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African Journal of Agricultural Research

Table of Contents: Volume 12 Number 17, 27 April, 2017

ARTICLES

Smallholder farming in Brazil: An overview for 2014 **1424**
Gabriel Paes Herrera, Reginaldo Brito da Costa, Paula Martin de Moraes,
Dany Rafael Fonseca Mendes and Michel Constantino

Cattle manure and liquid biofertilizer for biomass production of yellow passion fruit seedlings **1430**
Francisco Hélio Alves de Andrade, Anailson de Sousa Alves,
Carla Sabrina Pereira de Araújo, Valéria Fernandes de Oliveira Sousa,
Daivyd Silva de Oliveira, Miguel Avelino Barbosa Neto,
Anselmo Ferreira da Silva and Rafael Ramos de Morais

Exogenous ascorbic acid improved tolerance in maize (Zea mays L.) by increasing antioxidant activity under salinity stress **1437**
M. Billah, M. M. Rohman, N. Hossain and M. Shalim Uddin

Identification of pulmonary lesions in slaughtered cattle and associated risk factors, North West Ethiopia
1447
Teshale Bekelle, Mebrahtu Gebreyohannes and Abebe Tesfaye

Seasonality influence the nutrient content of litter fall in secondary forest in the Amazonian **1451**
Rosecélia Moreira da Silva CASTRO, Maria de Lourdes Pinheiro RUIVO, Possidônio
Guimarães RODRIGUES and Seidel Ferreira dos SANTOS

Effect of urea treatment and concentrate mix supplementations on feed intake and digestibility of Horro sheep fed cured maize husk (Zea Mays) at Bako, Western Ethiopia **1459**
Girma Defar, Mengistu Urgie and Solomon Melaku

Towards attaining equity and satisfaction in water allocation mechanism for irrigated agriculture in Northern Nigeria **1466**
Jeleel Abiola Balyaminu

Potential of antranilic diamides applied in seeds of soybeans with and without cry1ac protein for Helicoverpa armigera caterpillar control **1477**
Crislaine Sartori Suzana, Lilian Cris Dallagnol, Leticia Slongo Fortuna,
Raquel Damiani, Renan Teston and José Roberto Salvadori

African Journal of Agricultural Research

Table of Contents: Volume 12 Number 17, 27 April, 2017

Two new wheat varieties for irrigated conditions of Afghanistan **1483**
M. Q. Obaidi, Elias Mohmand, M. H. Azmatyar and Rajiv Sharma

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Full Length Research Paper

Smallholder farming in Brazil: An overview for 2014

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The goal of this paper is to provide an update on smallholder farming in Brazil. Instead of using data from the last available Agricultural Census (2006), a database from the Ministry of Agrarian Development for 2014 was used. These data are extracted from a tax form called "Declaração de Aptidão ao Pronaf-DAP" (Declaration of Aptitude to Pronaf) that is mandatory for all farmers in Brazil and is used as a source of information to screen smallholders, also called "family farmers" in Brazil, applying for special subsidized public funds available to those in this category. Therefore, the DAP is a valuable source of information regarding this sector. The results show that family farming in Brazil continues to grow and is concentrated in the Northeastern region. The South and Southeast have the highest yields per hectare, up to seven times more than the Northeast. Most of the land is in the hands of a small group concentrated in the Northeast, while most of the income is in the hands of a small group concentrated in the South.

Key words: Family farm, economy, Brazil, agriculture.

INTRODUCTION

The world's agricultural market is expected to continue to grow over the next decade as the world population grows at an exponential rate. Brazil is among the world's ten largest economies and has the fifth-largest surface area, and it plays an important role in agricultural exports in the international market. The country is the world's second-largest agricultural exporter and the leading supplier of sugar, orange juice and coffee; furthermore, it is a major exporter of soybeans, tobacco, maize and rice (OECD/FAO, 2015).

Family farms in Brazil represent more than 80% of production units and were responsible for 38% of the gross value of agricultural production in 2006, according to the Brazilian Institute of Geography and Statistics – IBGE (2006). There is no universal definition for family farming; for example, the Brazilian definition focuses on less affluent farms, while the US definition includes farms of all sizes, from farms with low revenue to those that are multi-million dollar enterprises. It is estimated that there are more than 570 million farms in the world, and more than 500 million of these are owned by families (Lowder et al., 2014). Brazilian law's main points for defining a family farm are as follows: a farm managed by the owner and his or her family; smaller than four fiscal modules

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(one module may be between 5 and 110 ha depending on the locality); mostly family rather than hired labor; and the family's main source of household income (Government of Brazil, 2006).

As reported by the OECD/FAO (2015), Brazil is projected to maintain its role as a leading supplier to international food and agriculture markets over the next decade, bringing new opportunities for family farmers. In Brazil, family agriculture has become stronger in the last few years due to the success of certain public policies implemented, which inspired other countries in Africa to adopt similar programs. One of them is The National Program for the Strengthening of family farming (PRONAF), which provides low-interest credit and whose resources reached BRL 25 billion in 2014.

To gain access to that credit and other benefits from the government, family farmers are asked to maintain a register in the Ministry of Agrarian Development (MDA). They must complete a form known as the "DAP" (Declaration of Aptitude to Pronaf), in which they provide detailed information about themselves and their properties, such as age, sex, schooling, area of the farm, number of crops produced, income of each crop, total income, number of workers and other income sources on-farm and off-farm, among others. There are approximately 5 million DAPs registered in the MDA database, which creates a plentiful source of information about family farming in Brazil. A survey with information as detailed as that obtained through the DAP is not possible even with the Agricultural Census.

Most of the studies about family farms conducted in Brazil are based on the Agricultural Census, which was last conducted in 2006. Studies using the information from the DAPs are still scarce due to the difficulty and bureaucracy involved in obtaining the data from the MDA. The Agricultural Census data, meanwhile, is easily accessed by everyone. Playing a major role in Brazil's economy and in the international market, family farms need proper attention. This article aims to generate a portrait of family farming in Brazil in 2014 using the information declared by the farmers on the DAP to offer an analysis with a new perspective and more updated and complete data.

MATERIALS AND METHODS

This article is based on information declared by family farmers on the DAP form obtained through the Ministry of Agrarian Development (MDA) from October 2014. Family farmers from every state in the country can fill in their declaration forms on authorized organizations and, after its correct completion, the form is immediately sent electronically to the MDA system. Subsequently, the DAP is checked to identify any mistakes or false information. The farmers must communicate any changes related to their properties and are not allowed to go for more than three years without updating their DAPs. Therefore, the data extracted from the system database contains information that may have been inserted on the same day or as far back as three years ago.

The method used to analyze the data was exploratory, with the purpose of verifying the behavior of family farming in 2014. To carry out the analysis, the database was refined by removing cases with missing values or very distorted values (outliers) to minimize errors in the results. Approximately 133 thousand DAPs were excluded, and the final database used for this study contained approximately 4.7 million cases.

The database analyses were conducted using the statistical software R (R Core Team, 2017), given its capacity to process large amounts of data.

RESULTS AND DISCUSSION

First, it is important to highlight that Brazil's size means that it contains many different climates, biomes and cultures, which affects agribusiness throughout the country. Therefore, it would not be correct to analyze the data and assume that the average values reflect the reality of the whole country. There are five main regions in Brazil, and each has its own importance, particular characteristics and productive structures. Thus, it is interesting to conduct analyses on a national level as well as on a regional level to develop a more micro perspective and better understand the reality of family farming in Brazil.

Going through the profile of the DAP owners, there are approximately 2.9 million males, representing 62.8%, and more than 1.7 million females, forming 37.2% of farmers. Studies conducted in Ghana, Kenya and Cotê D'ivoire demonstrated a higher number of male smallholder heads: 70, 80.6 and 85.2% respectively (Martey et al., 2012; Kiplimo et al., 2015; Lawin and Zongo, 2016). The age distribution is very wide-ranging, from 18 – the age of majority in Brazil - to 100 years old. Figure 1 indicates that most family farmers are between 20 and 55 years old. These results are similar to the mean age between 31 and 50 found by Kiplimo et al. (2015) in a study conducted with 600 family farmers in Kenya.

Schooling levels draw attention to the fact that most smallholders have a low level of education, ranging from having completed elementary school to literate, according to Figure 2. This scenario is true for all regions of the country, as none of them stands out with high levels of education. According to Lawin and Zongo (2016), most of agricultural household heads in Cotê D'Ivoire have not been to school and, as in Brazil, the level of education of family farmers is in general very low.

The results also show a low number of family farmers who are members of agricultural cooperatives, only 5%. Those who seek technical assistance or for formal education make up only 7.6%, and these numbers are similar to the ones found by Guanziroli et al. (2012). Partnership arrangements are considered to be the reason for the strengthening and resilience of smallholders in regions as eastern Spain and it is also regarded as a very important factor for family farmers in Ghana which mostly belong to a farmer association (Moreno-Perez et al., 2011; Martey et al., 2012). The results present evidence of the continuity of the profile of

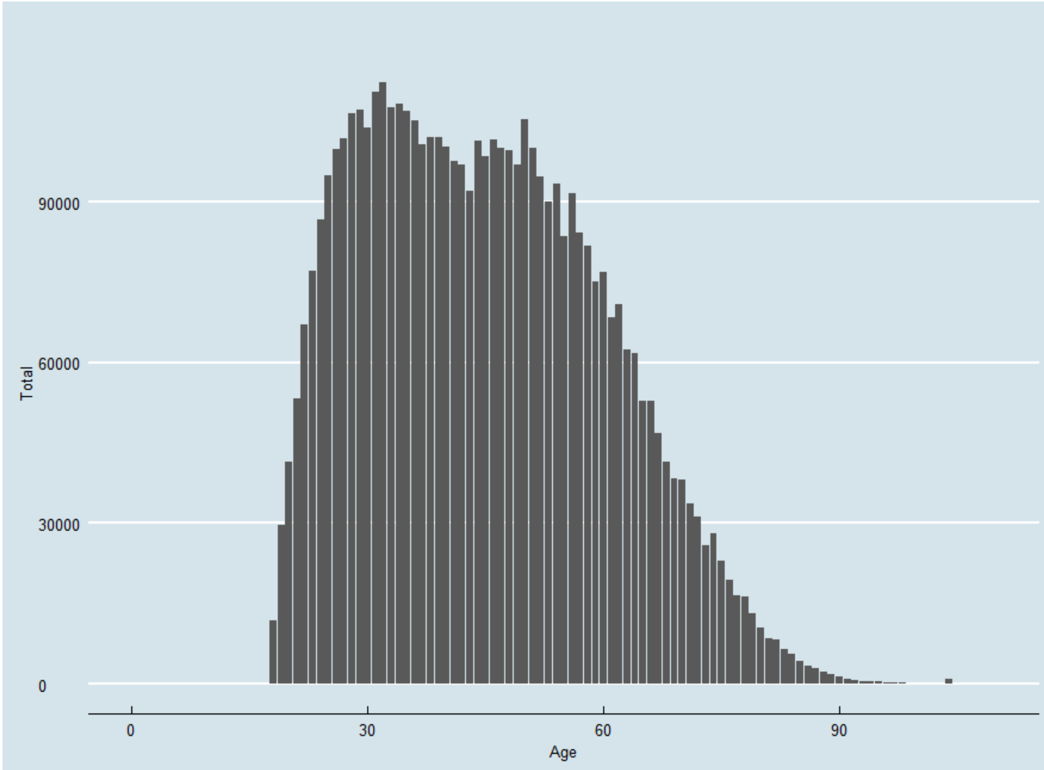

Figure 1. Age distribution of smallholder heads based on the Declaration of Aptitude to Pronaf (DAP).

