-4):185-199.
- Elfnech F, Tekalign T, Solomon W (2011). Processing quality of

- improved potato (*Solanum tuberosum* L.) cultivars as influenced by growing environment and blanching. *African Journal of Food Science* 5(6):324-332.
- FAOSTAT (2015). Statistics division. FAO. <http://faostat.fao.org/site/612/default.aspx#ancor> (accessed 27 November 2018).
- Getachew T, Belew D, Tulu S (2012). Yield and growth parameters of Potato (*Solanum tuberosum* L.) as influenced by intra row spacing and time of earthing up: In Boneya Degem District, Central Highlands of Ethiopia. *International Journal of Agriculture Research* 7(5):255-265.
- Haase NU (2004). Estimation of dry matter and starch concentration in potatoes by determination of under-water weight and near infrared spectroscopy. *Potato Research* 46:117-127.
- Habamu G, Wahassu M, Beneberu S (2016). Evaluation of Potato (*Solanum tuberosum* L.) Varieties for Yield and Yield Components in Eastern Ethiopia. *Journal of Biology, Agriculture and Healthcare* www.iiste.org, ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) 6:5.
- Kathiresan A (2011). Strategies for sustainable crop intensification in Rwanda: Shifting focus from producing enough to producing surplus. Kigali, Rwanda, Ministry of Agriculture and Animal Resources. http://www.gakenke.gov.rw/fileadmin/templates/DOCUMENT_Z_ABA_KOZI/abakozi/CIP_Strategies_2011.pdf
- Lutaladio N, Castaldi L (2009). Potato: The hidden treasure. *Journal of Food Composition and Analysis* 22(6):491-493.
- MINAGRI (2014). Annual Report for fiscal year 2013-2014. Kigali, Rwanda, Ministry of Agriculture and Animal Resources. <https://www2.ed.gov/about/reports/annual/index.html>
- Muhinyuza JB, Shimelis H, Melis R, Sibiya J, Ndambe Nzaramba M (2012). Participatory assessment of potato production constraints and trait preferences in potato cultivar development in Rwanda. *International Journal of Development and Sustainability Online* ISSN: 2168-8662 – www.isdsnet.com/ijds Volume 1 Number 2, September 2012 (In Press) ISDS Article ID: IJDS1208020.
- National Institute of Statistics (NISR) (2015). Seasonal Agricultural Survey 2015. National Institute of Statistics of Rwanda. Kigali Rwanda. Website: www.statistics.gov.rw.
- Paul HL (1985). Potato physiology. *Academic Press*. INC. Academic Press 602 p.
- Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM (2011). An introduction to GenStat for windows, 15th ed. Hemel Hempstead, UK.
- Pereira AS, Giovani OS, Carvalho ADF, Ponijaleki RS (2017). Performance of advanced potato clones: Plant vigor, tuber yield and specific gravity. *Horticultura Brasileira* 35:440-444.
- RAB (2014). Annual report. Rwanda Agriculture and Animal Resources Development Board (RAB). Kigali, Rwanda.
- Silva GO, Pereira AS, Suinaga FA, Carvalho ADF (2014). Yield performance of advanced clones of potato. *Horticultura Brasileira* 32:230-233.
- SPSS (2006). PASW Statistics for Windows SPSS Inc. SPSS Inc., Chicago, IL.
- Monares A (1984). Building an effective potato country programme: the case of Rwanda. *International Potato Centre* P 34.
- USAID (2016) Rwanda early generation seed study country report. <http://www.africaleadftf.org/wp-content/uploads/2016/09/Rwanda-EGSStudy-Final-Report.pdf>. Accessed on 26th January 2019.

Full Length Research Paper

Inoculation with entomopathogenic fungi reduces seed contamination, improves seed germination and growth of chilli seedlings

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The use of entomopathogenic fungi is a common practice for integrated pest management. It has recently been observed that they also play a role as growth promoters and plant disease antagonism. In this study, the effect of inoculation with two strains of *Beauveria bassiana* [(Bals.-Criv.) Vuill. 1912] (strains BB42 and BB09) and two strains of *Metarhizium anisopliae* [(Metschn.) Sorokin 1883] (strains MA25 and MA28) on the percentage of seed germination and development of chilli seedling (*Capsicum annuum* L.) was evaluated. In the *in vitro* test, we did not find significant differences between percentages of germination, but in the *in vivo* test, differences were significant, where sMA28 and BB09 strains obtained the highest germination percentage (85%). It was also found that seedlings inoculated with entomopathogenic fungi generate longer roots and produced more biomass in both tests, as well as lower percentages of contaminated seeds in *in vitro* and *in vivo* tests. All strains evaluated had inhibitory effects against two seed borne fungi isolated from contaminated seed, belong to genus *Alternaria* sp.

Key words: Germination, antagonisms, entomopathogenic fungi.

INTRODUCTION

Capsicum annuum L. is one of most important vegetable worldwide, with a global consumption of 400,000 t approximately, and it account of 16% of the world's total spice trade, but yields are usually very low (Olatunji and Afolayan, 2018). Research efforts to ensure sanity and quality of chilli seeds are of the main importance (Matthews et al., 2012). In optimum conditions, seeds

must be present with a high germination percentage and a good seedling development, however, seedlings are commonly affected by exposed biotic and abiotic factors (Penella and Calatayud, 2018). In chilli seeds, there have been isolated and identified several pathogens that can inhibit germination and generate infections, causing in some cases the death of the plant (Chigoziri and Ekefan,

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2013). Fungal diseases reduce yield losses of up to 50%, mainly seed and seedling rot, causing by fungi such as *Aspergillus niger* (Chauhan Rinkal et al., 2018).

Chilli production is also limited by low germination rate. In nature, seed borne microorganism have strongly associated with seed germination and seedling growth (Shearin et al., 2018). Different pre-germination treatments have been tried to increase the range of chilli seed germination, but it implies the use of chemical agents (Prado-Urbina et al., 2015). Microbial inoculation of seeds with fungi favours germination and emergency of the embryo (Lee et al., 2010). In *Opuntia*, artificial inoculation with native fungi of the soil rhizosphere (*Penicillium chrysogenum*, *Phoma* sp., and *Trichoderma* sp.) helped to break their dormancy, growth of pathogens was inhibited and germination percentage increased (Delgado-Sánchez et al., 2011, 2013). Another rhizosphere fungus *P. chrysogenum* improved seed germination, and reduced disease incidence and disease protection against plant pathogens (Murali et al., 2013).

Beauveria bassiana [(Bals.-Criv.) Vuill. 1912] and *Metarhizium anisopliae* [(Metschn.) Sorokin 1883] are more abundant entomopathogenic fungi (EF) in subtropical environments and their use is a common practice for integrated pest management (IPM) (Pérez-González et al., 2014). These fungi have an excellent biocontrol capacity against insects such as whitefly (*Bemisia tabaci*) in eggplants (Islam et al., 2010), fall armyworm (*Spodoptera frugiperda*) in *Zea mays* (Ramirez-Rodriguez and Sánchez-Peña, 2016), *B. tabaci* Gennadius (Aleyrodidae), potato/tomato psyllid, *Bactericera cockerelli* Sulc. (Triozidae), and western flower thrips, *Frankliniella occidentalis* (Pergande) in tomato (*Solanum lycopersicum* L.), (Ríos-Velasco et al., 2014) among others. They also have been shown to be a plant symbiont which have plant-root-promoting properties (Sasan and Bidochka, 2012) and antagonistic activity against plants pathogens (Jaber and Alananbeh, 2018) and we proposed that these can significantly improve germination and facilitate establishment of seedlings.

This study presents the first report about the effect of *B. bassiana* and *M. anisopliae* on the inhibition of seed borne fungi in chilli seeds. The effect of these fungi on the promotion and seedlings growth of *C. annuum* L. is also evaluated and discussed.

MATERIALS AND METHODS

Fungi strains

Four strains of entomopathogenic fungi were evaluated; two of *B. bassiana* (BB09 and BB42 strains), and two of *M. anisopliae* (MA28 and MA25 strains). Native strains used were BB42 strain, isolated from a bug *Lygus* sp. in El Copal, Guanajuato, and strain MA25, isolated from a white grub that was found in Puruaga town, Guanajuato, both provided by the Laboratory of Beneficial Organisms Reproduction, which belongs to the State Committee of

strains used were active ingredient in commercial products: BB09 strain (Bassianil Wp®) and MA28 strain (Metabich®). All mono-spore cultures were generated and were sown in Sabouraud Dextrose Agar (SDA) and incubated at $25 \pm 1^\circ\text{C}$ (Villegas-Rodriguez et al., 2014).

C. annuum L. seeds

In both experiments, we evaluated seeds of native poblano chilli pepper, native from the state of San Luis Potosi, Mexico.

In vitro inoculation of *C. annuum* L. seeds with entomopathogenic fungi

Chilli seeds were superficially disinfected by soaking for 5 min in 20% sodium hypochlorite (NaOCl) of purity (6% of free chlorine) after washing in 70% ethanol for 5 min. In following step, seeds were rinsed four times in sterile distilled water. One hundred seeds were allocated in 5 petri dishes (20 per dish) with Water Agar Medium. Each seed treated with EF was inoculated with 2 μL of conidia solution (final concentration, 1×10^8 spores ml^{-1}) and in the case of control seeds, 2 μL of INEX-A® solution at 0.2% were added to each one (Lohse et al., 2015). Each treatment was repeated five times. The petri dishes were kept in a growth chamber with a photoperiod of 16:8 (L:D) h. Germination percentage, length of roots and shoots, fresh weight and biomass production of the seedlings were measured after 15 days (Elena et al., 2011).

Trials of antagonisms among seed borne fungi and entomopathogenic fungi

Three seed borne fungi were isolated to non-inoculated seeds used in the *in vitro* test. Infected seeds were marked and placing to new petri dish with SDA and incubated at $25 \pm 1^\circ\text{C}$. Active discs of each strain of EF mixed with SDA medium were placed in one edge of the petri dishes, and we placed each seed borne fungi evaluated in the opposite edge. The petri dishes were maintained in a growth chamber at $25 \pm 1^\circ\text{C}$ and after fifteen days, antagonist effect of each strain of EF over the seed borne fungi was evaluated (Jaber and Alananbeh, 2018). The experiment was repeated three times.

Inoculation tests of *C. annuum* L. seeds with entomopathogenic fungi under greenhouse conditions

Chilli seeds were planted singly in pots of 100 ml containing sterile peat moss (Sunshine Grow Mix #3®, Sun Gro Horticulture Canada). 500 μL of solution containing 1×10^8 conidia ml^{-1} of each EF tested, and commercial surfactant solution INEX -A® at 0.2% in the case of control was applied directly to the substrate. When the seeds began their germination, after seven days, inoculations were carried out again (Farias et al., 2018). It was considered that the seed germinated when the cotyledons emerged on the surface of the soil. Dead plants that had presence of mycelium during the experiment were considered as contamination. Variables evaluated were length, biomass production and fresh weight in roots and shoots 45 days after germination (Moloinyane and Nchu, 2019).

Statistical analysis

One-way ANOVA analysis was conducted on the experimental data that are presented as the mean \pm standard error. Tukey's test was used to compare the treatment with a significant F value in the

ANOVA ($p \leq 0.05$). The analyses were performed with the Statistica software (ver. 7.0, StatSoft Inc., Tulsa, OK, USA).

RESULTS

In vitro inoculation of *C. annuum* L. seeds with entomopathogenic fungi

Although the statistical analysis did not show significant differences among treatments in ANOVA test at the level of 5%, germination of seeds inoculated with BB09 and MA28 strains was almost 10% greater than non-inoculated control and MA25 treatment. Strain BB42 had a germination percent greater than 80% as described in Figure 1. In the *in vitro* test, seeds inoculated with the strain BB42 generated seedlings with longer roots and shoots (5.18 and 1.51 cm, respectively), compared with shortest roots from the seedlings inoculated with the strain MA25 (3.8 cm), while shortest shoots belonged to the control and to the seeds inoculated with the strain MA28 (1.17 cm). Seedlings inoculated with *B. bassiana* had a better colour as well as a greater size compared with other ones as presented in Figure 2.

A higher root fresh weight was observed in the control seedlings compared to those inoculated with BB09, with means of 13.58 and 6.6 mg, respectively as described in Figure 3A. Seedlings inoculated with the strains BB09, MA28, BB42 and the control ones showed the lowest root dry weight with values oscillating among 1.36 and 1.42 mg, without finding statistically significant differences between their weights ($p < 0.05$) as described in Figure 3B.

Seedlings inoculated with BB42 and MA28 strains generated seedlings with the highest weight, with 30.18 and 28.37 mg, respectively followed by seedlings inoculated with BB09, which obtained a fresh weight of 25.61 mg, forming the same group with the control (26.85 mg). With respect to dry weight or shoots, all the seedlings inoculated with EF showed higher values than the control (3.22 mg). Seedlings inoculated with BB42 had the highest biomass production with 3.93 mg (Figure 3B).

The control treatment had 18% of contaminated seeds while the seeds inoculated with EF showed a lower contamination percentage than the control: 5% in the case of seeds inoculated with the strain BB42, 2% of seeds inoculated with MA25 and there was no contamination in seeds inoculated with the strains BB09 and MA28 as presented in Figure 1.

Antagonisms among seed borne fungi and entomopathogenic fungi in *C. annuum* L. seeds

Based on the results of microscopy, it was determined that these colonies belong to genus *Alternaria* sp. and *Cladosporium* sp. All EF strains had inhibitory effects on

seed borne fungi isolates one and three belong to genus *Alternaria* sp. (light brown and gray colonies, respectively) and MA28 and BB09 strain formed a characteristic halo zone surrounding the colony as shown in Figure 4. None of the EF isolates tested could inhibit the growth of colony 2, belonging to genus *Cladosporium* sp.

In vivo inoculation of *C. annuum* L. seeds with entomopathogenic fungi

Significant differences between treatments were found in the germination percentage of chilli seeds under greenhouse conditions in Tukey test. Seeds inoculated with strain MA25 showed the highest percentage of germination with 85%, and the lowest percentage obtained by seed inoculated with BB42 strain was 70% as shown in Figure 5. The highest percentage of contamination (50%) was observed on the control seedlings, unlike of EF inoculated seeds where percentage of contamination oscillated among 5 and 15% as presented in Figure 5.

With respect to the seedling development, seedlings inoculated with MA28 strain have the largest number of leaves (4.8), roots with lengths up to three times longer than the control (12.94 cm) and the higher height of the seedlings (4.03 cm). Control treatment obtained the lowest values for the number of leaves (1.6 cm), the length of the root (4.09 cm) and the height of the seedling (1.75 cm) as described in Figures 6 and 7.

Seedlings inoculated with the strain MA28 also showed the highest fresh weight (269.17 mg) and biomass production of roots and shoots, while seedlings inoculated with BB42, MA25 and BB09 strains got shoots with fresh weights that oscillated within 142 and 172 mg as presented in Figure 7A. Comparatively, seedlings inoculated with the strain BB09 and the control treatment produced the seedlings with the lowest fresh weights of roots (18.39 and 19.16 mg, respectively).

Regarding the dry weight of shoots, the control treatment produced the lowest value (4.91 mg), followed by the seedlings inoculated with the strains MA25, BB09 and MA25, with values at least twice as large as described in Figure 7B. Seedlings inoculated with the MA28 strain produced 23.22 mg of biomass, a value almost five times heavier than the control as shown in Figure 7B. In the case of root dry weight, control and treatments inoculated with BB09 produced similar values (3.34 and 4 mg), followed by the seedlings inoculated with MA25 and BB42 (6.33 and 8.36 mg). The heaviest root dry weight was produced by the seedlings inoculated with the MA28 strain with roots of 13.88 mg as shown in Figure 7B.

DISCUSSION

EF have been employed mainly to combat insect pests of several crops with economic importance (Villegas et al.,

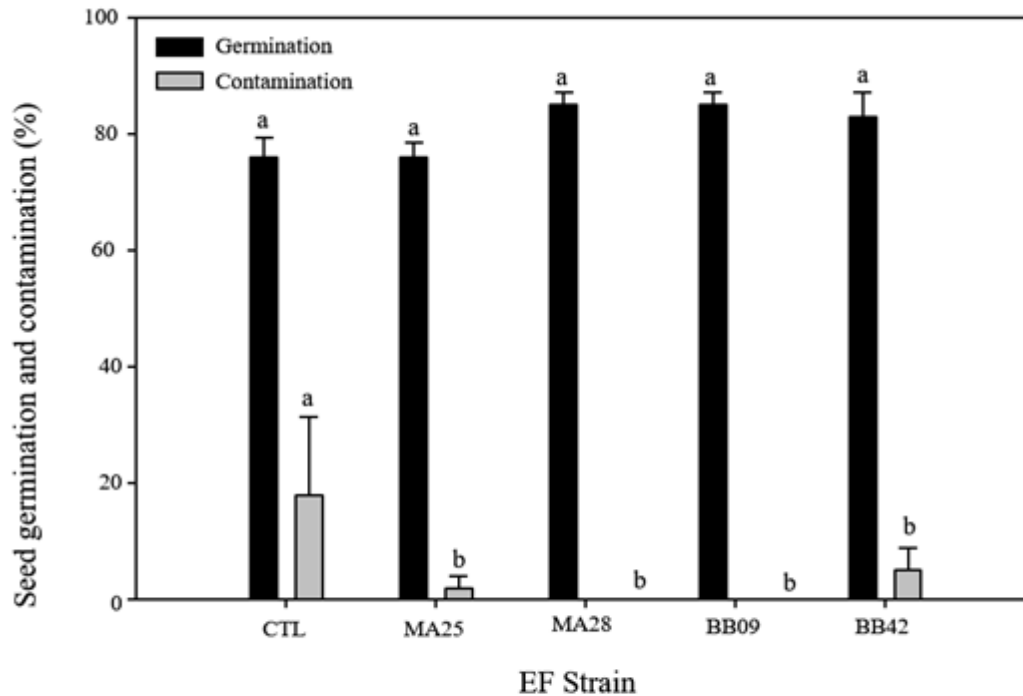


Figure 1. *In vitro* effect on the germination and contamination of chilli seeds inoculated with different isolates of entomopathogenic fungi (EF). CTL: Control; bb09: *Beauveria bassiana* strain 09; bb4: *Beauveria bassiana* strain 28. Different letters indicate significant difference (Tukey test; $p \leq 0.05$).

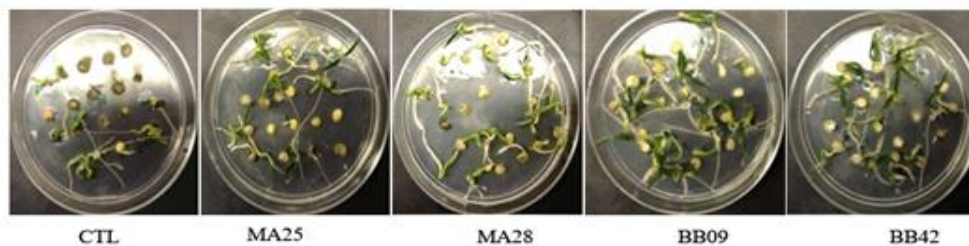
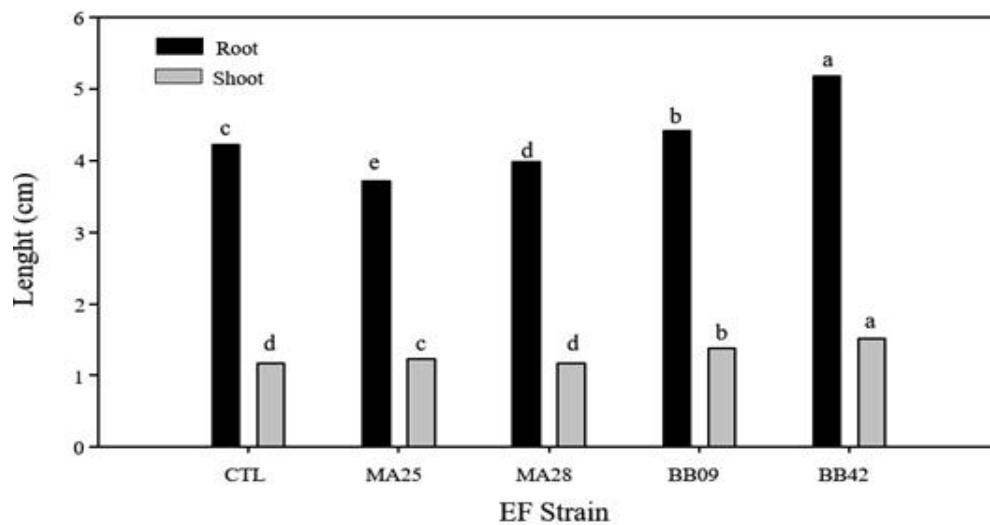


Figure 2. *In vitro* effect on the development of chilli seedlings inoculated with entomopathogenic fungi (EF). CTL: Control; BB09: *Beauveria bassiana* strain 09; BB42: *Beauveria bassiana* strain 42; MA25: *Metarhizium anisopliae* strain 25; MA28: *Metarhizium anisopliae* strain 28. Different letters indicate significant differences (Tukey test; $p \leq 0.05$).

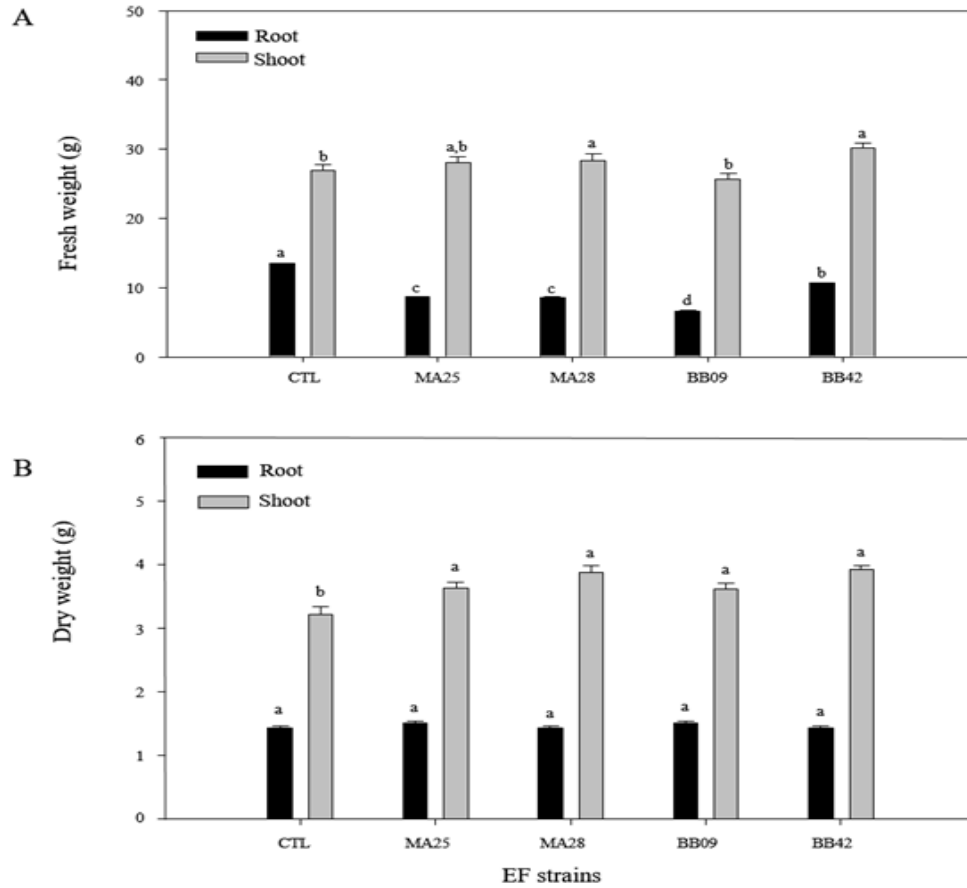


Figure 3. *In vitro* effect in the biomass production on chilli seedlings inoculated with different entomopathogenic fungi (EF). CTL: Control; BB09: *Beauveria bassiana* strain 09; BB42: *Beauveria bassiana* strain 42; MA25: *Metarhizium anisopliae* strain 25; MA28: *Metarhizium anisopliae* strain 28. Different letters indicate significant differences (Tukey test; $p \leq 0.05$).

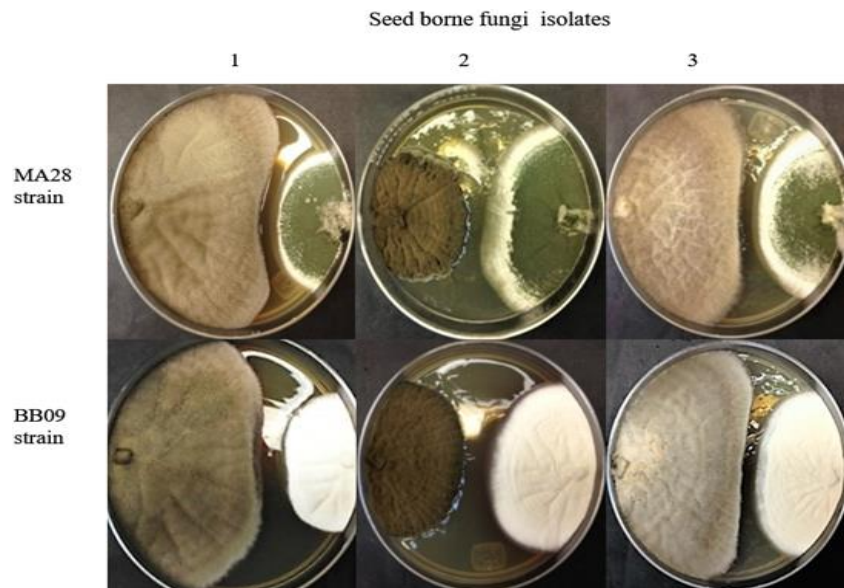


Figure 4. *In vitro* effect of entomopathogenic fungi strains on seed borne fungi isolated from seeds (1,2,3). *Metarhizium anisopliae* strain MA28, dark green mycelium; *Beauveria bassiana* strain BB09, white mycelium.

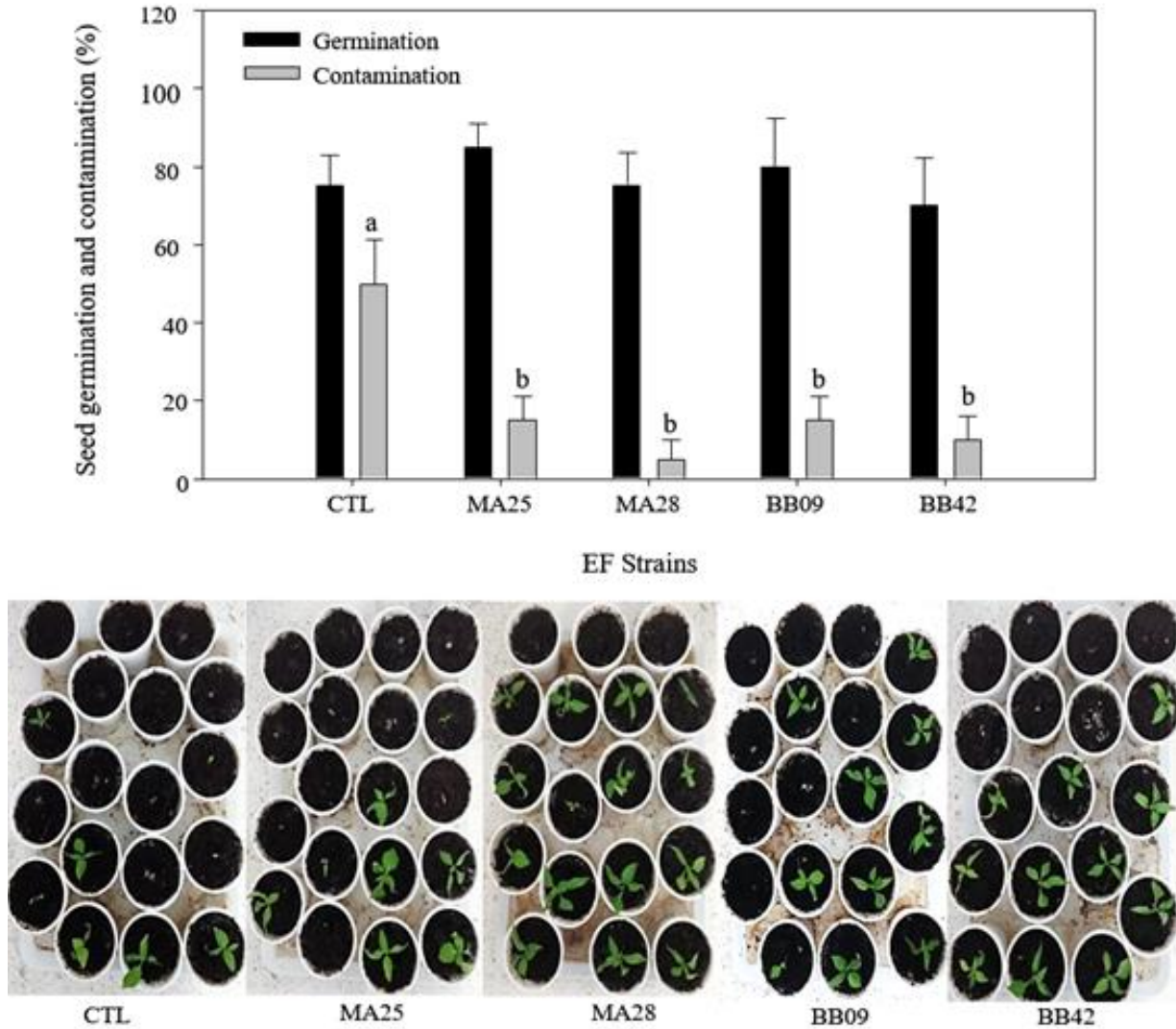


Figure 5. Effect of inoculation with entomopathogenic fungi (EF) on chili seedlings (*Capsicum annum* L.) germination and reduction of fungal seed contamination in substrate. CTL: Control; BB09: *Beauveria bassiana* strain 09; BB42: *Beauveria bassiana* strain 42; MA25: *Metarhizium anisopliae* strain 25; MA28: *Metarhizium anisopliae* strain 28. Different letters within each column indicate significant differences (Tukey test; $p \leq 0.05$).

2014; Maniani and Ekesi, 2013), in addition to be insect pests and plant pathogens antagonist, and their ability to promote plant growth (Rai et al., 2014). Our results showed that inoculation with EF produced seedlings with greater height and weight. Results from this study is in agree with others, where EF was able to promote plant growth parameters (Bamisile et al., 2018; Jaber and Enkerli, 2016, Tall and Meyling, 2018), but in other cases, differences in growth parameters were not significant (Tefera and Vidal, 2009).

Even though the effect of EF on the germination of *C. annum* L. was not significant in this study, a trend of germination increment was observed. At the end of greenhouse experiment, low germination and high contamination percentages was observed in control seedlings. From a practical perspective, this situation

would be a limiting factor because having enough healthy plants at the beginning of the production cycle is crucial. In our case, the chili seedlings inoculated with EF showed a higher survival percentage.

