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Full Length Research Paper

Analysis of light transmission ratio and yield advantages of pigeonpea in relation to intercrop and different plant population

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A field experiment was conducted at Tamil Nadu Agricultural University, Coimbatore, during *kharif* 2011 to optimize the spacing for medium maturation pigeonpea at different planting geometry and row proportions under intercropped and irrigated situation. The experiment was laid out in Randomized block Design with 3 replications. Adjacent to the treatment plots, sole pigeonpea and greengram were also raised in dummy plots with same management practices to calculate the yield advantages. The treatments comprised of planting geometry (row spacing of 90, 120, 150 and 180 cm at varied level of plant to plant spacing with 30, 45, and 60 cm) and different row proportions of pigeonpea + greengram (1:2, 1:3, 1:4 and 1:5) Pigeonpea sown with different spacing's under sole as well as intercropped with greengram showed positive effect on various agronomic traits and yield parameters for different planting geometry. Sowing of pigeonpea at 120 x 30 cm with 1:3 row proportions recorded significantly higher LER (1.52), ATER (1.15) and IER (1.29) was recorded. The lowest light transmission ratio of 34.7% was recorded with the above said treatment.

Key words: Pigeonpea, planting geometry, light transmission ratio, land equivalent ratio, area time equivalent ratio and income equivalent ratio.

INTRODUCTION

Pigeonpea (*Cajanus cajan* L.) is one of the major grain legume crop of tropical and subtropical regions and it is grown predominantly under rainfed conditions. India accounts for 90% of world's pigeonpea growing area and 85% of world's production of pigeonpea. In India, it is grown in an area of 4.5 M ha with an annual production of 3.3 MT and productivity of 799 kg ha^{-1}. When pigeonpea is grown as a sole crop, it is relatively inefficient because of its slow initial growth rate and low harvest index; therefore it is grown as intercrop, which helps in efficient utilization of available resources for enhancing the productivity and profit.

Pigeonpea is desirable for inter-cropping with different crops like cotton, sorghum, pearlmillet, greengram, blackgram, maize, soybean and groundnut for enhancing yield output and sustaining soil fertility. Greengram

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(*Vigna radiata* L.) also one of the most essential pulse crop in India because of its shorter growth duration, low water demand, low soil fertility and is privileged for consumption due to its soft digestibility and low yield of flatulence (Shil and Bandopadhyay, 2007).

Agronomic package like plant population is recognized to affect crop environment, which determine the yield components. Optimum population levels should be maintained to tap utmost natural resources such as nutrient, sunlight, soil moisture and to assure satisfactory yield (Sharifi et al., 2009). If plant population is lower than optimum, then per hectare production will be down and weeds will also be to a greater extent (Allard, 1999).

The low productivity of pigeonpea has been ascribed to the reality that large area is under rainfed situation raised in broader spacing. Under such situation, other leguminous crops such as greengram can be sprung up as an intercrop to heighten the productivity of the system. However, intercropping in pigeonpea may not be potential with conventional planting pattern of 90 × 30 cm. Thereby adoption of wider planting geometry pave a way for preserving the optimum plant population of pigeonpea and also it provides a chance for introducing an intercrop. Keeping above in perspective, a trial was carried out to study the effect of planting geometry on pigeonpea under intercropped and irrigated condition.

MATERIALS AND METHODS

Climate and soil

Field experiment was conducted during *kharif,* 2011, to optimize the spacing for medium maturation pigeonpea at different planting geometry and row proportions under intercropped and irrigated situation at Millet Breeding Station of TNAU, Coimbatore, Tamil Nadu (India) is situated at 11° North latitude and 77° East longitude at an altitude of 426.7 m above Mean Sea Level (MSL). The mean annual rainfall of Coimbatore is 657 mm distributed over 47 rainy days and the mean maximum and minimum temperatures were 31.5 and 21.4°C, respectively during the crop period.

The relative humidity ranged from 61 to 90% in the forenoon and 14 to 68% in the afternoon. The mean bright sunshine hours per day were 7.4 h with a mean solar radiation of 429 cal cm^{-2} day^{-1}. The meteorological data for the cropping season 2011 was recorded at the meteorological observatory of from Agro Meteorological Observatory at Tamil Nadu Agricultural University, Coimbatore. The sandy clay loam was alkaline (pH 8.32), medium in organic carbon (0.59%), available N (148 kg ha^{-1}), available P (26 kg ha^{-1}) and available K (307 kg ha^{-1}).

Treatment details

The treatments comprised of planting geometry (row spacing of 90, 120, 150 and 180 cm at varied level of plant to plant spacing with 30, 45, and 60 cm) and different row proportions of pigeonpea + greengram (1:2, 1:3, 1:4 and 1:5) were taken in randomized block design with three replications. The gross and net plot sizes were 18.0 x 8.4 m and 14.4 x 4.8 m respectively. Adjacent to the treatment plots, sole pigeonpea and greengram were also raised in dummy plots with same management practices to calculate the yield advantages. The pigeonpea variety CO 6 was chosen for the study. The COGG 973 of greengram was used as intercrop.

Agronomic packages

The crop was sown under irrigated condition. Hence to meet out the basic agronomic package the crop was fertilized with recommended dose of nutrients (25: 50: 25 kg of NPK ha^{-1}) through Urea, single super phosphate (SSP) and muriate of potash (MOP) and incorporated at the time of sowing. Urea is the cheapest source of nitrogen as far as farmer's point of view. The entire dose of NPK was applied as basal.

Growth and yield components were recorded in the five randomly selected plants. Net returns were calculated by deducing cost of cultivation from gross returns. The B:C ratio was worked out as a ratio of gross returns to cost of cultivation. Competitive indices, nutrient uptake and soil fertility status were also taken by following standard procedures. The crops were sown on 19th of August 2011 as per the treatments. Thinning was done at 20 DAS leaving one healthy seedling per hill. Pre-emergence herbicide of Pendimethalin 30% EC at 1.0 kg ha^{-1} was sprayed as on 3 DAS through the battery operated knapsack sprayer. Weeding was done in all the treatment plots on 35 DAS by hand hoe with the help of manual labour.

Sampling and measurements

Plants are harvested separately from the net plot area of each treatment and pods are separated, threshed, weighed and recorded as grain yield (kg ha^{-1}). The grain yield was recorded at 12% moisture level.

Computations for light transmission ratio and yield advantages

The yield differences among the treatments and sole plots were used to estimate the yield advantages due to different plant geometry. The light transmission ratio and yield advantages were calculated as proposed by Yoshida et al. (1972), Willey (1979) and Hiebsch and McCollum (1987).

$$\text{Light transmission ratio LTR (\%)} = \frac{Ii}{Io} \times 100$$

Where, Ii = Light intensity received at the ground level, Io = Light intensity received above the canopy of the corp.

$$\text{Land equivalent ratio (LER)} = LA + LB = \frac{YA}{SA} + \frac{YB}{SB}$$

Where, LA and LB are the LER for individual crops, YA and YB are the individual crop yield in intercropping, SA and SB are their sole crop yields.

$$\text{Area time equivalent ratio (ATER)} = \frac{(Ryc \times tc) + (Ryp \times tp)}{T}$$

Where, Ry = Relative yield of species c (main crop) and p (intercrop).

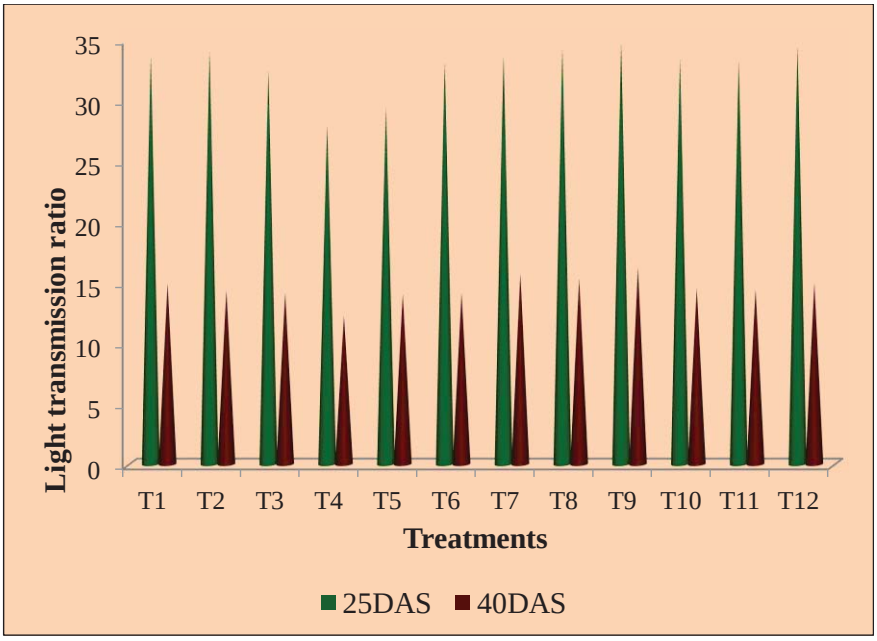

Figure 1. Effect of planting geometry and row proportions on light transmission ratio (LTR) of pigeonpea + greengram intercropping system.

$$Ry = \frac{\text{Yield of intercrop per hectare}}{\text{Yield of monocrop per hectare}}$$

t = duration (days) for species C and P, T = duration (days) for the intercropped system.

$$\text{Income equivalent ratio (IER)} = \frac{Iab}{Iaa} + \frac{Iba}{Ibb}$$

Where, Iaa : Gross income of component 'a' in pure stand, Ibb : Gross income of component 'b' in pure stand, Iab: Gross income of component 'a' in mixed stand with 'b', Iba: Gross income of component 'a' in mixed stand with 'b'.

Statistical analysis

The data were analyzed statistically following the procedure given by Gomez and Gomez (1984). Critical differences were worked out at five per cent probability level wherever the treatments were significant. The treatment differences that were non-significant were denoted as NS.

RESULTS

Light transmission ratio (LTR)

The light transmission ratio of intercropping system differed significantly due to planting geometries adopted for pigeonpea and row ratios of pigeonpea + greengram cultivars. During 25 DAS and 40 DAS among intercropped treatments, higher LTR of 34.7 and 16.2 was recorded in pigeonpea (150 x 60 cm) + greengram in 1:4 row proportion (T_9) followed by T_{12} (34.4) with 1:5 row proportion at 25 DAS. The lower LTR of 27.9 and 12.2 was recorded in intercropped pigeonpea (120 x 30 cm) with greengram 1:3 row proportion (T_4) at two stages (Figure 1).

Land equivalent ratio (LER)

The data obtained shows significant variations in land equivalent ratio due to cropping systems, planting geometry of pigeonpea and row proportions of pigeonpea and greengram (Figure 2). Among the intercropping treatments, comparatively highest LER (1.52) was recorded in pigeonpea (120 x 30 cm) + greengram in 1:3 row proportion (T_4). However, (T_{10}) pigeonpea (180 x 30 cm) + greengram in 1:5 (1.50), (T3) pigeonpea spacing (90 x 60 cm) (1.47) and pigeonpea (120 x 45 cm) + greengram 1:3 (T_5) remained on par with each other. Pigeonpea (90 x 30 cm) + greengram in 1:2 row proportion (T_1) recorded lower LER values (1.28).

Area time equivalent ratio (ATER)

Area time equivalent ratio showed significant variations due to cropping systems, row ratios of pigeonpea and greengram. Among the intercropped treatments, pigeonpea

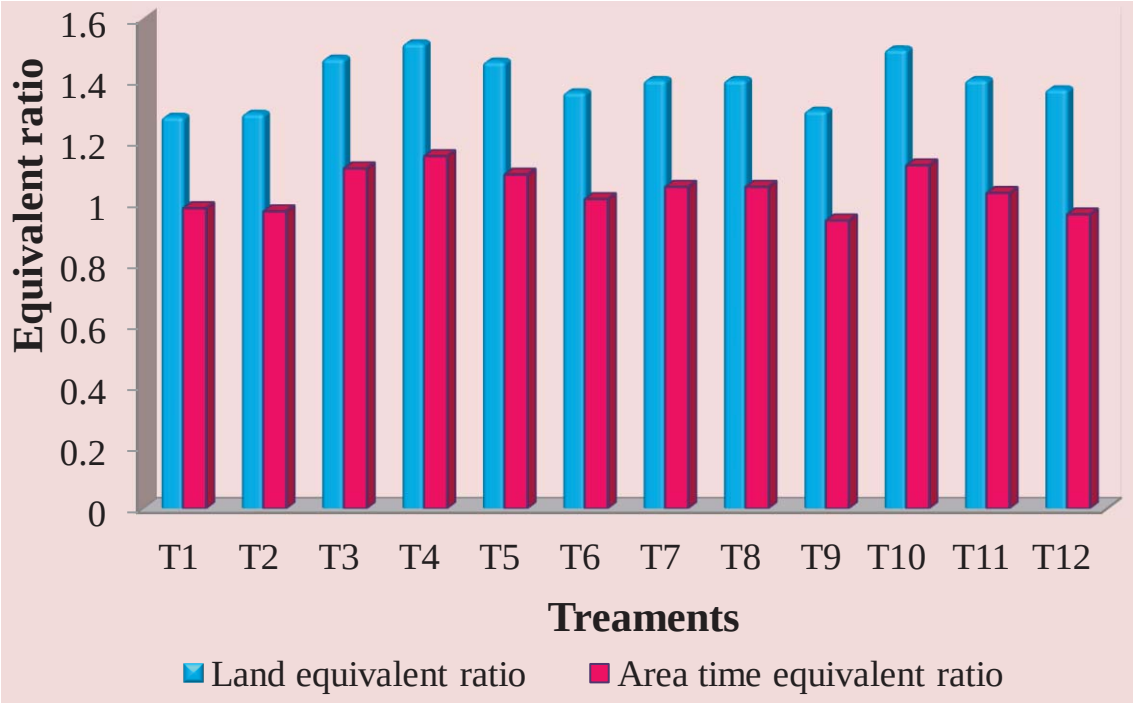

Figure 2. Effect of planting geometry and row proportions on land equivalent ratio (LER) and area time equivalent ratio (ATER) of pigeonpea + greengram intercropping system.

(120 x 30 cm) + greengram in different row ratios recorded significantly higher ATER (1.15) than all other intercropping treatments which was statistically on par with T_{10}, T_3 and T_5 treatments. Pigeonpea (150 x 60 cm) + greengram in 1:4 row ratio (T_9) recorded lower ATER (0.94).

Income equivalent ratio (IER)

Income equivalent ratio was higher (1.29) with a crop geometry of pigeonpea (120 x 30 cm) + greengram under 1:3 row proportion (T_4), followed by pigeonpea spacing of 90 x 60 cm with 1:2 row ratio (T_3) . The lowest IER (1.04) was recorded in 1:4 row ratio of pigeonpea (150 x 60 cm) + greengram (T_9).

DISCUSSION

Light transmission ratio (LTR)

The better use of resources can be supported by observations on LTR, which showed significant difference due to intercropping systems. The higher light interception in intercropping/mixed cropping was due to quick growth and good vegetative cover, which helped in better interception of light. The lower LTR values was observed in 1:3 row ratio with pigeonpea spacing of 120 x 30 cm which implies the highest light utilization efficiency as compared to other row proportions (Figure 1). This was due to better spatial use of light by leaf canopy or root system might have made better spatial use of nutrients and water. It is also clear from the investigation that, the yield advantage in intercropping/mixed cropping was due to better overall use of resources than when crops grown separately.

These factors hold well in present investigation in which pigeonpea has deep root system while greengram have shallow root system. This is in conformity with Rathod (2002) in pigeonpea + cowpea, Biru et al. (2004) in sorghum + legume and Mohan (2003) in maize intercropped with legumes in 1:2 row proportion.

Land equivalent ratio (LER)

Advantage of intercropping over sole cropping system is measured through LER varied from 1.28 to 1.52 due to different planting geometry and row proportion (Figure 2). Thus, biological efficiency of land under intercropping was higher as compared to sole greengram/pigeonpea and different planting geometries of pigeonpea intercropped with greengram. The higher LER under intercropping systems may be due to better planting geometry and spatial arrangements which might have avoided the coincidence of the peak period of growth of component crops. Intercropping of pigeonpea and greengram in 1:3 row ratio recorded higher yield LER of

Table 1. Effect of planting geometry and intercropping on income equivalent ratio of pigeonpea + greengram intercropping system.

Treatments	IER
T_1- Pigeonpea (90 x 30 cm) + Greengram (1:2)	1.13
T_2- Pigeonpea (90 x 45 cm) + Greengram (1:2)	1.11
T_3- Pigeonpea (90 x 60 cm) + Greengram (1:2)	1.27
T_4- Pigeonpea (120 x 30 cm) + Greengram (1:3)	1.29
T_5- Pigeonpea (120 x 45 cm) + Greengram (1:3)	1.21
T_6- Pigeonpea (120 x 60 cm) + Greengram (1:3)	1.13
T_7- Pigeonpea (150 x 30 cm) + Greengram (1:4)	1.16
T_8- Pigeonpea (150 x 45 cm) + Greengram (1:4)	1.14
T_9- Pigeonpea (150 x 60 cm) + Greengram (1:4)	1.04
T_{10}- Pigeonpea (180 x 30 cm) + Greengram (1:5)	1.20
T_{11}- Pigeonpea (180 x 45 cm) + Greengram (1:5)	1.11
T_{12}- Pigeonpea (180 x 60 cm) + Greengram (1:5)	1.05
SEd	0.05
CD (P=0.05)	0.10

Means are compared as per CD/LSD. Model used is Agress software.

1.52 over other system and the lowest one with 1:2 row proportion. It was concluded that, for producing the same amount of yield, 52% more area is required under sole crop system. This is in accordance with the findings of Prakash and Bhushan (2000) in pigeonpea/castor + greengram.

Area time equivalent ratio (ATER)

In the present investigation, ATER realized from intercropping systems of pigeonpea and greengram was significantly higher than that obtained from either sole crop of pigeonpea or greengram. Higher ATER (1.15) under intercropping of pigeonpea and greengram indicate that not only the efficient use of land, but efficient use of time. The extent of time utilization ranges from 9.5 per cent in 1:4 row proportions to 15 per cent in 1:3 row ratios (Figure 2). The observations in the present experiment are in agreement with the criteria set out earlier by Patil (2003) in little millet + pigeonpea.

Income equivalent ratio (IER)

IER values are higher in 120 x 30 cm spacing with 1:3 row proportion, due to high resource use efficiency and equivalent yield. This system gives 29% higher economic advantage over growing crops in pure stands (Table 1). The similar result was reported by Billore et al. (2009) in soybean + pigeonpea cropping system. Thus, it can be inferred from the above results, on the basis of agronomic as well as economic performance, sowing of pigeonpea (120 x 30 cm) + greengram intercropping in 1:3 row proportion under ridges and furrows land configuration proved to be more productive and remunerative and this salient finding will be useful for pigeonpea growers to enhance income under irrigated conditions of Tamil Nadu.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of indigenous technologies of fresh cocoyam (*Colocasia esculenta* (L.) Schott) Storage in Southeastern Nigeria

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Cocoyam (*Colocasia esculenta)* also referred to as taro is an herbaceous root crop and its non availability throughout the year has implicated lack of good storage methods. Survey and storage experiments were therefore, conducted at the Department of Crop Science, University of Nigeria, Nsukka to establish the scientific basis for adopting the indigenous technologies by the farmers and indentify the best among the technologies. The experiments were carried out in two phases. Phase (1) involved collection of farm level information on the existing fresh cocoyam preservation practices from the farmers through questionnaire interview while phase (2) involved the storage of cocoyam using some of the technologies based on their frequency of application by the communities. The storage experiment was laid out as 3 x 2 x 4 factorial in completely randomized design (CRD) and replicated four times. The storage techniques (treatments) were Pit + *Casia alata* + soil, Pit + *Jatropha curcas* leaf extracts + soil and no extract control. Twenty-four pits of each 0.7 m deep and 0.33 x 0.33 m^2 were dug at the experimental field under plantain and banana shades. Out of the 720 farmers sampled, 34.7% used Pit + *C. alata* leaf + soil to store their cocoyams while only 11% represent those who did not add botanicals. Weight loss from cocoyams treated with *C. alata* leaf extracts inside storage pits was significantly (P ≤ 0.05) lower than those treated with *J. curcas* and no treatment control. Similarly, treatment of cocoyams with both *J. curcas* and *C. alata* siginificantly (P ≤ 0.05) reduced rot incidence compared to no treatment control but there was no significant effects of these botanicals sprout weight. Among the leaves used for storage of cocoyam in the study area, *J. curcas* and *C. alata* leaf extracts outstandingly reduced post-harvest losses of cocoyam suggesting that these botanicals posses some anti fungal or anti bacterial properties that reduced rots in the stored cocoyam.

Key words: Cocoyam, botanical extracts, pit, shade, storage.

INTRODUCTION

Cocoyam (*Colocasia esculenta* (L) Schott) also referred to as taro (Dutta, 1990) is a herbaceous root crop, measuring about 0.5 to 2 m tall and belongs to the family of Araceae. It has underground round starchy corm which

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produces at its apex a whorl of large leaves with long robust petioles. The leaves are heart shaped and the corms which vary greatly in size are surrounded by a number of secondary corms (cormels). The root system is superficial and fibrous.

Cocoyam is believed to have originated from India and other parts of South East Asia (FAO, 1988). The countries popular with the cultivation of cocoyam are Hawaii, Japan, Ghana and Nigeria. Nigeria is the world's largest producer of cocoyam. The annual production figure for Nigeria is about 5 million metric tonnes which accounts for about 37% of total world output of cocoyam (FAO, 2007).

Cocoyam is cultivated mainly for the edible corms, the leaves, petioles and flowers are used in soup preparation (Eze and Maduewesi, 1990). The researchers noted that it is nutritionally superior to other roots and tubers in terms of digestibility, crude protein and mineral (Ca, Mg and P) contents. Cocoyam possesses the smallest starch grain size relative to other root and tuber (FAO, 2007) and makes it suitable for several food products especially as food for potentially allergic infants (gluten allergy), persons with gastro intestine disorders, diabetic patients etc. Increasing awareness and concern for environmental quality makes cocoyam starch granules superior to other sources of starch as agro-industrial material for the production of biodegradable plastics (FAO, 2007).

Chukwu and Nwosu (2008) noted that lack of good storage methods limit the availability of cocoyam all through the year. A preliminary survey that preceded this study showed that farmers commonly stored their cocoyam corms and cormels on raised platforms where they may remain in good condition for up to 3-4 months. Other storage techniques include packing on spots and dusted with wood ash, or leaving on heaps/ridges unharvested for 2 to 3 months but sprouting should not be allowed.

Under these conditions, high rate of rots could have been recorded. Cocoyam like other roots and tuber crops are liable to postharvest losses through a number of factors such as rots, sprouting, and respiration among others. Rots have been associated with microorganisms such as *Botryodiplodia theobromae* Pat, *Fusarium moniliforme* Var, *Penicillium oxalicum* Currie and Thom, *Aspergillus niger, Rhizoctonia spp* etc. (Okigbo and Ikedigwu, 2000). Some botanical extracts like *Jatropha curcas, Zingiber officinale, Azadiratha indica, Xylopia aethiopica* have been reported to have anti-microbial properties (Eze et al., 2006) and also effective in control of rots in yams. Therefore, there is need to investigate the use of botanicals and pit treatments for cocoyam storage.

The objective of this study therefore, was to evaluate indigenous technologies of cocoyam storage in Southeastern Nigeria in order to establish the scientific basis for adoption by farmers in the study area and to develop new technology to improve on what they have.

MATERIALS AND METHODS

The experiments were carried out in two phases:

Phase (1) involved collection of farm level information on the existing technologies of cocoyam preservation from the farmers through purposive questionnaire interview. Six local government areas in Enugu state were randomly selected for this study. They are the present Uzo-Uwani, Igbi-Etiti, Udenu, Igbo-Eze North, Igbo-Eze South and Nsukka local government areas. The selection was based on the practical evidence that these areas are centres of cocoyam production in Enugu state of Nigeria. Random samples of 120 farmers were selected from each of the six cocoyam production zones giving a total of 720 respondents. Some of the farmers interviewed were not well educated so the questions were read out to them and the answers were filled by the interviewer. Other sources of information on indigenous technologies of cocoyam storage were collected from literature and books.

Phase (2) of the experiment involved the storage of cocoyam (*Colocasia esculenta* (L) Schott) using some of the technologies based on their frequency of application by the communities. Selection of the investigated technologies was primarily to establish the scientific basis for adoption of such technologies in a wider horizon. The storage study was conducted in the storage field of the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka (Latitude 06°25'N, Longitude 07°24'E, Altitude 447.26 M above sea level). The storage field was under a plantation of banana and plantain mixture. The plantain and banana crops supply shade that cools the cocoyam storage environment. The experiment was carried out between the months of December 2011 and April, 2012.

Source of cocoyam and botanical extracts

Freshly harvested corms and cormels of cocoyam locally called "Ede Ofe" cultivar were collected from the experimental farm of the Department of Crop Science, University of Nigeria, Nsukka. The sound and clean cocoyams were cured under the sun for four days, and thereafter taken to the storage field where they were evaluated and graded visually to separate the wounded, rotted and disease or pest infested ones from the healthy ones. The healthy ones were separated into corms and cormels. The corms and cormels were put into bulks of 30 pieces each.

Preparation of botanical extracts

Based on survey information, the farmers in the study area put fresh leaves of some plants/botanicals on top of the cocoyams inside the pits before the pit is covered with soil. For purposes of convenience and in order to quantify the amount of these botanicals required for storing cocoyam in relation to the weight, the leaves of these botanicals were dried and prepared into powder. The fresh and mature leaves of the most frequently used botanicals of *Jatropha curcas* and *Casia alata* were harvested from the nearby village (Owerre-ezeorba); a village beside the UNN campus. The fresh leaves of *J. curcas* and *C. alata* were washed clean with tap water, rinsed with sterile distilled water and dried under shade for 7 days. The dried leaves were ground into fine powder using Thomas Wiley's Laboratory Mill Model 4 in the Physiology Laboratory of the Department of Crop Science UNN.

Storage experiment

The experiment was laid out as 3 x 2 x 4 factorial in completely randomized design (CRD) and replicated four times. The treatments

Table 1. Percentage distribution of farmers and indigenous technologies of cocoyam storage in southeastern Nigeria.

Indigenous technologies	Number of farmers	Percentage
Pit + wood ash + soil	70	9.7
Pit + *C. alata* leaf + soil	250	34.7
Pit + plantain leaf + soil	60	8.3
Pit + *J. curcas* +soil	220	30.5
Shade + plantain leaf	40	5.5
Pit + soil alone (control)	80	11
Total	720	100.0

were three storage techniques and two cocoyam storage organs replicated four times. The storage techniques were Pit + *C. alata* + soil, Pit + *J. curcas* leaf extracts + soil and no extract control. Twenty-four pits of each 0.7 m deep and 0.33 x 0.33 m^2 were dug at the experimental field under plantain and banana shades. Three hundred grammes of each of the botanical extracts were divided into two for each pit. The first 150 g of the botanical extracts were spread at the bottom of the pits to form beddings for the cocoyam while the other 150 g were sprinkled on top of the cocoyams. Each corm or cormel bulk containing 30 pieces was weighed and put at random into the designed pits. The pits were covered with the top soils (sandyloam) in a random manner. The cocoyams were stored for four months (December to April).

Measurements

At the end of storage the following parameters were collected using the formulae thus: Determination of post-harvest losses of cocoyam corms and cormels

$$\% \text{ Weight loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

$$\% \text{ physical cocoyam loss} = \frac{\text{Initial number of cocoyam – cocoyam after storage}}{\text{Initial number of cocoyam}} \times 100$$

$$\% \text{ sprout relative weight} = \frac{\text{Total weight of cocoyam – Weight of sprout}}{\text{Total weight of cocoyam}} \times 100$$

$$\% \text{ cocoyam rot} = \frac{\text{Total cocoyam rots}}{\text{Total cocoyam stored}} \times 100$$

Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) according to the procedure for CRD using the Statistical Analysis System (SAS) package (SAS, 1999). Mean separation was done by least significant difference (LSD).

RESULTS

Different technologies of cocoyam storage in southeastern Nigeria and percentage of farmers that practice them are shown in Table 1. Out of the 720 farmers sampled, 34.7% used Pit + *C. alata* leaf + soil to store their cocoyams while only 11% represent those who did not add botanicals in the pit for their cocoyam storage. However, the percentage storage in pits without addition of botanicals was higher than storage in shades covered with plantain leaves. Weight loss from cocoyams treated with *C. alata* leaf extracts inside storage pits were significantly ($P \leq 0.05$) lower than those treated with *J. curcas* and no treatment control (Table 2). Similarly, treatment of cocoyams with both *J. curcas* and *C. alata* siginificantly ($P \leq 0.05$) reduced rot incidence compared to no treatment control but there was no significant difference on the effects of *C. alata* and *J. curcas* on sprout relative weight. Application of botanical extracts of *J. curcas* to cocoyams stored in the pit significantly ($P \leq 0.05$) reduced weight loss, percent cocoyam rot and percent physical loss/total damage compared to cocoyams in the pit where no botanical was added. Again, the extracts of *C. alata* significantly reduced weight loss, percent rot and percent physical loss compared with no extract in the pit storage. Sprout relative weight was significantly ($P \leq 0.05$) lower with no extract pit storage compared to the cocoyams where extracts of *J. curcas* and *C. alata* were applied. The type of storage organ is important in the storability of cocoyams. Weight loss was significantly lower in stored corms than in the cormels (Table 3). Sprout relative weight varied between cormel and corm but the differences were not significant.

The combined effects of the storage treatments and storage organs on the physical characteristics of the stored cocoyams are shown in Table 4. The interaction effects of the two botanicals, *J. curcas* and *C. alata* and the two storage organs, corms and cormels significantly ($P \leq 0.05$) reduced weight loss, rot incidence and total damage compared to the combined effects of no extracts (pit + soil alone) and the storage organs. Similarly, combined effects of Pit + *C. alata* + soil and the corms or cormels significantly ($P \leq 0.05$) showed lower weight loss of cocoyam and lower sprout relative weight than the interaction effect of Pit + *J. curcas* + soil and corms or cormels. Correlation analysis revealed that sprout relative

Table 2. The effects of storage treatments on the physical characteristics of cocoyam in storage.

Storage treatments	Physical characteristics of cocoyam in storage			Sprout weight (kg)
	Weight loss (kg)	Rot incidence (%)	Total damage (%)	
Pit + soil alone	33.3	35.6	30.0	26.5
Pit + *J. curcsa* + soil	29.8	25.8	16.8	36.2
Pit + *C. alata* + soil	28.7	24.9	15.7	35.9
$LSD_{0.05}$	2.72	1.47	1.53	1.05

Table 3. The effects of storage organs on the postharvest physical characteristics of cocoyams.

Storage organ	Physical characteristics of corms and cormels in storage			Sprout weight (kg)
	Weight loss (kg)	Rot incidence (%)	Total damage (%)	
Cormel	17.65	25.10	7.89	37.6
Corm	15.10	26.67	15.86	37.85
$LSD_{0.05}$	0.95	0.87	0.25	ns

ns = Not significant.

Table 4. The interaction effects of storage treatments and storage organs on the physical characteristics of cocoyam in storage.

Storage treatments	Storage organ	Physical characteristics of cocoyam in storage			Sprout weight (kg)
		Weight loss (kg)	Rot incidence (%)	Total damage (%)	
Pit + soil alone	Cormel	32.31	32.35	30.75	25.50
Pit + soil alone	Corm	31.60	35.33	35.0	26.10
Pit + J.curcas + soil	Cormel	19.65	24.0	16.81	35.65
Pit + J.curcas + soil	Corm	16.90	24.95	17.75	34.67
Pit + C. alata + soil	Cormel	15.75	24.79	15.65	35.22
Pit + C. alata +soil	Corm	15.70	24.78	16.54	34.67
LSD0.05		2.50	3.70	4.80	2.59

Table 5. Correlation analysis for weight loss, sprout weight, total damage and rot of cocoyam in storage.

Variables	Weight loss	Sprout relative weight	Total damage	Rot incidence
Rot incidence	0.0754ns	0.8152**	0.9300***	-0.7865*
Weight loss		0.6557*	-0.6789	0.0042^{ns}
Sprout relative weight			0.8976**	0.2458^{ns}
Total damage				-0.3456^{ns}

*** Significant at P≤ 0.0001 ** Significant at P≤ 0.001 *significant at P≤ 0.05 ns = not significant.

weight was highly and positively related with rot incidence (r = 0.82) (Table 5). Total cocoyam damage was also highly and positively related to rot incidence and sprout relative weight.

DISCUSSION

Analysis of the survey data showed that farmers in the study area preserve their harvested cocoyam in a variety of ways/techniques. Some the techniques include under shade, storage in pits with addition of some botanicals, storage in pits without botanicals, storage in pits with addition of wood ashes among others. This is similar to the report of the Food and Agricultural Organization (FAO) (2003) that cocoyam is stored in a variety of traditional low-cost structures such as shade, hut and underground pits. The report also noted that cocoyams were stored in heaps in a shade and/or covered with straw or plantain leaves. In parts of southern China, it is

common practice to pile the corms in heaps and cover them with soil or seal them in leaf-lined pits in the ground (Plucknett and White, 1997).

The present study has revealed that farmers in the study area acquired the storage techniques from experience suggesting that no new technology has been developed. However, the result also confirmed that farmers learnt out of experience that the addition of plant leaves in the pit with cocoyam enhanced storability. Among the leaves used for storage of cocoyam in the study area, *J. curcas* and *C. alata* leaf extracts outstandingly reduced post-harvest losses of cocoyam suggesting that these botanicals posses some anti fungal or anti bacterial properties that reduced rots in the stored cocoyam. In the present study, the possibility of cocoyam to store for 16 weeks with less than 40% rot for corms and cormels without leaf extract treatments and less than 30% rot where leaf extracts were applied could be attributed to the effectiveness of these botanicals as anti rot causing organisms. This does not agree with Anaele and Nwauisi (2008) report that after 4 months of storage, 94.9% of the stored corms and 36.7% of the cormels got rotten in the pit storage. However, other workers have reported that it was possible to control postharvest rots in other plant products using botanical extracts/fungicides (Uzuegbu and Okoro, 1999). The higher percentage weight loss recorded for the cormels could be attributed to the smoothness of the skin which probably enhanced water evaporation. The corms recorded higher rots than the cormels. This is not surprising because when cormels were dethatched from corms at harvest they, the spots on the skin being the attachment points of the cormels, probably created entry points for rot causing organisms. It has been reported (Eze et al., 2006) that yam tubers with wound during storage rotted faster than tubers without bruises. Higher sprout relative weight in the corms was not surprising since the corms were bigger than cormels in size and expectedly produced more vigorous sprouts.

Storage of cocoyam in pit + *C. alata* + soil reduced weight loss and cocoyam rots in this study. This result suggests that *C. alata* may have some bio-activities on some rot causing organisms. Similar result on the control of weight loss using ashes from the bark of kolanut tree, neem tree and inflorescene of oil palm has been reported earlier (Eze, 1991).

Very high positive correlation existed between rot incidence and sprout relative weight (r = 0.82) and also between rot incidence and total damage (r = 0.93). These relationships can be explained in the light of report of yam tuber studies by Girardin et al. (1998) that yam is less susceptible to fungal attack than during germination. Shannen (2003) also noted that growth of sprouts increased the respiration rate of yam tuber and predisposed the tubers to invasion by micro-organisms that caused dehydration and rot of yam tuber in storage. It is possible that sprouting of the cocoyam corms and cormels in this study pre-disposed them to pathogenic invasion that caused rot incidence which also caused total damage at higher degree of microbial activities.

Conclusion

Cocoyam is an important root crop that has very little period of shelf life. Unfortunately, the preservation methods remain the traditional way and therefore, they are vulnerable to post-harvest losses after harvest. The low period of shelf life could be attributed to their high moisture contents and chemical composition. It can be concluded that cocoyam is one of the neglected crops in the southeastern Nigeria. Therefore, farmers, research scientists and policy makers should combine efforts to develop improved storage technologies of cocoyam preservation in order to make food available for feeding increasing human population.

Conflict of Interest

The authors have not declared any conflict of interest.

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Economic viability of cucumber cultivation under NVPH

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The economic analysis was conducted for cultivation of parthenocarpic cucumber cv. Dinamik under naturally ventilated polyhouse for two consecutive years, 2013 and 2014. The actual value of economic inputs along with subsidy component (65 and 75%) imparted by Government of Gujarat in coordination with Government of India was considered for fitting into simulation model. The differences in net realization of Rs. 371642.00 (BCR 1.36) and Rs. 164723.00 (BCR 0.55) for the years 2013 and 2014, respectively represented maximum dependency on prevailing market selling rate in respective years besides some minor difference in yield component. BCR of 2.03, 0.95 and 2.17, 1.03 could be anticipated by availing 65 and 75% subsidy in each individual year for a crop of short duration, thus opening new avenues for small farmers of the Gujarat, India.

Key words: Cucumber, parthenocarpic, protected cultivation, economics, subsidy.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an edible cucurbit popular throughout the world for its crisp texture and taste. Cucumber is truly a versatile vegetable because of wide range of uses from salads to pickles and digestive aids to beauty products. The caloric and nutritional value of cucumber is very low but it is a primary source of vitamins, minerals and fibre for human body (Keopraparl, 1997).

The annual production of cucumber in India is 698000 MT from 45000 ha area with productivity of 15.5 t ha^{-1} only during 2012-2013 (Anonymous, 2014). However, the major concerns are low productivity, diminishing return from farming as a whole and lack of awareness among the vegetable growers regarding scientific crop management and quality product (Chattopadhyay et al., 2007). The structure of land holding has also been changing very fast in India because of too much fragmentation leading to more percentage of small and marginal famers. With the advent of modern technologies, the scenario of vegetable industry in India is changing at a fast rate. Now, it is not only a question of providing enough vegetables for a balanced diet, but also to produce quality vegetables throughout the year that are acceptable and competitive in international market. But due to erratic behaviour of weather, the crops grown in open field are often exposed to fluctuating levels of temperature, humidity, wind flow etc., which ultimately affect the crop productivity and quality adversely.

Protected cultivation being the most efficient means to overcome climatic diversity, has the potential of fulfilling the requirements of small growers as it can increase the yield manifolds and at the same time improve the quality of the produce significantly as per the demand of the market. In the recent times, the introduction of partheno-

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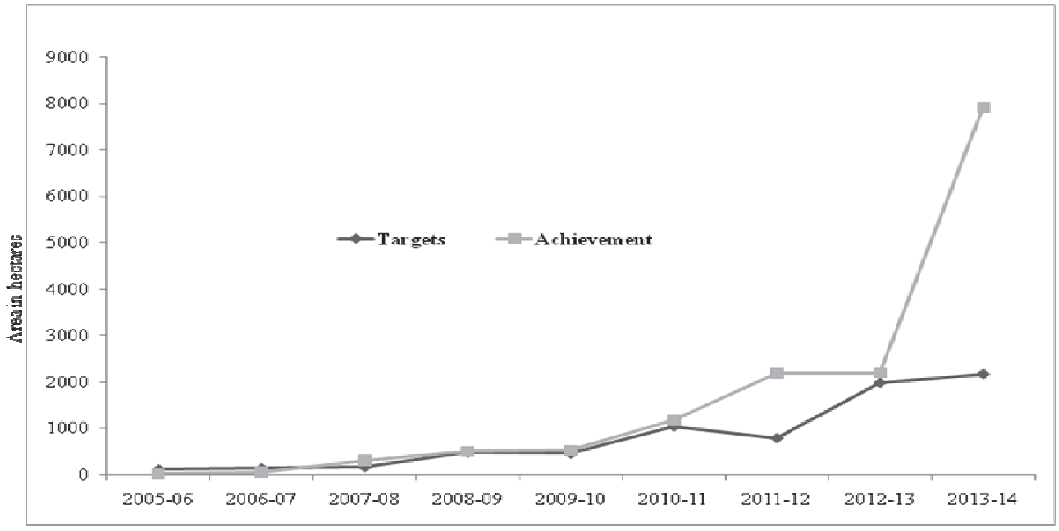

Figure 1. Phase-wise implementation of protected cultivation by Government of Gujarat, India. Source: (http://nhm.nic.in).

carpic varieties in cucumber has revolutionized its cultivation under protected culture in India. Simultaneously, implementation of protected cultivation through various financial schemes such as National Horticulture Mission (NHM), *Rashtriya Krishi Vikas Yojna* (RKVY) and many more at state level have bolstered the adoption of protected cultivation across the country. The Government of Gujarat have been implementing protected cultivation in phase-wise manner by fixing physical targets for each year. It is only due to these efforts that area under protected cultivation in Gujarat has increased from 18.08 (2005-2006) to 7900.74 ha (2013-2014) as illustrated in Figure 1.

The situation of market is always unpredictable and responds varyingly over a period of time even for same growing season in different years. The profitability of any project largely depends on financial gain over a period of time. So, under these circumstances it becomes very interesting to work out the economic feasibility of cucumber cultivation under protected cultivation by taking into account the subsidy factor.

MATERIALS AND METHODS

The economic analysis of two experiments laid under naturally ventilated polyhouse (NVPH) of 1,000 m^2 during April, 2013 and 2014, at Regional Horticultural Research Station, Navsari Agricultural University, Navsari (Gujarat) was carried out. The location is situated at latitude 20°57'N and longitude 72°54'E with an altitude of 12 m above the mean sea level characterized by high humid climate with high annual rainfall of 1,600 to 2,400 mm, mostly concentrated during monsoon from 2nd fortnight of June to September. More specifically, the area falls under Agro-Ecological Situation-III characterized by high humidity in the atmosphere during most parts of the year. A parthenocarpic cucumber cv. Dinamik (Yuksel Tohumculuk Ltd., Turkey) was grown on raised beds having dimensions of 100 of 100 x 40 x 50 cm (width, height and path) at the spacing of 60 x 45 cm and fertigated with N:P:K at the rate of 90÷75÷75 kg per hectare along with common doses of organic manures, *Trichoderma viridi, Pseudomonas inflorescens*.

The produce from both the years was marketed at *Shree* Navsari Jalalpore *Taluka* Horticulture Cooperative Society Ltd., Navsari, Gujarat and average selling rate was worked out accordingly. As the cropping period of cucumber under protected conditions varies from 105-120 days, so three crops per annum can successfully be taken up under agro-climatic conditions of South Gujarat, India. Therefore, data of single season have been considered to work out the annual account. To work out and simplify calculations, the data generated through accounting method was subjected to analysis as suggested by Berry et al. (1979) and Gittinger (1982). The actual values on fixed investment were subjected to amortized accounting by adopting certain assumptions (Table 1). As far as calculation of variable components is concerned, the prevailing market value at that point of time was accounted into analysis first for single season in each year and then converted into expected per annum value.

The component of protected cultivation is being strengthened under National Horticulture Mission by Government of India by imparting 50% subsidy to the farmers. Incentives in terms of subsidy to the tune of 65 and 75% are imparted by Government of Gujarat State (India) to encourage the farmers for adopting protected cultivation by adding its share of 15 and 25% in Central Government subsidy depending upon socio-economic status of the farmers. Therefore, an attempt has also been made to work out comparative trend of economic returns for cucumber cultivation under NVPH in each case (without subsidy, with 65 and 75% subsidy) for the respective year.

RESULTS AND DISCUSSION

Components of fixed cost

It was only the cost of structure, which made huge difference in economic gain for cucumber as protected cultivation is highly capital intensive farming requiring substantial investment during the initial period of establishment. Rezende et al. (2011) and Sreedhara et

Table 1. Adopted assumptions.

S/ No.	Particulars	Useful life (yrs)
1	Polyhouse Structure	10
2	Red soil*	10
3	Rice husk	3
4	Plant support system	5

*Conditional life of red soil has been considered equivalent to that of structure's life assuming that sufficient organic matter will be incorporated into it over the period of time.

al. (2013) had similar observations regarding expenditure incurred on fixed component, thus showing slow response of growers for adoption of this technology. Considering 10 years life of structure, the annual capital investment was divided equally and worked out to US$ 1511.48 per annum. With the involvement of Government in boosting this technology financially, the initial capital investment came down to US$ 529.02 and 377.87 only with 65 and 75% subsidy, respectively. So, it is recommended for the farmers to encash this facility to lower down the huge pressure of initial investment for erecting such structure to economize protected cultivation of crops to a greater level.

Generally, use of red soils for cultivation of crops under protected conditions is suggested because pH of such soils falls in neutral range thus making available most of nutrients applied to the plants. Nevertheless, farmers can use other types of soil owing to the availability and financial status, but care must be taken to amend the soils appropriately depending on the pH of soil and be enriched with organic matter periodically. Rice husk is an important component of protected cultivation for maintaining proper aeration in soil based growing media and also possesses antifungal properties because of the presence of silica. Rice husk is easily and reasonably available in this part of the country as rice is one of the commercial crops being grown in the region. It is also clearly depicted in Table 2 that annual cost of rice husk was very nominal (US$ 1333.00) and remained unchanged over the period of study. Cucumber being a viny crop, needs support to train the plants vertically and moreover, the concept of utilizing vertical space under protected structure is fully justified. The capital investment on this component was also found to be nominal (US$ 32.33) based on its expected life of five years. Going through above enumeration, it is undoubtedly evident that provisions made by the Government in this direction have truly lowered down the financial burden from the shoulders of farmers.

Components of variable cost

Practically, three crops in tandem can successfully be raised in NVPH under Agro-climatic Situations of this region making it possible to supply cucumbers throughout the year. The actual cost of individual components for single season in each year was taken into consideration for calculating the annual investment. The cost of seed, farmyard manure, formaldehyde, *T. viridi, P. inflorescens,* micro-nutrients, vermicompost and pesticides remained the same exhibiting negligible fluctuations in the price of these components in both the years. However, it was the other components like labour wages and packing material, which showed significant variation over the years. It is always recommended to follow sterilization of soil with formaldehyde or any other chemical once in a year to avoid the build up of soil borne pests. So, labour involved in its application made a difference during second year of cultivation as a result of hike in minimum wages. Rezende et al. (2005) also considered labour as the heavier component in total operational cost accounting for 20.6%. Similarly, Rodrigues et al. (1997) in an experiment under protected conditions observed 17% representation of labour in total operating cost. The raised beds were prepared in the first season and would not be dismantled and used with minor cultivation and levelling with labour involved in performing other operations.

The analysis of production system of cucumber under NVPH displayed overall expenditure to the extent of US$ 4428.68 (without subsidy), 3446.22 (with 65% subsidy), 3295.07 (with 75% subsidy) and 4813.89 (without subsidy), 3831.43 (with 65% subsidy), 3680.28 (with 75% subsidy) for respective years of study, 2013 and 2014. So, it could be envisaged that the subsidies imparted by Government of Gujarat made a huge difference particularly in the component of fixed cost. Matsunga et al. (1976) also highlighted the importance of all production factors with a greater emphasis on fixed cost for getting ultimate benefit from a crop. Singh and Kumar (2006) emphasized that economic feasibility of cucumber cultivation largely depends upon the basic cost of erection of greenhouse (Figure 2).

On the basis of average selling rate of cucumber to the tune of US$ 0.32 and 0.22 and average yield of 10.76 t and 11.42 t per 1000 m^2 in the respective years, there was a financial gain of US$ 6007.79 and 2662.84.00 in 2013 and 2014 without considering the subsidy component. Singh et al. (2005) and Sreedhara et al.

Table 2. Economic analysis of cucumber cultivation under naturally ventilated polyhouse.

S/ No.	Particulars	Year 2013-2014			Year 2014-2015		
		Actual	65% subsidy	75% subsidy	Actual	65% subsidy	75% subsidy
(A) Amortized Fixed Cost (US$)							
1.	Structure cost including HDPE sheet and drip irrigation system	1511.48	529.02	377.87	1511.48	529.02	377.87
2.	Red soil	116.39	116.39	116.39	116.39	116.39	116.39
3.	Rice Husk	21.55	21.55	21.55	21.55	21.55	21.55
4.	Plant support system	32.33	32.33	32.33	32.33	32.33	32.33
Total (A)		1681.75	699.29	548.14	1681.75	699.29	548.14
(B) Variable Cost (US$)							
1.	Seed	632.88	632.88	632.88	632.88	632.88	632.88
2.	FYM	45.59	45.59	45.59	45.59	45.59	45.59
3.	Formaldehyde	54.56	54.56	54.56	54.56	54.56	54.56
4.	Application of formaldehye	19.40	19.40	19.40	24.25	24.25	24.25
5.	*Trichoderma viridi*	1.94	1.94	1.94	1.94	1.94	1.94
6.	*Pseudomonas inflorescens*	1.94	1.94	1.94	1.94	1.94	1.94
7.	Micro-nutrients	43.65	43.65	43.65	43.65	43.65	43.65
8.	Vermicompost	77.59	77.59	77.59	77.59	77.59	77.59
9.	Bed preparation	38.80	38.80	38.80	48.50	48.50	48.50
10.	Labour	1396.70	1396.70	1396.70	1745.88	1745.88	1745.88
11.	Pesticides	48.50	48.50	48.50	48.50	48.50	48.50
12.	Fertilizer	208.28	208.28	208.28	215.45	215.45	215.45
13.	Packing	104.36	104.36	104.36	118.67	118.67	118.67
14.	Miscellaneous	72.74	72.74	72.74	72.74	72.74	72.74
Total (B)		2746.93	2746.93	2746.93	3132.14	3132.14	3132.14
Total Expenditure (A+B)		4428.68	3446.22	3295.07	4813.89	3831.43	3680.28
Yield (kg)		32280.00	32280.00	32280.00	34260.00	34260.00	34260.00
Selling Rate (US$/kg)		0.32	0.32	0.32	0.22	0.22	0.22
Gross Realization ($)		10436.47	10436.47	10436.47	7476.72	7476.72	7476.72
Net Realization ($)		6007.79	6990.25	7141.40	2662.84	3645.30	3796.44
Benefit-Cost Ratio		**1.36**	**2.03**	**2.17**	**0.55**	**0.95**	**1.03**

(2013) also substantiated for more remuneration from protected cultivation on account of 3 to 4 times higher and better quality yield. Although, yield of cucumber in 1000 m^2 area was higher during second of experimentation, but average selling rate came down thereby affecting

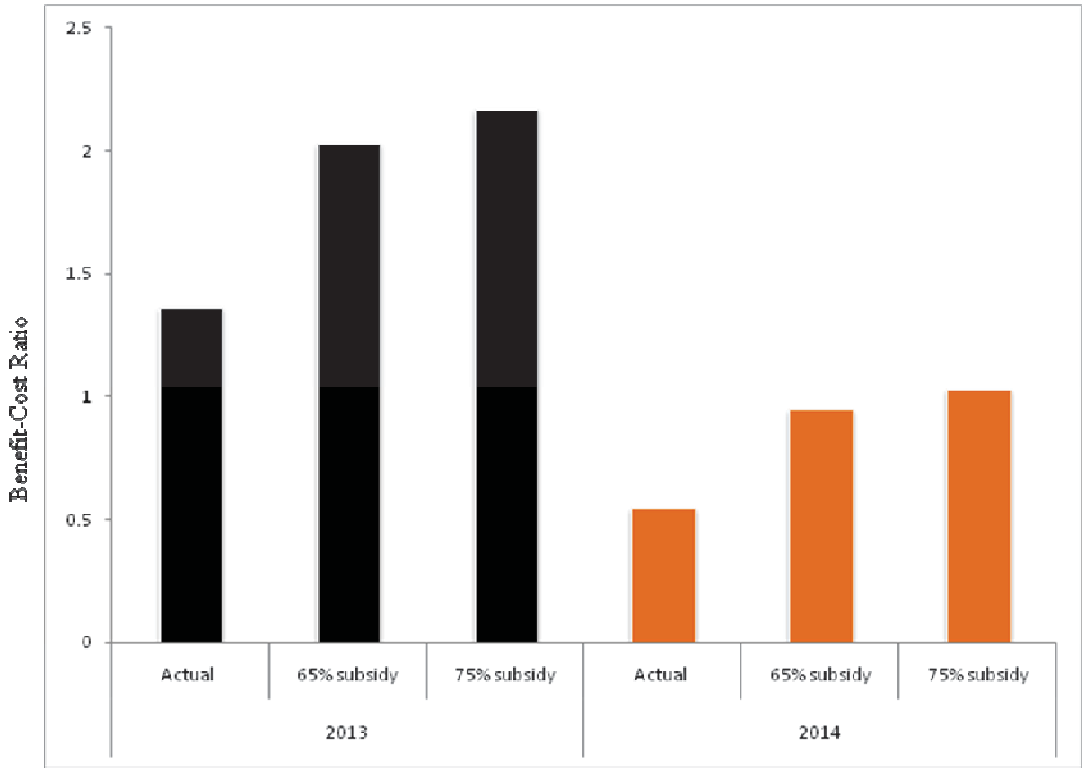

Figure 2. Estimation of benefit cost ratio under various economic situation.

net returns from the crop and highlighting the dynamics of market for the same period in different years. The earlier workers, Engindeniz and Gul (2009) also reviewed that production as well as market risks affect profitability and economic feasibility of vegetables grown under protected structure.

Yilmaz et al. (2005) drew a conclusion that role of local market is very important factor in marketing of greenhouse products and price difference in two production systems indicated that local market has reached to saturation in terms of meeting local demand. So, at this junction it becomes utmost important to encash bigger surrounding markets. Even though, farmers could get very good returns with benefit-cost ratio (BCR) of 2.03, 2.17 and 0.95, 1.03 in the years 2013 and 2014, respectively under different provisions of subsidies and is illustrated in Figure 2. Pozderec et al. (2010) had also highlighted the importance of protected cultivation for better economic returns in cucumber.

Conclusions

The economic analysis shows that cost of fixed component and selling rate of produce were the two important factors deciding net realization of the project. Although, BCR without subsidy was also very good for a crop of short duration under prevailing market price during the period of investigation, but it could be multiplied manifolds with the addition of subsidy component as per the socio-economic status of farmers. Looking in the uncertainty factor particularly in local market as observed in the study, it can opined that this instability must be handled smartly through a cluster approach by the farmers to encash either surrounding/ bigger or even export markets.

Conflict of Interest

The authors have not declared any conflict of interest.

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Chemical profile, somatic cell count and milk production of Holstein, Girolando and Jersolando cows

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The aim of this study was to evaluate the chemical profile, somatic cell count (SCC) and milk production of Holstein, Girolando and Jersolando cows. Fresh milk samples were collected from 34 high-production cows, averaging 31 L/day, belonging to three genetic groups (Holstein, Girolando and Jersolando). Experimental design was completely randomized, in which data collected were submitted to analysis of variance considering the effects of genetic group on milk quality and production. Correlations between milk volume and fat, protein, lactose, total dry extract, defatted dry extract and SCC were assessed. Jersolando herd produced milk with higher content of solids compared to the Holstein and Girolando. In relation to milk production, Girolando cows stood out over the others. Correlations between volume and fat, protein, total dry extract and defatted dry extract were negative; however, lactose showed positive correlation. The dairy system evaluated in this study demonstrated potential to meet the quality requirements of milk related to SCC in the current period and also from 2014, showing that large investments in facilities are not required to obtain quality milk.

Key words: Correlation, mastitis, racial group, productivity, milk quality.

INTRODUCTION

Milk composition has increasing importance for the dairy industry and producers, as it is directly related to processing, industrial production and price. In payment programs, parameters such as fat and protein contents, SCC, total bacterial count (TBC) and milk volume are evaluated.

In these programs, crossbreeding now has the possibility of becoming more popular, since payment is based on the content of solids, production and quality. In tropical countries, native breeds compromise the increase in milk production due to low production levels and European breeds due to adaptive difficulties.

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Crossing involving breeds of Indian origin (Zebu) and breeds of European origin (Taureans) provide the rational use of adaptation to the tropical climate of Indian breeds combined with the productive potential of European breeds.

The interest in crossing, in particular with the Jersey breed, is driven by the profitability potential (Lopez-Villalobos et al., 2000), fertility (Auldist et al., 2007) and longevity (Harris et al., 1996), and with the Holstein breed, the goal is to improve production yields, since this breed produces large milk volumes.

Zebu breeds, especially Gyr, play an important role in Brazilian dairy chain due to their good adaptability and performance under the management conditions prevailing in Brazil, both as a pure breed, as in crosses with specialized dairy breeds, especially Holstein (Santiago, 1975). Due to the importance of knowing the characteristics of the milk produced by animals of different genetic groups in order to fit to quality payment program, the aim of this study was to evaluate the chemical composition and SCC of milk, as well as the milk production of Holstein, Girolando and Jersolando cows, in the dry period.

MATERIALS AND METHODS

Study location and description

The study was conducted according to the ethical principles of animal experimentation of the "Ethics Research Committee with Animal Models", Federal Institute of Education, Science and Technology of Goiás - Rio Verde Campus - GO under No. 031/2012. The study was conducted on a dairy farm at the municipality of Rio Verde - Goiás, located on Highway GO 174, Km 05, which had a herd of 94 Holstein, Gyr Girolando and Jersolando cows divided into three different groups namely, animals with high (31 L milk / day), intermediate (16 L milk / day) and low (10 L milk / day) milk production. The milk produced in the farm was transferred to a dairy industry located in the city of Goiania - Goias. The study was conducted in the dry period.

Fresh milk samples were collected during morning milking (5:30 am) only from the first group (animals of high production), which had the same average milk production and received the same diet. The group consisted of 34 high-production cows, with average 31 L milk / day, belonging to three distinct genetic groups, which were selected from the blood degree as follows: Group 1 (13 Holstein cows), Group 2 (16 cows with genetic composition from ¾ to ⅞ Holstein / Gyr, called Girolando) and group 3 (five cows with genetic composition ¼ Holstein and ¾ Jersey, called Jersolando). All cows were on average at 100 days of lactation, 90 months of age and 510 kg of body weight.

Animals were submitted to two daily milkings in a mechanized milking system with closed circuit and six sets provided of collectors. Morning milking began at 5:30 am and the afternoon milking at 04:30 pm, lasting two and a half hours each milking. All animals were submitted to artificial insemination. Milk production was monitored throughout the experimental period (3 months) through milk meters connected to the milking machine in the morning and afternoon milking, totaling the daily production of animals. The result was expressed in kg.

Animals had access to clean water and balanced diet, offered according to the nutritional requirements and between milkings, cows remained on pasture that was not the main source of food but rather a resting place. Diets were formulated according to NRC (2001) for dairy cows with an average of producing 31 kg milk/day with 3.0% milk fat.

During the experimental period, cows received the following in the trough during milking (twice daily): 4 kg of feed, distributed into two milkings and 8 kg of feed and corn silage *ad libitum* in trough outside the milking room. The feed consisted of corn germ, soybean meal, corn grits, cotton cake, uremax, optigem and cooked soybean core, according to Table 1.

Sample collection and milk analysis

The collection procedure followed the norms of good milking practices: teats were washed with water and the first three milk jets were discarded in a black bottom mug to verify the presence of lumps, then, with the aid of an applicator, teats were immersed in pre-milking solution based on sodium hypochlorite and waiting 25 s to obtain total product efficiency. After cleaning, teats were dried with paper towels for the coupling of teatcups, milk samples were collected in individual collectors and after complete milking of animals, the amount of milk produced was individually recorded in field record; subsequently, milk was packaged in sterile flasks containing preservative Bronopol®, homogenized and stored in isothermal boxes containing ice and sent to the Laboratory of Milk Quality, Food Research Center, School of Veterinary and Animal Science, Federal University of Goiás (LQL / CPA / UFG) for the performance of electronic analyses to determine milk components (fat, protein, lactose, defatted dry extract (DDE), total dry extract (TDE) and SCC. At the end of milking, post-milking solution was used, whose base was 0.25% glycerin iodine.

Milk quality assessment

Proximate composition

Fat, protein and lactose contents, DDE, TDE were determined using the analytical principle based on the differential absorption of infrared waves by milk components using equipment MilkoScan 4000 (Foss Electric A / S. Hillerod, Denmark) (International Dairy Federation, 2000). The results were expressed as percentage (Table 1).

Somatic cell count

SCC, whose analytical principle is based on flow cytometry, was performed using equipment Fossomatic 5000 Basic (Foss Electric A / S. Hillerod, Denmark), (International Dairy Federation, 2006). The result was expressed in SC / ml.

Statistical analysis

Animals were divided into three groups according to breed. Group 1 (13 Holstein cows with 135 repetitions), Group 2 (16 Girolando cows with 162 repetitions) and Group 3 (05 Jersolando cows with 81 repetitions) totaling 34 animals. The experimental design was fully randomized and data collected were submitted to analysis of variance considering the effects of genetic group on milk quality and productivity. Analysis also used Tukey's test at 5% probability and SISVAR software (Ferreira, 2003). Linear correlations between milk volume and fat, protein, lactose contents, DDE, TDE and SCC were assessed. Statistical procedures were performed using the ASSISTAT software.

Table 1. Proximate and chemical composition of diets offered to cows.

Total diet ingredients	**%**
Corn silage	48.13
Corn germ	24.24
Soybean meal 44%	06.28
Corn grits	05.04
Cotton cake	07.14
Uremax	00.55
Optigem	01.10
Cooked soybean core	07.52
Total	100
Chemical composition of total diet	**%**
Crude protein	16.7
Ether extract	04.5
Neutral detergent fiber	31.9
Acid detergent fiber	16.8
Total digestible nutrients	69
Calcium	00.8
Phosphorus	00.4

Table 2. Influence of breed on chemical composition and milk production.