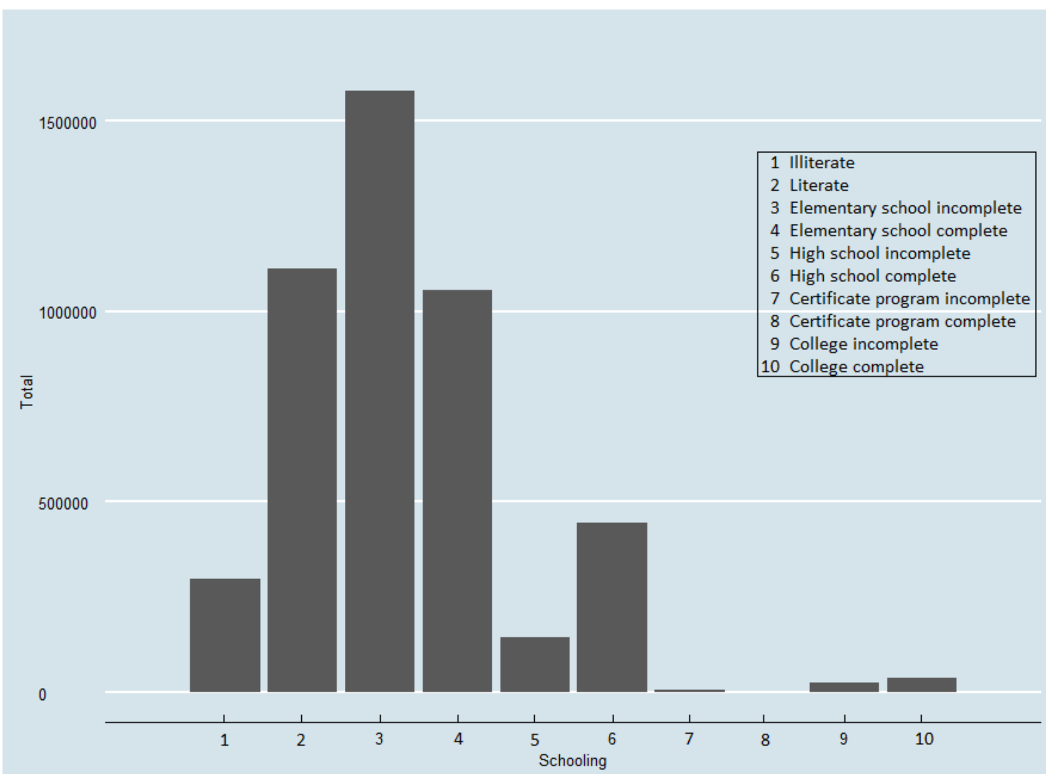

Figure 2. Schooling levels of smallholder heads.

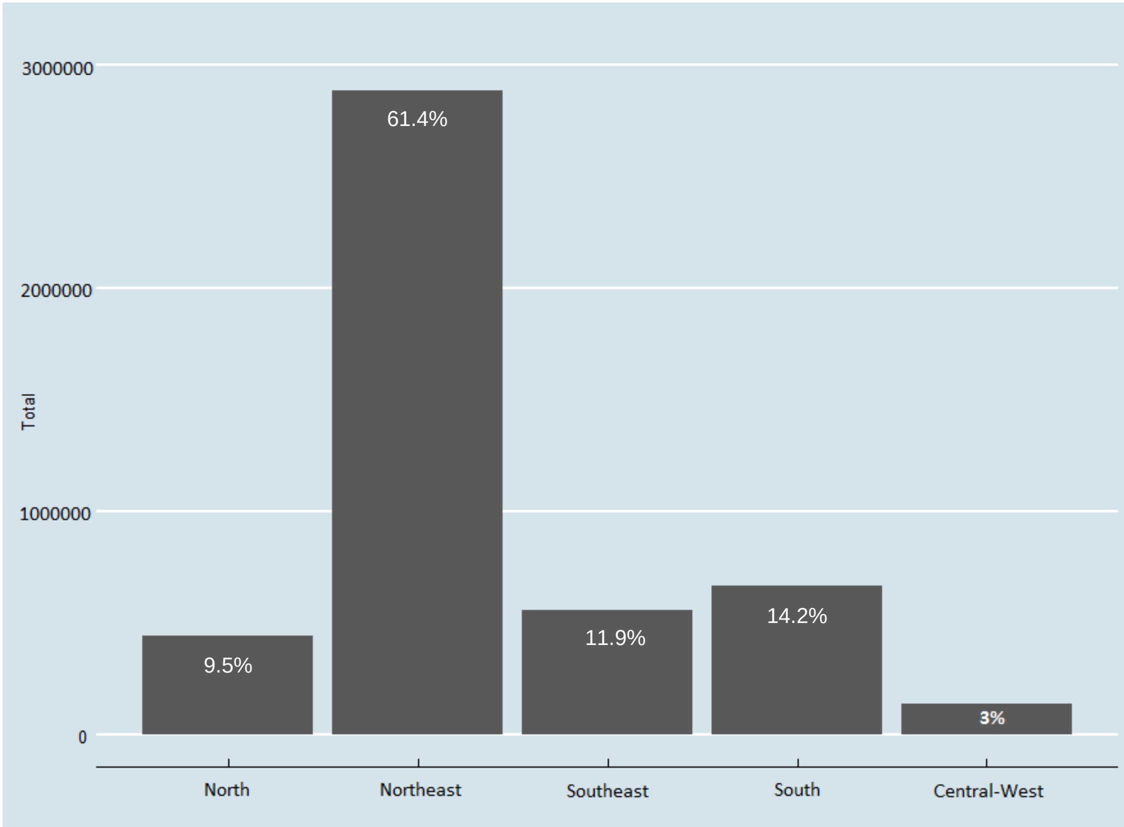

Figure 3. Distribution of family farms by main regions in Brazil.

family farmers in Brazil already described in the IBGE 2006 Agricultural Census.

The average size of smallholder's farms in Brazil is 19.06 ha, however there are major differences between the five main regions. The Central-West and North have the biggest averages, 41.07 ha and 39.67 ha respectively. Whereas the Southeast, Northeast and South have an average size of 17.08 ha, 16.02 ha and 15.51 ha respectively. Those results suggest that the average size of smallholder's farms in Brazil are bigger than those in other regions such as eastern Spain (5 ha), central-east Kenya (2 ha), Republic of Macedonia (1.7 ha) and Malawi (0.4 ha) (Moreno-Perez et al., 2011; Kikulwe et al., 2015; Angelovska and Ackovska, 2012; Denning et al., 2009).

According to the database, more than half (61.4%) of Brazilian family farmers are located in the Northeast region, as shown in Figure 3. On the Agricultural Census (2006) this amount was approximately 50.1%. Following in second place is the South region followed by the Southeast, North and Central-West, respectively. The Central-West region is known as an area of large industrial farms and for its focus on producing commodities for exportation, with little space for family farmers.

Notably, even though the large majority of family farmers are located in the Northeast, the region is not the leader in gross production value (GPV). Instead, the South region is responsible for the largest proportion of the GPV, approximately 38.6%. The GPV analyzed considers all on-farm income sources that include agriculture and livestock production, agro-industry, handicraft and agrotourism. This reveals greater production efficiency in the South, which can be seen on Figure 4. The difference between the regions in Brazil are impressive: while the South has an average productivity of BRL 3,225.55/ha, that of the Northeast region is BRL 410.57/ha. According to Guilhoto et al. (2007), the structure observed in the South is strongly related to the form of colonization of the region and to the culture that settled there due to the European immigration to Brazil.

Furthermore, the South, Southeast and Central-West regions are areas with a higher rainfall rate, better soil fertility and, consequently, more expensive land. On the other hand, the Northeast region is an area that experiences long dry periods and is less developed, with high levels of social inequality. The productivity increase in this region is strongly related to investments in irrigation.

Unfortunately, inequality is a constant problem in Brazil and is also present in family farming. The database shows that only 10.6% of family farmers own farms with

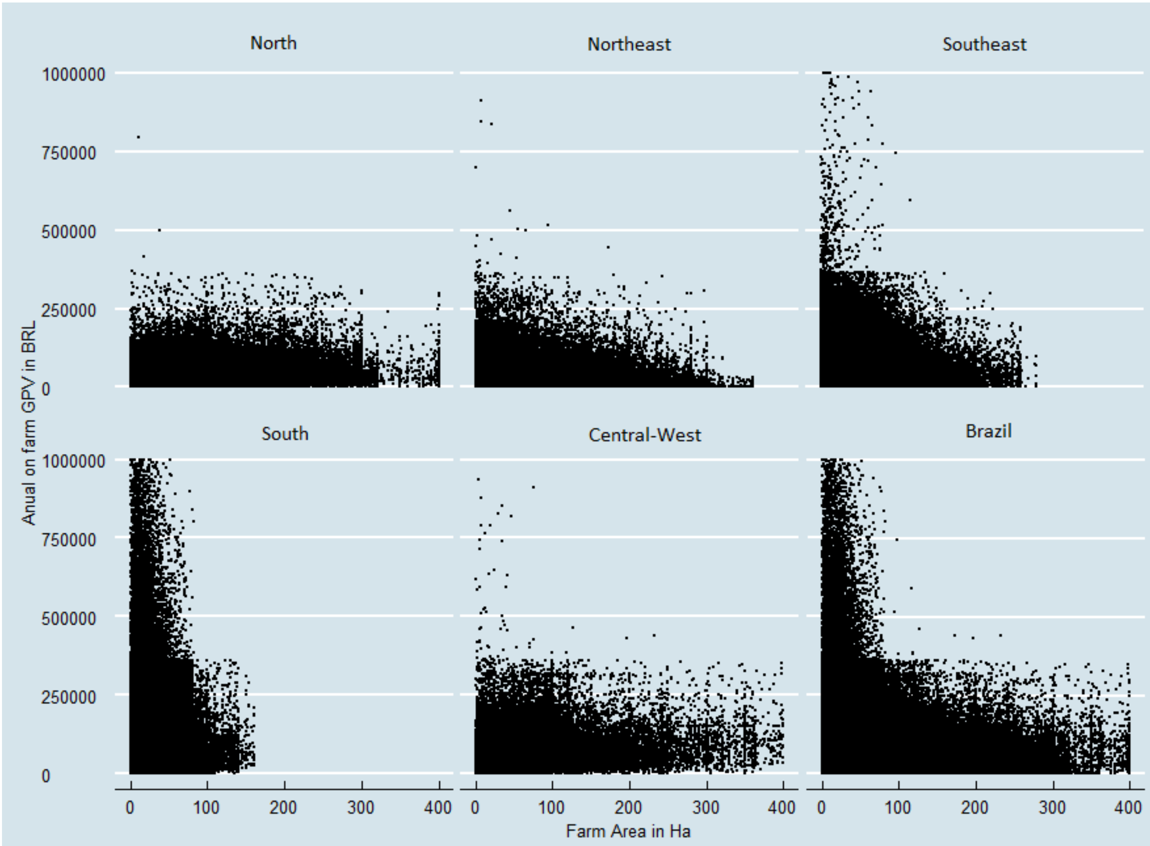

Figure 4. Relationships between farm area and gross production value (GPV) by main regions in Brazil.