With respect to the length of shoot and roots, no significant differences were found in *in vitro* test, but potted chili seedlings inoculated with MA28 strain was higher compared to other treatments. Sasan and Bidochka (2012) reported that *Metarhizium robertsii* colonized plant roots and stimulates the growth of lateral roots *in vitro* and *in vivo* test, using an appropriate fungal dose on soil is vital to ensure the plant growth proprieties of EF (Raya-Diaz et al., 2017). Substrate where the inoculated seeds are sown is another source of variation, and *Metarhizium* is not always successful in invading plant tissue and competing with seed borne fungus or

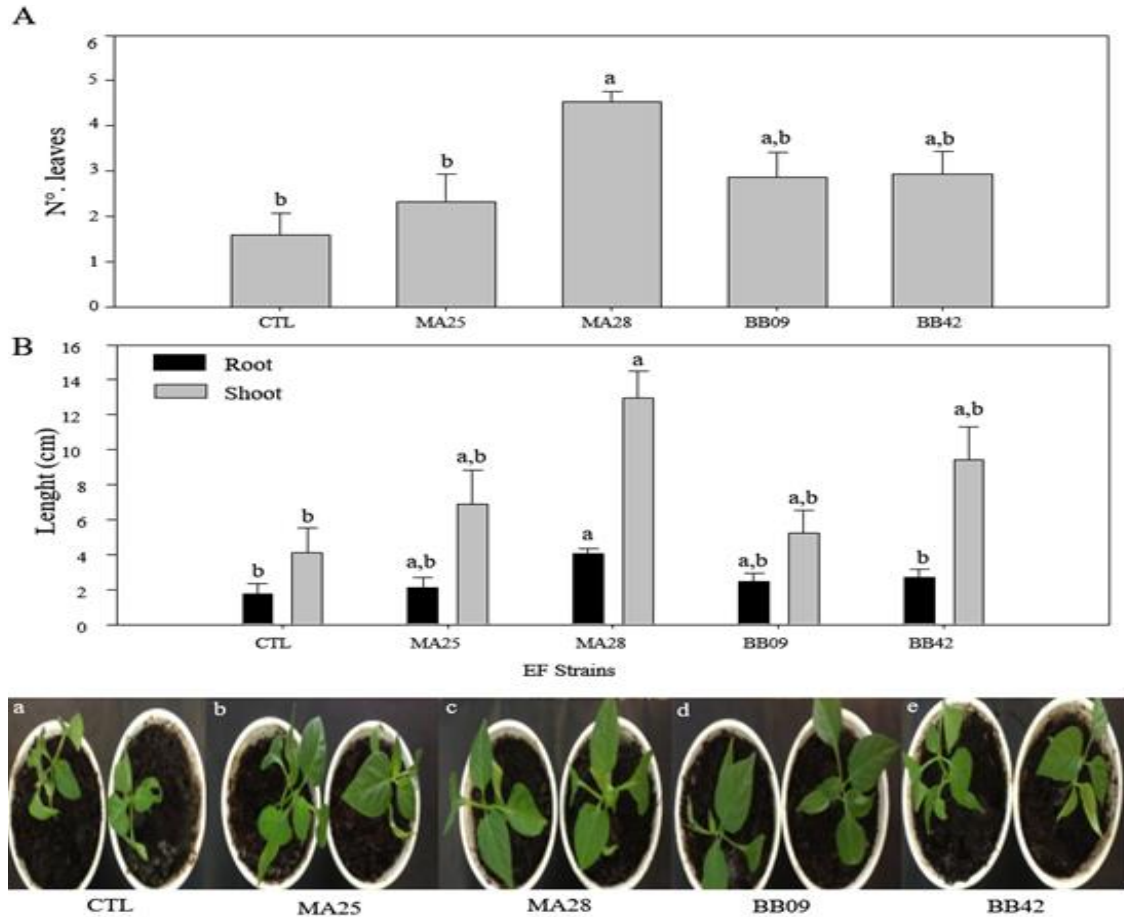


Figure 6. Effect on the development of potted chilli seedlings inoculated with entomopathogenic fungi (EF). a) Number of leaves and b) length of seedlings. CTL: Control; BB09: *Beauveria bassiana* strain 09; BB42: *Beauveria bassiana* strain 42; MA25: *Metarhizium anisopliae* strain 25; MA28: *Metarhizium anisopliae* strain 28. Different letters within each column indicate significant differences (Tukey test; $p \leq 0.05$).

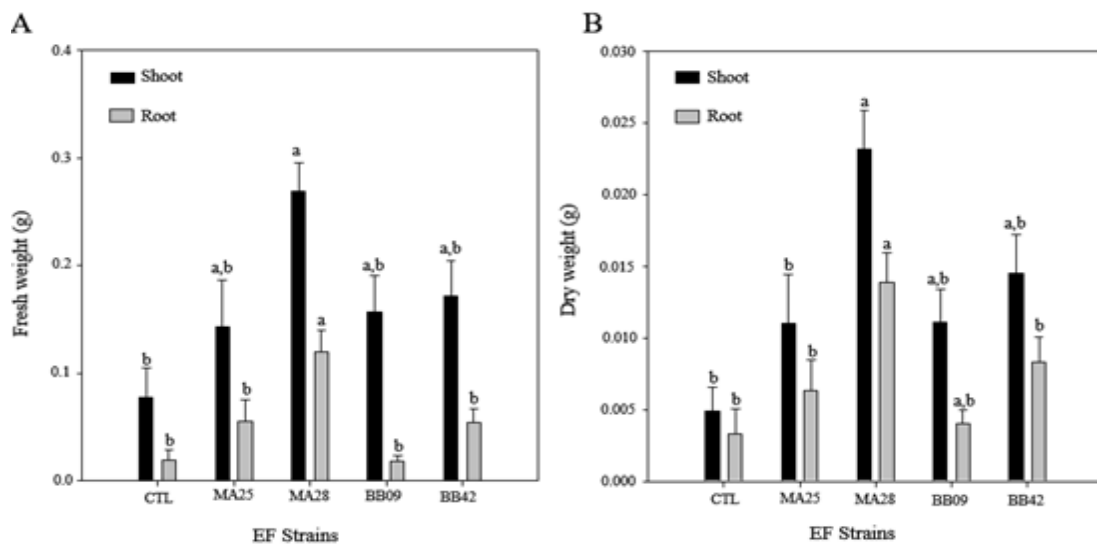


Figure 7. Effect on biomass production in potted chilli seedlings inoculated with entomopathogenic fungi. CTL: Control; BB09: *Beauveria bassiana* strain 09; BB42: *Beauveria bassiana* strain 42; MA25: *Metarhizium anisopliae* strain 25; MA28: *Metarhizium anisopliae* strain 28. Different letters indicate differences (Tukey test; $p \leq 0.05$).

other microorganism in the soil (Parsa et al., 2016).

Biological control of plant pathogens could complement chemical control, such as *P. chrysogenum* which can alter rhizosphere soil and becoming available soil nutrients and suppressing diseases in grasses (Murali et al., 2013). In this model fungal endophytes, that live asymptotically within plant tissues without causing symptoms of disease (Khan et al., 2016), modulate plant defensive hormones, repressed jasmonic acid and salicylic acid pathways and produce alkaloids which are related with plant defence (Bastias et al., 2017). In *Capsicum annuum* L., actinomycetes isolated by medicinal plants improved a growth parameter and there is evidence that these fungi produced indole-3-acetic acid, chitinase, can solubilise inorganic phosphorous and produce HCN (Passari et al., 2015).

EF fulfil very different functions (defence against pathogens, nutrient acquisition, symbiotic interactions) so they must produce many secondary metabolites (Macheleidt et al., 2016). Plants colonized by EF caused significantly differential accumulation of metabolites and may also influence how plants respond to plant pathogen (Dastogeer et al., 2017), and some of them can act against diverse soil pathogens such as *Pythium spp.*, *Rhizoctonia spp.* and *Fusarium spp.* affecting the canopy (Ownley and Gwinn, 2010). Isolates of *Metarhizium brunneum* and *B. bassiana* showed strong inhibition of the mycelial growth of olive pathogens *Verticillium dahlia* and *Phytophthora megasperma* (Lozano-Tovar et al., 2017). More important mechanism elicited by EF is induced systemic resistance (ISR), which includes reduction of disease symptoms in parts of the plant distant from the site where the inducing agent is active (Pieterse et al., 2014). It has been reported that *B. bassiana* induces systemic resistance against *Xanthomonas axonopodis* pv. *malvacearum* (bacterial blight) when inoculated on cotton and tomato seeds, and previously inoculated on tomato seeds, *B. bassiana* can induce resistance against soil pathogens such as *Rhizoctonia solani* and *Pythium myriotylum* (Ownley et al., 2008). Field research suggests that *B. bassiana* and *M. anisopliae* applied as endophyte in maize showed suppression of maize stem borer damage caused by *Chilo partellus* Swinhoe reported reduction in steam tunnelling in maize plant, mainly due to systematic activity of this EF isolates (Ramanujam et al., 2017).

In the present study, seeds inoculated with EF prevented contamination by inhibiting the growth of seed borne fungi; therefore it can be employed as symbiotic insecticides that may offer protection against plant pathogens as well as others EF (Jaber and Ownley 2017).

Conclusion

EF used in this work could ensure optimal, safe germination and seedlings production, even if the seeds used were contaminated. In general, seeds inoculated

with EF showed the biggest sizes and weights, and seedlings inoculated with BB42 and MA28 showed the seedlings with the biggest size and weight as shown in Figures 2 and 3). MA28 and BB42 strains would be an attractive alternative to be included in integrated pest management, but more research will be needed to determine other effects on the plant development and plant defence response.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Bamisile BS, Dash CK, Akutse KS, Keppan R, Afolabi OG, Hussain M, Qasim M, Wang L (2018). Prospects of endophytic fungal entomopathogens as biocontrol and plant growth promoting agents: an insight on how artificial inoculation methods affect endophytic colonization of host plants. *Microbiological Research* 217:34-50.
- Bastias DA, Martínez-Ghersa MA, Ballaré CL, Gundel PE (2017). Epichloë fungal endophytes and plant defenses: not just alkaloids. *Trends in Plant Science* 22(11):939-948.
- Chauhan Rinkal T, Patel PR, Thumar VM (2018). Occurrence of seed borne pathogens in Chilli (*Capsicum frutescens* L.) cv. GVC 111 in vitro. *International Journal of Chemical Studies* 6(2):1374-1376.
- Chigoziri E, Ekefan EB (2013). Seed borne fungi of chilli pepper (*Capsicum frutescens*) from pepper producing areas of Benue State, Nigeria. *Agriculture and Biology Journal of North America* 4:370-374.
- Dastogeer MGK, Li H, Sivasithamparam K, Jones GKM, Du X, Ren Y, Wyle SJ (2017). Metabolic responses of endophytic *Nicotiana benthamiana* plants experiencing water stress. *Environmental and Experimental Botany* 143:59-71.
- Delgado-Sánchez P, Jiménez-Bremont JF, Guerrero-González ML, Flores J (2013). Effect of fungi and light on seed germination of three *Opuntia* species from semiarid lands of central Mexico. *Journal of Plant Research* 126:643-649.
- Delgado-Sánchez P, Ortega-Amaro MA, Jiménez-Bremont JF, Flores J (2011). Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (*Cactaceae*). *Plant Biology* 13:154-159.
- Elena GJ, Beatriz PJ, Alejandro P, Lecuona RE (2011). *Metarhizium anisopliae* (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. *Advances in Biological Regulation* 5(1):22-27.
- Farias CP, Carvalho RC, Resende FM, Azevedo LC (2018). Consortium of five fungal isolates conditioning root growth and arbuscular mycorrhiza in soybean, corn, and sugarcane. *Anais da Academia Brasileira de Ciências* 90(4):3649-3660.
- Islam MT, Olleka A, Ren S (2010). Influence of neem on susceptibility of *Beauveria bassiana* and investigation of their combined efficacy against sweet potato whitefly, *Bemisia tabaci* on eggplant. *Pesticide Biochemistry and Physiology* 98:45-49.
- Jaber LR, Alananbeh KM (2018). Fungal entomopathogens as endophytes reduce several species of *Fusarium* causing crown and root rot in sweet pepper (*Capsicum annuum* L.). *Biological Control* 126:117-126.
- Jaber LR, Enkerli J (2016). Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. *Biological Control* 103:187-195.
- Jaber LR, Ownley BH (2017). Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? *Biological Control* 107:50-59.
- Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, AIMamar A, Waqas M, Asaf S, Elyassi A, MAbood F, Shin JH, Lee IJ (2016). Endophytic fungi from frankincense tree improve host growth and produces extracellular enzymes and indole acetic acid. *PLoS ONE*

- 11:e0158207. doi: 10.1371/journal.pone.0158207
- Lee JY, Kim YC, Han TH, Kim ST, Gi GY (2010). Study on increasing rose seed germination. *Acta Horticulturae* 855:183.
- Lozano-Tovar MD, Garrido-Jurado I, Quesada-Moraga E, Raya-Ortega MC (2017). *Metarhizium brunneum* and *Beauveria bassiana* release secondary metabolites with antagonistic activity against *Verticillium dahliae* and *Phytophthora megasperma* olive pathogens. *Crop Protection* 100:186-195.
- Macheleidt J, Mattern DJ, Fischer J, Netzker T, Weber J, Schroeckh V, Valiante V, Brakhage AA (2016). Regulation and Role of Fungal Secondary Metabolites. *Annual Review of Genetics* 50:371-392.
- Maniani NK, Ekesi S (2013). The use of entomopathogenic fungi in the control of tsetse flies. *Journal of Invertebrate Pathology* 112:83-88.
- Matthews S, Noli E, Demir I, Khajeh-Hosseini M, Wagner MH (2012). Evaluation of seed quality: from physiology to international standardization. *Seed Science Research* 22(S1):S69-S73.
- Moloinyane S, Nchu F (2019). The effects of endophytic *Beauveria bassiana* inoculation on infestation level of *Planococcus ficus*, growth and volatile constituents of potted greenhouse grapevine (*Vitis vinifera* L.). *Toxins* 11(2):72.
- Murali M, Sudisha J, Amruthesh KN, Ito SI, Shetty HS (2013). Rhizosphere fungus *Penicillium chrysogenum* promotes growth and induces defence-related genes and downy mildew disease resistance in pearl millet. *Plant Biology* 15(1):111-118.
- Olatunji TL, Afolayan AJ (2018). The suitability of chilli pepper (*Capsicum annuum* L.) for alleviating human micronutrient dietary deficiencies: A review. *Food Science and Nutrition* 6(8):2239-2251.
- Owley B, Griffin M, Klingeman M, Gwinn W, Moulton K, Pereira R (2008). *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of Invertebrate Pathology* 98:267-270.
- Owley BH, Gwinn KD (2010). Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. *BioControl* 55:113-128.
- Parsa S, García-Lemos AM, Castillo K, Ortiz V, López-Lavalle LAB, Braun J, Vega FE (2016). Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. *Fungal Biology* 120(5):783-790.
- Passari AK, Mishra VK, Gupta VK, Yadav MK, Saikia R, Singh BP (2015). *In vitro* and *in vivo* plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. *PLoS One* 10(9):e0139468.
- Penella C, Calatayud A (2018). Pepper crop under climate change: grafting as an environmentally friendly strategy. In *Climate Resilient Agriculture-Strategies and Perspectives*. InTech. <https://www.intechopen.com/books/climate-resilient-agriculture-strategies-and-perspectives/pepper-crop-under-climate-change-grafting-as-an-environmental-friendly-strategy>
- Pérez-González VH, Guzmán-Franco AW, Alatorre-Rosas R, Hernández-Lopez J, Hernández-Lopez A, Carrillo-Benitez MG, Baverstock J (2014). Specific diversity of the entomopathogenic fungi *Beauveria* and *Metarhizium* in Mexican agricultural soils. *Journal of Invertebrate Pathology* 119:54-61.
- Pieterse CM, Zamioudis RL, Berendsen DM, Weller SC, Van W, Bakker PA (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* 52:347-375.
- Prado-Urbina G, Lagunes-Espinoza LDC, García-López E, Bautista-Muñoz C, Camacho-Chiu W, Mirafuentes GF, Aguilar-Rincón VH (2015). Seed germination of wild chilli peppers in response to pre-germination treatments. *Ecosystems and Agricultural Resources* 2:139-149.
- Rai M, Rathod D, Agarkar G, Dar M (2014). Fungal growth promotor endophytes: a pragmatic approach towards sustainable food and agriculture. *Symbiosis* 62:63-79.
- Ramanujam B, Japur K, Poornesha B, Shylesha AN, Rangeshwaran R (2017). Field evaluation of endophytic entomopathogenic fungi against maize stem borer (*Chilo partellus*) (Crambidae: Lepidoptera). *Indian Journal of Agricultural Sciences* 87:1099-1103.
- Ramirez-Rodriguez D, Sánchez-Peña SR (2016). Endophytic *Beauveria bassiana* in *Zea mays*: Pathogenicity against Larvae of Fall Armyworm, *Spodoptera frugiperda*. *Southwest Entomologist* 41:875-878.
- Raya-Díaz S, Quesada-Moraga E, Barrón V, Del Campillo MC, Sánchez-Rodríguez AR (2017). Redefining the dose of the entomopathogenic fungus *Metarhizium brunneum* (Ascomycota, Hypocreales) to increase Fe bioavailability and promote plant growth in calcareous and sandy soils. *Plant and Soil* 418(1-2):387-404.
- Ríos-Velasco C, Pérez-Corral DA, Salas-marina MÁ, Berlanga-Reyes DI, Ornelas-Paz JJ, Muñoz CHA, Cambero-Campos J, Jacobo-Cuellar JL (2014). Pathogenicity of the Hypocreales Fungi *Beauveria bassiana* and *Metarhizium anisopliae* Against Insect Pests of Tomato. *Southwestern Entomologist* 39:739-750.
- Sasan RK, Bidochka MJ (2012). The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. *American Journal of Botany* 99:101-107.
- Shearin ZR, Filipek M, Desai R, Bickford WA, Kowalski KP, Clay K (2018). Fungal endophytes from seeds of invasive, non-native *Phragmites australis* and their potential role in germination and seedling growth. *Plant and Soil* 422(1-2):183-194.
- Tall S, Meyling NV (2018). Probiotics for plants? Growth promotion by the entomopathogenic fungus *Beauveria bassiana* depends on nutrient availability. *Microbial Ecology* pp. 1-7.
- Tefera T, Vidal S (2009). Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. *BioControl* 54:663-669.
- Villegas-Rodríguez F, Marín-Sánchez J, Delgado-Sánchez P, Torres-Castillo JA, Alvarado-Gómez OG (2014). Management of *Bactericera cockerelli* (Sulc) (Hemiptera: Trioziidae) in Greenhouses with Entomopathogenic Fungi (Hypocreales). *Southwestern Entomologist* 39:613-624.

Full Length Research Paper

Evaluation of the systemic action of neem (*Azadirachta indica* A. juss) seed products against the desert locust immature *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae)

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The current study was done to investigate the potential of the systemic growth regulatory effects of various neem seeds products against immature stages of desert locust infesting potted millet plants in Sudan. The tests also covered the stability of the systemic action of neem seed powder (NSP) under conditions of water stress. Some (33-80%) of the exposed nymphs developed to the third stage without further moulting. Medium lethal time for neem seed water extracts (NSWE), neem seed organic extract (NSOE) and NSP ranged from 166 to 248 hours. All neem seed product induced significant systemic antifeedant activity, ranging from 52 to 99% against the immatures. Based on these findings, NSP, the simplest form, was found to possess systemic activity comparable to complicated forms of neem products. NSP was stable under conditions of delayed watering up to 10 days and the latter had effects on development, mortality and feeding comparable to immediate watering. All aspects studied indicated the superior systemic activity of various neem seed products. The fact that they were able to delay development, prevent further moulting of instars, stabilize under conditions of delayed watering, enabled them to confine the desert locust to their breeding sites as immatures without threat of swarm formation and limited damage to local growers.

Key words: Desert locust, neem seed products, pearl millet, Sudan.

INTRODUCTION

Man suffers tremendous losses from feeding and other activities of insects. Many insects feed on the plants man cultivate, some feed on valuable stored materials, clothing, or wood, while others feed on man and other animals directly (Borror et al., 1981). Among these insects are locusts, the most historic world pest. Locusts

are extraordinary insect whose name is synonymous with famine among one-eighth of the world population (Baron, 1972; Edward, 2017). About 200 species of grasshoppers and locusts with different food preferences and geographical distribution are known to be agricultural pests, in Africa (Office of Technology Assessment, 1990).

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The most important locust species in Africa are the desert locusts, *Schistocerca gregaria gregaria* Forskal in east Africa and *S. gregaria flaviventris* Burmeister in South West Africa; the brown locust, *Locurta pardalina* Walker; the African migratory locust, *Locusta migratoria migratorioides*; the red locust, *Nomadacris septemfasciata* Serville and the tree locust, *Anacrieedium melanorhodon* Walker. Among these, the desert locust *S. gregaria* Forskal is the most important because of its serious damage to crops, and its vast invasion area, which covers 29,000,000 km² equivalent to 20% of the world land surface (Meinzingen, 1993; Showler, 2013). Over the years, man had faced desert locust plagues with the most futile attempts to control it. However, since early forties, synthetic insecticides have become the remedy; particularly, the long lasting chlorinated hydrocarbons, such as dieldrin, appear to be the ideal solution (Krall and Wilps, 1994). The extensive use of these substances has led to serious negative effects on the environment such as long environmental persistence, accumulation in food chain and body fats of higher animals as well as many cases of insect resistance. The universal banning of these compounds started in 1973 in USA placed a significant pressure on necessity of alternative control measures (Krall, 1994) with short persistence pesticides. These latter pesticides (of short environmental persistence) necessitate repeated blanket spray with negative impact on the environment and enormous increase in the cost of control, in addition to the enhanced risk to humans and other non-target organisms (Fadl-Elmawla 2004; Ilboudo et al., 2014).

Considering the ongoing threat of desert locust, hazards associated with conventional synthetic pesticide to the environment and the progressive increase in the cost of chemical control, the current study was initiated to throw light on possible alternative control measures that are effective, environmentally safe, and cheap. The use of microbial control agents as well as pheromone in combinations with fractional pesticide against locust were reported (Hosny, 2012; Bashir et al., 2016). Botanicals are groups of biodegradable pesticides with greater selectivity and low mammalian toxicity. They have received worldwide interest as one of the safe substitutes of conventional pesticides (Mamadou and Sarr, 2009; Hosny, 2012). The neem tree, *Azadirachta indica* A. Juss, out of 2000 plant species has been reported as one of the most promising source of natural pesticide (Maute et al., 2016). Neem tree is an abundant evergreen plant in various parts of Sudan. It was reported as an excellent source of botanical pesticides with multiple actions against various types of insects, virus, mite, nematode, fungal and bacterial management (Schmutterer, 2002; Krall and Wilps, 1994). Many workers (Elamin, 2002; Abd El Rheem, 2005; Hummel et al., 2012) confirmed its activity as contact and/or systemic insecticide, especially against immature stages. The systemic growth regulatory activity action of neem product, its availability and

cheapness under local condition makes it a suitable alternative of great potential for use in confinement of locust swarms in their breeding sites as immature. This initiated our interest for thorough investigation of neem products as systemic pesticides against immature stages of desert locust. Therefore, this study aim to evaluate the systemic activity of different neem seeds products on development, antifeedant activity and water-stress of the immature stages of desert locust.

MATERIALS AND METHODS

Rearing of the desert locust nymphs:

Egg laying tubes of 3.5 cm diameter and 10.5 cm deep, containing egg pods of desert locust *S. gregaria* were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Port Sudan, Sudan. The tubes were placed under rearing cages (54 cm x 42.5 cm x 42.5 cm) in a room measuring 2 x 5 m at room temperature of 26 ± 2°C during the day (11 hours), and 20 ± 2°C during the night (13 h). The relative humidity was 60-70%. Extra heat was provided by electric bulb (60 watt) placed at the top of the cage to enhance eggs hatching. Hatching instars were fed pear millet seedlings grown in pots of 7 cm diameter and 10 cm deep, plus wheat bran. The cages were cleaned daily.

Experimental cages

The cages used are of the standard type described by Harvey (1990), with some modifications. They measured 54 x 42.5 x 42.5 cm, with-stands of 15 cm. The front side was covered with plywood, with a 13.7 cm diameter opening fitted with cloth sleeves to facilitate feeding, cleaning and handling of insects. The other sides were fitted with wire mesh.

Preparation of natural products

Preparation of neem seeds powder (NSP)

Mature seeds of neem (*A. indica*) were collected in July from trees grown for shade at Shambat area. The seeds were left to dry under shade for 10 days. The dried seeds were crushed with sticks to remove the shell, while keeping the seed intact. The seed were then ground by pestle and mortar into fine powder, stored in tightly closed glass jar, wrapped with aluminum foil, and kept at room temperature until required for extraction and/or bioassay.

Preparation of neem seeds aqueous extracts (NSAE)

Neem seeds aqueous extracts were prepared following the method of Siddig (1991). Ten grams seed powder were soaked in 1000 ml distilled water, left for 24 h at room temperature, while thoroughly stirred by a piece of wood every eight hours for 5 min. The mixture (1% w/v) was then filtered through light cloth and the filtrate was used on the same day. Other concentrations (5, 10 and 20% w/v) were prepared following the same method.

Preparation of neem seeds organic extract (NSOE)

Prepared neem seeds powder (300 g) was placed in a Soxhlet apparatus. The powder was extracted for 12 h with 1000 ml of hexane (boiling point 67-79°C). The added hexane was removed

using rotary evaporator at 45°C. The obtained oil was kept in a flask, tightly closed, wrapped with aluminum foil and stored in the refrigerator at 5°C till needed for bioassay. Various dilutions needed (10, 15, and 20% v/v) were done by serial dilution.

Production of millet seedlings

Pearl millet seeds (local variety), were purchased from local market, grown in pots of 7 cm diameter and 10 cm deep with 200 g of alluvial soil mixed with sand (5:2 parts respectively) to improve soil texture, then two grams of seeds were sown per plot. Irrigation water (30 ml/pot) was given every 3 days.

The systemic action of neem seeds products on the development of the desert locust immatures

The following neem seeds products used were:

- (i). Neem seeds organic extract (NSOE), at concentrations (1, 5, 10 and 20% w/v).
- (ii). Neem seeds water extracts (NSWE), at concentrations (1, 5, 10 and 20% w/v).
- (iii). Neem seed powder (NSP), at concentrations (1, 5, 10 and 20% w/v).
- (iv). Azadirachtin (as azal) at the recommended dose (2 L/ha).
- (v). Carbofuran (as furadan) as standard systemic insecticide at the recommended dose (4 kg/ha).
- (vi). Control (potted pearl millet seedlings) without any treatment.

Chemical treatments were directly applied to the soil of 3 days old seedlings, and left for an extra 72 hours to allow the uptake of active ingredient (a-i) of the products by the seedlings. Five desert locust nymphs (2nd instar) were introduced into each cage, containing ten treated seedlings, and daily observed until the control treatments reached the adult stage (fledglings). Each treatment was replicated three times and units were assigned in randomized complete block design (RCBD). The daily observations included mortalities, deformation, developmental period and weight gain. Data were recorded every 24 h, through the entire developmental period. Necessary corrections were done according to Abbott's formula (1925).

$$P_1 = \frac{P_T - P_c}{100 - P_c} \times 100$$

Where;

P_1 : Corrected mortality%; P_T : Treated mortality and P_c : control mortality %.

Corrected mortality was subject to probit analysis.

Effects of water stress on the efficacy of the systemic action of neem seeds powder

Different concentrations (1, 5, 10 and 20% w/v) of neem seeds powder were applied to the soil prior sowing. Irrigation water (30 ml/pot) was given either immediately (NSP1) or after 7 days (NSP7) or 10 days (NSP10). Potted seedlings were maintained in cages placed in the Wire house, Faculty of Agriculture, University of Khartoum. Plants were left for 72 hours after irrigation to ensure the uptake of the active ingredient by the germinated seedlings. Five individuals of the desert locust nymphs (2nd instar), were introduced

separately into each cage. Units were assigned in randomized complete block design (RCBD), with three replicates. The development of the caged desert locust nymphs was observed until the control treatments reached the adult stage (fledglings). Daily observation includes mortalities, deformation and developmental period. The 24 hours mortality data was subject to necessary corrections according to Abbott's formula and the data was subject to probit analysis.

Systemic antifeedant action of different neem seeds products

The systemic antifeedant effect of neem seeds products on different nymphal instar (2nd, 3rd, 4th and 5th) of desert locust was studied. Soils of potted pearl millet seedlings were treated with different concentrations (1, 5, 10 and 20%) of various neem products (NSWE, NSOE, NSP, Azadirachtin 1% and carbofuran (as Furdan) 1%). Treated seedlings were left for 72 hours to allow uptake of the active ingredient. Patches of desert locust nymphs (5 each) were weighed and separately introduced into different cages. Treated seedlings were then chopped at soil level and provided as food to test insects. Experimental insects were starved for 24 hours prior to testing. Treated nymphs were daily provided with sufficient amount of seedlings.

In parallel, the similar amount of untreated seedlings were chopped and placed in a cage under the same condition to estimate natural water loss. Treatments were assigned in randomized complete block design (RCBD) with three replicates. The amount of food consumed by the different desert locust nymphs in various treatments was recorded after 24 hours by reweighing the remaining amount. Care was taken to correct the evaporation losses.

Statistical analysis

The results were statistically analyzed using F-test and means separated with the least significant difference test (LSD) at $p = 0.05\%$.

RESULTS

The effect of systemic action of neem seed products on development of desert locust nymphs

Nymphal duration

The effect of various neem seed products on developmental period was summarized in Table 1. All treatments were significantly different from the control. The results indicated that the nymphal duration progressively increased with the increase in concentration of neem seed products and the effects were dose-related.

The duration of nymphal instar (2nd) reared on pearl millet seedlings treated with different concentrations of soil applied neem seed water extracts (NSWE) were summarized in Table 1a. The increase in nymphal duration of the second nymphal instars were 24, 33, 43 and 24% for the concentrations 20, 10, 5 and 1%, respectively.

Only 40, 40, 53 and 80% of the test instars succeeded

Table 1. The systemic action of various neem seed products on the development of desert locust nymphs.

Conc. (%)	Duration				
	2 nd instar duration (days)	% moulted to 3 rd instar	3 rd instar duration (days)	Total duration (days)	%mortality 3 rd instar
(a) NSW					
20	9.50 ^c (24)	40	12.00 ^b (41)	21.5(33)	100
10	10.16 ^{bc} (33)	40	11.80 ^{bc} (39)	21.96(36)	100
5	11.00 ^b (43)	53	11.00 ^{bc} (29)	22(36)	100
1	9.50 ^c (24)	80	10.33 ^{bc} (18)	19.5(21)	100
Azal 1%	12.30 ^a (64)	73	28.667 ^a (237)	41(126)	100
0.0	7.667 ^d (0.0)	100	8.50 ^c (0.0)	16.16	6.6
Lsd	1.226		3.009		
(b) NSOE					
20	15.00 ^a (100)	47	11.00 ^{bc} (29)	26(62.5)	100
10	14.00 ^a (86.6)	33.3	12.00 ^b (41)	26(62.5)	100
5	9.50 ^c (27)	73	11.50 ^b (35)	21(31)	100
1	9.66 ^c (29)	53	10.50 ^{bc} (24)	20.16(26)	100
Azal 1%	12.33 ^b (64)	41	28.66 ^a (237)	41(126)	100
0.0	7.50 ^d (0.0)	100	8.5 ^c (0.0)	16	6.6
Lsd	1.116		2.905		
(c) NSP					
20	10.00 ^b (42)	60	12.00 ^b (41)	22(42)	100
10	8.25 ^c (17)	66	11.00 ^{bc} (29)	19.5(24)	100
5	10.00 ^b (42)	53	10.00 ^{bc} (18)	20(29)	100
1	12.00 ^a (70)	66	10.00 ^{bc} (18)	22(42)	100
Azal 1%	12.30 ^a (69)	73	28.66 ^a (237)	41(126)	100
0.0	7.50 ^b	100	8.50 ^c	16	6.6
Lsd	1.749		2.867		

Mean values having different letters in each column differ significantly ($P \leq 0.05$) according to LSD test.

Values between brackets represent the percentage increase in nymphal duration.

NSWE: Neem seed water extracts

NSOE: Neem seed organic extracts.

to moult to the third instar for the respective concentration of NSW 20, 10, 5 and 1%. The respective increase in the nymphal duration of the third instars was 41, 30, 29 and 18% for the concentrations 20, 10, 5 and 1%, respectively. No further moulting occurred and nymphs died as overaged instar. The increase in the total developmental period for the two stages ranges from 21-36%. All treatments were significantly different from the control. However, differences between the various concentrations were sometimes non-significant at $P = 0.05$.

The duration of the second nymphal instars reared on pearl millet seedlings treated with different concentrations of soil applied NSOE were summarized in Table 1b. The respective increases in nymphal duration were 100, 86, 27 and 29% for the 20, 10, 5 and 1% concentrations, respectively. Percentage instar succeeded to moult in the third stage, ranging from 33 to 73%. Those moulted instars experienced longer duration during the third instar

period of 29, 41, 35 and 24% for the 20, 10, 5 and 1% concentrations, respectively. Again, no further moulting occurred and nymphs died as overaged instars. The percentage increase in the total developmental period for the two stages (2nd and 3rd) were 62.5, 26, 31 and 26% for the 20, 10, 5 and 1% concentrations, respectively. All treatments were significantly different from the control ($P = 0.05$) (Table 1b). Table 1c shows the effect of neem seed powder (NSP) on the duration of the second nymphal instars reared on pearl millet seedlings treated with soil applied NSP. The respective increases in the duration of the second instar were 42, 17, 42 and 17% for the 20, 10, 5 and 1% concentrations, respectively. Lower concentration gave longer duration.