Breed	**Chemical composition (%)**					**Milk production (Kg)**
	Fat	**Protein**	**Lactose**	**TDE**	**DDE**	
Holstein(n=135)	2.93 [b]	3.03 [ns]	4.67 [b]	11.60 [b]	8.67 [b]	31.72 [b]
Girolando (n=162)	2.70 [c]	3.01 [ns]	4.59 [c]	11.27 [c]	8.57 [c]	34.40 [a]
Jersolando (n=81)	3.18 [a]	3.06 [ns]	4.80 [a]	12.04 [a]	8.86 [a]	22.36 [c]
Mean	**2.89**	**3.03**	**4.66**	**11.55**	**8.66**	**30.86**
CV (%)	**22.32**	**7.4**	**4.91**	**6.85**	**4.02**	**16.92**

Means followed by different letters in the column are statistically different * ($p<0.05$). * NS = not significant ($p>0.05$). TDE: Total Dry Extract; DDE: Defatted Dry Extract; CV: Coefficient of Variation.

RESULTS AND DISCUSSION

In Brazil, there are few studies on dairy cattle evaluating the productivity of cows, especially with Jersolando animals. The evaluation of the milk production of these animals in breeding systems used in Brazil, especially in southwest Goiás, enable obtaining results that demonstrate the productive potential and the characteristics of milk of these animals in this particular environment and management conditions.

Table 2 shows the fat, protein and lactose contents, TDE, DDE and milk production of Holstein, Girolando and Jersolando cows mechanically milked at a dairy farm in the Southwestern region of the state of Goiás. Regarding the chemical composition of milk, effect ($p<0.05$) of the different breeds on the fat and lactose levels, TDE and DDE was observed, as well as on milk volume (Table 2).

By analyzing the coefficient of variation (CV), it was observed that the experimental precision was adequate for all variables under study, and fat content was the most unstable response with respect to CV, equal to 22.32%, due to the increased variation range in relation to other components. The fat content of milk from Jersolando cows was higher (3.18%), followed by Holstein cows (2.93%) and Girolando cows, whose milk showed the lowest fat percentage (2.70%). The fat contents of 2.93 and 2.70%, which refer to Holstein and Girolando breeds respectively, are not within the limits established by Brazilian legislation, Normative Instruction 62 of 2011, which recommends minimum values of 3.0% (Brazil, 2011); however, the fat content of milk from Jersolando cows (3.18%) was above the minimum required by Normative Instruction 62 of 2011 (IN 62/2011).

This result may be related to the lower volume of milk produced by Jersolando cows, explaining the higher fat content, since as the production potential increased, the fat percentage decreased. Studies on the quality of milk from Jersolando cows are scarce and there are no scientific reports on the characteristics and volume of milk produced by these animals. Fat contents lower than those found in this experiment were observed by Raimondo et al. (2009) in a study conducted in the state of São Paulo with Jersey cows in the first month of lactation, obtaining 2.39 to 2.97% fat.

Similar to our findings, but with Jersey cows, Botaro et al. (2011), in a study in the state of São Paulo from 2007 to 2008 found higher fat contents in the milk from Jersey cows (3.97%) compared to Holstein and Girolando cows, which showed no significant difference from each other (3.54 and 3.45%, respectively). The result of this study in relation to the fat percentage of milk from Holstein cows (2.93%) was lower than that found by Santos et al. (2009), who observed high fat content in the milk from Holstein cows (4.30%) using soybean oil in the diets of cows during the transition period. Paula et al. (2008) and Stelzer et al. (2009) observed similar fat contents (3.40%), but higher than those found in this work.

Dairy breeds of European origin have specialized function for milk production and high feed utilization efficiency, but suffer from physiological and behavioral problems caused by heat stress, slowing production and consequently concentrating nutrients; on the other hand, animals of Indian origin are more adapted to tropical climate. The protein content in milk of the present study did not differ between breeds ($p>0.05$), regardless of genetic group evaluated, and the minimum protein content was achieved according to Brazilian legislation, which recommends values above 2.9%. Unlike this research, Botaro et al. (2011) observed significant differences among breeds (Jersey, Holstein and Girolando), where Jersey cows showed higher protein percentage in milk (3.38%) compared to the other groups that showed no difference from each other (3.21 and 3.22%, respectively).

As found in this study, Deitos et al. (2010) also observed no difference ($p>0.10$) between breeds (Holstein and Brown Swiss) in the state of Paraná, with mean values of 3.15 and 3.17% protein, respectively. Changes of protein content of milk are less significant and although influence total production, have little variation in milk (Dürr, 2002).

The average lactose content in milk was 4.80, 4.67 and 4.59% for Jersolando, Holstein and Girolando breeds, respectively, showing significant difference among themselves ($p<0.05$). Similarly to results obtained in this study, Deitos et al. (2010), in a study with 32 ½ blood Holstein and Brown Swiss cows found no variation between groups ($p>0.10$), with average content of 4.61% lactose in milk. Smaller lactose percentages were observed by Botaro et al. (2011), who obtained average lactose content in the milk of Holstein, Jersey and Girolando cows of 4.42, 4.30 and 4.45%, respectively.

In this study, it was observed that there was variation in the lactose percentages according to breed, and Jersolando cows showed lower production potential compared to the other breeds in this study, which may explain the higher concentration of lactose in the milk from these animals. Furthermore, the lactose levels in milk depend on the glucose produced in the liver from the propionic acid produced in the rumen, and this acid is produced in greater proportion when adequate amounts of concentrate is supplied to animals (Pereira, 2000) and milk collections in this work were carried out during the dry season when forage availability is scarce and animals receive concentrate-based diets. The Brazilian legislation does not establish minimum lactose content for refrigerated raw milk.

Total dry extract, which results were 12.04, 11.60 and 11.27% for Jersolando, Holstein and Girolando animals, respectively, showed significant difference among breeds ($p<0.05$). Data obtained in this study were lower than those found by Ponce (1996), who reported average values of 13.83% for Zebu cows due to the characteristics of the breed of raising the fat content in milk and consequently increase total solids.

Similar to results of this study, but with different breed, Deitos et al. (2010) also found significant difference ($p<0.10$) in the TDE content of milk from Brown Swiss (12.17%) and Holstein cows (11.72%). Total solids in milk represents the sum of all milk constituents (except water) and fat is most responsible for its change, thus, the results found in this study can be attributed to the higher fat and lactose contents present in milk from Jersolando and Holstein cows. The DDE percentage was influenced by breeds ($p<0.05$), and the highest DDE concentrations were observed in milk from Jersolando cows (8.85%) compared to Holstein (8.67%) and Girolando cows (8.57%).

In contrast to our study, Deitos et al. (2010) found no significant differences ($p>0.10$) in relation to DDE between genetic groups (Holstein and Brown Swiss), obtaining mean value of 8.75%. Defatted dry extract includes all milk constituents except fat, thus, it is related to the amount of milk nutrients aimed at cheese production. The differences among genetic groups evaluated in this study for DDE can be attributed to variations in the milk constituents of the different groups. Brazilian law recommends minimum DDE content of 8.4% for fresh milk (Brasil, 2011). Girolando cows demonstrated greater production potential (34.40 L milk /day) compared to Holstein (31.72 L milk/day) and Jersolando cows (22.36 L milk/day). These results confirm that Girolando cows produce higher milk volume due to the robustness of Gyr and good productivity of Holstein cows. Although animals of European origin are known for high production potential, in this study, Holstein and Jersolando cows showed lower productivity, and this

Table 3. Influence of breeds on somatic cell count of fresh milk.

Breeds	Somatic cell count	
	SC/ml x 1000	Log
Holstein	294.68 ^{ns}	2.16 ^{ns}
Girolando	333.86 ^{ns}	2.18 ^{ns}
Jersolando	454.12 ^{ns}	2.02 ^{ns}
Mean	**345.64**	**2.14**
CV (%)	**272.92**	**24.23**

*NS = not significant (p>0.05). CV: Coefficient of Variation.

result may have occurred due to the greater sensitivity of these animals to higher temperatures, since sampling was performed during the dry season. According to Aguiar and Baccari (2003), high temperatures associated with high humidity and intense solar radiation are responsible for decrease in milk production of cows of intermediate and high production.

Similarly to this study, Heins et al. (2008) in confined system in the United States, observed higher milk production in Holstein cows compared to ½ Holstein x Jersey (7705 vs.7147 kg). Several studies such as Lopez-Villalobos et al. (2000); Auldist et al. (2007) and Heins et al. (2008) found that Holstein x Jersey crossbred cows produced approximately 93% of the amount of milk of pure Holstein cows, regardless of production system. Thus, crossbreeding offers advantages such as complementarity and hybrid vigor and most crosses between specialized dairy animals are based on Holstein breed, with higher milk production, a feature that was not observed in this study due to the lower adaptation to the tropical climate of the southwestern region of the state of Goiás. Jersey, known for high concentration of solids in milk, as could be observed in this study, rapid maturity and higher fertility (Freyer et al., 2008) and Gyr, for good adaptability and performance under the management conditions prevailing in Brazil, also observed in this experiment.

Table 3 shows the SCC results of milk from Holstein, Girolando and Jersolando cows mechanically milked at a dairy farm in the Southwestern region of the state of Goiás, which also showed the SC values according to breed, with no significant results (p>0.05). The results are presented as arithmetic mean, but when analyzing the CV (272.92%), it was observed that the experimental accuracy was not appropriate due to the lack of normal distribution of data, so the data were transformed using the logarithmic function and also presenting in logarithm with CV (24.23%).

Regardless of genetic group evaluated, it was observed that the milk SCC is within values recommended by Normative Instruction 62 of 2011, which establishes maximum count of 600,000 SC/ml, up to June 30, 2014, when it will be reduced to 500,000 SC/ml; however, even considering that the average SCC values are within current legislation, improving measurement and mastitis control should be performed, since the milk from the mammary glands of healthy animals contains 50 to 200,000 SC/ml (Kitchen, 1981).

Results above those found in this study were found by Botaro et al. (2011), who obtained scores of 639,000 SC/ml.; 567,000 SC/ml and 578,000 SC/ml for Holstein, Jersey and Girolando cows, respectively.

According to Souza et al. (2005), factors such as milking system, type of equipment, cleaning and disinfection of teats, number of calving, stage of lactation and diet can influence the SCC in milk. Thus, the SCC present in milk is a general indicator of the mammary gland health, widely used as an indicator of subclinical mastitis, being also accepted as a standard measure for determining the quality of refrigerated raw milk (Tsenkova et al., 2001). Table 4 shows the results of the simple linear correlation analysis among variables milk volume and quality. There was a negative correlation (p<0.01) between milk volume and fat content of Holstein and Girolando cows. This result indicates that the higher the milk volume, the lower the fat content in milk due to the dilution effect; fat concentrations in this study tended to be lower as there was an increase in milk production in the different breeds analyzed (Table 3). For Jersolando cows, milk fat and volume were not correlated (p>0.05), and this may have occurred due to the decreased milk production observed in this group as well as to the higher fat percentage in milk, which is concentrated as a function of the lower milk volume.

Similarly, Ribeiro et al. (2009) found mean fat content of 4.42% in the milk from animals producing up to 10 kg/milk/day, demonstrating that in lower milk volume, fat concentration is higher. Negative correlation was also observed (p<0.05) between production volume and protein content of milk from Girolando and Jersolando cows, with no correlation for variables among Holstein cows (p>0.05). This result is also related to the higher milk volume produced, with average of 31 L of milk/day, diluting the protein content (Table 4). These data corroborate those found by Galvão Junior et al. (2010), who observed a decrease in the protein content as the production of animals increased from 3.88% protein in the milk of animals producing 8.41 L milk/day; 3.56%

Table 4. Linear correlation between volume and quality of milk from Holstein, Girolando and Jersolando cows.

Correlation	Breeds		
	Holstein	Girolando	Jersolando
Volume x Fat	-0.2396 **	-0.3243 **	-0. 0858 ns
Volume x Protein	-0.1635 ns	-0.1685 *	-0.2448 *
Volume x Lactose	0.1950 *	-0.0909 ns	0.0097 ns
Volume x TDE	-0.1727 *	-0.3317 **	-0.1336 ns
Volume x DDE	0. 0543 ns	-0. 1841 *	-0.1707 ns
Volume x SCC	-0.1487 ns	0.0654 ns	0.1341 ns

** Significant at 1% probability level ($p<0.01$). * Significant at 5% probability ($0.01<p<0.05$). NS: not significant ($p>0.05$). TDE: total dry extract; DDE: defatted dry extract; SCC: somatic cell count.

protein for milk production of 12.63 L/day and 3.43% protein when cows produced more milk (milk 20.28 L/day).

There was a positive correlation ($p<0.05$) between production and lactose content in milk from Holstein cows. For Girolando and Jersolando cows, variables milk volume and lactose were not correlated ($p>0.05$). The synthesis of lactose by the glandular epithelium significantly affects the amount of milk produced due to the critical role of lactose as an osmotic regulator of milk volume (Machado et al., 2000). This result demonstrates that the greater the milk volume, the higher the lactose concentration, because the higher the lactose synthesis, the larger the amount of water drained into alveolar cells. Since Holstein cows are known for their high production potential, this correlation could be observed in their milk. Unlike results obtained in this study, Galvão Junior et al. (2010) observed that the average lactose content (4.82%) was higher when animals produced lower milk volume (15 L milk/day).

Negative correlation was also observed between production volume and TDE content in milk from Holstein ($p<0.05$) and Girolando cows ($p<0.01$), and no correlation was found for this variable in the Jersolando herd ($p>0.05$). Increase in milk production tends to dilute components of the dry extract. Thus, variables negatively correlated with milk production in this study are mainly components of the milk dry extract (fat and protein).

Similarly to our study, Galvão Junior et al. (2010) observed correlation coefficient of -0.3358 for TDE, according to the average daily production of Zebu cows.

In this study, DDE was only correlated with milk volume in Girolando cows ($p<0.05$), thus, the variation in protein content observed in the milk from these animals can justify the decrease in DDE obtained in this study, since DDE includes all milk constituents except water and fat. As in this research, Galvão Junior et al. (2010) found negative correlation between milk volume and DDE (-0.4700) in zebu cows. There was no correlation ($p>0.05$) between milk volume and SCC in the different breeds. Corroborating the results observed in this study, Galvão Junior et al. (2010) found no correlation between milk production and SCC (0.0629) in zebu cows. There are several factors that affect SCC such as the level of infection of the mammary gland, season, stage of lactation and age; however, milk volume did not affect SCC of milk from cows studied in this research.

A peculiar feature of the Brazilian livestock, especially in dairy cows, is the great variability of production systems. This makes the correct choice of a certain breed or crossing of breeds for these herds even more difficult. Thus, the production potential of every breed and production systems used in the country should be studied for the correct selection of a particular breed for the different production systems. Since the choice of the genetic group is an optimization element, investigating the characteristics of the milk produced among the major milk-producing breeds in Brazil and used mainly in the southwestern of the State of Goiás is relevant, since payment programs evaluate quality parameters such as production, content of solids and SCC.

Conclusions

The results of this research indicated that the use of Jersolando cows in dairy herds is a good alternative to increase fat, protein and DDE contents, which are the main parameters used in payment programs for milk produced by dairy systems. In the environmental conditions of the southwestern region of Goiás, Girolando cows showed better milk production potential compared to Holstein and Jersolando cows. However, considering that payment programs in the southwestern region of Goiás prioritize volume and concentration of milk nutrients, Holstein cows may result in better financial returns to the dairy producer because these cows show good production potential and chemical composition of milk compared to Girolando cows. The dairy system evaluated in this study demonstrated potential to meet the quality requirements of milk related to SCC in the current period and also from 2014, showing that large investments in facilities are not required to obtain quality milk.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of biochar application on microbial biomass and enzymatic activities in degraded red soil

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To assess biochar effect on soil microbial biomass, community and enzymatic activities, degraded acidic soil was amended with three different rates (0.5, 1.0 and 2.0%) of oak wood biochar ($W_{0.5}$, $W_{1.0}$ and $W_{2.0}$) and bamboo biochar ($B_{0.5}$, $B_{1.0}$ and $B_{2.0}$), with control as 0%. The soil and the biochar were mixed thoroughly, wetted and incubated at a constant temperature of 25°C. The amended soil properties were evaluated after the 1st, 8th and 16th weeks of the incubation. It was found that soil pH, total organic carbon (TOC) and urease increased significantly with increasing biochar rate while the activity of acid phosphatase decreased, the reason can be the inverse correlation of this enzyme with soil pH. TOC had positive correlation with urease. The β-glucosidase correlated positively with dissolved organic carbon (DOC) and negatively with C/N, suggesting that mineralization of organic matter provides substrates for this enzyme. The highest microbial biomass C as well as total Phospholipid fatty acid analysis (PLFA) was observed at the lowest rates, particularly the treatment of W0.5 had higher relative abundance of soil bacteria, fungi and gram-positive bacteria. Our results suggest that biochar application improve the fertility of degraded red soil by increasing soil pH, TOC and DOC which, in turn, enhance soil enzymes, microbial biomass and community.

Key words: Biochar, enzymes, microbial biomass, microbial community, phospholipid fatty acids.

INTRODUCTION

Currently, in response to the need of more sustainable agricultural production and in order to tackle global warming, there are attempts to recreate Terra Preta (ancient soils amended with black carbon) (Glaser, 2007) by incorporating biochar to soils as means of increasing soil fertility and carbon sequestration (Lehmann et al., 2006). Biochar is the carbon-rich product obtained when biomass is heated in a closed container with little or no available air with the purpose to amend soil (Lehmann and Joseph, 2009).

Biochar has been widely and increasingly proposed as soil amendment (Lehmann and Joseph, 2009; Sohi et al., 2010). By increasing soil pH, biochar has been proved to ameliorate soil acidity (Yuan et al., 2011). This effect could particularly benefit China where soil acidification is a major problem in soils of intensive agricultural systems such as extremely leached red soils (Argi-Udic Ferrosols) and yellow soils (Ali- Periudic Argosols), the most acidic

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of them in south China are approaching pH values at which potentially toxic metals such as Al and Mn could be mobilized (Guo et al., 2010).

Interactions between biochar, soil, and microbes are known to occur within a short period of time after application to the soil (Lehmann and Joseph, 2009). Dissolution, hydrolysis, carbonation and decarbonation, hydration, and redox reactions are the major process affecting biochar weathering in soil, as well as interactions with soil biota. The rates at which these reactions occur depend on the nature of the reactions, type of biochar, and pedoclimatic conditions.

It is widely recognized that organic matter plays and essential role in a range of soil physical, chemical and biological process and that soil organic carbon is important in maintaining soil quality (Ghosh et al., 2012). Biochar, as a soil amendment, can increase concentrations of soil organic matter, especially water-extractable organic carbon (Lin et al., 2012), increase microbial biomass (Kolb et al., 2009), stimulate soil microbial activity (Lehmann et al., 2011), change microbial community in soil (Pietikainen et al., 2000; Wardle et al., 2011). Biochars application in soil can affect soil microbial community structure due to their high sorption capacity (Lehmann et al., 2011), changing the soil pH (Rousk et al., 2010) as well as modification of microbial environment (Jindo et al., 2012a). Painter (2001) reported that biochars may contain compounds such as polycyclic aromatic hydrocarbons and other toxic carbonyl compounds that can have bactericidal or fungicidal activity. However, Ogawa (1994) has shown that these substances can, and do, serve as C and energy sources for selected microbes.

Due to intensive agricultural activities along with abundant moisture and high temperature in the region, the red soil used in this experiment was low in carbon content (Zhang and Xu, 2005), and soil pH. Hence, we hypothesized that the application of biochar (pyrolyzed at 600°C with high pH value) improve soil organic carbon and soil pH and would have effect on soil enzymes, microbial biomass and community that support many key ecosystem functions essential for soil quality. Therefore, the objective of this study was to evaluate the effect of oak wood and bamboo biochar on soil pH, total organic carbon (TOC) dissolved organic carbon (DOC), soil enzymes, microbial biomass and community of soil.

MATERIALS AND METHODS

Soil sampling

A laboratory incubation was carried in the facilities of the College of Environmental and Resource Science, Soil Laboratory, Zhejiang University, Hangzhou, China. The experimental soil for this study was sampled at Meijiawu, suburban of Hangzhou. The soil is highly weathered (Plinthic Hapli Udic Ferrosols in Chinese Soil Taxonomic Classification System; and Typic plinthustults in Soil Taxonomy), derived from quaternary red clay and characterized by low pH. The Land use was tea plantation.

Biochar preparation and characterization

The biochars used in the incubation were purchased from a company located in Hangzhou city (Linan Yaoshi charcoal production Limited) and obtained from oak wood (*Quercus phillyraeoides*) and bamboo (*Phyllostachy edulis*) after pyrolysis at 600°C for 2 h. These feedstocks (oak wood and bamboo) were chosen to represent woody and grassy biomass. The pH was determined in deionized water at the ratio of 1:10 wt/v (Gaskin et al., 2008) by Orion 720 pH meter. The carbon, hydrogen, and nitrogen contents of the oak wood biochar were determined using a CHN elemental analyzer (Flash EA 1112, Thermo Finnigan). The oxygen content was estimated by mass difference (1000 – C, H, N and ash). The ash content was determined according to ASTM D-1762-84 (2007) by combusting the biochar at 750°C for 6 h in open crucibles on a dry weight basis. The BET (Brunauer-Emmet-Teller) surface areas was measured via N_2 adsorption multilayer theory using a Nova 2200e surface area analyzer (Quantachrome, Boynton Beach, FL) (Chen et al., 2008).

Incubation experiment

The soil sample was passed through 5 mm sieve and placed in the plastic pots, and oak wood biochar and bamboo biochar (sieved at 0.25 mm) were added to the soil (toal 2 kg). On basis of soil weight, the biochars were added with three different rates (0.5, 1.0 and 2.0%) of oak wood biochar ($W_{0.5}$, $W_{1.0}$ and $W_{2.0}$) and bamboo biochar ($B_{0.5}$, $B_{1.0}$ and $B_{2.0}$), with control as 0%. After mixing the soil and the biochar thoroughly, they were wetted with deionized water to about saturation of the experimental soil. All pots were covered with plastic film and then a small hole was made to allow gaseous exchange. The pots were incubated at a constant temperature of 25°C. Based on evaporation loss, the soil moisture was kept constant by regular weighing of the pots. For each treatment, triplicate samples were prepared. After Week 1, 8 and 16, the incubated soils were taken and separated into three groups: The first group samples were air dried and sieved with 2 mm and 0.25 mm. These samples used for analysis of chemical properties. The second groups were sieved with 2 mm to determine soil microbial biomass C and N and enzymatic activities. The third group were freeze-dried and preserved at -70°C in refrigerator, these group used to determine phospholipid fatty acids for microbial communities in soil.

Analysis

Soil basic properties

Particle size distribution was determined by pipette method. Soil pH was determined through a suspension sample with a soil (air-dried) to water (w/v) ratio of 1:2.5 and measured with Orion 720 pH meter (Pansu and Gautheyrou, 2006). Soil organic carbon was determined by dichromate oxidation (Nelson and Sommers, 1982). Total nitrogen (TN) in soil was measured using the Kjeldahl method after H_2SO_4 digestion in the presence of K_2SO_4-$CuSO_4$-Se catalyst (Bremner, 1996).

Dissolved organic carbon (DOC)

Soil dissolved organic carbon (DOC) was determined by the method of Jones and Willet (2006). The extracts were analyzed for carbon concentration with a multi N/C analyzer (Flash EA 1112,

Thermo Finnigan).

Enzyme activity

The activities of acid phosphatases were determined by a slightly modified method of Tabatabai and Bremner (1969) at pH 6.5 with p-nirophenyl phosphate (pNPP) solution used as substrate. β-glucosidase activity was measured following the method described by Tabatabai (1982). This method is based on the colorimetric estimation of the p-nitrophenol (PNP) formed by the hydrolysis of the p-nitro-phenyl- β-D-glucopiranoside (PNG) at 37°C for 1 h. The estimation of urease activity was carried out following the method described by Li (1996). Briefly, 10 g soil sample was taken into 100 ml conical flask, and 10 ml of (100 g L^{-1}) urea solution and 20 ml citric acid buffer (pH 6.7) were added into the flask. The soil sample was incubated at 37°C for 24 h. After incubation, the solution was diluted to 100 ml and filtered. Of the filtrate, 1 ml was taken into 50 ml volumetric flask, and 10 ml distilled water, 4 ml of sodium phenolate and 3 ml of sodium hypochlorite were added. Then, it was mixed and made the volume to 50 ml with distilled water, and absorbance of color was checked at 578 nm.

Microbial biomass C and N

Microbial biomass (C and N) were determined by fumigation extraction method (Vance et al., 1987). The extracts were measured for C and N concentration with a multi N/C analyzer (Flash EA 1112, Thermo Finnigan). Microbial biomass C was calculated as follows: microbial biomass C $=E_C/ K_{EC}$, where E_C= [(organic C extracted from fumigated soils) minus (organic C extracted from non-fumigated soils)] and K_{EC}= 0.45 (Wu et al., 1990). Microbial biomass N was calculated as follows: microbial biomass N $=E_N/K_{EN}$, where E_N= [(total N extracted from fumigated soils) minus (total N extracted from non-fumigated soils)] and K_{EN} = 0.54 (Brookes et al., 1985).

Phospholipid fatty acid analysis (PLFAs)

After 8 and 16 weeks of incubation, the incubated soil samples were sieved (<2 mm), freeze-dried and stored at -70°C. Using the freeze-dried soil samples, PLFAs were extracted and identified according to Wu et al. (2009). Lipids were extracted using a single-phase chloroform–methanol–citrate buffer system. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns (Supelco, Inc., Bellefonte, PA). After methylation of the polar lipids, PLFA methyl esters were separated and analysed in an Agilent 6890 N Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE). The fatty acid 19:0 was added as an internal standard before methylation and fatty acid methyl esters were identified automatically by the MIDI peak identification software.
The identified fatty acids were taken to represent different microbial groups: PLFA 18:1ω9c was taken as a fungal biomarker, monounsaturated and cyclopropyl fatty acids as Gram-negative bacteria biomarkers, iso- and anteiso-fatty acids as Gram-positive bacteria biomarkers, straight chain saturated fatty acids as bacteria biomarkers and carboxylic acids with a methyl function on the carbon chain as actinobacteria (Federle et al., 1986; Frostegard et al., 1993a, b; O'Leary and Wilkinson, 1988; Zelles, 1999; Zelles and Bai, 1994; Zogg et al., 1997). The ratio bacteria to fungi were determined. All results are given in nmol g^{-1}.

Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) using SAS statistical analysis software version 9.1. Microbial C and N, soil enzymes, soil pH, total organic carbon, total nitrogen and dissolved organic carbon were analyzed by two-factor ANOVA to compare treatments across time and one-factor ANOVA was deployed to compare treatment effects at any given time. The least significant difference (LSD at 0.05 level of probability) test was applied to assess the differences among the means. Principal component analysis (PCA) was performed on individual fatty acids. Pearson's coefficient analysis was used for correlation.

RESULTS AND DISSCUSION

Biochar effects on soil pH, DOC, total organic C and N

Incorporation of biochar to soils could result in an increase or decrease in soils pH, depending on the pH and liming value of the biochar (Lehmann et al., 2011). The soil used was degraded soil which had an acidic pH of 4.57, whereas the pH of oak wood and bamboo biochar (10.25 and 10.22, respectively) were basic (Table 1). Due to the dissolution of the alkaline minerals, the pH in the amended soils increased with increasing application rate with the highest pH value measured in W2.0 and the lowest in the control for each time of incubation (Table 2). In contrary, the pH decreased with increasing time of incubation. The maximum pH (4.87) was recorded at Week 1 and the lowest (4.46) was measured at Week 16 (Table 2). The reason for a pH decrease through incubation times can be oxidation of C to form acidic carboxyl groups as described by Lehmann et al. (2011).

The soil used in this experiment was highly degraded with low amount of C (5.5 g kg^{-1}), therefore amendment of this soil with organic matter is unquestionable. Biochar, pyrogenic organic matter (PyOM) (Santos et al., 2012) contains a considerable organic matter (Schmidt et al., 1999). Recent research findings also showed that biochar increase concentrations of soil organic matter (Lin et al., 2012). As shown in Table 2, there was a sharp increase in total organic C (TOC) with increasing biochar application, which was due to the high C content of the oak and bamboo biochars. Due to its sensitivity to heating, the N content of the biochars used in this experiment was low (Tyron, 1948). Hence, the C/N ratio kept the trend of total organic C, increased with increasing the application rate. As to time effect, both TOC and total N (TN) decreased with increasing time of incubations and the reverse was observed in C/N.

DOC represents a small proportion of soil organic matter, but is of significant importance in the soil ecosystem due to its mobility and reactivity (Lin et al., 2012). The bamboo biochar treated soils had higher DOC than the oak wood biochar (Table 2). The reason could be the higher labile organic carbon content in bamboo biochar than oak wood biochar. The DOC was higher in B1.0 (46.57 mg kg^{-1}) followed by B0.5 (43.14 mg kg^{-1}) at Week 1. Similarly, at Week 8, DOC was higher in B1.0

Table 1. Basic property of soil, oak wood and bamboo biochars.

Property	Soil	Oak wood biochar	Bamboo biochar
Sand %	22	ND	ND
Silt%	40	ND	ND
Clay%	38	ND	ND
Texture	Clay Loam	ND	ND
pH	4.57	10.25	10.22
Total C (g kg^{-1})	5.50	758.10	759.20
Total N (g kg^{-1})	0.90	6.40	11.60
Total P (mg kg^{-1})	881.28	897.90	1098.33
Total K (g kg^{-1})	12.70	9.94	19.76
Hydrogen (g kg^{-1})	ND	11.20	21.10
Oxygen (g kg^{-1})	ND	104.90	64.50
Ash (g kg^{-1})	ND	119.40	143.60
Surface area (m^2g^{-1})	ND	154.6	137.7

ND- not detected, Oxygen =1000- (C+N+H+ Ash).

Table 2. Soil pH, DOC, total organic C and N at different rate of biochar applications.

Treatment	pH	DOC (mg kg^{-1})	Total organic C (g kg^{-1})	Total N (g kg^{-1})	C/N
			Week 1		
Con	4.56±0.01^{f}	27.65±1.10^{e}	5.50±0.10^{g}	0.94±0.05^{d}	5.86±0.42^{g}
$W_{0.5}$	4.69±0.01^{c}	37.60±1.04^{c}	8.71±0.04^{e}	0.95±0.00^{cd}	9.17±0.04^{e}
$W_{1.0}$	4.74±0.01^{b}	36.33±0.76^{c}	15.21±0.02^{c}	0.95±0.02^{cd}	16.01±0.32^{c}
$W_{2.0}$	4.87±0.01^{a}	33.32±1.07^{d}	23.95±0.04^{a}	0.99±0.00^{c}	24.19±0.04^{a}
$B_{0.5}$	4.59±0.03^{e}	43.14±0.31^{b}	7.71±0.05^{f}	0.95±0.04^{cd}	8.13±0.40^{f}
$B_{1.0}$	4.66±0.02^{d}	46.57±0.04^{a}	11.21±0.01^{d}	1.06±0.02^{b}	10.58±0.19^{d}
$B_{2.0}$	4.68±0.01^{d}	42.34±0.34^{b}	20.55±0.04^{b}	1.14±0.02^{a}	18.03±0.28^{b}
			Week 8		
Con	4.50±0.13^{a}	18.37±0.23^{d}	5.47±0.02^{g}	0.94±0.01^{d}	5.82±0.04^{g}
$W_{0.5}$	4.56±0.10^{a}	19.10±0.60^{cd}	8.69±0.00^{e}	0.94±0.00^{d}	9.24±0.00^{e}
$W_{1.0}$	4.59±0.23^{a}	18.80±1.03^{cd}	15.19±0.02^{c}	0.94±0.00^{d}	16.16±0.02^{c}
$W_{2.0}$	4.67±0.08^{a}	15.40±0.45^{e}	23.95±0.04^{a}	0.98±0.01^{c}	24.44±0.29^{c}
$B_{0.5}$	4.52±0.07^{a}	20.43±0.28^{ab}	7.69±0.03^{f}	0.94±0.02^{d}	8.18±0.21^{f}
$B_{1.0}$	4.56±0.09^{a}	21.41±1.38^{a}	11.15±0.03^{d}	1.05±0.04^{b}	10.63±0.38^{d}
$B_{2.0}$	4.60±0.11^{a}	19.88±0.34^{bc}	20.54±0.01^{b}	1.12±0.00^{a}	18.34±0.01^{b}
			Week 16		
Con	4.46±0.08^{c}	21.95±0.05^{a}	5.45±0.01^{g}	0.93±0.01^{d}	5.86±0.07^{g}
$W_{0.5}$	4.50±0.04^{bc}	20.83±0.78^{b}	8.67±0.03^{e}	0.93±0.00^{d}	9.32±0.03^{e}
$W_{1.0}$	4.53±0.02^{b}	19.70±0.28^{c}	15.17±0.05^{c}	0.94±0.04^{cd}	16.16±0.74^{c}
$W_{2.0}$	4.65±0.03^{a}	16.36±0.42^{e}	23.94±0.03^{a}	0.97±0.01^{c}	24.68±0.29^{a}
$B_{0.5}$	4.47±0.03^{bc}	22.28±0.47^{a}	7.67±0.01^{f}	0.93±0.02^{d}	8.25±0.17^{f}
$B_{1.0}$	4.51±0.04^{bc}	18.30±0.40^{d}	11.10±0.05^{d}	1.03±0.01^{b}	10.78±0.15^{d}
$B_{2.0}$	4.53±0.01^{b}	18.24±0.70^{d}	20.51±0.01^{b}	1.11±0.00^{a}	18.48±0.01^{b}

DOC: dissolved organic carbon. All values were expressed as mean ± standard deviation n=3). Different letters in the same column for each of sampling time indicate significant differences ($p < 0.05$).

(21.41 mg kg^{-1}) followed by B0.5 (20.43 mg kg^{-1}). However, at Week 16, DOC decreased in increasing the biochar rate. The reason could be sorption of DOC into the biochar. Our previous study also showed that fixation of labile organic carbon with increased biochar application rates (Zhang et al., 2012). This is because

Table 3. Correlations between soil pH, DOC, C, N, microbial biomass and soil enzymes at different incubation times (n = 3).

Property	MBC	MBN	Urease	Acid Phosphatase	β-glucosidase
			Week 1		
pH	0.41	0.04	0.37	-0.88***	-0.88***
DOC	0.21	0.46*	0.66**	0.07	0.44*
TOC	0.04	-0.16	0.62**	-0.76***	-0.73***
TN	-0.37	-0.30	0.60**	-0.08	0.11
C/N	0.13	-0.11	0.56**	-0.82***	-0.81**
			Week 8		
pH	0.11	0.03	-0.00	-0.52*	-0.25
DOC	-0.16	0.09	0.53*	0.66**	0.79***
TOC	0.15	-0.09	0.25	-0.81***	0.71***
TN	-0.38	-0.35	0.75***	-0.10	0.06
C/N	0.23	-0.04	0.14	-0.85***	-0.77***
			Week16		
pH	0.37	0.13	0.25	-0.74***	-0.52*
DOC	-0.25	0.01	-0.50*	0.65**	0.52*
TOC	0.27	0.10	0.53*	-0.74***	-0.77***
TN	-0.16	-0.28	0.74***	-0.17	-0.22
C/N	0.33	0.15	0.45*	-0.77***	-0.80***

DOC: dissolved organic C; TOC: total organic C; MBC: microbial biomass C; MBN: microbial biomass N; ns: non significant. *, **, and *** are significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

high-temperature pyrolysis (>550°C) produces biochars that generally have high surface areas (Downie et al., 2009; Keiluweit et al., 2010), are good adsorbents (Mizuta et al., 2004). Time of incubations had significant effect on DOC. The addition of biochars to the soil increased DOC in the first week but decreased in later part of incubation. This is because DOC, biologically easily available form of carbon, may be consumed by microorganisms in early time of incubation.

Biochar effects on microbial biomass and enzymatic activities

Microbial biomass is responsible for organic matter decomposition in terrestrial ecosystems, and thus ultimately responsible for maintenance of nutrient release in soil and soil fertility (Guo et al., 2012). There were significant differences among the treatment in the microbial biomass C (MBC) and microbial biomass N (MBN) (Figures 1 and 2). The maximum microbial biomass C (Figure 1) and N (Figure 2) were measured in W0.5 and B0.5, respectively. The lowest MBC and MBN were measured in control. Both the MBC and MBN decreased with increasing time of incubations. Jindo et al. (2012b) also reported decrease of microbial biomass carbon after 150 days of composting biochar blended poultry manure compared to 35 days. DOC is labile form of soil organic matter, easily available for microorganisms. Thus, the less availability of DOC at Week 8 and 16 as compared to Week 1, could result in decrease in microbial biomass along with time of incubations.

Enzymes are the main mediators of soil biological processes, such as organic matter degradation, mineralization and nutrient cycling (Marx et al., 2001). In this study, there were significant effect of biochar on β-glucosidase, acid phosphatase and urase activities. Moreover, there was significant correlation among the soil properties (soil pH, TOC, DOC, C/N) that changed due to biochar amendment and enzymatic activities at different times of incubation (Table 3). As reviewed by Lehmann et al. (2011) application rates between 1 and 12 t h^{-1} will likely show significant decreases in the activity of some C-mineralizing enzymes. The application rate between 1 and 12 h^{-1} is in the range used in this study. The activity of β-glucosidase (one of C-mineralizing enzyme) was higher in B1.0 (61.05 µg PNP g-1 soil h^{-1}) followed by B0.5 (60.94 µg PNP g-1 soil h^{-1}) at Week 1, similarly at Week 8, it was higher in B1 (46.69 µg PNP g-1 soil h^{-1}) followed by B0.5 (45.78 µg PNP g-1 soil h^{-1}) whereas, at Week 16 the control (58.27 µg PNP g^{-1} soil h^{-1}) showed the highest value (Figure 3). However, at Week 16, no significant difference among the treatments except W1.0 (43.55 µg PNP g-1 soil h^{-1}) and W2.0 (35.62 µg PNP g^{-1} soil h^{-1}) which were lower than the rest of treatments. The reason can be a co-location of C and microorganisms on biochar surfaces that may improve efficiency and reduce the need for enzyme production as described by Lehmann et al. (2011).

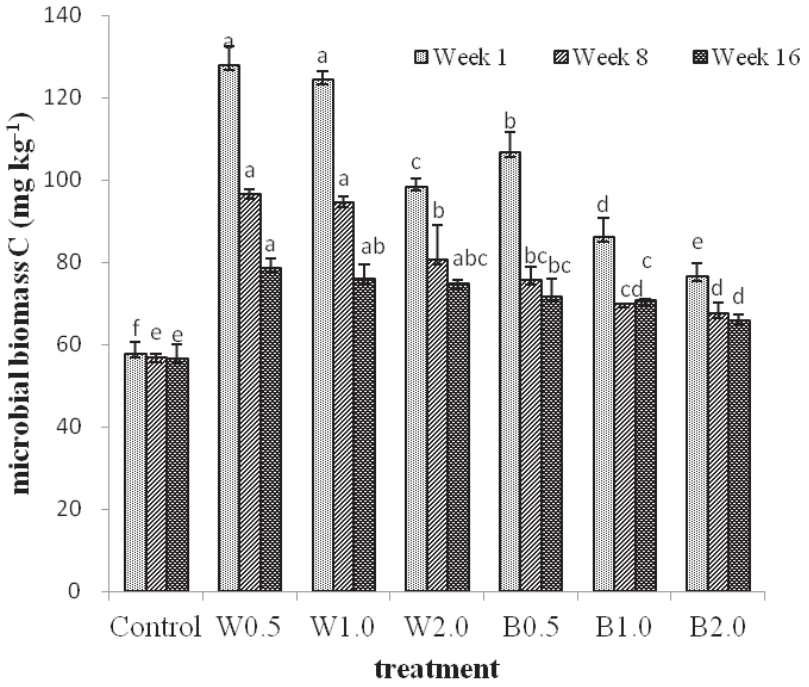

Figure 1. Soil microbial biomass C at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

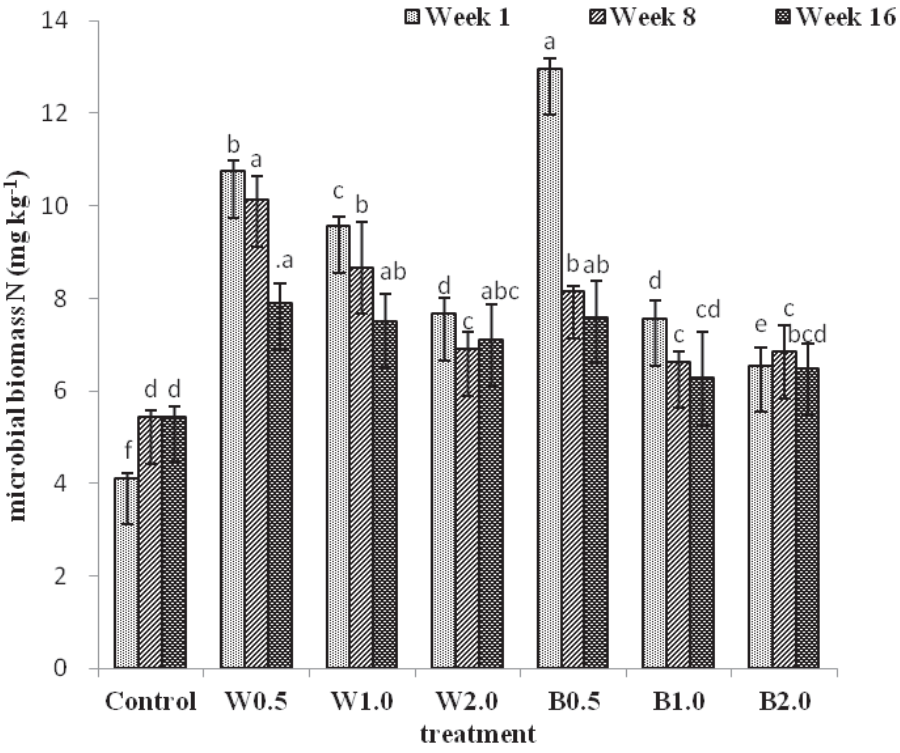

Figure 2. Soil microbial biomass N at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

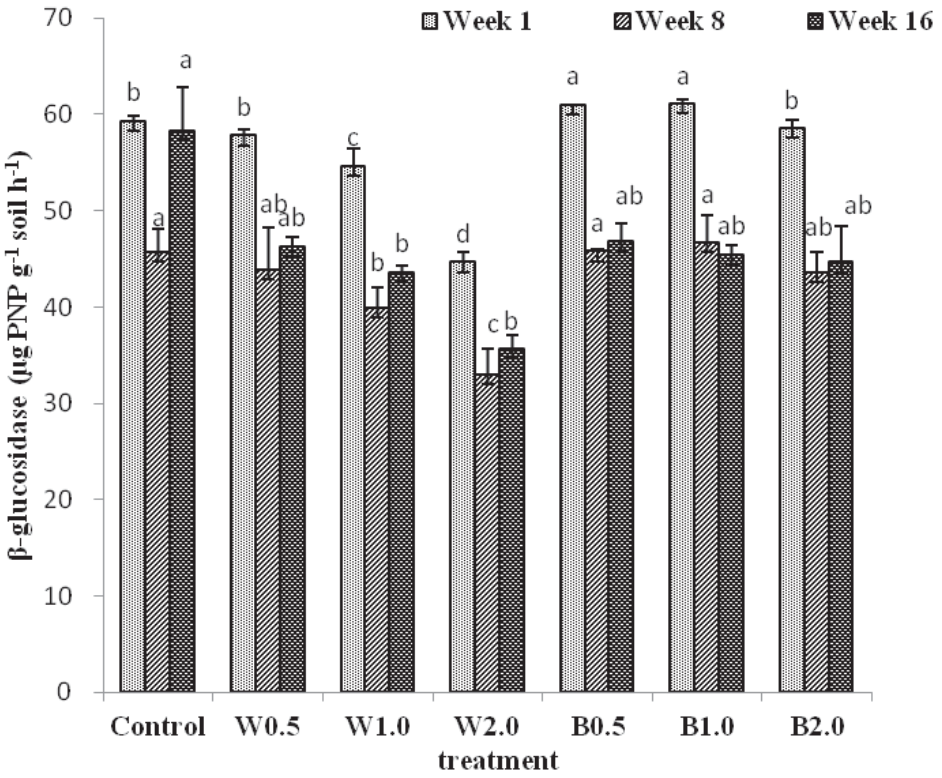

Figure 3. β-glucosidase activity at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

Our results agree with the results described by Paz-Ferreiro et al. (2012) who reported β-glucosidase activity decreased in biochar treated soil in comparison with the enzyme activity of the control soils. As to time effect, β-glucosidase showed the maximum activity at Week 1. The decrease of β-glucosidase to last two incubation times could be related to the decrease of DOC. This context is supported by the positive correlation of this enzyme with DOC (r=0.44*, r=0.79***, r=0.52* at Week 1, 8 and 16, respectively). Jindo et al. (2012b) also reported the decrease of the enzyme towards the end of the composting process. Moreover, the negative correlation of this enzyme with C/N (r=-0.81***, r=-0.77***, r=-0.80*** at Week 1, 8 and 16, respectively) implies mineralization of organic matter provides substrates for this enzyme.

Acid phosphatase play vital role in P cylces. This enzyme hydrolysis organic phosphourus compounds to different inorganic forms. Acid phosphatase is predominant in acidic soils (Eivazi and Tabatabai, 1977). In this study, at Week 1, acid phosphatase activity was higher in control (136.03 µg PNP g^{-1} soil h^{-1}) compared to biochar treated pots, however no significant difference with B0.5 (132.73 µg PNP g^{-1} soil h^{-1}) and B1.0 (130.14 µg PNP g^{-1} soil h^{-1}) was found (Figure 4). At Week 8, B1.0 (149.11 µg PNP g^{-1} soil h^{-1}) had higher activity followed by B0.5 (148.95 µg PNP g^{-1} soil h^{-1}), but they were not significantly different from the control. The control was higher at Week 16, however, it had no significant difference with the treatments except W1.0 (132.64 µg PNP g^{-1} soil h^{-1}), B2.0 (131.68 µg PNP g^{-1} soil h-1) and W2.0 (115.14 µg PNP g-1 soil h-1) which were lower than the rest of treatments. Due to the inverse correlation of acid phosphatase (r=-0.88***, r=0.52*, r=-0.74*** at Week 1, 8 and 16 respectively) with soil pH, its activity could decrease with increased rate of biochar application.

The urease activity is involved in the hydrolysis of C–N bonds of some amides and urea (Bremner and Mulvaney, 1978). Increasing biochar rates increased urease activities (Figure 5). At Week 1, the maximum urease activity was observed by W2.0 (943.63 µg NH_3-N g^{-1} soil 24 h^{-1}), however it had no significant difference with B2.0 (938.80 µg NH_3-N g^{-1} soil 24 h^{-1}) and B1.0 (930.21 µg NH_3-N g^{-1} soil 24 h^{-1}). The highest activity was observed in B2.0 (901.38 µg NH3-N g^{-1} soil 24 h^{-1}, at Week 8 and 797.72 µg NH_3-N g^{-1} soil 24 h^{-1}, at Week 16). The TOC could have contribution for increase of urease activity with increasing biochar rates. The positive correlation of urease activity with TOC (r=0.62**, r=0.25, 0.53*) support this context. This is because soil organic matter plays a vital role in protecting soil enzymes since they form complexes with clay and humus (Tabatabai, 1994).

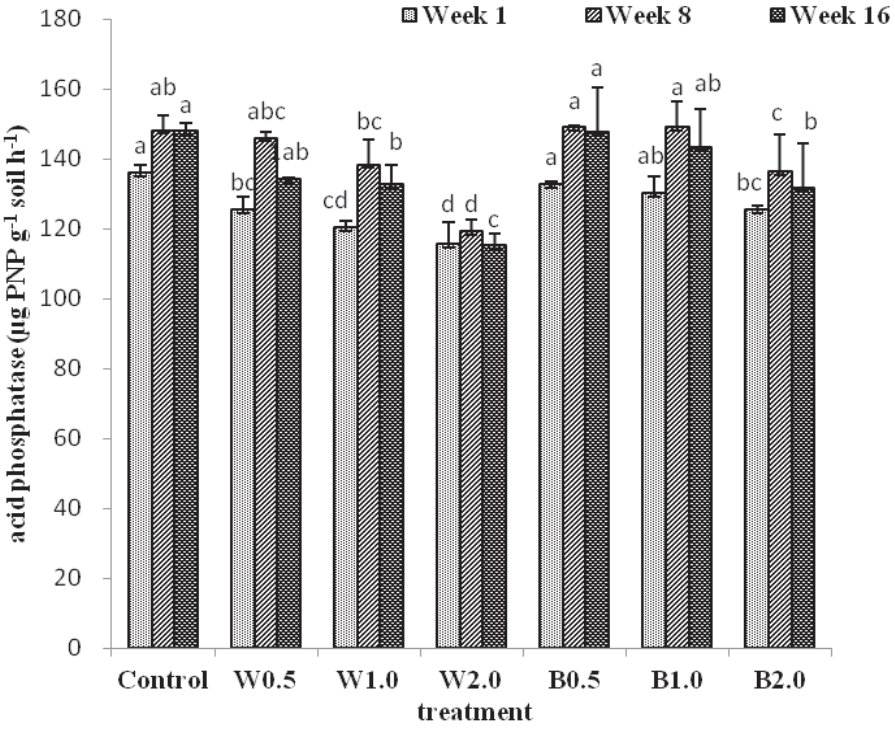

Figure 4. Acid phosphatase activity at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

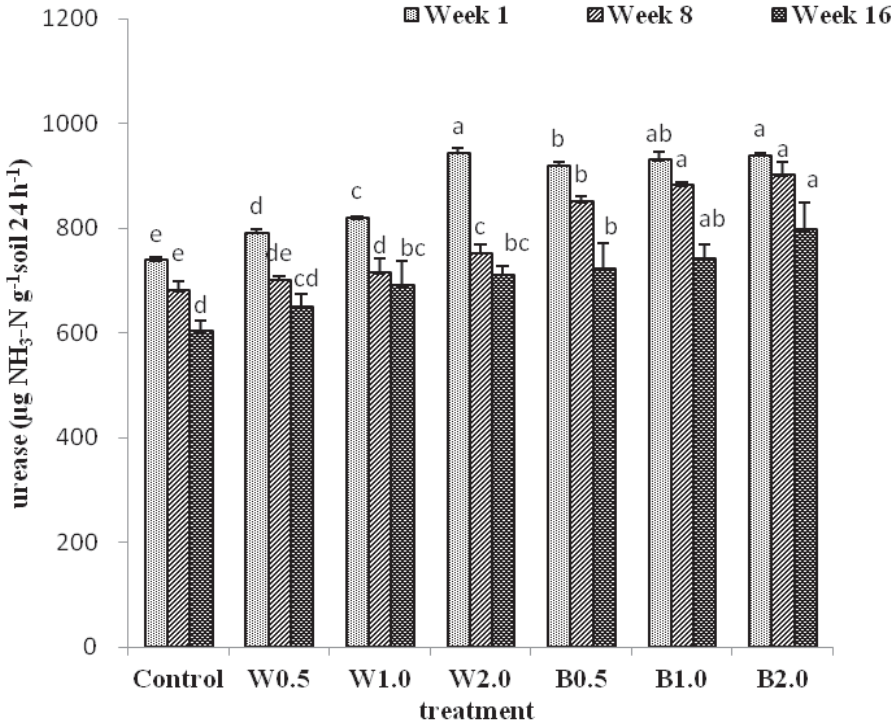

Figure 5. Urease activity at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

Table 4. The microbial biomass of the different PLFA groups in the soils incubated at the different biochar application rates, and their respective ratios.

Treatment	Total PLFA (nmol g^{-1})	Bacteria (nmol g^{-1})	Fungi (nmol g^{-1})	Actinomycetes (nmol g^{-1})	Gram+ (nmol g^{-1})	Gram- (nmol g^{-1})	Fungi/ Bacteria	Gram+ /Gram-
					Week 8			
Con	32.92±1.17^{b}	11.99±0.30^{b}	3.28±0.30^{a}	2.26±0.04^{ab}	5.81±0.59^{ab}	5.88±0.17^{c}d	0.27±0.03^{a}	0.99±0.13^{ab}
$W_{0.5}$	36.56±1.17^{a}	12.94±0.30^{a}	3.48±0.27^{a}	2.37±0.21^{ab}	6.30±0.04^{a}	6.31±0.23^{ab}	0.27±0.01^{a}	1.00±0.03^{ab}
$W_{1.0}$	33.06±1.28^{b}	12.23±0.43^{ab}	2.92±0.17^{ab}	2.19±0.05^{ab}	6.00±0.35^{a}	5.96±0.08^{bc}	0.23±0.01^{ab}	1.00±0.05^{ab}
$W_{2.0}$	29.35±0.68^{c}	10.35±0.25^{c}	2.60±0.18^{b}	1.88±0.04^{c}	5.10±0.05^{bc}	5.05±0.16e	0.25±0.01^{ab}	1.01±0.02^{ab}
$B_{0.5}$	34.33±0.24^{ab}	12.81±0.59^{ab}	2.67±0.26^{b}	2.17±0.17^{b}	6.24±0.29^{a}	5.67±0.28^{cd}	0.21±0.01^{b}	1.10±0.00^{a}
$B_{1.0}$	33.17±0.26^{b}	12.67±0.09^{ab}	3.08±0.34^{ab}	2.46±0.06^{a}	5.92±0.06^{ab}	6.47±0.16^{a}	0.24±0.03^{ab}	0.91±0.03^{b}
$B_{2.0}$	28.69±0.78^{c}	10.61±0.58^{c}	3.00±0.09^{ab}	2.18±0.15^{ab}	4.81±0.55^{c}	5.47±0.05d^{e}	0.28±0.02^{a}	0.88±0.09^{b}
					Week 16			
Con	31.22±2.76^{b}	11.41±1.24^{b}	2.96±0.07^{a}	2.22±0.04^{a}	5.64±0.90^{b}	5.51±0.33^{a}	0.26±0.02^{a}	1.02±0.10^{b}
$W_{0.5}$	36.11±0.23^{a}	13.66±0.22^{a}	3.25±0.15^{a}	2.32±0.00^{a}	7.34±0.04^{a}	5.99±0.05^{a}	0.23±0.01^{ab}	1.22±0.02^{a}
$W_{1.0}$	32.33±1.04^{ab}	11.93±0.10^{b}	2.95±0.04^{a}	2.16±0.05^{a}	6.35±0.06^{b}	5.37±0.12^{a}	0.25±0.00^{a}	1.18±0.04^{ab}
$W_{2.0}$	31.54±0.76^{b}	11.72±0.19^{b}	2.94±0.07^{a}	2.19±0.08^{a}	5.90±0.33^{b}	5.59±0.12^{a}	0.25±0.00^{a}	1.05±0.08^{ab}
$B_{0.5}$	32.11±0.13^{ab}	11.59±0.13^{b}	2.39±0.08^{b}	2.11±0.02^{a}	5.65±0.12^{b}	5.57±0.25^{a}	0.20±0.00^{b}	1.01±0.07^{b}
$B_{1.0}$	32.19±3.49^{ab}	11.72±0.95^{b}	3.03±0.32^{a}	2.25±0.21^{a}	5.94±0.22^{b}	5.54±0.68^{a}	0.25±0.01^{a}	1.07±0.09^{ab}
$B_{2.0}$	29.70±1.43^{b}	11.65±0.12^{b}	3.05±0.32^{a}	2.13±0.26^{a}	5.78±0.26^{b}	5.68±0.34^{a}	0.26±0.03^{a}	1.02±0.11^{b}

Gram+/Gram-: Gram-positive to Gram-negative bacteria PLFA ratio; Fung/Bacteria: Fungal to bacterial PLFA ratio. All values were expressed as mean ± standard deviation. Different letters in the same column for each of sampling times indicate significant differences ($p < 0.05$).

Biochar effects on microbial communities

Phospholipids are the key components of cellular membranes of all living cells, their composition are an important criterion to classify microbial groups and to evaluate their physiological conditions (Zelles, 1999). Microbial abundance in biochar amended soil has been determined by various methods, of which PLFA extraction is the one (Birk et al., 2009). As shown in Table 4, bacteria, fungi, and gram-positive bacteria were higher in W0.5 than the rest of the treatments at both times of incubation. This resulted in higher total PLFA (36.56 nmol g-1 soil at Week 8 and 36.11 nmol g^{-1} soil at Week 16) in W0.5 than the rest of treatments. Aromatic C and aliphatic C that are found in biochar as well as in soil are consumed by gram-positive bacteria (Fierer et al., 2003; Bird et al., 2011) which are the largest PLFA microbial groups present, resulted in higher bacteria and total PLFA in W0.5. This confirms reports of Farrell et al. (2013) who suggested bacteria are capable of rapidly metabolizing biochar-C, and increasing their community size relative to other microbial groups. The microbial groups were analyzed by principal component analysis and the contributions of the first two principal components (PCs) were 44.44 and 26.29%, respectively at Week 8 (Figure 6) and 38.75 and 28.88%, respectively at week 16 (Figure 7). The first two principal components accounted for 70.73% (at Week 8) and 67.63% (at Week 16) of the total variation. Treatment W0.5, to the right of PC 1, had higher relative abundance of bacteria, fungi, and gram-positive bacteria than the other treatments at Week 8 (Figure 6) and Week 16 (Figure 7). Moreover, the maximum total PLFA was measured at the lowest rates (W0.5 and B0.5) and the minimum at the highest rates (W2.0 and B2.0), indicating the optimum rate of biochar application for higher microbial biomass, at this specific soil is 0.5%. This implies that biochar at rate of 0.5% has more effect on microbial community. Labile components of biochar (DOC) and adsorbed volatile organic compounds, along with pH, are likely to be the major drivers of soil microbial community structure change when biochar is applied to soil (Farrell

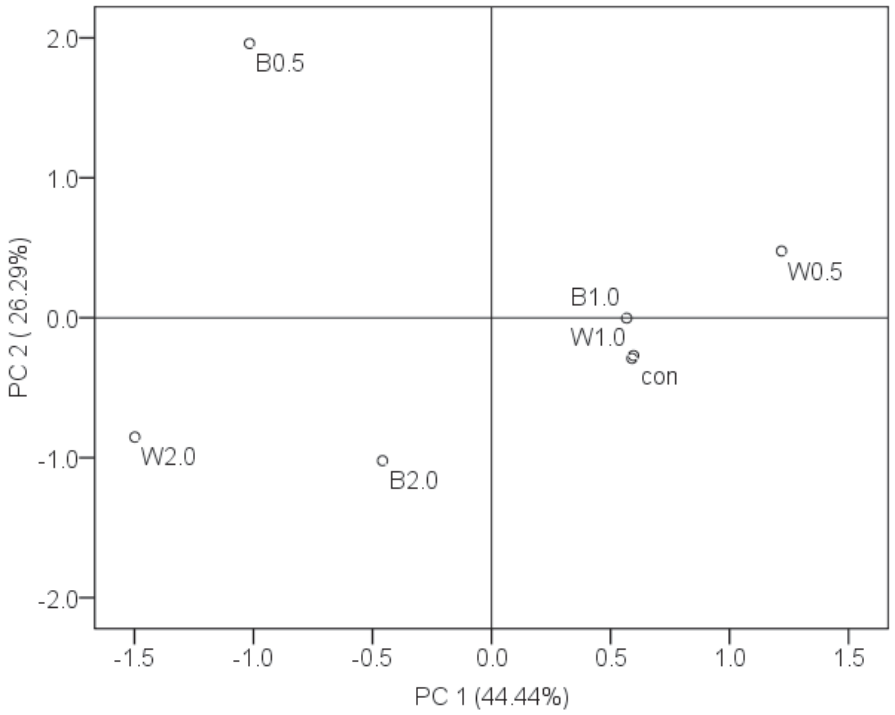

Figure 6. Principal component analysis (PCA) of microbial phospholipid fatty acids extracted from different treatments at week 8. Con= control, oak wood biochar at 0.5, 1.0, and 2.0% ($W_{0.5}$, $W_{1.0}$, and $W_{2.0}$, respectively) and bamboo biochar at 0.5, 1.0, and 2.0% ($B_{0.5}$, $B_{1.0}$, and $B_{2.0}$, respectively).