Table 1. Distribution of large properties and bigger incomes.

Region	Area more or equal to 50 ha				Income more or equal to BRL 50K			
	Quantity	%*	Area ha	%*	Quantity	%*	GPV	%*
North	131,320	2.8	13,160,219	14.7	33,617	0.7	2,748,977,336	3.2
Northeast	254,536	5.4	24,536,925	27.4	34,062	0.7	2,863,856,517	3.3
Southeast	44,173	0.9	3,652,725	4.1	135,131	2.9	12,907,695,540	14.9
South	29,587	0.6	1,946,875	2.2	200,104	4.3	23,583,617,590	27.3
Central-West	38,823	0.8	4,004,729	4.5	39,820	0.8	3,993,244,645	4.6
Total	**499,439**	**10.6**	**47,301,473**	**52.8**	**442,734**	**9.4**	**46,097,391,628**	**53.3**

* Percentage in relation to the total of family farmers analyzed.

an area greater than or equal to 50 ha and they occupy 52.8% of the total area owned by family farmers in Brazil. Most of these are located in the Northeast region, as shown on Table 1. It was found, however, that 9.4% of family farmers have 53.3% of the total annual GPV and the large majority of them are in the South and Southeast regions. Again, the superior capacity of production per hectare of the South and Southeast regions can be verified. Moreover, these figure draw attention to the fact that perhaps millions of hectares in the Northeast are not being used to their fullest capacity. Angelovska and Ackovska (2012) found a similar problem of uncultivated lands in the Republic of Macedonia, there, among other reasons; this problem is related to the lack of cooperativism amidst family farmers. This may also be the reason for the low productivity in large areas in the Northeast region, however further studies need to be conducted in order to diagnose the causes of this problem in the region.

A study conducted by Guanziroli et al. (2012) also found a small group of 400,000 family farmers that were responsible for 69.5% of the total production and concentrated most of the revenue, but this study did not specify where in Brazil this group was located.

Although we cannot confirm that all family farmers in Brazil are registered on MDA and have a DAP, the number of DAPs analyzed in this article (4.7 million) is greater than the total number of family farmers found by the 2006 Agricultural Census (4.3 million). It is possible to assume from these figures that the number of family farmers has been increasing in Brazil. More than half of them are located in the Northeast, which has the largest properties; however, this region has one of the lowest revenues, which clearly demonstrates a problem of inequality that has also been identified by other authors and still persists.

The huge productivity gap between regions needs to be carefully assessed. Brazil is expected to remain one of the largest agricultural exporters in the world and will therefore need to rely on family farm production, which has already proved to be voluminous and important for the country. One of the solutions may be investing in and encouraging the education of family farmers. Although the low level of schooling is prevalent in all states and some states have high levels of productivity per hectare despite low education levels, the promotion of education would be beneficial for the entire sector.

The problem of income concentration by a small portion of family farmers has already been described by Guanziroli et al. (2012), who attribute it to the fact that there are subgroups of family farmers: industrial, non-industrial and peasant. Industrial family farmers seem to have access to the most lines of credit. Therefore, it is necessary to adjust the public policies for strengthening family farming that are actually creating inequity and strengthening a small group rather than all.

Conclusions

The analyses show that family farming continues to grow and plays an important role in Brazilian agricultural production. The existence of a disproportional distribution of family farmers, which are highly concentrated in the Northeast region, was observed. Another main point is the low level of schooling found for the vast majority of family farmers in all regions of Brazil. In addition, the data revealed an enormous inequality in the distribution of land and income. Among Brazilian family farmers, 10.6%, mostly from the Northeast, own 52.8% of the land. In contrast, 9.4% of family farmers, mostly in the South, concentrate 53.3% of the total income of the sector.

Further studies are necessary to diagnose the causes of low productivity in the Northeast region, this may be a key point for increasing agricultural production of family farmers in Brazil. In addition, it is important that new studies continue updating the data of the sector rather than analyzing information from the Agricultural Census of 2006.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interest.

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Full Length Research Paper

Cattle manure and liquid biofertilizer for biomass production of yellow passion fruit seedlings

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This study aims to evaluate the production of biomass of different cattle manure, and biofertilizer concentrations. The experiment was conducted between April and June 2014 in a greenhouse at the seedling production nursery of the State University of Paraíba (UEPB), Catolé do Rocha, Paraíba (PB) state. The experiment was completely randomized in a 5 x 2 factorial design corresponding to five levels of cattle manure (0, 20, 40, 60 and 80% of the substrate volume) in the absence, and the presence of a biofertilizer. The propagation material was giant yellow passion fruits with 95 to 100% of purity purchased in the local market. After 60 days, the following variables were evaluated: root dry matter (RDM), plant dry matter (PDM), root biomass (RB), shoot biomass (SB), plant biomass (PB), biomass percentage (BP), effective leaf area (ELA) and leaf area ratio (LAR). The interaction between cattle manure and fertilizer concentrations was significant for root dry matter, mass of total plant dry matter, root biomass, shoot biomass and total plant biomass. In turn, cattle manure affected significantly biomass percentage, effective leaf area and leaf area ratio. Cattle manure and biofertilizer make the production of yellow passion fruit seedlings feasible.

Key words: *Passiflora edulis Sims f. flavicarpa Degener*., alternative sources, propagation, protected environment.

INTRODUCTION

The genus Passiflora contains the highest number of species in the *Passifloraceae* family, with approximately 400 species. 20 of them are restricted to Australia, China, India, Oceania Islands and neighboring regions, and Southeast Asia. Argentina, Chile and the United States account for the remainder species (Santos et al., 2012).

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Brazil has about 120 species, making it the country with the highest number of native species (Bernacci et al., 2003).

In order to obtain a satisfactory production of yellow passion fruits, good quality seedlings are needed. They must be vigorous, present good physiological characteristics and a well-developed root system, and have an adequate size. According to Costa et al. (2011), the use of appropriate techniques in seedling production is very important to promote healthy and vigorous plants for the formation of orchards, such as an improved production microclimate, volume of containers, substrates, irrigation and nutrition.

Among the techniques used in the development of seedlings, seedling production through organic inputs stands out because such inputs are low cost and easy to find, and meet the needs of the plants. According to Artur et al. (2007), organic inputs are sources of nutrients improving physical attributes and stimulating microbial processes. Among organic inputs, cattle manure and biofertilizers stand out.

Cattle manure not only improves physical, chemical and biological properties of the substrate, but also improves soil conditions. Studies were conducted aiming to quantify the optimal dose for the formation of seedlings of several cultures (Jiang et al., 2014).

Furthermore, cattle manure not only improves physical, chemical and biological properties of the substrate, but also improves soil conditions. It is therefore an important option to maintain sustainable agricultural practices (Larney and Angers, 2012). Studies were conducted aiming to quantify the optimal dose for the formation of seedlings of several cultures. Mesquita et al. (2012) concluded that, for the production of papaya seedlings, 80% of cattle manure should be incorporated into the substrate. Oliveira et al. (2009) found a better development of castor bean seedlings by using approximately 30% of cattle manure.

Biofertilizers based on cattle manure are also viable alternatives for seedling production. According to Bezerra et al. (2007), biofertilizers are considered metabolic activators, stimulating root growth and the development of plants. Biofertilizers are organic inputs that affect soil conditioning acting as microbial inoculant and corrective fertilizers (Gondim et al., 2010). Cavalcante et al. (2009) concluded that biofertilizers applied before sowing were effective in the early growth of yellow passion fruit seedlings by decreasing soil electrical conductivity. Martins et al. (2015) found that cattle manure alone, or its interaction with biofertilizers and/or inoculants, replaces mineral fertilization.

The quantification of cattle manure and biofertilizer concentrations may positively affect the formation of seedlings by enabling biomass production, and providing beneficial effects for the substrate (such as increase in organic matter, and favorable conditions for the development of the root system).

In this sense, the objective was to evaluate different concentrations of bovine manure in the presence and absence of biofertilizers in the biomass production of yellow passion fruit seedlings.

MATERIALS AND METHODS

Place of experiment

The experiment was conducted between April and June 2014 in a greenhouse at the seedling production nursery of the State University of Paraíba (UEPB), campus IV, Catolé do Rocha, Paraíba (PB) state (6°2’38" S, 37°44’48" W; altitude: 275 m). The nursery was set with shading, allowing 50% of light inside. The experiment was completely randomized in a 5 x 2 factorial design and five replications corresponding to five levels of cattle manure (0, 20, 40, 60 and 80% of the substrate volume) in the absence and the presence of a biofertilizer. The propagation material was giant yellow passion fruits with 95 to 100% of purity purchased in the local market.

Sowing and analysis

The seeds were sown in polyethylene bags with a capacity of 1 dm^3. Five seeds were sown per bag at 1 cm depth. The thinning was performed 24 days after sowing. Weed control was performed by hand weeding. The soil was classified as a eutrophic Fluvic Neosol (Embrapa, 2011), whose analysis, performed at the 0 to 20 cm layer, presented the following chemical characteristics: pH in H_2O: 8.2, EC: 1.53 dSm^{-1}, P: 3.27 cmolc dm^{-3}, K: 0.26 cmolc dm^{-3}, Ca: 5.09 cmolc dm^{-3}, Mg: 1.66 cmolc dm^{-3}, Al: 0.0 cmolc dm^{-3}, Na: 0.26 cmolc dm^{-3}, and 1.19% of organic matter.