Individuals succeeded to the next moult, ranging from 53 to 66%. The percent increase in duration of the third nymphal instar were 41, 29, 18 and 18% for the 20, 10, 5 and 1% concentrations, respectively. No further moult occurred and all individuals died as overaged nymphs.

The increase in the total developmental period (2nd and 3rd) ranges from 29 to 42%. All treatments were significantly different from the control. Statistical differences between various levels were sometimes noticed at $P = 0.05$ (Table 1c).

The soil applied Azadirachtin (Azal 1%) gave 64 and 237 % increase in the duration of the second and third nymphal instars, respectively. About 73% second instars moulted to third instar with no further moult and individual died as overaged nymphs. The total developmental period of the two stages were increased by 126 % as compared to the control. All nymphs exposed to pearl millet seedlings treated with soil applied Carbofuran died within 6 days following the application.

Mortality cases resulting from the systemic action

The mortality cases among various nymphal instars of desert locust fed on pearl millet seedlings treated with soil-applied neem seed products were given in Table 2. Table 2 showed the mortality cases noticed among various nymphal instar fed on pearl millet seedlings treated with soil applied neem seed water extract (NSWE). The different concentrations brought about significant deaths among test insects compared to the control. Mortality in most cases occurred before moulting. Mortality rate is dose-related with higher dosage generally resulting in higher and faster mortality rate compared to lower dosage. Complete mortality of test insects occurred between 15 and 24 days, depending on the concentration; while insects tested with Furadan died within 6 days. Those treated with Azal 1% reached 100% mortality after 33 days.

The mortality cases occurred among desert locust nymphal instars fed on pearl millet seedlings and treated with soil-applied neem seed organic extract (NSOE) are given in Table 2. All tested concentrations caused significant mortality compared to the control and response is dose-related; higher dosage gave quick and fast mortality. Complete mortality of treated insects was noticed between 18 and 24 days (Table 2) depending on the concentration. Table 2 showed the mortality of the desert locust nymphs treated with soil-applied neem seed powder (NSP). All treatments significantly differ from the control. The mortality rate is dose-related with higher doses resulting in higher and faster mortality rate compared to lower dosage. Complete mortality was noticed between 15-18 days, depending on the concentration.

Morphological deformations on the insect

The number of insects that showed damaged parts (antennae, hind legs, fore legs and wings) were ranged from 6.6 to 13%.

PROBIT ANALYSIS OF RELATIVE TOXICITIES OF DIFFERENT NEEM SEEDS PRODUCTS

Time response at 5%

Table 3a and Figure 1 showed the comparative time related toxicities data of the 5% concentration of NSWE, NSOE, NSP₁, NSP₇ and NSP₁₀. The corresponding percentage mortality responses are shown in Table 2. The results indicated that, the slope of the mortality regression lines (LT-P lines) were steep and positive, indicating homogenous population. The homogeneity is also evident from the narrow LT_{90}/LT_{50} values and fiducial limits. Chi-square values (2.6, 9.5, 6.5, 3.3 and 2.6 for NSWE, NSOE, NSP₁, NSP₇ and NSP₁₀ respectively) were small, indicating a good line fit and good execution. Responses appear more variable at LT_{10} , while they become close at the middle (LT_{50} - LT_{90}). The results indicated the following overall effectiveness $NSP_1 > NSP_7 > NSP_{10} > NSWE$. NSOE, with relative potencies (relative to LT_{50} of NSOE) 1.5, 1.3, 1.05, 1.01 and 1.0, respectively.

Time response at 10%

The time related toxicities data for the 10% concentration are given in Table 3b, and Figure 2. The test products showed a relative increase in efficiency with dose as evident from the decrease in LT_{50} , LT_{90} values compared to that of 5% concentration. Similar to the previous observation, test population was quite homogenous in its response as indicated by the steep line slopes, narrow LT_{90}/LT_{50} ratios and fiducial limits. Chi-square values (7.7, 12.5, 4.4, 5.2 and 3.8 for NSWE, NSOE, NSP₁, NSP₇ and NSP₁₀) were low, indicating good execution and line fit. The test neem seed products showed an overall order of effectiveness as follows $NSP_7 > NSP_1 > NSOE$. $NSP_{10} > NSWE$ (based on LT_{50} of NSWE).

The responses to test compounds appeared relatively closer at LT_{50} compared to LT_{10} and LT_{90} as indicated by the respective differences in values of LT_{10} and LT_{90} as well as line slope (Figure 2).

Time response at 20%

Table 3c and Figure 3 showed the comparative time related toxicities of the five-neem seed products to the test nymphs. The test compounds showed a relative increase in efficacy as evident from the lower values of LT_{50} , LT_{90} compared to those of 5 and 10% concentrations. The homogeneity of test population was evident by the narrow values of LT_{90}/LT_{50} ratios. The LT_{50} values ranges between 119 and 174 hours for various products. The results indicate that LT-P lines are almost

Table 2. Mortalities among desert locust nymphs (2nd instar) fed on pearl millet seedlings treated with soil applied by NSWE, NSOE and NSP.

Conc. (%)	Mortality through time (days)											
	3	6	9	12	15	18	21	24	27	30	33	
NSWE	20	0.33 ^a (6.6)	2.33 ^a (46.6)	2.667 ^a (53.3)	4.33 ^a (68.6)	5.00 ^a (100)						
	10	0.33 ^a (6.6)	1.33 ^{ab} (26.6)	2.33 ^{ab} (46.6)	4.33 ^a (68.6)	5.00 ^a (100)						
	5	0.33 ^a (6.6)	1.00 ^{ab} (20)	2.33 ^{ab} (46.6)	3.33 ^{ab} (66.6)	4.33 ^{ab} (86.6)	5.00 ^a (100)					
	1	0.33 ^a (6.6)	1.33 ^{ab} (26.6)	2.33 ^{ab} (46.6)	3.00 ^b (60)	4.00 ^a (80)	4.33 ^a (86.6)	4.66 ^a (93.3)	5.00 ^a (100)			
	Azal 1%	0.00 ⁻	0.00 ^b (-)	0.33 ^b (6.6)	1.00 ^c (20)	1.66 ^b (33)	2.66 ^b (53)	3.33 ^b (66.6)	3.33 ^b (66.6)	3.66 ^a (73)	4.00 ^a (80)	5.00 ^a (100)
	0.0	0.00 ⁻	0.00 ^a (-)	0.33 ^a (6.6)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^c (13)	0.66 ^c (13)	0.66 ^c (13)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)
	LSD	0.838	1.779	2.045	1.110	1.949	1.258	0.727	0.592	1.869	1.608	0.856
NSOE	20	1.667 ^a (33)	3.00 ^a (60)	4.66 ^a (93)	4.66 ^a (93)	4.66 ^a (93)	5.00 ^a (100)					
	10	1.00 ^{ab} (20)	2.00 ^{ab} (40)	2.66 ^b (53)	2.66 ^b (53)	3.33 ^{ab} (66.6)	3.66 ^{ab} (73)	5.00 ^a (100)				
	5	0.00 ^b (-)	2.00 ^{ab} (40)	2.33 ^{ab} (46.6)	2.33 ^b (46.6)	3.00 ^{bc} (60)	3.33 ^a (66.6)	5.00 ^a (100)				
	1	0.00 ^b (-)	0.33 ^{ab} (6.6)	1.00 ^{bc} (20)	2.00 ^{bc} (40)	2.66 ^{bc} (53)	2.66 ^a (53)	3.66 ^b (73)	5.00 ^a (100)			
	Azal %	0.00 ⁻	0.00 ^c (-)	0.33 ^c (6.6)	1.00 ^{cd} (20)	1.66 ^{cd} (33)	2.66 ^a (53)	3.33 ^b (66.6)	3.33 ^b (66.6)	3.66 ^a (73)	4.00 ^a (80)	5.00 ^a (100)
	0.0	0.00 ⁻	0.00 ^c (-)	0.33 ^c (6.6)	0.66 ^b (13)	0.66 ^d (13)	0.66 ^b (13)	0.66 ^c (13)	0.66 ^c (13)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)
	LSD	1.110	1.677	1.569	1.110	1.728	0.727	0.592	1.869	1.608	1.608	0.856
NSP	20	0.33 ^{bc} (6.6)	2.66 ^a (53)	3.33 ^a (4.6)	4.00 ^a (80)	5.00 ^a (100)						
	10	0.33 ^{bc} (6.6)	2.33 ^{ab} (46.6)	3.33 ^a (66.6)	3.66 ^a (73)	5.00 ^a (100)						
	5	1.33 ^a (27)	2.00 ^{ab} (40)	3.00 ^a (60)	4.00 ^a (80)	5.00 ^a (100)						
	1	1.00 ^{ab} (20)	1.66 ^b (33)	3.00 ^a (60)	3.00 ^a (60)	4.66 ^a (93)	5.00 ^a (100)					
	Azal %	0.00 ⁻	0.00 ⁻	0.33 ^b (6.6)	1.00 ^b (20)	1.66 ^b (33)	2.66 ^b (53)	3.33 ^a (66.6)	3.33 ^a (66.6)	3.66 ^a (73)	4.00 ^a (80)	5.00 ^a (100)
	0.0	0.00 ⁻	0.00 ⁻	0.33 ^b (6.6)	0.66 ^b (13)	0.66 ^c (13)	0.66 ^c (13)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)
	LSD	0.727	0.727	1.391	1.185	0.727	1.185	0.592	1.050	1.869	1.6082	0.856

-Mean values having different letters in each column differ significantly ($P \leq 0.05$) according to LSD test.

-Values between brackets represent the percentage mortality.

-The std systemic insecticide (Furdan) curve 100% mortality within 6 days.

-NSWE: Neem seed water extracts

-NSOE: Neem seed organic extracts

-NSP: Neem seed powder.

parallel with the following overall order of effectiveness; NSOE>NSP₇>NSP₁>NSWE.NSP¹⁰ with relative potencies of 1.46, 1.35, 1.02, 1.02 and 1.0, respectively based on LT₅₀ of NSP₁₀.

Systemic effects of neem seed products on food intake

Results summarized in Table 4 showed the

amount of food ingested by different nymphal instars of desert locust (2nd, 3rd, 4th and 5th) fed on pearl millet seedlings treated with various types of soil applied neem seed products viz., NSWE,

Table 3. Time response (mortality) data of the nymphal instar of desert locust reared on pearl millet seedling treated with soil applied neem seed products (at concentrations 5, 10 and 20%).

Insecticide	LT ₅₀	LT ₉₀	LT ₉₀ /LT ₅₀ ratio	Slope	Fiducial limits	Chi-square	D.F	Relative potency
based on NSP₁₀					(a) Conc. 5%			
NSWE	245	396	1.6	6.15	1.966	2.6	10	1.01
NSOE	248	504	2.0	4.17	1.969	9.5	10	1.00
NSP ₁	166	380	2.3	3.55	1.970	6.5	10	1.50
NSP ₇	186	313	1.7	5.66	1.973	3.3	10	1.3
NSP ₁₀	234	330	1.4	8.58	1.966	2.6	10	1.05
based on NSOE					(b) Conc. 10%			
NSWE	198	312	1.6	6.60	1.965	7.7	10	1.00
NSOE	192	611	3.2	2.53	1.975	12.5	10	1.03
NSP ₁	188	374	1.9	4.29	1.969	4.4	10	1.05
NSP ₇	167	271	1.66	6.11	1.966	5.2	10	1.2
NSP ₁₀	197	276	1.4	8.85	1.965	3.8	10	1.005
based on NSWE					(c) Conc. 20%			
NSWE	170	301	1.8	5.15	1.967	7.2	10	1.02
NSOE	119	272	2.3	3.6	1.971	12.2	10	1.46
NSP ₁	170	334	1.9	4.42	1.968	6.8	10	1.02
NSP ₇	128	226	1.8	5.09	1.967	3.3	10	1.35
NSP ₁₀	174	273	1.6	6.56	1.965	4.0	10	1.00

NSWE: Neem seed water extracts
 NSOE: Neem seed organic extracts
 NSP1: Effect of one day delayed watering of treated seedlings
 NSP7: Effect of 7 day delayed watering of treated seedlings
 NSP10: Effect of 10 days delayed watering of treated seedlings.

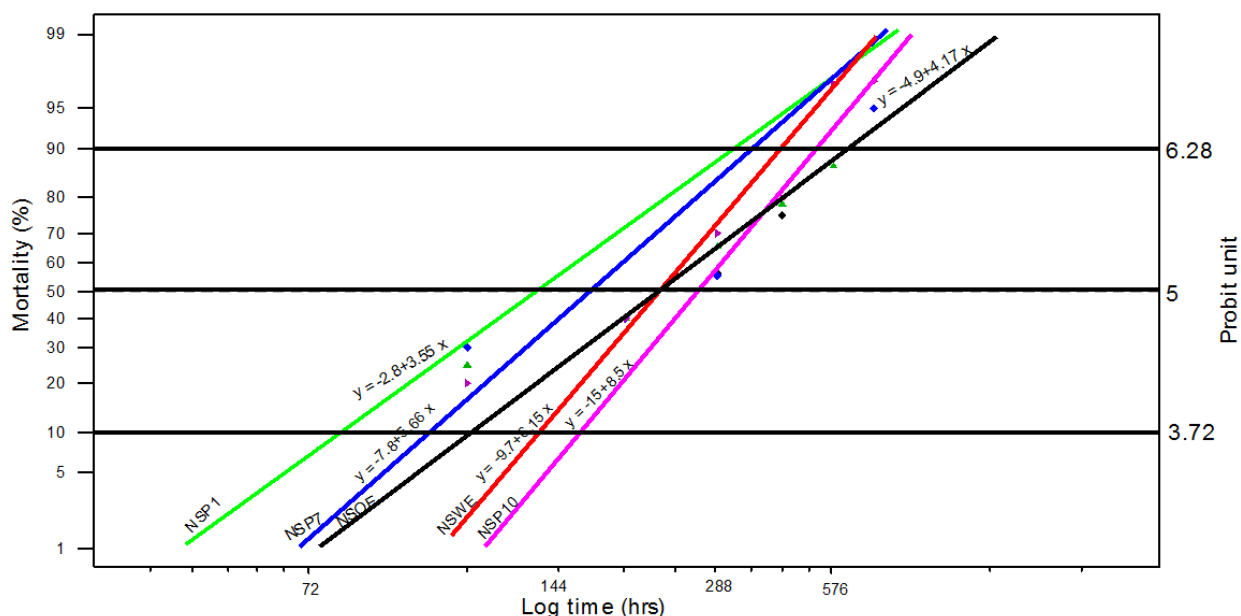


Figure 1. Logtime-Probit-line of the 2nd nymphal instar of Desert locust fed on pearl millet seedling treated with soil applied Neem seed products at concentration 5%.

NSOE and NSP at various concentrations (20, 10, 5 and 1%) and Azal 1% (as standard antifeedant).

The results clearly indicated that the amount of ingested food is inversely related to the dose. Increasing

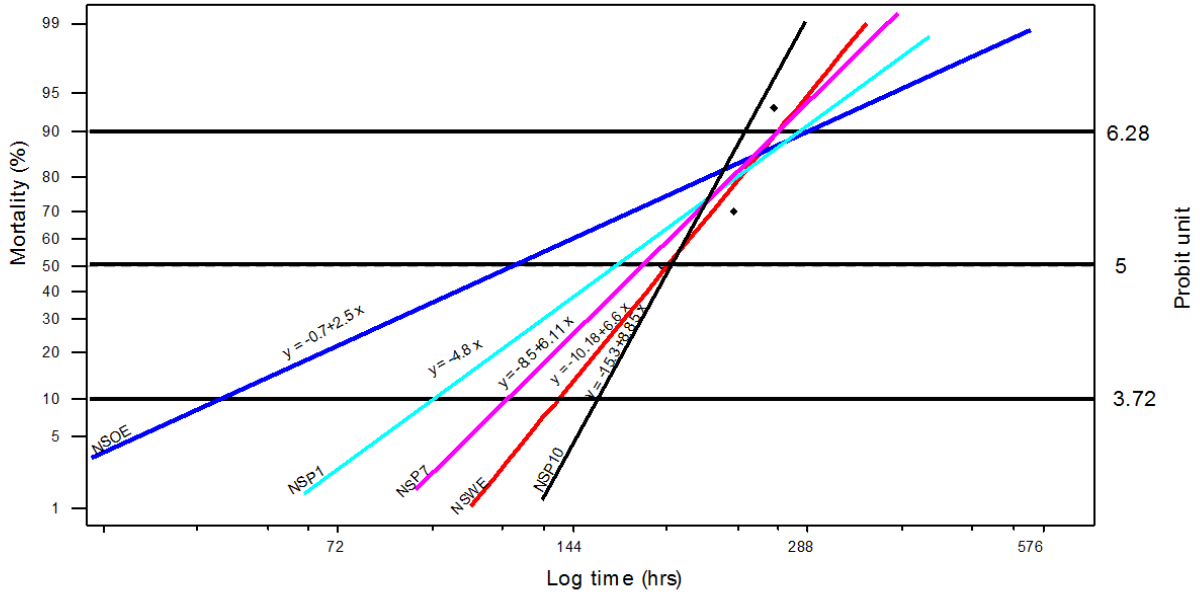


Figure 2. Logtime-Probit-line of the 2nd nymphal instar of Desert locust fed on pearl millet seedling treated with soil applied Neem seed products at concentration 10%.

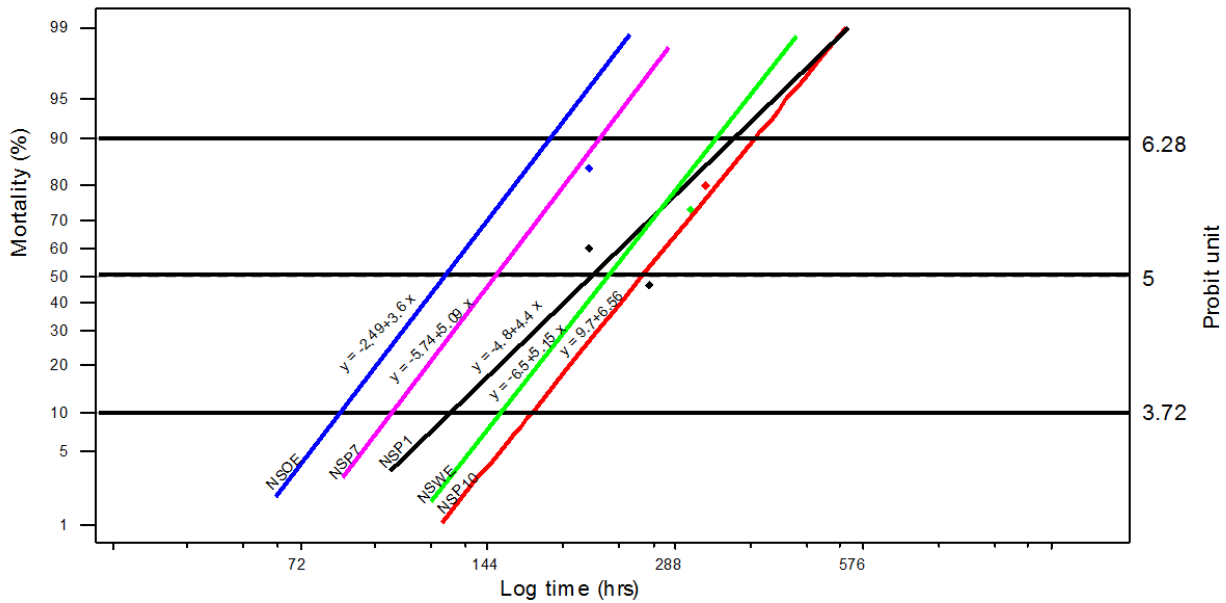


Figure 3. Logtime-Probit-line of the 2nd nymphal instar of Desert locust fed on pearl millet seedling treated with soil applied Neem seed products at 20% concentration.

the concentration of neem products results in progressive decrease for food ingested. The extent of reduction in food intake for the different neem treatments is extremely high, exceeding 52% at the lowest concentrations tested in various treatments. Neem seed water extracts causes reduction in food intake, ranging from 72-90.5, 86-93, 96-

99 and 97-99% for the second, third, fourth and fifth nymphal instars, respectively. The overall percentage reduction in food intake by all nymphal instars ranges from 86.75-95.4% for the various concentrations of neem seed water extracts. All concentrations significantly suppressed the feeding rate of test insects compared to

Table 4. Amount of ingested food and %reduction in food intake of various desert locust instars (2nd-5th) fed on pearl millet seedlings treated with soil applied neem seed products.

Conc. (%)	2 nd instar	3 rd instar	4 th instar	5 th instar
(a) NSWE				
20	0.267 ^d (90.5)	0.507 ^c (93)	0.333 ^b (99)	0.400 ^b (99)
10	0.33 ^d (89)	0.667 ^{bc} (91)	0.733 ^b (98)	0.900 ^b (98)
5	0.50 ^d (83)	0.920 ^{bc} (88)	0.900 ^b (97)	1.400 ^b (97.7)
1	0.80 ^c (72)	1.040 ^{bc} (86)	1.233 ^b (96)	1.600 ^b (97)
Azal 1%	1.33 ^b (50)	1.400 ^b (77)	2.467 ^b (90)	2.400 ^b (96)
0.0	2.867 ^a (-)	7.667 ^a (-)	31.4 ^a (-)	62.461 ^a (-)
Lsd	0.2813	0.7795	3.168	4.045
(b) NSOE				
20	0.267 ^c (90.5)	0.533 ^e (93.7)	1.333 ^b (96)	1.333 ^c (98)
10	0.467 ^c (83)	2.333 ^d (73)	3.533 ^b (89)	3.667 ^c (94)
5	0.933 ^b (66.7)	3.767 ^c (56)	5.067 ^b (84)	7.600 ^b (88)
1	1.333 ^b (52.5)	5.000 ^b (59)	6.067 ^b (81)	9.533 ^b (85)
Azal 1%	1.333 ^b (50)	1.400 ^{de} (77)	2.467 ^b (90)	2.400 ^c (96)
0.0	2.800 ^a (-)	8.533 ^a (-)	31.133 ^a (-)	62.677 ^a (-)
Lsd	0.4248	1.218	5.739	4.738
(c) NSP				
20	0.400 ^d (86)	0.600 ^c (92)	0.867 ^b (97)	0.733 ^d (99)
10	0.600 ^{cd} (79)	1.400 ^b (82)	1.033 ^b (96.7)	4.00 ^{cd} (94)
5	0.933 ^{bc} (67)	1.500 ^b (80)	1.267 ^b (95)	7.067 ^{bc} (87)
1	1.200 ^b (58)	1.800 ^b (76)	1.600 ^b (94.9)	8.733 ^b (86)
Azal 1%	1.333 ^b (50)	1.400 ^b (77)	2.467 ^b (90)	2.400 ^d (96)
0.0	2.867 ^a (-)	7.667 ^a (-)	31.600 ^a (-)	62.267 ^a (-)
Lsd	0.527	0.7209	2.957	3.667

Mean values having different letters in each column differ significantly ($P \leq 0.05$) according to Lsd test. Values between brackets represent the percentage reduction in food intake.

NSWE: Neem seed water extracts

NSOE: Neem seed organic extracts

NSP1: Neem seed powder.

the control. The reduction in food intake caused by NSWE is superior to that caused by Azal 1% (Table 4a).

Neem seed organic extracts induce reduction in feeding rate, ranging from 52.5-90.5, 59-93.7, 81-96 and 85-98% for the second, third, fourth and fifth instars, respectively. The overall percentage reduction in food intake by all nymphal instars ranges from 69.4-94.5% for the various concentrations of neem seed organic extracts. All concentrations of NSOE significantly suppressed the feeding rate of test insects compared to the control (Table 4b). The reduction in food intake caused by NSOE is superior to that caused by the standard (recommended) dose of Azal 1%.

Neem seed powder (Table 4c) cause's reduction in feeding rate, ranging from 58-85, 76-92, 94-97 and 86-99% for the second, third, fourth and fifth instars, respectively. The overall percentage reduction in food intake ranges from 78.7-93.5% for the various neem seed powder treatments. All neem seed powder (NSP) concentrations significantly suppressed the feeding rate

of test insect compared to the control. The reduction in feeding rate exceeds that caused by the standard dose of Azal 1%. Azadirachtin as Azal 1% at the recommended dose reduced the feeding rate by 50, 77, 90 and 90% for the second, third, fourth and fifth nymphal instars, respectively. It also showed a significant suppression in food intake rate of test insects compared to the control.

Effect of water stress on the efficacy of systemic action of NSP in nymphal duration increase

Table 5 showed the effect of water stress on the efficacy of the systemic action of neem seed powder. Watering was performed either immediately following sawing (NSP₁), after 7 days (NSP₇) or after 10 days (NSP₁₀). NSP₁ resulted in increased duration of the second nymphal instar by 47, 17, 42 and 70 hours for the concentrations of 20, 10, 5 and 1%, respectively (Table 5-a) as explained before; while NSP₇ resulted in

Table 5. The effect of various water regimes on the efficacy of NSP as related to development of 2nd instar

Conc. (%)	Duration				
	2 nd instar duration (days)	%moulted to 3 rd instar	3 rd instar duration (days)	Total duration (2 nd and 3 rd) (days)	%mortality 3 rd instar
(a) NSP1					
20	10.00 ^{ab} (47)	60	12.00 ^a (41)	22(42)	100
10	8.25 ^{bc} (17)	66	11.00 ^b (29)	19.5(24)	100
5	10.00 ^{ab} (42)	53	10.00 ^c (18)	20(29)	100
1	12.00 ^a (70)	66	10.00 ^c (18)	22(42)	100
0.0	7.06 ^b (-)	100	8.50 ^d (-)	15.5	6.6
Lsd	2.152		0.8420		
(b) NSP7					
20	10.00 ^a (33.3)	13.3	12.00 ^a (41)	22(37.5)	100
10	12.00 ^b (60)	13.3	11.00 ^a (29)	23(44)	100
5	11.00 ^b (47)	20	10.00 ^a (18)	21(31)	100
1	18.00 ^{ab} (140)	20	15.00 ^a (76)	33(106)	100
0.0	7.50 ^c (-)	100	8.50 ^a (-)	16	6.6
Lsd	1.684		5.119		
(c) NSP₁₀					
20	11.00 ^b (47)	13.3	12.00 ^a (41)	23(44)	100
10	8.00 ^b (7)	13.3	12.00 ^a (41)	20(25)	100
5	14.00 ^a (87)	20	11.00 ^b (29)	25(56)	100
1	17.33 ^a (131)	26.6	11.00 ^b (29)	28(75)	100
0.0	7.50 ^b (-)	100	8.50 ^c (-)	16(-)	6.6
Lsd	5.514		0.421		

Mean values having different letters in each column differ significantly ($P \leq 0.05$) according to Lsd test.

Values between brackets represent the percentage increase in nymphal duration.

NSP1: Effect of one day delayed watering of treated seedlings

NSP7: Effect of 7 day delayed watering of treated seedlings

NSP10: Effect of 10 days delayed watering of treated seedlings.

prolongation of the second instar duration by 33.3, 60, 47 and 140% for the 20, 10, 5 and 1% concentrations, respectively. Generally, effects were inversely related to dose. The individual succeeded to moult to the third instar, ranging from 7 to 13.3% (Table 5b). The increase in the duration of the third nymphal instars were 41, 29, 18 and 78% for the concentrations 20, 10, 5 and 1%, respectively. No further moult was observed. The increase in the total developmental period (2nd + 3rd) was 37.5, 44, 31 and 105% for the concentrations 20, 10, 5 and 1%, respectively. All concentrations were significantly different from the control.

The percentage increase in nymphal duration of the second nymphal instar exposed to NSP₁₀ treated seedlings was 47, 7, 87 and 131% for the concentrations 20, 10, 5 and 1%, respectively (Table 5-c). Prolongation was inversely related to dose. Percentage of the second instar moulted to the third stage, ranging from 7-13%. No further moult was recorded. On the other hand, the increase in the duration of the third nymphal instar

ranged from 29-51%, while the increase in the total period (2nd and 3rd) ranged from 25-75%. All treatments were significantly different from the control and effects were almost dose related.

Mortality cases

Mortality of desert locust nymphs fed on pearl millet seedlings treated with soil applied NSP watered after 7 days were given in Table 6-b. All concentrations gave significant deaths (at $P = 0.05$) among test insects compared to the control. The mortality is dose related; higher concentration resulted in higher mortality and at faster rate compared to lower dosage. In most cases, mortality occurred before moulting. Lower dosage cause death after a long period and insects died as overaged nymphs. Complete mortality of test insects occurred between 12-21 days depending on dosage. On the other hand, NSP₁₀ (Table 6c) induced significant mortality

Table 6. Mortality cases among desert locust nymph (2nd and 3rd) fed on pearl millet seedlings treated with soil applied NSP.

Conc. (%)	Mortality through time (days)							
	3	6	9	12	15	18	21	24
(a) NSP₁								
20	0.33 ^{bc} (6.6)	2.66 ^a (53)	3.33 ^a (66.6)	4.00 ^a (80)	4.00 ^a (80)			
10	0.33 ^b (6.6)	2.33 ^{ab} (46.6)	3.33 ^a (66.6)	3.66 ^a (73)	3.66 ^a (73)			
5	1.33 ^a (27)	2.00 ^{ab} (40)	3.00 ^a (60)	4.00 ^a (80)	4.00 ^a (80)			
1	1.00 ^a (20)	1.66 ^{ab} (33)	3.00 ^a (60)	3.00 ^a (60)	3.00 ^a (60)	5.00 ^a (100)		
0.0	0.00 ^b (-)	0.00 ^b (-)	0.33 ^b (6.6)	0.66 ^b (13)	0.66 ^b (26)	0.66 ^b (13)		
Lsd	0.8136	0.8136	1.409	1.3280	0.6635	0.8564		
(b) NSP₇								
20	1.33 ^a (26)	2.66 ^a (53)	4.66 ^a (93)	5.00 ^a (100)	-			
10	0.60 ^{ab} (13)	0.60 ^b (13)	4.33 ^{ab} (87)	4.66 ^{ab} (93)	5.00 ^a (100)			
5	0.60 ^{ab} (13)	1.66 ^{ab} (32)	4.33 ^{ab} (87)	4.33 ^{ab} (87)	5.00 ^a (100)			
1	0.30 ^b (6.6)	1.33 ^b (26)	3.33 ^b (67)	3.33 ^b (67)	3.33 ^b (67)	3.66 ^a (73)	5.00 ^a (100)	
0.0	0.00 ^b (-)	0.00 ^c (-)	0.33 ^c (6.6)	0.66 ^c (13)	0.66 ^c (13)	0.66 ^b (13)	0.66 ^b (13)	
Lsd	0.8136	0.9337	1.5062	0.4923	0.3828	0.5010	0.8564	
(c) NSP₁₀								
20	1.00 ^a (20)	1.67 ^a (33.4)	4.66 ^a (93)	4.66 ^a (93)	5.00 ^a (100)			
10	0.66 ^{ab} (13)	1.00 ^{ab} (20)	4.66 ^a (93)	4.66 ^a (93)	5.00 ^a (100)			
5	0.66 ^{ab} (13)	0.66 ^{bc} (13)	3.66 ^a (73)	4.00 ^a (80)	4.66 ^a (93)	4.66 ^a (93)	5.00 ^a (100)	
1	0.33 ^{ab} (6.6)	0.66 ^{bc} (13)	3.00 ^a (60)	3.33 ^a (66.6)	4.33 ^a (87)	4.33 ^a (87)	4.66 ^a (93)	5.00 ^a (100)
0.0	0.00 ^b (-)	0.00 ^b (-)	0.33 ^b (6)	0.66 ^b (66)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)	0.66 ^b (13)
Lsd	0.8136	0.8136	2.048	1.243	1.050	1.632	0.9413	0.8564

Mean values having different letters in each column differ significantly ($P \leq 0.05$) according to Lsd test.

Values between brackets represent the percentage mortality.

NSP₁: Effect of one day delayed watering of treated seedlings

NSP₇: Effect of 7 day delayed watering of treated seedlings

NSP₁₀: Effect of 10 days delayed watering of treated seedlings.

among desert locust nymphs with all treatments significantly different from the control. The mortality rate is dosage related and increases progressively with the increase in the dosage. Most of the mortality cases occurred before moulting. Lower doses resulted in late mortality and insects died as overaged nymphs. Probit analysis of mortality as related to dose or time was explained earlier.