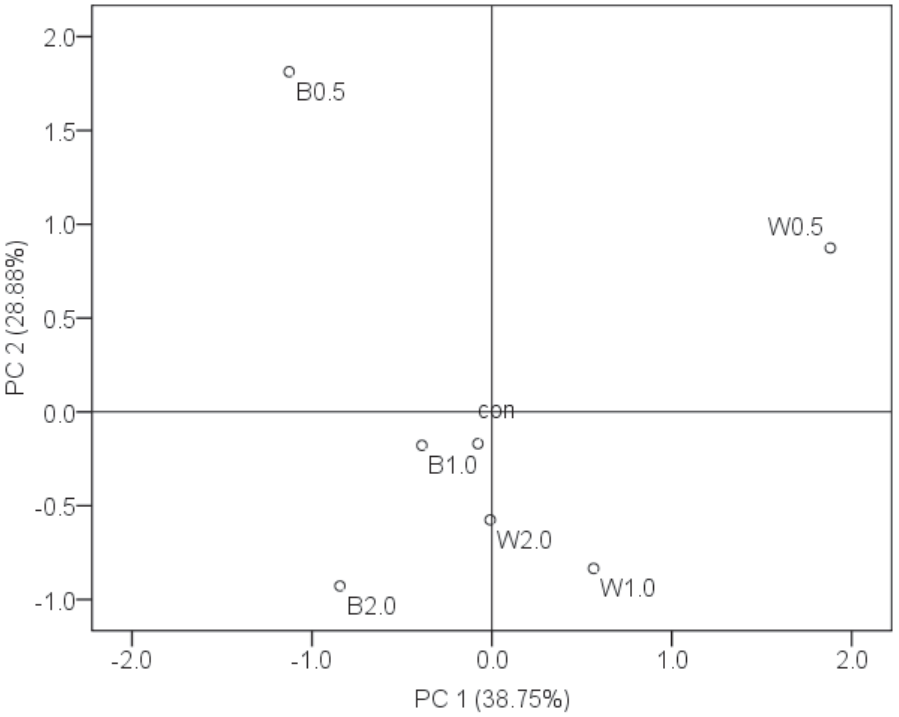

Figure 7. Principal component analysis (PCA) of microbial phospholipid fatty acids extracted from different treatments at week 16. Con= control, oak wood biochar at 0.5, 1.0, and 2.0% ($W_{0.5}$, $W_{1.0}$, and $W_{2.0}$, respectively) and bamboo biochar at 0.5, 1.0, and 2.0% ($B_{0.5}$, $B_{1.0}$, and $B_{2.0}$, respectively).

et al., 2013). In treatment B0.5, the fungi as well as fungi/ bacteria ratio was lower than the rest of treatments in both time of incubations, which could be related to the highest DOC which may favored bacteria than fungi, because bacteria are capable of metabolizing biochar-C than the other groups (Farrell et al., 2013). Incubation times had significant effect on microbial biomarkers gram-negative and gram-positive bacteria. Gram-positive bacteria increased at Week 16 compared to Week 8, whereas gram-negative bacteria decreased (Table 4).The decrease of gram-negative bacteria at Week 16, indicate that the soil microorganisms may have been energy-limited (due to the decrease of DOC) during the latter part of the incubation.

Conclusion

Biochar application significantly increased soil pH, organic C and dissolved organic C which created conducive environment and add substrates for microbial biomass and for enzyme production. Due to the low C/N of the soil, the lowest rates (W0.5 and B0.5), particularly W0.5, had higher microbial biomass and abundance than the highest rates. The inverse correlation of acid phosphatase with soil pH, the positive correlation of β-glucosidase with dissolved organic C as well as the positive correlation of total organic carbon with urease activities indicates the indirect effect of biochar on soil enzymes. The results from this study confirm that biochar application improve fertility of red soil by increasing the soil pH, organic carbon and dissolved organic carbon which, in turn, enhance the soil microbial biomass, soil enzymes and microbial community.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of some soil test methods in acid soils for available phosphorus for soybean of Imphal East District, Manipur (India)

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Evaluation of nutrient status in soil is important for nutritional, environmental and economical aspects. Phosphorus is a very important nutrient for leguminous crops. In order to evaluate the phosphorus availability, six soil test methods were carried out for predicting response of soybean to phosphorus application on twenty surface soils (0 - 15 cm) of Imphal East District, Manipur were studied. The suitability of these extractants was in the descending order: Bray P_1 > Mehlich P_1 > Bray P_2 > Troug P > Olsen P > Morgan P. Bray's P_1 extractable phosphorus showed the highest and positive correlations with phosphorus content (control), phosphorus uptake (control), Bray's percent yield and uptake. Therefore, this extractant may be used as an index of available phosphorus for soybean (JS-335) grown on acid soils of Imphal East District, Manipur, the critical level being 13 ppm (mg kg^{-1}). The critical limit of phosphorus concentration in plant at 40 days of planting was 0.22%.

Key words: Phosphorus, acid soils, Bray's percent yield, soybean, critical limit.

INTRODUCTION

Phosphorus deficiency in plants has been reported from various parts of India. The responses of oilseeds to applied phosphorus, particularly soybean had been reported by many workers. Soil testing allows one to access a soil's current nutrient status and decide on appropriate fertilizer rates to maximize crop production. But no information is available on the status of available phosphorus in these soils. The knowledge of relationship between soil test methods and soil phosphorus fractions help in selecting a suitable soil test method for a particular soil to grow the crop profitable. The most appropriate soil test method for a soil would be one which extracts predominantly that fraction of phosphorus playing the major role towards plant uptake. Therefore, the present investigation was planned to select the most promising extractant which may predict the availability of

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Table 1. Physical and chemical characteristics of the soils used for the pot study.

Soil characteristic	Mean	Range
pH	5.43	4.50 - 5.97
Organic carbon g kg^{-1}	16.30	9.80 - 23.00
Total N (%)	0.26	0.10 - 0.41
Available N (kg ha^{-1})	322.60	245.10 - 389.39
Available P_2O_5 (kg ha^{-1})	41.47	9.92 - 66.46
Available K_2O (kg ha^{-1})	208.64	163.29 - 252.67
Ca[cmol(p^+) kg^{-1} soil]	3.21	1.50 - 5.20
Mg[cmol(p^+) kg^{-1} soil]	3.10	1.41 - 5.10
Clay (%)	51.62	26.50 - 74.32
Silt	24.12	11.84 - 34.00
Sand	24.26	7.00 - 61.66

Table 2. Amount of available soil P content extracted by various methods (ppm).

Extractant	Range	Mean	References
Bray's P_1	4.43-29.67	18.49	Bray and Kurtz (1945)
Bray's P_2	7.71-27.32	20.17	Bray and Kurtz (1945)
0.5 M $NaHCO_3$	5.45-30.02	17.40	Olsen et al. (1954)
Truog-P Truog P	12.10-30.64	19.29	Truog (1930)
Morgan reagent	3.67-12.79	8.81	Morgan (1937)
Mehlich-P_1 Mehlich P_1	2.17-22.51	16.59	Mehlich (1978)

phosphorus to soybean (JS-335) grown in acid soils.

MATERIALS AND METHODS

Twenty soils samples (0 -15 cm) were collected from various cultivated fields of Imphal East District, Manipur. The physical and chemical characteristics of these soils were determined by standard methods (Jackson, 1973) and reported in Table 1. Four kilograms of soil was filled in earthen pots and phosphorus was applied at 0, 60 and 90 kg P_2O_5 ha^{-1} through single superphosphate. The treatments were replicated thrice in a completely randomized design. A basal dose of 20:60 kg NK ha^{-1} was applied in the form of urea, and muriate of potash in each pot. Soybean (var.JS-335) seeds were sown and thinned to six plants after ten days of sowing. The pots were irrigated with distilled water as and when required. The crop was harvested 40 days after germination.

The plant samples were washed in water and dried in oven at 65°C for 48 h and the dry matter yield was recorded. The samples were then powdered and requisite quantities of the same were digested in nitric - perchloric acid mixture. Phosphorus was determined by using vanadomolbdophosphoric acid reagent.

To test the suitability, six soil test methods were used (Table 2). The soil samples were shaken for two minutes with soil to solution ratio of 1:10. Extractable phosphorus was determined spectrophotometrically.

RESULTS AND DISCUSSION

Extractable phosphorus

The available P obtained with different chemical extractants revealed that the varying amounts of P extracted from different soils depended on the nature of extractant (Table 2). Based on the mean values of extractable P, the extractants were arranged in the following decreasing order: BrayP_2 > Truog P > BrayP_1 > Olsen P > Mehlich P_1> Morgan P. This was in conformity with the findings reported by Jaggi et al. (1990) and Ravindra and Ananthanarayana (1999). The higher solubility in Bray P_2 may be due to its relatively higher strength of acidity and complexing of Al^{3+} and Fe^{3+} ions with F^- ions and consequent release of P adsorbed by these trivalent ions (Ballard and Fiskell, 1974). The lowest quantity of P was extracted by Morgan reagent. This might be due to the presence of weekly buffered salt solution such as acetic acid-sodium acetate solution. Similar finding was also reported by Hesse (1971).

Correlation between different chemical extractants

The data on the simple correlation coefficients among the different methods of phosphorus extractants (Table 3) revealed that the extractants were closely interrelated. Such a close relationship between the different extractants suggested that these extractants were able to extract more or less the same forms of phosphorus indicating the existence of dynamic equilibrium among different forms of phosphorus but relatively to different

Table 3. Simple correlation coefficients among the different methods of phosphorus extraction.

Extractants	Bray-P_1 Bray P_1	Bray-P_2 Bray P_2	Olsen-P Olsen P	Mehlich-P_1 Mehlich P_1	Truog-P Truog P	Morgan-P Morgan P
Bray-P_1 Bray P_1	1	0.732**	0.680**	0.731**	0.704**	0.676**
Bray-P_2 Bray P_2		1	0.511*	0.721**	0.572**	0.539**
Olsen-P Olsen P			1	0.677**	0.713**	0.978**
Mehlich-P_1 Mehlich P_1				1	0.658**	0.698**
Truog-P Truog P					1	0.630**
Morgan-P Morgan P						1

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

Table 4. Simple correlation coefficient of S P extracted by various extractants with yield and uptake of soybean (JS-335).

Extractants	Yield parameter			
	P content (control)	Total P uptake (control)	Bray's % yield	Bray's % uptake
Bray's P_1	0.573**	0.687**	0.670**	0.709**
Bray's P_2	0.507*	0.563**	0.482*	0.451*
0.5 M $NaHCO_3$	0.433	0.512*	0.429	0.476*
Truog-P Truog P	0.445*	0.532**	0.463*	0.439
Morgan reagent	0.434	0.491*	0.381	0.467*
Mehlich-P_1 Mehlich P_1	0.497*	0.502*	0.563*	0.525*

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

degrees. Significant correlation between extractable phosphorus by all the procedures indicates that they extract similar pool of phosphorus in the soils but with varying degree. Similarly, Bhattacharya et al. (1990) and Jaggi (1991) reported that the transformation and availability of P in the soils was dependent on its various forms.

Correlations of soil P with yield and uptake

The simple correlation coefficients of coefficients between soil test results and yield were presented in Table 4. The data revealed that different soils significantly affected the dry matter production of soybean and also P uptake by soybean. The relative yield ranged from 46.70 to 95.32%, P content (control) 0.16 to 0.45%, and average P uptake from 6.81 to 24.67 mg pot^{-1}. Out of the six extractants, four were found to be significantly correlated with Bray's percent yield and five were also significantly correlated with P uptake by the crop. Among the extractants used for this investigation, Bray P_1 showed higher degree of co-efficient of correlation with Bray's percent yield as well as phosphorus uptake by the soybean, with 'r' values of 0.670** and 0.709**, respectively than the other extractants, that is, Bray P_1 > Mehlich P_1 > Bray P_2 > Troug P > Bray P_1 > Morgan P. So, Bray P_1 was the most suitable test for determining available soil phosphorus in the studied soil as the degree of co-efficient of correlation between the quantities of P extracted by this extractant and yield parameters were of higher order. Base on the simple correlation, it can be suggested that for acid soils of Imphal East District, Manipur, Bray-P_1 extractable

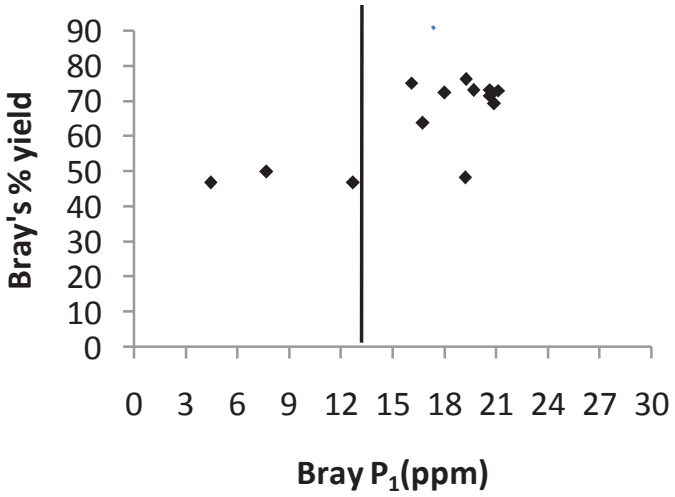

Figure 1. Relationship between soil P and relative yield of soybean.

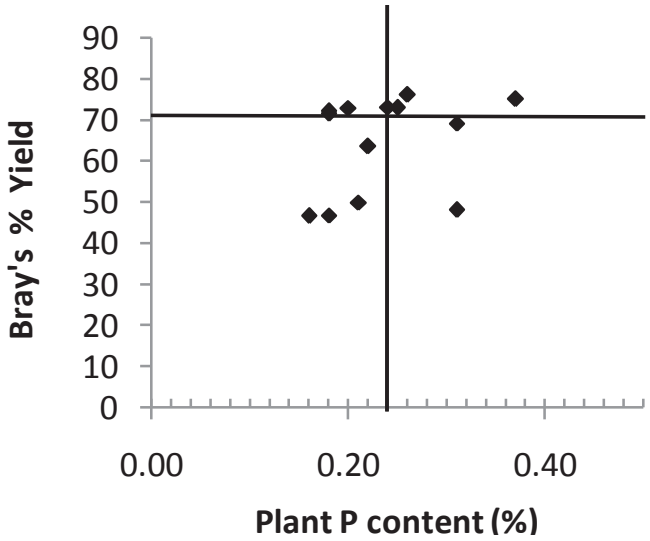

Figure 2. Relationship between the plant P concentration and relative yield of soybean.

phosphorus is found to be the suitable test for evaluation of available phosphorus in the soils for growing soybean plants.

Critical level of phosphorus

It was observed that the critical level of phosphorus in the soils for growing of soybean plants varied with the methods of phosphorus extraction. According to graphical procedure of Cate and Nelson (1965), the critical level of soils ranged from 13 to 20.2 ppm phosphorus depending upon the methods of phosphorus extraction. A high degree of correlation between Bray P_1 reagent extractable P and Bray's percent yield against Bray P_1 reagent extractable P was found to be 13 ppm (Figure 1) as the critical limit of available P in these soils for demarcating the phosphorus responsive soil from the non-responsive ones. Similar observations were also reported by Tandon (1987), Gupta and Vyas (1993), Mullen et al. (2009) and LaBarge (2013).

The result revealed that the critical level of phosphorus concentration in soybean plant was found to be 0.22% as shown in scatter diagram (Figure 2) partitioning the dimensional percentage yield versus phosphorus content in 40 days old soybean plants scattered into two groups. Similar observations were also reported by Ali et al. (2006) and Mallarino (2010).

Conflict of Interest

The authors have not declared any conflict of interest.

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Increasing farmers and breeders access to yam (*Dioscorea* spp) diversity: The case of Forest-Savannah Transition Agroecology

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A study was conducted in five yam-growing communities in the Forest Transition Agroecological Zone of Ghana to identify cultivated yam varieties, their distribution and intensity of cultivation and to document the rate and causes of landrace germplasm loss. Generally, the cultivated varieties ranged from 9 to 16 with a mean of 12. The most diversity was found in Sankore (Asunafo South District), followed by Sampa in the Jaman North District, Asantekwa in the Kintampo District, Ejura in the Ejura-Sekyedumase and Mim in the Atebubu/Amantin Districts with 16, 15, 12, 11 and 9 cultivated varieties respectively. *Dioscorea rotundata* is most widely cultivated species of yam followed by *Dioscorea alata*, *Dioscorea cayenensis*, *Dioscorea praehensilis* and *Dioscorea bulbifera* respectively. Factors such as good culinary characteristics, high yield, seed generation capacity, good storage characteristics and resistance to biotic and abiotic stresses were important criteria for selection of variety. The market and utilization were major determinants of continual cultivation of a variety or its neglect.

Key words: Agro-morphological, cultural, environmental, Ghana, technological.

INTRODUCTION

Yam (*Dioscorea* spp. of family Dioscoreaceae) is multi-species, polyploid in nature and vegetatively propagated crop. It is cultivated for its starchy tubers (both cultivated and wild) (Obidiegwu et al., 2009). The major edible yam species are *Dioscorea rotundata* P., *Dioscorea cayenensis* Lam., *Dioscorea dumetorum* (Kunth) Pax, *Dioscorea alata* L., *Dioscorea bulbifera* L., *Dioscorea esculenta* (Lour.) Burk, *Dioscorea trifida* L. and *Dioscorea nummularia* Lam (Alieu and Asiedu, 2011).

Yam is a staple food crop of over 300 million people in tropics and subtropics (Mignouna et al., 2003). Ghana is one of the most important yam producing countries in the world; it is the third (9%) behind Nigeria (66%) and Cote d'Ivoire (13%) in terms of production (FAO, 2009). It is an elite crop, preferred over other root and tuber crops in West Africa and it is the food of choice at

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festive occasions, and the only crop that is celebrated wherever it is cultivated (Coursey, 1967; Hahn et al., 1987). Resource poor people, especially women, derive a good income from its production, processing, and marketing (Dufie, 2009). It also constitutes a cheap source of carbohydrate in the diets of millions of people worldwide and in tropical West Africa it provides some 18 metric tonnes of food for people in the yam zones. Yam also constitutes 53% of total root and tuber consumption in West Africa (Asiedu and Otoo, 2009).

Yam, therefore, is not only an important stable crop in West Africa but also an important cash crop. It contributes 12% of dietary energy supply and of the major agricultural food items in Ghana traded in 2010; yams were among the most important crops (top exports) with the net trade (export) of 7030 tonnes valued $4245000 (FAO, 2010). The importance of yams to the Ghanaian economy can therefore not be over-emphasized.

Yam shows considerable diversity both at inter- and intraspecific levels (Okoli, 1991). The diversity under cultivation is further enhanced by the ongoing domestication of wild yam in various countries (Mignouna and Dansi, 2003; Scarcelli et al., 2006a). The diversity of yams in Ghana is poorly determined. Yam species generally, however, have adapted to different zones where they are often more abundant. *D. rotundata,* for instance, thrives well and is grown in the Guinea Savannah zone even though it can also be grown in the forest and Sudan Savannah zones. *D. alata*, *D. cayenensis*, *D. dumentorum* and *Dioscorea praehensilis* are mostly grown in the forest zone. In terms of utilization as food, *D. rotundata* is the most popular yam in Ghana followed by *D. alata*, *D. cayenensis*, *D. dumetorom*, *D. esculenta* and *D. praehensilis.*

Yam (*Dioscorea* spp.) belongs to the genus *Dioscorea*, representing more than 600 species worldwide (Coursey, 1967). The Dioscoreales are believed to be amongst the earliest angiosperms that originated in Southeast Asia, but followed a divergent evolution in three continents separated by the formation of the Atlantic Ocean and desiccation of the Middle East (Hahn, 1995). As a result, the major food species occur in three isolated centers: West Africa, Southeast Asia and tropical America (Alexander and Coursey, 1969).

Ghana however, is the leading exporter of the crop. It contributes about 17% of agricultural gross domestic product (GDP) and also plays a key role in guaranteeing household food security (Kenyon and Fowler, 2000). The crop occupies 11.6% of the total cropped area of Ghana and annual production is estimated to be 5.8 million metric tonnes in 2009 (FAO, 2009).

There are numerous yam species in Ghana including the *D. cayenensis and D. rotundata* as well as some wild species such as *D. praehensilis*. The relative importance of these species has not been determined hence the extent of their usage is not known resulting in loss of some landraces over time. For vegetative propagated crop such as yam, and being indigenous to the sub-region such an approach will greatly enhance the improvement of the crop. Germplasm of vegetatively propagated species generally often contains accessions which, although morphologically similar have different genetic origins and vice versa (Lebot et al., 1998). The first step in this direction is knowing the status of the gene base of the crop in the country especially at the Forest Savannah Transition where it is mostly cultivated, documenting the rate of loss and initiating action to conserve relevant germplasm.

The aim of the study therefore was to identify the cultivated varieties of yam and their distribution in different zones in the Forest-Savannah Transition Agroecology in Ghana, the extent of landraces loss, causes and farmers' variety preference criteria.

MATERIALS AND METHODS

Study area

The study was conducted in the forest-savannah transition zones in Ghana (Figure 1). Major yam growing communities were selected from each of the districts for the study. The study area covers all the tiers of yam production in the country. It included for instance, Mim in the Atebubu/Amantin District (average production per year of 250001-459860t), Asante Kwa in the Kintampo District, Sampa in the Jaman North District, Ejura in the Ejura-Sekyedumasi District (average production per year 100001-250000t) and Sankore in the Asunafu South District (average production per year 10001-25000t). Yam production is very important in these districts especially Ejura-Sekyedumasi, Kintampo and Atebubu/Amantin (Figure 1). The other districts were added in order to obtain as much biodiversity as possible. Selection of these districts was targeted at capturing as much diversity as possible.

To assess the diversity of yams at each location, a Participatory Rural Appraisal approach was used to obtain relevant information from the farmers. It also included focus group discussions and key informant interviews.

In each of the zones, a group of at least about 30 farmers were organized and taken through a discussion based on the checklist prepared (Appendix I). The information obtained was organized in a form that it could be well communicated. Gender and ethnic zones to effectively capture relevant data on parameters such as cooking quality and preferences disaggregated the participants.

The distribution and intensity of cultivation of cultivated varieties were documented. The rate of variety loss was assessed using 4×4 matrix system (Appendix II).

The study also carried out the following activities: an inventory of the cultivated varieties in the different yam production zones and assessment of their distribution and extent of cultivation; determination of the rate of landraces loss and its variation across villages; documentation of the reasons that underlie the landraces loss and its variation across diversity zones, identification of the different yam diversity zones in the country for development actions, identification and prioritization of the farmers' variety preference criteria across zones, and documentation of the cultivated varieties for the construction of national yam database.

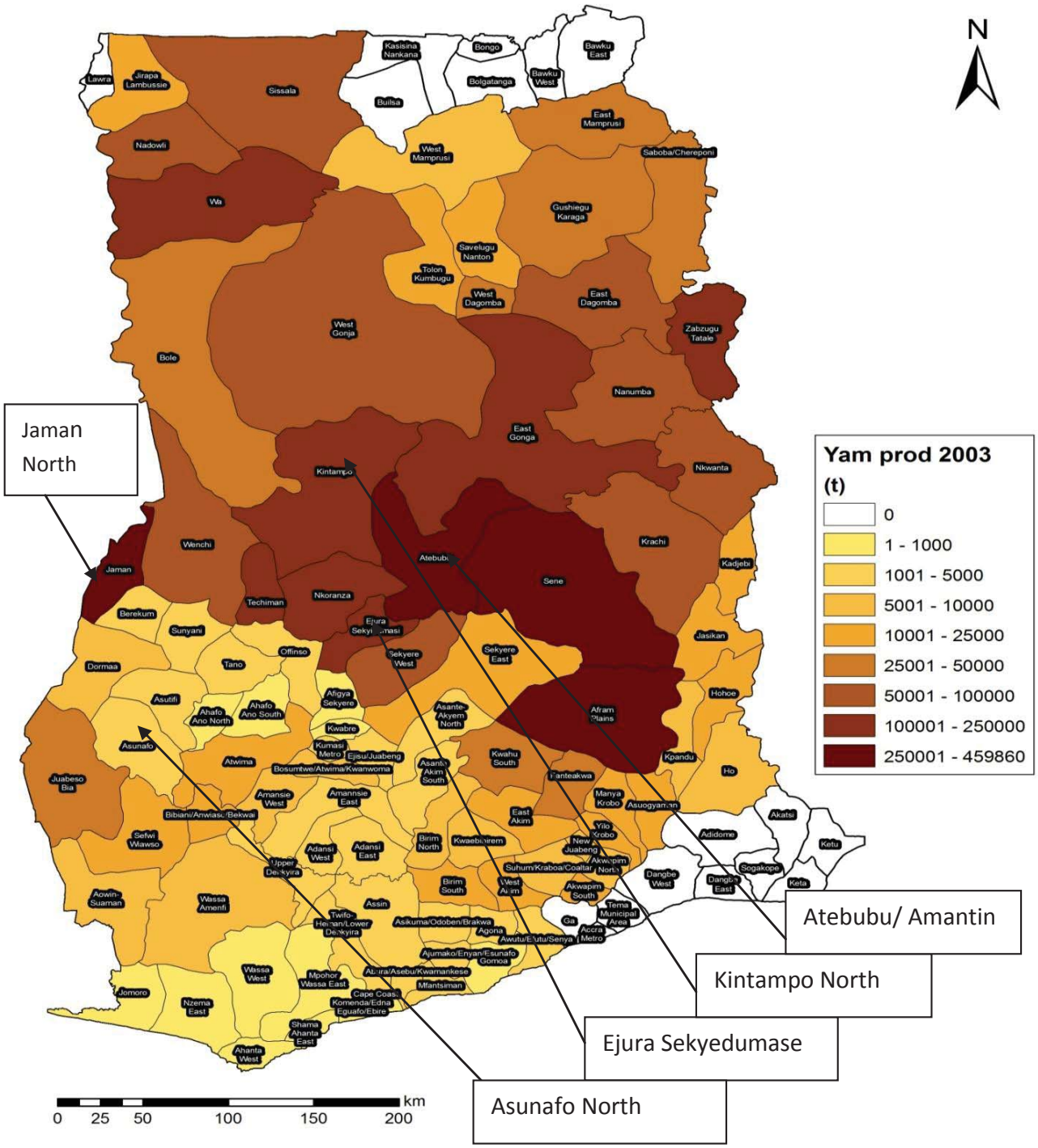

Figure 1. Study area.

RESULTS

Results of the comparison of the distribution and intensity of cultivation are shown in Table 1. In the Mim community in Atebubu-Amantin District, *D. alata* cv Matches and *D. rotundata* cv *Muchumudu* are widely cultivated by many households and in large acreages. *D. rotundata* cvs *Pona, and Serwaa* are however in danger of extinction, due to the few number of households cultivating it and on the small acreage of cultivation.

Similar trends were observed at Kintampo North District, where *Dioscorea alata* cv Matches and *D. rotundata* cv momnyowa are cultivated by many

Table 1. Distribution and intensity of yam varieties at various locations.

S/No.	Variety name	Species	Harvesting type	Distribution and extent	
				Household	Cultivated area
		Mim (Atebubu/Amantin District)			
1	Akaba	*D. alata*	Single	+	-
2	Matches	*D. alata*	Single	+	+
3	Labreko	*D. rotundata*	Double	-	+
4	Dente	*D. rotundata*	Single	+	-
5	Yesu mogya	*D. rotundata*	Double	+	-
6	Serwaa	*D. rotundata*	Single	-	-
7	Didi	*D. rotundata*	Double	+	-
8	Pona	*D. rotundata*	Double	-	-
9	Muchumudu	*D. rotundata*	Double	+	+
		Asante Kwa (Kintampo District)			
1	Afebetua	*D. rotundata*	Double	-	-
2	Akaba	*D. alata*	Single	-	-
3	Dansi	*D. alata*	Single	-	-
4	Dente	*D. rotundata*	Single	-	-
5	Dobare	*D. rotundata*	Double	-	-
6	Fuseni	*D. rotundata*	Double	-	-
7	Karangba	*D. cayenensis*	Single	-	-
8	Lele	*D. rotundata*	Double	+	-
9	Matches/Seiduble	*D. alata*	Single	+	+
10	Mmonyowa	*D. rotundata*	Double	+	+
11	Pona	*D. rotundata*	Double	+	-
12	Tela	*D. rotundata*	Double	-	-
		Sampa (Jaman North District)			
1	Afun/Kamba	*D. cayenensis*	Single	-	-
2	Akaba	*D. alata*	Single	+	-
3	Apoka/nkontina	*D. alata*	Single	-	-
4	Asamoah	*D. alata*	Single	-	-
5	Asobayere	*D. rotundata*	Single	-	-
6	Dente	*D. rotundata*	Single	-	-
7	Dobre	*D. rotundata*	Double	-	-
8	Enoti	*D. alata*	Single	-	-
9	Lele/nkasebayere	*D. rotundata*	Double	-	-
10	Lobi bayere	*D. rotundata*	Double	-	-
11	Matches	*D. alata*	Single	+	+
12	Pona	*D. rotundata*	Double	-	-
13	Teacher Takyie	*D. rotundata*	Double	-	-
14	Tempi	*D. rotundata*	Single	-	-
15	Tila	*D. rotundata*	Double	-	-
		Sankore (Asunafo South District)			
1	Afase pona	*D. alata*	Single	-	-
2	Afun	*D. cayenensis*	Single	+	-
3	Apoka	*D. alata*	Single	-	-
4	Asobayere	*D. rotundata*	Double	-	-
5	Dente	*D. rotundata*	Single	-	-
6	Entrentre	*D. alata*	Single	-	-
7	Esom ne hyen	*D. alata*	Single	-	-

Table 1. Contd.

8	Gonglogon	*D. alata*	Single	-	-
9	Guawa	*D. alata*	Single	-	-
10	Matches	*D. alata*	Single	+	+
11	Mensa	*D. rotundata*	Single	-	-
12	Muchumudu	*D. rotundata*	Double	-	-
13	Nnokoben	*D. rotundata*	Double	-	-
14	Pona	*D. rotundata*	Double	-	-
15	Serwaa	*D. rotundata*	Single	-	-
16	Soaba	*ariel yam*	Single	-	-
		Ejura (Ejura –Sekyedumasi district)			
1	Afebetua	*D. rotundata*	Single	-	-
2	Akaba	*D. alata*	Single	-	-
3	Ama Serwaa	*D. rotundata*	Single	+	+
4	Dente	*D. rotundata*	Single	+	+
5	Labreko	*D. rotundata*	Double	+	-
6	Lele	*D. rotundata*	Double	+	+
7	Matches	*D. alata*	Single	+	+
8	Nananto	*D. rotundata*	Single	-	-
9	Nentipo	*D. rotundata*	Single	-	-
10	Pona	*D. rotundata*	Double	+	-
11	Saate	*D. rotundata*	Double	-	-
12	Yesu Mogya	*D. rotundata*	Single	-	-

+: under household means lot of household, and under extent means cultivated in large acreages (greater than 2 acres), and –: under household is few household, and under extent is small acreages (less than 2 acres).

households and also in larger acreages; all other varieties are cultivated by few households and in small acreages. This trend was consistent for almost all locations.

In the Ejura Sekyedumase district, however, *D. alata* cv Matches, and *D. rotundata* cvs Dente, Lilee and Ama Serwaa are all cultivated by many household on large acreages. Varieties such as Akaba, Nananto, Nentipo, Yesu mogya *and* Afebetua have the potential of disappearing. *D. rotundata* cvs *Yesu mogya* and *Afebetua* are almost at the brink of extinction.

In the Mim community in Atebubu District, *D. rotundata* cvs *Pona, Serwaa and muchumudu* are also in danger of extinction, due to the low number of households cultivating it and the small acreage of cultivation. *D. alata* cv Matches is cultivated by many households and in large acreages.

At Sampa in the Jaman North district, there is no one variety that is cultivated by many households and on large acreage. Most of the yams varieties cultivated in the area are by few households and on small acreages. All other varieties except *D. rotundata* cv *Larebako* are cultivated in small acreages. *D. rotundata* cvs *Pona, Tila, Tempi, Teacher Takyi* and *Lobare* are gradually getting extinct. Some farmers also indicated their preference to *D. praehensilis.*

Similarly at Sankore, all the varieties are cultivated in small acreages with an exception of *D. cayenensis* cv *Afun;* all others are cultivated by a few households. Although this is not a major yam producing area, there are several yam germplasms in this area. *D. alata* cv Apoka, and *D. rotundata* cvs Dente, Nnokoben and Pona were listed as varieties on the brink of extinction.

A wide diversity of *D. rotundata* yam species was documented in the study areas; 78% in Mim, 58% in Asantekwa, 60% in Sampa, 44% in Sankore and 82% in Ejura. *D. alata* varieties were 22% in Mim, 33% in Asantekwa, 33% in Sampa, 44% in Sankore, 18% in Ejura. Only one variety of *D. cayenensis* (*D. cayenensis* cv Afun) was found at Sampa and Sankore. It was only at Sankore that *D. bulbifera* cv was documented as a cultivated species. The maturity period also correlated positively to the harvesting type (r=1), with all *D. rotundata* varieties except for *D. rotundata* cvs Dente, Serwa and Tempi, being early maturing and thus double harvested. *D. alata*, *D. cayenensis* and *D. bulbifera* were late maturing and thus singly harvested. Income and food security were major determinants of distribution and intensity of cultivation of a particular variety across all locations, gender and ethnic groupings.

The Table 2 shows the rate of variety loss in the studied communities. Results from Table 2 show that the

Table 2. Analysis of rate of yam variety loss at various locations.

Villages	Total number of varieties	Number of DHV varieties	Number of SHV varieties	Number of varieties in Q1	Number of varieties in Q2	Number of varieties in Q3	Number of varieties in Q4	Number of NIV	Rate of variety loss (RVL)
Mim	9	5	4	1	4	1	3	2	1
Asante Kwa	12	8	5	2	2	0	6	0	6
Sampa	15	9	6	0	3	0	12	**5**	7
Sankore	16	6	10	0	1	0	10	4	6
Ejura	12	4	6	4	3	0	5	2	3

DHV: Double harvest variety, SHV: single harvest variety, NIV: newly introduced variety, Q: quadrant, RVL = (Q4 – NIV)/Total number of varieties.

Table 3. Preference criteria of farmers in the selected localities.

Criteria	Ejura	Kintampo	Atebubu	Sankore	Sampa
Good culinary characteristics	x	xxx	x	x	x
Good yield	xxxx	xxxxx	xxx	xxx	xxx
High seed production capacity	xxxx	xxx	xxx	xxx	xxxxx
Low staking demand	xx	xxxx	xxxx	x	
Good post harvest storage characteristic of the tuber	xxxx	xx	xxx	xxx	xxxxx
Good quality of the cossettes					
Resistance of the cossettes to storage insects				xx	
Good storage characteristic of the fresh tuber	xxxx	xxxxx	xx	xxxx	xxx
Tolerance to high soil moisture	xxxx	xxx	x	xxxxx	x
Resistance to drought	xxxxx	xxx	xxxxx	xxx	xxx
Tolerance to poor soils	xxx	xxx	xxxxx	xxxxx	xxxxx
Resistance to pest and diseases	xxx	xxxxx	xxxx	xxx	xxxxx
Tolerance to weed	xx	xxx	xxxxx	x	xxx
Adaptability to all type of soil	xx	xx	x	x	xxx

x: indicates the level of importance, with x- least important and xxxxx- most important.

diversity is lower at Mim than the other locations. Although the number of newly introduced varieties is more at Sampa than the other locations, the rate of variety loss is also greater than all the other locations. This means that yam cultivation is expanding in the area than the other locations hence new varieties are being explored and those not satisfactory enough are quickly lost. There were however, no newly introduced varieties at Asante Kwa in the Kintampo district. The rate of variety loss is therefore the least at Mim in the Atebubu District.

To ascertain the reasons for preference of one variety to the other, assessment was done on farmer preference criteria ranking them in the order of importance (Table 3). From Table 3 above, it can be seen that good productivity, high seed production capacity, good post harvest storage characteristic of the tuber, tolerance to high soil moisture and resistance to drought are the most important criteria farmers look out for in selecting yam varieties in the Ejura area. In the Kintampo area, however, good culinary characteristics, good productivity, low staking demand, good storage characteristic of the fresh tuber and resistance to pest and diseases are the most prominent criteria. There is not much difference between criteria for Atebubu, Sankore and Sampa. Among these three locations, resistance to drought, resistance to pest and diseases, tolerance to weed, high seed production capacity and good post harvest storage characteristic of the tuber dominated.

From Table 4, the importance of agro-morphological and environmental (biotic and abiotic) factors to the farmers was more than all the other factors. Agro-morphological alone accounted for about 50% in terms of importance in about all locations except Kintampo where environmental factors dominated. Cultural factors however accounted for about 4.51% in the Kintampo district, 1.17 in the Ejura and 0.82% in the Atebubu districts.

DISCUSSION

Local yam classification systems exist in all yam-growing

Table 4. Percentage importance of technological, agromorphological, environmental and cultural factors to yam production.

Type of factors	Importance per zone (% of responses)				
	Ejura	Atebubu	Kintampo	Sampa	Asunafu South
Technological factors					
Poor quality of pounded yam	4.57	0.41	1.50	0.47	1.24
Difficulty in pounding of the boiled tuber	4.57	4.49	4.51	0.93	1.86
Low expansion capacity of the pounded yam	2.86	6.94	9.77	2.33	1.24
Frequent presence of lumps in the pounded yam	3.43	6.12	9.77	2.79	4.97
Total	**15.43**	**17.96**	**25.56**	**6.51**	**9.32**
Agromorphological factors					
Profuse branching of the tuber	8.57	6.94	1.50	8.37	6.21
Profuse thorniness of the tuber	8.00	0.82	1.50	8.37	11.18
Presence of red spots in the tuber flesh	6.86	6.94	2.26	8.37	11.18
Low productivity	5.71	6.94	3.76	7.44	7.45
Low seed tuber production capacity	8.00	6.53	1.50	5.58	11.18
High staking demand	6.86	6.94	3.01	8.37	1.24
Poor post-harvest storage characteristic of ware tubers	1.14	6.94	3.01	6.51	3.11
Poor post-harvest storage characteristic of seed tubers	0.57	6.94	3.76	5.58	0.62
Total	**45.71**	**48.98**	**20.30**	**58.60**	**52.17**
Environmental (biotic and abiotic) factors					
Poor adaptation to climate change	5.71	6.94	15.04	7.44	8.70
Susceptibility to poor soils	6.86	6.94	4.51	5.58	6.21
Soil selectivity	7.43	6.53	0.00	7.44	1.24
Susceptibility to pests and diseases	5.71	4.90	15.04	7.44	11.18
Susceptibility to weeds	11.43	6.94	15.04	6.98	11.18
Total	**37.14**	**32.24**	**49.62**	**34.88**	**38.51**

areas, and are often based on relevance or the characteristics of the individual yam variety. Previous studies revealed an intricate naming system where the most important or recognizable feature of the yam is used in naming it. These names are often descriptive. Farmers often preserve varieties they think is important and neglect unimportant varieties, leading to the loss of such varieties. The continuous existence of a particular variety, therefore, is an indication of its relevance in one way or the other to a particular individual or community.

D. rotundata cv Pona, for instance, is an early maturing variety, and therefore amenable to double harvesting. Many households cultivate it albeit in small areas. This was attributed to the poor storability of this variety and the high cost of its seed yam. Even though it is the yam that attracts the premium price, its poor storability means that it is often depleted very quickly after harvesting. The seed yam is also often not available making it very expensive and increasingly putting it out of reach for the resource-poor farmer.

D. rotundata cvs *Afebetua, Tela, Dobare, Fuseini* and *Dente* are cultivated by few households on small acreages. This was attributed to the relative low market value of these varieties compared pona, for instance, thus placing these varieties in danger of extinction if no serious measures are taken to collect and conserve.

The high patronage of *D. alata* cv *Matches* and *D. rotundata* cv *Mmonyiwa* in these locations could be attributable to the good storability of these two varieties. Farmers therefore can rely on these varieties for both home consumption and marketing during the hunger period. The former variety for instance, is known to have being introduced to Ghana from Cote D'ivoire. Due to its high propensity to establish, it was distributed using matchbox as the standard multiplication size. This, coupled with its late maturity and ability to be used for several food forms has enhanced its spread in the country.

To enhance germplasm preservation therefore, local agro-biodiveristy, decentralized participatory breeding could be the preferred approach to breeding (Kirsten vom Brocke et al., 2010).

In choosing varieties, it was clear that agro-morphological characteristics such as good culinary characteristics, good productivity, low staking demand, good storage characteristic of the fresh tuber and resistance to pest and diseases are the most prominent criteria. Hence any variety improvement programme that fails to take cognisance of these facts is bound to fail. It was therefore in agreement with Tamiru et al. (2007) assertion that often, overall structure of morphological diversity may be consistent with the local yam classification system.

Conclusions

A wide diversity of yams exists in the study area, however more and more of these diversity is getting extinct. There is the urgent need to collect and conserve these varieties. *D. rotundata* is most widely cultivated species of yam followed by *D. alata, D. cayenensis* and *D. bulbifera* respectively.

Conflict of Interest

The authors have not declared any conflict of interests.

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APPENDIX

APPENDIX I: VARIETY DOCUMENTATION (GUIDE OF DISCUSSION)

Generalities

- Vernacular name and naming language
- Other names and corresponding language of naming
- Meaning of the vernacular name
- Origin of variety (domestication, introduction from other country)
- Species

Agronomic characteristics

- Earliness (single harvest / double harvest)
- Relative Productivity (farmers' perception)
- Number of tubers per mound (indicate range when necessary)
- Relative length and size
- Adaptability to pour soil (put score)
- Preferred type of soil (clayey, sandy, chipping, etc.)
- Staking demand
- Adaptability to lowland (put score)
- Resistance to drought (behaviour in the following situations)
  - In case of delay of the rain
  - Regular but insufficient quantity of rain
  - Stop of the rain during vegetative growth
- Storage aptitude of fresh tuber (put score / duration of storage
  - Post harvest
  - In the mounds
- Storage aptitude of the cossettes (resistance to storage insects / put score)
- Susceptibility to weeds (put score)
- Resistance to yam nematodes
- Susceptibility to other biotic factors (scale insects, virus, termites, Mealybugs, Tuber beetles)
- Multiplication rate / Tuber seed production capacity

Culinary characteristics

- Coloration after peeling
- Poundability (discuss the following)
  - Easy to pound? If not why?
  - Quality (taste, elasticity, expansion, presence of eventual lumps)
- Quality of chips (taste, softness)
- Quality of boiled yam (taste, softness, become hard when cool?)
- Quality of the cossettes (flour, quality of the pate, etc.)

Particular utilisations and information

- Any particular use? (Cultural? Medicinal?)
- Market value
- History? Taboo and proscriptions? Etc.

APPENDIX II. ASSESSMENT OF YAM DIVERSITY AT COMMUNITY LEVEL

Objectives of the study

1. Carry out an inventory of the cultivated varieties in the different yam production zones and assess their distribution and extent.
2. Determine the rate of landraces loss and its variation across villages.
3. Understand the reasons that underlie the landraces loss and its variation across diversity zones.
4. Identify the different yam diversity zones in the country for development actions.
5. Identify and prioritize the farmers' variety preference criteria across zones.
6. Document the cultivated varieties for the construction of national yam database

Selection of the villages for the study

This is very important and the success of the study depends on that.

- Avoid selecting villages only along major roads.
  Take into account the necessity to have a very good geographical distribution of the selected sites: *country/ yam production zone* should be well *covered.*
- Consider the ethnic zones: *Preference criteria vary most of the time with the ethnic groups.*
- Do not forget agroecological zones (*humid zones; arid and semi arid zones*).

Diversity inventory and distribution and extent analysis

- This is done at community/village level and in group
- The group should be made of 30 to 40 farmers. Here, gender issue (sex and age) is important. *Women will give the best information on the cooking qualities; rare or old varieties are mainly produced by old farmers; newly introduced varieties are most often well known by young producers*, etc.
- Record the local generic name of: early maturing yam (double harvest), late maturing yam (single harvest), *D. alata, D. dumetorum, D. esculenta, D. bulbifera.* This is important as ignorance of this name create a lot of confusion leading to an underestimation of the real diversity. For example at Djougou region of northern Benin, all the early maturing varieties are called ***Noudouossé***. In a given village of this region, you may therefore by ignorance record the existence of only one variety named Noudossé while this village has really 12 early maturing landraces of different names.
- Based on our experiences, the expressions "**double harvest"** and "**single harvest"** should be used at the place of *early maturing* and *late maturing* respectively. In fact within the early maturing yams, farmers recognize some that are early and some that are late. This sometime lead to serious confusion when documenting the earliness of the varieties.
- Record now (*per category of yam*) the list of all the varieties cultivated in the village. Fill the three four columns of the following table:

N°	***Variety name***	***Species***	***Earliness***	***Distribution and extent***	
				Household	***Cultivated area***

- In the process of the four square analysis, take the parameter frequency *(relative frequency of the households cultivating the variety)* and assess the varieties with that. Farmers know the situation of all the varieties. Let them tell you in group (*and sometime after discussion between them*) if the variety **X** is cultivated by "*many households"* or just by "*few households".* Use the symbols **+** and – **to fill column 5**
- Repeat this exercise with the parameter *"cultivated area".* Use the symbols **+** and – **to fill column 6.** *Never combine the two parameters together in a single exercise*

  Summarize and restore the results to the farmers (*use paper of big size: 1m x1m for example*) for comments as follow:

QUADRANTS CLASSIFICATION METHOD FOR DETERMINING VARIETY LOSS

Quadrant 1: Many households, large area (++)	**Quadrant 2**: Many households, small area (+-)
Quadrant 3: Few households, large area (-+)	**Quadrant 4**: Few households, small area (--)

Varieties in quadrant 4 are those that are disappearing. Note however, that in this same quadrant are also found varieties that were newly introduced in the village.

Start filling this table (use Excel when back to office for your statistical analysis):

Villages	Total number of varieties	Number of DHV varieties	Number of SHV varieties	Number of varieties in Q1	Number of varieties in Q2	Number of varieties in Q3	Number of varieties in Q4	Number of NIV	Rate of variety loss (RVL)
Village 1									
Village 2									
.....									
Village n									

DHV: Double harvest variety, SHV: single harvest variety, NIV: newly introduced variety, Q: quadrant.

RVL = (Q4 – NIV)/Total number of varieties.

Reasons of variety loss

- Take one by one the varieties in quadrant 4 and ask farmer to give the reasons why they are being abandoned. Generally the reasons vary from variety to variety.
- Carry out individual survey with 20 farmers from the group. Let them tell you individually (isolate them) why, according to them, varieties are being disappeared.

Farmer preference criteria

- Carry out individual survey in each village.
- You can also use the matrices comparison method.

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Full Length Research Paper

Drought coping strategies at Lonhlupheko community, a semi-arid rural area in Swaziland

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Climate change predictions reflect that temperatures in Swaziland will increase by 2.5°C and annual rainfall could decrease by 100 mm by 2050. Drought frequency and intensity is likely to increase in future and its occurrence could not be divorced from climate change. A descriptive research using questionnaire survey procedures and personal interviews was designed to determine drought coping strategies at Lonhlupheko, a semi-arid area in Swaziland. The target population was 150 households with a sample size of 108 households. The data collected was both qualitative and quantitative in nature. Quantitative data were presented as percentages following analysis by Statistical Package for Social Sciences computer software. Results reflected the drought coping strategies practiced by households as vegetables marketing and selling (19.4%), labour for food and money (5.6%), brewing and selling traditional brew (9.3%). External and institutional support obtained by households included food rations and farming inputs from non governmental organizations (32.4%), free primary education and feeding schemes (8.3%). Adaptation measures proposed by households included provision of irrigation water (56.5%), construction of dams and structures for rainwater harvesting (23.1%), agricultural extension services revival (13.0%), access to loans for small and medium enterprises (5.5%) and drought tolerant crops promotion (1.9%).

Key words: Adaptation, coping, domestic water, drought mitigation, livelihood. strategies, semi-arid.

INTRODUCTION

Drought is considered one of the most severe and costly natural hazards, and it is the most important severe factor affecting world food security (Tallaksen, 2012). The most severe human consequences of drought are often found in arid and or semi-arid regions such as the Lowveld of Swaziland, where Lonhluphelo is located. This is evident where water availability is already low under normal conditions, and demand is close to, or exceeds natural availability, and society lacks the capacity to mitigate or adapt to drought. Drought is a direct effect of climate change (Kumwenda, 2012). Climate change has caused extreme weather events such as frequent droughts, floods, heat and cold waves (Mwase et al., 2012). Farmers and communities lack contextualized information on adaptation in order to cope with the effects of climate change.

Swaziland is a small country in southern Africa, covering an area of 17,364 km^2. It has a population of

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about 1,120,000. About 77% of the population lives in the rural areas, with 23% in urban areas. A large proportion of the rural population practice subsistence farming (Government of Swaziland, 2007). According to Manyatsi and Mwendera (2007), about 69% of the population lives below the poverty line of US$1 per day.

Maize dominates the fields of small holder farmers in Swaziland. Drought tolerant crops such as cassava and sorghum are infrequently grown. The country, which once was self-sufficient in food production and exported surpluses regionally, now imports 60% of its food requirements even in non-drought years. The total cereal requirement for the 2009/2010 season was 166,000 tons, while the local production was 82,000 tons (Shongwe, 2010). The government of Swaziland has encouraged sugarcane production on irrigated land in order to improve its foreign exchange earnings through exports of sugar-based products. Meanwhile, the production of maize remains mostly on non-irrigated land. Livestock are an important component of the agricultural industry in Swaziland, as about 60% of the total area is used for livestock grazing.

Swaziland is prone to climatic variability, and it manifests itself in a number of hydrological disasters including change in rainfall regime as well as extreme weather conditions. The most severe droughts in the country occurred in 1983, 1992, 2001, 2007 and 2008 (Manyatsi et al., 2010). It was reported that over 500 people lost their lives due to the drought of 1983. In 1992, about 90 000 cattle died in the country due to drought. The production of maize dropped by 70% in 2000 to 2005 due to the large arable land that was not cultivated due to delayed rains and a shortage of seeds and other farming inputs (IRIN, 2007).

Adaptation involves longer-term shifts in livelihood strategies to respond to change in the environment, while coping on the other hand involves temporary adjustment to respond to change or a short-term modification of livelihood activities in the face of a shock or stress (Mogotsi et al., 2011). The range of drought coping options available to a given household depends on the resources available to them. The coping strategies to drought may include selling of assets for food, reduced meals, limiting food portions, skipping entire meals, adults eating less, labour for food, consuming more than usual amounts of wild food, credit and reliance on relief support (Pandey and Bhandari, 2009; Tideman and Khatana, 2004).

The adaptation measures in the agriculture and food security sector in developing countries may include dam construction for irrigation, introduction of new crops, changes in planting and harvesting times, and educational and outreach programs on soil and water management (United Nations Framework Convention on Climate Change (UNFCCC), 2011).

The future of the global area equipped for irrigation could change putting more pressure on the water resources, though bringing the much needed relief against drought in arid and semi-arid regions of the world. In this regard, work by

Valipour (2014a) revealed that the changes of area equipped for irrigation in the world are 12.1 to 70.0% and 29.0 to 99.9% from 2011 to 2035 and 2060, respectively. Work by Valipour (2014b) also concluded that the trend of permanent crops per cultivated area (with the exception of Northern America), human development index, irrigation water requirement, percentage of total cultivated area drained was increasing in Americas.

The capacity to cope and adapt to new environments including those brought about by climate change embodies community survival and by extension sustainability. Failure on the other hand could bring untold human suffering with costly interventions if addressed later than on the onset, hence this study.

Objective

The objective of the study was to determine the drought coping strategies employed by households at Lonhlupheko, a semi-arid rural area in Swaziland.

METHODOLOGY

Study area and research design

Lonhlupheko is located 15 km west of Siteki in the Lowveld region of Swaziland (Figure 1). The community has about 150 households with a population of approximately 1290 people. The area has a good road network making it easily accessible to all the major towns and cities.

Lonhlupheko is a semi-arid area that was heavily affected by the drought of 2006/2007. It is a small rural area with no formal source of employment. Community facilities include a primary school, a high school, public clinic and three grocery shops. The natural dominant vegetation type is a combination of mixed savanna and Acacia savanna. However, there is some evidence of deforestation and vegetation degradation, as there are very few trees and bush in the communal land, compared to privately owned land that is fenced and well managed, adjacent to the study area. There is a pipe transporting water from a reservoir 15 km north-west of Lonhlupheko to Siteki which passes through.

The study was descriptive in nature. It utilized schedule questionnaire survey procedures and personal interviews for collecting primary data, while secondary data was collected through desk search.

Sampling procedure and data analysis

The target population was the 150 households obtained from a household list provided by the Chiefdom headman for Lonhlupheko. The sample size was 108 households determined from the sampling tables developed by Krejcie and Morgan (1970). These households were randomly selected from the list provided. The data collected was both qualitative and quantitative in nature. The quantitative data set were presented as percentages following analysis by Statistical Package for Social Sciences (SPSS) computer software. Qualitative data was described and summarized.

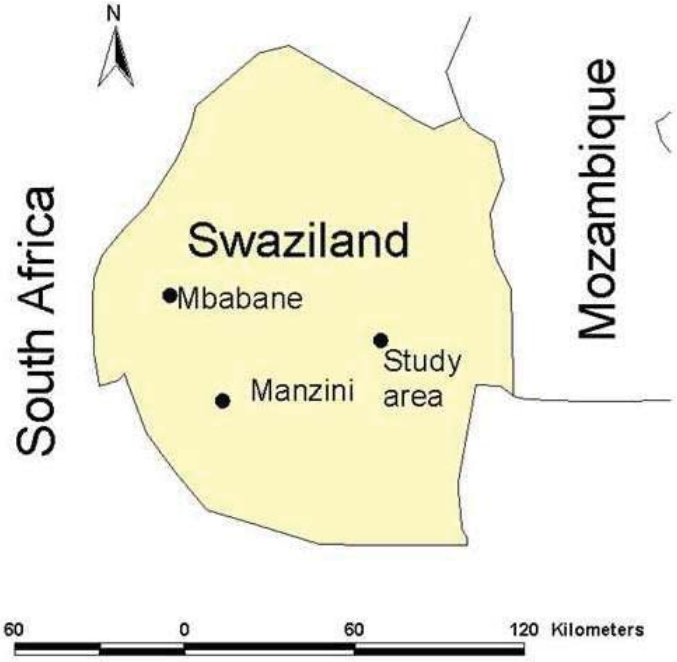

Figure 1. Map showing the location of Swaziland, major towns and the study area.

Table 1. Sources of domestic water used by households (N = 108).

Water source	N	%
Rooftop rainwater harvesting	49	45.4
Communal borehole	36	33.3
Communal tap	20	18.5
Dam	03	02.8
Total	**108**	**100**

RESULTS AND DISCUSSION

Water sources

The households identified rooftop rainwater harvesting, communal boreholes, communal taps and dams as their sources of water (Table 1). About 33% of the households used boreholes as their main source of water. The boreholes were drilled by the Ministry of Natural Resources and Energy to provide domestic water.

Most (45.4%) of the households used rooftop rainwater harvesting techniques for the collection of rainwater through gutters and downpipes from roof catchments to storage devices that ranged from 200 L containers to 10000 L water tanks. The rainwater was used to supplement water obtained from other water sources. The proportion of households practicing rooftop rainwater harvesting were higher (45.4%) than the national average of 1.3% (WHO and UNICEF, 2010), and the 8% reported by Singwane and Kunene (2010) for a community in the same ecological zone as the study area.

Communal borehole and communal tap were utilized by 33.3% (36) and 18.5% (20) households, respectively. The water from communal boreholes was abstracted by the use of hand pumps or electric pumps. Each household that accessed water from boreholes that used electric pumps paid about US$5 per month to cover the costs of electricity. The communal taps and stand pipes were installed by the Swaziland Water Services Corporation, a public enterprise that has the responsibility of supplying domestic water to urban areas and some designated rural areas (Swaziland Water Services Corporation, 2010). This water was potable as it was treated. The remaining 2.8% (3) households used dams as sources of domestic water. The water from the dam was not potable as it was not treated and thus likely to be contaminated. It is worth noting that the study area had no river or stream.

Table 2 reflected that a few (9.3%) households had running water inside their houses, while 28% had standpipes within their yards. Twenty-one (19.4%) of the households had to travel for over 200 m to collect their domestic water, with eight (7.4%) having to travel for over a kilometer to collect water from communal boreholes and dams.

Drought coping strategies utilized by households

The drought coping strategies employed at household level included marketing and selling vegetables, brewing and selling traditional brew, providing labour for food and money, dressmaking, selling second hand clothes, carpentry, collecting and selling thatching grass, cutting and selling of building timber, and other strategies (Table 3).

More than half (52.8%) of the households studied used coping strategies besides the ones specified. Marketing and selling of vegetables was the mostly (19.4%) employed drought coping strategy. Groceries such as sweets and biscuits were purchased in bulk from shops in town, and sold to the local community in small quantities, at higher prices. Vegetables were also bought in bulk and sold in small quantities at higher prices to make profit, a coping strategy reported by 19.4% households. Ten (9.3%) households indicated that they brewed and sold traditional brew to cope with the effects of drought. The sale of traditional brew without a permit is however illegal in Swaziland.

The female members of the households played a major role in drought coping strategies as they were involved in the majority of the strategies. Several livelihood strategies that were reported in other parts of Swaziland were not practiced in the study area, probably because the area lacked the natural resources to do so. These strategies included collection and sale of firewood, collection and sale of wild fruits, and traditional medicinal plants, and weaving handicraft from traditional plants (Manyatsi and Hlophe, 2010).

Table 2. Distance travelled to collect water for domestic use (N = 108).

Distance to water source	N	%
Inside the house	10	9.3
Within the homestead yard	30	27.8
Outside yard, less than 50 m	03	2.8
50 m - 99 m	08	7.4
100 - 199 m	11	10.2
200 - 499 m	21	19.4
500 - 1000 m	17	15.7
More than 1000 m	08	7.4
Total	**108**	**100**

Table 3. Drought coping strategies employed at household level (N=108).

Adaptation strategy	N	%
Marketing and selling vegetables	21	19.4
Brewing and selling traditional brew	10	9.3
Providing labour for food and money	06	5.6
Dressmaking	04	3.7
Selling second hand clothes	04	3.7
Carpentry	02	1.9
Collecting and selling thatching grass	02	1.9
Cutting and selling of building timber	02	1.9
Other (Strategies besides the ones above)	57	52.8
Total	**108**	**100**

Table 4. External and institutional support received to cope with drought (N = 108).

External and institutional support	N	%
Receiving food rations and farming inputs from NGOs	35	32.4
Benefiting from feeding schemes	21	19.4
Receiving crop seeds and fertilizer	15	13.9
Benefit from government paying school fees for OVC	13	12.0
Benefit from free primary education	09	8.3
Benefit from forming cooperatives	05	4.6
Benefit from government subsidized tractor service	04	3.7
Benefit from water delivered by the DMA	04	3.7
Benefit from government veterinary service	02	1.9
Total	**108**	**100**

OVC – Orphaned and vulnerable children; **DMA –** Disaster Management Agency.

External and institutional support to cope with drought

Table 4 shows the external and institutional support received by households to cope with drought. The majority of the households (32.4%) received food rations from non governmental organizations during drought years. The food rations consisted of maize, beans and cooking oil. The Non Governmental Organizations that distributed them were World Vision International and Swaziland Red Cross. They were made available through funds from the World Food Programme and the government of Swaziland. Fifteen (13.9%) of the household received seeds and fertilizer. The seeds supplied were those for sorghum, maize and beans to plant about half a hectare of land. Another 19.4% households benefited from school feeding schemes. Food in the form of soup, beans, and porridge was provided to school going children in order to improve their nutritional status. The elderly people (60 years and above) received monthly social grants of E200 (about US$ 25). They used the grants to purchase food and other household requirements.

The government of Swaziland paid fees in public schools for orphaned and vulnerable children, and 12.0% of the households benefited from that. The scheme has been operational since January 2007. Prior to that, guardians of orphaned and vulnerable children who did not have any sources of income had to sell their livestock, food and other assets in order to pay school fees. Free primary education in public schools was introduced in 2010, and nine (8.3%) households benefited from it. Currently government pays for children in years one to three under the free primary education program. In 2015 it will be rolled over to year seven, which is the last year for primary education. The government also operated a subsidized tractor hire scheme that ploughed for farmers in communal areas at subsidized cost. Four (3.7%) households were reported to have benefited from this service. The service charged for a commercial tractor is E200 (US$25) per hour, while the subsidized government tractor cost was E120 (US$15) per hour.

The Disaster Management Agency (DMA) under the Deputy Prime Minister's Office provided relief assistance to disaster victims, including those affected by drought. Four (3.7%) of the households studied stated that they benefited from potable water that was distributed by the agency during the drought of 2007/2008. The water was distributed by mobile water tankers to the affected communities.

The Ministry of Agriculture provided a free veterinary service to the rural community. The Veterinary Assistants advised the community on livestock production and health. They also gave advice on management of livestock and grazing areas. Two (1.9%) households reportedly benefited from the services provided by the Veterinary Assistants. Communities formed cooperatives through which they pooled financial resources and purchased farm inputs in bulk at reduced costs. Members of the cooperatives were also able to get soft loans from the cooperatives when they needed some finance. Five (4.6%) households indicated that they benefited from the

Table 5. Drought adaptation strategies proposed by households (N = 108).

Proposed adaptation strategy	N	%
Provision of water for irrigation	61	56.5
Construction of dams and structures for rainwater harvesting	25	23.1
Revival of agricultural extension services	14	13.0
Access to loans for small and medium enterprises	06	5.5
Promotion of drought tolerant crops	02	1.9
Total	**108**	**100**

cooperatives.