Cattle manure, from the Cattle Production Sector of UEPB, campus IV, Catolé do Rocha, PB, was tanned for 35 days. The analysis revealed the following chemical properties: N: 12.76 g kg^{-1}, P: 2.57 g kg^{-1}, K: 16.79 g kg^{-1}, Ca: 15.55 g kg^{-1}, Mg: 4.02 g kg^{-1}, Na: 5.59 g kg^{-1}, Zn: 60 mg kg^{-1}, Fe: 8,550 mg kg^{-1}, Mn: 325 mg kg^{-1}, soil organic matter: 396 g kg^{-1}, organic carbon: 229.7 g kg^{-1}, and C/N ratio: 18:1.

The material used for the production of the organic biofertilizer consisted of 70 kg of green manure of cows in lactation, 120 L of water, 4 kg of rock flour (MB4), 5 kg of legumes (beans), 3 kg of wood ash. 5 L of milk and 5 kg of sugar were also included to accelerate the metabolism of anaerobic bacteria for 35 days (SANTOS, 1992). The chemical composition of the biofertilizer was analyzed from the dry matter at the Soil Fertility Laboratory (LFS) of the Federal Rural University of Pernambuco (UFRP), and presented the following results: pH in H_2O: 5.25, EC: 7.1 dS m^{-1}, N: 0.8%, P: 403.4 mg dm^{-3}, K: 1.78 $cmol_c$ L^{-1}, Mg: 6.0 $cmol_c$ L^{-1}, and Ca: 5.4 $cmol_c$ L^{-1}.

The water supply was supplied twice a day, at 07:00 and 17:00, using a watering can of 16 L. The chemical analysis of the water used for irrigation showed the following attributes: pH: 8.13, EC: 0.99 dS m^{-1}, Ca: 1.305 mmol L^{-1}, Mg: 1.48 mmol L^{-1}, Na: 5.5 mmol L^{-1}, K: 0.49 mmolc L^{-1}, CO_3^{-2}: 0.44 mmolc L^{-1}, HCO_3^-: 3.67 mmolc L^{-1}, chlorides: 4.97 mmolc L^{-1}, and RAS: 3.29. The water was classified as C_3 according to Richards (1954).

Analyzed variables and statistical program

60 days after sowing, the following variables were evaluated: root dry matter (RDM), plant dry matter (PDM), root biomass (RB), shoot biomass (SB), plant biomass (PB), biomass percentage (BP),

effective leaf area (ELA) and leaf area ratio (LAR).

Root dry matter and plant dry matter were measured using the mass of dry matter of both variables by drying in a forced-air circulation oven at 65°C for 48 h. Then, it was weighed on an analytical balance according to the methodology adopted by Silva et al. (2006). Root biomass, shoot biomass and total plant biomass were determined by the difference between the fresh weight and the dry matter of the respective variables. Subsequently, the parameters specific leaf area, and leaf area ratio were determined according to Benincasa (2003), using the formulas:

$$ELA = \frac{TPLA}{LDM}$$

$$LAR = \frac{TPLA}{PDM}$$

SLA: Specific leaf area;
TPLA: Total plant leaf area;
LDM: Leaf dry matter;
LAR: Leaf area ratio;
PDM: Plant dry matter.

The data were submitted to analysis of variance using the F test $P<0.05$, and subsequently linear and quadratic regressions using the statistical analysis software SISVAR® (Ferreira, 2014).

RESULTS AND DISCUSSION

The interaction of the factors cattle manure x biofertilizer affected significantly mass of root dry matter, total plant dry matter, root biomass, shoot biomass and total plant biomass. In turn, cattle manure affected significantly biomass percentage, effective leaf area and leaf area ratio (Table 1).

60 days after sowing, the interaction between cattle manure and biofertilizer significantly affected root dry matter. An optimal concentration was found in the presence (B_1) and the absence (B_0) of biofertilizer using 55 and 50% of cattle manure, respectively. It is also possible to observe the highest RDMs: 1.69 g in the presence (B_1) and 1.08 g in the absence (B_1) of the biofertilizer. Thus, the presence (B_1) of biofertilizer at a concentration by derivation of 50% of cattle manure provided an increase of 36.09% when compared to the optimal concentration in the absence (B_0) of biofertilizer (Figure 1A).

Mesquita et al. (2012) reported divergent results when compared with the results of this research, which reported for castor bean plants a higher mass of root dry matter (RDM) at a concentration of 80% of cattle manure in a 2 dm^3 container. This higher root dry matter (RDM) of yellow passion fruits can be associated with the genetic potential of the culture because a high value was observed for the level estimated by derivation of concentrations of 55% in B_1 and 50% in B_0, results lower than the values found by Mesquita et al. (2012), that is, there was an economy of 45 to 50% of cattle manure. Passion fruit crops respond well to low concentrations of cattle manure in the substrate.

On the other hand, the interaction of 50% of cattle manure in the presence of the biofertilizer increased RDM, and may be associated with the provision of macro and micronutrients, apportionment of applications and its release during the crop cycle (Sediyama et al., 2014), or there could be a likely decomposition of organic matter by microorganisms present in the biofertilizer, releasing humic substances.

For mass of total plant dry matter (Figure 1B), an increase by 47.08% and 40% of cattle manure, respectively, was observed by derivation up to the optimal concentration in the presence (B_1) and the absence (B_0) of the biofertilizer, favoring 3.49 g and 2.92 g of PDM. Thus, the cattle manure concentration 47.08% used together with the biofertilizer promoted an increase of 0.57 g in PDM, equivalent to 16.33% when compared to the estimated concentration in the absence of the biofertilizer.

Cavalcante et al. (2010) also found a high PDM in the presence of the biofertilizer in relation to 0.5 dS/m³ water for guava seedlings. According to Sousa et al. (2013), the biofertilizer provides an increase in photosynthetic rate and chlorophyll. This results in a greater assimilation and fixation of CO_2, producing mass of total plant dry matter (PDM), which may have occurred in this study. Oliveira et al. (2009) reported a greater mass of total plant dry matter (PDM) at a concentration of 28.88% of cattle manure at the initial growth of castor beans. Rodrigues et al. (2008) studied the agronomic performance of arugula and observed high dry matter at a concentration of 53.20% of cattle manure.

Root biomass increased gradually when subjected to different concentrations of cattle manure in the presence (B_1) and the absence (B_0) of biofertilizer (Figure 1C). As the concentrations of manure increased, an increase in root biomass occurred. A value of 2.88 g was found in B_1 and 2.7 g was found in B_0 at a concentration of 80% of cattle manure. Organic matter favors the release of important nutrients such as nitrogen (N) and sulfur (S) (Araújo et al., 2010). When correlated with other factors, it expands the photosynthetic area, ensures the development of plants by vegetative growth and increases the productive potential of crops (Filgueira, 2000).

Shoot biomass was influenced by the interaction between concentrations of cattle manure x biofertilizer (Figure 1D). The highest value was measured in treatments with 41.66% of cattle manure in the presence (B_1) of the biofertilizer: 10.65 g of SB. On the other hand, the concentration of 50% of cattle manure without the biofertilizer resulted in 8.55 g, that is, a decrease of 19.72% in shoot biomass. Notably, the biofertilizer provided the greatest shoot biomass (SB) at a concentration of 50% of cattle manure. Yang et al. (2016) reported a high yield and a low incidence of pathogens in watermelons regarding the consistent application of cattle

Table 1. Results of analysis of variance of the morphological parameters root dry matter (RDM), shoot dry matter (SDM), root biomass (RB), shoot biomass (SB), total plant biomass (TPB), biomass percentage (BP), effective leaf area (ELA) and leaf area ratio (LAR) of seedlings subject to different cattle manure concentrations in the presence (B_1) and the absence (B_0) of biofertilizer in yellow passion fruit plants.

SV	DF	Mean square			
		RDM	PDM	RB	SB
Manure	4	2.048**	8.109**	4.142**	49.440**
Linear	1	4.084**	7.409**	16.265**	27.457**
Quadratic	1	3.921**	24.411**	0.101**	131.764**
Biofertilizer	1	0.576	0.006^{ns}	0.323**	2.402*
E x F	4	0.267**	1.056**	0.282**	12.153**
Residue	40	0.002	0.006	0.006	0.568
C.V.	-	4.95	3.28	4.16	10.18
Mean	-	1.00	2.45	2.00	7.40
SV	**DF**	**Mean square**			
		PB	**BP**	**ELA**	**LAR**
Manure	4	63.714**	686.473**	116,124.047**	51,229.617**
Linear	1	85.988**	2,028.421**	258,260.125**	70,978.017**
Quadratic	1	124.551**	32.198^{ns}	2,748.637^{ns}	11,081.391^{ns}
Fertilizer	1	0.963^{ns}	51.999^{ns}	7,755.853^{ns}	6,695.790^{ns}
E x F	4	12.968**	14.914^{ns}	12,924.060^{ns}	7,357.177^{ns}
Residue	40	0.572	14.986	7,845.311	4,497.752
C.V.	-	8.04	17.31	19.21	22.73
Mean	-	9.40	22.36	461.02	295.06

**, *: Significant at 1 and 5% by F test, respectively; NS: not significant (Source of variation (SV), Degree of freedom (DF), mean square (MS) and coefficient of variation (CV)).

manure in the rotation of Garlic with watermelons. According to the authors, this was due to improvements in the soil biological condition, such as microbial quantity and high levels of enzyme activity. In addition, soil characteristics such as lower levels of phenols, salts and increase in pH also played a relevant role in it. The concentrations of cattle manure affected significantly total plant biomass, biomass percentage, specific leaf area and leaf area ratio. The total plant biomass (Figure 2A) fitted to a quadratic polynomial model, obtaining an optimal point at the concentrations 47.73 and 73.91% in the presence (B_1) and the absence (B_0) of biofertilizer, respectively. It provided a PB of 9.33 g and 10.96 g, respectively. It is possible to observe a rationing of 26.16% of cattle manure when using biofertilizers, in addition to stimulating a high PB.

The highest volume of total plant biomass was obtained in the presence of the biofertilizer. According to Cavalcante et al. (2007), the biofertilizer acts positively because it is a source of bioactive compounds, which favors the release of humic substances and stimulates an increased activity of the enzyme reductase, reducing free amino acids. On the other hand, Freire et al. (2014) found a high potential quantum yield (Fv/Fm) by using biofertilizers based on cattle manure and a low volume of internal CO_2 of passion fruits. This may reflect directly in the production of biomass, as the highest Fv/Fm and the lowest rate of internal CO_2 may mean a high CO_2 fixation as a result of a high biomass.

The percentage of biomass increased with the gradual increase in cattle manure concentrations (Figure 2B). The maximum concentration obtained 29.75% of biomass. As a result, there was an increase of 59.16% when compared to the 0% increase of the organic input. The benefits of cattle manure, according to Oliveira et al. *(*2010), are probably related to the fact that, when supplied in adequate amounts, it may be able to meet the needs of the plants due to an increase in N, P and K contents available, being K the element whose content reaches high values in the soil.