Effects on food intake

Results summarized in Table 7 showed the amount of food ingested by different nymphal instar of desert locust. It is clear from the table that, the amount of food ingested is negatively related to the dose. Increasing the concentration of neem seed products resulted in progressive suppression in the amount of food ingested. This observation is true for all tested instars (2nd-5th nymphal instars of desert locust). The extent of suppression in food intake for NSP₇ and NSP₁₀ treatments is very high that is, more than 56% suppression was induced by the lower concentration

(1%). All treatments significantly suppressed the feeding rate of test insects compared to the control and gave similar results compared to NSP₇ treatments. For NSP₇, the reduction ranges from 57-85%, 76-91%, 95-97% and 85.1-98.5% for the 2nd, 3rd, 4th and 5th nymphal instars, respectively. While for the NSP₁₀, the respective reduction ranges from 56.5-84%, 76-92%, 95-97.1% and 84.1-98%.

Morphological deformations on the insect

Deformation cases were noticed on desert locust nymphs fed on pearl millet seedlings treated with soil applied NSP subjected to various types of water regimes (7 and 10 days). The percentages of damaged insects noticed in various treatments ranged from 6.6 to 13%. No deformation was noticed in the control counterparts.

DISCUSSION

An important tactical component in the preventive control strategy of desert locust is to locate hoppers during

Table 7. Amount of ingested food (mg)/insect and percentage reduction in food intake of various locust instars (2nd-5th) fed on pearl millet seedlings treated with soil applied NSP.

Conc. (%)	Instars			
	2 nd	3 rd	4 th	5 th
(a) NSP				
20	0.400 ^d (86)	0.600 ^c (92)	0.867 ^b (97)	0.733 ^d (99)
10	0.600 ^{cd} (79)	1.400 ^b (82)	1.033 ^b (96.7)	4.00 ^{cd} (94)
5	0.933 ^{bc} (67)	1.500 ^b (80)	1.267 ^b (95)	7.067 ^{bc} (87)
1	1.200 ^b (58)	1.800 ^b (76)	1.600 ^b (94.9)	8.733 ^b (86)
Azal 1%	1.333 ^b (50)	1.400 ^b (77)	2.467 ^b (90)	2.400 ^d (96)
0.0	2.867 ^a (-)	7.667 ^a (-)	31.600 ^a (-)	62.267 ^a (-)
Lsd	0.527	0.7209	2.957	3.667
(b) NSP₇				
20	0.443 ^d (85)	0.700 ^c (91)	0.867 ^b (97)	0.933 ^d (98.5)
10	0.640 ^{cd} (79)	1.600 ^b (79)	0.873 ^b (97)	5.333 ^c (91.5)
5	1.166 ^{bc} (61)	1.677 ^b (78.5)	1.267 ^b (96)	7.233 ^b (88)
1	1.300 ^b (57)	1.900 ^b (76)	1.600 ^b (95)	9.500 ^b (85)
0.0	3.000 ^a (-)	7.800 ^a (-)	31.600 ^a (-)	62.267 ^a (-)
Lsd	0.5489	0.6216	3.164	3.317
(c) NSP₁₀				
20	0.483 ^c (84)	0.657 ^c (92)	0.890 ^b (97)	1.067 ^d (98)
10	0.657 ^c (78.5)	1.650 ^b (80)	1.100 ^b (96.5)	5.967 ^c (90.5)
5	1.267 ^b (59)	1.700 ^b (79)	1.400 ^b (95.5)	7.667 ^b (88)
1	1.333 ^b (56.5)	1.967 ^b (76)	1.667 ^b (94.7)	10.067 ^b (84)
0.0	3.067 ^a (-)	8.167 ^a (-)	31.633 ^a (-)	63.200 ^a (-)
Lsd	0.5457	0.5585	3.091	2.934

Mean values having different letters in each column differ significantly ($P \leq 0.05$) according to Lsd test.

Values between brackets represent the percentage increase in nymphal duration.

NSP1: Effect of one day delayed watering of treated seedlings

NSP7: Effect of 7 day delayed watering of treated seedlings

NSP10: Effect of 10 days delayed watering of treated seedlings.

upsurges and mount control operations with pesticides to halt their exponential growth into large swarms (Magor et al., 2008; Leach et al., 2009; Lecoq, 2010). The strategy is built on improved knowledge of locust ecology, more efficient monitoring of pest levels in various areas, use of more effective pesticides, and improved application methods. The botanical compounds, although they are slow in action, are easily degradable under field conditions; thus requiring repeated spraying on crops.

One of the promising sources of natural products is neem tree. Neem seed products possess systemic activity, which was first proved by Gill and Lewis (1971), who reported a systemic antifeedant action of three neem products viz.; azadirachtin (tetracyclic triterpenoid), ethanolic extract from neem seeds, and aqueous suspension of ground seed kernel, as against the desert locust, *S. gregaria*.

The study of Gill and Lewis (1971) indicated that the desert locust caused slight damage to bean plants grown

in treated soil. Bean seedlings grown from seed soaked in a solution of 0.01% azadirachtin, 0.1% alcohol extract, or 0.1% aqueous kernel extract gave protection against *S. gregaria* adults for one week after germination. Ruscoe (1972) who found that azadirachtin is an ecdysteroids in habit, acts as ecdysone analogue, and ecdysone is a hydrophilic in nature also reported the systemic activity of neem products. Attri (1975) reported that the active principle of neem is hydrophilic rather than lipophilic in nature. Saxena (1984) found that, after oil expression from neem seeds, the seed residue (seedcake) gave good protection to crops when applied to the soil.

The fact that the extract can be taken by plants and confer protection from within is one of the neem's interesting and potential use. The various merits of the systemic actions of neem products such as enhanced margin of safety to human and environment, protection from photo degradation, the longer period of activity, the

reduction in number of sprays (thus cost) as well as the possible systemic growth regulatory and/or antifeedant activity initiated the authors interest to investigate the efficacy of its systemic action in the control of desert locust at immature stages. The idea of using systemic antifeedant/growth regulatory agent could prevent locust immatures from reaching adults stages, and this may result in confining the locust as immatures in their breeding sites, with little threat of swarm formation and limited damage to local growers. The preliminary work from this group on the antifeedant and growth regulatory effect and the work of previous authors (Govindachari et al. 2000; Elamin 2002; Schmutterer 2002; Abd El Rheem 2005; Mohamed 1999) on the subject further strengthen these goals.

In the current study, various aspects of systemic activity of different neem seed products (NSWE, NSOE, NSP and azadirachtin as azal 1%) were tested against various instars of desert locust. Aspects covered include evaluation of effects on development of immature, deformation, mortality and food intake. Effects of various water regimes on stability of powder products under pots conditions were also investigated.

Results indicated that, various neem seed products are capable of delaying the developmental period of all tested instars. The delay in development, which is dose-related in most cases, is very prominent in both instars tested (2nd and 3rd). The majority of tested 2nd instar individuals either never moult to the third stage, died as overaged 2nd instars, or moult to the third instar with no further moulting and died as overaged 3rd instar nymphs. Death may occur before or during moulting. Prolongation (delay) of development by neem seed products is well documented and current results agree with the findings of previous authors (Elamin, 2002; Abd El Rheem, 2005).

Complete mortalities of test nymphs subject to various treatments occurred within 2-3 weeks. Many of the test insects were deformed prior to death. Although most test insects died in stage treated, yet many of the nymphs, who succeeded to proceed to the next instars were unable to complete shedding their nymphal exuviae or moult to further instar, and others had some morphological abnormalities. All these observations agree with previous reports of Ruscoe (1972) and Nasseh et al. (1993).

Neem seed powder and neem water extract (the simplest forms of neem) gave comparable or sometimes superior results compared to NSOE and azal. Thus, deserve further investigation under field conditions. The facts that they are less expensive, simple to prepare and with comparable superior systemic action, which prevent further moulting give strong support to this argument.

Field application of these products especially in locust breeding areas could prevent swarm formation by keeping the locust in the early stages of development until they die as overaged immatures. The finding of the limited water stress experiments carried indicated the

stability of the powder for up to 10 days if the area receives no rains as would be explained later.

The time related insecticidal activities of neem seed products, were subject to probit analysis, the data indicated that all neem seed products have similar and relatively slow action. This is clear from the time response mortality data, which shows an increase in efficacy with the increase in exposure time of the nymph to treated pearl millet seedlings, evident from the decrease in the values of LT_{50} and LT_{90} . The line slopes for the concentrations 5, 10 and 20% were steep and positive, indicating a homogenous test population. The homogeneity of responses is also evident from the narrow LT_{90}/LT_{50} ratios and narrow fiducial limits. Suitable lines fit and good execution is also evident from the low chi-square values. The time response of late watering treatments (7 and 10 days) of NSP gave results comparable to those of immediate watering.

Active principle of neem could mostly kill test insects through feeding suppressing and/or growth regulatory effects on immatures stages. As reported by Ruscoe (1972) and Ruskin (1991) azadirachtin, the active principle of neem, is structurally similar to ecdysone, the ecdysteroid hormones controlling metamorphosis in insects. Azadirachtin affect the corpus allatum, which secrete vital hormones in insects. Azadirachtin blocks the secretion and release of ecdysone and this may delay the moulting process and therefore prolong the duration of the immature stages and causes the associate deformations and mortality before or during moulting process (Rembold et al. 1982).

Reduction in food intake might also lead to the prolongation of the nymphal period and deformation of test immatures, and they must reach a critical body weight for moulting (Elamin 2002). Azadirachtin seem to prolong the time needed by the immatures to gain the critical body weight through its suppression of feeding rate (Mohamed, 1999). The fact that test nymphs either died within the 2nd nymphal stage or succeeded to moult to the third stage without further moulting, could indicate also the role of amount of food consumed (as reflected by the weight gain) in triggering the moulting process. Individuals who are able to consume larger amount of food were able to moult, since they reached the critical body weight for moulting faster. Once the nymphs enter the 3rd instar and are continuously exposed to neem seed products through food ingestion, their feeding rate decrease and therefore, remain as immatures without further moulting. This argument needs further investigation for clarification. The results of the current study showed a dose dependent antifeedant effect of various neem seed products as indicated by the significant reduction in food intake. All forms of neem seeds products gave superior suppression of food intake compared to Azal 1%.

Pradhan et al. (1962); Gill and Lewis (1971) and Ridha et al. (2018) reported the antifeedant effect of neem. The

active antifeedant principle in neem includes Salanin, Salannol acetate, 3-deacetyl salannin, 4-epoxy azadirachtin, gedunin, nimbenin and deacetyl nimbinen (Schwinger et al., 1984). Neem exerts its antifeedant action through gustation at physiological site associated with chemoreceptors (Pradhan et al., 1962; Fagoonee, 1981; Gill and Lewis, 1971; Ridha et al., 2018). Beside the regulatory growth and the suppression in food intake, mortality might result from the direct toxic action, which was also reported as mode of action of neem (Fagoonee and Lauge 1981; Saxena and Khan, 1985).

The current study investigates the effect of various water regimes on stability of neem seeds products. The results indicated that applying neem seed powder to the soil and irrigation after a period of water stress up to 7 or 10 days do not adversely affect the efficacy of neem seed powder on the prolongation of the nymphal duration, mortality or deformation. This superior stability, though not tested more than 10 days, indicated the stability of neem seed powder for field application in locust breeding areas, where watering depends on sporadic irregular rainfall. Results reported here were very encouraging as it demonstrates the possibility to confine the locust in the breeding area as tiny immatures, which pose little hazards to crops in the breeding site and/or invasion zone (as no swarms will be formed).

Conclusion

The current study has shed light on the efficacy of systemic action of various neem seed products against immature stages of desert locust. Superior suppression of moulting with subsequent delay in development, deformation, mortality, suppression of feeding rate and stability of products under conditions of delayed rainfall were noticed in the current study. Results reported here indicate the suitability of soil applied (systemic) neem seed products in management of desert locust in their breeding site. Efficacy of seed treatment with the products was not tested for some logistic reasons, but it deserves investigation in future work. Other aspects related to the validation and use of these products under field conditions needs further investigation.

CONFLICT OF INTERESTS

The authors declare no competing financial interest.

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REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an

- insecticide. *Journal of Economic Entomology* 18:265-269.
- Abd El Rheem OSE (2005). Semi-field Evaluation of the Systemic Activity of Neem Seed Powder against Desert Locust Immatures [*Schistocerca gregaria* (Forsk.)] (Orthoptera: Acrididae). M.Sc. Thesis. University of Khartoum.
- Attri BS (1975). Utility of neem oil extractive as feeding deterrents to locust. *Indian Journal of Entomology* 37:417-419.
- Baron S (1972). The desert locust. Great Britain, Eyre Methren Ltd. 11 Newsletter fether lane Ec₄P4EE. pp. 14-52.
- Bashir M, Hassanali A, Korena H, Bashir W (2016). Semi-field evaluation of the effects of sub-lethal doses of pesticides, with and without adult *Schistocerca gregaria* pheromone, on hoppers. *The Netherlands Entomological Society Entomologia Experimentalis et Applicata* 160:72-76.
- Borror JD, DeLong MD, Triplehorn AC (1981). An introduction to the study of insect. 5th (end). Saunders College Publishing, Philadelphia, New York pp. 1-222.
- Edward D (2017). Plagues and Players: an environmental and scientific history of Australia's southern locusts. Ph.D. Australian National University.
<https://pdfs.semanticscholar.org/94ff/f0a71323a930641dc57365db62fe87619d81.pdf>
- Elamin MNE (2002). Evaluation of the systemic action of neem seed products against desert locust immatures *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae). M.Sc. Thesis. University of Khartoum.
- Fadl-Elmawla KM (2004). Impact of some Pesticides used in the Control of Desert Locust (*Schistocerca gregaria* Forskål) on some Non-target Arthropods in the Red Sea Coastal area of Sudan. M.Sc. Thesis. University of Khartoum.
- Fagoonee I, Lauge G (1981). Noxious effects of neem extracts on *Crociodolomia binotalis*. *Phytoparasitica* 9(2):111-118.
- Gill SJ, Lewis CT (1971). Systemic action of an insect feeding deterrent. *Nature* 232:402-403.
- Govindachari TR, Suresh G, Gopalakrishnan G, Wesley (2000). Insect antifeedant and growth regulating activities of neem seed oil ± the role of major tetranortriterpenoids. *Journal of Applied Entomology* 124: 287-291.
- Harvey AW (1990). Rearing and breeding of locusts in the laboratory. NRI. Natural Resources Institute Overseas Development Administration P 28.
- Hosny AKH (2012). Biological and Chemical Control of Desert Locust and Grasshoppers in Egypt. Ph.D. Dissertation. Cairo University.
- Hummel EH, Hein DF, Schmutterer (2012). The coming of age of azadirachtins and related tetranortriterpenoids. *Journal of Biopesticides* 5:82-87.
- Iboudo S, Toé AM, Ouédraogo R, Ouédraogo M, Guissou IP (2014). Ecological risk assessment of pesticide residues in water from desert locust area in Burkina Faso. *Research Journal of Environmental and Earth Sciences* 6:227-232.
- Krall S, Wilps H (1994). New trend in locust control. Deutsche Gesellschaft für technische Zusammenarbeit (GTZ) GmbH, Postfach 5180, 65726 Eschborn, Fedral Republic of Germany pp. 5-80."
- Leach AW, Mullie WC, Mumford JD, Waibel H (2009). Spatial and Historical Analysis of Pesticides Externalities in Locust Control in Senegal. FAO II International Workshop on the Future of Bio-Pesticides in Locust Control, FAO, Rome, Italy.
- Lecoq M (2010). Integrated pest management for locusts and grasshoppers: are alternatives to chemical pesticides credible? *Journal of Orthoptera Research* 19:131-132.
- Magor JI, Lecoq M, Hunter DM (2008). Preventive control and desert locust plagues. *Crop Protection* 27:1527-1533.
- Mamadou A, Sarr M (2009). Impact of two Insecticides Used in the Control of the Desert Locust on *Psammotermes hybostoma* Desneux (Isoptera: Rhinotermitidae) in Niger. *African Entomology* 17(2):147-153.
- Maute K, French K, Story P, Bull CM, Hose GC (2016). Effects of two locust control methods on wood-eating termites in arid Australia. *Journal of Insect Conservation* 20:107-118.
- Meinzingen WF (1993). A guide to migrant pest management in Africa. Food and Agriculture Organization of the United Nations (FAO) 1st (edn) pp. 1-36.

- Mohamed YS (1999). Evaluations of Efficacy of Neem and Sadom apple (Ushar) Products and its Control of Desert Locust (*Schistocera gregaria* Forskal) (Orthoptera-Aerididae) Under Laboratory Conditions. M.Sc. Thesis, University of Khartoum, Sudan.
- Nasseh OM, Feres T, Krall S (1993). Repellent effect of *Azadirachta indica* A. juss, on adults of *Schistocerca gregaria* (Forsk.)-investigation in the Republic of Niger. *Mitteilugen-der-Deustschen-Gesellschaft-fur-Allgemeine-und-Angewandtedte-Entomologie* 8(4-6):835-838.
- Office of Technology Assessment (1990). Past and current control method for locust and grasshopper. In "Plague) congress of the united State pp. 37-41.
- Pradhan S, Jotwani MG, Rai BK (1962). The neem seed deterrent to locust. *Indian Farming* 12:7-11.
- Rembold H, Sharma GK, Czoppelt CH and Schumutterer H (1982). Azadirichin: a potent insect growth regulator of plant origin. *Zeitschrift Fur Angewandte Entomologie*. *Journal of Applied Entomology* 93:12-17.
- Ridha K, Nurdin S, Rosnani N (2018). Antifeedant Activity from Neem Leaf Extract (*Azadirachta indica* A Juss). *Jurnal Natural* 18(1):7-10.
- Ruscoe CNE (1972). Growth disruption effect of an insect antifeedant. *Nature Neem Biology* 236:159-160.
- Ruskin FR (1991). Neem a tree for solving global problem. Report of an AdhOC Panel of the Barrd on Science and Technology for International Development National Research Council 4:23-77.
- Saxena RC, Khan ZR (1985). Electronically recorded disturbance in feeding behavior of *Nephotettix virescens* (Homoptera, Cicadellidae) on Neem oil treated rice plants. *Journal of economic entomology* 78:222-226.
- Saxena RC, Epino PB, Cheng-wen TU and Puma B (1984). Neem, chinaberry and Custard apple: antifeedant and insecticidal effects of seed oils on leaf hopper pests of rice and their natural enemies. Proceedings of the 2nd International Neem Conference Rauschhlzhausen pp. 403-417.
- Schmutterer H (2002). The neem tree *Azadirachta indica* A. Juss. and other meliaceous plants. Sources of unique natural products for integrated pest management, medicine, industry and other purposes. 2nd edition, Neem Foundation, Mumbai.
- Schwinger M, Ehhammer B, Kraus W (1984). Methodology of the *Epilachna varivestis* bioassay of antifeedants demonstrated with some compounds from *Azadirachta indica* and *Melia azaderach*. In: *Natural Pesticides from the Neem Tree and Other Tropical Plants*. Ed. by Schmutterer, H.; Ascher, K. R. S. Eschborn: GTZ pp. 181-197.
- Showler AT (2013). *The Desert Locust in Africa and Western Asia: Complexities of War, Politics, Perilous Terrain, and Development*. Radcliffe's IPM World Textbook, University of Minnesota.
- Siddig AS (1991). Evaluation of neem seed and leaf water extracts for the control of insect pest in Sudan. *Agricultural Research Corporation, Shambat Research Station Bulletin No. 6:1-39*.

Full Length Research Paper

Use of temporary immersion bioreactors on *in vitro* culture of cactus pear

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This work was conducted with the objective of evaluating the performance of cactus pear cultivars on *in vitro* culture using bioreactors and the conventional method. The treatments were arranged in a 3 × 2 factorial scheme with a completely randomized design, and the three cactus pear cultivars (*Orelha de elefante mexicana*, *Miúda* and *IPA-Sertânia*) were combined with two micropropagation methods (conventional and temporary immersion bioreactors), totaling six treatments with five replicates each. After 30 days, the following variables were evaluated: cladode length, fresh explant matter mass, number of shoots and number of roots. There was a significant interaction between the cultivar factor and the micropropagation method for the cladode length and fresh matter mass, with bioreactors being the most responsive. However, regarding to the number of shoots and number of roots, there was no interaction between these factors under the same level of significance, where the conventional crop stood out. The results indicate different morphogenetic responses among the tested cultivars, and specific *in vitro* propagation protocols should be developed for each one.

Key words: Micropropagation, bioreactor, plant productivity, cactus pear cultivars.

INTRODUCTION

The cactus pear, *Opuntia* and *Nopalea* species, are an important resource for the maintenance of livestock in

Brazilian semi-arid region, as well as food, guarantees water supply to animals during periods of drought (Frota

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et al., 2015). In general, cactus pear has low levels of dry matter (6.1 to 17.1%) and crude protein (2.9 to 6.0%) being rich in minerals, mainly calcium, potassium and magnesium.

According to Donato et al. (2017), the growing interest in cactus pear is due to productive security, associated to a set of measures necessary to reduce the risks of production's loss, and which enables the farmer to guarantee harvest. Already, Silva et al. (2017), said that choosing the appropriate cultivar, among other factors, can increase productive and qualitative safety, sustainability and resilience of activity, which is very important in a family farming crop.

The cactus pear cv. *Miúda* - IPA-100004 [*Nopalea cochenillifera* Salm-Dyck], *Orelha de elefante mexicana* - IPA-200016 [*Opuntia stricta* (Haw.) Haw] and *IPA-Sertânia* - IPA-200205 [*N. cochenillifera* Salm-Dyck] show good production of dry matter and are resistant to carmine scale (*Dactyloius opuntiae*), the main crop pest (Santos et al., 2006). These factors ensured the great acceptance by the rural producers and the increase in the cultivated area.

Cactus pear seedlings can be obtained from cladodes from a palm tree or through tissue culture. The latter provides uniform propagative material free of phytopathogenic agents, but they are still at a high price because of their production's cost.

Recent advances in biotechnology and plant tissue culture have provided new protocols and equipment aimed at minimizing production's costs, increasing seed multiplication *in vitro*, and reducing laboratory time. Among these equipment are bioreactors.

According to Teixeira (2002), bioreactors were developed from equipment known as fermenters, which were previously used in the cultivation of cells and microorganisms, aiming at the production of secondary metabolites and alkaloids for industrial purposes. Currently, they have been used in micropropagation of plants based on temporary or permanent immersion of vegetable tissues in liquid medium.

In the temporary immersion system the vegetable tissue is periodically bathed with the nutrient medium that is pumped into the container containing the explants and returning to the container that stores it, promotes the periodic exchange of the culture flask atmosphere (Fogaça et al., 2006). This renewal of the atmosphere provides a reduction of stressors, such as the gases accumulation.

Of the numerous advantages provided by temporary immersion bioreactors (TIB) and pointed out by Escalona et al. (1999) are renovation of the internal atmosphere, increased photosynthetic and respiration rates, improved nutrient uptake, increased plant multiplication rate and reduced crop manipulation.

There are reports of success in the use of TIB's for large-scale propagation of various species, such as banana (Lemos et al., 2001), sugarcane (Lorenzo et al.,

1998), orchid (Paek et al., 2001) and pineapple (Silva et al., 2007), presenting a multiplication rate higher than conventional micropropagation.

In this sense, the objective of this work was to analyze the performance of cactus pears explants cv. *Miúda*, cv. *Orelha de elefante mexicana* and cv. *IPA-Sertânia* micropropagated in a conventional way and with use of temporary immersion bioreactors.

MATERIALS AND METHODS

The experiment was conducted at the Plant Biotechnology Laboratory of Agricultural Research Company of Minas Gerais - EPAMIG, EPAMIG Norte - Gorutuba Experimental Field, Nova Porteirinha - MG, during the year 2018.

Cactus pear explants (cv. *Miúda*, cv. *Orelha de elefante mexicana* e cv. *IPA-Sertânia*) used in the experiment were obtained from the *in vitro* establishment in MS solid medium (Murashige and Skoog, 1962) of young cladodes buds supplemented with 30 g L⁻¹ sucrose, 0.1 g L⁻¹ inositol, 4 mg L⁻¹ benzylamine-6-purine (BAP) and 7 g L⁻¹ agar with pH adjusted to 5.8. This material remained in the growth room for 60 days, or until sprouting was emitted, under irradiance of 40 μmol m⁻² s⁻¹ provided by cold white light, with 16 h of photoperiod at 25 ± 2°C. Sprouting originated was subcultured in culture medium of the same composition for 90 days, reaching the fourth stage.

In vitro culture, two methods were used, the conventional one using the solid culture medium (7 g L⁻¹ agar) and the TIB's using the liquid medium with immersion of 3 min every 4 h. Explants of three cultivars of forage palm were used: cv. *Miúda*, cv. *Orelha de elefante mexicana* and cv. *IPA-Sertânia*.

The treatments were arranged in a 3 × 2 factorial scheme with a completely randomized design, and the three cactus pear cultivars were combined with the two micropropagation methods, thus totaling six treatments with five replications, in which each replicate was composed of two explants.

The explants had their length reduced to 15 mm (± 1 mm) and were then packed in temporary immersion bioreactors and in conventional subculture bottles.

In conventional micropropagation, 50 mL of MS culture medium supplemented with 30 g L⁻¹ sucrose, 0.1 g L⁻¹ inositol, 7 g L⁻¹ agar, 0.5 mg L⁻¹ of naphthalene-acetic acid (ANA) and 1 mg L⁻¹ of BAP.

A culture medium of similar composition was used in the temporary immersion bioreactor, but no agar was used and 200 mg L⁻¹ of ascorbic acid and 0.25 ml of Plant Preservative Mixture (PPM, Sigma) were added. The immersion time of the explants was adjusted to 3 min every 4 h. In both methods, the pH of the culture medium was adjusted to 5.8 and autoclaved at 120°C for 20 min.

The treatments were kept in a growth room, submitted to a 16-h photoperiod obtained from white LED lamps (40 μmol m⁻² s⁻¹) and a mean temperature of 25°C for a period of 30 days. At the end of this period, the following parameters were evaluated: cladode length (mm), fresh matter mass of the explants (g), number of shoots (unit) and number of roots (unit).

The data collected were submitted to analysis of variance (F < 0.05) by the statistical program Sisvar 5.6 (Ferreira, 2008) and, when significant, Test F was carried out for the method and T test for the cultivars. The mean values of the fresh mass of the explants, number of roots and number of shoots were transformed to (X + 0.5)^{0.5}, the latter analyzed at 10% significance.

RESULTS AND DISCUSSION

Based on the results obtained, the interaction between

Table 1. Mean values of forage palm cladode length (mm), obtained at 30 days of *in vitro* culture under different micropropagation methods and cultivars.

Method	Cultivars			
	OEM	Miúda	IPA-Sertânia	Average
Conventional	13.7 ^{Ba}	14.1 ^{Ba}	14.3 ^{Aa}	14.0
TIB	17.7 ^{Ab}	19.8 ^{Aa}	14.6 ^{Ac}	17.3
Average	15.7	16.9	14.4	-
CV (%)	8.05	-	-	-

Means followed by the same capital letter in the column and lowercase in the row do not differ by the T test at 5% significance.

Table 2. Mean values transformed to $(X + 0.5)^{0.5}$ of the fresh mass of forage palm explants (g), obtained at 30 days of *in vitro* culture under different micropropagation methods and cultivars.

Method	Cultivars			
	OEM	Miúda	IPA-Sertânia	Average
Conventional	0.16 ^{Ba}	0.23 ^{Aa}	0.21 ^{Aa}	0.20
TIB	0.60 ^{Aa}	0.29 ^{Ab}	0.15 ^{Ac}	0.34
Average	0.38	0.26	0.18	-
CV (%)	6.36	-	-	-

Means followed by the same capital letter in the column and lowercase in the row do not differ by the T test at 5% significance.

the factors cultivar and cultivation methods was observed when the cladode length and fresh mass were evaluated at 30 days (Tables 1 and 2).

In this study, it was observed that the use of the TIB provided better mean values for cladode length in the *Orelha de elefante mexicana* (OEM) and *Miúda* cultivars, but did not differ in the cultivar *IPA-Sertânia* (Table 1).

A similar event occurred when the fresh mass was evaluated, where OEM cultivation using bioreactor was more responsive and did not differ in the others (Table 2).

These results demonstrate that the use of bioreactors can increase the performance of cultivars *Orelha de elefante mexicana* and *Miúda* and indicate the tendency of the cultivars to respond differently to the techniques used. It is noteworthy that these belong to different species and genus and their responses *in vitro* culture are related to nutritional requirements and different morphogenetic characteristics.

Silva et al. (2017), when working with these three cactus pear cultivars comparing the growth, productivity and relationships with the meteorological variables, verified differences both morphologically and in the productive parameters. Also in this work, it was noticed that the OEM stands out as the one that produces the most fresh matter, revalidating the data obtained in the present work.

Nobel (2001) and Pimienta-Barrios et al. (2005) reported that in favorable times *Opuntia* species alter the pattern of CO₂ capture, thus promoting an increase in

growth, accumulation of reserves and increase in productivity. This factor may have influenced the performance of the *Orelha de elefante mexicana*, belonging to the genus *Opuntia*, on the others under the favorable conditions provided by the bioreactor, conditioning the production of almost four times more fresh mass than when compared to the other method.

Similar results were obtained by Medeiros (2011), working with different methods of *in vitro* cultivation combined with different doses of BAP in the micropropagation of cactus pear cv. *Orelha de elefante mexicana*, which observed the highest fresh mass gain of the shoots when using the temporary immersion system with high frequency of immersion.

Silva et al. (2017) evaluated the efficiency of water use and nutrient use of these cultivars, and observed that the *Orelha de elefante mexicana* and *IPA-Sertânia* stand out in the water use efficiency considering fresh mass production. In the efficiency of nutrient use, the genotypes had similar performances, with the exception of the element magnesium and sodium that was higher for *Orelha de elefante mexicana*.

According to Taiz and Zeiger (2004), magnesium is an important structural constituent of chlorophyll and acts on the activation of photosynthesis, respiration and nucleic acid synthesis enzymes. For Frizzone et al. (2005), increasing their uptake by the plant may promote increased crop productivity. However, sodium has great importance in the stomatal activity of CAM species, which

Table 3. Mean values transformed to $(X + 0.5)^{0.5}$ of the number of roots of forage palm (units), obtained at 30 days of *in vitro* cultivation under different cultivation methods and cultivars.

Parameter	Methods		OEM	Cultivars		CV (%)
	TIB	Conventional		Miúda	IPA-Sertânia	
Number of roots	0.26 ^B	1.20 ^A	0.20 ^B	0.90 ^{AB}	1.10 ^A	28.72

Means followed by the same letter in the line do not differ from each other by the F test for method and T test to cultivate, both at 5% significance.

gives greater efficiency in the use of water (Taiz and Zeiger, 2004), being essential to the survival of crops such as forage palm.

When analyzing the cultivar unfolding in each cultivation method, it was observed that the cultivars did not differ using the conventional method in both cladode length and fresh mass (Tables 1 and 2). For the cultivars in the bioreactor, it was observed that these differed from each other, standing out the *Orelha de elefante mexicana* and *Miúda* in the fresh mass and length of the cladodium, respectively.

This performance is probably related to the types of culture media used in both methods. On the one hand, in the conventional cultivation, the action of the gelling agent of the culture medium and the solid state that only allows the absorption of the nutrients by parts of the explant that are in direct contact with the medium, thus suppressing the development of the explants. On the other hand, the use of the liquid medium in the bioreactor and its greater absorption by the tissue and constant renewal of the air during the period of transfer of the medium.

Other authors have already demonstrated the superiority of bioreactors in relation to the conventional technique in hybrid clones of *Eucalyptus globulus* (Correia, 2011), in banana cv. Dwarf Cavendish (Farahani and Majd, 2012), in bamboo (Ribeiro et al., 2016) and in sugarcane (Matoso et al., 2017), which confirm the data presented in the present study. The results also identify the need for an adjustment in the *in vitro* propagation protocol for the cultivars in question, as they present individuals under the same conditions.

For the number of roots, there was no significant interaction between the cultivar factors and the cultivation method, but a significant difference was observed between the methods and the cultivars alone. Conventional *in vitro* cultivation provided the highest number of roots and the most responsive cultivar was *IPA-Sertânia* (Table 3).

Bioreactors, due to the use of liquid medium, provide a greater contact of the tissue of the explants with the medium and promote a greater absorption of nutrients and phytohormones. With the higher absorption of BAP, cytokinin responsible for cell division and shoot morphogenesis probably influenced the development of the roots due to the increase of its concentration in the

tissue, inhibiting the action of auxins and suppressing root initiation.