Drought adaptation measures proposed by households

The households proposed several drought adaptation measures that they believed would lead to sustainable development in the area, and improve their capacity to withstand the effects of drought. These included the provision of water for irrigation (56.5%), construction of dams and other structures to harvest and store rainwater (23.1%), revival of agricultural extension services (13.0%), access to loans for small and medium enterprises (5.5%), and promotion of drought tolerant crops (Table 5).

The terrain of the study area is flat and the soils are mostly suitable for irrigated agriculture (Murdoch, 1968). However, there was no source of surface water within the study area. The provision of water for large scale irrigation may not be technically and financially feasible, as it would require transfer of water from rivers and dams that are far away. Rainwater harvesting is not well developed in Swaziland and there is a potential to do so in the study area. The harvested water could be used to irrigate household or community vegetable gardens that could be a source of income.

The Ministry of Agriculture has an extension service that is responsible for giving advice to farmers on crop husbandry and the crops to grow under different climatic and environmental conditions. However, the service has not been functioning properly for over ten years due to a number of factors that include shortage of extension officers and lack of transport for the officers. Commercial banks and other financial institutions were reluctant to give credit to farmers and other rural dwellers because they lack collateral security. Six (5.5%) households indicated that the government should create a special fund that could avail loans to them for small developmental projects. Planting material for drought tolerant crops such as cassava, sorghum and sweet potatoes was not readily available in many parts of the country where they are required. One respondent suggested that the Ministry of Agriculture should ensure that the planting material is made available.

Conclusions

The drought coping strategies were identified by the 108 households studied as marketing and selling vegetables (19.4%), brewing and selling traditional brew (9.3%), providing labour for food and money (5.6%), dressmaking (3.7%), selling second hand clothes (3.7%), carpentry (1.9%), collecting and selling thatching grass (1.9%), cutting and selling of building timber (1.9%), and other strategies besides the ones above (52.8%).

The drought adaptation measures proposed by households included provision of water for irrigation (56.5%), construction of dams and other structures to harvest and store rainwater (23.1%), revival of agricultural extension services (13.0%), access to loans for small and medium enterprises (5.5%), and promotion of drought tolerant crops (1.9%).

The external and institutional support that were received to cope with drought were identified by the households as; receiving food rations (32.4%), benefiting from feeding schemes (19.4%), receiving crop seeds and fertilizer (13.9%), benefit from government paying school fees for OVC (12.0%), benefit from free primary education (8.3%), benefit from forming cooperatives (4.6%), benefit from government subsidized tractor service (3.7%), benefit from water delivered by the Disaster Management Agency (3.7), and benefit from government veterinary service (1.9%).

RECOMMENDATIONS

According to climate change predictions, temperatures in Swaziland will increase by 2.5oC and annual rainfall will decrease by about 100 mm by the year 2050. The frequency of drought and its intensity is likely to increase in the future. The occurrence of drought cannot be divorced from the effects of climate change. However, the country does not have a climate change policy, and it needs to develop one. The government may request for assistance in the form of funds and experts from international organizations such as UNDP (United Nations Development Programme) and UNFCCC. The National Meteorological Services department should produce simplified versions of seasonal weather forecast reports in order to benefit farmers.

The agricultural extension services need to be revitalized so that the officers could advise farmers on crop and livestock production. The officers should be sensitized and trained on subjects of climate change and drought, as well as interpretation of seasonal weather reports. The government should consider up-scaling the construction of small dams for rainwater harvesting in the semi-arid region of the country. The communities could use water from the small dams to produce crops and vegetables in order to improve their livelihoods. Maize is currently the staple food in Swaziland. Climate variability and drought has made the production of maize in the

semi-arid region not viable. Local people may have to change their eating habits and preferences, and consume drought tolerant crops such as cassava as a source of starch instead of maize. By extension, farmers should be encouraged to grow drought tolerant crops that could withstand the expected high temperatures. This should be complemented by crop breeding activities to avail the drought tolerant seed cultivars, an initiative that is lacking in the country as evident by the seed importations from South Africa, Zambia and Zimbabwe.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

The effect of temperature and cutting time on curd yield and quality made from goat, buffalo and cow milk

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For the present study, two species of lactating animals, that is, Bubalusbubalis (Buffalo) and Bosindicus (Cow) was selected. In both types of milk, highest curd yield at 34°C was obtained at 60 min of cutting time after the addition of rennet followed by 39°C at 60 min of cutting time while at 28°C and 60 min of cutting time the yield was low and cutting times. This may be due to the improper rearrangements of casein network and the minimum curd fat retention level. The % total solids of curd were maximum at 28°C and at 60 min of cutting time may be due to the lower fat losses in whey and %TS were lower at 34°C when the cutting time was 90 min after the rennet addition because of the higher moisture content retention in the casein network due to the elongation in the protein network rearrangement time. The whey fat losses and curd fat retention values were minimized and maximized, respectively, between 28 and 39°C. The whey fat losses (*WFL)* and curd fat retention (*CFR)* were minimum at 28°C and maximum at 39°C. Increasing cutting time at 28°C results in firm gel with a good capability of fat retention and will decrease fat losses in whey. However, at temperature more than 30°C, coarsening of the milk gel occurs more rapidly, permeability of the gel is greater and the microsyneresis can occur at longer aging times. All these factors decreased the ability of the curd to retain fat. These results showed that the retention of fat is dependent on relative rigidity and structure of network at cutting. Below 28°C, there will be less gel firmness before cutting, therefore, increase in temperature will increase the rate of curd firming at best gel will be formed at 35°C. Above 35°C, the network becomes more rigid, rapid coarsening occur and the gel is more porous and all these assists the release of fat in whey and also the fat has high mobility at higher temperature which results in increase fat losses.

Key word: Gelation temperature, optimum cutting time, curd yield and quality.

INTRODUCTION

The ratio of fat and protein is higher up to 40 to 60% in buffalo and goat milk as compared to cows' milk. The ash percentage was also found to be greater in buffalo and goat milk. It may be due to the high concentration of calcium bound to casein protein and different buffering capacity of both milk types. Due to the composition of

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buffalo and goat milk (higher solids), it is expected that they would give a higher curd yield than cows' milk (Hussain et al., 2011). The goat milk are higher in some free amino acids contents like taurine which is upto 9 mg/100 ml and plays an important role in cerebral, vision, cardiac function, detoxification and fatty acids accumulation . The calcium, phosphorus and magnesium content between soluble and colloidal phases of goat, cow and sheep milks are similar. It is considered that iron bioavailability is higher in goat milk than cows' milk due to the higher nucleotide content which contributes to better absorption in the intestines. Extensive studies have been carried out on cows' milk due to higher commercial production. The current increase in consumers and the importance of dairy products from goats and buffalo milk have resulted in further more research on goat and buffalo milk processing and further value addition of milk products.

The development of controlled instability in milk leads to the formation of curd by destabilizing the protein especially the casein and is mainly done by two process, that is, by traditional method, in which selected strains of lactic acid bacteria are used which reduce the pH and cause the precipitation of casein around their isoelectric point and the other method in which rennet/chymosin are used which are proteolytic enzymes and produce coagulum in the presence of calcium, both methods development of instability leading to coagulum/curd formation. The proteinase enzymes like chymosin/rennet are the major coagulating enzymes which act on the active side of the casein micelles called K-casein which lies on the outer surface of casein and causes instability in the casein structure and the inner reactive regions to calcium appears and causes the proteins particles to aggregate and as the aggregation proceeds, three dimensional macroscopic network of casein micelles forms throughout the milk which convert the milk physically to a semi firm viscoelastic gel called curd. After a predetermined time the curd is cut into small pieces which promote the expulsion of the containing water, lactose, small ion and some whey soluble proteins by the rearrangements of proteins called syneresis. Curd is cut after a specific period of time of enzymatic reaction which depends on operator judgment and on immanent assessment of textural and visual properties of curd. Delaying in cutting time results in higher moisture content in cheese due to higher firmness and retarded syneresis and too early cutting will result in low yield of final cheese product due to the losses of fat and curd particles in whey (Payne et al., 1998). These causes suggest the grandness of an objective method for best possible cutting time determination in cheese making. Determination of the most favorable cutting time is more important for the small and less automated cheese plants usual for goat cheese production, because of the large integrative variation between milk batches and the large variation in coagulation process.

The gelation/incubation temperature is an important parameter and has a great effect on the yield and quality of curd. Chymosin activity is highly dependent and sensitive to temperature. More proteolysis and super imposition of thermal effects occur at higher gelation temperatures. The curd moisture is affected by temperature, fat and protein percentage. Gelation temperature affects the contraction of gel matrix and the yield of curd. The yield, quality, curd moisture, and whey fat losses are affected by cutting times and lengthen the cutting time produces an overly firmness in curd, in which the network fails to rearrange, which thus increases curd moisture content and shattering of resultant curd will increase and cause more losses of fat and protein in whey (Castilloa et al., 2006).

The objectives of this study is to determine the upshot of different gelation/incubation temperatures and cutting times at constant pH on curd yield and quality and to conclude curd moisture, fat losses in whey and fat retention capability of curd at different incubation temperatures and cutting times.

MATERIALS AND METHODS

Animal selection

For the present study, three species of lactating animals, that is, Bubalusbubalis (Buffalo), Bosindicus (Cow) were selected in Dairy Training and Research Center and Capra hircus (Goat) was selected in Small Ruminant Training and Research Center, University of Veterinary and Animal Sciences Lahore, Ravi campus, Pattoki. The animals were free from disease indicating healthy conditions. Fresh milk samples from each animal were collected in plastic bottles after complete milking and proper mixing at morning time and transported to post graduate laboratory, Department of Dairy Technology, Ravi Campus Pattoki, UVAS.

Experimental design

The research experiment consisted of three phases, that is, phase-1, phase-2 and phase-3. All the experiments were performed in triplicate.

Phase-1

In Phase-1, milk normal composition was analyzed by Milkoscane, (WTO/Admin/DSR/04) present in the Quality Operational Laboratory, University of Veterinary and Animal Science Lahore. Milk samples from each milk type were transported in falcon tubes in thermo-flask containing ice pieces to the Quality Operational Laboratory. Each sample was analyzed in triplicate to avoid the chances of error. The Milkoscane results consisted of fat%, SNF%, density kg/m^3, lactose%, ash%, protein% and pH of milk. The samples were also analyzed by manual method for fat% and total solids.

Fat determination: Fat was determined by Gerber method, in which 10 ml 98% pure sulfuric acid is taken in butyrometer and 10.94 ml of milk sample is added at an angle so that the milk do not touch the sulfuric acid directly. Then 1 ml amyl alcohl is added to it. The sulfuric acid dissolves all the components other than fat. The amyl alcohol is used for the phase separation. After proper mixing of

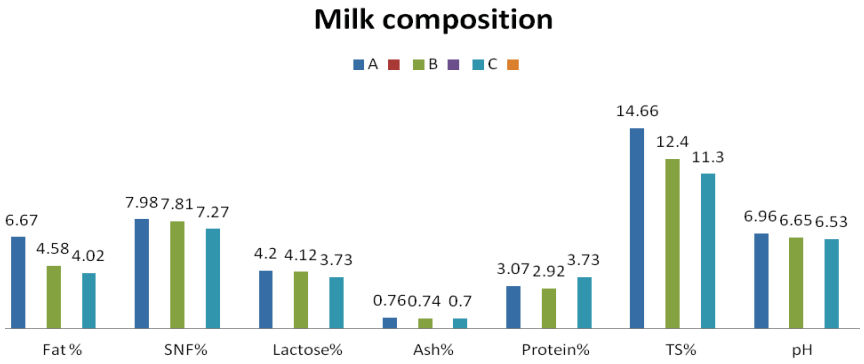

Figure 1. Comparison of three types of milk. A Buffalo milk; B, cows' milk; C, goat milk.

Table 1. Treatment phase

Sample No.	Sample weight (g)	Temperature (°C)	Chymosin (g)	Cutting time minutes
S. 1 (W)	300	28	0.06	30
S. 2 (X)	300	28	0.06	45
S. 3 (Y)	300	28	0.06	60
S. 4 (Z)	300	28	0.06	95

these reagents, centrifugation is done for five minutes at 1100 rpm. The fat is calculated in percentage.

Total solids determination: For total solids determination by manual method, empty, clean and dry crucible is weighed and then upto 5 gm sample is taken and kept at 102°C for overnight. The crucible is weight along with sample and the total solids are determined by the following formula:

% TS= (S+C) /Sample weight * 100
(S+C) = sample plus crucible weight after drying,
Sample weight= sample weight before drying

Phase-2 (Treatment phase)

Milk samples each of 300 ml were weight and pasteurized at 65°C for 30 min in a control water bath and then allowed to cool to 28°C. Each milk sample was treated separately, that is, first buffalo, then cows' and in the last goat milk was treated and analyzed. The milk samples were placed at gelation temperature of 28, 34 and 39°C in a temperature control water bath for 30, 45, 60 and 90 min. Chymosin/Rennet at 0.02% was added to the milk samples and the samples were thoroughly mixed and incubated as shown in Table 1.
The same procedure was repeated for 34 and 39°C. After the calculated time of chymosin addition, the curd was cut into10 to 12 mm or 1cm size pieces, using a specially designed laboratory scale curd cutter. Curd sample was healed at similar Gelation temperature for 10 min and then the samples were centrifuged by a centrifuge (Agilent series 1100) present in the Quality Operational Laboratory, University of Veterinary and Animal Sciences Lahore, to separate whey and curd and weight on electric balanc e and stored at freezing temperature for further analysis.

Phase-3 (Post treatment)

In phase-3, the physico-chemical parameters like fat, protein and total solids of curd and rennet whey were determined by manual methods and also by Milkoscane. The curd fat level was measured by Gerber method and the protein was determined by the formal titration method. Total solids were measured by taking accurately weighed up to 5 gm from each sample of different gelation temperatures in a clean dry crucible and were placed in hot air oven at 102°C over night. The curd was analyzed for yield on wet and dry bases, total whey fat losses and total fat retention by the formulas as follow:

Total whey fat losses in grams, WFL= (M-C) / 100 * F_w
Curd Yield on wet basis, % CY_{wb} = C / M *100
Curd yield on dry basis, % CY_{db}= (C*TS_c/ M*TS_m) *100
Curd fat retention, % CFR= (M*F_m –W*F_w) / M*F_m *100

C= Curd weight in grams, M=Milk weight in grams, W=Whey weight in grams, F_m=% milk fat, F_w= % whey fat, TS_c=curd % total solids, TS_m= Milk % total solids, *=Multiplication.

Statistical analysis

Data was statistically analyzed by applying two way analysis of variance (ANOVA) at a significant level of P<0. 05. The effect of different gelation temperatures and cutting times on curd yield, curd moisture, whey fat losses and casein fractions of the curd made from three different milk types and the mean will be calculated by Duncan Multiple Range Test (DMR).

RESULTS AND DISCUSSION

The curd was prepared from buffalo, cows' and goat milk. The milk samples were analyzed for its chemical composition such as total protein, fat, lactose, ash, total solids and pH before using for curd preparation. The milk curd was analyzed for physicochemical composition like

Table 2. Curd total solids, curd moisture, curd ash, whey total solids and whey ash of three experiments of Buffalo milk.

Samples	Curd %TS	Curd %moisture	Curd %Ash	Whey %TS	Whey %Ash
WA	54.46^{a}±2.69	45.54^{a}±2.69	3.08^{a}±0.08	8.03^{a}±0.24	0.74^{a}±0.01
XA	50.7^{b}±0.67	49.30^{b}±0.66	3.68^{c}±0.12	7.18^{b}±0.03	0.59^{b}±0.01
YA	69.08^{c}±2.22	30.92^{c}±2.22	3.22^{b}±0.05	7.59^{c}±0.046	0.68^{a}±0.02
ZA	54.55^{a}±1.13	45.45^{a}±1.12	3.6^{c}±0.15	7.23^{b}±0.65	0.7^{a}±0.005
WB	59.94^{d}±0.55	40.06^{d}±0.55	3.60^{c}±0.02	7.55^{c}±0.11	0.66^{a}±0.01
XB	58.80^{d}±0.64	41.20^{d}±0.64	3.50^{d}±0.01	7.56^{c}±0.01	0.76^{a}±0.02
YB	56.95^{a}±1.79	43.05^{a}±1.79	4.02^{e}±0.05	7.54^{c}±0.6	0.65^{a}±0.01
ZB	60.44^{d}±0.67	39.56^{d}±0.67	3.45^{d}±0.04	7.75^{c}±0.15	0.65^{a}±0.01
WC	60.04^{d}±0.55	39.96^{d}±0.55	4.50^{d}±0.04	7.21^{b}±0.46	0.59^{b}±0.02
XC	58.80^{d}±0.2	41.57^{d}±0.2	4.0^{e}7±0.03	6.65^{c}±0.06	0.6^{b}±0.01
YC	63.79^{e}±0.65	36.21^{e}±0.66	3.45^{d}±0.05	6.51^{c}±0.42	0.61^{b}±0.02
ZC	61.87^{e}±0.69	38.13^{e}±0.69	4.53^{d}±0.04	7.04^{b}±0.07	0.61^{b}±0.03

Means with different letter in a column are statistically different (P<0.05); **A, B, C**: 28, 34 and 39°C, respectively.

Table 3. Overall whey analysis buffalo milk.

Samples	%Fat	%SNF	%Lactose	%Ash	%protein	pH
WA	0.01^{a}±0.001	8.01^{a}±0.02	4.28^{a}±0.01	0.73^{a}±0.01	2.97^{a}±0.01	4.89^{a}±0.02
XA	0.06^{a}±0.01	4.15^{b}±0.05	2.2^{b}±0.01	0.36^{b}±0..02	1.56^{b}±0.03	4.92^{a}±0.03
YA	0.03^{a}±0.001	7.45^{a}±0.03	3.99^{a}±0.02	0.68^{a}±0.03	2.76^{a}±0.04	7.05^{b}±0.05
ZA	0.05^{a}±0.01	7.80^{a}±0.03	4.16^{a}±0.03	0.72^{a}±0.03	2.88^{a}±0.03	4.79^{a}±0.01
WB	0.14^{b}±0.01	5.56^{b}±2.86	3.84^{a}±0.03	0.65^{a}±0.01	2.66^{a}±0.01	5.42^{c}±0.03
XB	0.19^{b}±0.01	7.21^{a}±0.02	3.88^{a}±0.01	0.66^{a}±0.01	2.66^{a}±0.02	5.26^{c}±0.04
YB	0.25^{b}±0.02	7.21^{a}±0.02	3.84^{a}±0.01	0.66^{a}±0.01	2.65^{a}±0.02	5.15^{c}±0.05
ZB	0.26^{b}±0.01	7.19^{a}±0.01	3.82^{a}±0.01	0.65^{a}±0.01	2.66^{a}±0.03	5.8^{c}±0.03
XC	0.29^{b}±0.01	6.45^{c}±0.01	3.45^{a}±0.01	0.59^{a}±0.02	3.4^{a}±0.01	5.08^{a}±0.05
WC	0.15^{b}±0.02	6.47^{c}±0.03	3.44^{a}±0.01	0.61^{a}±0.02	2.4^{a}±0.01	5.05^{a}±0.07
YC	0.25^{b}±0.02	7.21^{a}±0.02	3.49^{a}±0.02	0.6^{a}±0.01	2.44^{a}±0.02	5.14^{a}±0.01
ZC	0.55^{c}±0.03	6.58^{c}±0.02	3.49^{a}±0.01	0.6^{a}±0.01	2.4^{a}±0.02	5.13^{a}±0.04

Means with different letter in a column are statistically different (P<0.05); A: B: C: 28, 34 and 39°C, respectively. W: X: Y: Z: Sample No. 1, 2, 3 and 4, respectively.

curd yield, whey yield, curd total solids, curd ash, and the physic-chemical composition such as protein, fat, lactose, ash, total solids and pH of whey was analyzed. The results of normal composition of milk and curd formation from three types of milk are discussed in detail in the study.

Physicochemical composition of milk

The composition of milk is not absolute as many factors influence the end product. These variations in milk composition can be related to genetics, environment, milk production, stage of lactation, species, disease, season, locality and age of the animal. Milk components, that is, protein and fat are the primary factors influencing the product quality and yield of the cheese, composition and characteristics. Fresh cows' and buffalo milk samples were procured from disease free animals for curd production. The results regarding the chemical composition of milk samples were shown in Table 2 to 4. The total solids content of buffalo milk were found to be higher when compared with cows' milk. This finding is fully agreement with the results of Hussain et al. (2011). The total protein, fat, and ash contents were found to be higher and significantly different than cows' milk. While fat and lactose contents were observed non-significantly different in both types of milk. The pH of all buffalo and cows' milk samples were found in the range of 6.63- 6.93 and 6.63 to 6.70 respectively (Table 2). These results were agreement with the previous studies of Hussain et al. (2011) and Ahmad et al. (2010).

Buffalo milk curd analysis

In buffalo milk, highest curd yield at 34°C was obtained at

Table 4. Buffalo milk curd and whey composition analysis.

Samples	WFL (g)	$\%CY_{wb}$	$\%CY_{db}$	%CFR
WA	0.025	15.837	61.596	99.8
XA	0.17	16.53	59.86	99
YA	0.73	18.83	92.91	99.5
ZA	0.13	20.3	79.11	99.2
WB	0.14	16.94	67.93	99.8
XB	0.46	18.70	73.53	97.93
YB	0.16	19.66	74.68	96.36
ZB	0.64	18.35	74.17	96.15
WC	0.38	17.39	77.79	97.69
XC	0.72	17.95	78.14	95.55
YC	1.36	18.31	74.17	96.15
ZC	1.4	16.18	75.61	91.5

WFL, Whey fat losses; CY_{wb}, Curd yield on wet bases; CY_{db}, curd yield on dry bases; CFR, Curd fat retention; A: B: C, 28, 34 and 39°C, respectively; W: X: Y: Z, Sample No. 1, 2, 3 and 4, respectively.

60 min of cutting time after the addition of rennet followed by 39°C at 60 min of cutting time while at 28°C and 60 min of cutting time the yield was lower as compared to the above two temperatures and cutting times. This is possible because `beyond 35°C, the fat boils and losses occur in whey, so the yield will be decreased. The lowest yield was obtained at 34°C when the cutting time was 45 min after the rennet addition. This may be due to the improper rearrangements of casein network and the minimum curd fat retention level. The %total solids of curd were maximum at 28°C and at 60 min of cutting time may be due to the lower fat losses in whey and %TS were lower at 34°C when the cutting time was 90 min after the rennet addition. This is because of the higher moisture content retention in the casein network due to the elongation in the protein network rearrangement time. These result correlated with the studies of Hussain et al. (2011) which shows that dynamic moduli of the curd is directly related with the gelation temperatures and at 28°C the curd yield was lower than 34 and 39°C. Their results also show that at 34°C curd has better moisture retention and less structural breakage. The whey fat losses and curd fat retention values were minimized and maximized, respectively, between 28 and 39°C which were selected for the research study.

This study shows that the best temperature for the formation of Mozzarella type cheese and other type cheese made from cow milk is 34°C which is better for the gel firmness, curd fat retention, optimum moisture content and final product yield and quality.

The whey fat losses *WFL* and curd fat retention *CFR* were minimum at 28°C and maximum at 39°C. At temperature below 28°C, the gel will be weak when cut. Therefore, increasing cutting time at 28°C results in firm gel with a good capability of fat retention and will decrease fat losses in whey. However, at temperature more than 30°C, coarsening of the milk gel occurs more rapidly, permeability of the gel is greater and the microsyneresis can occur at longer aging times. All these factors decrease the ability of the curd to retain fat. These results were broad supported by the studies of Fagan et al. (2007). Their results show that the retention of fat is dependent on relative rigidity and structure of network at cutting. At temperature below 28°C, there will be less gel firmness before cutting, therefore, increase in temperature will increase the rate of curd firming at best gel will be formed at 35°C. Above 35°C, the network becomes more rigid, rapid coarsening occur and the gel is more porous and all these assists the release of fat in whey and also the fat has high mobility at higher temperature which results in increase fat losses.

Cow milk curd analysis

The cow milk analysis show less difference as compared to buffalo milk. It may be due to the little difference in the total solids contents of both milks. The highest yield at gelation temperature of 28°C was obtained after 60 min of cutting the curd when the chymosin was added. This may be due to the proper rearrangements of casein matrix and the proper fat retention in the curd. At 34°C of gelation temperature, the yield was highest and at 39°C of gelation temperature the curd was also high at 60minutes of cutting time. These results show that the best cutting time for high yield of curd is 60 min after the addition of chymosin. The best yield was obtained at gelation temperature of 34°C. These results correlate with the results of Husain et al. (2011). The percent total solids were higher at 28°C and 90 min of temperature and time combination, followed by 34°C and 60 min and the lower at 39°C and 45 min of temperature and time combination, respectively. The whey fat losses were lower at 28, 34 and 39°C with the time relation of 30, 60

Table 5. Overall analysis of curd total solids, curd moisture, curd ash, whey total solids and whey ash of cow milk experiments.

Samples	Curd %TS	Curd %moisture	Curd %Ash	Whey %TS	Whey %Ash
WA	61.05^{a}±0.68	38.95^{a}±0.68	3.4^{a}±0.01	7.36^{a}±0.12	0.65^{a}±0.01
XA	63.17^{a}±0.52	36.83^{a}±0.52	2.64^{b}±0.05	7.38^{a}±0.01	0.51^{b}±0.01
YA	66.28^{b}±0.85	33.71^{b}±0.85	3.04^{c}±0.05	7.31^{a}±0.04	0.6^{a}±0.02
ZA	66.90^{b}±0.03	33.10^{b}±0.03	3.11^{a}±0.04	7.41^{a}±0.03	0.49^{b}±0.01
WB	46.85^{c}±0.71	53.13^{c}±0.73	3.24^{a}±0.04	7.42^{a}±0.06	0.65^{a}±0.01
XB	48.52^{d}±0.55	51.48^{d}±0.56	2.69^{b}±0.03	7.47^{a}±0.04	0.65^{a}±0.02
YB	64.7^{a}±0.88	35.3^{a}±0.88	2.62^{b}±0.03	7.47^{a}±0.05	0.63^{a}±0.01
ZB	63.51^{a}±0.76	36.49^{a}±0.76	2.77^{b}±0.06	7.56^{a}±0.15	0.33^{c}±0.01
WC	61.4^{a}±1.22	38.58^{a}±1.22	3.19^{a}±0.45	6.84^{b}±0.02	0.58^{a}±0.01
XC	63.52^{a}±0.71	36.48^{a}±0.71	4.05^{d}±0.63	6.81^{b}±0.12	0.57^{a}±0.02
YC	62.2^{a}±0.42	37.79^{a}±0.42	4.04^{d}±0.05	7.06^{c}±0.1	0.59^{a}±0.01
ZC	61.36^{a}±0.28	38.64^{a}±0.28	4.71^{d}±0.16	7.25^{a}±0.05	0.59^{a}±0.01

Means with different letter in a column are statistically different (P<0.05); A: B: C, 28, 34 and 39°C, respectively; W: X: Y: Z, Sample No. 1, 2, 3 and 4, respectively.

Table 6. Whey Analysis of three experiments of cow milk.

Samples	%Fat	%SNF	%Lactose	%Ash	%protein	pH
WA	0.05^{a}±0.05	7.05^{a}±0.02	3.74^{a}±0.04	0.64^{a}±0.01	2.05^{a}±0.04	6.75^{a}±0.05
XA	0.12^{b}±0.02	5.64^{b}±0.03	3.01^{b}±0.03	0.52^{b}±0.02	2.07^{a}±0.01	6.71^{a}±0.02
YA	0.08^{a}±0.02	6.58^{c}±0.02	3.37^{a}±0.31	0.60^{a}±0.02	2.61^{b}±0.02	6.70^{a}±0.04
ZA	0.15^{b}±0.02	5.36^{b}±0.03	2.83^{b}±0.03	0.50^{b}±0.03	2.00^{a}±0.03	6.69^{a}±0.01
WB	0.03^{a}±0.04	7.08^{a}±0.02	3.77^{a}±0.02	0.66^{a}±0.01	2.64^{b}±0.03	6.68^{a}±0.02
XB	0.06^{a}±0.03	6.98^{a}±0.03	3.67^{a}±0.04	0.63^{a}±0.02	2.58^{b}±0.02	6.70^{a}±0.03
YB	0.01^{a}±0.01	6.90^{a}±0.02	3.66^{a}±0.01	0.64^{a}±0.02	2.54^{b}±0.02	6.70^{a}±0.01
ZB	0.21^{c}±0.02	3.49^{d}±0.02	1.85^{c}±0.02	0.32^{c}±0.01	1.30^{c}±0.01	6.81^{a}±0.04
WC	0.15^{b}±0.02	6.40^{a}±0.02	3.40^{a}±0.01	0.58^{a}±0.01	2.36^{b}±0.01	6.62^{a}±0.04
XC	0.01^{a}±0.02	6.20^{a}±0.02	3.29^{a}±0.01	0.56^{b}±0.01	2.90^{d}±0.2	6.70^{a}±0.01
YC	0.37^{d}±0.04	6.45^{a}±0.02	3.39^{a}±0.02	0.59^{a}±0.02	2.38^{b}±0.02	6.69^{a}±0.02
ZC	0.48^{e}±-.02	6.40^{a}±0.01	3.41^{a}±0.01	0.59^{a}±0.01	2.36^{b}±0.01	6.70^{a}±0.01

Means with different letter in a column are statistically different (P<0.05); A: B: C, 28, 34 and 39^{o}C, respectively. W: X: Y: Z: Sample No. 1, 2, 3 and 4, respectively.

and 45 min, respectively, after the cutting of curd while the fat losses were higher at 28, 34 and 39°C at 90 min after the cutting of curd. These results coincide with the results of Fagan et al. (2007) (Tables 5 to 7).

Goat milk curd analysis

The goat milk shows different results as compared to buffalo milk. It is because of the total solids content difference of both milks. The highest yield was obtained at 28°C at 45 min of cutting time, at 34°C the highest yield was at 90 min and at 39°C the highest yield was also at 90 min of cutting time. The whey fat losses at 28°C were higher and the yield was lower at this time and temperature combination while at 28°C and 45 min of cutting time the whey fat %age was lower and the curd yield was highest at this combination. At 34°C and 90 min of cutting time, the whey fat losses were lower and the curd yield was higher while at 39°C and 90 min of cutting time. The total solids percentage was higher at 28°C and 90 min of cutting time followed by 34°C and 90 min and in the last at 39°C at 90 min. It is possible that at 28°C, the curd moisture retention was lower so the total solids were higher, followed by 34°C and the highest moisture retention was at 39°C at 90 min of cutting time (Tables 9 to 10).

Conflict of Interest

The authors have not declared any conflict of interest.

Table 7. Cows' milk curd and whey composition analysis.

Samples	WFL (gm)	%CY_{wb}	%CY_{db}	%CFR
WA	0.13	15.43	69.72	99.13
XA	0.13	15.79	73.85	98.28
YA	0.20	16.95	83.17	98.64
ZA	0.38	16.19	80.16	97.43
WB	0.08	9.60	31.84	99.45
XB	0.16	13.58	46.52	98.95
YB	0.02	21.16	99.37	99.84
ZB	0.51	20.60	92.33	99.61
WC	0.38	16.07	81.63	96.94
XC	0.03	17.29	90.84	99.94
YC	0.95	15.60	80.27	92.40
ZC	1.23	15.36	77.97	90.12

WFL, Whey fat losses; CY_{wb}, Curd yield on wet bases; CY_{db}, curd yield on dry bases; CFR, Curd fat retention A: B: C, 28, 34 and 39°C, respectively; W: X: Y: Z, Sample No. 1, 2, 3 and 4, respectively.

Table 8. Curd total solids, curd moisture, curd ash, whey total solids and whey ash analysis of three goat milk experiments.

Samples	Curd %TS	Curd %moisture	Curd %Ash	Whey %TS	Whey %Ash
WA	62.53^{a}±0.27	37.47^{a}±0.26	4.95^{a}±0.22	6.34^{a}±0.05	0.57^{a}±0.02
XA	62.12^{a}±0.1	37.87^{a}±0.1	4.67^{b}±0.1	6.57^{a}±0.02	0.57^{a}±0.01
YA	61.86^{a}±0.54	38.13^{a}±0.54	5.52^{c}±0.06	6.5^{a}±0.08	0.59^{a}±0.01
ZA	66.77^{b}±0.76	33.22^{b}±0.77	4.95^{a}±0.18	6.66^{a}±0.06	0.50^{b}±0.01
WB	57.73^{c}±0.44	42.34^{c}±0.44	3.75^{d}±0.07	7.42^{b}±0.67	0.67^{c}±0.02
XB	54.86^{d}±0.33	45.14^{d}±0.3	3.95^{d}±0.06	7.68^{b}±0.04	0.63^{c}±0.01
YB	56.4^{c}±0.19	43.6^{c}±0.19	3.49^{d}±0.4	7.97^{c}±0.01	0.69^{c}±1.32
ZB	58.99^{c}±0.8	40.01^{c}±0.8	3.63^{d}±0.14	8.06^{c}±0.1	0.65^{c}±0.01
WC	54.98^{d}±0.83	45.01^{d}±0.83	3.81^{d}±0.07	7.60^{b}±0.2	0.41^{d}±0.02
XC	54.65^{d}±0.24	45.36^{d}±0.24	3.88^{d}±0.06	8.35^{d}±0.19	0.63^{c}±0.01
YC	56.67^{c}±1.32	45.33^{c}±4.15	7.71e±0.12	8.03^{d}±0.07	0.65^{c}±0.02
ZC	57.12^{c}±0.76	42.88^{c}±0.77	4.09^{d}±0.21	8.21^{d}±0.19	0.65^{c}±0.01

Means with different letter in a column are statistically different (P<0.05); **A: B: C**, 28, 34 and 39°C, respectively; **W: X: Y: Z**, sample No. 1, 2, 3 and 4, respectively.

Table 9. Whey analysis of experiment of goat milk.

Samples	%Fat	%SNF	%Lactose	%Ash	%protein	pH
WA	0.34^{a}±0.01	6.21^{a}±0.01	3.29^{a}±0.01	0.59^{a}±0.02	2.3^{a}±0.01	6.68^{a}±0.03
XA	0.24^{b}±0.01	6.2^{a}±0.01	3.3^{a}±0.02	0.57^{a}±0.03	2.29^{a}±0.02	6.72^{a}±0.08
YA	0.30^{a}±0.01	6.27^{a}±0.04	3.31^{a}±0.01	0.58^{a}±0.01	2.31^{a}±0.02	6.7^{a}±0.01
ZA	0.30^{a}±0.03	5.45^{b}±0.06	2.9^{b}±0.01	0.51^{b}±0.02	2.04^{a}±0.05	6.71^{a}±0.02
WB	0.12^{c}±0.01	7.29^{c}±0.01	3.9^{c}±0.01	0.67^{c}±0.02	2.7^{b}±0.02	6.29^{b}±0.04
XB	0.4^{d}±0.02	6.76^{a}±0.03	3.64^{a}±0.04	0.61^{a}±0.02	2.49^{c}±0.01	6.69^{a}±0.01
YB	0.27^{b}±0.01	7.45^{c}±0.03	3.98^{c}±0.02	0.69^{c}±0.01	2.76^{b}±0.01	6.69^{a}±0.01
ZB	0.35^{a}±0.02	7.19^{c}±0.02	3.84^{c}±0.02	0.66^{c}±0.02	2.66^{b}±0.02	6.71^{a}±0.01
WC	0.59e±0.02	4.41^{d}±0.02	2.4^{b}±0.02	0.4^{d}±0.01	1.62^{d}±0.02	6.67^{a}±0.03
XC	0.7f±0.02	6.83^{a}±0.02	3.65^{a}±0.01	0.64^{c}±0.01	2.53^{c}±0.02	6.69^{a}±0.01
YC	0.61g±0.03	7.02^{c}±0.05	3.71^{a}±0.05	0.68^{c}±0.03	2.76^{b}±0.01	6.69^{a}±0.01
ZC	0.83h±0.02	7.0^{c}±0.05	3.73^{a}±0.03	0.65^{c}±0.05	2.61^{b}±0.02	6.6^{a}±0.03

Means with different letter in a column are statistically different (P<0.05); **A: B: C**, 28, 34 and 39°C, respectively; **W: X: Y: Z**, Sample No. 1, 2, 3 and 4, respectively.

Table 10. Goat milk curd and whey composition analysis.

Samples	WFL (gm)	$\%CY_{wb}$	$\%CY_{db}$	%CFR
WA	0.90	12.38	63.75	92.26
XA	0.60	17.11	87.56	94.83
YA	0.80	12.41	63.25	93.17
ZA	0.77	15.50	85.24	93.42
WB	0.30	18.54	76.39	97.54
XB	1.01	16.81	65.70	91.70
YB	0.68	17.40	69.96	94.44
ZB	1.03	21.27	90.97	91.55
WC	1.46	18.44	71.99	88.15
XC	1.75	17.57	68.20	85.79
YC	1.50	18.97	76.34	87.83
ZC	2.01	20.30	82.37	83.71

WFL, Whey fat losses; CY_{wb}, Curd yield on wet bases; CY_{db}, curd yield on dry bases; CFR, Curd fat retention; A: B: C, 28, 34 and 39°C, respectively;W: X: Y: Z, Sample No. 1, 2, 3 and 4, respectively.

Conflict of Interest

The authors have not declared any conflict of interest.

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Production practices of potato *(Solanum tuberosum* L.) by farmers in Mzimba District, Northern Malawi

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Potato (*Solanum tuberosum* [L.]) is one of the food and cash crops in Malawi. Almost all potato farmers in the country are smallholders with low productivity. In order to develop appropriate interventions to improve productivity, it is important to first understand farmers' characteristics and prevailing production practices. This study was therefore carried out in October 2010 in Champhira Extension Planning Area (EPA) in Mzimba District, Malawi with main objective of documenting key baseline information characterising smallholder potato growers and their production practices in the area. The survey was conducted in five randomly selected villages in Kazingilila Section in the same EPA whereby a total of 50 households (10 household from each village) were interviewed using a pre-tested questionnaire. The questionnaire was designed to gather information on social and demographic characteristics of households, farm size, knowledge and experience with potato production, soil fertility management practices, perceived market for potatoes and challenges. In addition, interviews with government extension officers residing in the area were carried out as key informants for general information about the status of potato production in the study area. Results indicate that total land holding size is between 0.65 and 1.01 ha and farmers allocate on average 0.08 ha to potato production. Results also show that farmers were growing Rosita as the only recognised and released variety in Malawi. Also, 80% of respondents were recycling their own seed with very limited knowledge of proper seed selection. On soil fertility management practices, 98% of farmers were applying chemical fertilisers mainly as 23:21:0 + 4 S and CAN or Urea. Sixty eight percent of farmers were also applying manure as a source of organic fertilizer to their crop. However, the average yield was 6 tons/ha, compare national average of 11.9 t/ha in 2008. Farmers and extension staff mentioned pests and diseases, limited access to improved varieties and clean seed, low soil fertility and limited knowledge of crop management as main constraints that affect potato production in the area. Based on these results technical issues, areas of research interventions and recommendations for improving potato production in the area have been indicated.

Key words: Potato, Malawi, soil fertility management, production constraints.

INTRODUCTION

Potato is increasingly becoming an important food and cash crop in Malawi. In 2007 Malawi was ranked the second largest potato producer in Africa with 2.85 million tonnes harvested (FAOSTAT, 2008). Potato production

also ranks fourth when compared to other major food crops produced in Malawi thus cassava, maize and sweet potato (Ministry of Agriculture and Food Security (FAOSTAT, 2008). Potato is the main cash crop and second major food crop after maize in the major growing districts of Malawi (Demo et al., 2009b), which are: Ntcheu, Dedza, Neno, Mchinji, Mzimba and Ntchisi. These are suitable areas for potato production because of their high altitude with cool climate and adequate rainfall (MoAFS, 2005).

Almost all potato growers in Malawi are small scale farmers (Demo et al., 2009a). Despite general increase in potato production in Malawi for the past years, the average productivity at national level is still very poor as compared to other countries. For instance, the national average yields for some countries such as South Africa and Egypt are 34 and 24.8 tonnes/ha respectively whereas with Malawi it is 11.9 tonnes/ha (FAOSTAT, 2008). The national average potato yield of 11.9 tons/ha is still very low against a potential of 40 tonnes/ha (Soko, 2004). The national aim is to increase production to 20 t/ha (MoAFS, 2005). Also, the current national potato production is still below market demand (Demo et al., 2009c). The quality of tubers obtained is also generally very low due to some factors such as small tuber sizes, bruises on tubers, tuber diseases and rotting (Soko, 2004; MoAFS, 2005). There are a number of constraints that negatively affect potato production in Malawi and these include lack of quality seed potato, declining soil fertility and structure due to poor management practice, diseases such as late blight, bacterial wilt and viruses, and limited knowledge of farmers, extension staff and existing research technicians on improved production (Demo et al., 2009c, 2007).

In order to improve potato productivity in Malawi, there is need to address some of these already identified constraints through research and other interventions. However, to develop appropriate interventions such as a research programme for addressing these constraints like poor soil fertility management, it is important to first understand farmer characteristics and prevailing soil fertility management practices. This is because soil fertility is one of the major factors that affect the yield and quality of the potato because the crop requires high amounts of nitrogen, phosphorus, potassium, magnesium and calcium (Adhikari and Sharma, 2004; Hossain et al., 2003; Gathungu et al., 2000). This is why a baseline survey of similar nature was conducted in Mzimba District, Malawi in 2010 in order to document key baseline information characterising potato production in the study area, with focus on soil fertility management practices. Mzimba District is one of the areas in Malawi where potatoes are important. Farmers are motivated by a steady market along the main Lilongwe - Mzuzu road.

MATERIALS AND METHODS

Bio-physical and socio-economic background to study area

The baseline survey was conducted in October, 2010 during 2010/2011 growing season. The study area Mzimba District, Champhira Extension Planning Area (EPA) located at 12° 24'S and 33° 38'E and lies at altitude ranging from 1216 to 1338 m. The organisation structure of extension service in Malawi starts from national level (Ministry of Agriculture and Food Security) → Agriculture Development Division (ADD) → District Agriculture Development Office (DADO) → Extension Planning Area (EPA) → Section. The EPA is headed by an Agricultural Extension Development Coordinator (AEDC) while a Section is headed by an Agricultural Extension Development Officer (AEDO).

Champhira EPA was preferred for the study because of senior author's work experience in the area whereby poor yields and quality of potato tubers was observed. It is also one of the major potato producing areas in the district. Farmers plant the crop at least twice a year. First planting is done around November/December depending on the on-set of rains and this is referred to as a summer crop. The second planting is done around March/April in 'wetland or dambo, areas (usually the crop relies on residual moisture) and this is referred to as a winter crop.

Study design

The survey was purposively conducted in Kazingilila Section because it is the main potato production area in the EPA. From a complete list of 64 villages under Kazingilila Section, five villages namely Chang'ombe, Chinombo, Psyutu, Kasoti and Zifere Chisi were selected using a simple random sampling method. In each village, 10 households were randomly selected for the interview from a complete list of households who grew potato. The number of households sampled from five villages was 50 from a total number of 121 households. The data from the households was collected using a pre-tested structured questionnaire. A questionnaire was designed to gather information on socio and demographic characteristics of households, farm size, knowledge and experience with potato production, soil fertility management practices, perceived market for potatoes and challenges. The questionnaire was administered on a one-to-one basis such that questions were well clarified. In addition, interviews with AEDOs and AEDC residing in the area were carried out as key informants for general information about the status of potato production in the study area.

Conversion of measures and weights

Farmers were also asked to provide information on potato yields using their local units for the past three seasons. Farmers were able to recall the number of ox-carts and pails harvested per unit area of land. One ox-cart full of potato was estimated to be weighing 400 Kg while a standard pail was equivalent to 20 Kg.

Data analysis

Data collected were analysed for descriptive statistics such as means, frequencies and percentages using Statistical Package for Social Sciences (SPSS) Computer Package 16th Edition.

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Table 1. Characteristics of the respondents.

Variable	Frequency	Percent
Gender of household head		
Male	40	80
Female	10	20
Education level		
None	1	2
Primary	36	72
Secondary	13	26
Age group of household heads		
Less than 30	7	14
30 - 40	18	36
40 - 50	15	30
Over 50	10	20
Household size		
Less than 5	11	22
5 - 10	36	72
Over 10	1	2

RESULTS

Characteristics of respondents

Socio and demographic characteristics of respondents are shown in Table 1. Most of the households were male headed (80%). The majority of respondents (72%) had household size between 5 and 10 members with average of 6.5. Most of household heads (66%) were between the ages of 30 and 50 years. Moreover, most of the respondents (72%) had primary education which is an indication that most farmers had a fairly good education level to understand basic farming practices.

Potato production

General information on potato production

The study found that 60% of respondents had total land holding size between 0.65 and 1.01 ha (Table 2). However, the average land size allocated to potato production for the summer crop was about 0.11 ha with ranges from 0.04 to 0.4 ha. For the winter crop, the average land size was 0.08 ha with majority (51.4%) growing on 0.04 ha of land. Most of respondents explained that they allocated less land to potato farming because of lack of adequate potato seed and limited land near water sources for winter farming. When asked how they perceive their own trend of potato production over the years, only 27.1% of the respondents indicated that it had increased while 35.4% claimed that it had decreased and 37.5% indicated that it had remained constant. The respondents attributed the decrease to pest and diseases, poor markets, low yields, and limited access to clean seed.

Results also show that 74% of respondents grow the crop twice in a year while 22% grow three times in a year (Table 2) through bucket irrigation. The majority of these farmers plant their summer crop in November/December and their winter crop in March/April. However, 76% of households dry plant their summer crop in order to reduce the work load as more field crops are planted in November/December. Results also show that the farmers were growing Rosita as the only recognised and released variety in Malawi (Table 2). Farmers were also growing two cultivars known in their language as Betane and Mbeya because of their source. Betane came from neighbouring Zambia while Mbeya was brought from Tanzania through traders at their Jenda produce market. Between Betane and Mbeya, 42% of the respondents preferred Betane because they claimed it was high yielding with big tuber sizes and tolerant to diseases unlike Mbeya which was perceived to be very susceptible to diseases and pests. On frequency of seed sources, 80% of respondents were recycling their own seed.

Soil fertility management practices, yield and constraints

On soil fertility management, the majority of respondents (68%) applied manure to their potato crop especially during the winter. The majority of respondents (44%)

Table 2. General information on potato production by respondents (n = 50).

Variable	Frequency	Percent (%)
Total land holding size		
0.04 ha to 0.61 ha	8	16
0.65 ha to 1.01 ha	30	60
> 1.01 ha	12	24
Varieties grown		
Rosita alone	18	36
Rosita and Betane[1]	21	42
Rosita , Betane[1] and Mbeya[2]	7	14
Betane[1] + Mbeya[2]	2	4
Betane[1]	2	4
Favourite variety		
Rosita	26	52
Mbeya[2]	3	6
Betane[1]	21	42
Frequency of seed sources		
Own	40	80
Neighbour	8	16
Market	2	4
Number of harvests per year		
Once	1	2
Twice	37	74
3 times	11	22
4 times	1	2

Betane[1] is a cultivar sourced from Zambia; Mbeya[2] is a cultivar sourced from Tanzania.

use fresh manure from khola without composting it (Table 3). On average, farmers applied an estimated rate of 6 tonnes of manure per ha.

The results also showed that 98% of respondents were also applying chemical fertilisers. On types of inorganic fertilisers, 70% of respondents applied 23:21:0 + 4 S alone as basal dressing and applied a mixture of 23:21:0 + 4 S and CAN or Urea as top dressing (Table 3). Only 4% of respondents used Compound D (8-18-15 + 6 S) fertiliser that contained potassium. On average, potato farmers in the study area were applying three 50 kg bags of 23:21:0 + 4 S and two 50 kg bags of CAN per ha.

The average total yield for the respondents was 6 tons/ha. However, the farmers complained of small size of potato tubers they usually harvest from their fields despite the application of both manure and inorganic fertilisers. When asked how they perceive market of potato in the area, 52% of the respondents were of the opinion that market for potato was not good especially for summer crop because of poor prices.

On production constraints, both farmers and agriculture extension staff in the area indicated that the major challenges were pests (such as red spider mite, aphids and cut worms) and diseases (bacterial wilt (*Ralstonia solanacearum*) and viruses), low soil fertility, limited technical knowledge for crop management, limited access to improved varieties and clean seed, poor marketing and lack of capital for inputs. When these constraints were ranked by respondents, the problem of pests (red spider mite and cut worms) and disease (bacterial wilt) emerged to be the major problem seconded by limited access to improved varieties and clean seed. However, all respondents and extension staff admitted that smallholder farmers harvest very low yields without application of any form of fertiliser to a potato crop although low soil fertility problem was ranked third.

DISCUSSION

Table 1 show that the average household size of 6.5 persons for the study area was above the national average household size of 4.6, and literacy level of 72% was above the national average of 64% for persons aged 6 years and above (National Statistics Office, 2009). The high literacy level for farmers is considered as one of

Table 3. Prevailing soil fertility management practices among respondents (n=50).

Variable	Frequency	Percent (%)
Use of manure		
Do not apply any type of manure	16	32
Apply fresh manure from khola	22	44
Apply pit manure (compost)	12	24
Type of fertilisers applied		
Basal dressing		
23:21:0 + 4S alone	35	70
23:21:0 + 4S and CAN	6	14
23:21:0 + 4S and Urea	7	10
D Compound alone	1	2
D Compound + Urea or CAN	1	2
Top dressing		
CAN alone	5	10
Urea alone	4	8
23:21:0 + 4 S and Urea or CAN	31	62
D compound + Urea or CAN	2	4
Do not top dress	8	16

variables that positively affect adoption of agricultural technologies (Doss, 2003). Due to this high literacy level, improved potato production methods can be extended to the farmers through reading materials such as pamphlets, leaflets and other aids (Demo et al., 2008). Also, the study recorded a higher % of men (80 %) engaged in potato production. This reflects that potato production is taken as a business enterprise, as men tend engage in income generating activities to fend for the family. This result is consistent with the findings of Takane (2008) who reported that compared to female-headed households, male headed households had higher own-farm income, land holding size, years of education, maize productivity, fertilizer use growing more tobacco, a leadig cash crop in Malawi.

Table 2 indicate that smallholder farmers were growing Rosita as the only recognised and released variety in Malawi. The smallholder farmers in the study area had very limited access to improved varieties such as Violet, Lady Rosetta, among others (MoAFS, 2005). However, both smallholder farmers and government extension staff claimed that Rosita variety was tolerant to most pests and diseases that affect the crop in the area. Their perception about Rosita variety being tolerant to some diseases agrees with what Tusiime et al. (1996) observed in Uganda during their screening of several potato genotypes for resistance to bacterial wilt (*Ralstonia solanacearum*) disease and Rosita was among the five genotypes that maintained high level of resistance to the disease for three seasons. Similarly, Demo et al. (2008) reported that potato farmers in Dedza and Ntcheu perceived that Rosita variety was resistant to late blight disease caused by *Phytophthora Infestans*. However, farmers in the study area claimed that the performance of Rosita variety has declined over years and that it was becoming more susceptive to pests and diseases. This could be attributed to limited access to clean seed as farmers recycle their own seed with limited technical knowledge of positive and negative seed selection technology. The recycling of own seed without proper selection and isolation encourages build up of diseases.

Table 3 indicate the majority of farmers were applying both manure and chemical fertilisers to the crop. This was an encouraging practice because increase of the nutrients in the soil can increase plant uptake and encourage potato haulm growth which increases both the photosynthetic and assimilation rates that lead to increase in total yield and yield components (Latif et al, 2011; Hossain et al., 2003, Gathunga et al., 2000). Manure application is widely recommended in potato production (MoAFS, 2005; Rolo, 2001). Information on the rates applied was not obtained; however, based on the potato yields in the area, the amounts are likely to be inadequate. However, use of suitable and recommended chemical fertilisers such as compound D (8-18-15 + 6 S) that contain potassium is very limited and only 2% of respondents were using this type of fertiliser (Table 3). The results found in this study are similar to those reported by Demo et al. (2008) who found out that, out of 81 potato farmers interviewed in Dedza and Ntcheu, over 90% of farmers were applying 23:21:0 + 4 S and CAN. Lack of K application in potatoes could also be part of the problems responsible for the current low yields and poor quality of potatoes in the study area. This is because potassium is the nutrient taken up by the tubers in the greatest amount; usually one and half times as much as

N, four or five times as much as P and appreciable amounts of calcium, magnesium and sulphur (Perrenoud, 1983; Rolo, 2001). Satyanarayana and Arora (1985) also reported that insufficient K results in reduced potato yield and smaller-sized tubers while Latif et al. (2011) reported that application of right amount of K per ha improves potato yields and tuber size. Potassium K is important in photosynthesis, increasing enzyme activity; improving synthesis of proteins, carbohydrates and fats, translocation of assimilates from leaves to tubers, ability to resist pests and diseases (Latif et al., 2011).

CONCLUSION AND RECOMMENDATIONS

The findings have shown that potato farmers in the study area have limited access to improved varieties and clean seed. The majority of farmers were applying both manure and chemical fertilisers to the crop but continue getting poor yields and quality. Thus is also need for a research programme to focus and demonstrate on soil fertility management. Most of the seed planted was recycled hence a need for a introducing a seed system that promotes access to clean seed. There is also need for general promotion of good agricultural practices for potato such as positive/negative seed selection, application of recommended chemical fertilisers, integrated pest and disease management.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Control of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) with entomopathogenic fungus and insecticides using two sampling methods on sugarcane fields

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The sampling of *M. fimbriolata* aims to estimate the population level and optimum timing for spittlebug control in the sugarcane fields. Thus, this study was conducted to determine the cost and efficiency of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) and insecticides utilized in *M. fimbriolata* control using two sampling techniques. The experiments were performed between November 2012 and April 2013, in a sugarcane field. In each experiment, apart from the control, treatments included Thiamethoxan (250 g ha^{-1}), Imidacloprid (700 g ha^{-1}), PL 43 (*M. anisopliae* - 2.0×10^{12} con mL^{-1}), ESALQ E9 (*M. anisopliae* -2.1×10^{12} con mL^{-1}) and IBCB 425 (*M. anisopliae* - 1.4×10^{12} con mL^{-1}). In the first experiment, the *M. fimbriolata* nymphs and adults were monitored, while on the other experiment, the nymphs sampled were the small, medium, large, and the adults of *M. fimbriolata* with objective was to identify the timing of the application of treatments. In general, by monitoring the small, medium and large nymphs and the adult spittlebugs, the timing of the application was optimized, which increased the efficiency of *M. fimbriolata* control.

Key words: Biological control, imidacloprid, *Mahanarva fimbriolata*, *Metarhizium anisopliae*, thiamethoxan.

INTRODUCTION

Mahanarva fimbriolata (Stål, 1854) (Hemiptera: Cercopidae) is one of the major pests in sugarcane fields of Brazil (Dinardo-Miranda et al., 2008; Tiago et al., 2012; Garcia et al., 2011). Nymphs and adults of *M. fimbriolata* can cause injuries in sugarcane plants compromising the productivity and quality of this crop (Dinardo-Miranda et al., 2004a, b; Madaleno et al., 2008; Carvalho et al., 2011; Korndörfer et al., 2011).

In order to reduce the *M. fimbriolata* populations (Dinardo-Miranda et al., 2004a, b; Loureiro et al., 2005;

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Cuarán et al., 2012) insecticides and entomopathogenic fungi like *Metarhizium anisopliae* (Metschnikoff, 1879; Sorokin, 1883) (Hypocreales: Clavicipitaceae) are used to protect the sugarcane plantations. Control of the spittlebug begins with monitoring the pest, immediately after the first spring rains (Almeida et al., 2007). Using the conventional sampling method, the *M. fimbriolata* nymphs and adults are monitored at 3 points per hectare

on 2 linear feet of row sugarcane planting and an ideal frequency of 15 days (Mendonça, 2005; Dinardo-Miranda et al., 2007).

However, the sampling of the nymphs and adults can impede the identification of the end of the life cycle as well as the timing of control of *M. fimbriolata* (Kassab et al., 2012). Highly infested areas could render the sample data unreliable, while regular rainfall could trigger the resurgence of the spittlebug populations, negating the effects of the insecticide and/or bioinsecticide applications during the control of *M. fimbriolata*. Therefore, Kassab et al. (2012) suggested modifying the *M. fimbriolata* sampling by monitoring the small nymphs (up to 5 mm), medium (6-10 mm) and large ones (over 10 mm) as well as the adult spittlebugs. This proposal enables the observation of the end of the cycle of *M. fimbriolata* generations and the best timing at which to control this pest, the period when the large nymphs are more numerous than the small and medium nymphs. It is at this time, which the *M. fimbriolata* adults can also be sampled in the pest population, which may indicate that eggs of the spittlebug had favorable to emergence of this insect.

Regardless, the new sampling method proposed for *M. fimbriolata* (Kassab et al., 2012) is yet to be compared with the conventional method (Mendonça, 2005; Dinardo-Miranda et al., 2007) and this indicates the importance of understanding the efficiency of the entomopathogenic fungi and insecticides in the control of *M. fimbriolata* by employing both these methods.

The aim of this study was evaluating the cost and efficiency of *M. anisopliae* and the insecticides used in controlling *M. fimbriolata* employing two sampling methods.

MATERIALS AND METHODS

A sugarcane field owned by Energética Santa Helena Ltda., a company in Nova Andradina, Mato Grosso do Sul State, Brazil was the site where the experiments were conducted between November 2012 and April 2013. The experimental area (S 22º13´58´´, W 53º20´34´´ and 380 m asl) was planted with the SP81-3250 variety of sugarcane, with no defects in the sprouting plants.

In this study, we followed the randomized blocks design (RBD) with six treatments and four replications of each, that is, with and without the conventional sampling of *M. fimbriolata*. The plots included 10 lines of sugarcane spaced 1.4 m apart and 10 m long, an area of 140 m^2.

The treatments in each experiment were represented by the control, Thiamethoxan (250 g ha^{-1}), Imidacloprid (700 g ha^{-1}), PL 43 (*M. anisopliae* - 2.0×10^{12} con mL^{-1}), ESALQ E9 (*M. anisopliae* - 2.1×10^{12} con mL^{-1}) and IBCB 425 (*M. anisopliae* - 1.4×10^{12} con mL^{-1}). The isolates of entomopathogenic fungi were used according to the manufacturers' recommendation. The isolates PL 43, ESALQ E9 and IBCB 425 are present in the commercial products Biometha WP Plus®, Metarril WP® and Metiê WP®, respectively. The manufacturers of products are “Biotech Controle Biológico Ltda” (Biometha WP Plus®), K oppert Biological Systems (Metarril WP®) and “Ballagro Agro Tecnologia Ltda” (Metiê WP®).

Using the conventional sampling method, the experiment with *M. fimbriolata* was performed with the weekly monitoring of the spittlebug nymphs and adults (Mendonça, 2005; Dinardo-Miranda et al., 2007). The first application of control for *M. fimbriolata* was done on 23 November, 2012 and the second on 15 Janand, 2013, when the degree of control of *M. fimbriolata* was achieved (Mendonça, 2005). To conduct the experiment with the proposed new sampling method, weekly sampling was done of the small, medium and large nymphs and adult individuals of *M. fimbriolata* to identify the end of the cycle of the spittlebug generations (Kassab et al., 2012) which occurred on 15 January 2013.

Coastal sprayers calibrated for a flow rate of 150 L ha^{-1} (Mendonça, 2005) were used and the insecticide was directed at the stump bases so that 30% of the spray volume reached the stems and 70% reached the sugarcane plant roots (Loureiro et al., 2005). The surfactant Tween® (0.01% polysorbate 80) was used to treat the fungal suspensions.

The *M. fimbriolata* nymphs were sampled every two weeks, up to 60 days after treatment (DAT) in the two linear meters of the furrow-planted sugarcane, in each plot, in the experiments using the two sampling methods. The *M. fimbriolata* nymphs found on the basal sugarcane internodes were counted after removing the residual straw.

Climatic conditions including average temperature, relative humidity and rainfall were represented with data from INMET (Instituto Nacional de Meteorologia) (Figure 1) to determine the relationship between the abiotic factors and *M. fimbriolata* infestation. The total value of recoverable sugar (TRS) according to Landell et al. (1999) was obtained from the sugarcane stalks randomly selected on 15 April 2013.

The tonne per ha value (TRS × TRS quote), estimated a yield per ha [68 tonnes (productivity of Mato Grosso do Sul State (Unica, 2013) × value of a tonne per ha], maintenance costs of the sugarcane (MCS) without the product and the cost involved in the application of *M. fimbriolata* control (Udop, 2013), cost control (including product and application expenses) and earnings per ha (estimated production per ha − MCS) were calculated in dollars (US$) for both the experiments. The expenses for the services outsourced and purchase of products to improve the sugarcane plantation were obtained from consulting firms and the UDOP agricultural database of 2013 (Table 1).

The population data for the *M. fimbriolata* nymphs recorded between 23 November 2012 and 15 January 2013 were subjected to the analysis of variance and the means were compared using the Scott-Knott test at 5% probability. Further, using Abbott's formula (Abbott, 1925) treatment efficiency was calculated and the means were compared by the Scott-Knott test at 5% probability. The average of the *M. fimbriolata* nymphs and the efficiency control (Abbott, 1925), post the 15 January 2013 treatment in the experiments following the two sampling methods were compared by the analysis of variance (ANOVA - F TEST) at 5% probability.