On the other hand, different cattle manure concentrations, regarding SLA and LAR resulted in a decrease as the concentrations of cattle manure increased (Figure 2C and D). This was expected because the obtained biomass percentage presented an inverse behavior. Bezerra et al. (2016) evaluating the growth of two genotypes of yellow passion fruit under a salinity condition considering specific leaf area do not support this, as the authors found a positive linear tendency.

According to the authors, ELA is an indicator of leaf

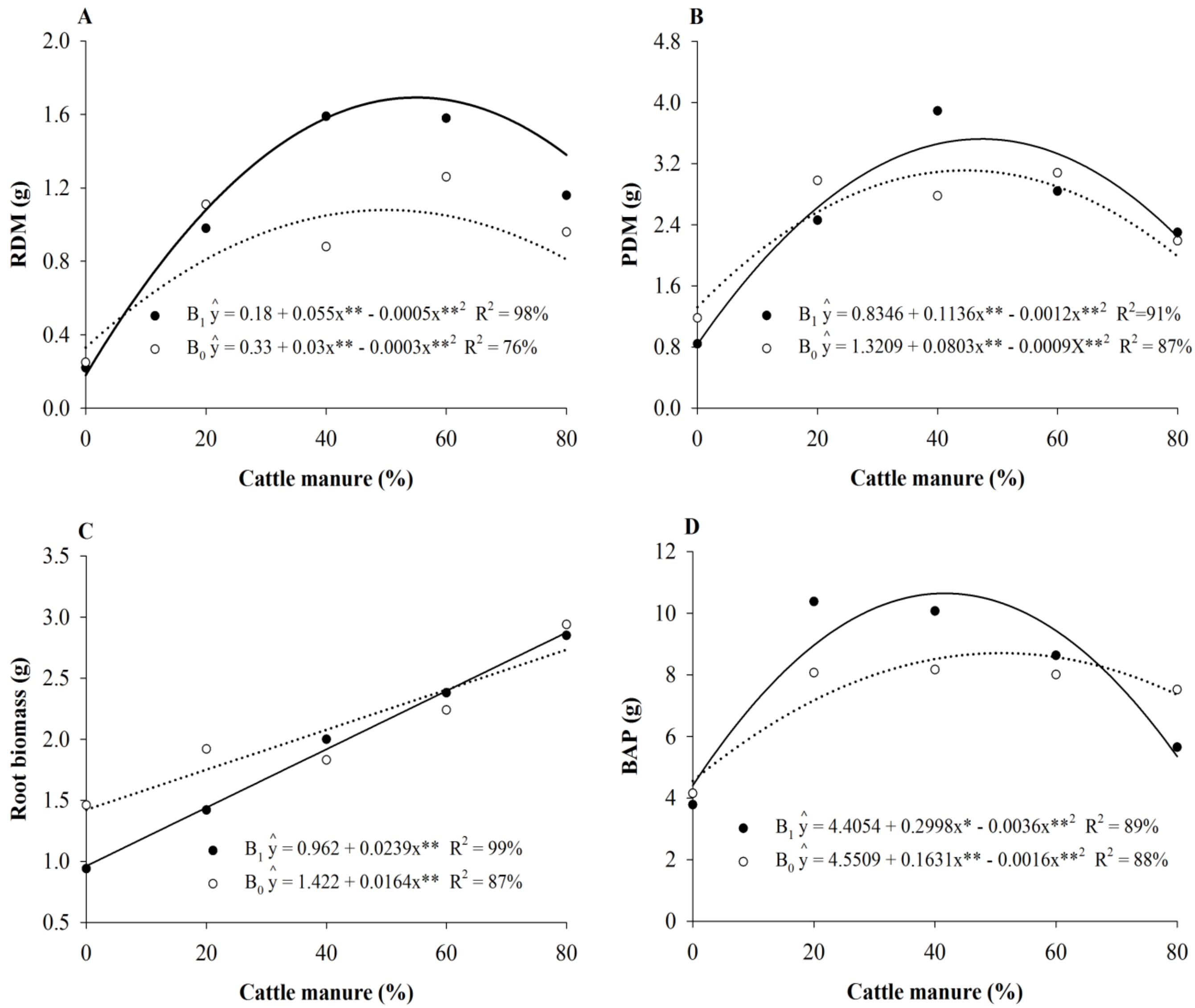

Figure 1. (A) Root dry matter (RDM), (B) plant dry matter (PDM), (C) root biomass, and (D) Biomass of the aerial part (BAP) in function of cattle manure concentrations in the presence (B_1) and absence (B_0) of the biofertilizer in yellow passion fruit plants.

thickness, the lower the value, the thicker the leaf. It indicates a small leaf area and a greater leaf dry matter, making it advantageous because leaves become more resistant to light intensity and more efficient in the absorption of photons and CO_2, resulting in high net photosynthesis and biomass production. SLA is given by the ratio between total leaf area and leaf dry matter (Dias-Filho, 1997).

LAR expresses the useful leaf area for photosynthesis. It is a morpho-physiological component for it is the ratio between leaf area (area responsible for intercepting light energy and CO_2) and total dry matter (a result of photosynthesis) (Silva et al., 2006). The decreasing linear tendency of leaf area ratio means that more mass was distributed on stems and roots, making it a positive point, because the allocation of dry matter to roots enables a greater absorption and translocation of humic substances from cattle manure.

Conclusion

The use of cattle manure isolated and/or interacting with liquid biofertilizers becomes feasible for the production of yellow passion fruit seedlings. Concentrations between 40 and 50% of cattle manure provide high root dry matter, plant dry matter, shoot biomass and biomass percentage in yellow passion fruit plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

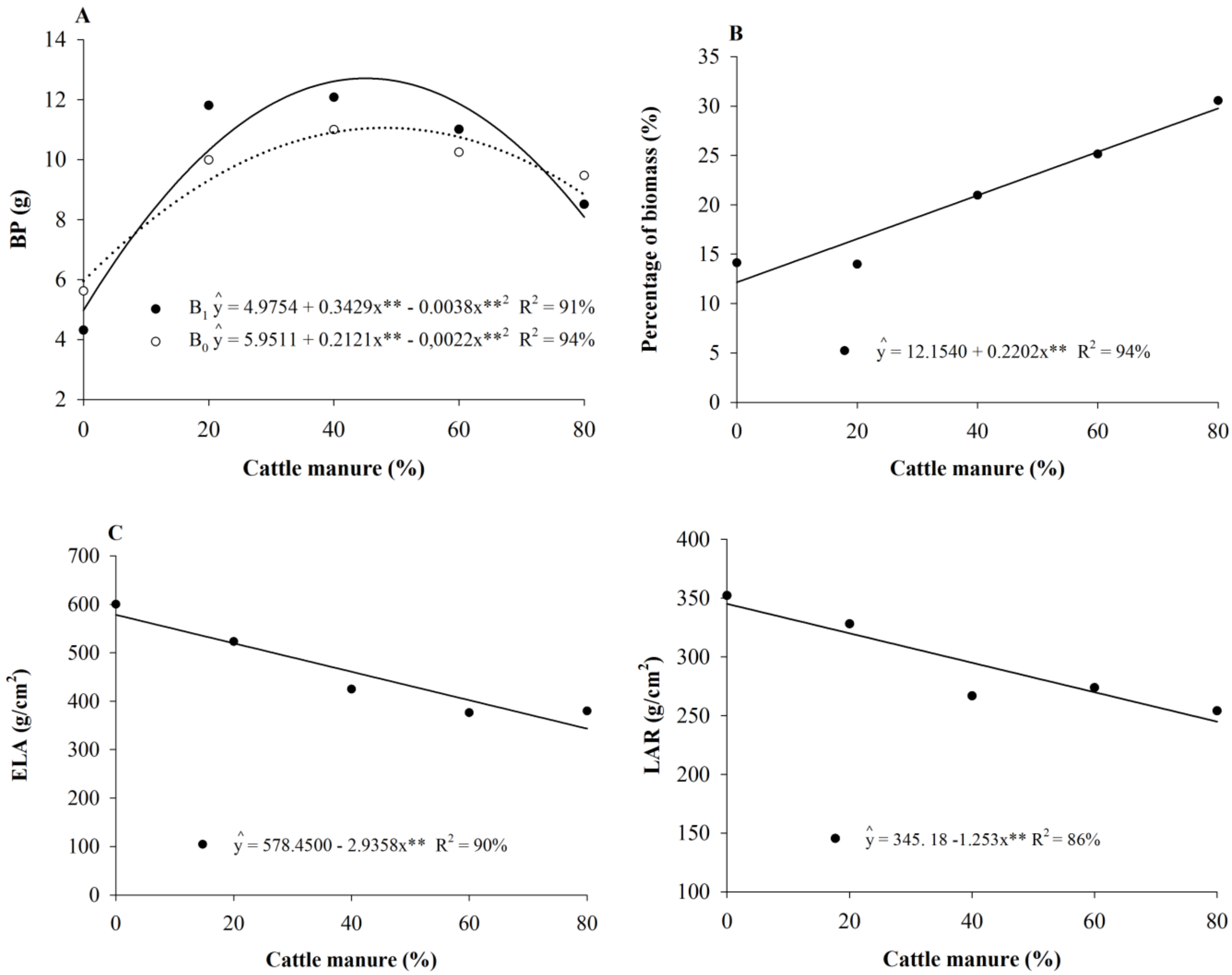

Figure 2. (A) Total plant biomass (TPB), (B) biomass percentage (BP), (C) specific leaf area (SLA), and (D) leaf area ratio (LAR) in function of cattle manure concentrations in the presence (B_1) and the absence (B_0) of the biofertilizer in yellow passion fruit plants.

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Full Length Research Paper

Exogenous ascorbic acid improved tolerance in maize (*Zea mays* L.) by increasing antioxidant activity under salinity stress

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Salinity causes additional manufacture of reactive oxygen species (ROS) in plants, and ascorbate plays important role in maintaining of ROS scavenging antioxidant enzymes. In this study, the role of exogenous ascorbic acid (AsA) was examined on growth, chlorophyll and oxidative stress related enzymatic and non-enzymatic antioxidants in three maize hybrids under NaCl mediated salt stress. In hydroponic culture, AsA was applied at 0.5 and 1.0 mM with and without 12 dSm^{-1} NaCl, each treatment comprised two independent experiments with three replications. After four weeks, plants were harvested for recording growth and biochemical parameters. Root length, shoot length, dry matter accumulation, chlorophyll (*Chl a* and *Chl b*), AsA, reduced glutathione (GSH) and activity of ascorbate peroxidase (APX) were markedly reduced by salt stress, while H_2O_2 and Malondialdehyde (MDA) content were increased significantly. Exogenous application of AsA in saline treatment significantly improved root length, shoot length, dry matter accumulation, chlorophyll, AsA, GSH and APX activity while it decreased the contents of oxidized glutathione (GSSG) significantly in all the hybrids. However, content of dehydroascorbate (DHA) was reduced only in 900M Gold and PS-999. On the other hand, activity of monodehydroascorbatereductase (MDHAR) was increased only in Super gold and 900M gold (by 0.5 mM AsA) while dehydroascorbatereductase (DHAR) activity increased in Super gold only. The results of the present study evidently concluded that exogenous AsA application responded differentially in maize genotypes under salt stress and mitigated the negative effects of salinity.