Araújo et al. (2008), working with the *in vitro* multiplication of 'smooth cayenne' pineapple using different concentrations of BAP and ANA in the culture medium, observed that the non-use of BAP stimulated the rooting and the greater growth of the explants. For them the availability and interaction of auxins and cytokinins regulate the formation of root, shoot and callus. Taiz and Zeiger (2004) reiterate this theory by saying that plant morphogenesis is guided by the balance of auxins/cytokinins, which in high/low favor rooting and the reverse balance promotes aerial part formation.

The cultivar *IPA-Sertânia* was the most responsive to the development of roots differing from the *Orelha de elefante mexicana*, which shows that besides the cultivation method, the cultivar also influences the *in vitro* morphogenesis of forage palm, indicating the need to establish specific protocols for each one.

Similar results were observed by Frota et al. (2004), which worked with proliferation and rooting of 10 clones of cactus pear [*Opuntia ficus-indica* (L.) Mill.] under different growth regulators and found different responses to rooting and sprouting among the clones. Alves et al. (2013) also observed variability of response among the studied genotypes to benzyladenine (BA) concentrations in budding induction, thus corroborating with the results found in the present study.

According to Vasconcelos et al. (2010), in addition to the type of growth regulators, their concentration and combination with other regulators, the response of explants *in vitro* also varies among species, variety, age and physiological state of the material used.

For the number of shoots, there was no interaction between the cultivar and method factors, being these analyzed in isolation. There was a significant difference in the cultivation method factor, with conventional cultivation giving the highest number of shoots. The cultivars did not differ from each other (Table 4).

The lower contact surface between the medium and the explant in conventional culture restricts the uptake of the growth regulators present in the medium. In this sense, the lower concentration of BAP in the tissue probably benefited shoot formation, since the plant growth regulators have activity under low concentrations.

For Taiz and Zeiger (2004), the effect of cytokinins is

Table 4. Mean values transformed to $(X + 0.5)^{0.5}$ of the number of forage palm shoots (units), obtained at 30 days of *in vitro* cultivation under different cultivation methods and cultivars.

Parameter	Methods			Cultivars		CV (%)
	TIB	Conventional	OEM	Miúda	IPA-Sertânia	
Number of shoots	0.0 ^B	0.2 ^A	0.2 ^A	0.1 ^A	0.0 ^A	19.69

Means followed by the same letter in the line do not differ from each other by the F test for cultivation method and T test to cultivate, both at 10% significance.

regulated by the enzyme cytokinin oxidase present in many plant tissues. It irreversibly inactivates cytokinins when high concentrations of these hormones are found in tissues.

Similar results were obtained by Medeiros (2011), working with different methods of *in vitro* cultivation combined with different doses of BAP in the micropropagation of cactus pear cv. *Orelha de elefante mexicana*, where they observed that in the treatments that had greater availability of the cytokinin and contact with the vegetal tissue caused a decrease in the emission of shoots with the increase in the concentration of BAP. For them the reduction of the BAP availability in the medium, either by the addition of the agar or reduction in the frequency of immersion, reduces the phytotoxic effect of the regulator even with the increase of the concentration of the same in the medium.

The greater exploitation of the medium by the explants, provided in the temporary immersion system of the bioreactors, can promote reduction in the cost of production of the seedlings *in vitro*, since it induces the use of formulations of culture media with lower concentrations of the reagents. However, it is necessary to adjust the concentrations of the phyto regulators for the propagation of the cactus pear cultivars using this cultivation method.

Conclusions

Temporary immersion bioreactors are a promising alternative to the propagation of cactus pear *in vitro*. The tested cultivars presented different performances for the number of roots, cladode length and fresh mass.

The cultivar *Orelha de elefante mexicana* had the best performance using the temporary immersion bioreactors.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alves Fal, Soares WS, Fernandes YTD, Rêgo MM (2013). Efeito de benziladenina na regeneração de duas variedades de palma

- forrageira (*Opuntia* spp.). Scientia Plena 9(6):34-49.
- Araújo RF, Siqueira DL, Cecon PR (2008). Multiplicação *in vitro* do abacaxizeiro 'smooth cayenne' utilizando benzilaminopurina (BAP) e ácido naftalenoacético (ANA). Revista Ceres 5(2):455-460.
- Correia ACG (2011). Micropropagação em biorreatores de imersão temporária e enraizamento de miniestacas e microestacas de clones híbridos de *Eucalyptus globulus*. Dissertação (Magister Scientiae) – Programa de Pós-Graduação em Ciência Florestal, Universidade Federal de Viçosa, Viçosa.
- Donato SLR, Arantes Ade M, Brito CFB, Rodrigues MG, Silva JÁ da, Donato PER (2017). Cultivo e utilização da palma forrageira. Informe Agropecuário 38(296):07-20.
- Escalona M, Lorenzo JC, Gonzalez BL, Danquita M, Gonzales JL, Desjardins Y, Borroto CG (1999). Pineapple (*Ananas comosus* (L.) Merr.) micropropagation in temporary immersion systems. Plant Cell Reports 18(1):743-748.
- Farahani F, Majd A (2012). Comparison of liquid culture methods and effect of temporary immersion bioreactor on growth and multiplication of banana (Musa, cv. Dwarf Cavendish). African Journal of Biotechnology 11(33):8302-8308.
- Ferreira DF (2008). SISVAR: um programa para análises e ensino de estatística. Revista Symposium 6(2):36-41.
- Fogaça LA, Dortzbach D, Alves AC, Pedrotti EL (2006). Características morfológicas de brotações de *Agapanthus umbellatus* var minor multiplicadas em biorreator de imersão temporária. Plant Cell Culture Micropropagation 2(2):80-87.
- Frizzone JA, Cardoso SS, Rezende R (2005). Produtividade e qualidade de frutos de meloeiro cultivado em ambiente protegido com aplicação de dióxido de carbono e de potássio via água de irrigação. Acta Scientiarum Agronomy 27(3):707-717.
- Frota HM, Carneiro MSS, Llamoca-Zárate RM, Campos FAP, Peixoto MJA (2004). Proliferação e enraizamento *in vitro* de brotos de palma forrageira – *Opuntia ficus-indica* (L.) MILL. Acta Scientiarum. Biological Sciences 26(2):235-238.
- Frota MNL, Carneiro MSS, Carvalho GMC, Neto RBA (2015). Palma forrageira na alimentação animal. Teresina: Embrapa Meio-Norte. 47 p. (Documentos/Embrapa Meio-Norte, 233).
- Lemos EEP, Ferreira MS, De Alencar LMC, Oliveira JGL, Magalhães VS (2001). Micropropagação de clones de banana cv. Terra em biorreator de imersão temporária. Revista Brasileira de Fruticultura 23(3):482-487.
- Lorenzo JC, González BL, Escalona M, Teisson C, Espinosa P, Bottoto C (1998). Sugarcane shoot formation in an improved temporary immersion system. Plant Cell Tissue and Organ Culture 54(23):197-200.
- Matoso ES, Domini L, Mascarenhas LS, Fehrenbach GW, Varnes LS, Silva SDA (2017). Multiplicação de plântulas de cana-de-açúcar e efeito da inoculação de bactérias diazotróficas durante a fase de enraizamento *in vitro*. Revista da Jornada da Pós-Graduação e Pesquisa – CONGREGA 1(1):2096-2109.
- Medeiros EC (2011). Aspectos bioquímicos e fisiológicos da palma forrageira *Opuntia stricta* Haw sob distintos sistemas de cultivo *in vitro*. Dissertação (Mestrado em Química) – Departamento de Química, Universidade Federal Rural de Pernambuco, Recife.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15(2):473-497.
- Nobel OS (2001). Biologia ambiental. In: Barbera, G., Inglese, P.,

- Pimenta-Barrios, E. P. (Ed.). Agroecologia, cultivo e uso da palma forrageira. [João Pessoa]: SEBRAE-PB: FAO. pp. 36-48.
- Paek KY, Hahan EJ, Son SH (2001). Application of bioreactors for large-scale micropropagation systems of plants. *In vitro Cellular Developmental Biology Plant* 37(2):149-157.
- Pimenta-Barrios E, Zañudo-Hernandez J, Rosas-Espinoza VC, Valenzuela-Tapia A, Nobel PS (2005). Young daughter cladodes affect CO₂ uptake by mother cladodes of *Opuntia ficus-indica*. *Annals of Botany* 95(2):363.
- Ribeiro AS, Brondani GE, Tormen GCR, Figueiredo AJR (2016). Cultivo *in vitro* de bambu em diferentes sistemas de propagação. *Nativa* 4(1):15-18.
- Santos DC, Farias I, Lira MA, Santos MVF, Arruda GP, Coelho RSB, Dias FM, Melo JN (2006). Manejo e utilização da palma forrageira (*Opuntia* e *Nopalea*) em Pernambuco. 48p. (IPA. Documentos, 30).
- Silva AB, Pasqual M, Teixeira JB, Araújo AG (2007). Métodos de micropropagação do abacaxizeiro. *Pesquisa Agropecuária Brasileira* 42(9):1257-1260.
- Silva JA da, Donato SLR, Donato PER, Rodrigues MG (2017). Cultivo e utilização da palma forrageira. *Informe Agropecuário* 38(296):34-45.
- Taiz L, Zeiger E (2004). *Fisiologia Vegetal*. 3.ed. Porto Alegre: Editora Artmed 719 p.
- Teixeira JB (2002). Biorreatores. *Biotecnologia Ciência & Desenvolvimento* 4(3):36-41.
- Vasconcelos AG, Donato VM, Brito JZ, Lira MA (2010). Cultura de tecidos vegetais: técnicas utilizadas para multiplicação acelerada da palma forrageira. Figueiredo MVB, Burity HA, Oliveira JP, Santos CERS, Stamford NP. *Biotecnologia aplicada à agricultura: textos de apoio e protocolos experimentais*. Brasília: Embrapa Informações Tecnológicas 4(3):653-677.

Full Length Research Paper

Wheat response to water stress condition at different growth stages in Amibara, Ethiopia

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To evaluate the effect of water stress at different crop growth stages on yield, yield components and water use efficiency, a field experiment was carried out in 2015/2016, 2016/2017 and 2017/2018 for bread wheat (Gambo variety) at Werer Agricultural Research Center. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications having 15 experimental treatments. Combination of water stress with crop growth stages was applied in the major investigation of the experiment. The highest grain yield was obtained from the control treatment irrigation at all stages (no stress). Stressing irrigation water at initial stages and maturity stages gave second highest yield (29.23 and 28.57 qt/ha) and lower water use efficiency (0.43 and 0.35 kg/m³) respectively. While irrigating only at the initial stage gave highest water use efficiency (0.79 kg/m³) and lowest grain yield. Application of irrigation water at all stages except initial and maturity gives optimum grain yield (26.73 qt/ha) and water use efficiency (0.62 kg/m³) for the study area. Therefore, wheat should not be stressed at development and mid stage to obtain optimum water use efficiency (WUE) without a significant grain yield reduction.

Key words: Grain yield, growth stages, water use efficiency, irrigation.

INTRODUCTION

Agricultural sector plays a major role in poverty reduction for sub-Saharan African countries; almost half of its population currently remains under poverty line (World Bank, 2016). The agriculture sector is not only the determinant of economic growth but also an activity of essential importance in social development, being the largest sector that contributes to almost two third of employment and gross national income of these nation.

Among scarce natural resource water is mainly used by irrigated agriculture. Of the total water withdrawals 70% and more than 60 to 80% of total water consumptive use

is utilized by irrigation (Huffaker and Hamilton, 2007). By 2025, the irrigated land should have to increase by more than 20% and the irrigated crop yield should be increased by 40% to secure food for about 8 billion people (Lascano and Sojka, 2007). Considering this fact, the productivity of agricultural water is highly demanding investigation to gain experience in improving performance, efficiency and profitability of utilized water for irrigation (Sleper et al., 2007).

The target crop wheat (*Triticum aestivum* L.) is one of the vital food crops in the world with an average yield of

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3.00 ton/ha (FAO, 2013). The production of wheat in Africa lays under 10-20% of its potential. Wheat is widely produced in Ethiopian highlands and mid-altitudes. Out of 18 major Agroecological zones in the country, it is grown in more than eight of the Ethiopian zones (ATA, 2014). The production trend of wheat in Ethiopia is increased by 45% from 2.92 million tons in 2012 to 4.23 million tons in 2016 and area of cultivation increased by 16% from 1.43 million ha in 2012 to 1.66 million ha in 2016, and the national average wheat productivity increased by 21% from 2.1 t/ha in 2012 to 2.54 t/ha in 2016. The country also has the potential of 300,000 ha of land for irrigated wheat production in the lowlands and 1.66 million hectares which are currently developed under rain fed. Recent research for development initiatives across Africa indicates that physically possible and economically profitable to grow more wheat and attain greater wheat self-sufficiency through effective application of proven, scalable and transformative wheat technologies (Wheat for Africa Updates, 2017).

Among the agricultural operations, irrigation is determinant of yield level; but it is a very decisive limiting factor whenever water is applied insufficiently. Under any case, application of irrigation water should be managed with intelligence to make the best use of it. Poor management of irrigation water has serious adverse effects, such as crop water stress due to waterlogging and hypoxia in root zone, nutrient loss with drainage water, pollution, water loss, soil salinity and increased susceptibility of crops to root diseases. Therefore, given the fact that proper utilization of irrigation water is a challenge, and of importance for irrigated wheat crop production to ensure food self-sufficiency for the country, it is paramount to generate technologies, knowledge and information suitable for sustainable use of soil and water resources. As a result, there is a promise of improving irrigated crop productivity under arid and semi-arid regions, thus further increasing the effectiveness and efficiency of the national endeavor. The objective of this experiment is to identify the stages of crop growth that are sensitive to water stress; to determine critical times for application of irrigation water in similar areas where there is limited water resources, and to determine the water productivity of wheat crop under stressed conditions.

MATERIALS AND METHODS

Description of study area

The study was conducted at Werer Agricultural Research Center Ethiopia, located at 9°16'N latitude and 40°9'E longitude, with a mean altitude of 750m m.a.s.l. The soil at the experimental site was Vertisol with bulk density of 1.17 g/cm³. The field capacity and permanent wilting point on a mass basis were 46 and 30.4%, respectively. The climate of the area is characterized as semi-arid with bi-modal low and erratic rainfall pattern, with annual average of 590 mm. The mean temperature varies from 26.7 to 40.8°C. The total monthly rainfalls of the study area during the growing season

are described in Table 1.

Experimental design

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications having 15 experimental treatments using bread wheat (Gambo variety). A Combination of water stress with the crop growth stages was applied in the major investigation of the experiment.

Soil-water measurement

Irrigation water was applied as per the treatment to refill the crop root zone depth close to field capacity. The amount of irrigation water applied at each irrigation was measured using Parshall flume. Soil moisture content before irrigation was monitored gravimetrically at different depths intervals up to maximum root depth to determine optimal irrigation scheduling. Each of the treatments received an irrigation depth of 54 mm for establishment. The appropriate growing stages date and establishment date were obtained from FAO 56. The treatment description, combinations, irrigation depth and number of irrigations for each treatment are described in Table 2.

Crops and management

The 'Gambo=Quaiu # 2' wheat variety, which is recommended for lowlands and irrigated farming system was used for the trial. Sowing generally starts at mid of October and harvesting in early January. The experimental plot size was 5x10m sown in eight ridges with two side plants. The samples were taken manually from the inside of six ridges from each experimental plot.

Assessment of water productivity

Water productivity has been estimated as a ratio of grain yield to the total crop evapotranspiration (ET_c) through the growing season and it has been calculated using the following equation (Zwart and Bastiaanssen, 2004).

$$CWP = (Y/ET)$$

Where, CWP is crop water productivity (kg/m³), Y is crop yield (kg/ha) and ET is the seasonal crop water consumption by evapotranspiration (m³/ha).

Statistical analysis

Yield and yield components data, also water productivity data were analyzed using statistical analysis system (SAS package) version 9. The Generalized Linear Model (GLM) procedure was applied for the analysis of variance. Mean comparisons were carried out to estimate the differences between treatments. Least significance difference (LSD) at 5% probability level was used to compare treatments.

RESULTS AND DISCUSSION

Plant height

Water stress at different growth stages showed a very high significant difference on height of wheat ($p < 0.000$, Table 3). The highest plant height (57.72 cm) was determined at a treatment of irrigating all stages except

Table 1. Total monthly rainfall of the study area during cropping season.

Month	Rainfall (mm) during cropping season		
	2015/2016	2016/2017	2017/2018
October	0	31.2	0
November	6.5	21.2	0
December	0	0	0
January	0.5	0	0
Total	7	52.4	0
Effective rainfall	0.3	26.3	0

Source: Werer Agricultural Research Center Agro-Meteorological Observatory Station.

Table 2. Treatments combinations, irrigation depth and number of irrigations.

Treatment	Growth stage				Irrigation depth (mm)	Number of irrigations	Total irrigation amount (mm)
	Initial	Development	Mid-season	Maturity			
Irrigated at all growth stages	1	1	1	1	54.76	15	821.4
Irrigated at all stages, except the initial stage	0	1	1	1	54.76	13	711.88
Irrigated at all stages, except the development stage	1	0	1	1	54.76	11	602.36
Irrigated at all stages, except the mid- stage	1	1	0	1	54.76	11	602.36
Irrigated at all stages, except the maturity stage	1	1	1	0	54.76	13	711.88
Irrigated at all stages, except initial and development	0	0	1	1	54.76	8	438.08
Irrigated at all stages, except initial and mid	0	1	0	1	54.76	8	438.08
Irrigated at all stages, except initial and maturity	0	1	1	0	54.76	8	438.08
Irrigated at all stages, except development and mid	1	0	0	1	54.76	6	328.56
Irrigated at all stages, except development and maturity	1	0	1	0	54.76	8	438.08
Irrigated at all stages, except mid and maturity	1	1	0	0	54.76	8	438.08
Irrigated only at maturity stage	0	0	0	1	54.76	3	164.28
Irrigated only at mid stage	0	0	1	0	54.76	5	273.8
Irrigated only at development stage	0	1	0	0	54.76	5	273.8
Irrigated only at initial stage	1	0	0	0	54.76	3	164.28

1 means irrigated and 0 means not irrigated during the crop growth stages.

maturity (stressing at maturity) and has no significant differences with a treatment of irrigating all growth stages (no stressing). The minimum plant height (31.42 cm) was determined at a treatment of irrigating only at maturity stage and this is significantly lower than all other treatments.

Effective tiller

Water stress at different growth stages of wheat has shown a significant difference in effective tiller. The highest effective tiller (8.25) was determined at a treatment of irrigating all stages except initial stage

(stressing at initial stage) and the minimum (4.72) was determined at a treatment of irrigating only the maturity stage. Both have a significant difference with the other treatments.

Spike length

Spike length was highly affected by water stress at different growth stages of wheat ($p < .0001$; Table 3). The maximum spike length (8.90 cm) was determined when all stages were irrigated except the mid-season stage; and it is significantly different from all the other treatments. The minimum spike length (6.08 cm) was

Table 3. The 15 irrigation treatments on wheat yield and its components.

Treatment	PH (cm)	ET	SL (cm)	NS/S	NK/S	GY (qt/ha)	WUE (kg/m ³)
Irrigated at all growth stage	56.92 ^a	6.61 ^{abc}	8.75 ^{ab}	14.64^a	32.53 ^{ab}	29.93^a	0.36 ^{ef}
Irrigated at all stages, except the initial stage	56.69 ^{ab}	8.25^a	8.42 ^{ab}	13.44 ^{abc}	31.97 ^{abc}	29.23 ^a	0.43 ^{bcdef}
Irrigated at all stages, except the development stage	50.00 ^{abcde}	6.64 ^{abc}	8.71 ^{ab}	13.5 ^{abc}	28.68 ^{abcd}	25.46 ^{ab}	0.43 ^{bcdef}
Irrigated at all stages, except the mid- stage	52.50 ^{abcde}	7.56 ^{ab}	8.90^a	14.33 ^{ab}	31.37 ^{abc}	19.43 ^{bcd}	0.33 ^f
Irrigated at all stages, except the maturity stage	57.72^a	6.03 ^{bc}	8.69 ^{ab}	14.83 ^a	35.23^a	28.24 ^a	0.40 ^{def}
Irrigated at all stages, except initial and development	44.17 ^{de}	7.53 ^{ab}	7.64 ^b	12.19 ^{bc}	25.51 ^{cde}	15.07 ^{cde}	0.33 ^f
Irrigated at all stages, except initial and mid	49.61 ^{abcde}	6.08 ^{bc}	8.71 ^{ab}	12.94 ^{abc}	28.57 ^{abcd}	15.07 ^{cde}	0.35 ^f
Irrigated at all stages, except initial and maturity	53.81 ^{abcd}	7.11 ^{ab}	8.50 ^{ab}	13.17 ^{abc}	27.03 ^{bcde}	26.73^{ab}	0.62^{ab}
Irrigated at all stages, except development and mid	45.64 ^{cde}	6.42 ^{abc}	8.57 ^{ab}	13.83 ^{abc}	23.61 ^{de}	13.41 ^{de}	0.41 ^{cdef}
Irrigated at all stages, except development and maturity	50.61 ^{abcde}	6.56 ^{abc}	8.71 ^{ab}	14.28 ^{ab}	29.22 ^{abcd}	24.18 ^{ab}	0.56 ^{bcd}
Irrigated at all stages, except mid and maturity	54.83 ^{abc}	6.89 ^{ab}	8.44 ^{ab}	14.17 ^{abc}	27.98 ^{bcd}	22.66 ^{abc}	0.50 ^{bcd}
Irrigated only at maturity stage	31.42 ^f	4.72 ^c	6.08^c	9.69 ^d	20.17 ^e	9.72 ^e	0.60 ^{abc}
Irrigated only at mid stage	43.39 ^e	6.69 ^{ab}	7.78 ^{ab}	12.53 ^{abc}	23.07 ^{de}	12.89 ^{de}	0.46 ^{bcdef}
Irrigated only at development stage	47.19 ^{bcde}	6.89 ^{ab}	7.93 ^{ab}	11.81 ^{cd}	23.91 ^{de}	15.54 ^{cde}	0.56 ^{bcd}
Irrigated only at initial stage	46.69 ^{cde}	6.69 ^{ab}	8.75 ^{ab}	13.94 ^{abc}	26.23 ^{bcde}	12.90 ^{de}	0.79^a
CV (%)	21.02	31.07	15.76	19.23	26.89	41.09	45.19
LSD (0.05)	9.698	1.947	1.222	2.386	6.948	7.685	0.201
p	<.0001	.0596	<.0001	.0003	<.0001	<.0001	<.0001

PH: Plant height, ET: Effective tiller, SL: Spike length, NS/S: Number of spikelets per spike, NK/S: Number of kernels per spike, GY: Grain yield, and WUE: Water use efficiency. Means followed by different letters in a column differ significantly and those followed by same letter are not significantly different at $p < 0.05$ level of significance. Bold font entries highlight the most significant results of interest.

determined when the irrigation treatment was applied only at a maturity stage.

Number of spikelet per spike

The number of spikelets per spike of wheat was significantly influenced by water stress at different growth stages (Table 3). The highest number of spikelets per spike (14.83) was determined at irrigation of all stages except maturity, and this has no significant difference with treatment of irrigation at all stages. The minimum number of spikelets per spike (6.08) was determined when irrigation was applied only at the maturity stage.

Number of kernel per spike

The number of kernels per spike of wheat was highly significantly influenced when water stress was applied at different growth stages ($p < 0.0001$, Table 3). The maximum number of kernel per spike (29.22) was determined from treatment of irrigation of all stages except maturity (stressing at maturity) and has shown significance difference from the other fourteen treatments (Table 3). The minimum number of kernels per spike (20.17) was determined at irrigation only at the maturity stage (stressing all stages except maturity).

Grain yield

Water stress at different growth stages showed a very highly significant influence on grain yield of wheat ($p < 0.0001$, Table 3). The highest grain yield (29.93 qt/ha) was obtained at a treatment of irrigation of all stages (no stress) and this has no significance differences with the treatment of irrigation of all stages except initial stage (stressing at initial stage) and irrigation of all stages except maturity (stressing at maturity) treatment (Table 3). The minimum grain yield (9.72 qt/ha) was obtained at a treatment of irrigation only at maturity stage. Three treatments, irrigation all stages except development, irrigation all stages except initial and maturity, and irrigation all stages except development and maturity showed statistically no significance difference on grain yield of wheat. The grain yield of wheat is reduced with increased stress, whereas the water use efficiency increased with stress level increased (Meskelu et al., 2017). The relationship between grain yield, water use efficiency and seasonal irrigation depth are illustrated in Figure 1.

Water use efficiency (WUE)

The water use efficiency of wheat was significantly influenced due to water stress at different growth stages

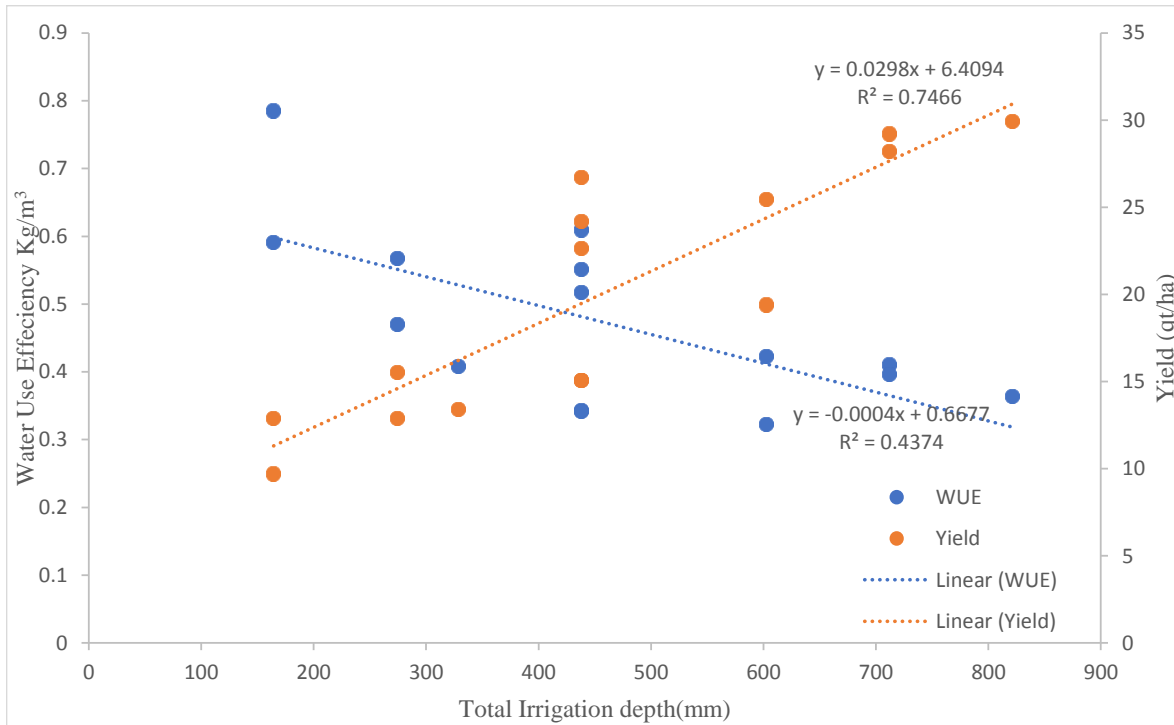


Figure 1. Relationship between grain yield, water use efficiency and seasonal irrigation depth. The r-square value for the regression estimate for yield ($r^2 \sim 0.75$) is substantially higher than that for the regression estimate of WUE ($r^2 \sim 0.44$) and reflects the greater scatter in the plotted points for WUE compared to yield.

($p < 0.001$). The WUE level depends on the controlled range of water stress applied at different crop growth stages. The results showed that the highest water use efficiency (0.79 kg/m^3) was obtained using a treatment of irrigation only at the initial stage. Treatments such as, stressing at mid, initial and development, initial and mid stages show statistically no significance differences and gave the lowest water use efficiencies ($0.33 - 0.35 \text{ kg/m}^3$). Irrigation at all stages (no stress) treatments showed the second lowest water use efficiency (0.36 kg/m^3). This result also is consistency with previous experiments conducted at different countries on wheat crops (Galavi and Moghaddam, 2012; Pradhan et al., 2013).

Conclusion

Poor irrigation water management has adverse effects on water productivity, crop yield, yield components and environment. Irrigation water application at different crop growth stages determines the grain yield and water use efficiency. Irrigating wheat only at the initial stage gives a high-water use efficiency, but lower yield; and irrigation at all stages gave one of the lower water use efficiency values. In general, treatments applying irrigation at all stages, except initial and maturity, give optimal yield and better water use efficiency than the remainder of

treatments in the experiment. So, stressing irrigation water at initial and maturity growth stages of wheat (Gambo variety) cultivated under irrigation shows better performance of the study area, in both yield and water use efficiency.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

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REFERENCES

- ATA (2014). Agricultural transformation agency. Wheat Technology Implementation Manual, Addis Ababa, Ethiopia.
- FAO (2013). Food and Agricultural Organization. World Food and Agriculture Statistical Yearbook, Rome, Italy.
- Galavi M, Moghaddam HA (2012). Influence of deficit irrigation during

- growth stages on water use efficiency (WUE) and production of wheat cultivars under field conditions. *International Research Journal of Applied and Basic Sciences* 3(10):2071-2078.
- Huffaker R, Hamilton J (2007). Conflict. In: *Irrigation of Agricultural Crops* (Lascano, R.J., and Sojka, R.E. eds.), 2nd edition, Agronomy Monograph no. 30. ASA-CSSA-SSSA publishing.
- Lascano RJ, Sojka RE (2007). Preface. In: *Irrigation of agricultural crops* (Lascano, R.J., and Sojka, R.E. eds.), 2nd edition, Agronomy Monograph no. 30. ASA-CSSA-SSSA publishing, *Agricultural Water Management*. p. 664.
- Meskelu E, Woldemichael A, Hordofa T (2017). Effect of Moisture Stress on Yield and Water Use Efficiency of Irrigated Wheat (*Triticum aestivum* L.) at Melkassa, Ethiopia. *Academic Research Journal of Agricultural Science and Research* 5(2):90-97.
- Pradhan S, Chopra UK, Bandyopadhyay KK, Singh R, Jain AK, Chand I (2013). Effect of water and nitrogen management on water productivity and nitrogen use efficiency of wheat in a semi-arid environment. *International Journal of Agriculture and Food Science Technology* 4(7):727-732.
- Sleper DA, Fales SL, Collins ME (2007). Foreword. In: *Irrigation of agricultural crops* (R.J. Lascano and R.E. Sojka, eds.), 2nd edition, Agronomy Monograph no. 30. ASA-CSSA-SSSA publishing, 664p.
- Wheat for Africa Updates (2017). Unlocking the potential of wheat in sub-Saharan Africa. A Newsletter of the SARD-SC Wheat program, an initiative funded by African Development Bank, AfDB, Implemented by ICARDA, Issue No.05.
- World Bank (2016). *Poverty and Shared Prosperity 2016: Taking on Inequality*. <http://www.worldbank.org/en/publication/poverty-and-shared-prosperity-2016>.
- Zwart SJ, Bastiaanssen WGM (2004). Review of measured crop water productivity values for irrigated wheat, rice, cotton and maize. *Agricultural Water Management* 69(2):115-133.

Full Length Research Paper

Soil chemical indicators and nutrient cycling variations across sequential years of rice cultivation: A case study of floodplain conditions of the Amazon, Brazil

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To meet the rising food demand for next generations, soils are needed to stand biological yield and promote animal, plant and human welfare. In this logic, the aim of the study was to determine the effects of different soil uses; (a) successive irrigated rice planting and (b) different soil management practices onto soil chemical properties and nutrient cycling. The study was carried out in areas with dissimilar years of irrigated rice farming, managed with conventional tillage (CT) and minimum cultivation (MC). Having as a reference a native vegetation plot, areas with 2, 4, 9, 14 and 26 years of different management such as conventional tillage or minimum cultivation were evaluated. Soil samplings were performed in the 0 - 0.10, 0.10 - 0.20 and 0.20 - 0.30 m layers. Three sub samples per layer were collected within each replicate and then condensed to the combined sample; straw sampling was also performed. Total nitrogen (TN) and total organic carbon (TOC) were determined by dry digestion in a Vario El III elemental. Highest TOC results were obtained in Ar9 and Ar14 areas in 0-0.10 and 0.10-0.20 m layers; straw production varied from 7.61 to 8.94 Mg ha⁻¹. Ar2 presented higher value in relation to others; nevertheless these variations were not significant. Still, it was observed that the Ar9 and Ar14 areas presented a higher total biomass production (grains and straw) than those found in Ar4, Ar2 and Ar26; so, these variations did not differ. Conversion of native ecosystems to irrigated rice production areas significantly affects soil fertility.