RESULTS

Experiment with conventional sampling: between 23 November 2012 and 15 January 2013

The number of nymphs and efficiency of *M. fimbriolata*

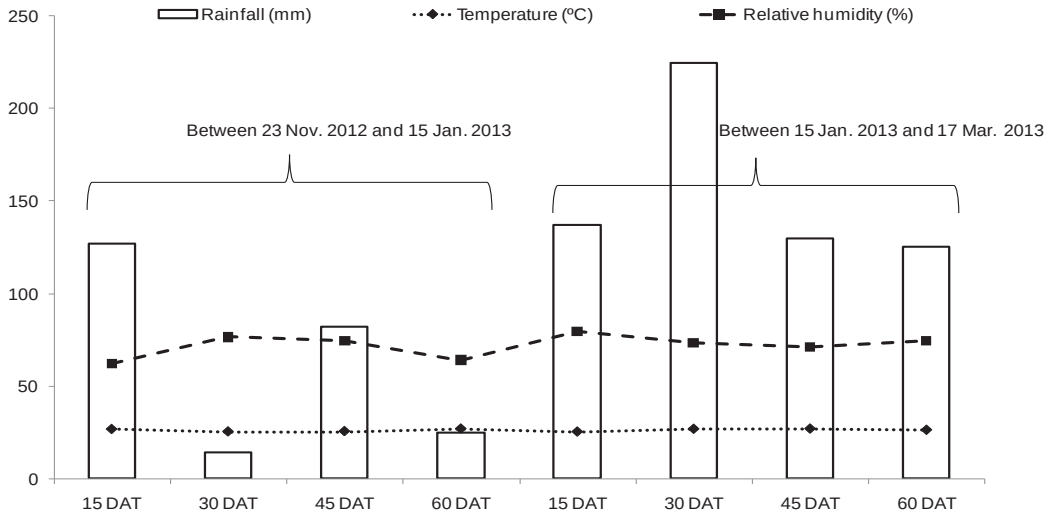

Figure 1. Rainfall (mm), average temperature (°C) and relative humidity (%) in the experimental area.

Table 1. Maintenance costs of sugarcane (MCS) per ha.

MCS	Value (US$)
Agricultural inputs for cultivation	548.50
Mechanized operations (applications)	59.78
Manpower	16.01
Administrative expenses	21.30
Total	645.59

control per treatment did not differ in the ratings of 15 DAT, 30 DAT and 45 DAT (Table 2 and Figures 2A, B and C). At 60 DAT, the treatments with the fungus *M. anisopliae* showed no difference and the nymphal infestations of *M. fimbriolata* were 6.50±1.75, 8.25±1.86 and 9.00±2.04 with the isolates IBCB 425, PL 43 and ESALQ E9, respectively (Table 2). Furthermore, at 60 DAT, the control efficiency of the *M. fimbriolata* recorded nymphs showed similarity between the treatments with isolates IBCB 425, PL 43 and ESALQ E9 with values of 33.75±13.34, 38.75±11.55 and 44.00±14.47. At 60 DAT, the insecticide Imidacloprid and thiamethoxan attained the highest efficiencies and lowest infestations of the *M. fimbriolata* nymphs.

Conventional sampling × new sampling method: Between 15 January 2013 and 17 March 2013

There was no difference in the number of nymphs of *M. fimbriolata* between the treatments during the experiments, both with and without the conventional sampling, at 15 and 30 DAT (Table 3). The control efficiency of the *M. fimbriolata* nymphs did not differ between treatments with and without conventional sampling in the assessments at 15 and 30 DAT (Figures 3A and B).

At 45 and 60 DAT, all the treatments utilizing the new sampling method showed significantly less infestation (Table 3). The treatment efficiency of the new sampling method was higher when compared with the conventional method (Figures 3C and D). Imidacloprid insecticide and the isolate IBCB 425 of *M. anisopliae* were which achieved the highest efficiencies of *M. fimbriolata* control at 45 and 60 DAT (Figures 3C and D). The increase in the number of *M. fimbriolata* nymphs parasitized by *M. anisopliae* (Figure 4), at 45 and 60 DAT, was due to the higher rainfall (mm), temperature (°C) and relative humidity (%) (Figure 1).

The total recoverable sugar (TRS) values, which included the expenses incurred per tonne and the earnings received per ha were higher in all the treatments employing the new sampling method (Table 4). The values regarding the acquisition and application of the products in the treatments with the conventional method were higher than in the other method, because two applications had been done for the control of *M. fimbriolata,* and therefore, lesser profits were made with the conventional method (Table 4).

DISCUSSION

Experiment with conventional sampling: Between 23 November 2012 and 15 January 2013

The lower variation recorded regarding the number of *M. fimbriolata* nymphs following the conventional sampling method, for 15 DAT, 30 DAT and 45 DAT can be related to the monitoring method used for this insect. In this method, the application of the insecticides and entomopathogenic fungi can occur in the initial stage of the first cycle of the spittlebug generations, that is, September/October (Almeida et al., 2007). During this

Table 2. Infestation by the *Mahanarva fimbriolata* (Hemiptera: Cercopidae) nymphs in the treatments with Thiamethoxan, Imidacloprid and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) following the conventional sampling method from 23 November 2013 to 15 January 2013.

Treatments	15 DAT	30 DAT	45 DAT	60 DAT
Control (untreated)	4.50±0.64^{a}	1.50±0.85^{a}	5.25±1.37^{a}	14.00±2.80^{a}
Thiamethoxan (250 g ha^{-1})	1.50±1.19^{b}	1.25±0.47^{a}	1.50±0.28^{b}	2.50±1.55^{b}
Imidacloprid (700 g ha^{-1})	1.75±0.75^{b}	1.00±0.49^{a}	1.50±0.28^{b}	0.75±1.55^{b}
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	0.75±0.47^{b}	2.00±0.67^{a}	3.00±0.81^{b}	8.25±1.86^{a}
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	1.75±1.03^{b}	0.25±1.18^{a}	3.00±0.78^{b}	9.00±2.04^{a}
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	1.50±0.85^{b}	2.00±1.02^{a}	1.75±0.75^{b}	6.50±1.75^{a}
CV	67.32	83.58	71.48	56.20

Means followed by the same letter per column were compared using the Scott-Knott test at 5% probability; CV, coefficient of variation; DAT, number of days after treatment; M.a., *M. anisopliae*.

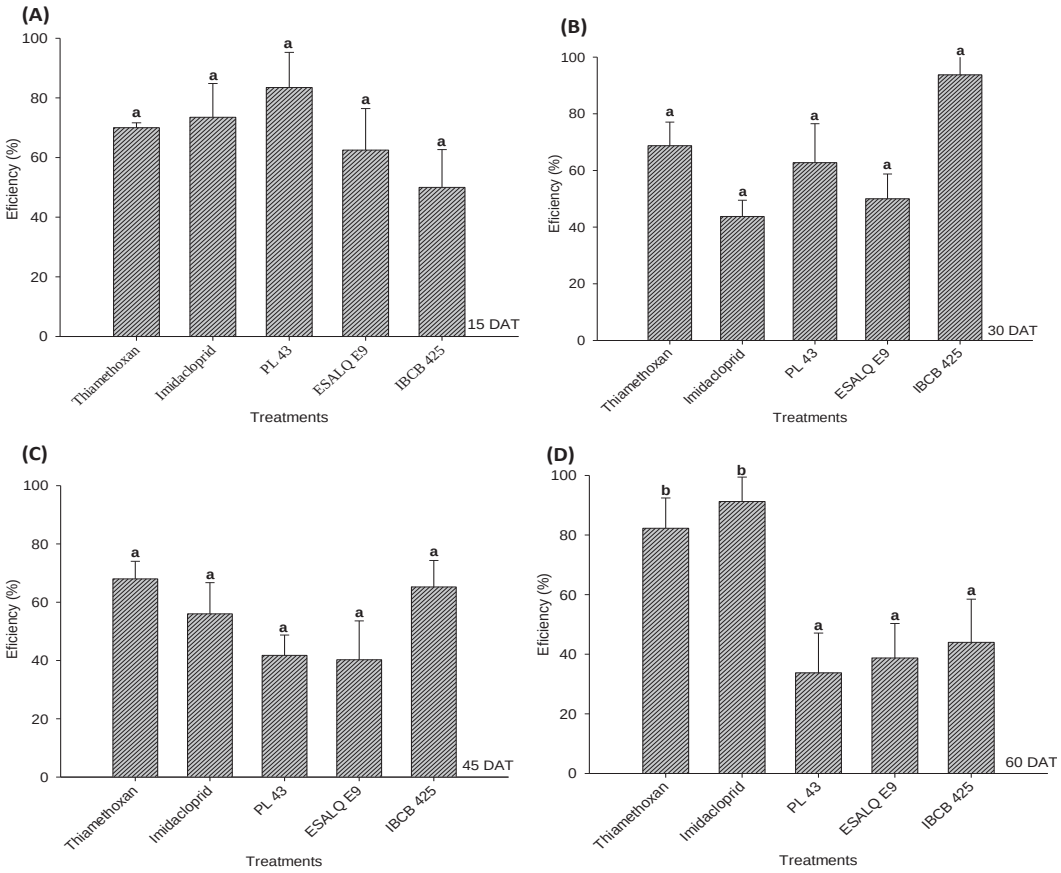

Figure 2. Efficiencies in control of *M. fimbriolata* (Hemiptera: Cercopidae) nymphs in treatments with Thiamethoxan, Imidacloprid and *M. anisopliae* (Hypocreales: Clavicipitaceae) using the conventional method of sampling from 23 November 2013 to 15 January 2013. Means followed by the same letter, in each bar, were compared using the Scott-Knott test at 5% probability

period, most of the *M. fimbriolata* diapausing eggs which are not hatched and due to the greater volume and regularity of rainfall between November and December can increase the possibility of reapplication of these products to control this pest (Kassab et al., 2012).

At 60 DAT, a lesser degree of infestations of the *M. fimbriolata* nymphs was obtained with Imidacloprid and thiamethoxan possibly as a result of the action of these insecticides, which may have contributed to a more efficient control of the spittlebug. The chemical molecule has a greater residual effect than that of the entomopathogenic fungi, which implies that this product

Table 3. Comparative infestation of the *Mahanarva fimbriolata* nymphs (Hemiptera: Cercopidae) between experiments with the conventional method and the new sampling proposal from 15 Jan. 2013 to 17 Mar. 2013.

Treatments	15 DAT		30 DAT	
	AC	NPA	AC	NPA
Thiamethoxan (250 g ha^{-1})	0.50±0.25^a	1.00±0.57^a	0.25±0.25^a	0.25±0.25^a
Imidacloprid (700 g ha^{-1})	0.50±0.50^a	0.50±0.28^a	0.00±0.00^a	0.75±0.47^a
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	1.50±3.71^a	2.50±1.04^a	3.00±0.43^a	2.25±0.94^a
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	1.25±0.47^a	2.25±0.71^a	1.50±1.19^a	1.50±0.64^a
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	2.25±0.75^a	2.05±0.85^a	1.75±0.81^a	2.00±0.85^a

Treatments	45 DAT		60 DAT	
	AC	NPA	AC	NPA
Thiamethoxan (250 g ha^{-1})	19.50±3.43^a	4.50±1.87^b	22.50±2.22^a	7.25±1.93^b
Imidacloprid (700 g ha^{-1})	15.00±0.50^a	3.75±2.49^b	23.75±2.27^a	9.25±0.85^b
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	14.25±2.09^a	4.25±2.78^b	22.00±2.31^a	11.50±1.47^b
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	13.25±1.75^a	5.50±1.32^b	28.50±3.12^a	10.25±1.08^b
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	10.75±2.21^a	3.25±2.13^b	18.50±1.93^a	9.50±0.75^b

Means followed by the same letter, in each line, were compared using F test at 5%; DAT, Number of days after treatment; AC, sampling conventional; NPA, new sampling proposal; M.a., *M. anisopliae*.

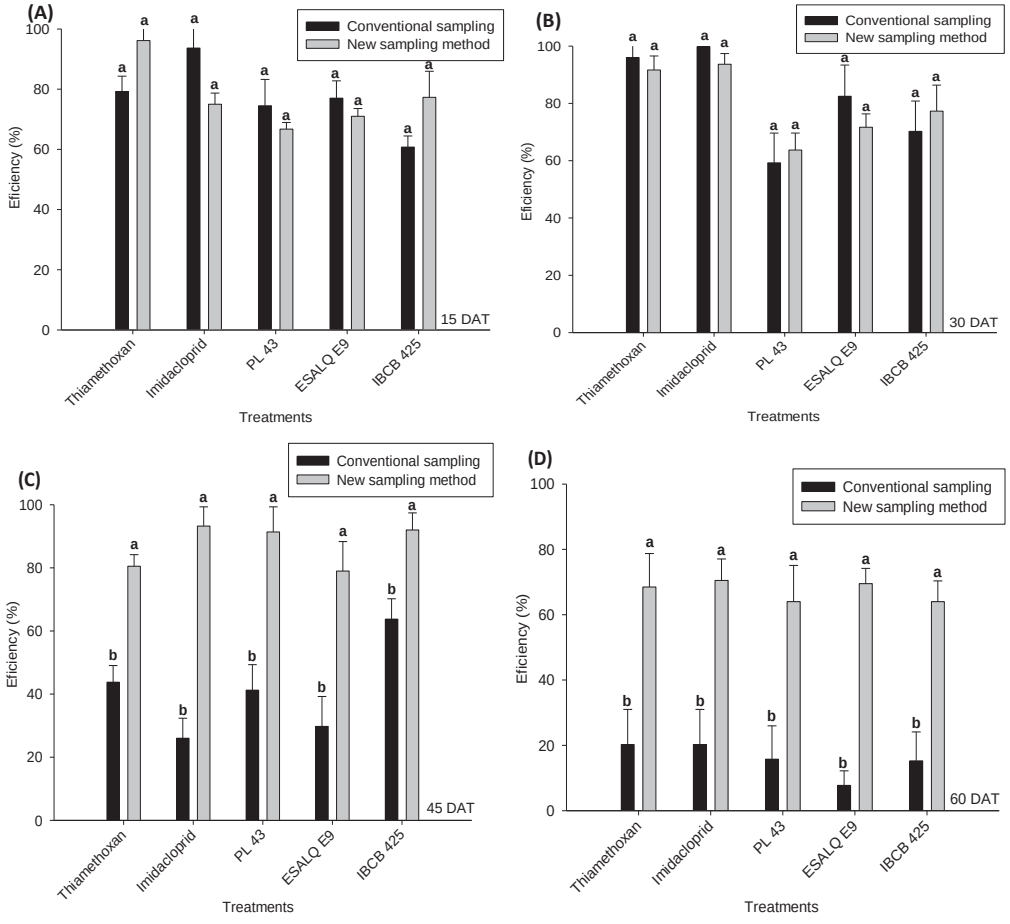

Figure 3. Comparison of the efficiency of control of *Mahanarva fimbriolata* (Hemiptera: Cercopidae) nymphs utilizing the conventional method and new proposal of sampling from 15 Jan. 2013 to 17 Mar. 2013. Means followed by the same letter, in each bar, were compared by the F test at 5%.

Table 4. Total recoverable sugar (TRS), TRS cost (Cot. TRS), price per tonne (Tonne. Price.), production estimate per ha (Prod. ha), maintenance cost of sugarcane plantations (MCS), cost control of *Mahanarva fimbriolata* (Cost. Contr.), profit per ha (L. ha) in the treatments with insecticides and *M. anisopliae* (Hypocreales: Clavicipitaceae) utilizing the conventional and proposed new sampling methods with the values expressed in dollars (US$).

Conventional sampling							
Treatments	**TRS**	**Cot. TRS**	**Tonne. Price.**	**Prod. ha**	**MCS**	**Cost. Contr.**	**L. ha**
Thiamethoxan (250 g ha^{-1})	88	0.19	16.72	1136.96	645.59	107.65	383.72
Imidacloprid (700 g ha^{-1})	90.72	0.19	17.23	1171.64	645.59	58.51	467.54
PL 43 ($2,0\times10^{12}$ con mL^{-1} of M.a)	85.02	0.19	16.15	1098.20	645.59	68.10	384.51
ESALQ E9 ($2,1\times10^{12}$ con mL^{-1} of M.a)	89.8	0.19	17.06	1160.08	645.59	62.12	452.37
IBCB 425 ($1,4\times10^{12}$ con mL^{-1} of M.a)	91.32	0.19	17.35	1179.80	645.59	59.57	474.64
New sampling method							
Treatments	**TRS**	**Cot. TRS**	**Tonne. Price.**	**Prod. ha**	**MCS**	**Cost. Contr.**	**L. ha**
Thiamethoxan (250 g ha^{-1})	89.23	0.19	16.95	1152.60	645.59	53.82	453.19
Imidacloprid (700 g ha^{-1})	94.57	0.19	17.96	1221.28	645.59	29.15	546.54
PL 43 ($2,0\times10^{12}$ con mL^{-1} of M.a)	90.31	0.19	17.15	1166.20	645.59	34.05	486.56
ESALQ E9 ($2,1\times10^{12}$ con mL^{-1} of M.a)	96.16	0.19	18.27	1242.36	645.59	31.06	565.71
IBCB 425 ($1,4\times10^{12}$ con mL^{-1} of M.a)	97.09	0.19	18.44	1253.92	645.59	29.80	578.53

Plant age = 8 months; Cot. TRS (Quotation) - "União dos Produtores de Bioenergia" (UDOP); Price Tonne. = TRS × Cot. TRS; Prod. Ha = value of tonne. × 68 (average production of Mato Grosso do Sul State); MCS = maintenance cost of sugarcane fields without product and application for control of *M. fimbriolata*; Cost. Contr. = Product and ground application; L. ha = Prod. ha - (MCS + Cost Control); M.a.-*M. anisopliae*.

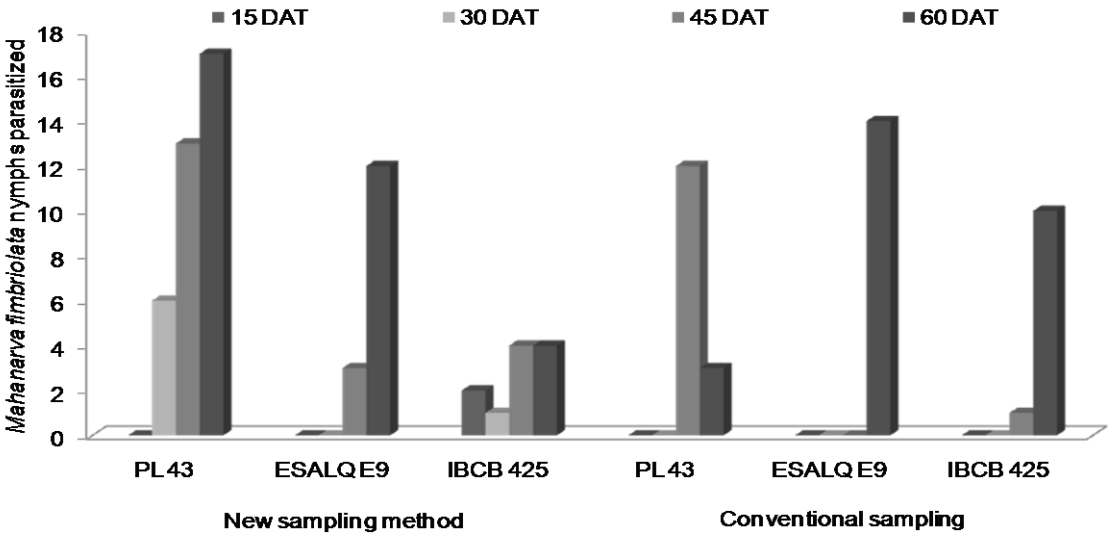

Figure 4. *M. fimbriolata* (Hemiptera: Cercopidae) nymphs parasitized by *M. anisopliae* (Hypocreales: Clavicipitaceae) in the experiments implementing the new proposed and conventional sampling methods from 15 January 2013 to 17 March 2013.

can persist longer on the crop and more effectively control this pest (Dinardo-Miranda et al., 2004b; Carvalho et al., 2011).

The entomopathogenic fungi, however, lose their viability after the application which could influence the effectiveness of the treatments on the isolates of *M. anisopliae* (Lopes et al., 2011; Guerrero-Guerra et al., 2013).

There were fewer *M. fimbriolata* nymphs parasitized by *M. anisopliae* in the experiment following the conventional sampling method, which can be explained by the timing of the treatment application. The conventional sampling method not considers the biotic potential of the area (Kassab et al., 2012) and the insecticide and/or bioinsecticide application in *M. fimbriolata* control occurs in the low density nymphs

(Mendonça, 2005). This simply means that if, in the first application, during spittlebug control the temperature and humidity favor the development of the *M. fimbriolata* nymphs (Freire et al., 1968), new applications of biological and chemical insecticides will be necessary. This is similar to what was observed in this experiment with the first application in *M. fimbriolata* control on 22 Nov. 2012, followed by the second application on 15 January, 2013.

Conventional sampling × new sampling method: between 15 January 2013 and 17 March 2013

The number of *M. fimbriolata* nymphs did not differ between the treatments followed in the two sampling methods, at 15 and 30 DAT. This indicates that the entomopathogenic fungi and insecticides may have a protracted effect in the field (Dinardo-Miranda et al., 2004a; Loureiro et al., 2005; Tiago et al., 2012). However, although generalizations cannot be made, the cumulative effect is controlled by the weather, the active constituent of the insecticide, the chemical mode of action, and the *M. anisopliae* isolated to control *M. fimbriolata* (Dinardo-Miranda et al., 2004a; Loureiro et al., 2005). On other hand, the genetic constitution of the pest population (Quinelato et al., 2012), adaptations and mechanisms of insect resistance (Dubovskiy et al., 2013) may influence the efficiency of the control techniques.

At 45 and 60 DAT, the treatments utilizing the new sampling technique were significantly less infested and revealed greater efficiency in the control of *M. fimbriolata*. The insecticide Imidacloprid and the isolate of *M. anisopliae* (IBCB 425) showed the highest control efficiency. This result was anticipated, as the late application of the insecticides to control the *M. fimbriolata* reduced the spittlebug infestations and increased the sugarcane productivity (Dinardo-Miranda et al., 2004a; Madaleno et al., 2008). Furthermore, the application occurred towards the end of the cycle of *M. fimbriolata* generations and, it is now accepted, that the normal and diapausing eggs of this pest, experienced conditions suitable for the emergence of nymphs which, in turn, may have enabled greater control efficiency (Kassab et al., 2012).

The number of *M. fimbriolata* of nymphs parasitized by *M. anisopliae* rose with the increase in rainfall (mm), temperature (°C) and relative humidity, which can be explained by the action of the climate-dependent entomopathogenic fungi (Almeida et al., 2007). The entomopathogenic fungi can also increase their density in the crop by infecting healthy individuals (Bruck, 2005, Bruck and Donahu 2007). Furthermore, the insects destroyed by *M. anisopliae* remain in the field, which can reduce the possibility of the resurgence of these pests (Guerrero-Guerra et al., 2013).

The value of the total recoverable sugar (TRS), the expenses per tonne and the earnings per ha were higher in all the treatments which employed the new sampling method. Moreover, the isolates PL 43 and IBCB 425 obtained a higher TRS value, which may be a result of the action of the *Metarhizium* ssp. to translocate the nitrogen of the parasitized insect to the plants (Behie et al., 2012). Besides, plant age can also influence the sugarcane productivity (Dinardo-Miranda et al., 2008). Thus, the plants at a more advanced developmental stage may show a higher TRS yield. The TRS value was estimated using 8-month-old plants, although it may be higher for the older individuals.

Careful monitoring of the small, medium and large nymphs and the *M. fimbriolata* adults can optimize the time of application and raise the efficiency of spittlebug control. Besides, from this study, monitoring the *M. fimbriolata* populations is best done after the first spring rains, following the diapause period of the spittlebug eggs. Areas with a history of *M. fimbriolata* infestations should be given priority in the monitoring programs of this pest and the timing of the insecticide application must be synchronized with the end of the spittlebug lifecycle, thus, lowering the likelihood of reapplication of insecticides in its control.

Conclusion

The use of *M. anisopliae* and insecticides along with monitoring the small, medium and large nymphs and adult *M. fimbriolata* is the most suitable method to control the spittlebug populations by providing greater efficiency and lower cost per hectare.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Agricultural yield components of dual purpose wheat cv. BRS Tarumã under cutting and nitrogen fertilization handlings

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This experiment aimed to study the effects on grain and forage productivity in dual-purpose wheat cv. BRS Tarumã under cutting handlings and nitrogen fertilization. The experiment was conducted in area of Rhodic Hapludox in the city of Marechal Cândido Rondon, Paraná State, in a randomized block design with 12 treatments and four replications. The treatments consisted of surface nitrogen levels (0, 60, 120 and 180 kg ha^{-1}) and number of cuttings accomplished (0, 1 and 2). The topdressing was split in two applications for handling of just a cutting (27 and 65 days after sowing - DAS), and three applications for handling with two cuttings (27, 65 and 96 days after sowing - DAS). Data were subjected to analysis of variance ($p \leq 0.05$). The use of one or two cuttings and the comparison between the first and second cuttings were done by F test (5%) whereas nitrogen doses were studied by regression analysis. For all variables, there was significant interaction of nitrogen doses in surface and the number of cuttings. Grain yield and its yield components were responsive to levels of nitrogen fertilization in surface as well as forage production and its quality, but the same factors were reduced with the use of cuttings. This handling increased the dry matter content and crude protein in grains.

Key words: Crop-livestock integration, *Triticum aestivum*, nitrogen, yield components, proximate composition.

INTRODUCTION

The cultivation of winter cereal enables forage production with low cost and high nutritional value (Scheffer-Basso et al., 2004), and when dual purpose cereals are sown there is also the possibility of grains production. However the forage yield and its quality can be significantly affected due to cuttings and grazing handlings and fertilization adopted.

The nitrogen fertilization is the most limiting factor in the production of grain and forage and appropriate levels of this nutrient increment the forage and grains production of dual-purpose wheat (Zagonel et al., 2002). One of the main factors observed in any crop is the

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fertilization (Lopes, 1996). Nitrogen (N) is an essential element for plants as it participates in a series of metabolic pathways. It is also a constituent of important biomolecules such as Adenosine triphosphate (ATP), nonphosphorylated nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), chlorophyll, storage proteins, nucleic acids and enzymes (Harper, 1994). Rarely, the level with higher production has the highest economic efficiency.

Nitrogen fertilization is particularly important for the wheat crop since among the nutrients that influence its performance, nitrogen is one of the most absorbed during the development cycle of the plant (Scalco et al., 2003). The use of wheat cultivars responsive to nitrogen fertilization is essential to obtain high productivity. However, fertilization requires care regarding the time and levels of application (Teixeira Filho et al., 2009).

The application of nitrogen is essential to increase grain yield (Silva et al., 2005), increasing the number of grains per spike and spike number per area (Sangoi et al., 2007). Oliveira (2009) obtained a grain yield of 2,250 kg ha^{-1} with the wheat BRS Tarumã with two cuttings and surface nitrogen fertilization in the amount of 150 kg ha^{-1}, using urea (45 g kg^{-1}) as a nitrogen source.

We aimed to evaluate different nitrogen levels applied in surface on the yield components of productivity of dual purpose wheat.

MATERIALS AND METHODS

The experiment was accomplished under field conditions, in the Experimental Station Professor Antônio Carlos dos Santos Pessoa, at Universidade Estadual do Oeste do Paraná, *campus* Marechal Cândido Rondon, at the following geographic coordinates: latitude 24° 33' 40'' S, longitude 54° 04' 12'' W and altitude of 420 m.

The local climate is classified according to Koppen as Cfa type, sub-tropical with rains well distributed throughout the year and hot summers (IAPAR, 2012). The average temperature of the coldest quarter vary between 17 and 18°C, of the hotter quarter vary between 28 and 29°C and the annual temperature is between 22 and 23°C. Climatic data were obtained from automatic meteorological station located 200 m from the experimental area.

The experimental area soil was classified as Rhodic Hapludox (EMBRAPA, 2006). Soil samples were collected from 0 to 0.20 m for its chemical analysis. The results were: pH in $CaCl_2$: 5.43; P (Mehlich): 20.20 mg dm^{-3}; K: 0.37 $cmol_c$ dm^{-3}; Ca^{2+}: 4.87 $cmol_c$ dm^{-3}; Mg^{2+}: 0.58 $cmol_c$ dm^{-3}; Al^{3+}: 0.40 $cmol_c$ dm^{-3}; H+Al: 6.48 $cmol_c$ dm^{-3}; SB: 6.82 $cmol_c$ dm^{-3}; CTC: 12.30 $cmol_c$ dm^{-3}, V: 55.28%, organic matter: 25.29 g dm^{-3} and clay: 650 g kg^{-1}. The experiment was accomplished in randomized block design with four replications, and was composed of 48 experimental plots, which had dimensions of 4 × 5 (20 m²), totaling an area of 960 m².

In order to study the chemical composition and forage production in the first and second cuttings as for the sum of both cuttings and the handling system with the use of no one, one or two cuttings, for comparison of one or two cuttings in the wheat handling, we adopted the randomized block design with four surface nitrogen levels (0, 60, 120 and 180 kg ha^{-1}) and three handling systems for forage production (0, 1 and 2 cuttings). Wheat (*Triticum sativum*) cv. BRS Tarumã of dual purpose wassown on 6th May, 2011 with precision seeder attached to the tractor in rows spaced 0.17 m apart. The seed density used was 350 to 400 viable seeds per square meter (CBPTT, 2010). As basis, 200 kg ha^{-1} of 00-20-15 (N - P_2O_5 - K_2O) fertilization was used, following the recommendations of Brazilian Commission for Research in Wheat and Triticale (2011). The nitrogen surface fertilization was held at tillering stage (Zadoks et al., 1974), using urea as a nitrogen source (45 g kg^{-1} of N), and the applications were made under favorable climatic conditions at dosages of 0, 60, 120 and 180 kg ha^{-1}. The nitrogen surface fertilization was split in two applications for the handling with just one cutting (27 and 65 DAS), and three applications for handling with two cuttings (27, 65 and 96 DAS).

During the culture development there was an application of fungicide and insecticide as a preventive method being performed 30 DAS. The active principles Azoxystrobin and Cyproconazole and Lambda-cyhalothrin were used at dosages of 300 and 150 mL ha^{-1} respectively diluted in 180 L ha^{-1} spray volume.

The cuttings were done with a costal mowing at a height of 30 cm and preserving residue at around 8 to 10 cm. After the cuttings the green mass was removed from the plots by using a rake and plastic bags. All samples were transported to the Animal Nutrition Laboratory of Universidade Estadual do Oeste do Paraná at *campus* Marechal Cândido Rondon for subsequent evaluations.

The crop was harvested at 169 DAS after sowing being sampled the four central lines with three feet long (2.04 m^2) with the aid of scissors and the samples were placed in plastic bags until the time of trail. To calculate productivity the samples were weighed on analytical balance, then threshed and after calibration were extrapolated to kg ha^{-1}. To obtain the 1000 grain weight eight samples with 100 grains were collected and the same were weighed on an analytical balance, and their values were extrapolated for 1000 grain weight. For spike weight, 15 samples from each experimental plot were sampled and weighed on an analytical balance.

The number of spikelets per spike was obtained by counting in fifteen spikes collected from base to apex of the spike. For the number of grains present in each spikelet, the spikelets were threshed separately and it was counted, and for weighing the spike grains the same threshed samples were used and weighed in an analytical balance. The dry matter production was estimated with the usage of metallic square with a known area (0.25 m^2) randomly placed once in each plot. The samples were collected using a cleaver and then packed in paper bags, weighed and placed in an oven with forced ventilation and maintained at a temperature of 55ºC for 72 h for drying. After drying, the samples were weighed and from data obtained the dry matter production was calculated, being expressed in kg ha^{-1}.

After drying the samples were ground in a Willey mill with 30 mesh sieve and then were subjected to laboratory procedures for evaluation of crude protein (CP) according to AOAC (1990), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991), neutral detergent insoluble protein (NDIP - expressed in g kg^{-1} of CP), acid detergent insoluble protein (ADIP - expressed in g kg^{-1} of CP), lignin and hemicellulose (Silva and Queiroz, 2006).

Data were subjected to analysis of variance ($p \leq 0.05$). The use of one or two cuttings and the first and second cuttings were compared by F test (5%) and nitrogen doses were studied by regression analysis which regression equations were adjusted by choosing the mean model of highest coefficient of determination (R^2) (Figure 1 and Table 1).

RESULTS AND DISCUSSION

Grains productivity and yield components

There was a significant effect of nitrogen levels for

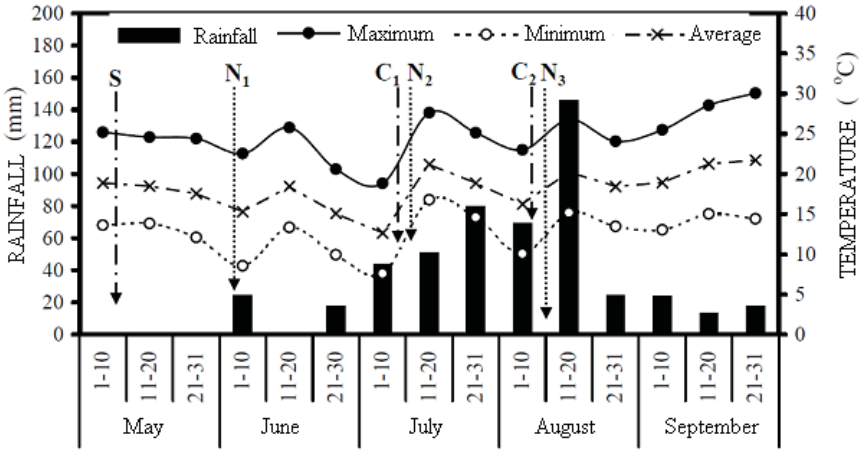

Figure 1. Climatic data of the experimental period. S: sowing; N_1; N_2 and N_3 nitrogen application on tillering, after the first and second cuttings, respectively; C_1 and C_2 first and second cuttings respectively (Marechal Cândido Rondon, 2011).

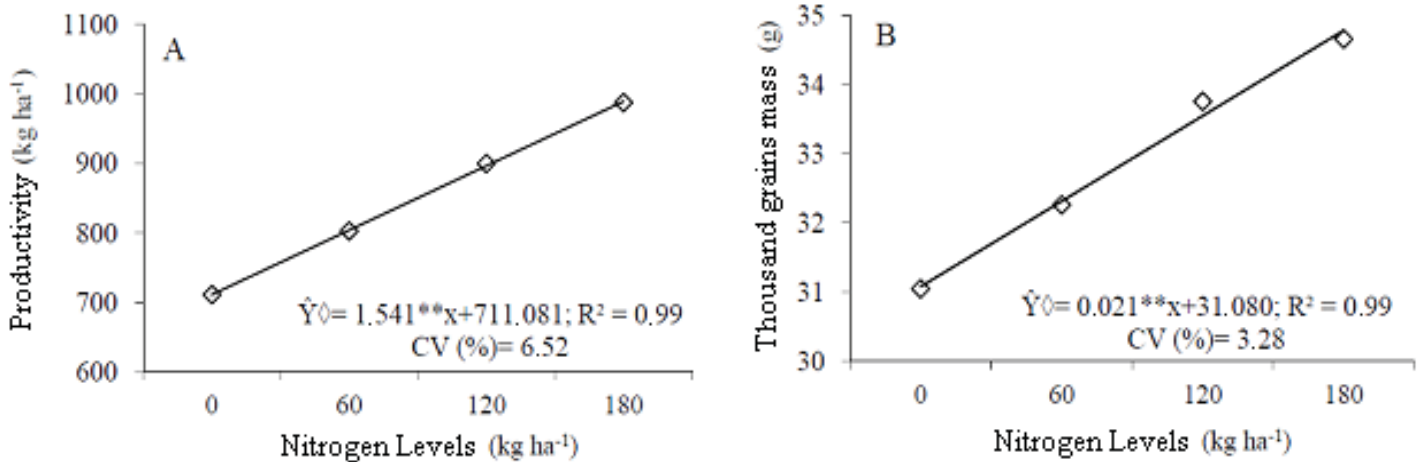

Figure 2. Grains productivity and 100 grains mass of dual purpose wheat BRS Tarumã under increasing levels of nitrogen. ** Significant at 1% of probability by t test. CV (%): Coefficient of variation.

isolated factors on grain productivity, 1000 grains mass, spikes weight, number of spikelets per spike, number of grains per spikelet, number of grains per spike and crude protein content in the grains of dual purpose wheat BRS Tarumã (Figures 2, 3 and 4; and Table 2) and there was no significant difference for interaction.

The grains productivity (Figure 2A) and 1000 grains mass (Figure 2B) had adjustment to the positive linear regression model in response to nitrogen fertilization. This result was expected since nitrogen is among the most absorbed nutrients during the development cycle of wheat (Scalco et al., 2003). Teixeira Filho et al. (2009) also observed positive responses of nitrogen application in wheat, with productivity increases, while Boschini et al. (2011) have observed increases in wheat productivity up to the level of 200 kg ha^{-1}.

The spikes weight (Figure 3A), number of spikelets per spike (Figure 3B), number of grains per spikelet (Figure 3C) and number of grains per spike (Figure 3D) also showed a better fit to the linear regression model with the application of nitrogen levels. This behavior was expected, since as in wheat the number of flowers per spikelet and spikelets per spike depends on nutritional and environmental factors (Aude et al., 1994).

Nitrogen fertilization improves maintenance of higher leaf area in plants, contributing to improvement in yield components of wheat and it becomes important because green leaf area represents the active photosynthetic tissue, providing greater partitioning of assimilates in the grain filling (Silva et al., 2006).

Decreases observed in grains productivity may be related to the plant ability to recover after defoliation due

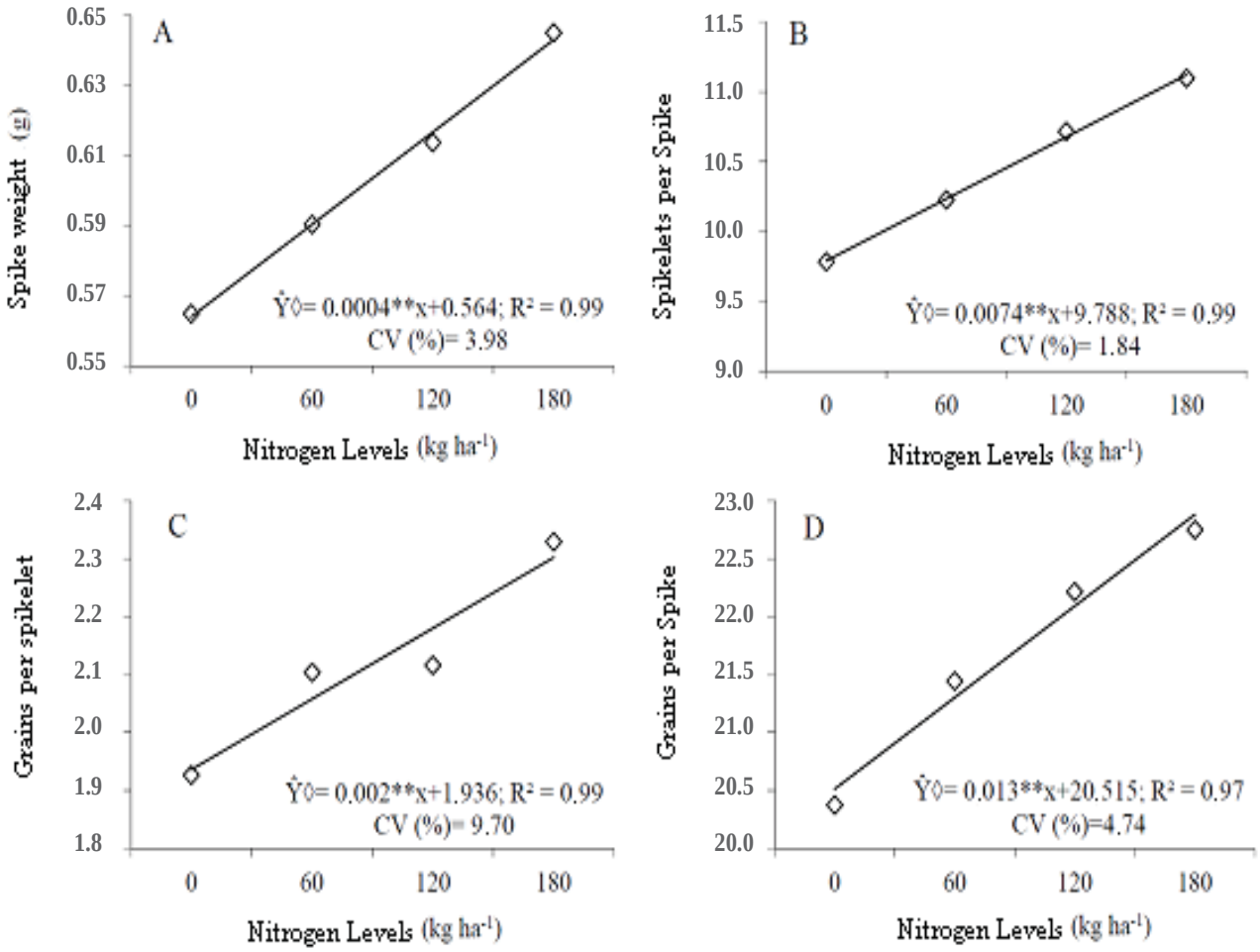

Figure 3. Grains yield components of dual purpose wheat BRS Tarumã under increasing levels of nitrogen. **Significant at 1% of probability by t test. CV (%): Coefficient of variation.

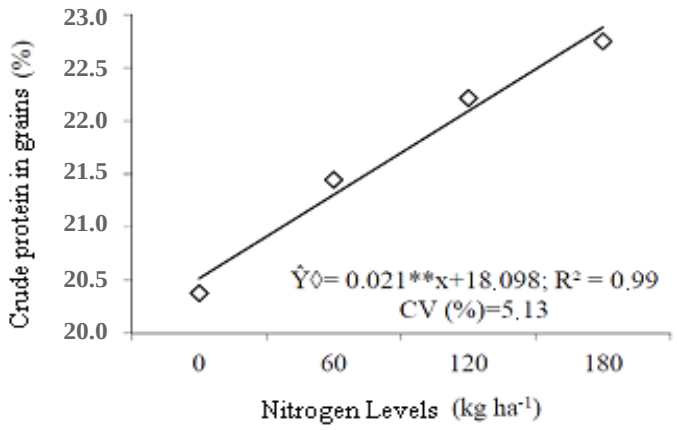

Figure 4. Crude protein content in grains of dual purpose wheat BRS Tarumã under increasing levels of nitrogen. ** Significant at 1% of probability by t test. CV (%): Coefficient of variation.

to the reduction in the rate of the whole plant photosynthesis (Parsons et al., 1988). Bortolini et al. (2004) studied the wheat BR 35 and also observed a reduction in grains production with increasing number of cutting to the forage harvest.

When the cutting handlings were compared, significant differences between treatments were found. The spikes weight and grains number per spikelet were higher in uncut handling compared to the others, while the spikes number was reduced with the increase in the cutting number. The number of grains per spikelet was higher in uncut handling, and lower in the handling of two cuttings, however both them did not differ from the handling with just one cutting (Table 2).

The damage observed on grains yield components when wheat was submitted to cuttings are due to the apical meristem removal. According to Hendrickson et al. (2005), when the apical meristem of plants is removed by cutting, the appearance of side tiller is induced and plants produce smaller spikes, with less grains or no grains. Hastenpflug et al. (2011) observed a reduction in spikes weight, number of spikelets per spike and number of

Table 1. Treatments, number of cuttings and handling of nitrogen fertilization in surface.

Treatments	Number of cuttings	Nitrogen fertilization in surface (kg ha^{-1})		
		Tillering (27 DAS)	After 1st cutting (65 DAS)	After 2nd cutting (96 DAS)
1	0	0	0	0
2	0	60	0	0
3	0	120	0	0
4	0	180	0	0
5	1	0	0	0
6	1	30	30	0
7	1	60	60	0
8	1	90	90	0
9	2	0	0	0
10	2	20	20	20
11	2	40	40	40
12	2	60	60	60

Table 2. Grains yield components and productivity of dual purpose wheat BRS Tarumã under different cutting handlings.

Cuttings	Productivity (kg ha^{-1})	MMG (g)	PE (g)	E/E	G/Es	G/Ep	PB (%)
0	1000^{a}	33.37^{ns}	0.63^{a}	10.77^{a}	2.25^{a}	22.29^{a}	20.14^{b}
1	881^{b}	32.90	0.60^{b}	10.51^{b}	2.04^{b}	21.61^{ab}	20.47^{b}
2	668^{c}	32.51	0.58^{b}	10.08^{c}	2.06^{b}	21.20^{b}	21.75^{a}
CV (%)	6.52	3.28	3.98	1.84	9.70	4.74	5.13

^{ns} Not significant. Means followed by different letters in the column differ by Tukey test (5%). MMG: 1000 grains mass; PE: spike weight; E/E: number of spikelets per spike; G/Es: number of grains per spikelet; G/Ep: number of grains per spike; PB: crude protein content in grains.

grains per spikelet with an increase in cutting number on wheat BRS Tarumã.

Forage productivity and chemical composition

The crude protein content in grains increased linearly with the application of nitrogen levels (Figure 4), and when the cutting handlings were compared, an increase in protein content was observed as the cutting number increased (Table 2). It was expected that nitrogen fertilization would raise the crude protein content of wheat grains due to higher nutrient availability in the soil, which led to greater absorption, metabolism and synthesis of amino acids and proteins by fertilized plants.

The increase of crude protein content in the grains as the number of cuttings increased can be related to the reduction in grains production (Figure 2A), providing a higher crude protein content in the grains mass produced.

A higher crude protein content in grains is a positive aspect because it increases the nutritional value of grains for both human consumption and animal feed. This inverse relationship between productivity and protein content in grains is explained by the higher energy waste which plant needs to form proteins, what can compromise the accumulation of carbohydrates in the grains (Sangoi et al., 2007).

There was effect of the factors interaction for the productivity of green and dry matter (Figure 5). When the handlings were compared the use of two cuttings provided productivity of green and dry matter (sum of two cuttings) higher than only one cutting (Figure 5A and 5B). This higher total forage productivity when wheat was subjected to two cuttings was due to the stimulation of tillering promoted by the cutting and to dry matter productivity in the second growth period between the first and second cutting.

About nitrogen fertilization, in both handlings a linear increase in green and dry matter productivity in response to increased levels of nitrogen was observed (Figure 5).

Bortolini et al. (2004) studied dual purpose cereals and also observed higher dry matter productivity when using two cuttings. The dry matter productivity obtained for only one cutting is similar to those observed by Fontaneli et al. (2007) who studied cultivars of dual purpose wheat.

When comparing the first and second cuttings within the two cuttings handling, there was an interaction of

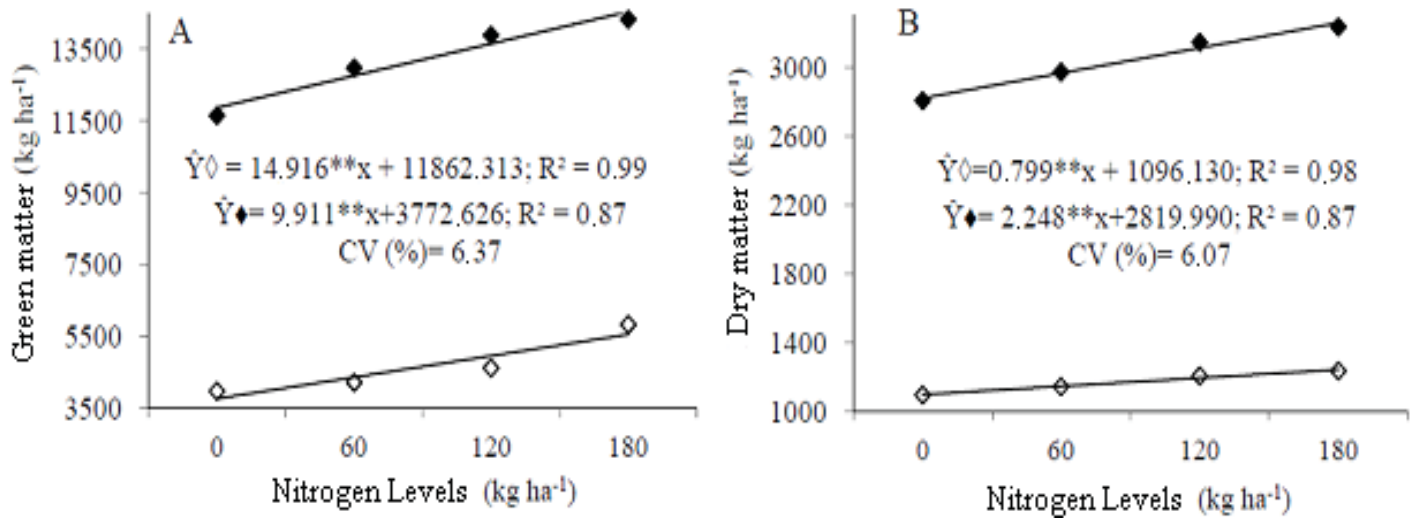

Figure 5. Total productivity of green (A) and dry matter (B) of dual purpose wheat BRS Tarumã under increasing levels of nitrogen and subjected to one (◊) or two (♦) cuttings, and average values (□). (**'* significant at 1 and 5% of probability by t test, respectively).

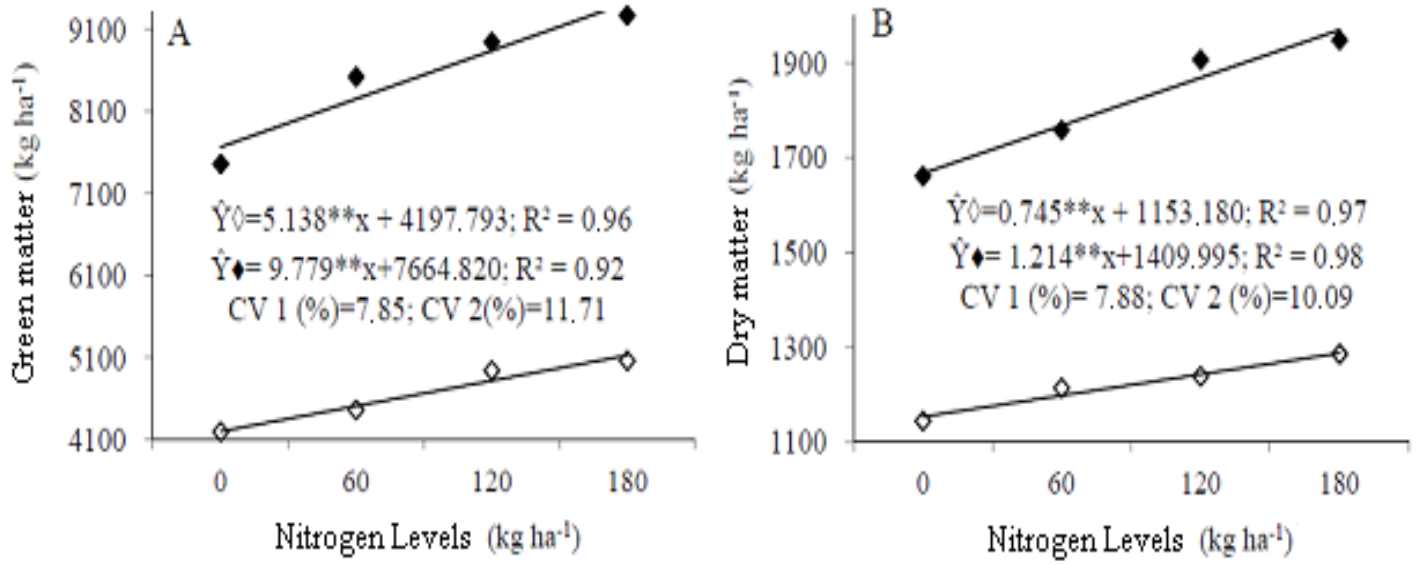

Figure 6. Productivity of green (A) and dry matter (B) of dual purpose wheat under increasing levels of nitrogen at the first (◊) and second (♦) cuttings. (**'* significant at 1 and 5% of probability by t test, respectively).

factors, with higher productivity of green and dry matter in the first cut compared to the second (Figure 6A and 6B). Bortolini et al. (2004) also observed a reduction in productivity of dry matter from the first to the second cutting when they studied dual purpose cereals subjected to one or two cuttings. In the case of nitrogen fertilization, either in the first or the second cutting, a linear increase in the productivity of green and dry matter with increasing levels of nitrogen was observed.

About the chemical composition, there was no significant difference among one or two cuttings in wheat for concentrations of NDF and ADF, which showed a linear decrease with the nitrogen increase (Figure 7A and 7B). The decrease in NDF and ADF contents, according to Grise et al. (2001), can be attributed to the higher participation of leaves over the stems in the forage once the leaves have a higher nutritional value.

Hemicellulose and lignin concentrations were altered by the interaction of factors, but both also showed a linear decrease in response to increased nitrogen levels (Figure 7C and 7D), with reduction in forage produced by wheat subjected to only one cutting.

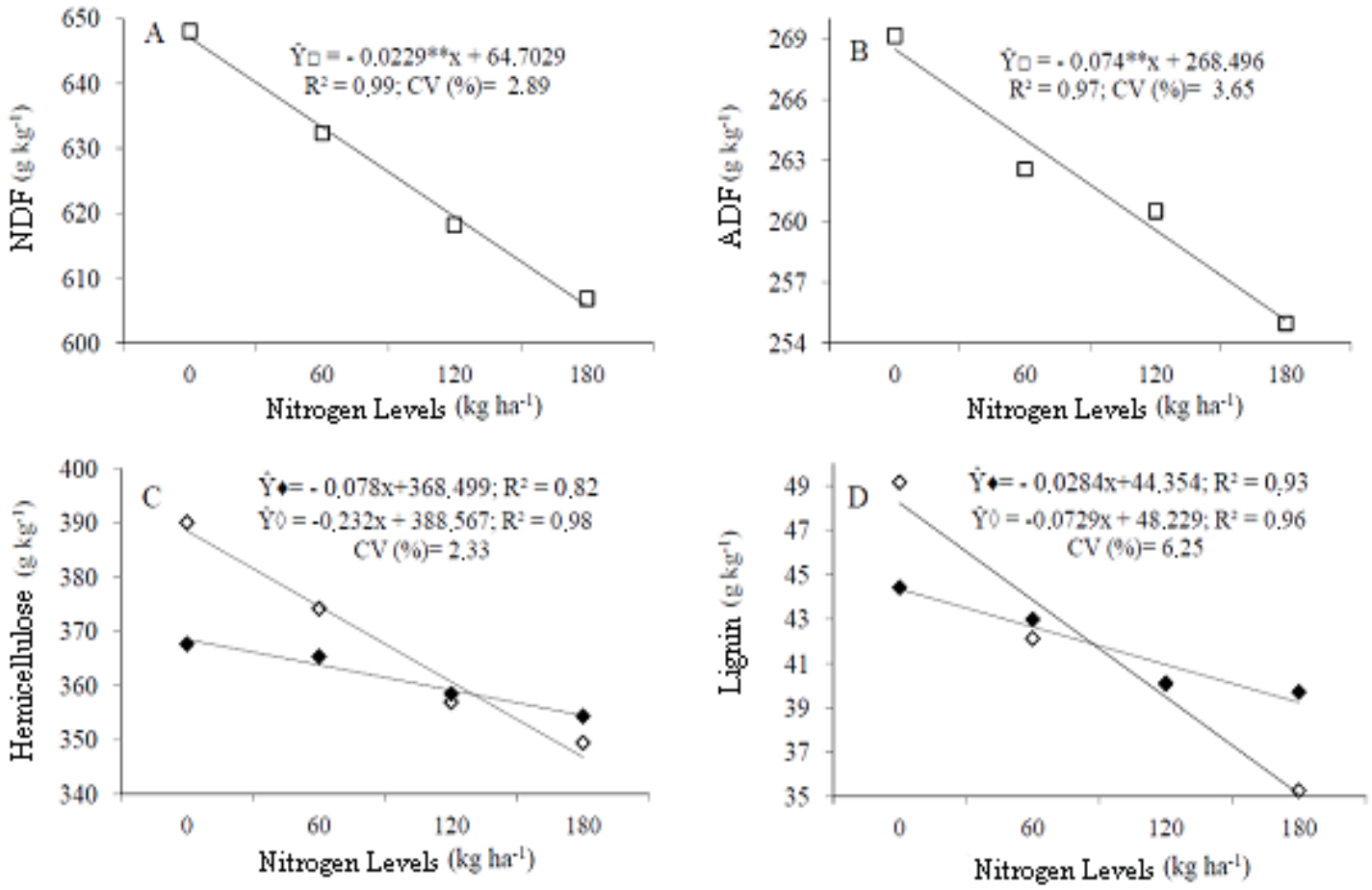

Figure 7. Fibrous components in the forage produced by dual purpose wheat BRS Tarumã under increasing levels of nitrogen and subjected to one (◊) or two (♦) cuttings, and average values (□). (**'*significant at 1and 5% of probability by t test, respectively).

About the crude protein contents, there was effect of factors interaction, however in both handlings with one or two cuttings the values increased in response to nitrogen levels (Figure 8A). The NDF adhered protein however was only affected by nitrogen levels and showed a linear decrease with the nitrogen levels increase (Figure 9B).

Though the Food and Drug Administration (FDA) adhered protein was affected by the interaction of factors, it was higher in the forage obtained by applying just one cutting to wheat. Both with the use of one or two cuttings a linear decrease was observed at concentrations of PIDA with increasing levels of nitrogen applied (Figure 8C).

After studying the chemical composition of forage produced by wheat subjected to two cuttings, in the first and second cuttings there was an interaction of factors for concentrations of NDF, ADF, hemicellulose and lignin (Figure 9). At first cutting the NDF was reduced with the nitrogen application, however in the second cutting the data fitted to a quadratic regression model and the NDF concentration increased up to 69 kg ha^{-1}, with subsequent decrease (Figure 9A). NDF values are within the recommended range (550 to 600 g kg^{-1}) for ruminants feeding (Mertens, 1994).

Concentrations of ADF and hemicellulose decreased in the first cutting, but increased in the second cutting in response to nitrogen levels applied (Figure 9B and 9C). Meinerz et al. (2011) also observed increase in FDA contents from the first to the second cutting in wheat BRS Tarumã. Fontanelli et al. (2007) studied dual purpose cereals and found NDF and ADF contents similar to those observed in this study. As the hemicellulose is NDF component (van Soest et al., 1991), its decrease with the nitrogen increasing levels in the first cutting and increasing in the second cutting is consistent.

At first cutting the lignin concentration decreased with nitrogen levels, however in the second cutting the data had no adjustment to the regression models studied (Figure 9D). Lignin is one of three compounds that bind to form the fiber fraction of forages and is considered the main limiting factor for digestibility (van Soest et al., 1994). In crude protein concentration there was significant effect of the factors interaction with higher crude protein content in the first cutting in relation to the

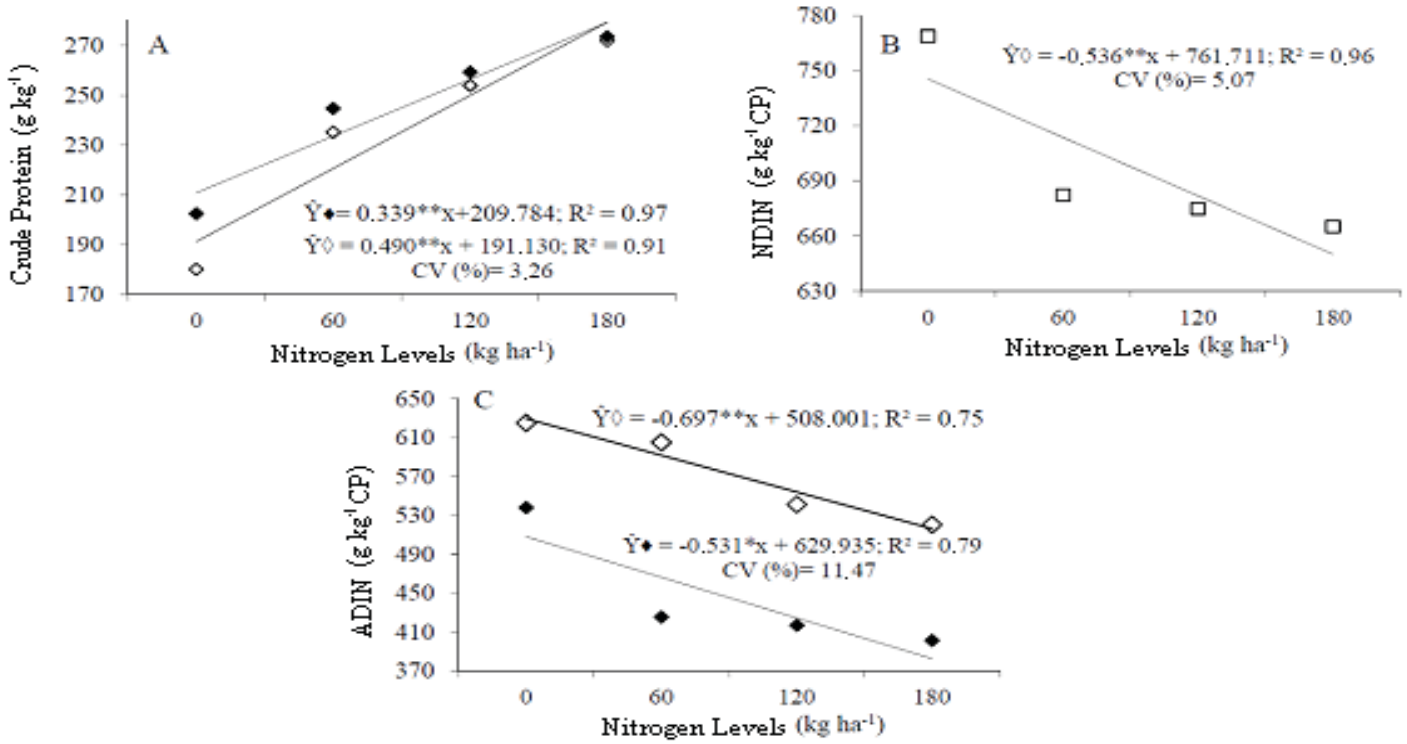

Figure 8. Chemical composition of dual purpose wheat under increasing levels of nitrogen and subjected to one (◊) or two (♦) cuttings and average values (□). (**;*significant at 1and 5% of probability by t test, respectively).