Key words: Maize, salinity, reactive oxygen species, antioxidant, ascorbic acid.

INTRODUCTION

Salt-stress is one of the most prime hindrances in salt affected area of the world for crop production. At present, nearly 6.5% of whole area of the world and around 20% of the cultured land is affected by salinity (Hakim et al., 2014). Salinity changes various physiological and biochemical characteristics which reduce the plant growth

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and yield production (Munns, 2005). Plant grown at high salt stress in the soil brings about hyperosmolarity, ion disequilibrium, nutrient imbalance and creation of reactive oxygen species (ROS) through molecular damage (Nawaz et al., 2010). The excess gathering of ROS at various abiotic stresses in plants which are extremely reactive and noxious and encourage to damage of carbohydrates, proteins, lipids and DNA which ultimately consequences to oxidative stress (Gill and Tuteja, 2010). Hydrogen peroxide (H_2O_2), produced by salt-stress, is the most stable among all the ROS and reacts with above molecules. Using enzymatic and nonenzymatic antioxidants treatment, these ROS would be managed strategically within a fine influential range (Bose et al., 2013). Moreover, exogenous antioxidant overcomes the low production of growth regulators at stress condition (Ejaz et al., 2012).

The importance and changes in ascorbic acid (AsA) levels in plant cells in response to varying environmental stress conditions are well established (Noctor and Foyer, 1998; Venkatesh and Park, 2014). Functionally, it is an essential metabolite which operates as antioxidant and acts as a significant protagonist of several plant species for salt tolerance (Hameed et al., 2012; Ozgur et al., 2013). Actually, it associates with H_2O_2 metabolism as well as lipid hydroxyl peroxidase and also reacts with various sorts of biotic actions in plant as a patron or receptor in electron transportation system and also as an enzyme co-factor (Conklin, 2001), this approach effectively minimizes the stress impact of salinity and encourage the plant growth. However, exogenous claim of non-enzymatic antioxidant is an imperious suppository for salt sensitive variety.

Several investigations were performed on different plant or crop that exhibited the application of exogenous AsA significantly mitigated salt stress effect and promoted growth and yield (Ejaz et al., 2012; Abou-Leila et al., 2012; Hameed et al., 2015). Nevertheless, few reports are available on the exogenous role of AsA in mitigating salinity-mediated oxidative damage. Therefore, this investigation was carried out to apprehend the role of exogenous AsA application on maize growth and physiological responses to salt stress.

MATERIALS AND METHODS

Plant materials

Three commercial maize hybrids, that is, Super gold, 900M gold and PS-999 were used as experimental materials.

Plant growth condition

The phenotyping was carried out in the green house of Plant Breeding Division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701 in hydroponic culture, where the temperature was maintained around 28 to 30°C for 14 h, and 22°C for 10 h under light and dark conditions, respectively. The relative humidity and light intensity of the greenhouse room were 50% and 657 µmol $m^{-2}s^{-1}$, respectively. The study was accompanied with completely randomized design (CRD), specifically, four treatments (Control, 12 dSm^{-1} NaCl, 12 dSm^{-1} NaCl +0.5 mM AsA and 12 dSm^{-1} NaCl +1.0 mM AsA) were set and two independent experiments, each containing three replications, were conducted. Seeds were sown under water saturated quartz granule after surface sterilization (with 0.05% $HgCl_2$ for 10 min) and washed three times with deionized water and covered with black polyethylene for 4 days for germination and kept under light for another 6 days. After the exclusion of endosperm, ten days old seedlings were wrapped with sponge tightly and put in the hole of the cover. Each cover has six holes, where six plants were put into for transplanting in hydroponic pot containing half-strength Hoagland solution. The continuous air was supplied to the nutrient solution for ventilation and the nutrient solution was changed every four days. Salt treatments were applied and changed both normal and treated solution every four days interval. During the changing time of solution, the pot was washed with a brush for minimizing the contamination of fungal growth. Culture duration was almost five weeks in the nutrients solution. After five weeks, the seedlings were taken out for measurement of growth parameters and biochemical analysis.

Growth parameter measurement

Root length and shoot length were measured during harvesting whereas total dry matters were determined after keeping the samples in oven at 60°C for seven days.

Biochemical analysis

Measurement of H_2O_2

H_2O_2 was assessed following the method of Yu et al. (2003).

Measurement of lipid peroxidation

The concentrations of lipid peroxidation were estimated by determining MDA, a disintegration product of the peroxided polyunsaturated fatty acid constituent of the membrane lipid with thiobarbituric acid (TBA) as the reactive material succeeding the way of Heath and Packer (1968).

Chlorophyll measurement

Extraction and determination of Chlorophyll (*Chl a* and *Chl b*) were done according to the method of Arnon (1949). *Chl* were computed via following equations and articulated in mg g^{-1} fresh weight (FW).

$$Chl\ a\ (mg\ g^{-1}) = (0.0127) \times (A_{663}) - (0.00269) \times (A_{645})$$
$$Chl\ b\ (mg\ g^{-1}) = (0.0229) \times (A_{645}) - (0.00468) \times (A_{663})$$

Extraction and measurement of ascorbate and glutathione

Ascorbate levels were examined following the process of Huang et al. (2005). The glutathione mere was analyzed using with Yu et al. (2003) methods.

Protein determination

Following to the method of Bradford (1976), the protein levels in the leaf excerpts were measured by BSA as a protein standard.

Enzyme extraction and analyzes

Enzyme extraction was executed affording to the technique as labeled by Hossain et al. (2005). APX (EC: 1.11.1.11) and DHAR (EC: 1.8.5.1) activities were assayed following the technique of Nakano and Asada (1981). MDHAR (EC: 1.6.5.4) activity was computed by the method followed by Hossain et al. (2010).

Statistical analysis

The statistical analysis was completed following complete randomized design (CRD) and the mean differences were compared by Tukey's test using Statistical Tool for Agricultural Research (STAR) Version 2.0.1 for window (IRRI, 2014). Data of mean ± standard error (SE) were recorded from two independent experiments with three replications and P≤0.05 was considered as significant.

RESULTS

Growth parameters

Salt stress significantly decreased all the studied growth parameters (Figure 1A to C). However, application of 0.5 mM AsA in salt treatment markedly increased root length, shoot length and dry matter accumulation in compared to salt stress, though 1.0 mM AsA application showed non-significant effects. Root length reduced near about 50% as compared to control of all maize hybrids under salt stress and presence of 0.5 mM AsA increased root length by 16.69% in Super gold, 17.17% in 900M gold and 19.17% in PS-999 compare to that in stress condition, while slight improvement (3.25% in 900M gold, 2.77% in Super gold and 1.37% in PS-999) was recorded in 1.0 mM AsA application. Salinity reduced shoot length by 30.29% in Super gold, 41.80% in 900M gold and 37.84% in PS-999, while application of 0.5 mM AsA improved 12.19, 14.55 and 11.95% in Super Gold, 900M gold and PS-999, respectively, overstress condition. On the other hand, seedlings treated with 1.0 mM AsA showed relatively lower growth than those treated with 0.5 mM AsA. Dry matter accumulation declined above 50% in all maize genotypes under salt stress, but exogenous AsA (0.5 mM) application increased the accumulation by 28.25, 26.86 and 30.51% in Super gold, 900M gold and PS-999, respectively.

Chl contents

The present study showed that *Chl* contents (*Chl a* and *Chl b*) were decreased significantly by salt stress and exogenous AsA (0.5 mM) application in salt treatment remarkably increased the contents of both *Chl a* and *Chl b* in all genotypes (Table 1). However, 1.0 mM AsA decreased the *Chl* contents compared to those at 0.5 mM AsA.

Salinity decreased *Chl a* content by 23.18% in Super gold, 26.86% in 900M gold and 21.68% in PS-999 while exogenous AsA (0.5 mM) increased the content by 9.55, 15.30 and 16.81% in Super gold, 900M gold and PS-999, respectively, over salt treatment without AsA. In contrast, an increase of *Chl a* and *Chl b* in the application of 1.0 mM AsA was comparatively lower than in the application of 0.5 mM AsA. At the same time, salinity decreased the level of *Chl b* by 29.63% in Super gold, 35.50% in 900M gold and 42.01% in PS-999 whereas 0.50 mM increased the content by 41.59%, 50.44% and 55.50% in Super gold, 900M gold and PS-999, respectively, and 1.0 mM AsA increased the level by 32.66, 27.58 and 37.50%, respectively.

H_2O_2 and Malondialdehyde (MDA) contents

The levels of H_2O_2 and MDA amplified enormously in salt-stressed seedlings, but the exogenous application of AsA reduced the contents in all studied maize hybrids (Figure 2A and B). As compare to normal condition, the concentrations of H_2O_2 under salt-stress were higher by 106.97% in Super gold, 154.47% in 900M gold and 79.65% in PS-999, while the level of MDA increased by 134.21% in Super gold, 156.66% in 900M gold and 95.43% in PS-990. Application of 0.5 Mm AsA reduced the contents of H_2O_2 and MDA more efficiently than in the application of 1.0 mM AsA, although the significant difference was not found between the treatments. As compared to salinity, application of 0.5 mM AsA decreased the content of H_2O_2 by 20.85, 21.73 and 21.29% in Super gold, 900M gold and PS-999, respectively, while it decreased the content MDA by 33.36, 29.12 and 15.05% in Super gold, 900M gold and PS-999, respectively.

Non-enzymatic antioxidant contents

The present study showed that salt stress significantly reduced ascorbate content in the seedlings of maize hybrids compared to respective controls (Figure 3A). Exogenous application of AsA in saline treatment sharply increased the level (Figure 3A). The highest positive effect was observed with 1.0 mM AsA (119.35, 187.67 and 135.62% increment in Super gold, 900M gold and PS-999 respectively) application as compared to o.5 mM AsA (29.82, 81.30 and 116.85% in Super gold, 900M gold and PS-999, respectively) in all the maize hybrids.

Unlike AsA, DHA content increased dramatically under saline stress as compared to control in all genotypes (Figure 3B). On the other hand, DHA content reduced by 22.87% in 900M gold and 16.53% in PS-999 by 0.5 mM AsA while the content reduced by 37.72% in 900M gold and 21.02% in PS-99 by 1.0 mM AsA in compared to salinity treatment alone. In contrast, exogenous AsA increased DHA content in Super gold.