Key words: Total organic carbon, soil management, irrigated rice.

INTRODUCTION

In order to meet the growing demand for food, upsurge production by 40% without adversely affect non-

renewable natural resources available by 2030 its needed, thus requires soils that support biological

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productivity, maintain environmental values and provision animal, plant and human healthiness (Asmamaw, 2017). Nowadays soils are subjected to processes that guarantee or reduce crop yields and their sustainability. However, processes involved, such as fertilization and nutrient losses by crops, may induce since soil alterations, in time, to accumulation of nutrients or soil fatigue, with direct responses on crop productivity and soil biology (Moreira and Kasuya, 2016).

Consequently, depletion degree of soil organic matter content (SOM) fractions depends on the net balance between carbon inputs and outputs (Schadel and Luo, 2012). Studies have revealed that reduction of crop rotation and soil protection with harvest residues may boost carbon fractions interaction with mineral particle surfaces and develop stability and nutrient accumulation in the surface layer (Briedis et al., 2018).

In this framework, the conversion of native areas to intensive irrigated rice production fields may affect plant nutrients availability (Acton and Gregorich, 2012) due to conventional soil preparation, total or partial exclusion of crop residues and insufficient exchange of nutrients lost by erosion and gasiform emissions, leading to SOM degeneration (Dang et al., 2015), thus dropping, as the lone source of nitrogen reserve, much of the phosphorus, sulfur, cation exchange capacity (CEC), exchangeable bases, and accessibility of other nutrients, while increasing redox potential (Moreira and Kasuya, 2016; Du et al., 2019). Hence, the maintenance of rice straw at soil surface, besides supporting soil carbon increase, reduces fertilizer inputs by the use of nutrients freely available in the straw for the next harvest since the high C:N ratio and the presence of polymers such as cellulose and lignin in the straw can act as a natural barrier and retard its mineralization (de Figueiredo et al., 2015; Jeong et al., 2016). Irrigated rice crop systems with reduced cropping activities over time can increase soil fertility, increase organic matter content and total and organic phosphorus content. Based on the above, the aim of the study was to determine the effects sequential years of irrigated rice soil use and different soil management practices on chemical properties and nutrient cycling under the floodplain conditions of the Amazon.

MATERIALS AND METHODS

Area of study and experimental design

The study was carried out at the commercial plantation "O Paraiso Farm" located in Roraima state, Brazil, between 3° 19' 01.56" N, 60° 23' 43.65" W and 68 m altitude (Figure 1), Aw (tropical rainy) predominant climate according to Köppen's classification, an average annual air temperature of 27.2°C, a fluctuating precipitation of 1,500 to 2,000 mm and a soil type classified as Typic Fluvaquents (Batista et al., 2018). The study was conducted after the 2016 - 2017 harvest, in areas with different years of use with irrigated rice cultivation and soil management. Two-year (Ar2 - with two consecutive years of conventional tillage), 4 years (Ar4 - with

two consecutive years of conventional tillage, followed by one year with minimum cultivation following with one year conventional tillage), 9 years (Ar9 - succession of three consecutive years of conventional tillage, for three years with minimum cultivation), 14 years (Ar14 - succession of three consecutive years of conventional tillage for three years with minimum cultivation), 26 years (Ar26 - succession of conventional tillage for two consecutive years, with one year with minimal cultivation) areas were evaluated. As reference, an area of native vegetation (Nat.V), located near the cultivated areas was used. Soil conventional tillage consisted of disc plowing at a depth of 0.20 m, followed by two tills, leveling with a flatten and a roller. The establishment level curves and construction plots were later done. In minimum cultivation soils, preparation operations after harvesting were reduced in relation to the conventional tillage system, plowing and leveling in areas with micro-relief imperfections was performed. The experimental design was a randomized complete block with four replications. Blocks had an area of 10,000 m² and the replicates 2,500 m². Planted cultivars, sowing dates, sowing densities and fertilization rates varied for each of the soil management systems used in the study areas (Table 1).

Concurrently with the seeding, initial fertilization 5-25-25 formula was carried out. On the other hand, urea as cover fertilization was divided into three stages of rice development, beginning of tillering (V4), maximum tillering (V8) and at the beginning of panicle development (R0).

Plant materials and soil samplings

Straw sampling was performed one day after harvesting using a 1 x 1 m wood square (Neto et al., 2015), launched at sunset at three different points per replicate. The samples were then conditioned in properly identified fiber bags and oven dried at 55°C. Rice straw chemical analysis was carried out according to EMBRAPA (2009) standard procedures. Grain production data were considered to determine harvest index according to the equation proposed by Yoshida (1981). For soil sampling, mini-trenches were opened in early straw sampled areas. Samplings were performed in the 0 - 0.10, 0.10 - 0.20 and 0.20 - 0.30 m layers. Three sub samples per layer were collected within each replicate and then condensed to the combined sample. Total Nitrogen (TN) and Total Organic Carbon (TOC) were determined by dry digestion in a Vario El III elemental analyzer (Nelson and Sommers, 1996). Available Phosphorus (P), exchangeable Potassium (K⁺), Manganese (Mn²⁺), Iron (Fe³⁺) and Zinc (Zn²⁺) were extracted by double acid solution (HCl 0.05 M + H₂SO₄ 0.0125 M), P analyzed by UV-Visible spectrometry, Potassium (K⁺) by photometry of Flame and Manganese (Mg²⁺) and Zinc (Zn²⁺) by Atomic Absorption Spectrometry. The exchangeable Calcium (Ca²⁺) and Magnesium (Mg²⁺) were extracted by Complexometric with ethylenediaminetetraacetic acid (EDTA). The exchangeable Aluminum (Al³⁺) (KCl 1 mol L⁻¹) and Potential Acidity (H+Al) (Calcium Acetate) were determined according to methodologies proposed by Tedesco et al. (1995). Organic Phosphorus (OP), Inorganic Phosphorus (IP) and Total Phosphorus (TP) were determined according to the procedures proposed by Bowman (1989) modified by Guerra (1993).

Statistical analysis

Statistical analyzes and graphs were performed in R version 3.3.2 (R Development Core Team, 2017) (of parametric statistics) and SigmaPlot software version 11.0 (SigmaPlot, 2008). For each indicator the Shapiro-Wilk test was performed to evaluate the normality of the errors. To determine soil management effects on chemical indicators and nutrient cycling, the results were submitted

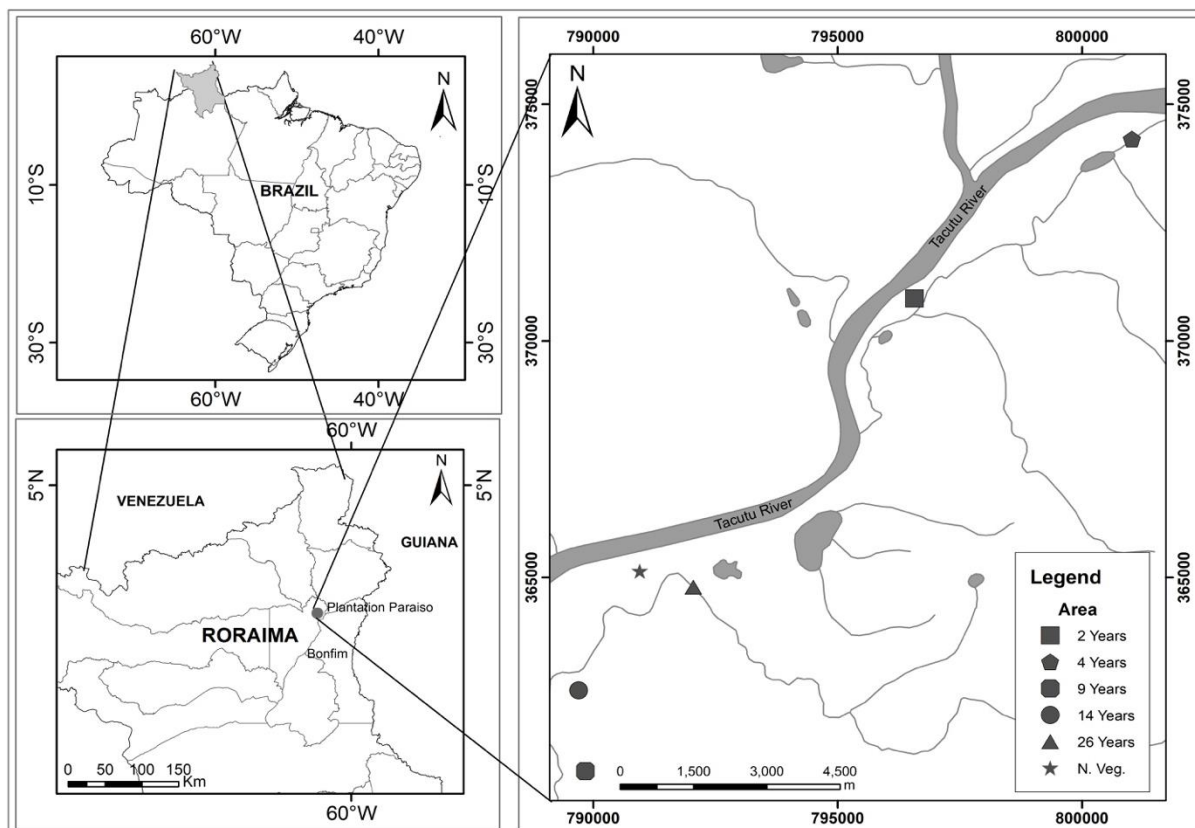


Figure 1. Location of the experimental area.

Table 1. Evaluated areas cultivars, sowing dates and densities and fertilization rates.

Area	Cultivar	Sowing date	Sowing density (kg ha ⁻¹)	Initial fertilization (5-25-25) (kg ha ⁻¹)	Cover fertilization (urea) (kg ha ⁻¹)
Ar2	IRGA 429	15/12/2016	80	450	330
Ar4	IRGA 429	29/11/2016	80	450	330
Ar9	IRGA 424	16/11/2016	135	450	330
Ar14	IRGA 424	17/10/2016	142	475	340
Ar26	IRGA 424	27/09/2016	132	468	340

to variance analysis using a 5% probability Tukey test. Pearson correlation and principal component analyzes were also performed.

RESULTS AND DISCUSSION

Organic carbon

The highest TOC results (Table 2) were obtained in Ar9 and Ar14 areas in 0-0.10 and 0.10-0.20 m layers, these values differed statistically from each other, yet, significant differences were observed when compared with the values found in other areas cultivated with irrigated rice and native vegetation. In the 0.20-0.30 m layer, the

highest TOC concentration was observed in Ar14 and deferred significantly from the concentrations found in other areas. TOC concentrations observed in the 9 and 14 year-old areas, alongside the evaluated profile, are due to reduced soil disturbance and superficial layer harvest residues maintenance, consequently favoring the interaction of TOC fractions with surface of colloidal particles of the soil, resulting in the formation of highly stable complexes between organic functional groups and functional groups present on the surface of minerals, which prevents or limits the access of microorganisms and enzymes to organic compounds located inside the aggregates (Yoo et al., 2011), and increases carbon

Table 2. Chemical indicators of six evaluated areas.

Area	pH (H ₂ O)	TOC (gkg ⁻¹)	TN (gkg ⁻¹)	K ⁺ (cmol _c kg ⁻¹)	Ca ²⁺ (cmol _c kg ⁻¹)	Mg ²⁺ (cmol _c kg ⁻¹)	Al ³⁺ (cmol _c kg ⁻¹)	H+Al (cmol _c kg ⁻¹)
0.0-0.10 m								
Ar2	4.30	7.35 ^d	0.60 ^b	0.08 ^d	0.66 ^b	0.10 ^b	1.36 ^{bc}	5.90 ^{bc}
Ar4	4.14	11.20 ^c	0.90 ^{ab}	0.10 ^{cd}	0.40 ^{bc}	0.15 ^b	1.83 ^b	9.60 ^a
Ar9	4.08	14.10 ^a	1.10 ^a	0.19 ^a	1.01 ^a	0.22 ^a	1.26 ^{bc}	9.93 ^a
Ar14	4.15	12.48 ^b	1.00 ^a	0.14 ^b	0.99 ^a	0.25 ^a	2.84 ^a	9.08 ^{ab}
Ar26	4.19	10.60 ^c	0.90 ^{ab}	0.11 ^{cd}	0.65 ^b	0.10 ^b	1.16 ^{bc}	5.82 ^c
N.Veg	4.32	10.28 ^c	0.78 ^{ab}	0.12 ^{bc}	0.43 ^c	0.17 ^b	0.69 ^c	3.58 ^c
CV(%)	ns	4,77	16.69	9.67	12.12	18.21	19.83	21.29
0.10-0.20 m								
Ar2	4.19	5.30 ^d	0.45 ^{bc}	0.07 ^c	0.55 ^{bc}	0.07 ^c	1.80 ^{bc}	5.67 ^b
Ar4	4.10	7.03 ^c	0.58 ^{bc}	0.10 ^b	0.40 ^{bc}	0.14 ^b	1.96 ^b	9.32 ^a
Ar9	4.15	12.30 ^a	0.95 ^a	0.16 ^a	0.94 ^a	0.20 ^a	1.76 ^{bc}	9.94 ^a
Ar14	4.00	9.30 ^b	0.70 ^{ab}	0.17 ^a	0.91 ^a	0.17 ^{ab}	3.94 ^a	9.14 ^a
Ar26	4.29	6.50 ^c	0.55 ^{bc}	0.09 ^{bc}	0.63 ^b	0.15 ^{ab}	1.25 ^c	4.07 ^{bc}
N.Veg	4.35	5.18 ^d	0.35 ^c	0.11 ^{ab}	0.40 ^c	0.16 ^b	0.34 ^d	2.15 ^c
CV(%)	ns	5.70	22.64	8.73	16.37	17.04	16.92	22.71
0.20-0.30 m								
Ar2	4.17	4.68 ^c	0.40 ^{bc}	0.07 ^c	0.46 ^b	0.06 ^d	2.16 ^b	6.83 ^b
Ar4	4.00	5.38 ^{bc}	0.43 ^{bc}	0.09 ^{bc}	0.30 ^c	0.11 ^{cd}	2.36 ^b	9.35 ^{ab}
Ar9	4.00	5.63 ^b	0.48 ^{ab}	0.12 ^a	0.67 ^a	0.18 ^{ab}	2.63 ^b	10.50 ^a
Ar14	4.00	8.58 ^a	0.70 ^a	0.11 ^{ab}	0.72 ^a	0.13 ^{bc}	3.93 ^a	9.12 ^{ab}
Ar26	4.15	2.88 ^d	0.18 ^c	0.08 ^c	0.43 ^{bc}	0.21 ^a	0.90 ^c	2.09 ^c
N.Veg	4.19	3.05 ^d	0.23 ^{bc}	0.11 ^{ab}	0.39 ^{bc}	0.13 ^{bc}	0.30 ^c	1.28 ^c
CV(%)	ns	8.22	28,26	12,21	12.04	19.89	19.51	21.43

Means followed by the same lowercase letter in the column did not differ significantly by the Tukey's test ($p < 0.05$). Where: TOC = Total Organic Carbon, TN = Total Nitrogen, K⁺ = exchangeable Potassium, Ca²⁺ = exchangeable Calcium, Mg²⁺ = exchangeable Magnesium, Al³⁺ = exchangeable Aluminum, H+Al = potential acidity, ns= Not significant.

fractions that are available and stabilized over time (Wang et al., 2014).

Adverse situation was observed in recent rice cultivated areas, as a result of the greater release of C to the atmosphere, through vegetation burning and less organic matter accumulation due to the continuous soil turning (Briedis et al., 2018). It was substantiated that the TOC values decreased in all the areas in relation to the depth, so the areas with conventional soil preparation presented a more uniform distribution in the studied profile, that is, in response to crop residues incorporation (Machado et al., 2011), aiding the reduction of more labile carbon fractions in the lowest layers (0.05 - 0.10 m), determining a potential condition of higher methane (CH₄) emissions onto the atmosphere in areas with conventional planting in relation to less disturbed soils (Nascimento et al., 2009; Qiu et al., 2018). This fact is explained by the incorporation of a soil corrector (limestone) previous to the implementation of a minimum crop system, in this process crop residues are incorporated in deeper layers.

Native vegetation did not present higher TOC contents in the superficial layer in relation to the older cultivated

rice areas, though, TOC stratification was verified in the layer 0-0.10 m, as a result to surface organic matter accumulation (Leal et al., 2015). In this context, soil surface crop residues maintenance over time has benefits in fertility in terms of storage of TOC and TN (Mazzoncini et al., 2016), since these elements are the main soil organic matter components (Jia et al., 2017), so it was observed that Ar9 and Ar14 presented the highest levels of TN. Consequently, the release of mineral N depends on the recalcitrance and resistance of soil organic matter (SOM) to microbial attack, being this process dependent on soil type and management, microbial activity and environmental conditions (Mazzoncini et al., 2016).

Potassium (K⁺), Calcium (Ca²⁺), Magnesium (Mg²⁺) and Aluminum (Al³⁺)

K⁺, Ca²⁺ and Mg²⁺ values found were considered low (CFSEMG, 1999), as a result of the high degree of withstanding of these soils. Therefore, Al³⁺ and H⁺

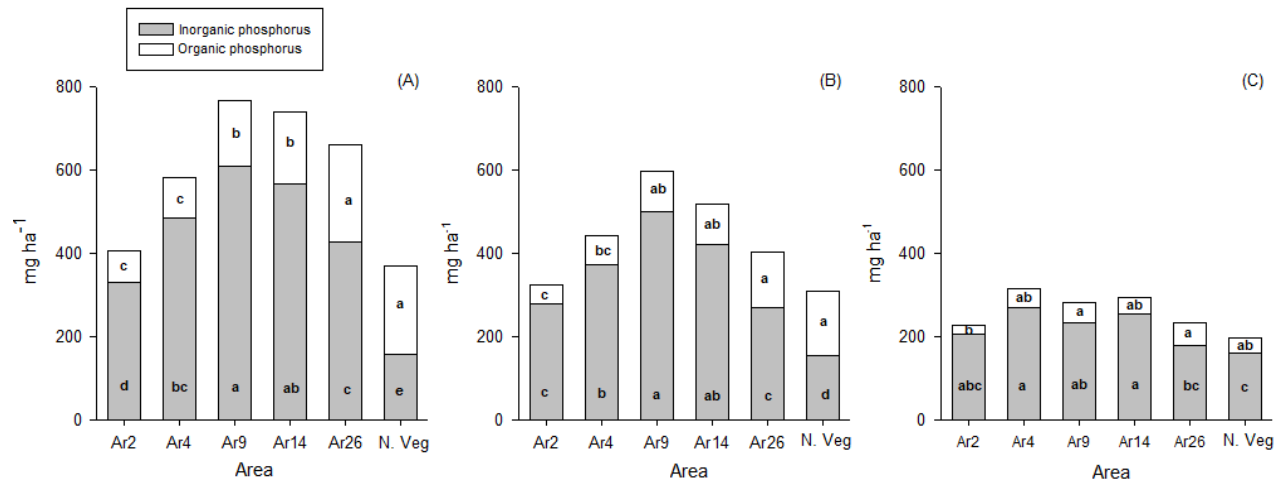


Figure 2. Phosphorus fractionation in the 0-0.10 m (A); 0.10-0.20 m (B) and 0.20-0.30 m (C) layers, in areas with different years of cultivated rice and handling. Bars followed by the same lowercase letter did not differ significantly by the Tukey's test ($p < 0.05$).

become dominant in the exchange complex. Yet, this situation is upturned hours after the soil is flooded and changes occur on the oxy-reducing system, related to these changes, there is a pH increase in values close to neutrality in acid soils due to their reduction. As a result, nutrients such as K^+ , Ca^{2+} and Mg^{2+} increase their availability (Kögel-Knabner et al., 2010; Lee et al., 2011).

Organic and inorganic phosphorus

In relation to inorganic phosphorus (IP) (Figure 2), the highest levels were found in Ar9 and Ar14, being statistically equal, consequently Ar9 value differed significantly from the results observed in Ar4, Ar26, Ar2 and native vegetation. Observed amplitude in IP contents may be due to the clay content and the mineralogical constitution of the soil, since they are relevant in the adsorption of P in Fe, Al, Ca, silica clays, oxides (Chen et al., 2013; Gonzalez-Rodriguez and Fernandez-Marcos, 2018), and even with SOM, through cation bridges, among others. Contrariwise, Ar26 presented lower values of IP in the 0-0.20 m layer, in relation to those found in Ar9 and Ar14 areas. This fact was probably due to mineral predominance of components with lower specific adsorption surface, therefore favoring the increase of IP lability as phosphate fertilizer was added due to saturation of adsorption sites (Tokura et al., 2011).

As for organic phosphorus (OP), contents increased in relation to the years of use with irrigated rice. Accordingly, the availability of P to plants depends on the transformation of OP into IP, this process involves a group of enzymes (phosphatases) produced by plants and microorganisms. Thus, this OP to IP transformation process to the plant has a reduced importance in

cultivated soils, since in these soils large doses of soluble P are added. In this context, Evald (2016) found higher activity of acid phosphatase enzymes in older areas cultivated with rice, in relation to areas of up to 8 years of use, possibly being influenced by the maintenance of crop residues on the soil surface and ongoing fertilization with phosphate sources. Ar2 and Ar4 presented lower levels of OP, this fact can be related to conventional management of soil and SOM content, since in revolved soils, OP mineralization increases, and as a result, the transformation of IP is mineralized in non-labile forms, resulting in exhaustion of P in a short period of time, if phosphate fertilizers are not incorporated (Audette et al., 2016). TP contents showed significant differences between evaluated areas, probably induced by physical and chemical characteristics of soils. Therefore, rice cultivated areas presented the highest P percentages in the inorganic fraction, this may be due to the continuous phosphate fertilization, and in relation to the native vegetation, a higher value of OP was observed in relation to IP, as a result of plant residue decomposition and microbial tissue (McLaren et al., 2015).

Still, the continuous addition of phosphate fertilizers in sufficient quantities over time, increase the availability of P in the soil due to organic and inorganic fractions of P have the capacity to supply this element to the plants. Hence, the results obtained in the evaluated areas showed a linearity relation between the phosphorus availability and the years of soil use with irrigated rice (Figure 3).

Micronutrients

In relation to Zinc (Zn^{2+}) (Figure 4A), in the 0-0.10 and

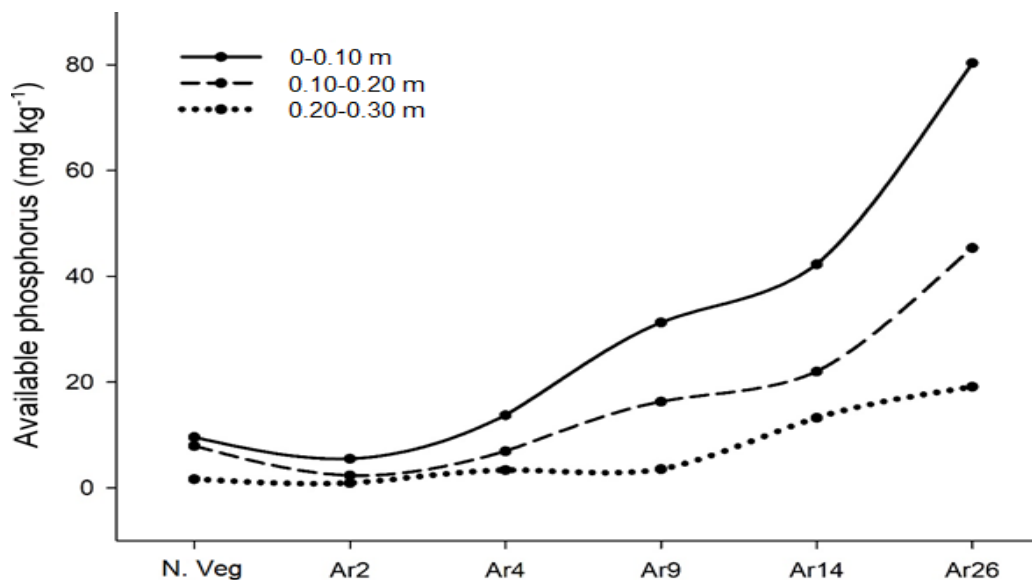


Figure 3. Phosphorus availability in 0-0.10, 0.10-0.20 and 0.20-0.30 m layers, in areas with different years with cultivated rice and soil management.

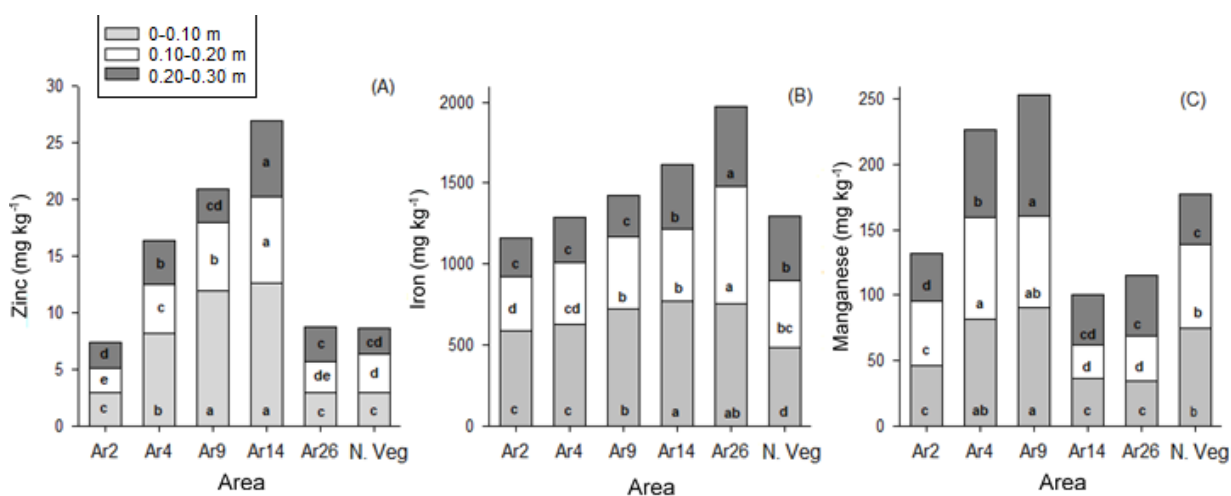


Figure 4. Zinc (A), Iron (B) and Manganese (C) values in 0-0.10 m, 0.10-0.20 m and 0.20-0.30 m layers. Bars followed by the same lowercase letter in the layer do not differ significantly by the Tukey's test ($p < 0.05$).

0.10-0.20 m layers, the highest values were observed in the Ar14 and Ar9 areas, being different from the others. The 0.20-0.30 m Ar14 layer presented the highest value along the profile studied. However, the high values of Zn^{2+} found are due to $pH < 5.0$ (Havlin et al., 2005). Inversely, flooded soils periodically exhibit changes in the redox potential (Eh) and consequently changes in soil pH may decrease the labile Zn fractions at less labile fractions (Kashem and Singh, 2001) and affect normal plant development (Dobermann and Fairhurst, 2000; Impa et al., 2012). In this scenario, the amplitude of the Zn^{2+} contents, in addition to the changes in Eh, may be related

to SOM content, microbial activity in the rhizosphere, concentrations of macro nutrients (especially P), soil moisture, and other factors that control Zn^{2+} availability for plants (Alloway, 2009).

For iron (Fe^{3+}) (Figure 4B), highest contents were observed in Ar14 and Ar26 areas in 0-0.10 m layer, though, in 0.10-0.20 and 0.20-0.30 m layers, the oldest cultivated area had the highest value with 724.66 and 492.08 $mg\ kg^{-1}$ respectively and presented a significant difference when compared with others. In this sense, the values obtained in studied areas were higher than the rice critical limit ($300\ mg\ kg^{-1}\ Fe^{3+}$), and under flooded

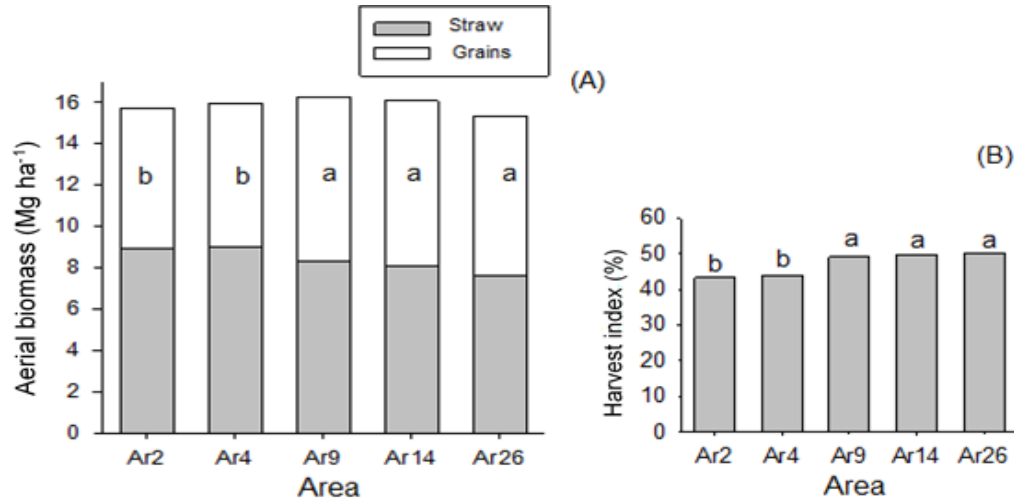


Figure 5. Aerial biomass production (A) and harvest index (B) in areas with different years of land use and management. Bars followed by the same lowercase letter did not differ significantly by the Tukey's test ($p < 0.05$).

conditions the availability of this element increases and subsequently affects the approachability of other nutrients (Mn^{2+} , Zn^{2+} and K^+) by antagonistic interactions or by Fe^{2+} accumulation in the root system, as well as by the excessive absorption by the plant, affecting normal development (Becker and Asch, 2005). Thus, rice plants Fe^{3+} toxicity expression severity has been related different factors such as: Material source, Fe^{3+} contents, soil pH, SOM and others. Amongst these factors, the material source plays a fundamental role in minerals surface Fe^{3+} retention, which explains why, in soils with predominantly kaolinite, toxicity symptoms occur more commonly than soils with a clay smectite predominance (Favre et al., 2002; Becker and Asch, 2005).

However, rice plants developed mechanisms (rhizosphere oxidation via aerenchyma, selectivity and root and stem membrane retention) to survive in soils with high Fe^{2+} concentrations (Nava and Bohnen, 2002; Becker and Asch, 2005). In relation to the Manganese (Mn^{2+}) (Figure 4C) Ar9 showed the highest value along the evaluated profile, then, Mn availability depends on OM contents, while sometimes soils with high OM content present less availability of this element, as observed in Ar14. However, soils under flooded conditions some Mn minerals dissolve and become available to plants (Jones and Jacobsen, 2009).

Aerial biomass production and nutrient cycling

Straw production varied from 7.61 to 8.94 $Mg\ ha^{-1}$ (Figure 5). Ar2 presented higher value in relation to others; formerly these variations were not significant. Though, it was observed that the Ar9 and Ar14 areas presented a higher total biomass production (grains and straw) than those found in Ar4, Ar2 and Ar26, so, these variations did

not differ ($p > 0.05$).

The grain-straw ratio varied from 43.20 to 50.29%, with Ar26 being the one with the highest harvest index (HI), that is, 50% of the total aerial biomass corresponded to grain yield and 50% of straw (Khush, 2005). However, this value did not differ from the HI obtained in Ar14 and Ar9. New rice cultivated areas presented a lower HI, probably due to the different sowing dates, since rice productivity is influenced by factors such as meteorological variables and soil management (Ntanos and Koutroubas, 2002; Katsura et al., 2008; Huang et al., 2013). Therefore, to evaluate varieties responses to climatic factors can lead to an improvement of applied nutrients utilization by plants in early stages, thus improving the potential of high yield cultivars (Ohsumi et al., 2014; Rabaioli et al., 2008). In this context, Ar14 presented higher N translocation in the anthesis phase for grain formation in relation to Ar2 (Table 3). In this scenario, the upkeep of crop residues, in addition to soil quality improving, is a source of nutrients for the next harvest, since many of the minerals absorbed by plants remain in the straw after harvest, recovering around 35% of N, 30% of P, 85% of K and 45% of sulfur (S). Much of these elements can be recycled by plants after decomposition (Byous et al., 2004).