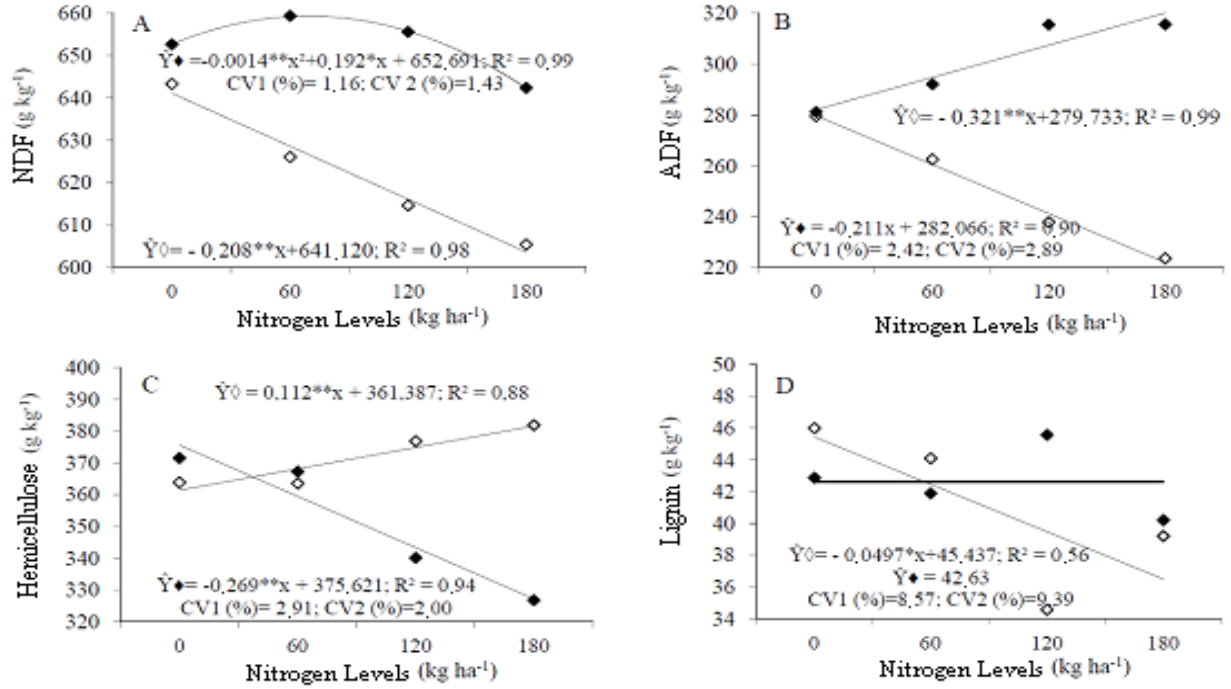

Figure 9. Fibrous components in the forage produced by dual purpose wheat BRS Tarumã under increasing levels of nitrogen and subjected at first (◊) or second (♦) cuttings, or for average values (□). (**;*;^{ns} significant at 1 and 5% of probability or not significant by t test, respectively).

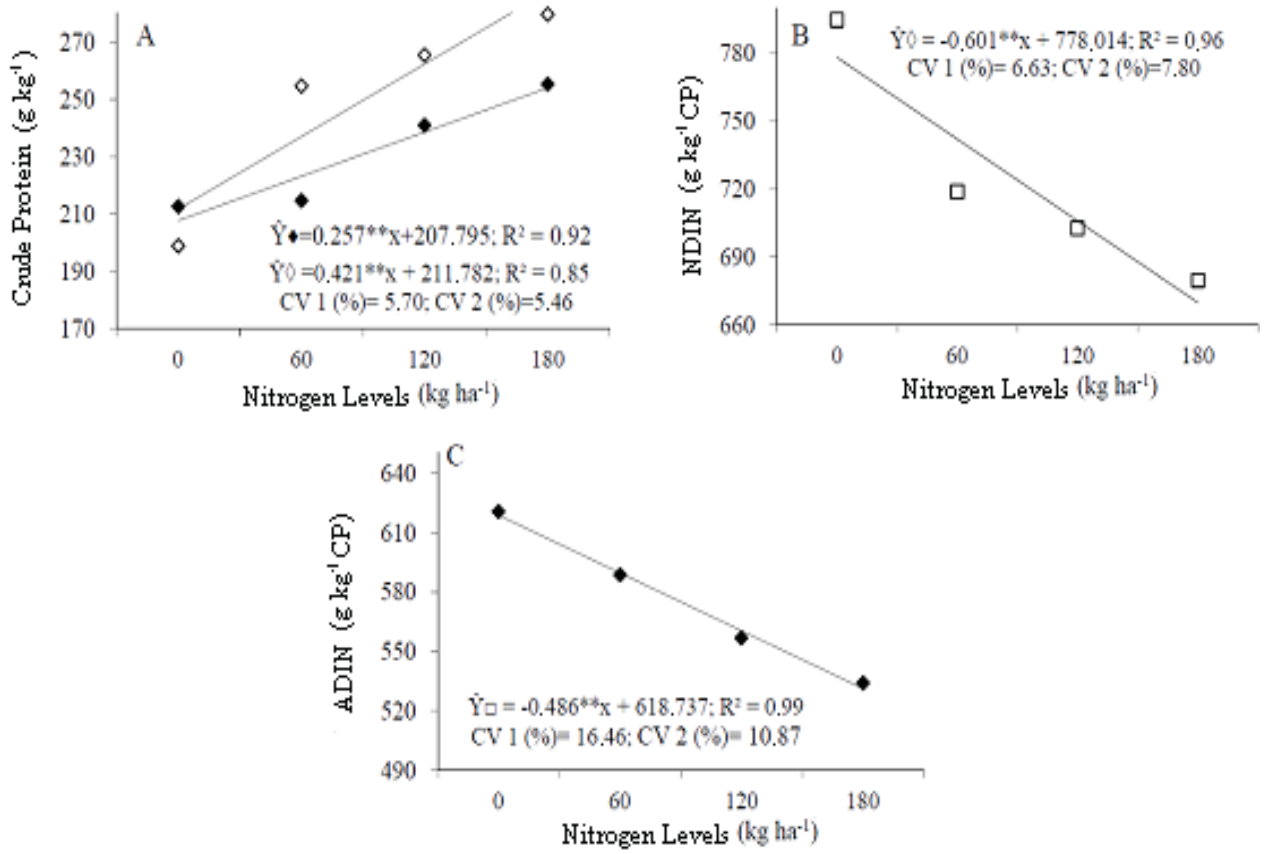

Figure 10. Proteic components in the forage produced by dual purpose wheat BRS Tarumã under increasing levels of nitrogen and subjected to one (◊) or two (♦) cuttings, and average values (□). (**;* significant at 1 and 5% of probability by t test, respectively).

second cutting. As for nitrogen levels, either in the first or the second cutting a linear increase was observed with increasing levels (Figure 10A).

The NDIP levels did not follow the behavior observed for crude protein, and were similar in both cuttings, with linear decrease as nitrogen levels increased (Figure 10B). That is, the increasing protein concentration in dry matter of forage produced by dual purpose wheat did not increase the NDF adhered protein. For the PIDA (ADF adhered crude protein) similar behavior was observed with significance only of nitrogen levels and linear reduction in PIDA contents with increasing levels (Figure 10C).

Quantification of CP associated with NDF is important in studies of forages due to the relationships that this fraction has with nutrients digestibility and intake (Aguiar et al., 2006). Crude protein cannot be considered a homogeneous nutritional fraction because if so can lead to distortions in estimates of apparent digestible fraction from the chemical composition of feed produced in tropical conditions (Detman et al., 2008). According to Silva et al. (2006), the NDIP may be present naturally in plants or can be considered an estimate of heat damage.

Conclusion

The dual purpose wheat BRS Tarumã is responsive to nitrogen fertilization in relation to increasing doses of surface application once its productivity and grains yield components increased by this handling.

The usage of one or two cuttings reduces the productivity and affects the yield of production components, but the use of cuttings increased crude protein content in the grains.

The adoption of two cuttings for production of forage wheat Tarumã BRS provides greater dry matter production without harming the nutritional value of forage produced.

When submitted to two cuttings for the production of forage, the wheat Tarumã BRS provides higher forage production in the second cutting when compared to the first, however with lower nutritional value.

Conflict of Interest

The authors have not declared any conflict of interests.

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Characteristics of maize production irrigation and time-varying doses of nitrogen

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Maize grown during the off season is a viable alternative for the state of Mato Grosso, Brazil. Therefore, the objective of the present study was to investigate the influence of the duration of irrigation with nitrogen doses on the yield characteristics of maize. The experiment was conducted in a greenhouse using a randomized completely block design with a factorial 5×5 matrix corresponding to 5 irrigation durations, the soil water tension at 15, 25, 35, 45 and 55 kPa and five doses of nitrogen (0, 50, 100, 150 and 200 mg dm^{-3}), with four replications. The experimental units were represented by plastic pots with a capacity of 18 dm^{3} of soil. We evaluated the productive characteristics of the maize plant. The data were submitted to the F test and polynomial regression. The increased dry mass of the shoot, the root dry mass and the ear length were obtained from the plant under irrigation when the soil reached a 15 kPa tension at doses of 100 and 150 mg dm^{-3} of nitrogen. For the weight of 100 grains, no interaction between the factors was found, providing a greater grain yield with 15 kPa and 200 mg dm^{-3} of nitrogen alone.

Key words: *Zea mays* L., tensiometer, nitrogen fertilization.

INTRODUCTION

Due to the global importance, maize (*Zea mays* L.) is one of the most studied plant species. In constantly seeking improvement, programs seek more productive and economically profitable varieties (Carvalho et al., 2008). In many breeding programs, cultivars with additional resistant to water stress and greater efficiency in the use of nitrogen are desired. According to Amin (2011) the crop has a wider range of uses including: human food, industrial processed food production of starch and used as forage to feed animals.

Maize has undergone major changes in management and cultivation in Brazil, which have resulted in significant increases in the grain yield (Von Pinho et al., 2009). Despite these transformations, Brazil still presents a low average productivity (3,620 kg ha^{-1}) compared to that of China (5,560 kg ha^{-1}) and the U.S. (9,660 kg ha^{-1}) (Agrianual, 2011).

The key to enabling increased production is irrigation management, which consists of determining when and with how much to irrigate (Bernardo et al., 2006).

Irrigation management aims to meet the water requirement of the crop without deficit or excess (Gomes

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Table 1. Chemical and textural characteristics of oxisol collected from 0.0-0.20 m in an area of Cerrado vegetation.

Characteristics	Value
Particle size distribution (g kg^{-1})	
Sand	496
Silt	83
Clay	421
pH ($CaCl_2$)	4.0
Phosphorus (mg dm^{-3})	1.1
Potassium (mg dm^{-3})	22
Organic matter (g dm^{-3})	12.8
Calcium + Magnesium (cmolc dm^{-3})	0.3
Calcium (cmolc dm^{-3})	0.2
Magnesium (cmolc dm^{-3})	0.1
Aluminium (cmolc dm^{-3})	0.9
Hydrogen (cmolc dm^{-3})	2.8
Sum of base (cmolc dm^{-3})	0.4
Cation exchange capacity (cmolc dm^{-3})	4.3
Bases saturation (%)	8.9
Aluminium saturation (%)	77.4

and Testezlar, 2004).

The productivity of maize under water deficit may be higher or lower than normal, depending on the intensity and timing of water stress (Cunha and Bergamaschi, 1992). Moreover, maize progressively responds to fertilization because the other factors are at optimum levels, and nitrogen is the nutrient that elicits the greatest response with regard to increasing the grain yield (Biscaro et al., 2011). However, the rational use of nitrogen fertilizer is critical, not only to increase production but also to decrease the cost of production (Fageria et al., 2007).

The monitoring of the soil moisture in the root zone of greatest activity has been recommended to verify the effectiveness of irrigation (Azevedo and Silva, 1999). This monitoring is necessary because dry soil cannot supply enough power to meet the growing needs of the crop (Wolfe et al., 2008), directly affecting the production.

Water, being one of the most essential factors for agricultural production, should be used rationally because its lack or excess significantly affects crop yields, making appropriate management necessary to maximize production (Morais et al., 2008) and decrease production costs. Therefore, the objective of this study is to verify the productive characteristics of maize as influenced by irrigation and nitrogen fertilization in the soil of the Brazilian Cerrado.

MATERIALS AND METHODS

The experiment was conducted from March to June 2012 in a greenhouse at the Institute of Agricultural Science and Technology, Federal University of Mato Grosso (UFMT), Campus of Rondonópolis, in the city of Rondonópolis-MT, which is located at an altitude of 281 m, 16°28'17''S latitude and 54°28'17"W longitude, with an Aw climate type, Köppen classification.

The experimental design was a randomized block in a 5×5 factorial scheme with four replications. The treatments consisted of five durations of irrigation, five soil water tensions (15, 25, 35, 45 and 55 kPa) and five doses of nitrogen (0, 50, 100, 150 and 200 mg dm^{-3}), totaling 25 treatments for a total of 100 plots. The experimental units were represented by plastic pots with a capacity of 18 dm^3 of soil.

During the experiment, the average temperature and relative humidity inside the greenhouse were 28.65°C and 73.8%, respectively.

The soil was collected from an area of Cerrado vegetation that is classified as Dystrophic Red sandy loam texture (Embrapa, 2006), and the 0.0 to 0.20 m layer was passed through a 4 mm aperture sieve and homogenized for insertion into the experimental units. A soil chemical analysis showed the following chemical characteristics (Table 1). The base saturation increased to 60% (Sousa and Lobato, 2002), applying limestone and incubating in the soil for 30 days to maintain the soil moisture at 60% of the field capacity.

Tensiometers were installed at a 0.20 m depth, near the effective area of the root system and near the center of the vessel using an auger screw. Five tensiometers were installed in each block, totaling 20 monitoring units. The tensiometers were installed in the treatments with a nitrogen rate of 100 mg dm^{-3} (reference dose) (Figure 1), which was used as an indicator of the duration of irrigation and the irrigation duration for each analyzed tension (15, 25, 35, 45 and 55 kPa).

The soil water retention curve was analyzed in the laboratory of hydraulic UFMT, Campus Rondonópolis, MT, Brazil. The soil was collected randomly in the very experimental unit using a volumetric ring (ring Köpeck) and subjected to low tension, the voltage and extraction chamber Richards table and high tension (Libardi, 2005). The retention results were interpolated by the Van Genuchten

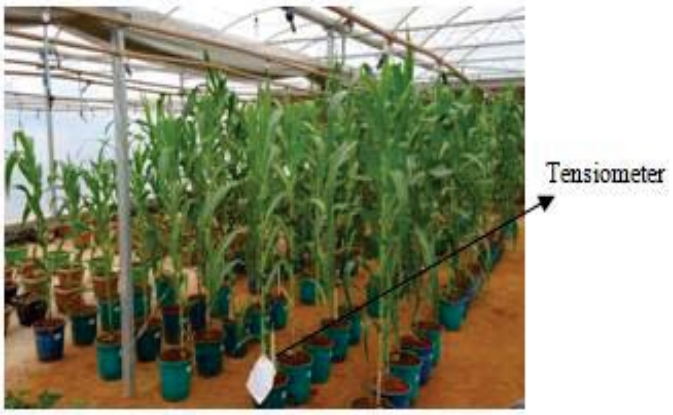

Figure 1. Overview of maize experiment in a greenhouse at 21 days after plant emergence.

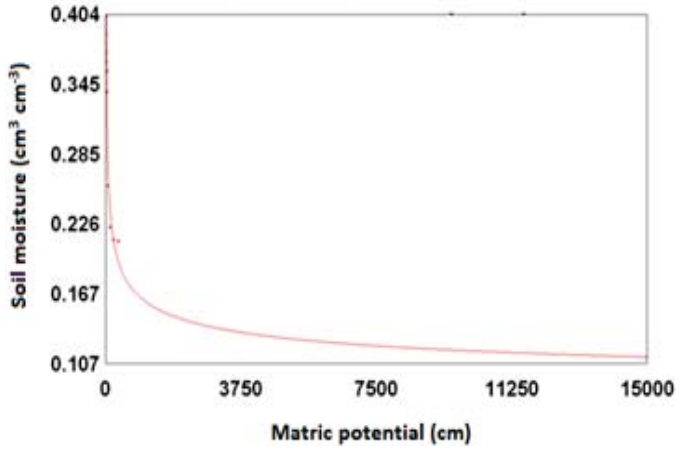

Figure 2. Water retention curve.

equation (Equation 1) (Figure 2) using the computer program Soil Water Retention Curve (SWRC, version 3.0), which was developed by Dourado Neto et al. (2000).

$$\theta = \frac{0.468}{[1+(0.0573|\Psi m|)^{0.3545}]^{0.5724}} \quad (1)$$

θ is the soil moisture (cm^3 cm^{-3}); Ψm is the soil water tension (cm).

The soil water tension during irrigation was monitored using a digital tensiometer with a sensitivity of 0.1 kPa. The duration and the volume of irrigation water replacement were defined based on the evaluation of the soil water tension. The irrigation was performed after three tensiometers for each of the four existing tension treatments reached their limits.

The volume of water that was applied by irrigation was calculated based on the soil-water retention curve, and all of the irrigations increased the soil moisture at field capacity (10 kPa) as in Bernardo et al. (2006).

With the observed tensions, the corresponding humidities were calculated from the retention curve. With these values and the corresponding moisture at field capacity, the volume replacement was calculated (Equation 2).

$$V = (\theta cc - \theta f) \times 18{,}000 \quad (2)$$

V is the volume of water, (cm^3); θcc is the soil moisture at field capacity (cm^3 cm^{-3}); θf is the moisture from the retention curve according to the observed tension (cm^3 cm^{-3}).

The maize crop was fertilized using 160 mg dm^{-3} of phosphate fertilizer (P_2O_5) and 75 mg dm^{-3} of potassium fertilizer (K_2O), using as sources the simple superphosphate and potassium chloride, respectively, as adapted from Bonfim-Silva et al. (2011).

The plants were fertilized with micronutrients 17 days after plant emergence (DAPE) according Bonfim-Silva and Monteiro (2010) using 1.39 mg dm^{-3} of H_3BO_3 (boric acid), 2.61 mg dm^{-3} of $CuCl_2.2H_2O$ (copper chloride dihydrate), 2.03 mg dm^{-3} of $ZnCl_2$ (zinc chloride) and 0.36 mg dm^{-3} of MoO_3 (molybdic acid).

In nitrogen fertilization, urea was applied in solution form and split into three applications: 50% with 5 to 7 fully expanded leaves, 25% with 9 to 12 fully expanded leaves and 25% with 16 fully expanded leaves.

The maize hybrid that was used in the experiment was DKB 390 PRO, which is classified as a simple hybrid with early maturity, high thatched sanity and good lodging resistance and grain production.

The seeds were sown on February 28, 2012 with five seeds per pot at a depth of 5 cm. The first thinning rushed 4 DAPE leaving two plants for pot. At 11 DAPE, the first nitrogen fertilization was performed with 50% of the doses of each treatment, and after the second thinning, leaving only one plant per pot. From planting until 32 DAPE, the soil was maintained at 10 kPa (field capacity), and the treatments with tension measurement began in this period. The tensions were monitored twice daily, in the morning and afternoon. Following each reading, irrigation was performed, when necessary, by hand watering.

The plants were harvested at R6 stage of physiological maturity of maize at 122 DAPE, when all of the plants were already in the process of natural senescence of the finalized leaves.

We evaluated the dry mass of the shoot (DMS), which included the dry mass of the leaves, dry mass of the stalk, dry mass of the tassel and dry mass of the cob. After the insertion of the material in an oven at 65°C to constant weight, the obtained material was analyzed. For the root dry mass (RDM), the roots were washed, dried for 24 h and later placed in paper bags, identified and transferred to the stove to a constant mass at 65°C and further verified by digital scales. The insertion of the first spike (FS) was verified from the ground up to ear height in the stem. The spike length (SL) was measured using a millimeter ruler, and before weighing to determine the weight of 100 grains, the moisture was adjusted to 13%.

The data were subjected to an analysis of variance by the F test and were found to be significant when, in the regression analyses, a 5% probability was found using the Sisvar program (Ferreira, 2008).

RESULTS AND DISCUSSION

There was an interaction between the nitrogen and the duration of irrigation in the dry mass of the shoots of maize. During irrigation (water tension in the soil), doses of 50, 100 and 150 mg dm^{-3} of nitrogen were significant, adjusting to the decreasing linear regression model (Figure 3). The largest decrease in the dry mass of the shoots occurred at a dose of 100 mg dm^{-3} of nitrogen, with a 39.62% loss in the dry mass of the shoots under irrigation occurred when the soil reached 15 to 55 kPa. The same nitrogen rate increased dry mass of the shoots with 251.10 g $plant^{-1}$ when the irrigation reached 15 kPa.

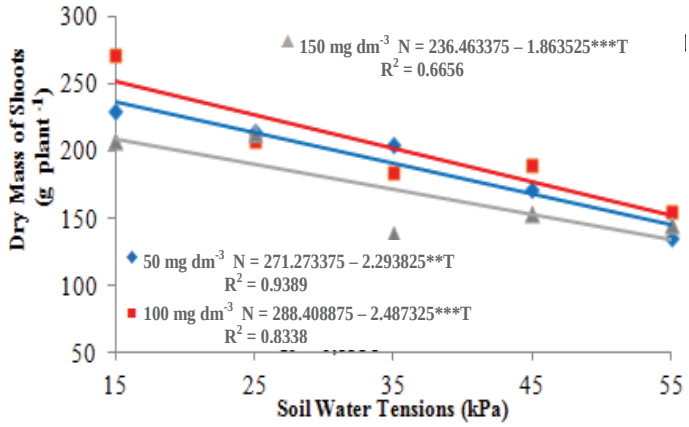

Figure 3. Dry mass shoots maize according to the tensions soil water mass at doses of nitrogen 50, 100 and 150 mg dm^{-3}. N - Nitrogen. T – Tension. ***, ** Significant at 0.1 and 1%, respectively.

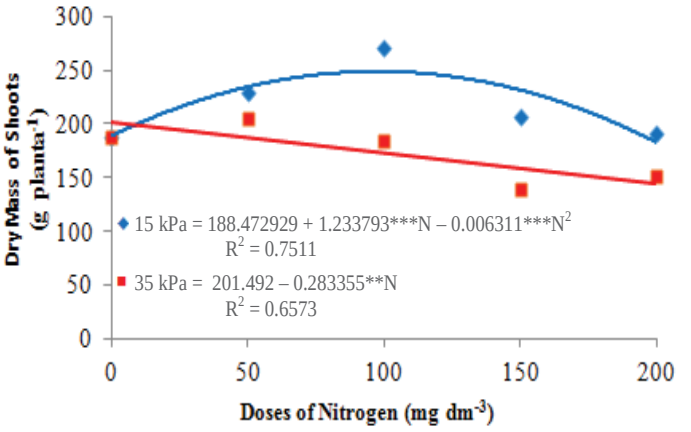

Figure 4. Dry mass of shoots maize according to the doses of nitrogen, in soil water tensions of 15 and 35 kPa. N – Nitrogen. ***, ** Significant at 0.1 and 1%, respectively.

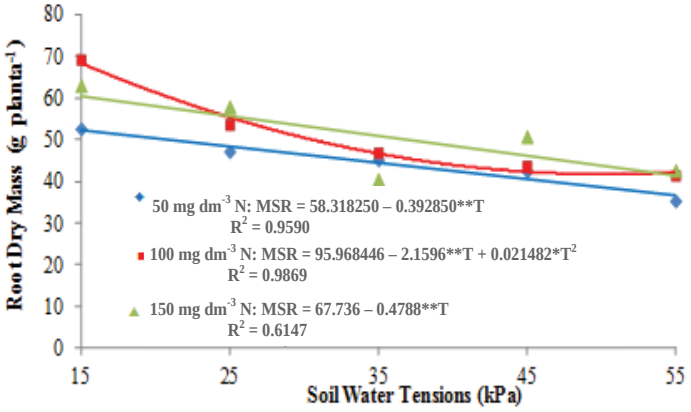

Figure 5. Root dry weight maize according to the tensions soil water mass at doses of nitrogen 50, 100 and 150 mg dm^{-3}. T – Tension. N - Nitrogen. **, * Significant at 1 and 5%, respectively.

There was a reduction of 38.74 and 35.75% of the dry mass of shoots at doses of 50 and 150 mg dm^{-3} of nitrogen, respectively, the shortest irrigation interval (15 kPa) for the largest range of evaluated irrigation (55 kPa). Therefore, it appears that smaller strains showed the highest production of dry mass in the aerial parts of the maize, demonstrating the importance of maintaining the moisture level close to field capacity. These results agree with those obtained by Guimarães et al. (2012), which verified the reduction in the total dry mass under water deficit in the three growth stages of maize, lasting 40 days at the vegetative stage, 75 days at the flowering stage and 52 days at grain filling stage. Nascimento (2008) observed an increase of 33.3% in the dry mass of the shoots of sorghum that were treated with greater water availability in the soil (field capacity) compared to treatment with a lower availability of soil water (40% of the field capacity). Pegorare et al. (2009) also found that the dry mass of the shoot was adjusted to the linear regression model, increasing with higher water levels in the soil for the maize crop.

Irrigation was performed when the soil reached the tensions 15 and 35 kPa, which were significant in the unfolding of nitrogen in the dry matter production of the shoots (Figure 4). The lowest pressure (15 kPa) at the beginning of irrigation was adjusted to the quadratic regression model, with the increased dry mass of the shoots (248.77 g), which occurred at a dose of 97.75 mg dm^{-3} of nitrogen. When the irrigation was performed at the time that the tension reached 35 kPa, the treatment without nitrogen fertilization produced higher growth in the shoots (201.49 g), with a decrease in the dry matter production of the shoots of 28.13% for the highest dose of applied nitrogen (200 mg dm^{-3}).

Galvão et al. (2009) found that the levels of nitrogen in the dry mass of the shoots were not significantly reduced when this nutrient was applied for a longer duration and are in the range that is considered adequate for maize in an oxisol. However Safari et al. (2014) testing doses 0 to 250 kg ha^{-1} observed that the dry weight increase linearly until the last dose. Carvalho et al. (2011) evaluated the maize response to the DBK 390 nitrogen, which fit to a linear model, with the increased dry weight of the shoots when subjected to a dose of 160 kg ha^{-1}. The photosynthetic activity and consequently the dry matter production can be determined by the level of available nitrogen (Jeuffroy et al., 2002).

The root dry weight also showed a significant interaction between the evaluated factors. In the analysis of the unfolding of soil water tension for nitrogen levels, the doses of 50, 100 and 150 mg dm^{-3} of nitrogen significantly influenced the root dry mass (Figure 5). At a nitrogen dose of 50 mg dm^{-3}, its interaction with the water tension in the soil for the duration of irrigation in the dry root mass of the maize was fit to a decreasing linear regression model, exhibiting a decrease of 29.97% in the dry mass of the roots when comparing treatments at

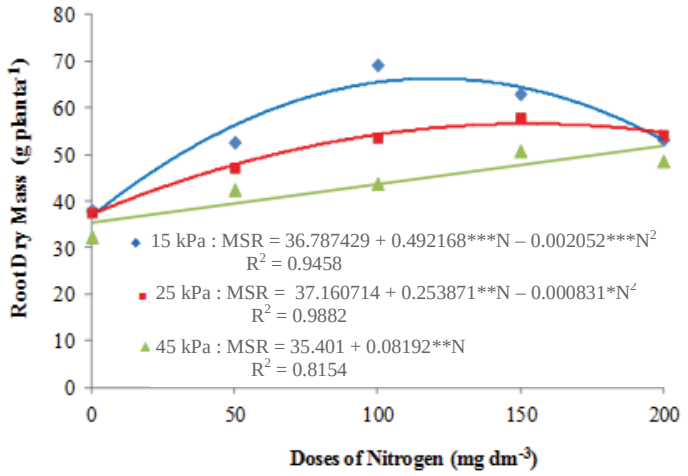

Figure 6. Root dry mass maize according to the doses of nitrogen, in soil water tensions of 15, 25 and 45 kPa. N – Nitrogen. ***, **, * Significant at 0.1, 1 and 5%, respectively.

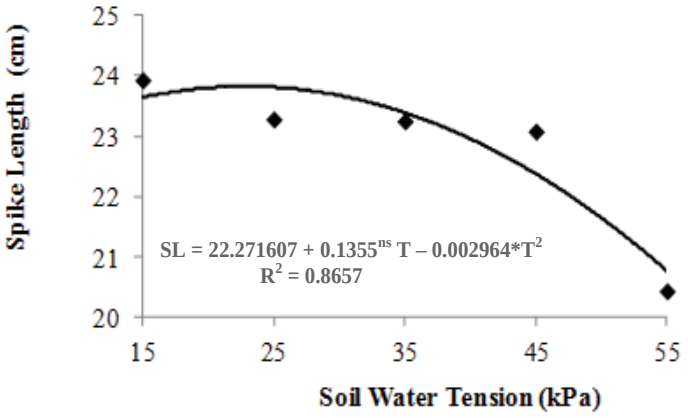

Figure 7. Spike length (SL) maize according to the soil water tension (kPa). T-Tension. ^{ns} Not Significant. * Significant at 5%.

higher voltages, which yielded the lowest production of the root dry mass. The dose of 150 mg dm^{-3} of nitrogen was adjusted to the decreasing linear regression model, experiencing a decrease of 31.63% in the dry mass of the root from the lowest to the highest rated voltage. The increased dry mass of the roots was obtained at a nitrogen dose of 100 mg dm^{-3}, with an interaction of 15 kPa tension at the beginning of irrigation, producing 68.41 g, but when independent of fertilizer applied, the best results of the root dry mass were provided when the plant was subjected to irrigation when the soil water tension reached 15 kPa.

Conte et al. (2009) found no significant effect on the root dry mass in irrigated and non-irrigated maize, and the authors attributed this result to the high coefficient of variation of 36.0%. Analyzing the results of the soil water stress for irrigation in relation to fertilization with nitrogen for the production of the root dry mass of maize, only the voltages of 15. 25 and 45 kPa were significant (Figure 6).

Analyzing the treatment which irrigated to the proper tension of 15 kPa, the values were fit to the quadratic regression model, with the highest production (66.30 g) observed when applied to 119.92 mg dm^{-3} of nitrogen; after this dose, the production reduced. Only 45 kPa irrigation fitted to the growing linear regression model, providing a 31.64% increase in the dry root mass when comparing the treatment without nitrogen fertilization with the highest applied dose (200 mg dm^{-3}). Soares et al. (2009) found no significant difference in the overall mean root dry mass between two nitrogen doses (0 and 2 mmol L^{-1}) for six inbred maize in nutrient solution. Sangoi et al. (2009) analyzed four doses of nitrogen in various soil types and found in most soils that the highest dose, 200 kg ha^{-1}, provided the lowest root growth.

The insertion of the first spike did not influence the nitrogen and irrigation duration, which varied independently between 107 and 140 cm, possibly an intrinsic characteristic of the hybrid DKB 390 PRO. Casagrande and Filho (2002) also found no difference in the insertion of the spike and nitrogen rates (0, 30, 60 and 90 kg ha^{-1}) in the form of urea. According to Siqueira et al. (2009), the insertion of the spike contributes greatly to the layering and occurs because the higher the plant is, the more susceptible it is to lodging. However, Campos et al. (2010), in verifying the relationship between plant height and ear insertion with bedding plants and breaking forty-nine commercial cultivars in five regions found no relationship between the plant height and the ear insertion rates with lodging.

For the spike length, the effect of irrigation duration and nitrogen were isolated, both of which fit within the quadratic regression model. Regarding the tensions, the largest ear length (23.82 cm) was obtained when the irrigation began with a tension of 22.86 kPa (Figure 7). Silva et al. (2012) in evaluating various genotypes of maize under severe water stress found that the spike length ranged from 12.8 to 9.05 cm among 36 genotypes. In agreement with the results of Blanco et al. (2011) using the hybrid AG1051, the ear length is set in a linear way with irrigation up to 220% of the reference evapotranspiration (ETo), 24.8 cm long.

Analyzing the nitrogen doses, the maximum spike length (24.06 cm) was provided by the dose of 143.91 mg dm^{-3} of nitrogen (Figure 8).

These results corroborate those of Ferreira et al. (2010), who also observed a quadratic response for this variable to the nitrogen dose of 250 kg ha^{-1}. However, Mendonça et al. (1999) in evaluating doses of nitrogen from 48.79 to 319.47 kg ha^{-1} found that higher doses produced the greatest ear lengths and adjusted to a linear regression model. The average length of the cob can interfere directly with the number of kernels per row and, consequently, the corn yield (Kappes et al., 2009). It is therefore important that the assessment is performed.

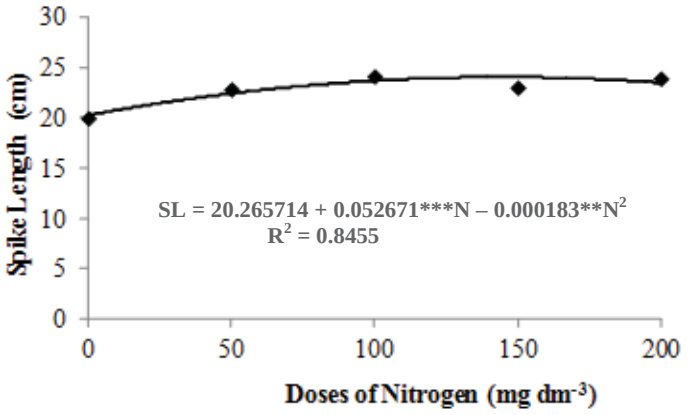

Figure 8. Spike length maize according to doses of nitrogen (mg dm^{-3}). N-Nitrogen.***, ** Significant at 0.1 and 1%, respectively.

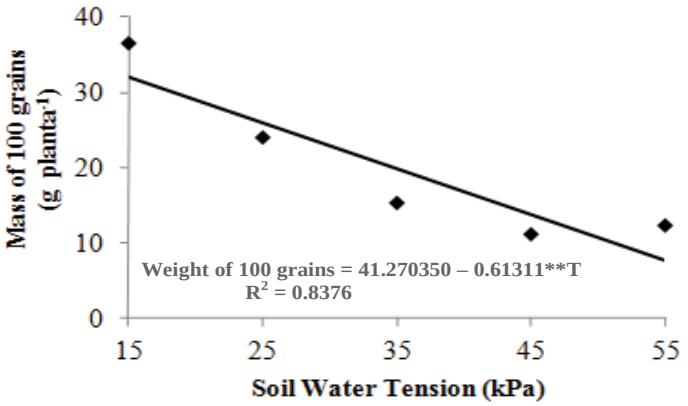

Figure 9. Weight of 100 grains corn according to soil water tension (kPa). T-Tension.** Significant at 1%.

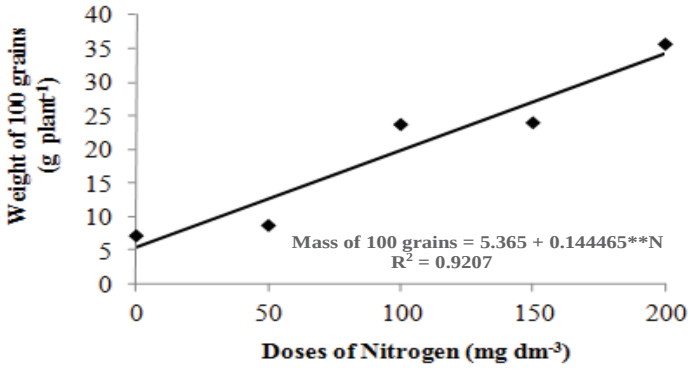

Figure 10. Weight of 100 grains corn according to doses of nitrogen (mg dm^{-3}). N- nitrogen.** Significant at 1%.

Therefore, more frequent irrigations and lower water tension in the soil, combined with the nitrogen rate of 143.91 mg dm^{-3}, performs better for the ear length, which could be indicative of increased productivity. In the weight of 100 grains, there was a significance effect of strains that were isolated from the soil water on the duration irrigation and nitrogen, and the results for the grain weight set the linear regression model. The voltage of the soil water during irrigation influenced the weight of 100 grains, obtaining an R^2 of 83.76% for this variable (Figure 9), for which there was a decrease of 76.46% in the weight of 100 grains when comparing the lowest with the highest measured voltage in the soil water. Therefore, it appears that the lowest pressure (15 kPa) for the duration of irrigation provided the best conditions for obtaining the largest weight production of 100 grains (32.07 g $plant^{-1}$).

The corn could reduce the grain yield during drought stress during the critical period of the crop, which occurs from tasseling to the top of kernel from 60 to 80 days after emergence (Rossetti and Centurion, 2013). Bergonci et al. (2001) verified a reduction of up to 63.30% when the cultivation of irrigated corn was compared with that of non-irrigated corn, indicating the importance of supplemental irrigation on the yield of corn grain. Fang et al. (2010) obtained the best results for wheat production while conserving water in the soil during the reproductive phase, as well as higher water availability during grain filling. Aydinsakir et al. (2013), analyzing the production and quality of maize subjected to five different water availability, observed that plant height, ear diameter, length, number of grains, weight of 1000 grains and grain protein were reduced with increasing water deficit.

Regarding the doses of nitrogen, the same adjusted linear regression model (Figure 10) increased the production of grain weight at the dose of 200 mg dm^{-3} of nitrogen applied, with an increase of 84.34% in the weight of 100 grains when compared to the treatment without nitrogen fertilizer at the higher dose of the experimental range, indicating the importance of nitrogen fertilizer for increasing the grain yield of maize.

The same linear regression model was obtained by Queiroz et al. (2011) with the application of nitrogen up to 160 kg ha^{-1}, using polymerized urea as a source. The results also corroborate Fernandes et al., (2005), who, when analyzing this variable at the dose of 180 kg ha^{-1}, obtained a 32.91 g weight of 100 grains, this value being close to that in this experiment. Ferreira et al. (2010) obtained quadratic regression models for analyzing the weight of 100 grains at doses 0 to 250 kg ha^{-1} of nitrogen, which verified the greater weight of 100 grains (26.37 g) with a nitrogen dose equivalent to 158 kg ha^{-1}. The application of 200 kg ha^{-1} of nitrogen provided the highest yield, promoting an increase in the grain yield of only 8% compared to 150 kg ha^{-1} and increasing the productivity by 53% compared to the treatment without nitrogen in an experiment conducted by Souza et al. (2011). Araújo et al. (2004) found an increase of 21.84% in grain production when comparing the treatment without

nitrogen fertilization to treatment with 240 kg ha^{-1} nitrogen. Carvalho et al. (2001) and Mohammadi et al. (2003) found that the weight of grains is the most important component for predicting corn production. According to Borrás and Otegui (2001), the weight of grains is the component of production that is less affected by changes in management practices and fertilization, which were not observed in the present study. According Genc et al. (2013), both nitrogen and water deficit can be checked by the use of chlorophyll reflectance reader and the data processed in the stove when there are indices.

Conclusions

The increased dry weight of the shoot, root dry weight and ear length were obtained from the interaction of 15 kPa tension with the nitrogen dose of 100 to 150 mg dm^{-3}. The weight of 100 grains had a higher weight when the corn plant was subjected to 15 kPa tension at a nitrogen dose of 200 mg dm^{-3}, with no interaction between the variables.

Conflict of Interest

The authors have not declared any conflict of interest.

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Analysis of effect of government's trade policy on rice supply in three local government areas of Cross River State, Nigeria

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This study was carried out to analyse the effect of government's trade policy on the supply of rice (imported and local) brands in three local government areas of Cross River State, Nigeria. The study captured two periods, July 2004 to February 2005, during which the Federal Government place Tariff on the importation of rice at 75% and the second period; March 2005 to October 2005 where the tariff was raised to 100% (Guardian Newspaper, 2003). Data for this study were collected from purposively selected rice traders in three case-study markets of Watt, Ika-Ika Oqua and Aningeje Markets representing Calabar South, Calabar Municipality and Akamkpa Local Government Areas Respectively. The data was collected over the period of 64 weeks. During the July 2004 to February 2005 period, 34,878.50 and 30,623.20 kg constituting 53.24 and 46.75% respectively of the total quantities of imported and local rice brands was bought for sale across the three markets. During the March 2005 to October 2005 period, the Federal Government raised the tariff on rice importation from 75 to 100%; this reflected in the quantity of imported and local rice bought for sale across the three markets given as 24,519.09 and 24,185.04 kg respectively constituting 50.34 and 49.66%, implying that increase in tariff rate from 75 to 100% was effective in altering the share of imported and local rice brands in the total quantities of rice bought for sale cross the three markets. The study recommends that tariff should be implemented strictly so as to help set up domestic rice production which would be sustained if Government embarks on research into development of high yielding rice varieties that will be supplied to farmers at low cost.

Key words: Importation, rice supply, tariff, trade policy.

INTRODUCTION

Nigeria is the most populous country in Africa with an estimated population of 160 million and annual growth rate of 2.5 vis-a-vis the population of Africa which is estimated to be about 1 billion in 2013 (World Bank Development Report, 2013).

Going by the figures stated above, Nigeria, unlike other developing countries in Africa is faced with the problem of producing food in sufficient quantity and quality to feed her teaming population. If this problem is to be addressed, then effort should be geared owards

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Table 1. Rice trend in Nigeria and the rest of West Africa.

Indicators	Means			
	1961-1975	1976-1983	1984-1995	1996-1999
Nigeria				
Production	332,800	806,222	2,306,794	3,189,833
Import	2,036	420,756	334,974	525,307
Self reliance ratio	99%	54%	77%	79%
Total consumption	178,199	833,640	1,599,609	2,248,113
Per capita consumption	3	12	18	22
West Africa				
Production	1,779,376	2,344,073	2,822,635	4,041,384
Import	416,183	894,073	1,760,884	2,107,146
Self reliance ratio	65%	56%	42%	50%
Total consumption	1,178,753	1,950,821	2,973,885	3,985,721
Per capita consumption	21	27	30	34

Source: Compiled from FAO-Agraostat.

formulation and implementation of policies that would increase agricultural productivity over a long period of time.

Godwin and Olaf (2001) stated that equitable and sustainable economic development cannot ignore basic food commodities since they play essential role in economic development as their availability and cost impinge directly on food security, expenditure and income of household particularly among the poorer segment of the population. Rice is a staple grain that is consumed globally. In Nigeria, the status of rice in the average diet has been transformed from being a luxury food item to that of a staple, taking place of other meals like cassava and yam (Daramola, 2005).

The continuous increase in the consumption of rice in Nigeria has made the country to depend on massive importation of the product from other part of the world to augment domestic production which is far below quantity demanded. Nigeria currently imports rice and pays for such imports in foreign currency which goes a long way to deplete our foreign reserve (Abebe, 2005). Given the precarious balance of payment position of the country, there is need for government to put in place suitable policies that would drive the rice industry in the country. Nigeria imports about one million metric tons of milled rice per annum which translate to a total expenditure of US$300 million per annum (Akande, 2014). Comparatively, the demand for rice has been on the increase at a much faster rate in Nigeria than in other West African countries since the mid 1970s. During the 1960s, Nigeria had the lowest per capita annual consumption of rice in the whole of the West African sub-region averaging 3 kg; soon after that period, the per-capita consumption level of rice in Nigeria grew significantly at 7.3% per annum. Despite the catching up of Nigeria's per-capita rice consumption with the rest of west Africa, Nigeria's consumption level still lag the rest of the sub-region as shown on Table 1.

Several factors have been suggested as being responsible for increase rice consumption in Nigeria, amongst them is urbanization. Rice is easy to prepare compared to other cereals thereby reducing the core of food preparation thus fitting more easily in the urban lifestyles of the rich and poor. In a bid to curb excessive importation of rice through the porous borders of the country and to pursue the food sufficiency objective, Government adopted policies that provide both price and non-price incentives to which agricultural output and sales should respond; these includes fixing of guaranteed minimum buying prices, (incentive product pricing), input prices subsidies, agricultural export promotion, via favourable tariff, quota and outright ban on importation of some food items (Akanji, 1995).

In spite all these incentives, self-sufficiency in food production in Nigeria is still an issue of crucial concern. This is evident in the decreasing trend in the growth rate of output of staple food prices and the rising import value of food items in the country over time (Anyanwu, 1997). This trend is as shown in Table 2.

The Federal Government, in a bid to curb the massive influx of imported rice into the country and to encourage domestic rice production have on several occasions formulated policies such as tariff, quota and outright ban (FAOSTAT, 2001).

Nigeria's rice policy can clearly be associated with three different periods in the history of the country, that includes pre-band, band and post-band periods.

i) Pre-band period (1971-1988): This refers to the era prior to the introduction of absolute quantitative restriction

Table 2. Mean quantity of rice produced and imported in Nigeria (1961-2000).

Rice quantity	1961-1970	1971-1980	1981-1990	1991-2000
Quantity imported (Tons)	1,187.20	205,906.80	390,488.80	466,043.60
Quantity produced (Tons)	176,154.70	355,644.40	1,172,674.20	1,878,785.00
Total quantity (Tons)	177,341.90	561,551.20	1,563,163.00	2,344,828.60

Source: Computed from FAOSTAT Database (2001). http.//oryza.com/Africa/Nigeria/index.shtml.

Table 3. Taxonomy of Nigeria's trade policy on rice.

Periods	Policy measures
Prior to April 1974	66.6% tariff
April 1974 - April 1975	**20% Tariff**
April 1975 - April 1978	10% tariff
April 1978 - June 1978	20%
June 1978 - October 1978	19%
October 1978 - April 1979	Imports in containers under 50 kg were banned
April 1979	Imports under restricted license only Government Agencies
September, 1979	6 months ban on all rice imports
January, 1980	Import license issued for 200,000 tonnes of rice
October 1980	Rice under general import license with no quantitative restriction
December 1980	Presidential Task Force(PTF) on rice was created and it used the Nigeria National Supply Company to issue allocations to customers and traders.
May, 1982	PTF commenced issuance of allocations directly to customers and traders in addition to those issued by NNSC
January, 1984	PTF disbanded. Rice importation placed under general license restrictions
October, 1985	Importation of rice and maize banned
July, 1986	Introduction of SAP and the abolition of Commodity boards to provide Production incentives to farmers through increased producer prices.
1995	100%
1996	50%
1998	50%
1999	50%
2000	50%
2001	85%

Source: Federal Government Budgets, 1984-1986, 1995-2000; SAP and Nigerian Economy (1987).

on rice imports.

ii) Band period (1986-1995): During this period, it was illegal to import rice into the country, hence the institution of absolute ban on rice importation.

iii) Post- band period (1995-till date): Here, quantitative restriction on rice importation was lifted while the country adopted a more liberal trade policy.

The ban on rice importation came into effect in 1985. This policy was anticipated to stimulate domestic production through increase in price of the commodity. The introduction of Structural Adjustment Programme (SAP) in 1986 reinforced the ban which was already in place, this to a large extent encouraged domestic production of rice (CBN/NISER, 1992).

During the 1970s and 1980s, increased export earnings coupled with the highly over valued naira exchange rate made it possible for Nigeria to finance huge food imports and this consequently helped depress domestic prices. Large importation of food items especially rice was allowed into the Country at relatively cheap prices (Anyanwu, 1997). This eroded the competitiveness of domestically produced rice and serve as major disincentive to rice farmers in Nigeria. Table 3 provide a summary of Nigeria's trade policy on rice covering the three (3) periods stated above.

Despite the various policies that the Federal Government has instituted prior to this time to ensure self-sufficiency in rice production, imported rice still flood the Nigerian rice markets making it difficult for the nation

to feel the impact of these policies, hence the need for this study. This study seeks to ascertain the impact of the Federal Government of Nigeria's rice trade policy on the local production of rice. The study compares the quantities of imported and local rice brands bought for sale between July 2004 and February 2005 (32 weeks) with those bought between March 2005 and October 2005 (32 weeks) to see if there is a difference between them owing to the fact that there was a structural change between these two periods, since (tariff on rice importation was raised from 75 to 100%).

The specific objectives of the study are to:

(i) Find out how the rice markets are organised and structured in Watt, Ika-Ika Oqua and Aningeje markets in Cross River State.
(ii) Compare the quantities of imported and local rice brands bought for sale between July 2004 and October 2005(32 weeks).

RESEARCH METHODOLOGY

The data for this study were collected from purposively selected traders across the three case-study markets of Watt, Ika-Ika Oqua and Aningeje markets from whom the quantities and prices of local and imported rice brands that were sold in the three markets were collected. Z-test was used to compare the differences between the mean quantities of imported and local rice brands bought for sale between July 2004 to February 2005 with those bought for sale between March 2005 to October 2005. The model took the form specified below

$$\text{z-cal} = \frac{X_1 - X}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n}}}$$

Where,
X_1 = Mean quantities of imported rice brands bought for sale during the July 2004 to February 2005 period.
X_2 = Mean quantities of imported rice brands bought for sale during the March 2005 to October 2005.
S_1^2 = Variance of the mean quantities of imported rice brands bought for sale during the July 2004 to February 2005 period.
S_2^2 = Variance of the mean quantities of imported rice brands bought for sale during March 2005 to October 2005 period.
n_1 = Number of observations (weeks) during the July 2004 to February 2005 period.
n_2 = Number of observations (weeks) during the March 2005 to October 2005 period

RESULTS AND DISCUSSION

Rice Market Organisations and structure in the three case study markets

The imported and local rice market in Watt, Ika-Ika Oqua and Aningeje markets are organised by traders who deal on rice trade. The organizations differ from one market to the other even though their operations appear similar.

Rice traders in Watt Market belong to a multipurpose co-operative society, which is a sub union of the Watt Market Food Stuff Union. The union charges ₦500 (Five Hundred Naira) as registration fee which is non-refundable and a daily due of ₦40 (Forty Naira). A defaulting member is expected to pay fines ranging from ₦100 (One Hundred Naira) to ₦2,000 (Two Thousand Naira) depending on the gravity of the offence committed.

In Ika-Ika Oqua Market, the registration fee for new member is ₦4,000 (Four Thousand Naira), daily due of ₦40 (Forty Naira) and fines for offenders ranging from ₦50 (Fifty Naira) to ₦1,500 (One Thousand Five Hundred Naira).

Aningeje Market, with relatively small number of traders, has low charges for all her fees and dues. This is connected with the fact that business activities taking place in this market is quite low when compared to those that take place in Watt and Ika-Ika Oqua Markets. Sequel to this fact, the registration fee is ₦2,000 (Two Thousand Naira), the daily due is ₦30 (Thirty Naira) while fines for defaulters ranges from ₦50 (Fifty Naira) to ₦1,000 (One Thousand Naira). The similarities and differences among these markets are displayed in Table 4.

The activities of union operating in the different markets imposes restrictions on the entry of non-members into the business and as a result of this, only registered members of the unions are allowed to operate freely in the market thus making the market assume an oligopolistic status. These unions on the other hand, serve as source of credit to her members; provide welfare packages to members during social functions like marriages, naming ceremonies and burials. The market union also regulates the activities of the traders in the different markets by imposing fines on offenders and daily dues to be paid by each member of the union including the registration fee for any person willing to belong to the market unions; these fees, dues and fines differ from one market to the other as shown in Table 4.

Quantity of imported and local rice brands sold in the three case study markets

Table 5 above shows the quantities of imported and local rice brands bought for sale during July 2004 to February 2005 period and March 2005 to October 2005 period.

During the July 2004 to February 2005 period, when the tariff on rice importation was 75%, about 34,897.50 and 30,623.20 kg bags of imported and local rice, respectively were bought constituting 53.24 and 46.75% of the total quantity of imported and local rice brands (65,520.76) bags bought. During the March 2005 to October 2005 period where the tariff was raised to 100%, the mean quantity of imported and local rice bought for sale was 24,519.09 and 24,185.41 kg bags respectively constituting about 50.34 and 49.66% of the total quantity of rice (48,704.49 kg) bags bought across the three case study markets.

Table 4. Rice Market Organizations and structures in the three markets.

Analytical parameters	Watt market (Calabar South)	Ika-Ika Oqua market (Calabar Municipality)	Aningeje market (Akamkpa Local Government Area)
Market Days	Daily market throughout the week	Daily market throughout the week	Once a week (Saturdays)
Name of Union	Rice Multipurpose Co-Operative Society	Rice Wholesale Association	Rice Wholesale Food Stuff Union
Number of Market Store	45 Lockup- Stores	30 Lock-up Stores	20 Lock-up Stores
Registration Fee	₦5,000.00 (Five Thousand Naira)	₦4,000.00 (Four Thousand Naira)	₦2,000.00 (Two Thousand Naira)
Daily Levy	₦40.00 (Forty Naira)	₦40.00 (Forty Naira)	₦ 30.00 (Thirty Naira)
Fines	₦100 – ₦2,000 (One Hundred Naira to Two Thousand Naira)	₦50 – ₦l,500 (Fifty Naira to One Thousand Five Hundred Naira)	₦50 – ₦1,000 (Fifty Naira to One Thousand Naira)

Source: Compiled from Field Study (2005).

Table 5. Mean quantities of imported and local rice brands bought for sale across the three markets.

Duration	Mean quantity of imported rice bought for sale (kg)	Percentage of imported rice bought for sale (%)	Mean quantity of local rice bought for sale (kg)	Percentage of local rice bought for sale (%)	Total quantity of rice imported and local bought for sale (kg)
July 2004 to February 2005	34,897.50 (3159.16)	53.26	30,623.26 (2,765.26)	46.75	65,520.76 (2,925.05)
March 2005 to October 2005	24,519.09 (2,456.3)	50.34	24,185.41 (921.02)	49.66	48,704.50 (1,506.60)

Source: Compiled from Field Study (2004/2005). Figure in parenthesis are standard deviation.

Table 6. Analysis of variance table for mean quantities of imported and local rice brands bought for sale across the three markets.

Source of variability	SS	DF	MS	F-Cal	F-Critical
Rows	1791.631	1	895.82	115.59**	1.81
Columns	250.512	1	50.10	6.46**	3.14
Error	1200.502	155	7.75		
Total	3242.644	191			

Source: Market Survey (2005/2006). Rows represent the two periods (July 2004 - February 2005). Columns represent the quantities of the imported and local brands of rice bought in the individual markets. **,* denotes significance at the 1, and 5% levels, respectively.

The analysis of variance result presented in Table 6 indicates that there exist significant differences among the mean weekly quantities of local and imported rice bought from the three markets at 1% level of significance. This is evident from the fact that the value of the F-calculated (6.46.) is higher than the F-tabulated (3.14) and having estimated the least significant difference of observation (1.97) which was used to compare the differences among the individuals means, it was clear that each of the mean weekly quantity of rice bought form the three markets differ significantly from one another.

The result of the Z-test shows that the quantity of imported rice bought for sale across the three markets during the July 2004 to February 2005 period (34,897.50 kg bags) representing 53.26% of the total quantity of rice bought for sale during this period is significantly different from that which was bought during the March 2005 to October 2005 period (24,519.09 kg bags) representing (50.34%) of the total quantity of rice bought, implying that the increase in tariff from 75 to 100% reduced the quantity of imported rice bought across the three markets which may have caused the reduction in the total quantity of rice(imported and local) bought for sale across the three markets from 65,520.76 kg bags during the July 2004 to February 2005 period to 48,704.50 kg in March 2005 to October 2005.

RECOMMENDATIONS

Based on the findings of the study, the following recommendations are proffered;

i) If tariff is well implemented by Government of the day, it will go a long way to increase domestic production of rice.
ii) The quality of local rice should be improved in order to

increase the price competitiveness of the local rice to those of the imported rice. This will encourage consumers to buy more of the local rice as the difference between the local and imported rice quality would be negligible. This increase in demand of local rice will now serve as a boost towards increase rice production by the farmers. With this increase, the per capita income of the farmers will be raised.

iii) This result shows that the increase in tariff from 75 to 100% during these two time periods brought about a 2.90% reduction in the percentage of imported rice bought for sale and 2.91% increase in the percentage of local rice bought for sale in these three markets. This implies that if tariff is effectively instituted with serious surveillance across the nation's borders to ensure strict compliance coupled with consistency in policy formulation, production of local rice would surely be boosted in Nigeria.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Infection of rose flowers by *Botrytis cinerea* under different temperatures and petal wetness

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The effect of temperature and petal wetness on the infection of *Botrytis cinerea* in rose flowers was studied by combining temperatures of 10, 15, 20 and 25°C with periods of petal wetness of 8, 16, 24 and 32 h. An increase in the severity of gray mold was observed when petal wetness period increased. Lower level of disease severity was verified at 10°C for all wetness periods as well as for the temperature of 25°C and wetness period of eight hours. In this wetness period, the lowest severity indexes were observed in all the temperatures tested. The maximum disease severity was observed at 20°C with 24 h of petal wetness. A model of multiple regression analysis was tested to associate temperature and wetness period. A quadratic effect of temperature was observed, which was overcome by free water time. The results show that infection on the rose flower petals depends on the period of wetness of the petals; maximum estimated severity occurred at 25°C with 32 h of petal wetness; the temperature of 10°C can reduce the severity of gray independently of the petal wetness period; at higher temperatures high disease severity is dependent on the wetness on the petals.

Key words: Disease severity, flowers, gray mold, *Rosa hybrid*.

INTRODUCTION

Roses (*Rosa hybrida*) are affected by several diseases that cause important losses. Gray mold caused by *Botrytis cinerea* is one of the most important diseases of this crop. The pathogen has worldwide occurrence and infects a great number of hosts including violet, begonia, chrysanthemum, gerbera, dahlia, geranium and tulip (Agrios, 1988; Jarvis, 1977; Marrois et al., 1988; Terry and Joyce, 2004).

B. cinerea occurs in various countries worldwide where roses are cultivated. This fungus is especially important because it causes petal spots, which evolves to rot petal areas reducing flower quality and yield (Volpin and Elad, 1991). In countries with usually moderate climate it was observed that epidemics caused by *B. cinerea* are more likely to occur under high relative humidity and low temperatures (Phillips and Margosan, 1985; Elad and Volpin, 1991; Hammer and Marois, 1988; Agrios, 1988). At the extent of our knowledge, in Brazil , there are no

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studies about the influence of climatic conditions on gray mold incidence in rose.

Although in Minas Gerais State roses are cultivated in greenhouses, where environmental conditions may be controlled to some extent, a high incidence of gray mold has been observed during rainy seasons. This happen perhaps because temperature is normally low and relative humidity is high in this period of the year. The petals sometimes do not present symptoms during harvest. Although the petals are handled at temperatures between 2 and 10°C after harvest, higher incidence of gray mold has been observed, mainly during transport and storage. The conidia of *B. cinerea* have an important function in gray mold epidemics (Jarvis, 1977; Braun and Sutton, 1987; Braun and Sutton, 1988, Church, 1992). Infections initiated by conidia can be completed in about eight hours, if high humidity and low temperature are available (Baker, 1946, Mackeen, 1974). However, in geranium there was an increase in the severity of gray mold from 10 to 25°C and the optimum temperature for maximum disease severity was 25°C (Hyre, 1972).

Immersion in water is probably the main requirement for conidia germination of *B. cinerea* (Jarvis, 1977). This fact is associated partly with low content of water in the conidia (Yarwood cited by Blakeman, 1980). The period of wetness in relation to infection of *B. cinerea* depends on the host. Bulger et al. (1987) reported an increase in the infection of strawberry flowers after an increase in the period of wetness, independent of the temperature.The purpose of this work was to study the influence of different temperatures associated with different periods of petal wetness on the infection of *B. cinerea.*

MATERIALS AND METHODS

Rose petals of the variety Kiss were collected from commercial greenhouses in Antonio Carlos county, in Minas Gerais, Brazil. To avoid the occurrence of latent infection from natural inoculum, three petals were cut around each bloom. The stems were immersed in tap water in plastic bags to a depth of 15 cm and then transported to the laboratory. The isolates of *B. cinerea* used in the work were cultivated in test-tubes at 20°C under 24 h fluorescent light, for 14 days (Bulger et al., 1987). The conidia were removed with distilled water using a small brush, and the inoculum concentration was adjusted to 2×10^5 conidia/ml. For inoculation a De Vilbiss atomizer run by compressed air was used. The inoculum suspension was sprayed on the petals until the petal surface was completely covered, without dripping. Afterwards, the bags were covered with damp plastic bags to ensure high levels of relative humidity and to maintain wetness on the plant tissue during the period to be assessed.