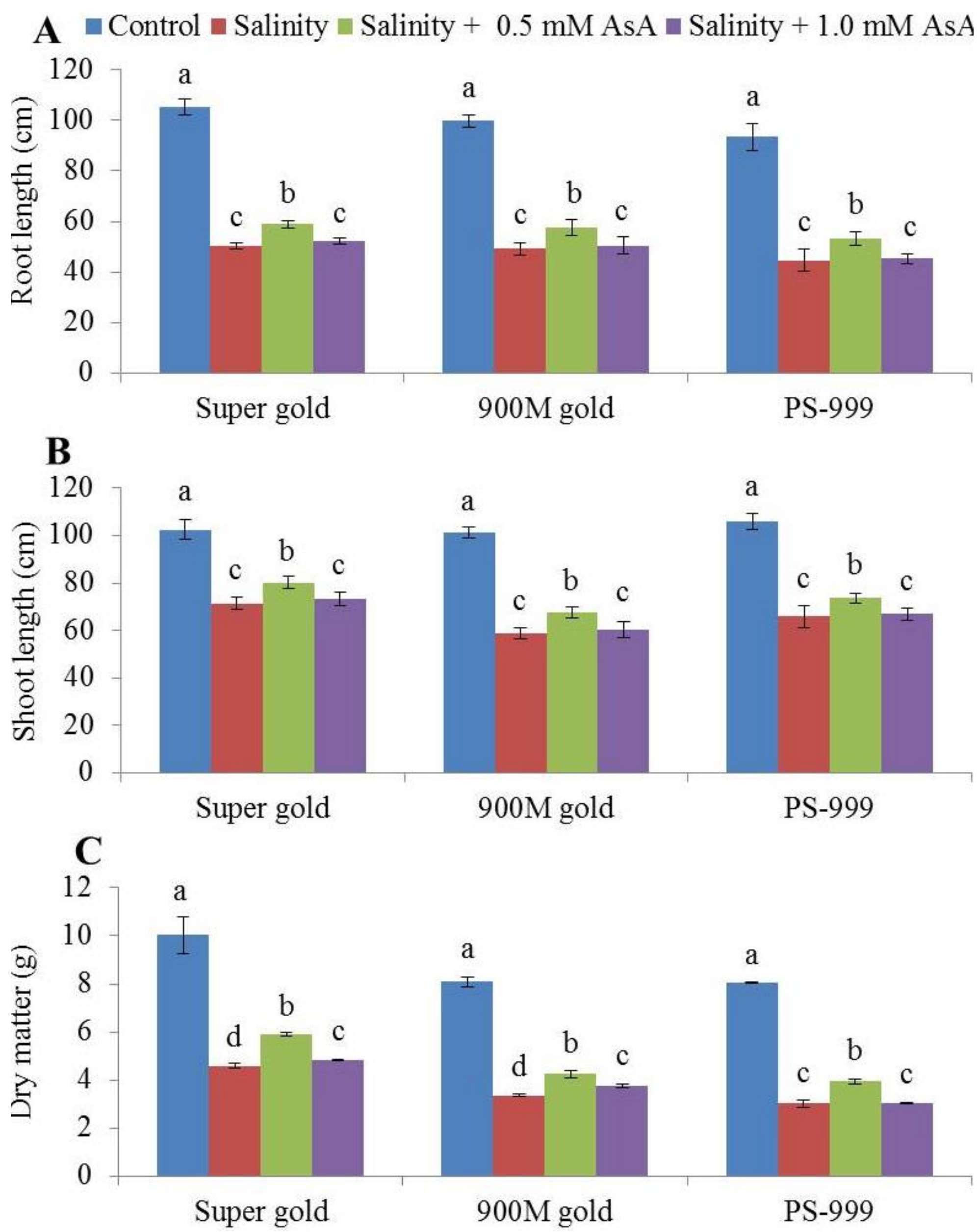

Figure 1. Effect of salinity and AsA on root length (A), shoot length (B) and dry matter accumulation (C) in maze seedlings. Bars denote mean ± SE. Dissimilar letters among different treatments within a variety are significant at P≤0.05%.

Table 1. Impact of AsA on *Chl* contents in leaves of maize hybrids grown under salt stress.

Treatments	*Chl a*			*Chl b*		
	Super gold	900M gold	PS-999	Super gold	900M gold	PS-999
Control	0.67 ± 0.038^a	0.71 ± 0.030^a	0.65 ± 0.029^a	0.88 ± 0.016^a	0.87 ± 0.025^a	0.90 ± 0.037^a
Saline	0.51 ± 0.033^c	0.52 ± 0.035^c	0.51 ± 0.049^c	0.62 ± 0.025^d	0.56 ± 0.039^d	0.52 ± 0.038^d
Saline + 0.5 mM AsA	0.56 ± 0.022^b	0.60 ± 0.050^b	0.60 ± 0.016^b	0.88 ± 0.036^b	0.84 ± 0.038^b	0.81 ± 0.030^b
Saline + 1.0 mM AsA	0.53 ± 0.026^c	0.53 ± 0.055^c	0.52 ± 0.019^c	0.82 ± 0.026^c	0.71 ± 0.058^c	0.71 ± 0.027^c

Data are mean of two independent experiments with three replicates ± SE. Dissimilar letters among different treatments within a variety are significant at P≤0.05%.

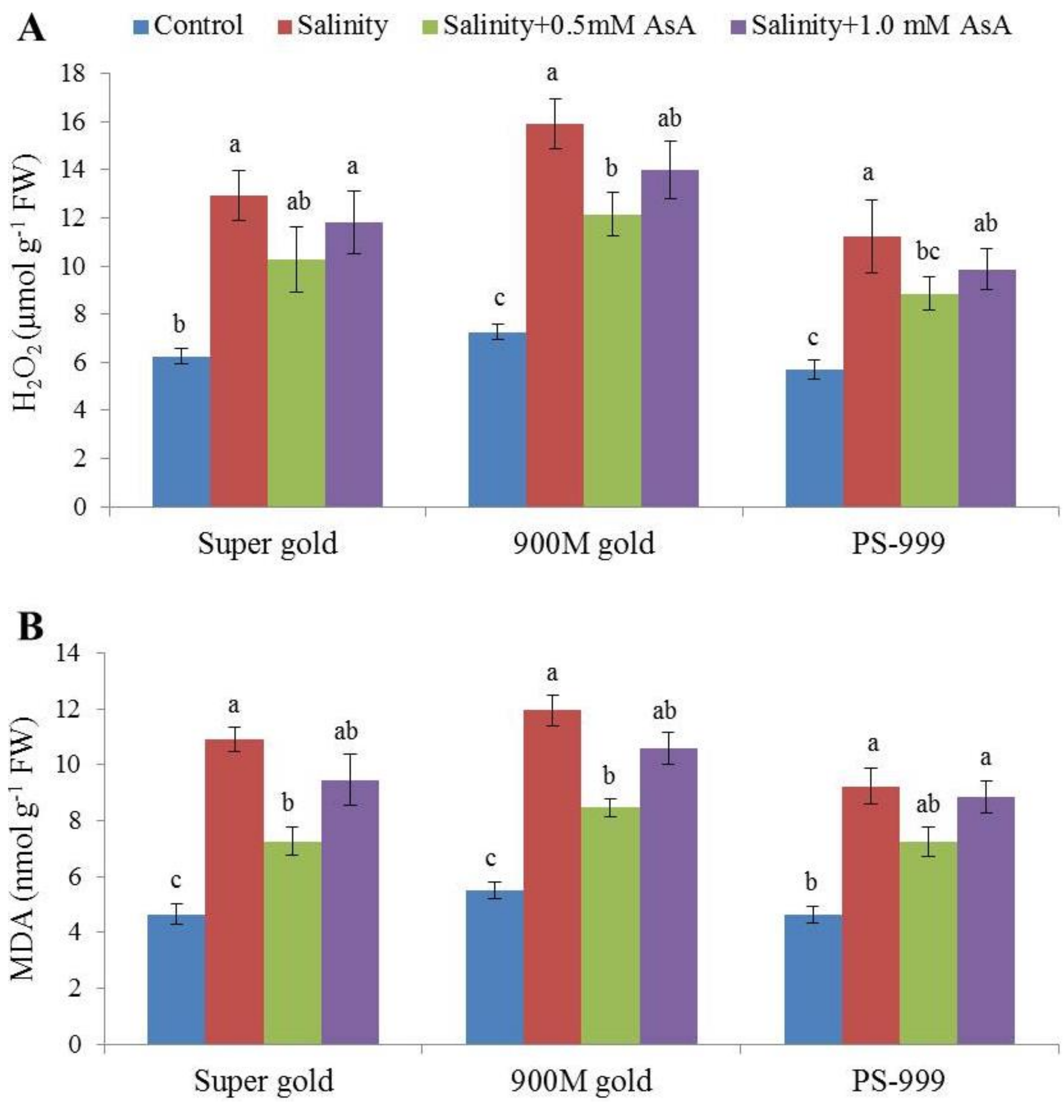

Figure 2. Effect of salinity and exogenous AsA application on H_2O_2 (A) and MDA (B). Bars denote mean ± standard error. Dissimilar letters among different treatments within a variety are significant at P≤0.05%.

Reduced glutathione (GSH) content decreased significantly by salinity in all maize hybrids under salt stress over control (Figure 3C). Due to the application of AsA, GSH improved remarkably in all genotypes. However, 1.0 mM AsA caused the highest increase in GSH by 60.37% in Super gold, 62.62% in 900M gold and 73.16% in PS-999 over the content under salinity.

The content of GSSG increased dramatically under saline conditions in compared to control in all three maize hybrids while exogenous AsA treatments resulted in a decrease in the content of GSSG (Figure 3D). Salt stress increased GSSG content by 305.16% in Super gold, 160.81% in 900M gold and 250.74% in PS-999 whereas, 0.5 mM AsA reduced the content by 33.54, 36.29 and 32.62% in Super gold, 900M gold and PS-999, respectively, and 1.0 mM AsA reduced the content by 39.40, 40.44 and 24.20%, respectively.

Activity of enzymatic antioxidant

Salinity caused the substantial diminution in the activity of APX in all of maize leaves hybrids. Application of AsA in saline solution improved the APX activity in all the genotypes (Figure 4A). Application of 0.5 mM AsA amended more APX activity than 1.0 mM AsA while compare to the stress condition, 0.5 mM AsA promoted the activity by 58.44, 52.29 and 40.63% in Super gold, 900M gold and PS-999, respectively. On the other hand, 1.0 mM AsA increased the activity by 38.61, 39.12 and 21.32% in Super gold, 900M gold and PS-999, respectively.