In relation to P, the highest levels of this element in rice straw were observed in Ar9 and Ar26 (Table 4). However, accumulations of P in crop residues depend on the opportune availability of P and the initial development of the plants (Dobermann and Fairhurst, 2000). For K^+ , was observed that Ar4 and Ar14 presented the highest accumulations in the straw, however, the highest return rate in relation to the amount of fertilizer applied varied from 76% found in Ar26 to 137% presented in Ar4. This fact may be related to K increased availability or increased solution diffusivity (mobility) under flooded

Table 3. Rice straw nutrient content in areas with different years of use and management.

Area	N	P	K	Ca	Mg	Fe	Mn	Zn	C/N
Ar2	7.82 ^a	0.72 ^d	11.60 ^b	0.15 ^b	0.03	110.00	52.50	20.93	60.78 ^c
Ar4	5.85 ^{bc}	0.97 ^b	17.18 ^a	0.09 ^c	0.03	97.50	47.50	20.49	81.77 ^b
Ar9	6.57 ^b	1.19 ^a	11.58 ^b	0.15 ^b	0.03	120.00	60.00	20.97	72.41 ^b
Ar14	5.25 ^c	0.85 ^c	15.78 ^a	0.13 ^b	0.04	137.50	55.00	20.78	96.02 ^a
Ar26	6.18 ^b	1.14 ^a	11.83 ^b	0.18 ^a	0.03	102.50	55.00	21.11	81.77 ^b
CV (%)	5.22	5.47	8.81	6.70	Ns	ns	ns	ns	5.80

Means followed by the same lowercase letter in the column did not differ significantly by the Tukey's test ($p < 0.05$). ns= Not significant

Table 4. Rice straw soil incorporated fertilizer dose and nutrient return in different years of use and management areas.

Area	Mineral fertilization (kg ha ⁻¹)			Return of nutrients to straw (kg ha ⁻¹)							
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	CaO	MgO	Fe ₂ O ₃	MnO	ZnO
Ar2	174.60	112.50	112.50	69.91	6.44	103.70	1.34	0.27	0.98	0.45	0.18
Ar4	174.60	112.50	112.50	52.53	8.71	154.28	0.81	0.27	0.81	0.45	0.18
Ar9	174.60	112.50	112.50	54.40	9.85	95.88	1.24	0.25	0.99	0.50	0.17
Ar14	180.15	118.75	118.75	42.42	6.87	127.50	1.05	0.32	1.13	0.48	0.16
Ar26	179.80	117.00	117.00	47.03	8.68	90.03	1.37	0.23	0.76	0.46	0.15

conditions (Kögel-Knabner et al., 2010). Although K trade for grain formation is small, the demand for the plant is great (Yamada and Roberts, 2005), and around 85% of the absorbed K remains in the vegetative parts (Byous et al., 2004). Thus, maintenance of crop residues results in a long-term source of nutrients (Behera and Shukla, 2015), and can substitute significant amounts of nitrogen and potassium fertilizers without negative effects on productivity (Huang et al., 2013).

A negative effect of residue retention on crop yield is the microbial immobilization of N during the establishment of the crop (before tillering), which can reduce N uptake and affect crop growth. However, the immobilization of N can be avoided when nitrogen fertilizers are applied in sufficient doses (Huang et al., 2013), or in some cases, the early application of nitrogen fertilizers may result in a more efficient increase in rice crop productivity. In general terms, efficient soil management, water regime, crop residues and fertilizers, contribute to the production of dry mass and support soil microbial activity, resulting in increased carbon stocks (Briedis et al., 2018; Wang et al., 2018) and soil nutrients (Mazzoncini et al., 2016). Positive correlations ($p \leq 0.01$) were observed between SOC with TN, TP and IP, for the 0-0.10, 0.10-0.20 and 0.20-0.30 m layers, however, the SOC and OP were correlated ($p \leq 0.05$) only in the 0-0.10 m layer. Positive significant relationships ($p \leq 0.01$) between SOC with Potassium (K⁺), Calcium (Ca²⁺), Magnesium (Mg²⁺) and Zn (Zn²⁺) were also presented in the 0-0.10 and 0.10-0.20 m layers. The 0.20-0.30 m layer was only correlated with Ca²⁺ and Zn²⁺. This is due to SOC interaction with the soil mineral particles, allowing it to function as nutrient reservoir and energy for the plants

(Bandyopadhyay et al., 2010, Yu et al., 2017). Principal component analyzes (PCA) showed that new cultivated areas had a decline in soil fertility, due to the constant turning of the arable layer and the rapid mineralization of SOM. However, in Ar9 and Ar14, that were managed with minimal cultivation, a close relation with the evaluated indicators was shown, thus demonstrating that the reduction of the disturbance of the arable layer and the conservation of the harvest residues at the soil surface contribute to the fertility of the soil (Yang et al., 2005, Wang et al., 2014, Qiu et al., 2018).

Conclusion

Conversion of native ecosystems to irrigated rice production areas significantly affects soil fertility. Nevertheless, the less disturbed rice production and the maintenance of the straw in the superficial layers result in an effective strategy to promote the increase SOC stocks, therefore improving the soil quality over time.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Acton DF, Gregorich LJ (2012). The health of our soils: toward sustainable agriculture in Canada. The health of our soils: toward sustainable agriculture in Canada. http://sis.agr.gc.ca/cansis/publications/manuals/1995-health/The_Health_of_Our_Soils.pdf

- Alloway B (2009). Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health* 5:537-548.
- Asmamaw DK (2017). A Critical Review of the Water Balance and Agronomic Effects of Conservation Tillage under Rain-fed Agriculture in Ethiopia. *Land Degradation and Development*. <https://doi.org/10.1002/ldr.2587>
- Audette Y, O'Halloran IP, Paul Voroney R (2016). Kinetics of phosphorus forms applied as inorganic and organic amendments to a calcareous soil. *Geoderma* 262:119-124.
- Bandyopadhyay P, Saha S, Mani P, Mandal B (2010). Effect of organic inputs on aggregate-associated organic carbon concentration under long-term rice-wheat cropping system. *Geoderma* 154:379-386.
- Batista K, Lumbreiras J, Coelho M, Oliveira V, Vale Junior J (2018). Guia de campo da XI Reunião Brasileira de Classificação e Correlação de Solos: RCC de Roraima. 15 jan.
- Becker M, Asch F (2005). Iron toxicity in rice - conditions and management concepts. *Journal of Plant Nutrition and Soil Science* 168:558-573.
- Behera S, Shukla A (2015). Spatial distribution of surface soil acidity, electrical conductivity, soil organic carbon content and exchangeable potassium, calcium and magnesium in some cropped acid soils of India. *Land Degradation and Development* 26:71-79.
- Bowman R (1989). Sequential extraction procedure with concentrated sulfuric acid and diluted base for soil organic phosphorus. *Soil Science Society of America Journal* 53:262-366.
- Briedis C, Sá J, Lal R, Tivet F, Franchini J (2018). How does no-till deliver carbon stabilization and saturation in highly weathered soils? *Catena* 163:13-23.
- Byous E, Williams J, Jones G, Horwath W (2004). Nutrient requirements of rice with alternative straw management. *Better Crops* 36:6-11.
- Comissão de Fertilidade do Solo do Estado de Minas Gerais (CFSEMG) (1999). Recomendações para o uso de corretivos e fertilizantes em Minas Gerais. In: Apresentação dos resultados das análises de solos. 5a Aproximação. Viçosa, MG. P. 310.
- Chen K, Zhao K, Zhang H, Sun Q, Wu Z, Zhou Y, Zhong Y, Ke F (2013). Phosphorus removal from aqueous solutions using a synthesized adsorbent prepared from mineralized refuse and sewage sludge. *Environmental Technology* 34(9-12):1489-1496.
- Dang YP, Moody PW, Bell MJ, Seymour NP, Dalal RC, Freebairn DM, Walker SR (2015). Strategic tillage in no-till farming systems in Australia's northern grains-growing regions: II. Implications for agronomy, soil and environment. *Soil and Tillage Research* 152:115-123.
- de Figueiredo EB, Panosso AR, Reicosky DC, La Scala N (2015). Short-term CO₂-C emissions from soil prior to sugarcane (*Saccharum* spp.) replanting in southern Brazil. *Global Change Biology Bioenergy* 7(2):316-327.
- Dobermann A, Fairhurst T (2000). Rice: Nutrient Disorders and Nutrient Management. Handbook Series, Potash and Phosphate Institute (PPI), Potash and Phosphate Institute of Canada (PPIC) and International Rice Research Institute, Philippines, P 191. http://books.irri.org/9810427425_content.pdf
- Du H, Wang T, Xue X, Li S (2019). Estimation of soil organic carbon, nitrogen, and phosphorus losses induced by wind erosion in Northern China. *Land Degradation and Development*. <https://doi.org/10.1002/ldr.3288>
- Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) (2009). Manual de análises químicas de solos, plantas e fertilizantes. 2. ed. Brasília P 627.
- Evald A (2016). Qualidade do solo na cultura do arroz cultivado em várzea roraimense. 2016. Dissertação (Mestrado). Faculdade de Agronomia, Universidade Federal de Roraima. Boa Vista. 84f.
- Favre F, Tessier D, Abdelmoula M, Génin J, Gates W, Boivin P (2002). Iron reduction and changes in cation exchange capacity in intermittently waterlogged soil. *European Journal of Soil Science* 53:175-183.
- Gonzalez-Rodriguez S, Fernandez-Marcos ML (2018). Phosphate sorption and desorption by two contrasting volcanic soils of equatorial Africa. *PeerJ*. 6:e5820.
- Guerra J (1993). Produção sazonal de *Brachiaria decumbens* Stapf. Conteúdo de fósforo orgânico e microbiano em solos tropicais de baixa fertilidade natural. 1993. Tese (Doutorado). Universidade Federal Rural do Rio de Janeiro – UFRRJ. 234f.
- Havlin J, Beaton J, Tisdale S, Nelson W (2005). Soil Fertility and Fertilizers. 7 ed. NJ. Pearson Prentice Hall P 515.
- Huang S, Zeng Y, Wu J, Shi Q, Pan X (2013). Effect of crop residue retention on rice yield in China: A meta-analysis. *Field Crops Research* 154:188-194.
- Impa S, Sarah E, Beebout J (2012). Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. *Plant Soil* 361:3-41.
- Jeong CY, Dodla SK, Wang JJ (2016). Fundamental and molecular composition characteristics of biochars produced from sugarcane and rice crop residues and by-products. *Chemosphere* 142:4-13
- Jia Z, Kuzyakov Y, Myrold D, Tiedje J (2017). Soil Organic Carbon in a Changing World. *Pedosphere* 27(5):789-791
- Jones C, Jacobsen J (2009). Micronutrients: cycling, testing and fertilizer recommendations. *Nutrient Management Module* 7:2-4.
- Kashem M, Singh B (2001). Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. *Nutrient Cycling in Agroecosystems* 61:247-255.
- Katsura K, Maeda S, Lubis I, Horie T, Cao W, Shiraiwa T (2008). The high yield of irrigated rice in Yunnan, China. 'a cross-location analysis'. *Field Crops Research* 107:1-11.
- Khush G (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology* 59:1-6.
- Kögel-Knabner I, Amelung W, Cao Z, Fiedler S, Frenzel P (2010). Biogeochemistry of paddy soils. *Geoderma* 157:1-14.
- Leal OA, Castilhos RMV, Pauletto EA, Pinto LFS, Pillon CN, Penning LH, Santos DC (2015). Organic Matter Fractions and Quality of the Surface Layer of a Constructed and Vegetated Soil After Coal Mining. II - Physical Compartments and Carbon Management Index. *Revista Brasileira de Ciência do Solo* 39:3.
- Lee CH, Hong CO, Kim SY, Schumacher T, Kim PJ (2011). Reduction of phosphorus release by liming from temporary flooded rice rotational system in greenhouse upland soil. *Ecological Engineering* 37(8):1239-1243
- Machado R, Vargas C, Pauletto E, Pillon C, Dos Anjos L (2011). Conteúdo de carbono orgânico em Planossolo Háptico sob sistemas de manejo do arroz irrigado. *Revista Brasileira de Ciência do Solo* 35:1769-1775.
- Mazzoncini M, Antichi D, Di Bene C, Risaliti R, Petri M, Bonari E (2016). Soil carbon and nitrogen changes after 28 years of no-tillage management under Mediterranean conditions. *European Journal of Agronomy*. 77, 156–165.
- McLaren TI, Smernik RJ, McLaughlin MJ, McBeath TM, Kirby JK, Simpson RJ, Guppy CN, Doolette AL, Richardson AE (2015). Complex Forms of Soil Organic Phosphorus-A Major Component of Soil Phosphorus. *Environmental Science and Technology* 49(22):13238-1345.
- Moreira F, Kasuya M (2016). Fertilidade e biologia do solo: Integração e tecnologia para todos. ed. Viçosa: Sociedade Brasileira de Ciência do Solo P 592.
- Nascimento P, Bayer C, Netto S, França L (2009). Sistemas de manejo e a matéria orgânica de solo de várzea com cultivo de arroz. *Revista Brasileira de Ciência do Solo* 33:1821-1827.
- Nava G, Bohnen H (2002). Oxidação de ferro em raízes de dois cultivares de arroz em solução de solo inundado. *Revista Brasileira de Ciência do Solo* 26(2):325-332.
- Neto T, Taketomi Y, Junior R, Oliveira D, Gallo J, Tanabe C, Martins I, Da-Silva A (2015). Quantificação de restos culturais e concentração de nutrientes na cultura do milho no Município de Belterra. *Espago Científico* 14:60-68.
- Ntanos D, Koutroubas S (2002). Dry matter and N accumulation and translocation for Indica and Japonica rice under Mediterranean conditions. *Field Crops Research* 74:93-101.
- Ohsumi A, Furuhashi M, Matsumura O (2014). Climatic responses of biomass production and grain yield in Japanese high-yielding rice cultivars under different transplanting times. *Field Crops Research* 168:38-47.
- Qiu H, Ge T, Liu J, Chen X, Hu Y, Wu J, Su Y, Kuzyakov Y (2018). Effects of biotic and abiotic factors on soil organic matter mineralization: Experiments and structural modeling analysis.

- European Journal of Soil Biology 84:27-34.
- R Development Core Team (2017). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project>.
- Rabaioli C, Merchesan E, Rossato T, Teló G, Arosemena D (2008). Influência da aplicação de nitrogênio e fungicida no estágio de emborrachamento sobre o desempenho agronômico do arroz irrigado. *Bragantia* 67:153-159.
- Schadel C, Luo Y (2012). Biofuels and Ecosystem Carbon Balance Under Global Change. In *Environmental Impact of Biofuels*. <https://www.intechopen.com/books/environmental-impact-of-biofuels/biofuels-and-ecosystem-carbon-balance-under-global-change>
- SigmaPlot (2008). *Statistics for user's guide*, Chicago, Systat Software Inc, P. 578.
- Tedesco M, Gianello C, Bissani C, Bohnen H, Volkweiss S (1995). *Análise de solo, plantas e outros materiais*. 2 ed. Porto Alegre: Universidade Federal do Rio Grande do Sul. [https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=295881&biblioteca=vazio&busca=autoria:"TEDESCO,%20M.%20J."&qFace ts=autoria:"TEDESCO,%20M.%20J."&sort=&paginacao=t&paginaAtual=1](https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=295881&biblioteca=vazio&busca=autoria:)
- Tokura M, Furtini N, Carneiro L, Curi N, Lopes S, Alovise A (2011). Dinâmica das formas de fósforo em solos de textura e mineralogia contrastantes cultivados com arroz. *Acta Scientiarum. Agronomy* 33(1):171-179.
- Wang H, Guan D, Zhang R, Chen Y, Hu Y, Xiao L (2014). Soil aggregates and organic carbon affected by the land use change from rice paddy to vegetable field. *Ecological Engineering* 70:206-211.
- Wang Y, Wang Z, Zhang Q, Hu, N, Li Z (2018). Long-term effects of nitrogen fertilization on aggregation and localization of carbon, nitrogen and microbial activities in soil. *Science of The Total Environment* 624:1131-1139.
- Yamada T, Roberts T (2005). *Anais do Simpósio sobre Potássio na Agricultura Brasileira*. Piracicaba P 841.
- Yang C, Yang L, Ouyang Z (2005). Organic carbon and its fractions in paddy soil as affected by different nutrient and water regimes. *Geoderma* 124(1):133-142.
- Yoo G, Yang X, Wander MM (2011). Influence of soil aggregation on SOC sequestration: A preliminary model of SOC protection by aggregate dynamics. *Ecological Engineering*. <https://doi.org/10.1016/j.ecoleng.2010.12.016>
- Yoshida S (1981). *Fundamentals of Rice Crop Science*. Los Baños: International Rice Research. Institute P 269.
- Yu Z, Zhang J, Zhang C, Xin X, Li H (2017). The coupling effects of soil organic matter and particle interaction forces on soil aggregate stability. *Soil and Tillage Research* 174:251-260.

Full Length Research Paper

Local knowledge of pumpkin production, performance and utilization systems for value addition avenues from selected agro-ecological zones of Uganda

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Pumpkin is one of the underutilized fruit vegetables in Uganda despite the fact that it has wide spectrum of both nutritional and medicinal values. A survey was carried out to document local knowledge of pumpkin production; performance and utilization systems to aid the selection of pumpkin varieties to be used for particular value addition avenues. It was observed that twelve varieties of pumpkin are commonly grown and they are mainly distinguished by their skin colour, texture of epicarp and shape. Pumpkin production levels on large scale, small scale and subsistence were at 27.8, 66.7 and 5.6% respectively. Farmers reported that 'pumpkin seeds are usually sown directly into the main garden;' thus, nursery beds are rarely used by the farmers. Farmers appreciate the crop's nutritional and medicinal values, in addition to being a source of livelihood. Pumpkin fruit vegetable is multi-purpose in that all the parts of the plant can be consumed thus minimizing food wastage and also the plant can stay long on the shelf thus has the potential to act as a food security crop. From the survey, it was observed that mostly elderly people above 46 years were involved in pumpkin cultivation; youths were less involved and pumpkin cultivation is mainly practiced on a small scale. The youths need to be educated and encouraged to get involved in pumpkin cultivation since it can reduce poverty levels, food insecurity and malnutrition.

Key words: Agro-ecological zone, local knowledge, medicinal value, nutritional value, pumpkin, variety.

INTRODUCTION

Cucurbita species are indigenous to America. The cultivated species are now widely disseminated throughout tropical, subtropical and temperate regions of the world (Whitaker and Bemis, 1975), including Africa where they have become naturalized and are categorized among indigenous vegetables (Abukutsa-Onyango, 2007). Pumpkins can grow well in almost all African countries and these include, Nigeria, Zimbabwe, Malawi,

Zambia, Uganda, South Africa, Kenya (Ngwerume and Grubben, 2004). For Uganda in particular, the districts that grow pumpkins include Kabale, Jinja, Mbale, Mityana, Mubende, Luwero, Nakaseke, Kabarole, Arua among others. There are quite many species of pumpkin in the world and these include *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita mixta* and *Cucurbita facifola* (Whitaker and Bemis, 1975). C.

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moschata, *C. pepo*, *C. maxima* are the mostly commonly domesticated species of pumpkin worldwide (Martins et al., 2015). Pumpkin has a position of high value among the Cucurbitaceous vegetables, due to its long shelf life, long period of availability, high nutritive estimates, medicinal properties, and better transport qualities. It is used at both mature and immature stages as a vegetable. It is also consumed after processing (Ravani and Joshi, 2014).

Conservation of plant genetic resources in East African countries has greatly given priority to cultivated mainstream food crops and less emphasis has been put on the underutilized crops like pumpkins (Hamisy et al., 2002). As a result, there has been over reliance on a few staple food crops like maize and cassava and to a lesser extent millet, sorghum, potatoes and bananas which lack most of the nutrients required for human health contributing highly to malnutrition. Consequently, traditional crops such as pumpkins, which are endowed with nutrients, are so far not highly considered by the smallholder farmers in the region (Ondigi et al., 2008). Furthermore, the pumpkin landraces in Uganda are known by vernacular names such as Nsujju (Luganda), Ebyozi and Ebihaza (Rukiga and Runyakole) which hinders easy the identification of pumpkin landraces with good characteristics due to language barrier since Uganda has a wide diversity of languages (UBOS, 2014).

With time, if no effort is made to document the knowledge on pumpkin production, utilization and conservation there would be knowledge erosion especially on the local pumpkin varieties grown in Uganda. In addition, farmers will not be able to identify the best varieties to grow for commercial purposes in order to consequently meet the quality standard for different value addition avenues and also to know the most yielding variety. Therefore, the main objective of this study is to evaluate and document local knowledge on pumpkin production systems, utilization and conservation status in four agro-ecological zones of Uganda.

METHODOLOGY

Study site

The study was conducted in six districts from five agro-ecological zones of Uganda. These were Southwestern highlands (Kabale and Kanungu districts), Western mid-altitude farmlands and Semiliki flats (Masindi and Mubende districts), Western medium-high farmlands (Kabarole district) and Lake Victoria and Mbale farmlands (Mityana district) as represented in Figure 1. Villages namely, Mwangyale and Nyakibande in Kabale, Rugyeyo in Kanungu, Kihubba and Bugyenje in Masindi, Butologo in Mubende, Nyamiseke in Kabarole, Nkokonjeru and Kalangalo in Mityana were visited.

Sampling and data collection

Non-probability methods that is to say, purposive sampling coupled with snowball were applied to recruit participants for the study

(Acharya et al., 2013). A total of 36 pumpkin farmers were involved. Cross sectional surveys, focus group discussions, rapid participatory observation appraisals, and face to face interviews were used to gather information on knowledge about pumpkin production, performance and utilization systems in four agro-ecological zones of Uganda. A questionnaire consisting of structured and semi-structured items in line with pumpkin growing, performance and utilization was designed to guide the interviews. Photographs of the pumpkin landraces grown were taken using a digital camera (Johnson and Turner, 2003).

Data analysis

The qualitative data obtained from the narrative interviews and participatory observation appraisals were coded and entered into IBM Statistical Package for Social Scientists (SPSS) software 20.0 (2011). The data were analyzed to generate numerical interpretation and percentages of the multiple responses obtained about local knowledge on production and utilization systems of pumpkin landraces in Uganda. Data were presented in form of tables and graphs using Microsoft excel professional plus 2016 version. General information that was common for almost all the farmers was not coded but rather qualitatively analyzed and presented as a narrative following the guidelines by Bogdan and Biklen (2007).

RESULTS

The information on local knowledge of pumpkin farmers on pumpkin production, performance and utilization systems is reported as follows:

Pumpkin production system in Uganda

Socio-economic information of pumpkin farmers

As indicated in Table 1, the male respondents (52.8%) were slightly more than the female respondents (47.2%). A considerable number of the farmers interviewed were educated at least up to primary level (61.1 %) and the majority of the farmers were above 46 years of age (50.0%) which shows that the youth are less involved in pumpkin cultivation. Additionally, most of the respondents practiced pumpkin growing on a small scale (66.7%) or intercropped (63.9%) with other crops such as sorghum, banana, beans and maize. Some of the farmers grew pumpkins alone on a large scale (27.8%).

Reasons for cultivating pumpkin crop

Farmers' responses indicated that pumpkins in the past were mainly grown for domestic consumption though of recent they are being grown as a source of income to earn a living. From the analysis, the major reason for cultivating pumpkin was food security (46.2%), the second reason for growing pumpkin was income generation (43.6%), then the fact that the crop is easy to cultivate (5.1%) and can be used as an animal feed

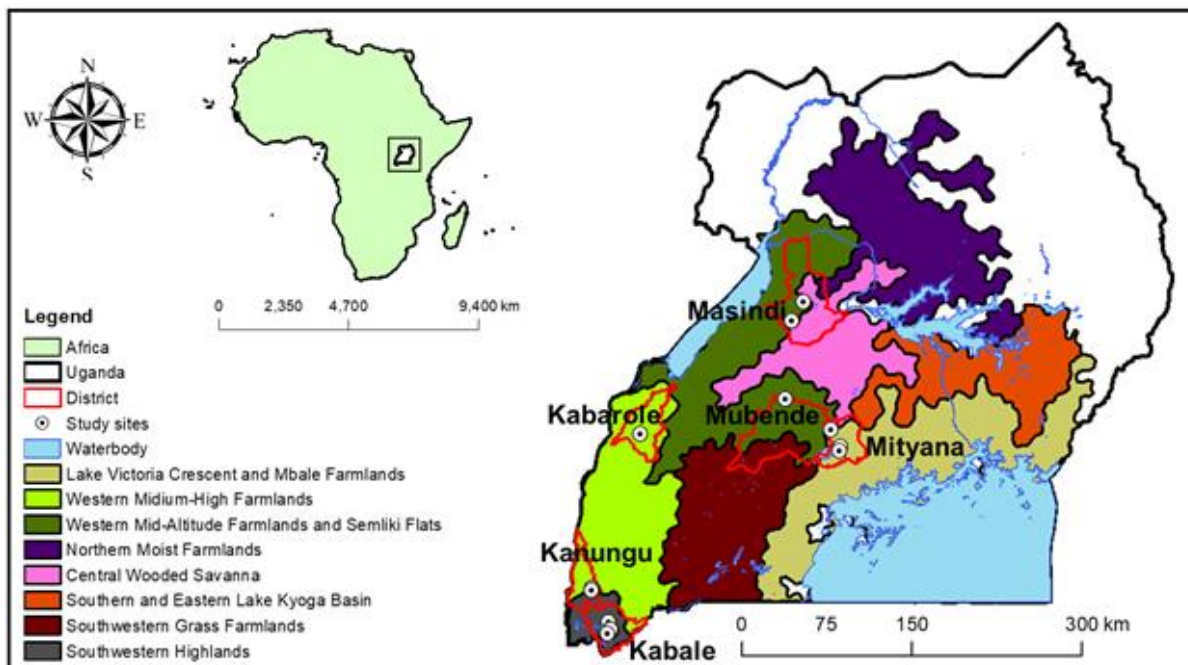


Figure 1. Map showing sites from which data was collected,
Source: Drawn using Quantum Geographical Information System software, version 2.18.1

Table 1. Socio-economic information of pumpkin farmers (n=36).

Demographic	Category	Percentage
Sex of farmer	Male	52.8
	Female	47.2
Education level	Primary	61.1
	Secondary	27.8
	Tertiary	11.1
Type of farming	Large scale farmer	27.8
	Small scale farmer	66.7
	Subsistence farmer	5.6
Farming practices	Monocropping	36.1
	Intercropping	63.9
Age of respondent	18-25	5.6
	26-35	22.2
	36-45	22.2
	46 and above	50.0

(5.1%) for example, for pigs.

Pumpkin cultivation practices

The pumpkin seeds were mainly obtained from some of

the mature harvested pumpkin fruits, some farmers reported that they buy the seeds from known pumpkin farmers who have knowledge about the most preferred pumpkin varieties. Some farmers obtained seeds from friends who had previously planted delicious pumpkin varieties. The farmers also reported that the pumpkin

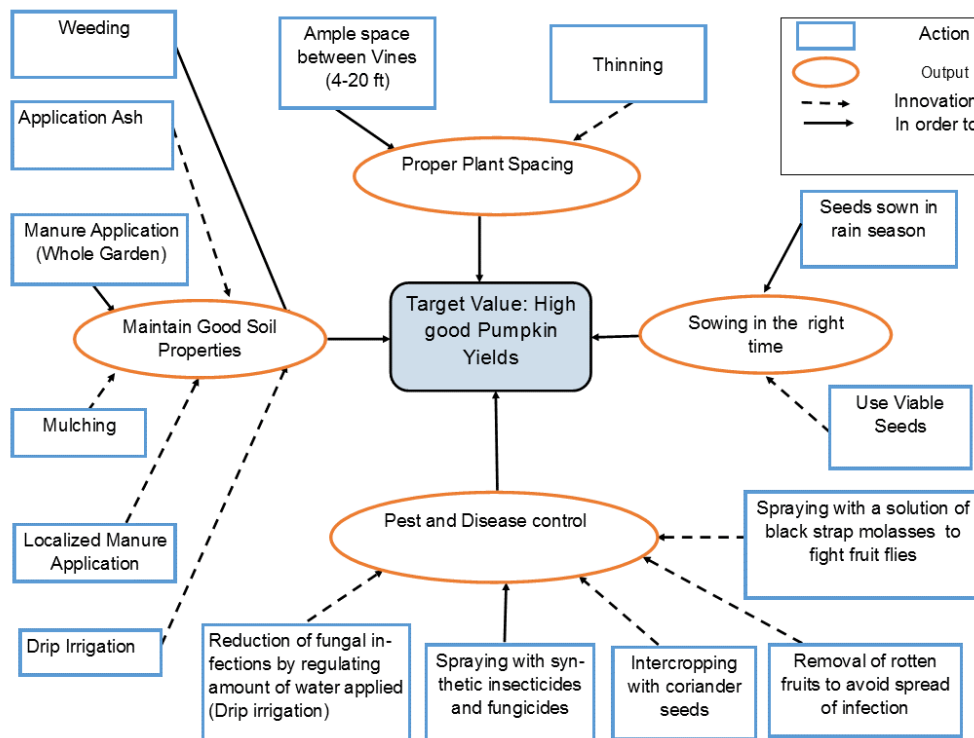


Figure 2. Simplified representation of routine control rules to i) ensure proper plant spacing, ii) sow seeds in the right time, iii) maintain of good soil properties and iv) control pest and disease, Source: Adapted and modified control loop model from Restrepo et al. (2016).

seeds are usually sown directly into the main garden, thus nursery beds are rarely used especially by small scale and subsistence farmers. Some pumpkin farmers who grow pumpkins on a large scale sometimes plant some pumpkin seeds in a nursery bed which are later transplanted to the main garden to fill up the gaps where seeds did not germinate after direct sowing. Usually decomposing animal wastes such as goat dung, cow dung, or chicken droppings are used as manure, which is first mixed with soil dug from a hole 30 cm deep and 30 cm wide. Thereafter, about 2-5 pumpkin seeds are planted in the hole where manure was applied at 3-5 cm depth. While digging the holes where seeds are to be planted, ample spacing should be emphasised since it contributes to good and high pumpkin yields. Spacing of about 4-20 feet is normally used depending on size of someone’s land. On average, one hole can be 10 feet away from the other to provide adequate space for the pumpkin vines to creep so as to produce a reasonable number of fruits. Sowing is mainly done during the rainy season, that is to say, from mid-February to early June for the first season, and from September to December for the second season. The first season usually has short rainfalls so the yields are not as high as those of the second wet season that normally has relatively prolonged rainfalls. Furthermore, some farmers irrigate their crops during the dry season to ensure continuous pumpkin

production and supply to markets. The pumpkin seeds take about 7-14 days to germinate depending on the variety sown and prevailing environmental conditions. Farmers also reported that the pumpkins take 3-12 months from the time of sowing to mature. The maturation time depends on the geographical location (environment) for instance, nature of soil, rainfall received, temperature and also the variety grown. Pumpkin farmers carry out some routine actions that they believe directly contribute to the final output of good high pumpkin yields. The routine activities carried out by pumpkin farmers were grouped as i) proper plant spacing, ii) sowing seeds in the right time, iii) maintenance of good soil properties and iv) pest and disease control. Farmers carry out particular actions in order to achieve the sub-goals which in turn enable them to achieve the target value as indicated in Figure 2.