The bags with the rose petals were transferred to growth chambers (Fanem BOD, São Paulo, Brazil) adjusted to various temperature levels. The effects of temperature (10, 15, 20 and 25°C) and of petal wetness duration (8, 16, 24 and 32 h) on infection by *B. cinerea* were studied. After each period of wetness the bags with the flower were removed from the incubators, the plastic bags were removed and the surfaces of the petals were dried using an aerator. Afterwards the plastic bags were transferred to a growth chamber at 20°C.

Forty-eight hours after removing the last treatment from the chamber, the severity of gray mold was assessed in two petals per bloom using a scale based on the scales of Horsfall and Barratt (1945) and Redmond et al. (1987) in which 1 = 0%, 2 = 0-2%, 3 = 2-5%, 4 = 5-10%, 6 = 15-25%, 7 = 25-50%, 8 = 50-75%, 9= 75-100 and 10 = 100 % of area of the petals with symptoms. There were three repetitions for each treatment in which a bag with eight blooms was a repetition. A control was inoculated with distilled water for each temperature vs. wetness combination. A multiple regression model was applied to the data in which the temperature and the wetness period were considered the independent variables.

$$Y = \beta_0 + \beta_{1T} + \beta_{2M} + \beta_3(T^2) + \beta_4 (M^2) + \beta_5 TM$$

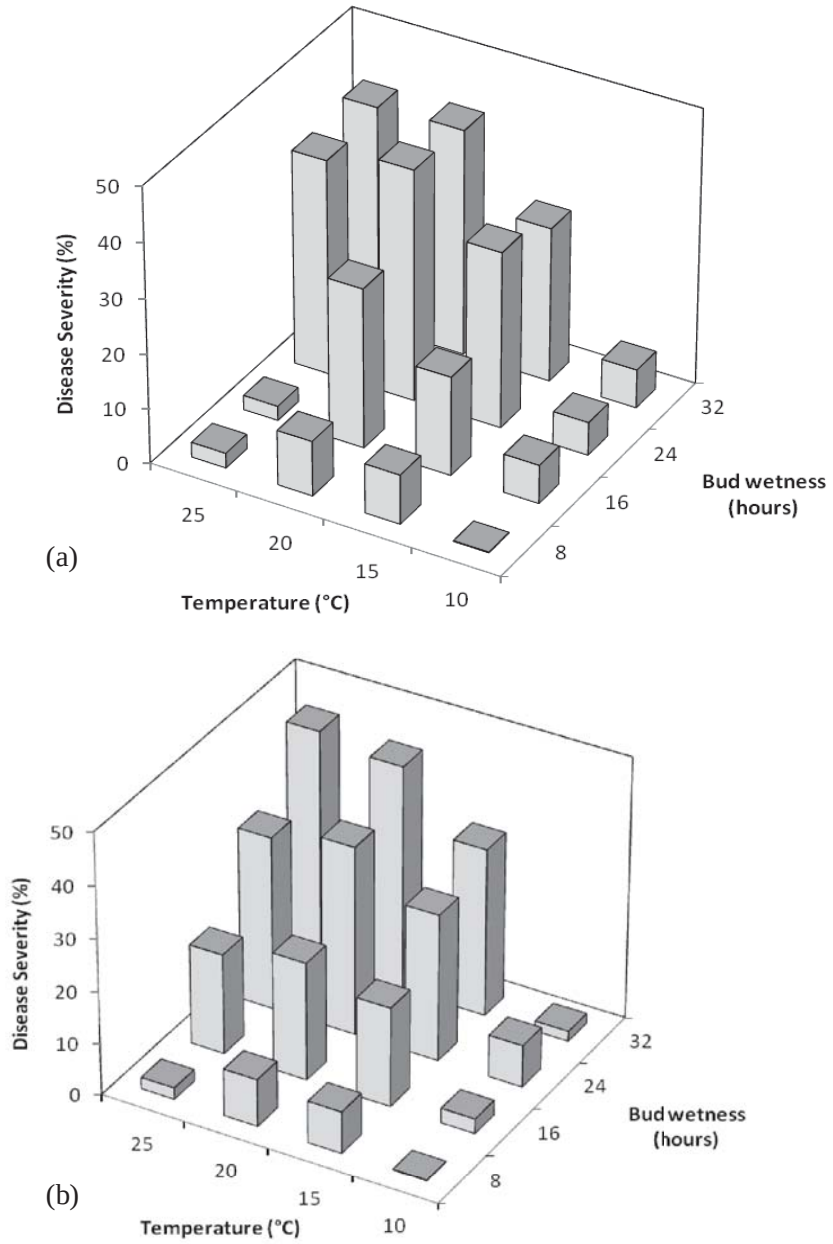
Where: Y = disease severity; β_0 = constant; $\beta_1, \beta_2, \beta_3, \beta_4$ and β_5 = regression coefficients; T = temperature (°C); W= wetness period of the petals (hours), the t test ($P \leq 0.05$) was used for the regression coefficients.

RESULTS

There was an increase in disease severity due to the association of different temperatures with increasing wetness periods. Lower severity indexes occurred at 10°C for all wetness periods. Similar results were observed at 25°C for the wetness periods of eight and 16 h. The higher severity values occurred at 24 and 32 h of wetness and maximum severity was observed at 20°C with 24 h or more of petal wetness (Figure 1a). After this period and under the same temperature, the disease severity showed a tendency to reduce. At 16 h of petal wetness disease severity increased up to 35% for the temperature of 20°C, dropping drastically to around 12% at 25°C. Similar results were estimated by linear regression analysis, where a quadratic effect of temperature on disease severity was observed with the increase in the wetness period. Results show that, starting at 15°C, the longer the wetness period the greater the severity. The maximum disease severity estimated was at 25°C after 32 h of petal wetness (Figure 1b). The lower severity indexes were estimated at 10 and 25°C and eight hours of wetness.

DISCUSSION

Lower temperatures are probably more critical to the pathogen and, at higher temperatures, there is a compensation phenomenon (Rottem, 1978) due to the increase in the wetness period. That is, at low temperatures the disease severity has a tendency to remains low, whereas at higher temperatures the reduction of the disease severity index depends on the wetness of the petals. It is possible that the pathogen may infect the flower petals in the field, before harvest, where environmental conditions are favorable. Nevertheless, these conditions have to be maintained if the pathogen needs to grow and to sporulate on the host tissues. If after field infection favorable environmental conditions are not available, the pathogen can remain



$$\hat{Y} = -67.3838 + 7.37037T + 0.8449124M - 0.23071T^2 - 0.042806M^2 + 0.114877TM$$
$$R^2 = 0.80$$

Figure 1. Effect of the temperature and wetness period on the infection of rose flower petals by *Botrytis cinerea.* (a) Observed and (b) estimated.

latent in the tissues of the petals until conditions improve (Elad, 1988; Elad and Volpin, 1991).

The temperature of 10°C is less favorable to mycelial grow of *B. cinerea* than temperatures between 25 and 30°C (Araújo et al., 2005). In post-harvest handling, temperatures between 2 and 10°C can be unfavorable to *B. cinerea.* However, in this work, the low severity values observed at the temperature of 10°C do not necessarily represent small losses caused by gray mold after harvest. The majority of losses caused by *B. cinerea* occur in the packing house and during transport to the market (Elad, 1988; Hammer and Marois, 1988). Therefore, it is important to consider the latent infection, which is imperceptible during the harvest, but can develop under humid conditions in mature flowers (Volpin and Elad, 1991). In post harvest, due to low temperature

in the packinghouse and in the transportation vehicle, the pathogen stays latent in the tissues of the petals. When the petals are put in bags at room temperature (20 to 25°C) the pathogen is provided with excellent conditions for renewed development, and symptoms of gray mold reappear. It is not yet known exactly how long *B. cinerea* can survive under latent conditions in the tissues of the host. Therefore, if it is considered that the infection normally occurs from the beginning of the opening of the sepals and that between this period and the harvest there is a period of six to eight days, it is probable that the infection stays latent for more than 10 days. Araújo et al. (2005) used infected material from greenhouses and maintained it at 4°C observing that the latent period lasted for up to 15 days after harvesting.

Barners and Shaw (2002) observed that the latent infection by *B. cinerea* was frequently detected in young *Primula* x *polyantha* (horticultural hybrid polyanthus) plants. Genetically marked isolates were used to demonstrate that conidial inoculum applied to young plants generally did not result in disease appearing on the leaves until flowering, regardless of when the plants were inoculated. Problems with gray mold are frequently reported during storage and transportation. In this case, it is probable that prolonged periods of wetness may increase severity of the disease, even at lower temperatures. If the petals are not infected, but there are conidia on their surfaces, they may cause infection under prolonged periods of wetness, which will cause losses of the rose flowers. Under a longer wetness period it is possible to increase the range of appropriate temperature for infection by *Botrytis* species. Shoemaker and Lorbeer (1977) observed that infection by *Botrytis squamosa* on onions could occur between 9 and 23°C, when they are exposed to water for about 40 h.

The temperature and wetness period are considered important variables for the infection of onion by *B. squamosa*. Alderman and Lacy (1983) observed the necessity of at least six hours of exposure to water for successful infection development of leaf blight in onion. In their work fewer lesions occurred under six hours of wetness than at 20°C, but more lesions were verified under 12 or more hours of wetness at the same temperature. Similar results were published by Nelson (1951) who demonstrated that infection of grapes by *B. cinerea* was higher after 21 h of wetness at 12 and 24°C, and by Ramsey and Lorbeer (1986) who found that between six and 24 h of wetness at 21°C are needed for infection of onion flowers by *B. squamosa*, *B. allii* or *B. cinerea* as well as 12 to 48 h are necessary for flower blight. The results of the present paper show a similar tendency to previous reported works, especially in relation to a more prolonged wetness period and the ideal temperature range for infection by *B. cinerea*. Although gray mold maximum disease severity was observed at 20°C and 24 h of petal wetness, through the regression equation the maximum disease severity was estimated to occur at 25°C and 32 h of petal wetness.

Under higher temperatures, the flower petals have a tendency to age more quickly or to liberate exudates with more nutrients, which favor the infectiveness by conidia (Blakeman, 1980, cited by Kerssies, 1992). Therefore, anything that can reduce the physiological stress level of rose petals may result in reduced losses due to gray mold. The implementation of control measures in the field must take into account knowledge about environmental favorable conditions for infection by *B. cinerea*. From the results of the present work it is possible to deduce that periods of more than 8 ho with relative humidity above 90% and temperatures in the interval of 15 to 20°C, or more than 16 hours of 90% RH and temperatures higher than 15°C, are favorable to the incidence of gray mold in rose flowers.

Considering the losses induced by *B. cinerea* during transportation and storage, and the low disease severity at 10°C observed in the present paper, it is important to determine the capacity of the pathogen to cause damage to rose petals under lower temperatures, including its effect on latent infections.

The implementation of measures to reduce the level of stress on the petals after harvest and to minimize the effects of latent infection must be encouraged. These studies must consider post-harvest practices of control, management of temperature and humidity in the packing house and prolongation of pot life of the flowers. In field conditions, it is important to observe environmental conditions constantly, so as to identify the most favorable period for incidence of gray mold, and thus to introduce control measures as quickly as possible.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Socio-economic impacts of wetland cultivation in South-Bench, Southwest Ethiopia

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Wetlands provide several ecological and socio-economic benefits. However, in southwestern Ethiopia, the conversion of wetland to agricultural land is substantial. Hence, the aim of the study was to identify the socio-economic impacts of wetland cultivation. The impacts were assessed through focus group discussion and semi-structured questionnaire of 252 households. According to the respondents, the results indicated that the shortage of subsistence food (65.5%), shortage of cropland (64%), declining of upland crop productivity (63.5%) and increasing demand of agricultural products produced in wetland (40.48%) were the driving forces for wetland conversion and cultivation. The majority (65.48%) of the households benefited from wetland cultivation through growing different crops. However, cultivation of wetlands created deterioration of socio-economical valuable ecological factors. Among the ecological degradation, about 61.21% of households interviewed perceive the degradation of quality and quantity of domestic use of water, 91.27% perceives the decrease of grass for thatching, 100% of interviewed households perceive the loss of grass for plastering, and also for fodder. This affects the livelihood of the community through ailing from water born disease and increased cost for wastewater treatment, increasing cost of construction, reduction of milk and milk products, and to a decrease in number of livestock of 42.86, 61.51, 93.25 and 68% of interviewed households, respectively. Therefore, wetland management needs legal supports and institutions, planning of wise use and strategies for improving the productivity of upland cropland and for minimizing the load on wetland utilization for cultivation.

Key words: Impact, wetland cultivation, wetland benefit, perception, South-Bench district, Ethiopia.

INTRODUCTION

The global wetland area is generally estimated to be 4 to 6% of the land surface of the earth (7 to 9 million km^2) (Mitsch and Gosselink, 2000). Wetlands are among the most biologically productive ecosystems as they are rich in species diversity and habitats (Mironga, 2005; Mwakaje, 2009). Wetlands support millions of people, not only to the local population living in their periphery but also to the national, regional as well as global outside the wetland (Ramachandra et al., 2011; Roy et al., 2012; Hagos et al., 2014). According to assessment of Costanza et al. (1997), the dollar value of wetlands worldwide was estimated to be $14.9 trillion. However,

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this important resource is so fragile and has suffered deterioration due to human activities such as cultivation, grazing, urbanizations and industrializations, water abstraction among others (Wuver and Attuquayefio, 2006; Crecious and Lazarus, 2013; Hagos et al., 2014). Besides, Sakataka and Namisiko (2014) also reported that wetland encroachment and subsequent degradation is caused by land hunger due to fast rising population. Moreover, Musamba et al. (2011) indicated that the wetland was found to be decreasing in size every year due to various socio-economic activities.

It is difficult to say much information about wetland ecosystem in developing country like Ethiopia due to limited studies of cause, consequence and remedial of wetland degradation. However, some studies (e.g. Abunje, 2003; Ayalew, 2010) estimated that Ethiopian wetlands cover an area of 13,699 km^2 area, or roughly 2% of the country's land surface. According to Dixon and Wood (2003) report, wetlands are becoming increasingly important and recognized as vital natural resources because wetlands have wide range of environmental functions and produce multiple products that are socially and economically beneficial to local communities of Ethiopia. But, there was high degradation of wetlands due to increasing demand of wetland cultivation (Legesse, 2007; Mellese, 2008). Cultivation of wetlands has existed in highland of Ethiopia for at least eight decades (Dixon and Wood, 2003; Mulugeta, 2004), with an average cultivation of 23% of the total wetland area (Mulugeta, 2004). Wetlands are ranked amongst the most highly threatened ecosystems in Ethiopia and unfortunately the degradation and loss of wetlands are continuing (Hagos et al., 2014).

Drainage and soil improvement mostly involved with wetlands cultivation have often totally destroyed their ecological character and the ecosystem services that go with it (Afework et al., 2005; Verhoeven and Setter, 2010). Disturbance to wetlands especially from agricultural activity is often considered as causes for degradation of wetland hydrology (Dixon, 2002), and elimination of native species and introduction of weedy species (Zedler and Kercher, 2004; Handa et al., 2012; Kassahun et al., 2014) which generally reduces the value of ecological and socio-economical of the wetland for wetland dependent species (Collins, 2005). Moreover, degradation and losses of wetlands linked to drainage causes the losses of the resources collected from the wetlands, increase the scarcity of thatching reeds, change in water quantity and quality, decrease in crop and livestock production, and loss of biodiversity which directly and indirectly affects the livelihoods of communities (Afework, 2003; Legesse, 2005, 2007).

In southwestern Ethiopia, the effect of considerable conversion of wetlands to cultivated land is received with little attention by decision maker and community. For such problems, research based information is vital to enhance awareness of the stakeholder on wetland management and for designing strategies and implementation of sustainable utilization of wetland resources. Although, the research on consequence of change of wetland to agricultural land on soil physicochemical and plant biodiversity of this area was recently conducted by Kassahun et al. (2014) shows that the degradation of soil fertility and loss of ecological valuable plant species. However, scientific information of the effect of human induced wetland deterioration on socio-economic is not available. Therefore, the objective of this study was to identify the socio-economic impacts of wetland cultivation.

MATERIALS AND METHODS

Descriptions of the study site

The study area is located in southwest of Ethiopia and nearly 586 km away from Addis Ababa, the Federal Capital of Ethiopia (Figure 1). The geographic location of the study area is between 29°23' 13.401" and 29°41' 37.004" east latitude and between 6°43' 55.916" and 6°59' 42.775" north longitude. The area receives annual rainfall of nearly 1,000 to 1,452 mm and much of it falls during March - November. The mean annual minimum and maximum temperature is 18 and 25°C, respectively. The altitudes range from 1,000 to 2,200 m above sea level with undulating plains and mountains.

The study conducted in two *kebeles* (peasant associations) selected purposively among 25 *kebele*s was based on the relative extent of wetland coverage and wetland use. Households were the basic sampling unit for individual interviews. Households were selected using systematic random sampling technique and the numbers of sample size from each *kebele* was proportion to total households of each *kebeles*. The formula of Krejcie and Morgan (1970) was used to determine a sample size of 252 (Zemika = 139 and Ketea = 113) from the total of 734 (Zemika = 405 and Ketea = 329) household heads.

$$s = \frac{x^2 NP(1-P)}{d^2(N-1) + x^2 P(1-P)}$$

Where: s = required sample size; x^2 = the table value of chi-square for 1 degree of freedom at the desired confidence level (3.841); N = the population size; P = the population proportion (assumed to be P = 0.50 since this would provide the maximum sample size); d = the degree of accuracy expressed as a proportion (0.05).

Semi-structured questionnaires were used for household interviews and focus group discussions (FGD). FGD was conducted with two groups of farmers and a total of 17 farmers from each *kebeles*. This was done to secure additional information on the extent and trends of wetland cultivation and its impact. The questionnaire consisted of a wide range of issues such as household characteristics, wetland resource use, and access to wetland resources, wetland cultivation, and wetland resource degradation and its consequences. The formal survey was conducted by trained enumerators who have knowledge about the study areas and speak and write the local language.

Data analysis

Qualitative responses were summarized, categorized and coded into numeric values. The data was summarized using Statistical package for social science, version 16.0 (SPSS). Descriptive statistics, charts, frequencies tables and graphs were used to present the results. The data obtained from FGD and field observation were written in the form verbal/narrative information.

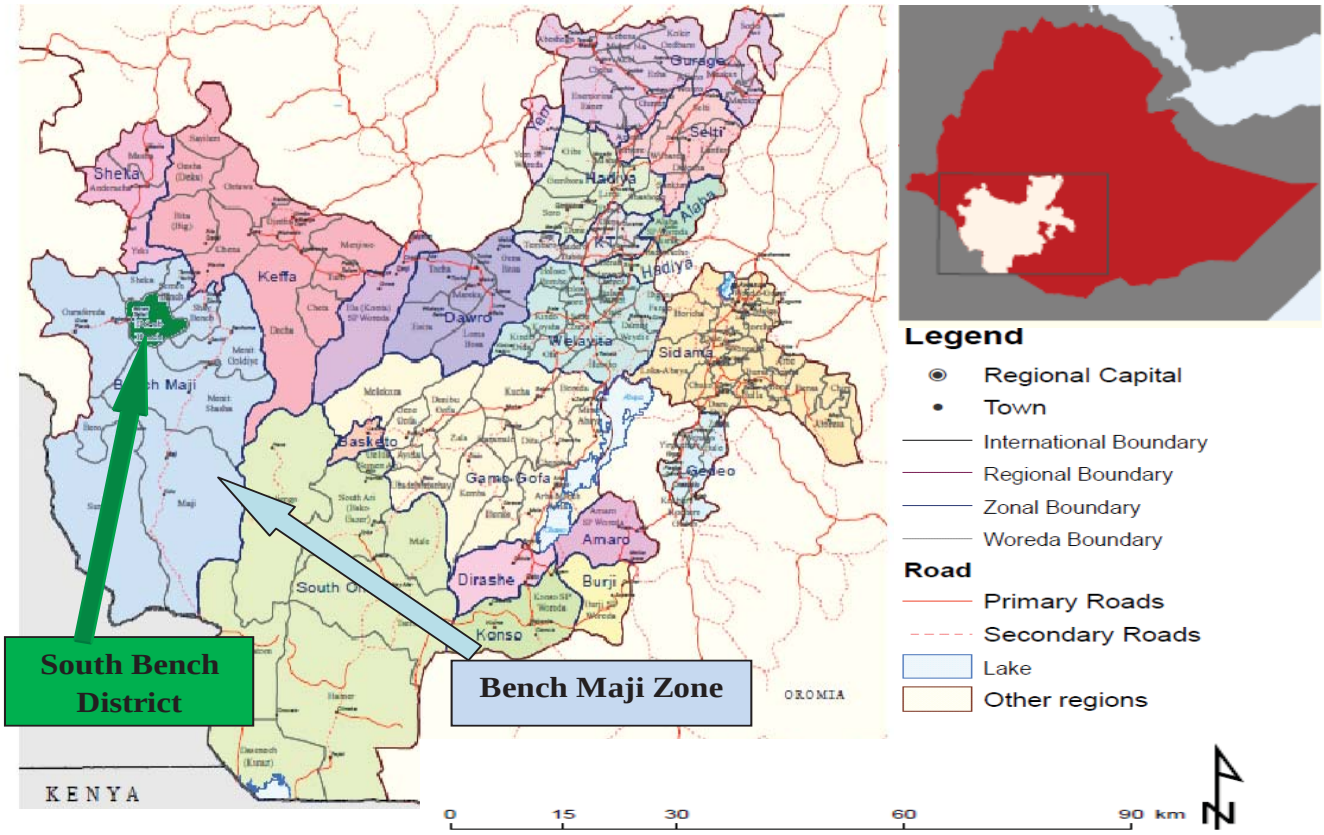

Figure 1. Map of the study area. Source: UN OCHA (2011).

RESULTS AND DISCUSSION

Household's characteristics

The result of household characteristics indicated that 37.7% of the households had a family size of 4 to 6, 28.2% had 7 to 9, 20.6% had more than or equal to 10 and 15.5% had 1 to 3. The sex composition of the sampled households head showed that 94.8% of them were male and 5.2% of them were female. Among the households, 99.2% of them were married and 0.8% was unmarried. The age structure of household heads indicated that the majority of the household heads (52%) were in the age group of 26 to 35 years, followed by 36 to 45 years (29.37%), 16 to 25 years (8.73%), and then 46 to 55 years (3.17%). Education background of 74.6, 10.7, 9.9 and 4.8% of the households were primary education, illiterate, adult literacy and secondary school, respectively. The landholding size of 59.52% of the respondents had ≥ 1 ha, 22.22% had 0.6 t 1.0 ha, 11.11% had 0.26 to 0.50 ha and 7.14% had ≤ 0.25 ha. Notable amount of households (40.48%) hand less than 1 ha and hence, this was an indicator of shortage of cropland that drives the farmers to change wetland into agricultural land.

Socio-economic benefits of wetlands in the community

Socio-economic benefits of wetlands in the study area were very diverse and greatly contribute to the livelihoods of the community. Such contributions do not only involve food security and income generation through farming, but also some specific goods and services such as collection of building material, medicinal plants, grazing areas. Greater number of households (65.48%) interviewed were perceived benefit from wetland cultivation. About 23.4% of them collected medicinal plant from wetland in their lifetime, 91.27% collect wetland grass for thatching, 69.84% of them used domestic water from wetlands and springs near wetlands, 91.67% of them used water from wetlands for livestock while 100% of them used grasses from wetland for plastering wall and for fodder (Table 1).
The result suggested that wetlands are the most important resource for the livelihoods of the community and the dependence of the community on wetlands resources were higher than reported in some other area of the country. For instance, in Jimma area 50% of households used wetlands for cultivation of crops and vegetables and 38% of households collects grasses from wetlands for different purposes (Hayal, 2006). In

Table 1. Some socio-economic benefits of wetland in study area.

Benefit	Respondents	
	Frequency	Percentage
Cultivation	165	65.48
Medicinal plant	59	23.4
Thatching grass	230	91.27
Domestic water	176	69.84
Dry season grazing	103	40.87
Water for livestock	231	91.67
Grass for plastering wall	252	100
Collecting fodder for livestock	252	100

Respondents: Ketea = 113; Zemika = 139 and Total = 252.

Table 2. Driving forces for wetland cultivation in the study area.

Reason for wetland cultivation	Respondents	
	Frequency	Percentage
Shortage subsistence food	165	65.48
High demand of agricultural products produced in wetlands	102	40.48
Declining productivity upland	160	63.49
Shortage cropland	161	63.89

Respondents: Ketea = 113; Zemika = 139 and Total = 252.

Illuababora zone, as high as 57% of households used wetland water from nearby springs for domestic water supply and 69% of households used C*yperus latifolius* or reed from wetlands for thatching purpose (Ethiopia Wetland and Natural Resource Association-EWNRA, 2003). Similarly, Afework (2003) reported that about 10% of the community used wetland for cultivation while Solomon (2004) reported that about 50% of the people used wetlands for cultivation and about 75% of the household depend on the reeds for thatching roofs.

Socio-economic impacts of Wetland cultivation

Impact of Wetlands cultivation on food security and income generation

The result of study shows that farmers of this area were growing taro (*Colocasia esculenta*), banana, sugarcane, maize and vegetables mostly to secure their food shortage and income generation (Figure 3). However, the most widely grown crops in wetlands are taro (*C. esculenta*) and maize. According to the respondents (Table 2), the major driving force for wetland cultivations were associated with shortage subsistence food (65.48%), high demand of agricultural products grown in wetland (40.48%), declining of upland crop productivity (63.49%) and shortage of cropland (63.89%) associated with increasing number of population. In line with this, Hengsdijk et al. (2008) reported that subsistent farming practices in the basin of Hawassa and Zway wetlands of Ethiopia for example are disturbing the respective wetlands. Besides, Sakataka and Namisiko (2014) reported that wetland degradation is caused by land shortage due to fast rising population in Kenya.

According to the households of the study area, wetlands are the only productive land for taro (*C. esculenta*) cultivation, which is a major staple food in the study area. They reported that taro (*C. esculenta*) cultivation in upland were almost stopped due the loss or decline of its productivity, hence all inhabitant were searching for wetland plots for this purposes. During household survey, about 34% of the farmers who did not have plots of wetlands for cultivation indicated that they were interested in doing so. This is because they were attracted by the income generated from the sale wetland crop. In addition to this, they indicated that wetland cultivators were more advantageous as they solve their problems related with crop land shortage, crop failure and food shortage. This suggested that the demand for

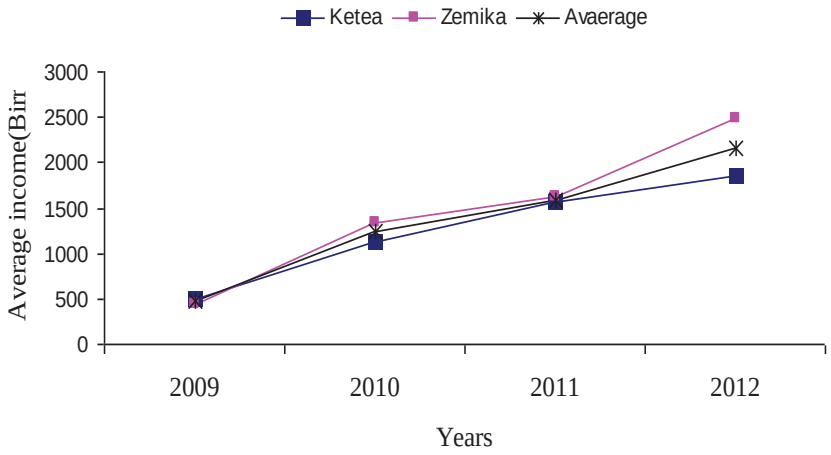

Figure 2. Trends in Income generation from the sale crops cultivated in wetlands from year 2009-2012.

wetland cultivation in the study area is high. This is similar to the observation of Solomon (2004), he indicated that pent-up demands for wetland cultivation in Kemise (Illuababora area) is so large and is an imminent threat to the sustainable use of these resources. Besides, according to Hagos et al. (2014), cultivation on the periphery of the wetlands and cropping in the wetlands has been identified as a threat for the survival of wetlands in Ethiopia.

Moreover, the key problem associated with distribution of plots of wetlands for cultivation, especially in recent years were illegal encroachment. Moreover, farmers with upland not boarded with wetlands are also disadvantageous. Generally, less control over wetland under communal and government were the main cause for illegal wetland holding for cultivation and unsustainable utilization of wetland resources in the study area.

While wetlands of this area are also the most threatened like other wetlands in Ethiopia, households obtained benefit from cultivation of wetlands. The income estimated from 2009 to 2012 (Figure 2), by wetland users from the sale of banana, sugarcane (*Saccharum officinarum*), dry season maize, vegetable and eucalyptus (*Eucalyptus spp*) showed an increase in average income from 468 to 2163 birr (23.4 to 108$). This was the net income wetlands cultivators gained from wetland cultivation when compared with non-wetland cultivator. Hence, cultivation of wetland resolves some of the socio-economic problems such as shortage of land for crop cultivation and crop failure, shortage of subsistence food and costs needed and contributed in reducing poverty.

However, farmers during group discussion indicated that the productivity of taro (*C. esculenta*) noticeably decreased when they compared it with the past after drainage practices were introduced. Due to this, they shifted from previously used wetlands for taro (*C. esculenta*) to sugar cane (*S. officinarum*), vegetable, banana and eucalyptus (*Eucalyptus spp*) to increase income from the sale of the crops.

Impact of wetland cultivation on wetland uses

Despite the fact that wetlands in the study area are the sources for various resources that were directly or indirectly used for different socioeconomic purposes, the survey result indicated that the resources degraded due to unsustainable utilization. The perception of households on the availability and conditions of wetland resources indicated that all (100%) the respondents reported that wetlands from which they collect different resources for their livelihoods shrunk due to conversion of wetland into agricultural land. This is in contrast to the result reported by Hayal (2006) who reported that about 48.68% of the households in Jimma area stated that the wetlands were expanding in size. However, this result agrees with earlier findings of EWNRA (2003), they reported that drainage of wetlands was one of the causes for the drying up of wetland spring and causes for the decline in water supply in Illuababora zone. In other parts of East Africa, for example, due to socioeconomic pressures on wetlands in Tanzania, the wetland areas along Lake Victoria changed into other land use and at an average rate of 6.5 ha yr^{-1} of wetland deterioration (Musamba et al., 2011).

The community in the study area recognized that the redaction in quality of water, using their own indigenous knowledge like color and taste of water, disease causing macro organism and large particles in the water, and increasing growth of algal in the vicinity of wetlands. The households living around wetlands also observed that large areas of wetlands drawdown. About 61.21% of the respondents perceived that the water they used for domestic purpose decreased in quantity and quality

Table 3. Perception of farmers on wetland resource condition in two *kebeles*.

Impact of wetland cultivation	Respondents	
	Frequency	Percentage
Shrinking of wetlands area	252	100
Decreasing access to wetland grass	224	88.89
Decreasing dry season water for livestock	199	78.97
Decreasing of drinking water quality and quantity	154	61.21
Loss of thatching grass	230	91.27
Decreasing grass for plastering wall	252	100
Decreasing grass for livestock fodder	252	100
Decrease in dry season grazing land	91	36.11
Ailing by water born diseases and increased in associated cost	108	42.86
Increasing cost for construction	155	61.51
Decreasing milk and milk production	235	93.25
Decreasing number of livestock	172	68.25
Hindering from keeping wealth's livestock & benefiting	23	9.13

Respondents: Ketea = 113; Zemika = 139 and Total = 252.

especially during dry period while only 22(8.63%) households assume the water they use is safe and clean for drinking (Table 3). Besides, 88.89% of the farmers indicated that, they had limited access to wetland grass because of conversion of wetlands to agricultural land and illegal holding of communal wetland by individuals. The household who depends on wetland grass for thatching (91.27%) and grass for plastering and fodder (100%) perceived that wetland grasses such as C*. latifolius* (used for thatching roof), *Triumfetta spp* (used for fodder, making rope and sources of income) and *Leersia hexandra* (used for plastering walls, thatching and grass for livestock) decreased or lost. This is similar to the observation in Illuababora Zone, southwest Ethiopia, in which an economically significance plant for thatching such as C*. latifolius* has disappeared from all of the drained and cultivated wetlands (Legesse, 2007). Similarly, Solomon (2004) reported that about 45% of the respondents felt that there was a reduced vegetation cover and amount of water in the wetlands in the same area. Moreover, from 103(40.87%) households used wetlands for dry season grazing, 91(36.11%) of them indicated that the wetlands area used for grazing decreased due to cultivation while 12(4.76%) indicted that they did not observed any change. Similar finding reported by Barakagira and Kateyo (2008) indicated that wetland drainage led to the reduction and in some cases complete disappearance of biodiversity such as medicinal herbs, raw materials for building and crafts.

The result of the study presented in Table 3 shows that wetland cultivation which causes diseases incidence on the community directly or indirectly depends on the wetland. About 43% of households interviewed were ailing by diarrhea and associated diseases frequently. Besides, the households noticed that the cost expenditure for treatment and buying medicine increased after wetlands were changed due to cultivation. The result was similar with the findings of Barakagira and Kateyo (2008), they indicated that wetland drainage for search for more arable land has impacted the livelihood and water supply of surrounding community as diseases causing organisms has not filtered off and then the health hazards to people such as stomach upsets now have more common than before; wetlands were drained and increased the cost for medicine (Metronidazol) in Uganda.

The data collected from Ketea Health Center indicated that from the total of 386 patients, 376 in year 2012 and from 195 patients in year 2013 (September to March) all of them were associated with water born diseases like typhoid fever*,* giardiasis and amebiasis (Figure 4). This indicated that people living in study area are at risk for water born diseases due to contaminated water and it may be associated with the fact that degradation of wetlands area reduced its ability to encouraging nutrients retention to sediments and taking up nutrients in plant biomass (Fisher and Acreman, 2004). Moreover, drained wetlands are less effective at regulating stream flow and purifying water, because the drainage channels speed up the movement of water through the wetland (Collins, 2005). Furthermore, the problem could be associated with the loss or reduction of some plant species, for instance, *C. latifolius,* which can play an important role in water purification (Jahn, 1981).

The result also revealed that, the current wetland utilization reduced the resources used for construction. About 61.51% of households were forced to buy substitute grass for thatching (locally known as *p'xxea:*- grass) and plastering (locally known as *gach push:* - remains of *teff (Eragrostis tef*)). Besides , the loss of

Figure 3. Converted wetland area to different cropland (top left: sugarcane; top right: maize; bottom left: sugarcane, banana and taro; bottom right: taro and eucalyptus tree).

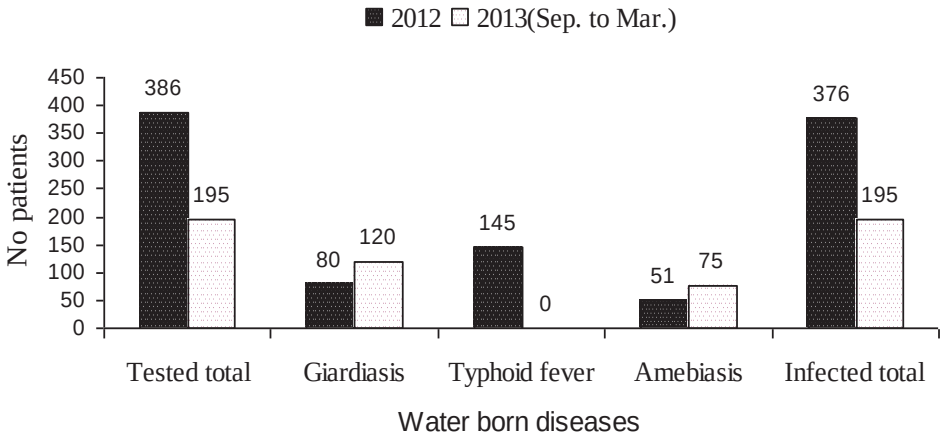

Figure 4. Patients treated for water born diseases in Ketea Health Center in year 2012-2013.

wetland grasses, reduction of grazing land and limiting access to collection of grass for livestock due cultivation and illegal conversion of communal and government owned wetland to private ownerships had impact on livestock production. During group discussion, farmers' reported that, the quality of grass (grass selected specially for high milk production, high quality cheese and butter) and quantity were lost due to wetland degradation and has induced the reduction of milk and milk products. Accordingly, 93.25% of respondents reported that the milk and milk products was decreased, 68.25% of farmers were forced to decrease the number of livestock and while 9.13% of them were hindered from keeping wealth's livestock in their homes and loss benefit from it such as sharing milk and milk product, sharing lamb and calves and used oxen for draught, respectively. They also indicated that their income also decreased due to the above problem.

Generally, the result indicated that, despite the fact that wetlands cultivation benefits majority of the community

through provision of fertile and additional land for ensuring subsistence food and increasing income generation, its unsustainable use had negative impact on the resources consumed for multipurpose use. For instance, EWNRA (2003), Afework (2007) and Tariku and Abebayehu (2011) indicated that planting of plants in and around wetlands such as eucalyptus (*Eucalyptus spp*), banana and sugarcane had destructive effect on water supply. Similarly, different studies in the country (Afework, 2003; Legesse, 2005, 2007) showed that degradation and losses of wetlands linked to drainage had caused the losses of resources collected from the wetlands, increase the scarcity of thatching reeds, change in water quantity and quality, decrease in crop and livestock production, and loss of biodiversity which directly and indirectly affects the livelihoods of communities.

According to Nonga et al. (2010), land use and habitat change in simplification of the ecosystem to increase the economic value of the services such as intensive agriculture can alter ecosystems and reduce their capacity to provide a broad range of services provided by wetlands. Moreover, McCartney et al. (2010) also indicated that if wetlands are not used sustainably, the functions which support agriculture, as well as other food security and ecosystem services, including water-related services, are undermined and can have profound social and economic consequence for people dependent on ecosystem services other than those provided directly by agriculture.

Conclusion

Majority of local peoples were dependant on wetlands resource directly and indirectly for their livelihood through non-cultivation and cultivation. However, the unsustainable use of wetlands for cultivation was creating the degradation and/or loss of wetlands system and their precious resources. The major problem associated with unsustainable utilization of wetland for cultivation is lack of proper ownership structure and legal supports over illegal holding of wetlands and its utilization. Therefore, wetland management needs strong legal supports and institutions that could define ownership structure and legal basis for planning and implementation of wise use strategy that integrates biophysical and socioeconomic aspects of wetland utilization. Moreover, strategies that can improve the productivity of upland cropland such as improvement in the management of the soil resources for sustainable agricultural use could help to reduce the burden on wetland use and protect biological diversity from agricultural expansion wetland.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of magnetic field on germination, seedling growth and cytogenetic of onion (*Allium cepa L.*)

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Magnetic field is considered a simple and cheap method for stimulation of germination process compared to traditional chemical processes. In this research, laboratory experiment was conducted at Seed Technology Unit, Mansoura, Egypt to evaluate the effect of magnetic field on germination, seedling growth and cytogenetic characters of fresh and carry over (old) onion seeds (c.v.Giza Red). Seeds were magnetically pretreated by different magnetic field (0.03 or 0.06 T) using static magnetic device for different periods time (30, 60 and 90 mint). The obtained results indicated that magnetic field treatment increased all germination and seedling growth characters compared with control. Exposed fresh and carry over seeds to 0.06 T with 30 mint gave the heights values of germination percentage, germination rate, speed germination index and seedling growth parameters, that is, seedling length, seedling dry weight, seedling vigor I and seedling vigor II. Whereas, mean germination time was decreased using 0.03 T with 60 mint gave maximum values in carry over seeds and with 60 min in new seeds. Also, the results showed significant increase in mitotic activity and chromosomal aberration after 30 min treatments for both doses of fresh seeds and with 0.06 T of carry over seeds, while relative division rate (RDR) gave positive values and little disturbance in mitotic phase index. However, the percentage of mitotic abnormalities increased after all exposure treatments. It could be concluded that utilization of magnetic field could enhancement of germination of onion seeds but we need to be carry out more experiments to make magnetic map for all varieties of onion.

Key words: Magnetic field, germination, mitotic activity, phase index, relative division rate, mitotic aberrations.

INTRODUCTION

Onions are an important food crop worldwide. Since many years one has been observing problems with storing onion seeds. Seeds generally have a relatively short storage life and viability decreases rapidly. The main reasons of low quality of onion seed listed: Long flowering period resulted in different stages of seed maturity in the umbel and suboptimal storage conditions as high temperature and relative humidity (Brocklehurst, et al., 1985).

For this reason, for many years there have been

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research works to improve seed germination and to prolong their use for sowing (Kelly, 1998). The improvement in seed germination have been achieved by different pre-sowing treatments including various physical factors such as electric field, magnetic field, laser radiation and microwave radiation (Pietruszewski and Kania, 2010). Over the years, the effects of magnetic fields on plant life have been the subject of different research studies. The first studies were conducted by Savostin (1930) who reported 100% increase in the rate of elongation of seedlings under the influence of magnetic condition. Recently, many authors have reported that magnetic field was affective on seed germination, seedling growth, reproduction and growth of meristem cells and chlorophyll quantities (Namba et al., 1995; Atak et al., 1997; Reina et al., 2001; Amera and Hozayn, 2010a, b; Hozayn et al., 2014). Magnetic field had a positive effect on photochemical activity, respiration ratio and enzyme activity (Martinez et al., 2000; Phirke et al., 1996; Carbonell et al., 2002). The reason of this effect can be searched in the presence of paramagnetic properties in chloroplast which can cause an acceleration of seeds metabolism by magnetic treatment (Aladjadjiyan and Ylieva, 2003). Physiological mechanisms of magnetic field on germination and seedling growth are not completely understood. Magnetic field treatment of seeds leads to acceleration of plants growth, proteins biosynthesis and root development (Kordas, 2002). Racuciu et al. (2008) reported that the activities of some enzymes were increased by exposure to magnetic field. Copeland and Donald (1995) reported that the effectiveness of the magnetic field stimulation is evaluated based on two parameters. These are: Energy of germination and capacity of germination. A result of higher energy of germination is often, stronger development of a radicle, increased fresh mass of the whole seedling and thereafter a plant. This, usually, results in better plant useful characters, e.g. yield of roots, bulbs or leaves (Kubisz et al., 2012) found that exposure onion seeds to magnetic field (20 mT) for 60 min increased their germination % from 4.6 control to 22%.

Recently some magnetic devices have been developed by Magnetic technologies L.L.C., Box 27559, Dubai, UAE with the claim that after passing seed through these devices get bio-stimulated. This magnetized seed exhibits better results in terms of germination and plant growth. Keeping in view their claim, the current study was undertaken to determine the influence of magnetic treatment on germination, seedling growth rate and cytogenetic in onion.

MATERIALS AND METHODS

This study was conducted at Seed Technology Unit, Mansoura, Egypt to evaluate the effect of magnetic field on germination characters of fresh and carry over (old) onion seeds. Onion seeds (c.v.Giza Red) production of season 2012 (carry over seed) and 2013 (fresh seed) were obtained from Onion Research Department, Field Crops Institute, Agriculture Research Centre, Giza, Egypt. Seeds immersed in 5% NaOCl (Sodium hypochloride solution) for 5 min to avoid fungal invasion. Seeds were exposed to magnetic field through rimming it in static magnetic device (Magnetic Technologies L.L.C) (Figure 1) with 0.03 or 0.06 T for different periods time (30, 60 and 90 min). Germination tests were performed according to ISTA (1999), while 300 seed of onion were sown in 3 replicates in 20±1°C in sterilized Petri dishes covered at the bottom with two sheets of Whitman filter paper that had been autoclaved and germination was performed daily to study the following characters:

1. Germination percentage defined as the total number of normal seedlings at the end of the test after twelve days.
2. Germination rate (GR): It was defined according to the following formula of Bartlet (1937):

$$GR = a + (a + b) + (a + b + c) \ldots\ldots\ldots (a + b + c + m)/n\ (a + b + c + m)$$

Where a, b, c are No. of seedlings in the first, second and third count, m is No. of seedlings in final count, n is the number of counts.
3. Speed germination index (SGI): It was calculated as described in the Association of Official Seed Analysis (AOSA, 1983) by following formula:

$$SGI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \ldots + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

4. Mean germination time (MGT): It was calculated based on the equation of Ellis and Roberts (1981):

$$MGT = \Sigma Dn/\Sigma n$$

Where (n) is the number of seeds, which were newly germinated on day, D is number of days counted from the beginning of germination.
5. Seedlings length (cm): It was measured of ten normal seedling 12 days after planting.
6. Seedlings dry weight (gm): Ten normal seedlings 12 days after planting, the seedlings were dried in hot-air oven at 85°C for 12 h to obtain the seedlings dry weight (g).
7. Seedling vigor was calculated following Abdul Baki and Anderson (1973) as:

Vigor index I = Germination (%) × Seedling length (Root + Shoot)
Vigor index II = Germination (%) × Seedling dry weight (Root + Shoot)

Cytogenetic analysis

After both fresh and carry over geminating roots were grown until they reached about 1 to 1.5 cm in length. The root tips were then used for cytogenetic investigation and analysis. Roots were fixed in ethanol : acetic acid (3:1) for 24 h, hydrolysed in 1 M HCl for 10 min then stained with aceto-orcien for 24 h. Root tips were cut off in a drop of 45% acetic acid, macerated and squashed (Sharma and Sharma, 1980). Three replicates were performed for each mentioned treatments and scoring about 2000 cells was done from at least 5 roots of each replicate. Mitotic Index (MI), frequencies of mitotic phases (prophase, metaphase, anaphase and telophase), relative division rate (Relative division rate (RDR)) calculated by the formula (Hoda et al., 1991):

$$RDR\% = \frac{\text{\% of dividing cells in treated sample - \% of dividing cells in control sample}}{\text{100 - \% of dividing cells in control bulbs}} \times 100$$

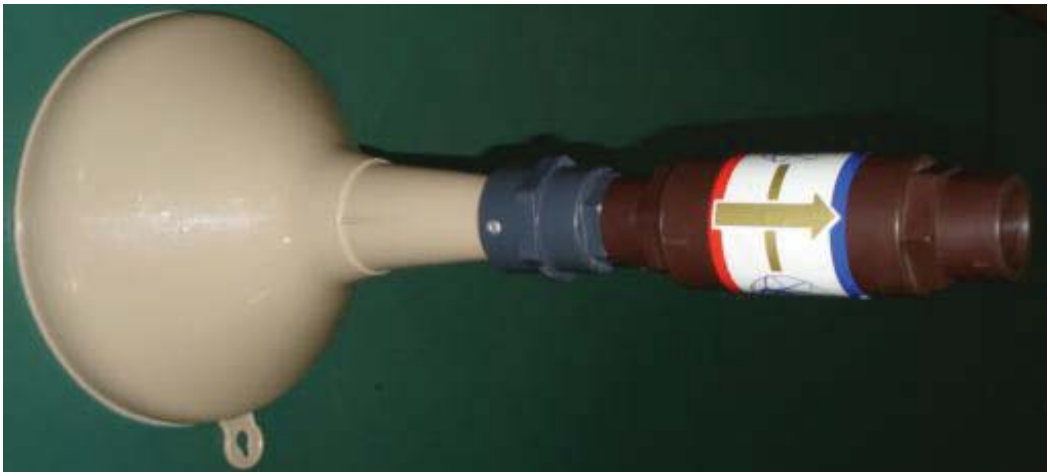

Figure 1. Magnetic devices used to treat onion seed.

Mitotic abnormalities were used as endpoints for determination of cytotogenetic effects. The MI was calculated as the ratio between the number of mitotic cells and the total number of scored cells and expressed as percentage. The frequency of mitotic abnormalities was expressed as a percentage in relation to the number of cells in mitosis. The most frequent abnormalities are shown in photomicrographs.

Statistical analysis

Data were statically analyzed using an analysis of variance (ANOVA) of completely randomized design (MSTAT-C v. 3.1., 1988). Least Significant Difference (LSD) was applied to compare mean values.

RESULTS

Germination traits

General results showed that magnetic field enhanced seed germination and seedling growth parameters comparing with untreated seeds.

Data presented in Table 1 showed that significant effect on Germination % (G), Germination Rate (GR) and Speed Germination Index (SGI) and Mean Germination Time (GMT) occurred by exposes carry over and new onion seeds for two magnetic field (0.03T and 0.06 T) at different time exposure (30, 60 and 90 min) compared to untreated seeds. Regarding germination percentage, the highest values achieved by exposing seeds to 0.06T for 30 mint in new and carry over onion seeds where G (%) increased from 82 and 54% in control to 90 and 70% on new and carry over seed, respectively. Germination rate increased from 0.729 in control to 0.793 when exposed to 0.03 T at 30 or 60 min on new seeds and from 0.688 to 0.739 when exposed to 0.03 T at 30 min on carry over seed. SGI increased from 23.48 to 35.72 in new seed and from 18.44 to 22.96 in old seed. Whereas, using 0.03 T with 30 min gave the maximum increase in G%, GR and SGI on new seeds and with 60 min on old seeds. Data in the same table show that significant decrease in mean germination time in magnetically treated onion seeds. Whereas the minimum MGT 2.87 and 3.45 were occurred from treated seed with 0.06 T for 30 min in new and carry over seeds. MGT decreased (3.35 to 3.43) in 0.03T with 30 min in new seed and (3.32 to 3.80) with 60 min in carry over seed.

It could be concluded that, using 0.03T with 30 min gave the maximum increase regarding new seeds and with 60 min on carry over seeds in above mentioned characters compared to untreated treatment.

Seedling growth

Regarding to seedling growth characters, results clear that a positive effect of different magnetic treatments on seedling length and dry weight, seedling vigor and seedling vigor index compared with untreated treatment (Table 2). The maximum increases were resulted from using 0.06T for 30 min in new and carry over seeds in all mentioned parameters. These increases reached to 58.50, 46.66, 49.13 and 74.15% in new seed and to 109.52, 72.72, 78.54 and 115.90% in carry over seed at the above parameters, respectively compared with control treatment. On the other hand, exposing seeds to 0.03 T gave the highest values with 30 min on new seeds and with 60 min on carry over seeds.

Cytogenetic analysis

Mitotic index (MI) results showed significant increase after all magnetic treatments for both fresh and carry over seeds as shown in Table 3. For fresh seeds, exposed for 30 min gave the highest values in both doses 0.03T and

Table 1. Effect of magnetic field treatments on germination traits of fresh and carry over onion seeds.

Character		Germination (G) (%)		Germination rate (GR)		Speed germination index (GRI)		Mean germination time (MGT) (day)	
Treatment		**Fresh**	**Carry over**	**Fresh**	**Carry over**	**Fresh**	**Carry over**	**Fresh**	**Carry over**
Control		82.00	46.00	0.70	0.69	23.48	18.44	3.43	3.80
	30 min	90.00	64.00	0.74	0.70	28.56	19.60	3.35	3.50
0.03 T	60 min	90.00	66.00	0.78	0.73	28.56	20.60	3.86	3.32
	90 min	90.00	56.00	0.70	0.68	26.32	18.44	4.38	3.85
	30 min	90.00	70.00	0.79	0.74	35.72	22.96	2.87	3.45
0.06 T	60 min	90.00	58.00	0.79	0.72	34.12	22.72	2.87	3.48
	90 min	88.00	56.00	0.72	0.68	28.92	21.40	3.57	3.88
F significant		**		**		**		**	
$LSD_{5\%}$		12.19		0.04		0.81		0.28	
CV (%)		9.85		2.56		7.6		4.66	

$LSD_{5\%}$= Least significance difference at probability 5% level; CV= coefficient of variation; T = Teslla.

Table 2. Effect of magnetic field treatments on seedling traits of fresh and carry over onion seeds.

Character		Seedling length (cm)		Seedling dry wt. (g)		Seedling Vigor		Seedling vigor index	
Treatment		**Fresh**	**Carry over**	**Fresh**	**Carry over**	**Fresh**	**Carry over**	**Fresh**	**Carry over**
Control		10.00	6.30	0.016	0.011	1.32	0.62	821.00	352.80
	30 min	10.65	6.65	0.015	0.018	1.34	1.13	958.20	428.40
0.03 T	60 min	9.55	6.45	0.012	0.016	1.09	1.05	845.40	424.60
	90 min	7.90	7.65	0.010	0.019	0.93	0.88	701.40	346.40
	30 min	15.85	13.20	0.022	0.019	1.97	1.11	1429.80	762.00
0.06 T	60 min	13.65	8.70	0.019	0.016	1.68	0.91	1230.60	487.20
	90 min	11.80	9.05	0.017	0.013	1.53	0.89	1036.80	631.60
F significant		**		**		**		**	
$LSD_{5\%}$		1.76		0.002		0.26		155.45	
CV (%)		10.7		6.37		13.64		12.44	

$LSD_{5\%}$= Least significance difference at probability 5% level; CV= coefficient of variation; T = Teslla.

0.06T which recorded values (13.30 and 14.13), respectively compared to control (10.42), while exposing carry over seed for 30 and 60 min gave the highest values at 0.03T (11.15) and 0.06T (10.68). Relative division rate (Relative division rate (RDR)) gave positive values after all magnetic treatments for both seeds coordinate with the increase in mitotic index and germination (Table 3). In the same table, phase index showed little disturbance decreasing or increasing in their values. Where in fresh seeds (0.03 T for 60 min) treatment found to have the pronounced change in prophase which decreased about 9.52% compared to control as well as in carry over seeds (0.06 T for 90 min) treatment was increased about 25.71% compared to control.

Relative division rate (RDR) had positive values after all magnetic treatments (Table 4), where the maximum increase was recorded in fresh seed which exposed to 0.06 T for 30 min. Data in the same table show significant increases in Aberrations index (AI) (chromosomal aberrations) under magnetic treatments compared to control. Where, exposing fresh seeds to 0.06T for 90 min treatment had the highest aberration with (3.05) compared to control (0.27) as well as for carry over seeds at 0.06 T with 90 min treatment (2.50) value compared to control (1.20). Generally, the recorded aberration percentage in this study found to be not lethal. Stickiness and micronucleus were the most recorded chromosomal aberration types; more pronounced were observed when both fresh and carry over seeds exposed to 0.06 T for 90 min, laggard chromosome and binucleolate cell also recorded (Table 4 and Plate 1).

Magnetic field treatments improved germination parameters of fresh and carry over onion seeds (Table 1).

Table 3. Effect of magnetic field treatments on mitotic index (MI), aberration index (AI), relative division rate (RDR) and phase index (P=Prophase, M=Metaphase, A=Anaphase and T=Telophase) of fresh and carry over onion seeds.

Treatment			MI	AI (%)	RDR	Phase index			
Age	Dose	Time				Pro	M	A	T
Fresh	Control		10.42	0.27	--	18.58	32.04	27.84	21.55
	0.03 T	30 min	13.13	0.63	3.02	17.31	34.29	29.29	19.10
		60 min	12.57	0.90	2.40	16.81	35.21	30.10	17.87
		90 min	11.05	1.15	0.70	19.64	34.56	26.91	18.89
	0.06 T	30 min	14.13	1.91	4.14	20.77	33.52	25.05	20.66
		60 min	13.04	2.38	2.92	19.58	34.64	26.68	19.10
		90 min	12.15	3.05	1.93	16.56	36.80	27.65	18.99
Carry over	Control		8.52	1.25	--	19.85	33.80	26.13	20.22
	0.03 T	30 min	9.88	1.24	1.49	18.74	33.61	28.05	19.60
		60 min	10.68	1.54	2.36	18.83	34.49	29.79	16.97
		90 min	9.01	1.57	0.53	19.53	34.22	28.96	16.90
	0.06 T	30 min	11.15	1.77	2.87	21.62	34.15	24.71	19.53
		60 min	9.99	1.95	1.61	17.55	35.89	28.71	17.85
		90 min	9.61	2.50	1.19	15.75	37.03	29.45	17.77
F significant			**	**		**	**	**	**
$LSD_{5\%}$			1.15	0.86		0.27	0.61	1.04	0.89
CV (%)			3.63	1.85		1.45	23.85	3.32	1.54

$LSD_{5\%}$= Least significance difference at probability 5% level; CV= coefficient of variation; T = Teslla.

Table 4. Effect of magnetic field treatments on chromosomal aberrations (CA) of fresh and carry over onion seeds.

Treatment			Total cell count	Miotic cell count	Type of aberration					
Age	Dose	Time			Sticky	Lag	Bi-nucleus	Micro-nucleus	Other	Total
Fresh	Control		6010	626	-	-	-	1	1	2
	0.03 T	30 min	6072	797	1	-	-	1	1	3
		60 min	6056	761	2	1	1	1	2	7
		90 min	6001	663	1	1	-	2	3	7
	0.06 T	30 min	6100	862	4	2	3	3	5	17
		60 min	6031	786	5	2	3	5	4	19
		90 min	6028	732	7	4	3	5	3	22
Carry over	Control		6011	512	2	-	1	2	2	7
	0.03 T	30 min	6034	596	2	-	2	1	2	7
		60 min	6082	650	3	1	2	2	2	10
		90 min	6100	550	3	1	1	1	3	9
	0.06 T	30 min	6051	675	4	2	2	1	3	12
		60 min	6038	603	3	2	2	2	3	12
		90 min	6075	584	5	3	3	2	2	15

Studies made on various plants have shown that positive effects of magnetic field on seed germination. Kubisz et al. (2012) showed that low frequency of magnetic field (20 mT) can be successfully used to improve germination of onion (4.6 to 22%). Also, they recorded that treated onion seeds (cultivar Eureka) with 20 mT for 60 min increased their energy of germination from 40 to 63%, which improves evenness of plants emergences in the

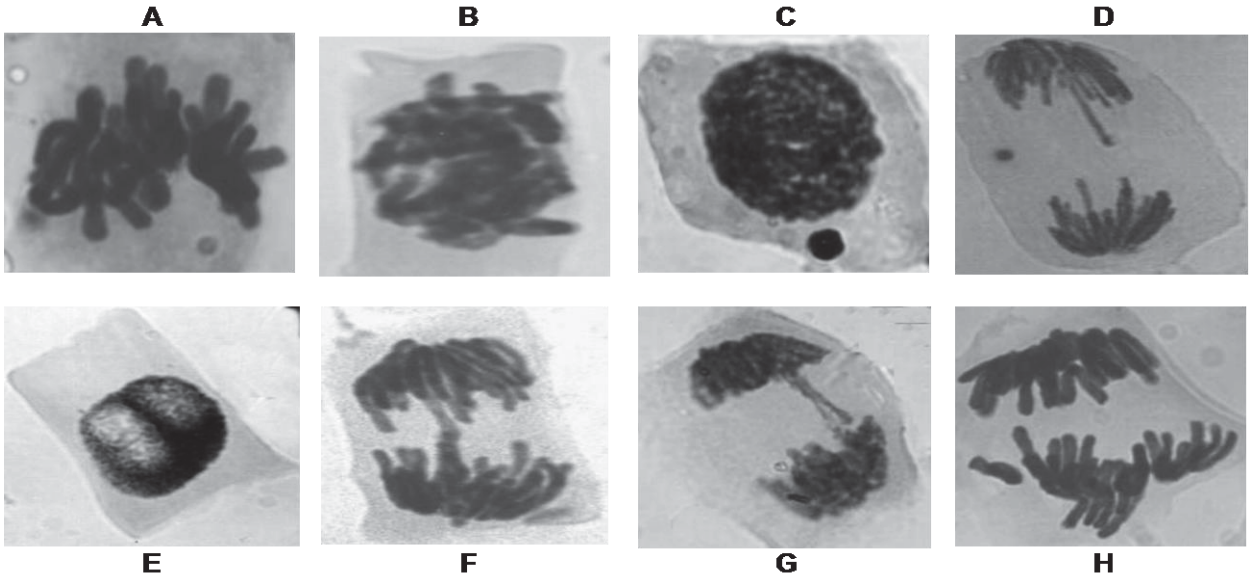

Plate 1. Types of chromosomal aberrations resulted from the treatment of *Allium cepa* seeds (fresh and carry over) with magnetic field, (a-b) stickiness, (c-d) micronucleus, (e) binucleolate nucleus, (f-g) bridge and (h) laggard chromosome.

field and has a significant importance for horticultural practice. Celestino et al. (2000) observed that germination was faster for seeds exposed to the magnetic field than those in the control group, and germination percentage increased. Alexander and Doijode (1995) reported that pre-germination treatment improved the germination and seedling vigor of low viability onion and rice seeds. In treated wheat seeds germination was raised from 45.3 to 49.3% (Ijaz et al, .2012). Flórez et al. (2007) observed an increase for initial growth stages and an early sprouting of rice and maize seeds exposed to 125 and 250 mT stationary magnetic fields. Magnetic field treatment of seeds leads to acceleration of plants growth, proteins biosynthesis and root development (Kordas, 2002). Carbonell et al. (2002, 2004). found that magnetic treatment produced a biostimulation of the germination. The activities of some enzymes were increased by exposure to magnetic field (Racuciu et al., 2008). Stress enzyme like APX and SOD increased in seedling which was grown from pretreated seeds, these stress enzymes have antioxidant mechanism they scavenge free radicals and decrease oxidative stress (Azita and Ahmad, 2009). Ahmad et al. (2010) showed that Mean germination time (MGT) significantly increased when the time of seed exposed at magnetic field treatments increased, about 3 and 2 h respectively for Omid and BCR wheat cultivars.

Regarding to seedling growth, our results clear that a positive effect on testing parameters seedling length, seedling dry weight, seedling vigor and seedling vigor index (Table 2). Similar results were obtained by Kubisz et al. (2012), they observed that clear differences in the length of radicals and fresh mass of seedling, longer radicals and bigger seedling than the ones from the control on the onion seeds exposed to magnetic field. Waleed et al. (2013) showed that root length, length of radical, dry weight of root and radical increased by 18, 12, 0.52 and 43%, respectively when exposed wheat seeds to (50 mT/30 min). Azita and Majd (2009) showed that the Lentil seedlings from seeds magnetically pretreated grew taller and heavier than untreated controls, they showed greatly improved root characteristics. Magnetic field treatment of seeds led to acceleration of plants' growth, protein biosynthesis and root development (Hirota et al., 1999; Pe-uelas et al., 2004; Amera and Hozayn, 2010a, b; Hozayn et al., 2014). Aladjaadjiyan (2005) concluded that magnetic field increased the shoot and root regeneration rate and their fresh weight in soybean and paulownia organ cultures. Azita and Ahmad (2009) suggested that pretreated plants by magnetic fields are more resistant against harmful environmental factors. Regarding cytogenetic parameters, magnetic treatment at low frequencies exerts significant increase the mitotic index in meristematic cells of *Allium cepa* and induces chromosomal aberration (Tables 3 and 4 and Plate 1). Similar results were recorded by Tkalec et al. (2009); they showed a significant increase of mitotic index in *A. cepa* roots after 900 MHz electromagnetic field exposure as well as mitotic abnormalities increased. Aksoy et al. (2010) found that mitotic index of *A. cepa* was significantly increased at 0, 10 and 25 m distance from magnetic field treatments compared with control, MI analysis showed significantly increased the cell division a dose dependent manner in *A. cepa* L. and in *Triticum baeoticum* Boiss. As well as they found differences in mitotic phases in both

plants. Marcano et al. (2004) considered mitotic index a parameter that allows one to estimate the frequency of cellular division. Root growth depends on mitotic activity and cell elongation (Evseeva et al., 2005); these processes could be influenced by magnetic field. Moreover, Marcano et al. (2004) considered mitotic index a parameter that allows one to estimate the frequency of cellular division. Root growth depends on mitotic activity and cell elongation (Evseeva et al., 2005); these processes could be influenced by magnetic field.

Aberration percentage in this study is found to be not lethal. The chromosomal aberrations resulting from magnetic exposure might be due to the direct interaction with moving electrons within DNA (Blank and Goodman, 1998). Eren et al. (2010) found various types of mitotic defects in MF-exposed lentil roots, such as stickiness, c-mitosis, micronuclei, double nuclei. Induction of chromosome aberrations, such as stickiness, has long been known to occur in response to many environmental agents, including chemical mutagens (Yumurtacı et al., 2007; Türkoğlu, 2009) and certain kinds of magnetic or electric fields (Rapley et al., 1988). Chromosomal aberrations (CA) occur due to lesions in both DNA and chromosomal spindle protein causing genetic damage (Amin, 2002), and may be induced by other factors, such as DNA breaks, inhibition of DNA synthesis and replication of altered DNA. Cell metabolism is sensitive to a range of nonspecific weak treatments that do not directly affect receptors or any other specialized cell structures (Racuciu, 2011). While Tkalec et al. (2009) found exposure of *A. cepa* to magnetic field under most of the test conditions induced a significant increase of mitotic activity in root tips of *A. cepa* compared with control. This study may proof that MFs altered rates of DNA, RNA, and protein synthesis as other previous studies (Goodman et al., 1993; Greene et al., 1993; Zhao et al., 1999). Magnetic fields affect the synthesis of DNA and RNA as well as the cellular proliferation. EMFs in both extremely low frequencies activate the cellular stress response, a protective mechanism that induces the expression of stress response genes (Ruediger, 2009). Consequently, MFs alters gene expression, protein biosynthesis, enzyme activity, cell reproduction and cellular metabolism (Nirmala and Rao, 1996). Studies on the meristematic cells of plants have shown that magnetic field effects normal metabolisms and has impact on cellular division (Belyavskaya et al., 1992; Dhawi et al., 2009).

Different studies evident that MFs of different intensities causes certain genotoxic effects in plants. During mitosis and meiosis both these fields are supposed to cause a number of chromosomal aberrations including stickiness, lagging chromosomes, micronuclei formation, bridges, multipolar division etc. (Racuciu et al., 2009; Aksoy et al., 2010; Zaidi et al., 2012; Zaidi et al., 2012), Induction of different types of chromosomal aberrations (Garcia-Secrado and Monteagudo, 1991; Nordenson et al., 1994). Stickiness may be due to degradation or polymerization of chromosomal DNA (Darlington and Mc-Leich, 1951) or may be due to defective functioning of one or two types of specific nonhistone proteins involving chromosome organization (Turkoglu, 2007). Some chromosomal aberrations induced by suitable MF exposure time of plant seeds may persist in the next generations so that some phenotypic characters may be modified (Racuciu, 2011) and these modifications could be observed following the plant development, some of them being benefit for the cultivation of this species (Attia et al., 2014). This way, the extremely low frequency MF could represent the molecular basis of a putative tool in the biotechnology of plant growth, an important species in the human life, with the advantages of being less toxic and most easy to manipulate in comparison to ionizing radiation for instance (Racuciu, 2011). Chromosomal aberrations that could cause delayed prophase and/or metaphase leading to an increased mitotic index (Evseeva et al., 2005).

Conclusion

The obtained results indicated that magnetic field exposure increased all germination characters compared with control. Exposed carry over and fresh onion seeds to 0.06 T with 30 mint gave the greatest values of germination percentage, germination rate, germination energy, speed germination index and seedling vigor (seedling length (cm), seedling dry weight (g). seedling vigor I and seedling vigor II). Whereas, Mean germination time was decreased. Using 0.03T with 60 mint gave maximum values in carry over seeds and with 60 min in new seeds. Significant increase in mitotic activity and chromosomal aberration after treatment with both doses for 30 min in fresh seeds and with 0.06 T in carry over seeds, while Relative division rate (RDR) gave positive values and little disturbance in mitotic phase index. However, the percentage of mitotic abnormalities increased after all exposure treatments. Hence, change in protein biosynthesis. The previous results indicate the change in cellular proliferation at low magnetic frequencies.

Conflict of Interest

The authors have not declared any conflict of interest.

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Review of patents on life cycle assessment

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Many tools are developed and used in order to evaluate the environmental impacts in products and services. One tool that is gaining prominence is the Life Cycle Assessment (LCA), which can analyze environmental aspects and impacts along the life cycle of product. However, not much is known about the patent generation through the use of LCA. In this sense, this paper aims to investigate LCA studies that generated patents through innovation in both products and processes. To reach the proposed objective, three patents basis data were used: WIPO, INPI and EPO. Eleven different patents requested involving LCA applications were found. The LCA and innovation generation need to be more explored, expanding the vision of organizations for this issue. LCA has a great potential to improve the environmental performance of products, processes and services and, at the same time, can assist in innovations generation and in technological development through patents requests.

Key words: Life Cycle Assessment (LCA), innovation, patents.

INTRODUCTION

Environmentally correct studies about the environmental performance are in evidence through awareness campaigns, and show that companies are increasingly treating and valuing environmental information combined with the life cycle of products to meet the demand of market consumers of products.

Thus, the life cycle of products has evolved in a context where demands for health and environmental requirements are raised by various stakeholders such as authorities, companies and social organizations, increasing demands for environmentally oriented products and targeting innovations through technological changes with the lowest possible environmental impact (Tidd et al., 2005; Azapagic, 1999).

In this context, several methods are being developed to assess the environmental impacts in the life cycle of products, processes or activities for the purpose of regulation and support in business decisions. One method that is gaining prominence is the Life Cycle Assessment (LCA) which has been used as a management tool in an integrated, systematic and multidisciplinary way, quantifying environmental loads and their potential impacts along the life cycle of a product, process or activity (Pieragostini et al., 2012;

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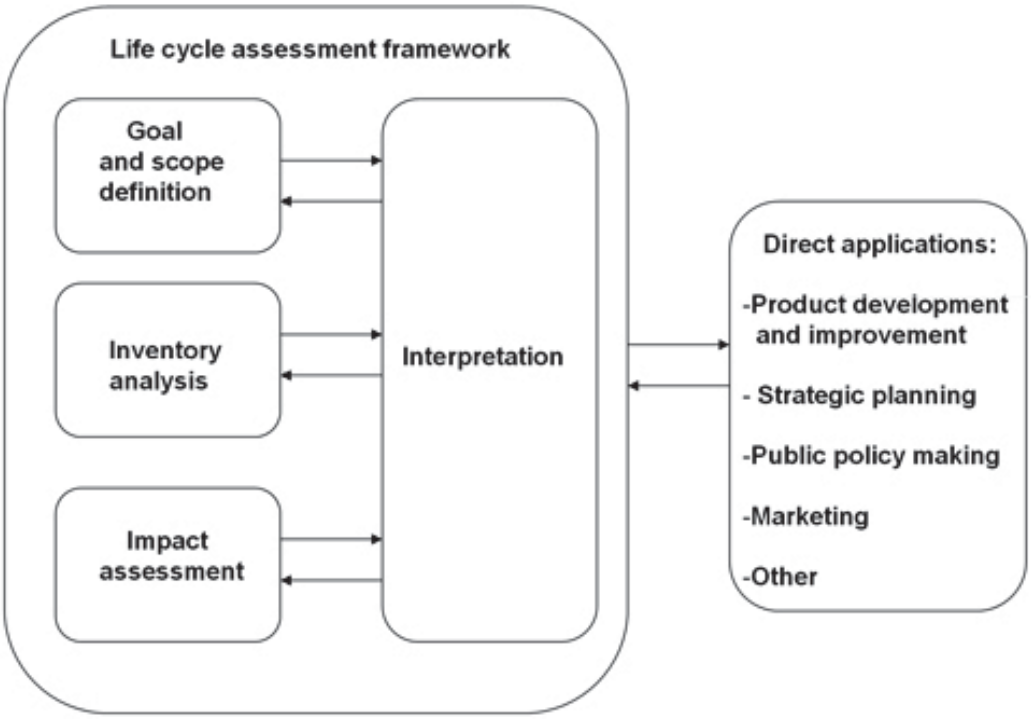

Figure 1. Phases of life cycle assessment. Source: Database of the ISO (2006a).

Wonglimpiyarat, 2005).

This indication resulting from LCA and the search for improvements in the production process enables that at the same time that companies seek to improve the environmental performance of its products this can result in technological innovations for the industry. Many authors complement their studies saying that LCA is a crucial tool for companies wishing to reach results of excellence and to facilitate green innovations.

Therefore, according to Oliveira et al. (2010), these new technologies can be identified through the analysis of patent applications in some fields.

In this context, the aim of this article was to conduct a survey of LCA studies that generated patents through innovation in both products and processes.

LIFE CYCLE ASSESSMENT (LCA)

LCA is a technical tool of analytical approach and managerial character which contributes to the evaluation of the environmental aspects and potential impacts associated with a product or activity during its life cycle (Garraín et al., 2010; Lofgre et al., 2011; Chauhan et al., 2011; Abdallah et al., 2012).

In general terms according to Lofgren et al. (2011), the aim in relation to the use of LCA is to satisfy customers orders and simultaneously achieve an ideal balance between the product and the environment. The methodology (LCA) has developed and matured over the last decades. Current activities about databases, quality assurance, consistency and harmonization of methods contributed to this.

The LCA methodology is internationally structured and standardized by the International Organization for Standardization (ISO), belonging to the series ISO 14040 and 14044 (ISO 2006a, b). Both are considered the main and most important standards for environmental assessment based on the life cycle of product.

The methodology consists of four phases: aim definition and scope, inventory analysis, impact assessment and interpretation. The iteration between these phases can be observed in Figure 1.

The first phase of the LCA comprises the definition of the objective and scope. The goal of an LCA study should unequivocally declare the intended application, the reasons for conducting the study and target audience ISO 14040 (2009a). The scope includes the cover of the study. According to Sherwani et al. (2010), Pieragostini et al. (2012), Zhou et al. (2011) and Roy et al. (2009), the system boundaries, the functional unit and the system limits should be stated clearly in the scope of the product system to be studied.

The analysis of the inventory, the second stage of LCA, involves an extensive database of materials involved in the product or system including the surveying, the collecting and analyzing data required for LCA (Jijakli, 2012; Olsen et al., 2001; Guinée et al., 2011).

The phase of impact assessment consists in studying the significance of environmental impacts from the inventory data. At the end of the impact assessment of the life cycle, the final result is an environmental profile of the product system under study, as defined in the aim and scope. These results are interpreted in the last phase of the methodological framework of life cycle assessment (Benetto and Klemes, 2009).

The last stage of evaluating the structure of the life cycle, comprises the interpretation. In this phase, the information from the inventory analysis and impact assessment system is evaluated, and conclusions in accordance with the objectives outlined in the first phase of the study are proposed (Benetto and Klemes, 2009; Jijakli, 2012; ISO 1440, 2009).

The authors Garraín et al. (2010), Lofgre et al. (2011) and Chauhan et al. (2011) add that life cycle analysis is a technical tool with analytical approach and managerial character, which contributes to the evaluation of the environmental aspects and potential impacts, associated with a product or activity during its life cycle, through the obtained data.

Life cycle assessment (LCA) as a tool for generating innovation

Nowadays, there is a strong pressure to innovate around the world. The innovation has been regarded by researchers as a strategic issue, a key for business success in an increasingly competitive environment as a result of intense technological development and the expectation of new products by consumers. This leads the companies to the need for constantly innovate.

According to Cramer and Tukker (2006), environmental demands are largely focused on innovation through adaptation of processes, products and services due to the regulations, considering that the best results are expected in relation to environmental aspects, at lower costs, thereby extending the range of answers to all stages of the life cycle.

For the process of technological innovation in organizations which results in sustainable products and processes, Figueirêdo et al. (2010) emphasizes that the availability and use of methodological tools which facilitate the consideration of environmental issues in every stage of this process is necessary.

In parallel with this, many methods are being developed to assess the environmental impacts of products for regulation and support in business decisions about the product improvement generating innovations (Krozer, 2008; Guziana, 2011). In this sense, one tool that is gaining prominence is the Life Cycle Assessment (LCA), which can provide fundamental technological shifts in production and in life cycle of products and services.

The LCA allows obtaining knowledge of the life cycle of the product as a whole, covering all stages of the product development process, including inputs and outputs in each process step. This makes possible to identify the unfavorable points of the product related to environmental aspects. Thus, the search for alternatives to improve the environmental performance of this product may result in innovations to the industry (ISO, 2009a; Lofgren et al., 2011).

According to Demuner et al. (2011) and Moen and Jorgensen (2010), the competitiveness of a company is related to its ability to research and implement innovative opportunities. Chiou et al. (2011), Tseng (2010) and Yung et al. (2011) reinforce that LCA is, in general, an innovative tool for industries.

The operationalization of the production system allied the innovations, which aim to meet the dimensions of sustainability and are considered imminent trends for organizations that want to remain competitive and consolidate good results. On this aspect, Luz (2011) states in his study, which evaluates the contribution of indicators obtained from the analysis of the life cycle on the generation of innovation in the industry, that the use of LCA may result in innovations and consequently improving competitiveness.

Corroborating with this thought, Kemp et al. (1999), Chiou et al. (2011), Tseng (2010) and Yung et al. (2011) emphasize that LCA is presented as a tool for assessing the environmental impact, able to recognize the multifaceted nature of environmental innovation; promoting improvements composed by the product innovation, process innovation and innovation in services. With the purposes of reducing the negative impacts and risks to the environment.

In this aspect, Madival et al. (2009), Rousset et al. (2011), Rodríguez et al. (2011), Li et al. (2010) and Abdallah et al. (2012) highlight the concerning of the LCA application in industry in which it is possible to observe the generation of technological changes both in product, process and service. These innovations may result in patents through the use of this methodology.

Study of patents related to LCA

A patent is an exclusive right granted for an invention, which is a product or a process that provides, in general, a new way of doing something, or offers a new technical solution to a problem (WIPO, 2013).

The patents provide incentives to individuals by offering them recognition for their creativity and material reward for their marketable inventions. These incentives encourage innovation, which assures that the quality of human life is continuously enhanced.

According to the World Intellectual Property Organization (WIPO), the number of patent applications has grown around 1.5 million each year, resulting in more than 500.000 patents granted. Companies in the United

States, Japan and Europe are increasingly using this instrument as strategic input.

METHODOLOGY

In order to verify if LCA studies generate patents through its innovations, a research at the patents basis “WIPO” - Word Intellectual Property Organization was carried out. The option to use the WIPO was due to the fact that it is a broad tool of patents research, whose mission is to promote innovation and creativity for economic, social and cultural development of all countries through a balanced and effective intellectual (patents, copyrights, trademarks, designs, etc.) international property. Searches were also conducted at INPI - National Institute of Industrial Property (INPI), which is responsible for the improvement, dissemination and management of the Brazilian system of granting and guarantee of intellectual property rights for industry and EPO - European Patents Office, which contains all information publicly available on European patent applications. The study was conducted in March 2013 and the keyword searched was "life cycle assessment (LCA)."

This analysis involved the acquisition of information, such as the nationality of the applicant, the nature of the application, the priority date and associated international patent classifications, as well as the title of technology. This research aimed to map the information protected in Brazil and consequently analyzing its importance on a global scale.

RESULTS AND DISCUSSION

Among the three basis of patents surveyed only at the base, WIPO eleven patent requests related to the topic ACV were found.

The first patent request occurred in 2001 in Japan, with the title: "Method for using a feature analysis of two-dimensional energy sources expressed by an influence of the life cycle of the environment and life cycle and costs of a system", whose aim was to provide information about the influences of energy sources in an environment, through analyzing the influence of environmental life cycle costs and life cycle through the LCA.

In the following year, Japan entered with two new patent requests: "Evaluation system of environmental effects of products and services"; a system that is able to objectively evaluate the recycling which is susceptible to subjectivity in an LCA, and to show quantitatively the total environmental load from products and services. The other patent application, "Process Evaluation System", aims to calculate and assess the value of environmental load of the production process based on the content of the production process and Life Cycle Assessment - LCA, through CAD and CAM system. The application was required by Fuji Electric Co Ltd. The same company has filled its second patent application in 2005, "Life Cycle Evaluation System"; this system aims to quickly modify the design in order to make the life cycle longer through the CAD system and LCA.

Later in 2005, Japan held two more patent applications: "Water in oil, aerosol type product", in order to provide an emulsion that is preferable from the point of view of life cycle assessment (LCA), required by the company Daizo: KK and "System and Method for Evaluation of the Life Cycle" which allows provision of an LCA system capable of simultaneously evaluating the environmental impact of the life cycle of the product and energy consumption of the facilities, referring to the company Hitachi Ltd.

The Republic of Korea requested this year, its first patent application: "Method for assessment of the integration for eco-design of an automobile through an integrated index, including recycling". This method evaluates the recycling (via LCA data and disassembly) of the automobile, and analyzes a weak point in order to increase the recycling of a design step from a concept of concurrent engineering, as the Hyundai Motor Company conducted the application.

In 2007, the company Nippon Oil Corp., installed in Japan, held the patent application: "Method for production of hydrogen to produce reformed gasoline and for production of aromatic hydrocarbons" aiming to provide a method to produce hydrogen, sufficiently effective to reduce LCA CO_2.

In 2009, the company Hitachi Ltd., made its second patent application: "System support to the project", in which an environmental assessment and system design support are provided and a database is obtained by the Association of Product Classification Information, in order to solve some problems of LCA methodology.

In 2010, the first patent application was required via PCT (Cooperation Treaty Patent) comprising a multilateral treaty which allows requirement of patent protection for an invention simultaneously in many countries through the filling of a single international patent application. In this sense, the patent entitled "System support for the project through sustainable project" aims to create an environment of social participation based on integrated software with the design of sustainable products and tools from LCA and processes.

In 2012, the Republic of Korea held its second patent application, by the Research Foundation and Business Sungkyunkwan University, called: "Method for the use of a feature of two-dimensional energy sources analysis expressed by an influence of the environment life cycle and the costs of a system life cycle", capable of providing information about the influences of energy sources in an environment, analyzing the influence through the environmental life cycle and life cycle costs through the LCA.

Table 1 illustrates the information described above, relating the year, the patent classification, the applicant company and the country.

According to the INPI - National Institute for Intellectual Property, the patents have different classifications. Hereafter the classification of patent applications found are described:

- The class G06F covers equipment or methods of digital

Table 1. Relationship title, purpose, classification, applicant and the year of filing. Source: Own Authors (2013).

Title	Purpose	Classification	Applicant / Inventor	Year
Method and device for evaluating environmental influence by evaluating the life cycle.	Evaluate the loads through databases with one of its key elements an inventory to calculate and evaluate the consumption of resources and energy.	G06F	National Institute of Advanced Industrial Science and Technology, Ministry of Economy, Trade and Industry (METI)	2001
Evaluation system of environmental effects of products and services.	Objectively evaluate recycling in an LCA and quantitatively show the total environmental load of products and services.	G06Q	Kankyo Technos KK	2002
Evaluation system of processes.	Calculate and evaluate the environmental load value of manufacturing process based on LCA through CAD and CAM.	G05B	Fuji Electric Co Ltd.	2002
Life cycle evaluation system .	Fast modification project by LCA.	G06F	Fuji Electric Co Ltd.	2005
Water in oil, aerosol product.	Develop a sustainable product from the point of view of ACV.	B65D	Daizo: KK.	2005
System and Life Cycle Assessment method.	LCA applied to the product and the energy consumption of facilities.	G05B	Hitachi Ltd.	2005
Evaluation method of integration for the eco-design of an automobile by an integrated index including recycling.	The objective is to evaluate the car recycling through LCA data and disassemblying.	G05F	Hyundai Motor Company	2005
Hydrogen production method for producing reformed gasoline and producing aromatic hydrocarbons.	This new method allows to innovate the hydrogen production process, effective enough to reduce the environmental impacts by evaluating the life cycle (LCA).	C01B	Nippon Oil Corp	2007
Support system for the project.	An environmental assessment through LCA and design support system are provided in which a database is obtained by associating the product rating information.	G06F	Hitachi Ltd.	2009
Support system for the project through sustainable design.	Create an environment of social participation based on an integrated software to design sustainable products, LCA tools and processes.	G06F	Sustainable Minds llc	2010
A method for analyzing the use of energy sources two-dimensionally expressed by an influence of the environment life cycle and the life cycle costs of a system.	Provide information on the influence of energy sources in an environment, analyzing the influence by environmental life cycle and the life cycle costs through LCA.	G06Q	Research and Business Foundation, Sungkyunkwan University	2012
Method and system to assess the environmental impact of a processing device.	Develop a method and system for evaluating the environmental impact of process equipment item via ACV.	G06G	Ebner Fritz F	2013
Data Management for Life Cycle Assessment (LCA).	Provide inventory data life cycle (ICV) via a secure structure for a data center.	G06F	Huizenga Neil Gregory	2013

computing or data processing, specially adapted for specific applications;
- The class G06Q comprises systems or data processing methods, specially adapted for administrative, commercial, financial, management, supervision or prediction purposes; systems or methods specially adapted for administrative, commercial, financial, management, supervision or prediction purposes, not otherwise provided for;
- The class G05B comprises the control and regulation. This class includes methods, systems, and devices for control in general;
- The class C01B includes nonmetallic elements and their compounds;
- The class B65D covers containers for storage or transport of articles or materials.

Japan stands out among the patent applications analyzed in this study, due to the rate of technological development and research of the country. Thus, Japan has demonstrated its concern for sustainability, seeking innovation through ways that enable and feature the minimization of environmental impacts caused by developed products, processes and services. Moreover, Demuner et al. (2011) justifies a strong interest of Japanese companies to invest and

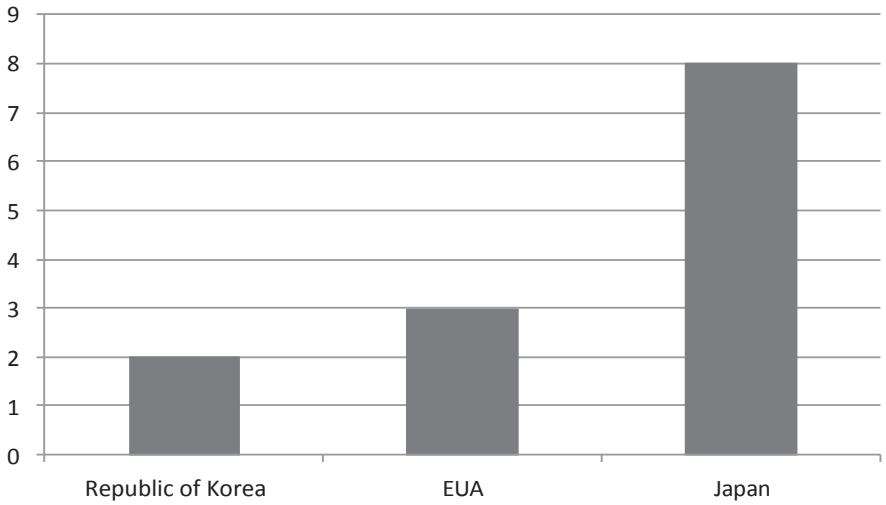

Figure 2. Value of countries and number of patent applications. Source: Database of the WIPO (2013).

develop its own technology. Sánchez et al. (2011) provided evidence that the best performing companies have an innovative behavior. Following Japan is the United States of America especially by applying for a patent via PCT and lastly is the Republic of Korea.

Among the pending applications for patents, only one is linked to a university. The industries represent the remainder of patent applications belonging to different sectors: automotive, electronics, energy, software, petrochemicals, gas and coal. These industries seek the commitment to innovation and environmental sustainability.

Figure 2 illustrates the relationship of the countries with the number of patent applications, highlighting the patents ratings required by Japan. As highlighted in Figure 2, the largest number of patent applications is represented in Japan whose focus is information technology applications related to software integrated with the design of sustainable products through LCA tools and systems support projects.

The Graph 1 illustrates the amount of patent applications between 2001 and 2013. You can see that the first application for patent related LCA occurred in 2001, with a significant increase by the year 2005, which had the highest number of patent applications. This year there was a decline in the requests, returning to growth only in 2013.

Conclusions

This article was developed with the intention of checking LCA studies which enabled the generation of innovations through patents generated by the application of this methodology.

It was noticed that the LCA has great potential to assist in the generation of innovation and patents, but this issue needs to be explored further, expanding the vision of organizations for that matter, since, in a study of LCA, there are generated indicators that enable a vision system as a whole; generating information which can be used to guide the actions and decisions of the companies. To enable us evaluate the implementation of generation of innovation in order to promote significant improvements aiming to enhance or reduce its impact in the environment and still be useful for the strategic planning of the company allowing the identification of innovation opportunities that had not yet been exploited.

Therefore, companies should incorporate monitoring systems and signs of change through LCA studies. Where, at the same time as they seek to produce in sustainable way, companies can use the LCA strategically and become more competitive and innovative in the market.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Natural seed germination and seedling dynamics in cultivated population of *Cassia fistula* Linn. in Nigeria

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For the first time, natural seed germination in cultivated population of *Cassia fistula* Linn. is reported in this paper. Natural seed germination occurred under many mother trees at Covenant University, Ota, Ogun State, Nigeria; while no germination occurred under some other mother trees. Random soil samples were taken in representative areas and analysed. Seedling dynamics were also investigated by fencing the seedlings around five mother trees, mapping and following their survival with time. Soil samples obtained where there was germination are loose, black soils with high moisture and humus contents and mean pH of 7.2 while soil samples obtained where there was no germination are hard, red lateritic clay soils with significantly lower moisture and humus contents and mean pH of 6.4. The seedlings study showed that some seedlings were more or less in a straight line from broken and rotted pods buried in the soil or covered in debris. Other seedlings were single, in pairs or in groups of three or four. A seed germinated within a pod and grew out of a small hole in the pod, with the radicle coming out first and by the next day, the cotyledons emerged. In the first few weeks of study, the number of seedlings in each fenced area increased with time, thereafter the number decreased accompanied by yellowing of leaves. There was also increased growth of surrounding weeds mainly grasses, as well as about 60% reduction in light intensity on the seedling floor due to the development of new foliage on the mother trees. The reduced number of seedlings under the mother trees was attributed to increased inter specific competition as well as reduced light intensity reaching the seedlings since *C. fistula* is not shade tolerant. Consequently, cultivated *C. fistula* like the natural population may not regenerate well in nature and its continued existence may depend on artificial conservation through its use as ornamental and medicinal plant.

Key words: Natural germination, seedling dynamics, cultivated population, *Cassia fistula,* Nigeria.

INTRODUCTION

Cassia fistula Linn. Is an ornamental and medicinal deciduous tree commonly called “golden shower tree” and “Indian Laburnum”. It is of the large genus Cassia and it belongs to the subfamily Caesalpiniaceae and the family Fabaceae (Sartorelli et al., 2009). Its origin is in the Indian subcontinent and is distributed in various tropical regions including Asia, South America, Australia and Africa (Orwa et al., 2009). It is widely used for landscaping

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not only because of its golden yellow flowers which last for several months, but it also has landscaping properties like drought tolerance and low maintenance requirement (Ghouse et al., 1980). As a result of these, *C. fistula* is the dominant landscaping species at Covenant University, Ota, Ogun State, Nigeria. It flowers nearly all year round (August – June) and produces enormous amounts of seeds that do not germinate readily in nature. There are many publications about the species from the tropical and subtropical regions (Nalawadi et al. 1977; Babely and Kandya 1988; Todaria and Negim 1992; Aref, 2002; Karaboon et al. 2005; Al-Menaie et al., 2010) but virtually none from sub-Saharan Africa. The species thus became our research plant to provide some data from this region.

Seed dormancy in *Cassia fistula* Linn. is a widely investigated and well documented aspect of the species from virtually all tropical regions; namely Nalawadi et al. (1977) in India; Babely and Kandya (1988) in Egypt; Karaboon et al. (2005) in Thailand; Al-Menaie et al. (2010) in Kuwait and only recently Babalola et al. (2014) in Nigeria. Several experiments have been carried out to break the dormancy of the seeds in view of the fact that the species has many uses, in particular as an ornamental and medicinal plant. The cheapest and probably the most efficient method to break seed dormancy in the species is by immersion of the fresh seeds in hot water (80 to 100°C) for about 2 to 5 min or in concentrated sulphuric acid for about 5 min (Babely and Kandya, 1988; Karaboon et al., 2005; Babalola et al., 2014).

Even though many germination experiments in the laboratory have been reported, there are only few reports about the regeneration of *C. fistula* in nature (Pokhriyal et al., 2010; Ballabha et al., 2013). There is virtually no report of natural germination (regeneration) in cultivated population of *C. fistula.* It was therefore of scientific interest to investigate natural seed germination (regeneration) if any, in cultivated population of *C. fistula.* This paper reports the investigation of natural seed germination of the species under cultivated mother trees at Covenant University, Ota, Ogun State, Nigeria, where the species is the dominant ornamental plant; about 250 individuals having been planted since the founding of the University in 2002. In addition, the dynamics of five different seedling populations under five mother trees were monitored to determine their survival with time. Analysis of soil samples taken under the five mother trees where there was regeneration and under another five trees where there was no regeneration was carried out to determine the possible reasons for the situation.

MATERIALS AND METHODS

Study site

The cultivated population of *C. fistula* is situated at Covenant University, Ota, Ogun State, SW Nigeria. Ota lies at longitude 03.20E and latitude 07.45N. Mean annual rainfall is about 1370 mm falling for about ten months in the year (February – November). Mean maximum temperature is 32°C and mean minimum temperature is 27°C. The University is on a flat plain on an elevation of 24 m above sea level. The original vegetation was tropical rain forest and there are still relics of tropical rain forest trees like *Ceiba petandria, Tetracarpidium conophorum* and *Chlorophora excelsa* on the University campus. All the 244 flowering cultivated *C. fistula* trees along the major roads on the University campus were numbered and tagged. Seedlings at all stages of development were carefully checked for, underneath the canopy of each tree.

Seedling dynamics

Areas under 5 mother trees, numbers 3, 4, 7, 196 and 213, with germinated seedlings were fenced off with wire net to prevent any anthropogenic activities. Each area was about 60 m^2. The seedlings were then counted and mapped; the growth and survival of the seedlings were followed over time. Every two weeks, the five selected tree sites were visited to map new seedlings as well as record the survival and morphology of the old seedlings. These observations were carried out for five months, May to October 2014.

Soil analysis

Three random soil samples were taken from under each of the five trees where regeneration occurred and had been fenced off for seedling dynamics study (trees number 3, 4, 7, 196 and 213). In addition, under each of five other trees where there was no regeneration (trees number 78, 198, 199, 234 and 235), three random soil samples were also taken. This was to determine what might be responsible for lack of regeneration. The three soil samples taken under each tree were bulked for analysis and the following soil characteristics were determined (i) colour (ii) type (iii) pH (iv) moisture content and (v) humus content.

RESULTS AND DISCUSSION

Of the 244 trees investigated, there was seedling regeneration under 190 trees. Forty trees could not regenerate because their surroundings were paved with concrete as walk-way. Only ten trees actually did not regenerate. Under many trees that regenerated, it was noticed that pods had been cracked open or rotted under the mother trees. Some seedlings were just sprouting from the ground with yellow cotyledons only (Figure 1a). On closer inspection, many more seedlings with 2, 4 or 6 paired green leaflets were discovered (Figure 1b) indicating that they had germinated sometime earlier before our inspection. This indicated that even though *C. fistula* seeds show dormancy, yet, they are capable of natural germination (regeneration), a condition never before reported in cultivated population of *C. fistula.*

Most of the seeds that germinated were under debris or buried in loose, dark and wet humus soil with high moisture content (Table 1 and Figure 1a) which ensured that they had water which is essential for germination. A high percentage of the seedlings was noted to be growing in small soil depressions which may contain

Figure 1. (a) Three young seedlings (I – III) of *C. fistula* just sprouting from the broken pods, (b) A group of four seedlings at the two or three paired leaves stage.

Table 1. Analysis of soil samples taken from cultivated *C. fistula* population at Covenant University, Ota, Nigeria.

	Tree number	Colour	Type	pH	Moisture content (%)	Humus content (%)
A	3	Grey	Silt	6.99 ± 0.4	16.85 ± 0.11	25.04 ± 0.24
	4	Black	Humic	7.07 ± 0.2	15.93 ± 0.20	18.09 ± 0.17
	129	Black	Humic	7.38 ± 0.5	22.53 ± 0.17	20.76 ± 0.66
	199	Greyish Black	Silt	7.11 ± 0.2	17.45 ± 0.12	22.74 ± 0.76
	200	Black	Humic	7.25 ± 0.3	18.21 ± 0.23	24.17 ± 0.88
Mean				7.16 ± 0.2	18.19 ± 2.56	22.16 ± 2.79
B	79	Reddish brown	Lateritic	6.14 ± 0.2	10.27 ± 0.14	12.56 ± 0.27
	198	Reddish	Clayey	6.53 ± 0.3	7.88 ± 0.11	11.38 ± 0.18
	199	Reddish	Lateritic	6.88 ± 0.1	8.05 ± 0.23	10.96 ± 0.23
	234	Reddish	Lateritic	6.96 ± 0.2	8.21 ± 0.10	12.95 ± 0.60
	235	Brown	Clayey	5.88 ± 0.2	7.53 ± 0.31	13.44 ± 0.08
Mean				6.48 ± 0.5	8.39 ± 1.08	12.26 ± 1.05

A: Soil samples under trees with seedlings regeneration, B: Soil samples under trees without seedlings regeneration.

relatively more moisture than the surrounding. Air and temperature were not limiting factors. Thus the seeds had the conditions necessary for germination (Mayer and Poljakoff-Mayber, 1982)

In some cases, the seedlings were in more or less a straight line showing their positions when they were seeds in the pods. The seedlings in a straight line were nearly at the same developmental stage indicating that the seeds germinated about the same time. Obviously, the seeds must have been scarified by some physical factors. These may include scarification by the broken pod fragments and/or soil particles (Brown, 1972) or the rupturing of the hard seed coat by continuous wet condition of the soil during the rainy season (Brown, 1972; Villiers, 1972). An interesting and unusual germination was observed in one instance; the radicle of a germinating seed was found protruding from a hole in a pod and bending towards the soil (Figure 2a). The next day, the cotyledons grew out of the hole and the seedling stood erect in the soil (Figure 2b). This confirmed that seeds of cultivated *C. fistula* can and do germinate in nature. It also showed that the pods must be broken or decayed to allow the seeds access to germination factors and before the seedlings could emerge. It is also clear

(A) (B)

Figure 2. (a) The radicle of a seedling protruding from a hole in the pod of *C. fistula* and just touching the soil. To the left of the seedling is the big black ant and to the right is the hole in the pod, (b) The seedling fully emerged from the hole in the pod. On the pod is the hole through which the seedling emerged.

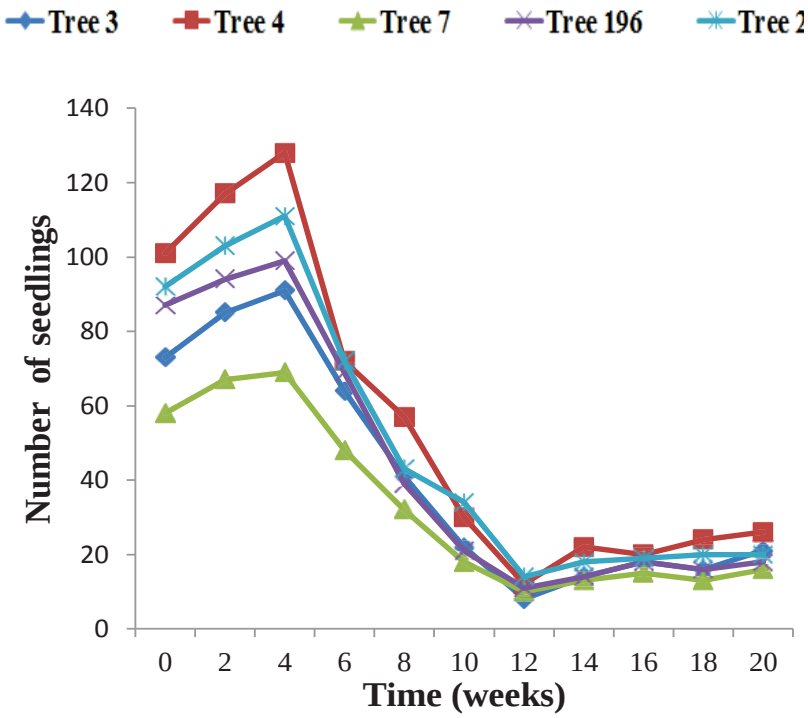

Figure 3. Seedling dynamics of *C. fistula* under five mother trees in Covenant University, Ota, Nigeria.

that the pods must somehow delay seed germination, probably to a more favourable time, until they break or decay. The hole in the pod was thought to have been made by the big black ants *Polyrhachis vicina* Roger (Figure 2a) which are very common in the area. An experiment is already in progress to determine how long it takes for pods to degrade or rot to a level that can allow seeds to germinate and grow out of the pods. The result will be related to the length of time it takes for the seeds to lose viability (Babalola et al., 2014).

Figure 3 showed that in all the investigated five sites with seedlings, there was increased number of seedlings in the first month, most likely due to the wet weather in May-June which aided more germination. Afterwards,

there was reduced number of seedlings culminating in the lowest values during the dry August break in rainfall (week 12, Figure 3). Thereafter, there was a slight increase in seedling number which more or less levelled off. Just as the seedlings were growing and more seeds were germinating, surrounding weeds, mainly grasses, were also flourishing. Consequently, there was increased competition all around. At the same time, the crowns of the mother plants became denser as a result of the development and growth of new foliage, thus the amount of light reaching the ground floor and the seedlings was severely reduced. Mean light intensity in the open was 20860 watts while on the seedling floor it was 8178 watts, showing a reduction of about 60%. Yellowing of the seedling leaves was also observed before the seedlings died.

Aref (2002) reported that reduced light intensity has a deleterious effect on seed germination and seedling growth of *C. fistula*. Alvarez-Buylla and Martinez-Ramous (1992) indicated that reduced light was one of the factors that led to the mortality of young plants. Similarly, Seiwa (1998) reported abrupt decrease in *Acer mono* in understory sites, but continued increase in gaps and forest edge. Thus the drastic reduction in the seedling population of *C. fistula* with time under the mother trees could be as a result of increased inter specific competition as well as reduced light intensity. The seedling regeneration may be explained by the differences between the soil samples; the loose, dark humus soil with high moisture and organic contents was found under the mother trees where there was regeneration and the hard, red lateritic soil with low moisture and organic contents was under the trees which had no regeneration (Table 1). It is easier for the pods to be buried and decayed in the moist, humus soil than to do so in the hard, less moist red lateritic soil. Humus binds soil particles and holds soil moisture (Nalawadi et al., 1977). The low soil moisture in the red lateritic soils may militate against seed germination and establishment. The similar soil pH values (Table 1) may not have much effect on regeneration.

If the sampling for seedlings had been carried out only in May/June, *C. fistula* could have been classified as having good regeneration (Pokhriyal et al., 2010); but this could have been otherwise if sampling was done in October (Ballabha et al., 2013). Thus, sampling should be carried out over a period of time by researchers in other to correctly determine the quality of regeneration of a tree. Nonetheless, this investigation has clearly shown that like naturally growing population of *C. fistula,* cultivated population may regenerate under suitable conditions. While the regeneration of *C. fistula* in nature is either to replace old dying mother trees or to provide resources, it is not clear what the use of regenerated seedlings in cultivated population would be as they may eventually be shaded out by the mother tree.

Observations over several years need to be carried out with the regenerated seedlings. On the other hand, layering from the roots of the mother trees of *C. fistula* has been observed, as seen in the cultivated population at Covenant University, to be very successful in providing new, strong and vigorously growing shoots along with the mother trees.

A species like *C. fistula* that has been cultivated and nurtured over many generations may have lost its competitive ability. Thus, even as *C. fistula* seeds are able to germinate in nature, the seedlings may not be able to survive to sapling stage and reach maturity on their own in nature (Ballabha et al., 2013) due to their poor survival with the low light intensity prevailing under the mother trees (Aref, 2002). Thus the perpetuity of *C. fistula* may depend on its use as ornamental and medicinal plant or on the occasional dispersal of the seeds to more favourable open spaces. Consequently, since *C. fistula* may not perpetuate itself well in nature, it appears that artificial regeneration by cultivation may be the major way to conserve the species.

Conflict of Interest

The authors have not declared any conflict of interest.

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Review

Seasonality and management of stone weevil, *Aubeus himalayanus* Voss (Curculionidae: Coleoptera): An emerging pest of Indian Jujube (*Ziziphus mauritiana* L.)

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The ber (*Ziziphus mauritiana* Lamarck) is an important fruit crop grown in arid and semi arid regions of India and elsewhere. Nutritionally, the fruits are rich in protein, phosphorus, calcium, carotene and vitamin C. In India, productivity tends to be low because of various biotic stresses such as pests and diseases. Of the various insect pests infesting ber, the stone weevil *Aubeus himalayanus* Voss (Coleoptera: Curculionidae) is a new threat to ber cultivation in India. This was recorded as a new pest of ber in India for the first time in the state of Andhra Pradesh in 1994. Serious attacks were later reported in Rahuri, Maharashtra and Jobner, in 1996, and in Karnataka during 1998. Recently, infestations of this pest were reported in Bikaner and Rajasthan in 2010 and in Bangladesh in 2009. The females lay their eggs on the stylar end of fruits, or rarely on the distal end. The newly emerged white coloured grubs enter the seeds by making a puncture in the endocarp of the immature fruits, and starts feeding on the soft seed coat. Later it enters the endosperm, feeds on it, and pupates within the seed. The weevil completes its life within a single fruit. The infestation results in severe fruit drop at the initial stage of fruit set. The damage intensity of the stone weevil varies with cultivars; stoned varieties are preferred more than the pulp rich varieties. Periodical monitoring of orchards for weevil incidence is essential for timely implementation of control strategies. Delayed detection makes management difficult as they enter the seed.

Key words: Ber, *Ziziphus mauritiana*, stone weevil, *Aubeus himalayanus.*

INTRODUCTION

The ber (*Ziziphus mauritiana* Lamarck) also called desert apple, jujube, Chinese apple, Badari (Sanskrit), Kul or Boroi, Ber (Hindi), Dongs, Bor, Beri, Indian plum, Permseret (Anguilla) is a tropical fruit tree species, belonging to the family Rhamnaceae (Balikai, 2013). In India it occupies a large area (22,000 ha) and it is popular dry land fruit crop for arid and semi-arid region of India (Jamandar et al., 2009) and most of the cultivated areas are confined to the states of Rajasthan, Haryana, Punjab, Gujarat, Maharashtra and Uttar Pradesh in India. To some extent its cultivation is also done in the states of Tamil Nadu, Andhra Pradesh, Karnataka, Bihar, Chhattisgarh, Madhya Pradesh, Assam and West Bengal. The fruits are quite nutritious, rich in vitamin C,

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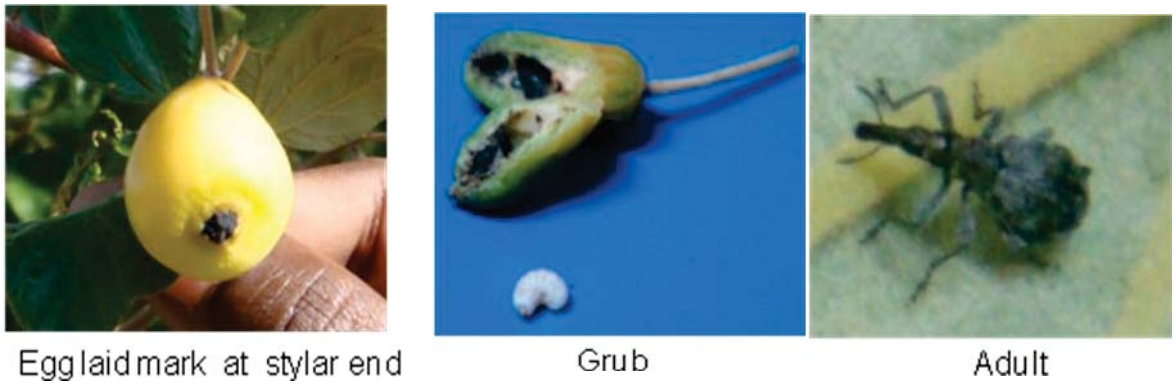

Figure 1. Damage symptoms of stone weevil, *Aubeus himalayanus.*

second only to aonla and guava, and much higher than citrus and apple (Khera and Singh, 1976). The crop is gaining popularity among the growers because of its adaptability to adverse climatic conditions and good returns. However, the crop suffers great losses due to insect pests and diseases (Singh, 2008). The introduction of improved varieties and irrigation led to unpredictable changes in insect pests in this crop. In India, more than 130 species of insect pests were found to attack ber (Lakra and Bhatti, 1985), but very few reach the pest status. Jothi and Tandon (1995) reported 17 insect species, and Balikai (1998) reported a total of 22 insect and non-insect species in Karnataka. Likewise, Kavitha and Savithri (2002) documented about 23 insect species on ber from the state of Andhra Pradesh. However, pests such as the fruit fly, *Carpomyia vesuviana* Costa, chafer beetles, *Holotrichia consanguinea* Blanch and bark eating caterpillars, *Indarbela tetraonis* Moore; *Indarbela quadrinotata* Walker, and the ber butterfly, *Tarucus theophrastus* Fabricius are the major pests of ber in India, causing significant yield losses (Sharma and Bal, 2009; Karuppaiah et al., 2010).

In addition to these, the ber stone weevil, *Aubeus himalayanus* Voss (Coleoptera: Curculionidae) appears to be an emerging pest that has been reported from various regions of India (Balikai et al., 1998; Karuppaiah et al., 2010; Balikai et al., 2013). The pest is an emerging threat for ber production in India, especially in the northern region. However, in India available studies are very few and there is need to investigate the biology and management of this pest. In this study we reviewed the available information about this emerging pest, in order to present the data available to begin developing control strategies against this hardy insect in the near future.

PEST DESCRIPTION AND DISTRIBUTION

The adult weevils are small, dark in colour, and with a snout. The grubs are white in colour with red colour marking on the body, c-shaped with brown coloured head capsule and are found inside the seeds (Balikai et al., 2009; Karuppaiah et al., 2010, 2011). The activity of the adults could be observed in the field during the morning and evening hours. The adult female weevil lays their eggs mostly on the stylar end; rarely on the distal end of fruits, and then covers the punctures with a brown encrustation. Upon hatching, the grubs enter the seed by through the endocarp of the immature fruit, and starts feeding on the soft seed coating. Later it enters the endosperm moving downward. After entering the seed, it starts feeding on the inner content of the seed, and pupates within the seed in hollow galleries, which has at this stage been completely eaten away. The weevil completes its life within a single fruit (Karuppaiah et al., 2010) (Figure 1). At the time of fruit maturity, infested fruits had a grub, a pupa or an adult. The infestation occurs in all the fruit stages; however it is prevalent in pea to pebble-size fruits. The attacked fruit had an abnormally enlarged calyx, and nearly half of the fruit towards the petiole turned reddish brown, with a rough surface. The remaining half portion towards the navel region remained greenish in colour. The infested fruits fail to attain full maturity and never increase in size more than pebble. This is because although the pest fed only on the seed portion of the developing fruit, it arrested any further development of the attacked fruit. The entry hole was healed up and closed while the exit hole was clearly seen (Balikai et al., 2009). The biology of this pest has not been studied so far.

Severe infestations of *A. himalayanus* have been reported from all major ber growing regions of India (Figure 2). It was recorded as a new pest of ber for the first time from the state of Andhra Pradesh in India (Gour and Sriramulu, 1994). Later, in Rahuri of Maharashtra and Jobner, Rajasthan during 1996 (Pareek and Nath, 1996), Karnataka (Balikai et al., 1998), and recently in Bikaner, district of Rajasthan (Karuppaiah et al., 2010, 2012). Severe damage of this pest at early stages of fruit development has also been reported from Bangladesh

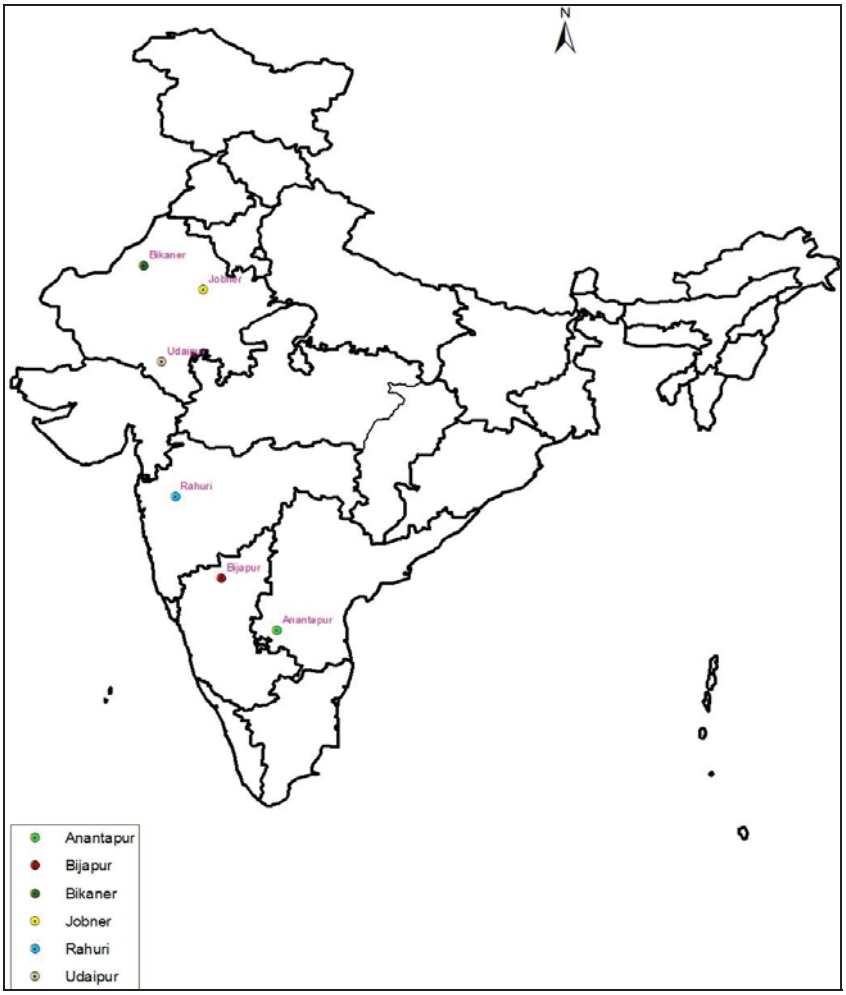

Figure 2. Distribution map for stone weevil in India.

(Qumruzzaman et al., 2009) (Table 1).

SEASONALITY

The activity of the weevil starts in the month of September and adult female lays their eggs from the blooming stage onwards. Severe damage could be observed during October, which is the fruiting month. The incidence could be noticed till the end of fruiting (October to February). In western Rajasthan, India, the pest was initially recorded during the first fortnight of October on cv Seb, and the second fortnight of October on cv Gola. The greatest infestation was registered during the first fortnight of December (Figures 3 and 4). Immature fruit drop was most pronounced during the second fortnight of November. The damage was severe in early variety cv Seb (Karuppaiah et al., 2010). The damage was noticed during July to August in Karnataka, and maximum damage was observed during July to August. Irrespective of varieties, the incidence was recorded during the months of July to November (Balikai, 2009). The extent of damage also found to be associated with the pruning dates. Severe infestation of stone weevil among early pruned ber cultivar Umran were reported by Biradar et al. (2001). The intra tree distribution pattern of stone weevil incidence revealed non significant difference with different directions of the plant; however the incidence in different strata of the plant showed significant differences. The branches which are examined just above the ground (0-1 m), showed maximum (33%) infestation as compared to the branches at 1-2 m and above 2 m high from the ground. The branches near the ground might be suitable for adults, emerging from residues or surviving population in the tree. Generally infestation decreased from lower branches to top branches (Karuppaiah et al., 2010).

VARIETAL PREFERENCE

The influence of biophysical factors of ber fruits plays a

Table 1. Details of stone weevil incidence reported from different regions of India and Bangladesh.

S/N	Location	References
	India	
1	Anantpur (Andhra Pradesh)	Gour and Sriramulu (1994)
2	Rahuri (Maharasthra)	Pareek and Nath (1996)
3	Bijapur (Karnataka)	Balikai et al. (1998)
4	Jobner (Rajasthan)	Pareek and Nath (1996)
5	Udaipur (Rajasthan)	Srivastava and Nanda (1983)
6	Bikaner, (Rajasthan)	Karuppaiah et al. (2010)
7	Bangladesh	Qumruzzaman et al. (2009)

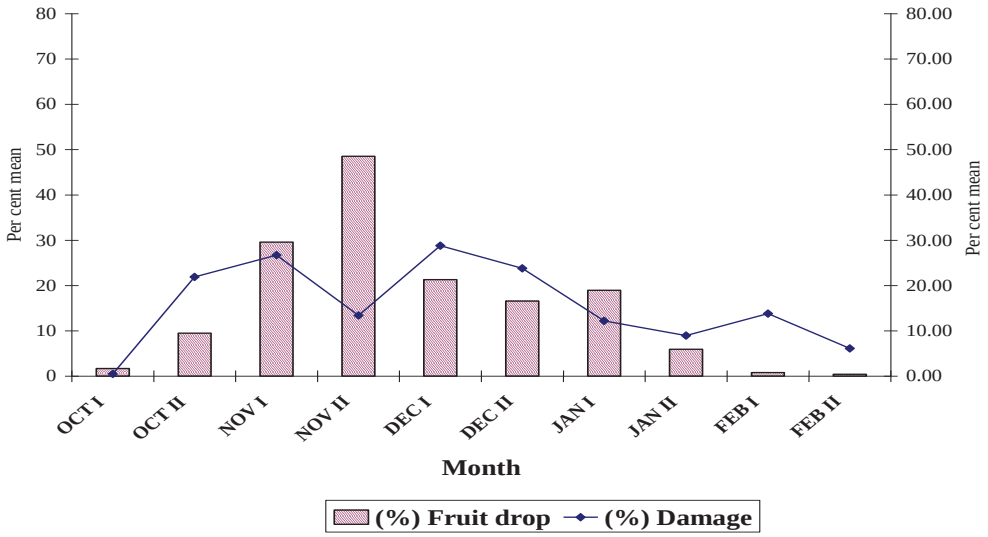

Figure 3. Fruits drop Vs. Stone weevil incidence on ber (Gola).

major role on damage intensity of ber stone weevil. The variety with high pulp stone ratio is attacked more than the lower pulp stone ratio and varieties like Umran and Seb seem to be more attractive for the egg-laying females. The mean damage of stone weevil was up to 23.63% in Gola and 43.28% in Seb at Rajasthan. Fruit dropping was more in Seb (73.48%) than the Gola (48.52%) cv (Karuppaiah et al., 2010). The maximum infestation of stone weevil was registered in cv Seb, followed by Mundia and Umran, and it reduce the yield an average of 10.3% (Srivastava and Nanda, 1983).The damage was severe in the cultivar Umran and the percent abnormal fruits due to weevil damage were between 5 and 10%. We speculate that the higher seed content was probably the reason for these higher damage levels (Balikai, 2009). Among the seven different cultivars observed for fruit weevil incidence, the maximum infestation was recorded in the cultivar Umran (38.9%) followed by Thar Sevika (35.75%), Seb (35.08%), Thar Bhubhraj (28.18%), Gola (24.59%), Goma Kirti (21.65%) and Banarasi Kadaka (7.05%). The correlation between the bio-physical characters, pulp: stone ratio (PSR) of *ber* fruits reveals positive correlation with weevil infestation. The varieties with higher proportion of stone are more preferred by the adult weevil for egg laying and results suggests bio-physical characters of cultivars play major role in fruit weevil infestation (Karuppaiah et al., 2014).

MANAGEMENT

As the damage is hidden, the periodical monitoring of adult weevil activity will help detect the infestation on time. For its management, the spray schedule must be started from flowering to fruit set stage only. Spraying of Carbaryl 50 WDP 0.1% just before the fruit setting and repeat the sprays at three weeks interval was found effective and showed least adverse effect on honey bees activity (AICRP, 2004). Collection and destruction of adult

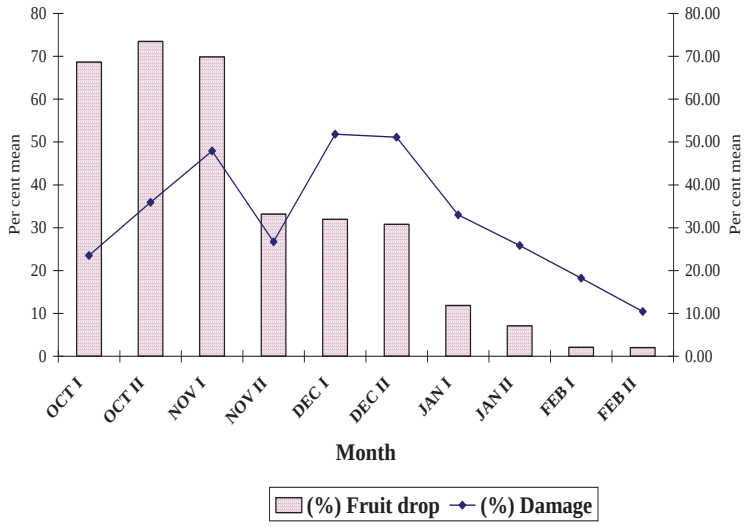

Figure 4. Fruits drop Vs. Stone weevil incidence on ber (Seb).

weevil immediately after detection can also reduce the population. Infested dropped fruits should be collected and burned to break the generation cycle (Karuppaiah, 2013). Application of spinosad 2.5 SC found to give significant reduction in weevil incidence and indoxacarb 14.5 EC, NSKE 5% and azadirachtin 2000 and 1000 ppm also found to be superior over control treatment in minimizing the weevil incidence (AICRP, 2012).

CONCLUSION

Ber stone weevil is a new threat to ber production in India as it causes direct loss to the fruit yield. The last few decades the incidence of this pest has been reported in various in few packets ber growing region of India in a sporadic manner. However, presently it seems to be regular pest of ber and severe damage has also been reported in major ber growing region of India. The distribution of stone weevil incidence reveals that the transport of planting material from one place to another place could be the reason for the pest establishment (Qumruzzaman et al., 2009). In India, hardly any studies have been conducted and little information is available about this emerging pest. While looking nature and intensity of damage it needs to be studied critically and being economically important, the detailed investigation on the biology and management protocol need to be developed for the successful management under changing climatic scenario.

Conflict of Interest

The authors have not declared any conflict of interest.

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