Almost all the interviewed participants reported that they applied manure before sowing the pumpkin seeds (27.0%). Most of the farmers weeded their gardens to remove unwanted plants (21.3%). A reasonable number of farmers reported that they mulched their gardens (15.6%). Some farmers reported that they spray insecticides to kill the pests such as fruit flies that would lead to heavy economic losses (10.7%). The other practices carried out by farmers to ensure good yield are good spacing (2.5%), removing rotten fruits (9.0%),

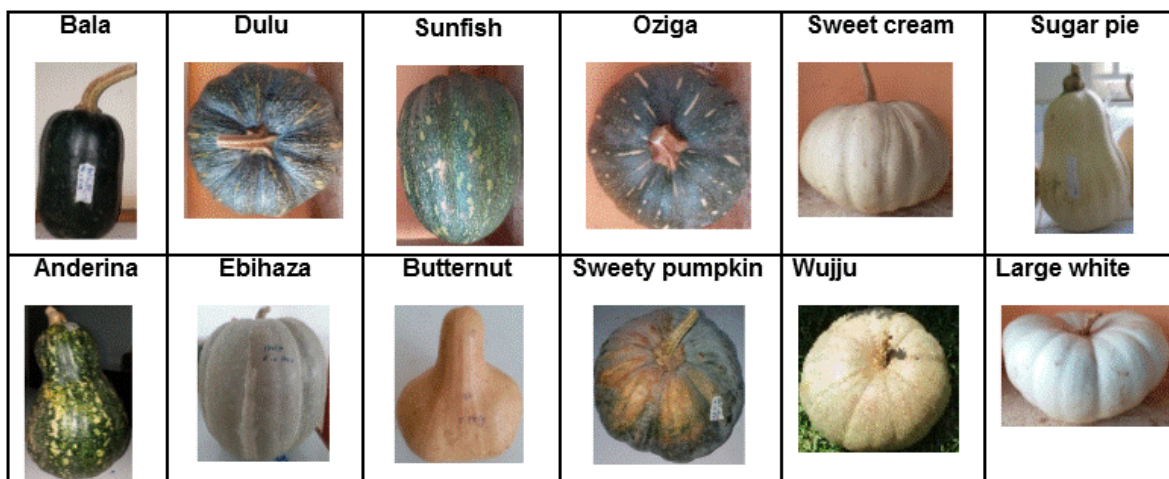


Figure 3. Pumpkin varieties commonly grown in Uganda.
Source: Nakazibwe (2017).

applying ash (0.8%), application of coriander seeds in the hole before sowing (1.6%), application of inorganic fertilizers such as leaf and flower boosters among others (4.9%), thinning (4.1%) and using good seed (2.5%) as shown in Figure 2.

Pumpkin varieties grown

Results from the interviews show that there is a wide range of pumpkin varieties grown in Uganda. These were summarized into twelve categories as revealed in Figure 3. The most cultivated pumpkin variety was Oziga (Luganda) (24.0%), followed by Dulu (Luganda) (22.6%), then Ebihaza (Rukiga/ Runyankole) (15.1%), Sweet cream (12.3%), Bala (Luganda) (11.0%), Butternut (4.1%), Wujju (Luganda) (2.7%), Anderina (Luganda) and Large white pumpkin (2.1%) respectively. The least cultivated variety was Sugar pie and Sweet pumpkin (0.7%) respectively.

Oziga was described as a medium sized pumpkin, with a globular shape; has secondary skin colour; is green with cream patches and soft fruit epicarp. Bala has a dark green skin colour without any patches. Its shape can vary from flattened to oblong and the fruit epicarp can be soft or hard. Dulu has a green primary skin colour with cream patches but with a hard fruit epicarp hence usually stays longer on shelf. Sunfish has a green primary skin colour with yellowish mottles, usually a soft fruit epicarp. The shape can be globular or oblong. Sweet cream has a cream fruit skin colour with no mottles and the shape is usually cylindrical or round. The fruit epicarp is frequently soft. Sugar pie has a cream fruit skin colour with no mottles, the shape is mainly pyriform and the fruit epicarp is frequently soft. Anderina has a green primary fruit skin colour with cream mottles, pyriform or dumbbell shape and the fruit epicarp is usually soft. Ebihaza has a gray

primary skin colour with stripes and no mottles; the shape can be oblong or globular and the fruit epicarp can be hard or soft. Butternut has a tan skin colour without mottles; the shape is pyriform and with frequently a soft fruit epicarp. Sweet pumpkin has a dark green skin colour without mottles while immature and tends to orange when mature. It has a flattened fruit shape and the fruit epicarp is often soft. Large white has a white skin colour, flattened fruit shape and a soft fruit epicarp. Wujju has a gray primary skin colour with stripes between the ridges, the shape is mainly globular and the fruit epicarp is often soft.

Socio-cultural values associated to pumpkin cultivation

Some farmers reported that there are some socio-cultural values associated with pumpkin farming specifically; i) liquid from the node on the vine can treat red eyes. ii) Women in their menstruation periods should not go to the pumpkin garden since all the fruits in the garden may rot. iii) It is not good to point at the young fruits because they may rot. (iv) If one touches the young fruit, it rots. v) If one adds grasshopper wings to their garden, the vine would produce more fruits. (vi) If one throws rat dung to the garden, more fruits would be obtained. vii) On the other hand, many farmers did not attach any socio-cultural values to pumpkin cultivation. viii) If one fell sick and yet they have pumpkin flowers in the garden, they would not die but rather recover from the sickness.

Pumpkin maturity indicators

Farmers reported that there are certain parameters that are commonly observed to determine maturity of the

pumpkins namely; (i) the fruit epicarp hardens and its colour becomes very pale. (ii) Fruit stalk changes from dark green to a lighter colour for example, yellowish in some varieties while light green in others. (iii) The remains of the flower completely fall off from the fruit. (iv) Leaves dry and fall off from the vine. (v) The stem colour changes from dark green to brownish, fruit stalk dries and hardens (vi) For 'Kihaza,' fruit stalk develops cracks, and when hit with one's fore finger, the fruit produces a high pitch sound.

Pumpkin post-harvest handling to prolong shelf life

During harvest, since the pumpkins on one vine usually do not mature at the same time, the person harvesting should put the following precautions into consideration to minimize damage to both the vine and the harvested fruit so as to prolong the shelf life of the fruit. This is to also ensure that the fruit retains its appealing characteristics to the buyer/consumer. The precautions include; (i) proper breakage of fruit from the vine without damaging the vine, for example, by using a knife. (ii) Avoid stepping on the vines while harvesting to ensure that the remaining fruits on the vine grow up to maturity. (iii) Keeping the stalk intact to avoid microbial attack through careful handling and good transportation. (iv) Do not expose fruits to sunshine which catalyzes fruit quality deterioration thus, store them in a cool place. v) Avoid harvesting pumpkins when they are too mature since this reduces the fruit's shelf life in storage. (vi) While in storage, it is not good to pile fruits on one another this may cause rotting of some fruits especially those with a soft epicarp due to the pressure exerted on the lower fruit. (vii) Providing good aeration in the store to prolong the shelf life of the fruits. (viii) Using wooden stalls overlaid with dry grass or spread dry banana for storage of pumpkins to regulate temperature thus, minimizing rapid fruit deterioration. (ix) Avoiding storage of pumpkins on bare ground in the store, this tends to compromise the shelf life of the pumpkins.

Pumpkin spoilage indicators

Farmers also reported that the pumpkin fruit can stay for a long period of time on the shelf (1-8 months) especially for the fruits with hard epicarps. In case the pumpkin loses its fruit stalk during carriage, it may not stay for long in storage, thus it easily deteriorates. While in storage, the following indicators can be used to tell if the pumpkin has gone bad: (i) loss of fruit stalk, the fruit stalk easily gets detached from the fruit, (ii) when the fruit is shaken, it sounds as though it has a liquid in it, (iii) the fruit skin texture becomes soft and appears as though it has got wrinkles, (iv) finger nail easily penetrates when pressed and the fruit has a bad smell, (v) the fruit becomes very pale, (vi) fluid oozes out of the fruit, (vii) a pumpkin with

deep scars is susceptible to microbial attack and (viii) the fruit slightly loses weight.

Performance and conservation status of pumpkin (Cucurbita spp)

Pumpkin commercial production in Uganda is still on a very small scale as specified in Table 1 above. Additionally, some farmers believe that pumpkin production is gradually increasing as awareness about the benefits of pumpkin consumption is being emphasized among different communities to deal with malnutrition, food insecurity and poverty. Although the pumpkin fruit vegetable has nutritional and medicinal values, from the survey, no National Agricultural Research Institute has supported the farmers to address the challenges faced by pumpkin farmers since pumpkin is not one of the main stream crops grown in Uganda. Farmers testified that about 3-50 fruits can be harvested from a pumpkin vine in a given season; nevertheless the harvest depends on variety cultivated and soil quality. Some farmers reported that it is not easy to keep track of the number of harvested fruits per vine since the vines intertwine in the garden. Fruits harvested also depend on the prevailing environmental conditions such as rainfall received. Some farmers observed that big sized fruits lead to production of fewer fruits per vine (about 8 fruits) while the small sized fruits lead to production of more fruits per vine (15-20 fruits).

Source of market and mode of transportation of pumpkin produce

From the interviews, farmers reported that the major source of market for the pumpkins grown were vendors (47.7%), who in turn sold the pumpkins to different markets both local and across borders such as Rwanda and Southern Sudan. The second source of market for pumpkins were the neighbours of the farmers (46.2%), who do not grow pumpkins but are interested in consuming pumpkin. The least source of market was the hotels and events such as parties (weddings) (6.2%). The mode of transportation of the mature pumpkins from the garden to the respective markets ranged from vehicles (59.1%), followed by motor cycle (34.1%), then motorcycle (4.5%) and very few farmers transported pumpkins to markets on foot (2.3%) especially for the farmers whose market sources were near where they cultivate from.

Challenges faced by farmers during pumpkin cultivation

The farmers reported that there are a number of challenges associated with pumpkin cultivation namely;

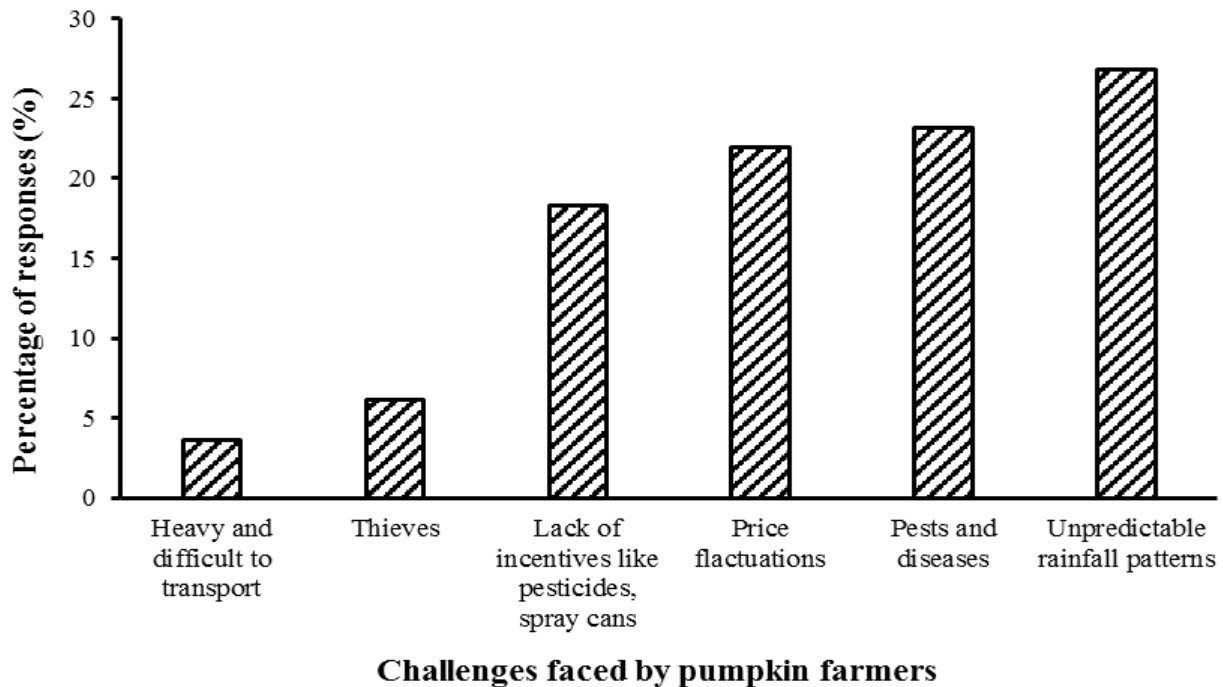


Figure 4. Major challenges faced by pumpkin farmers.

unpredictable rainfall patterns (26.8%), pests and diseases (23.2%), price fluctuations (22.0%), lack of incentives like pesticides (18.3%), thieves (6.1%), and the pumpkin heads are heavy and difficult to transport (3.7%) as shown in Figure 4.

Utilization of pumpkin in Uganda

Pumpkin parts consumed

The most consumed part of the pumpkin was the pulp (32.1%), followed by the seeds (31.3%), then the leaves (26.8%), and the least consumed part was the flowers (9.8%). Farmers reported that the most preferred pumpkin varieties were Oziga (Luganda), Sweet cream, Bala and Dulu. There are quite a number of value addition avenues associated to pumpkin consumption as a way of minimizing post-harvest losses especially during the periods of high production, and demand fluctuation. The pumpkin value avenues are discussed in Table 2.

Modes of preparation of pumpkin for consumption:

There are different modes of preparing the different pumpkin parts for consumption namely, boiling pulp alone (10.5%), steaming the pulp (16.0%), boiling pulp with already cooked beans (5.5%), roasting the pulp in a local oven (1.7%), steaming the leaves as vegetables (8.3%),

cooking the leaves with other sauce like beans (15.5%), roasting the seeds (19.3%), cooking seed flour as sauce (8.8%), cooking young fruits/flowers with leaves as vegetables (5.5%), cooking young fruits/flowers with other sauce such as ground nuts (8.8%). These are also highlighted in Table 2 with their respective value addition avenues.

Medicinal values associated to pumpkins

Farmers reported that there are quite number of medicinal values associated with pumpkin consumption, such as boosting immune system (40.0%), improving someone's eye sight (11.1%), improving erectile functionality in men (8.9%), unblocking sperm ducts in men (7.8%), treating abdominal disorders (6.7%), good memory (6.7%), relieving diabetes (6.7%), strengthening bones (4.4%), improving muscle activity (2.2%), treating epilepsy (2.2%), preventing some cancers (2.2%) and curing allergies (1.1%) as described in Table 2.

DISCUSSION

Socio-economic information of pumpkin farmers

The male respondents were more than women especially in commercial pumpkin production because usually, men are the heads of the family and this is in line with the

Table 2. Pumpkin modes of utilization and possible value addition avenue.

Mode of utilization	Pumpkin part consumed	Method of preparation for consumption	Pumpkin value addition avenue	Basis for value addition
Food	Pulp	(i) Boiling pulp alone (ii) Steaming the pulp	Pumpkin soup	Nutritious and delicious
		Boiling the pulp with the right amount of sugar	Pumpkin jam	Nutritious and delicious
		Roasting pulp in local oven	Confectionery like baking pumpkin cakes, bread among others.	The appealing pumpkin flour flavour gives fortified wheat a sweeter taste and makes it more nutritious.
		Boiling pulp with already cooked beans	Fortification of other stew like beans	Pumpkin contains micronutrients such as β -carotene that the beans do not have.
		Squeezing the pulp to obtain juice.	(i) Pumpkin juice (ii) Pumpkin wine	Sweet pumpkins have high sugar content thus good sources of pumpkin juice and wine
		Drying the sliced pumpkin pulp and then grinding them into powder	Pumpkin pulp flour	The appealing pumpkin flour flavour gives fortified wheat a sweeter taste and makes it more nutritious.
	Leaves	Slicing pumpkin pulp	Pumpkin salads	Pumpkins are nutritious and can be consumed in their raw form.
		Steaming the leaves as vegetables	Pumpkin leaf flour	The dry leaf flour has a long shelf life thus ensuring continuous supply any time, even in time of scarcity.
	Seeds	Cooking the leaves with other sauce like beans	(i) Fortification of other stew like beans	(i) Pumpkin leaves contain micronutrients such as folic acid that the beans may not have.
		Roasting the seeds	(ii) Packed ready to eat roasted seeds snacks. (iii) Pumpkin seed oil	(ii) To get higher profit returns other than selling a whole pumpkin with its seeds.
		Drying the seeds and grinding to make flour	Seed flour applied to tea as a spice.	(ii) High mineral content such as Zinc, Calcium and potassium.
	Flowers/ young pumpkins	Cooking seed flour as sauce	Fortification of other stew like ground nuts	
(i) Cooking young fruits/flowers with leaves as vegetables (i) Cooking young fruits/flowers with other sauce such as ground nuts		(i) Dried flower flour (ii) Dried slices of young pumpkins	To increase shelf life but also to ensure continuous supply even during off peak seasons.	
Medicinal values	Leaves	Crush and mix clean leaves with sieved ash and water	Pumpkin leaf syrup	(i) Presence of bio-active components in the leaves that can cure allergies. (ii) Presence of minerals such as iron, calcium, zinc, potassium. The leaves are also a good source of vitamins and folic acid.
	Pulp	Steaming the pulp	(i) Instant porridge. (ii) Weaning meal for babies.	Presence of vitamins, minerals and micronutrients such as β -carotene which is a strong anti-oxidant.
		Squeezing the pulp to obtain juice.	Pumpkin juice	Presence of micronutrients such as β -carotene which is a strong anti-oxidant.
	Seeds	Drying the seeds and grinding to make flour	Seed flour applied to tea as a spice.	(i) High Zinc content (ii) Presence of bio-active components

findings of Ondigi et al. (2008). The responsibility of family heads is to work hard and provide food and other basic needs for the survival of their family members (Furstenberg, 1998). On the other hand, some families are headed by women who have to supply the needs of their families. Most of the respondents at least had a minimal level of education of primary. Many of the respondents were in very remote (rural) areas where poverty levels are quite high; it is most likely that they dropped out of school due to lack of incentives to attain higher levels of education. Agriculture is practiced to obtain food for home consumption and the surplus is sold to get income to meet some of their crucial basic needs. This is in line with the observations made by Gollin and Rogerson (2010). The pumpkins are grown alongside other crops such as sorghum, millet, matooke, maize beans among others (Ngwerume and Grubben, 2004; MAAIF, 2003, UBOS, 2010), since pumpkin is considered as a supplementary food crop and food security crop consumed more often during periods of food scarcity by farmers (Ondigi et al., 2008). Thus there were more respondents who practiced intercropping than monocropping.

Pumpkin cultivation is mainly on small scale (less than half an acre of area cultivated) for one farmer, while some farmers practiced pumpkin production on a commercial large scale of at least a hectare of cultivated area. This trend is similar to cultivation of other food crops in Uganda (MAFAP, 2013). Pumpkin production in Uganda is still very low due to the fact that it is not one of the mainstream crops grown in the country hence less emphasis is put in place to encourage its cultivation. Furthermore, most of the respondents reported that they do not have plenty of land to carry out monocultural pumpkin production due to the increasing human population that competes for the limited land resource. Most of the respondents were above 46 years of age with very few participants in the age bracket of 18-25 years. This is perhaps because knowledge about the benefits of pumpkin consumption to the human body has not been well passed on from elders to the young generation thus the young people tend to neglect growing pumpkins. Needless to say, most of the young people tend to migrate from the rural areas to urban areas to look for other jobs to earn a living rather than depending on agriculture since it is generally perceived that the agriculture has low rewards as noted by Ahaibwe et al. (2013). Some youths move to urban areas for better social amenities such as education services and they eventually do not return to rural areas to practice agriculture.

Pumpkin production systems, performance and utilization in Uganda

Pumpkin is mainly cultivated as a food security crop due

its ability to stay on the shelf for quite a long period of time provided it is harvested when mature and good storage measures are put in place as reported by farmers. The pumpkins also act as food supplements for animals such as pigs, because they are nutritious as also noted by Prohens-Tomás and Nuez (2007). Farmers mainly use vehicles to transport their pumpkins to markets because the pumpkins are heavy, also to minimize post-harvest loss due to damage imposed on the fruit especially through loss of fruit stalk. A vehicle is a better means of transport than a bicycle or motor cycle. Some farmers who cannot afford to hire vehicles to transport their produce, especially subsistence farmers, use motorcycles, bicycles or even walk to the market if it is in the neighborhood.

Several practices are carried out by farmers to ensure that they obtain high pumpkin yields and among these, application of manure ranked highest. Manure mainly animal wastes such as goat dung and cow dung are applied to increase the organic matter in the soil. Manure enhances good soil quality for agricultural productivity and environmental quality to be sustained for future generations (Reeves, 1997). Weeding is carried out to remove unwanted plants which are potential competitors for the little available nutrients to ensure that the available nutrients in the soil are only utilized by plants of interest to the farmers. Mulching is done as one of the measures of conservation agriculture, using mainly dry grass to maintain soil moisture and also mulches prevent direct contact of the developing pumpkin fruit with the ground. Some farmers spray their pumpkins with synthetic insecticides and fungicides to control pests and diseases that can lower the crop's productivity despite their side effects as also noted by Wilson and Tisdell (2001) and Walter et al. (2004). But some farmers use organic and less harmful methods to repel insects such as planting the seeds with a regulated amount of coat meal as also noted by Dubey et al. (2010). Some farmers apply some ash to the soil before planting the seeds to increase the soil pH (Qin et al., 2017) especially during the rainy season when ferric soils tend to be too acidic (Young, 1974). This is to favour proper crop growth since the suitable pH for good pumpkin yields ranges from 6.0 to 6.5 (Kemble et al., 2000).

Several pumpkin varieties are grown by farmers. The several pumpkin varieties observed are attributed to the fact that the pumpkin is a monoecious plant thus they experience high rates of cross pollination. These pollination events can change the genetic identity of populations, giving rise to quite a number of varieties among the open pollinated populations (Robinson and Decker-Walters, 1997) such as those owned by many farmers in Uganda. Most of these were mainly known by local names described by farmers based on the fruit skin colour, shape and texture of fruit epicarp as also stated by Prohens-Tomás and Nuez (2007). According to the farmers' responses, Oziga was the most preferred variety

because it is generally a sweet variety with less water content. This eases the drying process during value addition for flour production unlike some varieties that have much more water content.

The major sources of market are the vendors who buy pumpkin heads from the farmers at low farm gate prices and in turn they make reasonable profits. This implies that pumpkin growing is a source of people's livelihood. The least sources of market as reported are events such as weddings because they are not held on a daily basis. Also, when served, pumpkin is a supplementary food. In addition, some people think that it is consumed by the vulnerable groups that are to say children and women as also reported by Ondigi et al. (2008).

The major challenges faced by pumpkin farmers are unpredictable rainfall patterns due to the effect of anthropogenic activities such as deforestation. This has greatly led to a shift in the climatic conditions and in turn has a negative impact on agricultural yields as noted by Adams et al. (1998) and Parry et al. (2004). The other big challenge is pests and diseases. This also lowers the pumpkin yields since some farmers especially in remote villages are not aware of the various pests and diseases that attack pumpkins. The most common pests are fruit flies as noted also by Ngoro et al. (2007). They attack the young fruit causing rotting of the fruit as their larvae develop within the fruit, aphids which suck fluids from leaves. The most common diseases are fungal infections such as powdery mildew, downy mildew which are very severe during humid conditions as also reported by Ngwerume and Grubben (2004).

Most of the farmers reported that they consume pumpkins mainly to boost their immune system and that of their family members. This can mainly be attributed to the presence of vital minerals, and micronutrients like vitamins and carotenoids of which many such as zinc, manganese, β -carotene, vitamin E, vitamin C among others are good antioxidants that render powerless the harm caused by free radicals. This eventually would result in a wide range of chronic and common diseases; for example coronary heart disease, malaria, diabetes, arthritis to mention a few (Tolonen, 1990). A deficiency of vitamins results in reduced resistance to disease (Finch and Onn, 1996; Holick and Holick, 2006; Makariou et al., 2011; Kitson and Roberts, 2012). Good eye sight is owing to the fact that pumpkins contain high amounts of β -carotene (Sharma and Rao, 2013). β -carotene is one of the few carotenoids that have provitamin A activity. β -carotene has 100% provitamin A activity better than α -carotene that has approximately 53% provitamin A activity. Also vitamin E and vitamin C are essential for visual health (Lien and Hammond, 2011). According to the review made by Caili et al. (2006), pumpkin is endowed with antidiabetic, antihypertension, antitumor, immunomodulation, antibacterial, antihypercholesterolemia, intestinal, antiinflammation and antialgic properties which further explains the medicinal values associated with pumpkin consumption.

Conclusion

Pumpkin cultivation in Uganda is mainly practiced on a small scale. Large scale commercial pumpkin production is still very low. From the survey, it was observed that mainly the elderly people above 46 years were involved in pumpkin cultivation and the youths were less involved. Pumpkin is a multi-purpose fruit vegetable in that all the parts of the plant can be consumed thus minimizing food wastage and also the plant can stay for quite long in shelf thus has the potential to act as a food security crop. There is a wide diversity of pumpkin varieties grown in Uganda thus this can form a good basis for pumpkin crop improvement. There are some attempts of pumpkin value addition taking place though still on a very small scale and in only a few places in Uganda such as Mityana. Nonetheless, it is important to note that conservation of the local pumpkin germplasm in Uganda has not been emphasized by the National Agricultural Research Organization since it is not one of their priority crops and therefore, this poses a risk of genetic erosion of some pumpkin landraces as exotic varieties are being introduced in the market.

Recommendations

More sensitization needs to be extended to the communities in Uganda about the relevance of consuming fruits and vegetables, the pumpkin being one of them as one of the efforts for human health improvement and fighting of malnutrition. Youths need to be encouraged and educated about pumpkin cultivation and on the different value addition avenues for pumpkin that can be potential sources of income and general socio-economic development. Farmers should be encouraged to keep records about their produce in order to track the progress of their agribusiness. There is need for farmers to form saving cooperative associations to gather resources together in order to expand the small and medium enterprises on pumpkin value addition to improve the income attained. This knowledge of pumpkin value addition needs to be shared with the local communities in other regions of Uganda to minimize post-harvest losses when there is plenty of pumpkin production, and in turn, this would improve on the general socio-economic wellbeing of the farmers involved. There is a need to set up a germplasm bank for the Ugandan pumpkin landraces for conservation and for future crop improvement to address challenges such as pests and diseases. A cost benefit economic analysis of value addition avenues needs to be studied to determine which avenue would give farmers the best income profit for their pumpkin produce.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Abukutsa-Onyango M (2007). The Diversity of Cultivated African Leafy Vegetables in Three Communities in Western Kenya. *African Journal of Food Agriculture Nutrition and Development* 7(3):1-15.
- Acharya AS, Prakash A, Nigam A (2013). Sampling: Why and How of it? *Indian Journal of Medical Speciaties* 4(2):330-333.
- Adams RM, Hurd BH, Lenhart S, Leary N (1998). Effects of global climate change on agriculture: an interpretative Review. *Climate Research* 11:19-30.
- Ahaibwe G, Mbowe S, Lwanga MM (2013). Youth Engagement in Agriculture in Uganda : Challenges and Prospects, P. 106.
- Bogdan RC, Biklen SK (2007). *Qualitative Research for Education: An Introduction to Theory and Methods* (5th ed.). Pearson Education, Inc.
- Caili FU, Huan SHI, Quanhong LI (2006). A Review on Pharmacological Activities and Utilization Technologies of Pumpkin. *Plant Foods for Human Nutrition* 61(2):70-77.
- Dubey NK, Shukla R, Kumar A, Singh P, Prakash B (2010). Prospects of botanical pesticides in sustainable agriculture. *Current Science* 98(4):479-480.
- Finch JM, Onn NRG (1996). Effects of selenium and vitamin E on the immune responses of domestic animals. *Research in Veterinary Science* 60(2):97-106.
- Furstenberg FF (1998). Social capital and the role of fathers in the family. *Men in Families: When Do They Get Involved? What Difference Does It Make*, pp. 295-301.
- Gollin D, Rogerson R (2010). Agriculture, roads, and economic development in Uganda. NBER Working Papers 15863. National Bureau of Economic Research, Inc.
- Hamisy WC, Makundi AH, Marandu D, Nkya MJ (2002). Evaluation of five accessions of *Cucurbita maxima* collected from different ecological zones in Tanzania. In: *The Second International Workshop on Plant Genetic Resources and Biotechnology Report* (Arusha, Tanzania, pp. 6-10.
- Holick MF, Holick MF (2006). Resurrection of vitamin D deficiency and rickets Find the latest version: Science in medicine Resurrection of vitamin D deficiency and rickets. *Journal of Clinical Investigation* 116(8):2062-2072.
- Johnson B, Turner LA (2003). Data Collection Strategies in Mixed Methods Research. In: Tashakkori, A.M. and Teddlie, C.B., Eds., *Handbook of Mixed Methods in Social and Behavioral Research*, SAGE Publications, Thousand Oaks, pp. 297-319.
- Kemble JM, Sikora EJ, Zehnder GW, Bauske E (2000). Guide to commercial pumpkin and winter squash production. Alabama Cooperative Extension System. ANR-1041http://www.aces.edu/pubs/docs/A/ANR-1041/ANR_1041.
- Kitson MT, Roberts SK (2012). Review D-livering the message: The importance of vitamin D status in chronic liver disease. *Journal of Hepatology* 57(4):897-909.
- Lien EL, Hammond BR (2011). Nutritional influences on visual development and function. *Progress in Retinal and Eye Research* 30(3):188-203.
- MAAIF (2003). *The Uganda Food and Nutrition Policy*.
- MAFAP (2013). *Review of food and agricultural polices in Uganda. MAFAP Country Report Series*, FAO, Rome, Italy.
- Makariou S, Liberopoulo EN, Elisaf M, Challa A (2011). European Journal of Internal Medicine Novel roles of vitamin D in disease: What is new in 2011? *European Journal of Internal Medicine* 22(4):355-362.
- Martins S, Ribeiro De Carvalho C, Carnide V (2015). Assessing Phenotypic Diversity of *Cucurbita* Portuguese Germplasm. *The Journal of Agriculture and Forestry* 61(1):27-33.
- Ndoro OF, Madakadze RM, Kageler S, Mashingaidze AB (2007). Indigenous knowledge of the traditional vegetable pumpkin (*Cucurbita maxima* / *moschata*) from Zimbabwe. *African Journal of Agricultural Research* 2(12):649-655
- Ngwerume FC, Grubben GJH (2004). *Cucurbita maxima* Duchesne. Record from PROTA4U. Grubben, GJH and Denton, OA (Editors). PROTA.
- Ondigi AN, Toili WW, Ijani ASM, Omuterema SO (2008). Comparative analysis of production practices and utilization of pumpkins (*Cucurbita pepo* and *Cucurbita maxima*) by smallholder farmers in the Lake Victoria Basin, East Africa. *African Journal of Environmental Science and Technology* 2(10):296-304.
- Parry ML, Rosenzweig C, Iglesias A, Livermore M, Fischer G (2004). Effects of climate change on global food production under SRES emissions and socio-economic scenarios. *Global Environmental* 14(1):53-67.
- Prohens-Tomás J, Nuez F (2007). *Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae*. Springer Science and Business Media P 1.
- Qin J, Frederik M, Ekelund F, Rønn R, Christensen S, Groot GA, Mortensen LH, Skov S, Krogh PH (2017). Wood ash application increases pH but does not harm the soil. *Environmental Pollution* 224:581-589.
- Ravani A, Joshi DC (2014). Processing for Value Addition of Underutilized Fruit Crops. *Trends Postharvest Technology* 2:15-21.
- Reeves DW (1997). The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil and Tillage Research* 43:131-167.
- Restrepo MJ, Lelea MA, Kaufmann B (2016). Second-Order Cybernetic Analysis to Re-construct Farmers' Rationale When Regulating Milk Production. *Systemic Practice and Action Research* 29(5):449-468.
- Robinson RW, Decker-Walters DS (1997). *Cucurbits*. Cab International, Wallingford, Oxon, New York 226 p.
- Sharma S, Rao R (2013). Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. *International Food Research Journal* 20(5):2309-2316.
- SPSS IBM (2011). *20.0 for Windows. Computer Software*. Chicago, IL: SPSS Inc.
- Tolonen M (1990). *Vitamins and minerals in health and nutrition*. Elsevier.
- UBOS (2010). *Uganda Census of Agriculture 2008/2009. Crop Area and Production Report (Vol. IV)*.
- UBOS (2014). *The National Population and Housing Census 2014-Main Report*, Kampala, Uganda P 71.
- Walter M, Harris-virgin P, Thomas W, Tate G, Waipara NW, Langford G (2004). Agrochemicals suitable for downy mildew control in New Zealand boysenberry production. *Crop Protection* 23(4):327-333.
- Whitaker TW, Bemis WP (1975). Origin and Evolution of the Cultivated *Cucurbita*. *Bulletin of the Torrey Botanical Club* 102(6):362-368
- Wilson C, Tisdell C (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics* 39:449-460.
- Wortmann CS, Eledu CS (1999). Uganda's agroecological zones: A guide for planners and policy marker. <https://cgspace.cgiar.org/handle/10568/54311>
- Young A (1974). Some Aspects of Tropical Soils. *Geography* 59(3):233-239.

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