In the case of MDHAR and DHAR, the activities varied with genotypes in both normal and saline condition (Figure 4B and C). Salinity decreased the activity of MDHAR in Super Gold and 900M gold while it increased

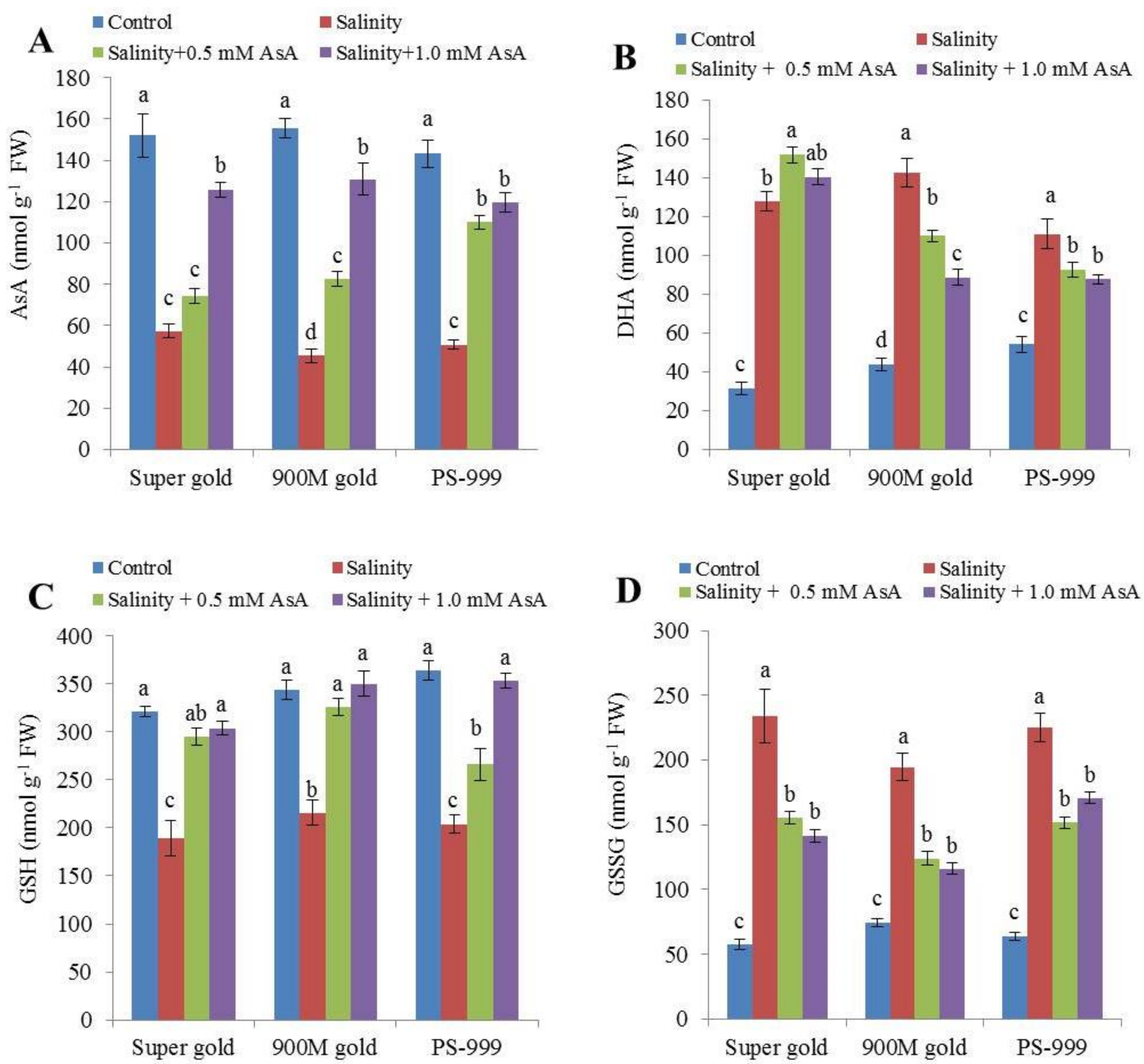

Figure 3. Effect of salinity and exogenous AsA application on endogenous AsA (A), DHA (B), GSH (C) and GSSG (D) contents. Bars denote mean ± standard error. Dissimilar letters among different treatments within a variety are significant at P≤0.05%.

the activity in PS-999. On the other hand, DHAR activity was decreased only in 900M gold. Application of 0.5 mM AsA increased MDHAR activity in Super gold whereas the activity increased with the concentration of AsA when compared to salt treatment. On the other hand, DHAR activity decreased in AsA treated seedlings of 900M gold and PS-999, but increased in seedlings of Super gold.

DISCUSSION

The results attained in the present investigation noticeably exhibited that the three maize genotypes revealed a remarkable decrease in root length, shoot length and dry matter accumulation under salinity (Figure 1A to C). However, exogenous AsA (0.5 mM) treatment significantly improved the all studied growth parameters (Figure 1), signifying useful role of AsA in maize under salinity. It is well recognized that Na^+ is a toxic element which hinders the different metabolic activities at higher concentration (Gul et al., 2015). Higher gathering of Na^+ and Cl^- ions in the cytoplasm due to noxiousness, the creation of ROS under salt-stress, nutritional imbalance and desiccation of the tissues via the low water potential are the four salt stress factors that affect the plant responses which caused by the intrusion of salty ions through crucial nutrients in fixation and translocation progressions (Arab and Ehsanpour, 2006). Moreover, during salt stress, osmotic and ionic effects decrease the plant growth (Munns, 2002; Ghoulam et al., 2002). Root

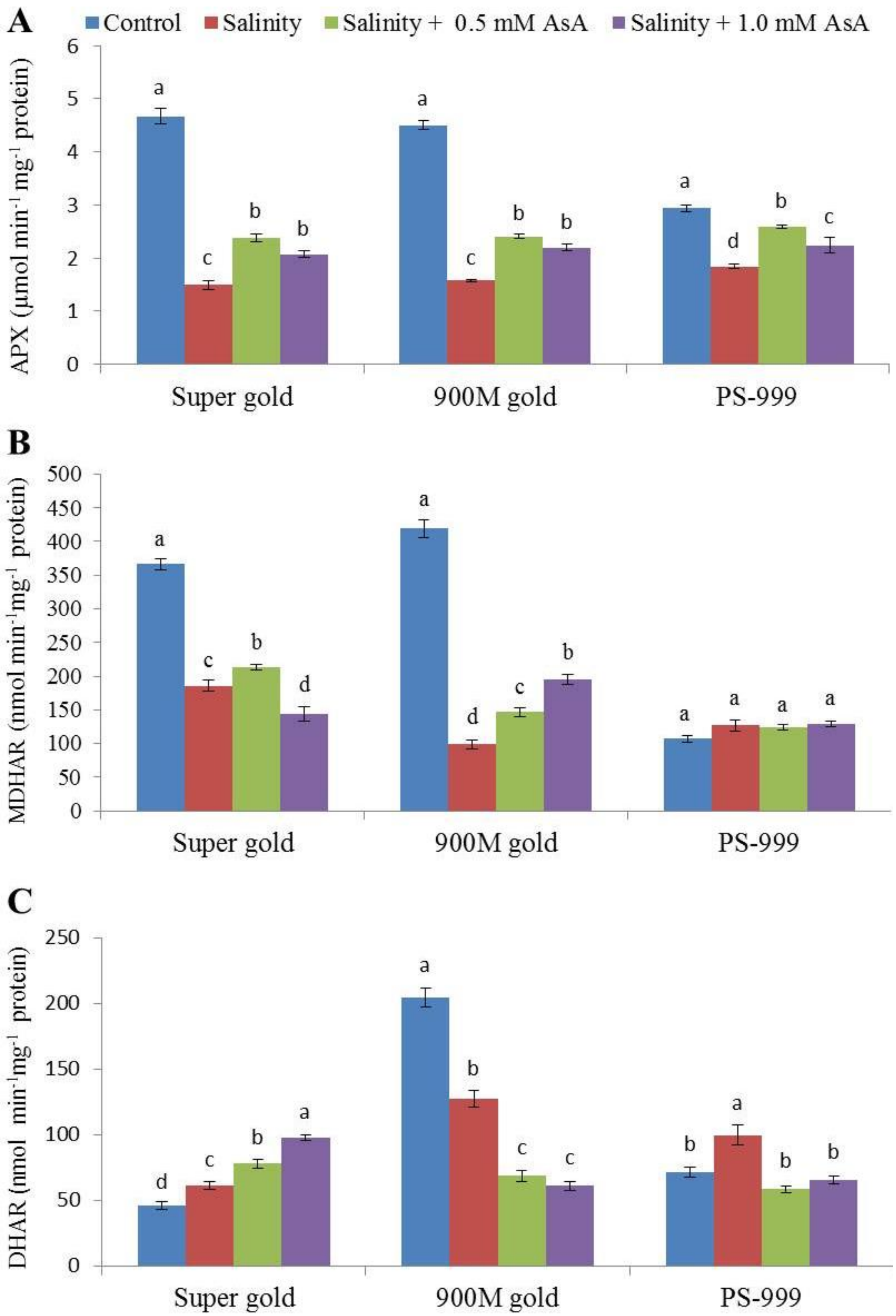

Figure 4. Effect of salinity and application of AsA on APX (A), MDHAR (B) and DHAR activity (C). Bars denote mean ± standard error. Dissimilar letters of different treatments for same variety on bars represent their comparative significance at P≤0.05%.

growth is sensitive to high salt concentrations solution (Akram et al., 2010) and roots are sharply reduced by salinity (Cramer et al., 1988; Ashraf et al., 2005). With increasing salinity, reduction in plant height is the distinctive influence of the toxic ions accumulation in cells which rigorously disturb cell division and expansion (Munns, 1993). Biomass accumulation was meticulously affected due to increasing salinity thus; fresh and dry weights were decreased (Akram et al., 2010). Similar result was shown from the previous experiment (Majeed et al., 2014; Hoque et al., 2015). Promotion of plant growth as a result of AsA treatment has also been described in several plant species (Tuna et al., 2013 in maize; Gul et al., 2015 in Guar; Ejaz et al., 2012 in sugarcane; Alhasnawi et al., 2015 in rice).

In the present observation, salt stress sharply reduced the photosynthetic pigment (*Chl a* and *Chl b*) whereas the exogenous treatment of AsA appreciably delayed the loss of chlorophyll contents of all maize hybrids (Table 1). The reduction in leaf *Chl* content probably decline due to biosynthesis or amplified degradation of chlorophyll at salt stress (Gul et al., 2015). The most probable reason for chlorophyll degradation is a production of proteolytic enzymes such as chlorophyllase (Zhao et al., 2007) and substantial accumulation of H_2O_2 (Tuna et al., 2013). Similar finding was investigated by Tuna et al. (2013). Therefore, our investigation revealed that exogenous AsA application significantly improved *Chl* content of maize hybrids (Table 1) which indicated that AsA protects photosynthetic pigment apparatus. This result was supported by the previous experiment of different crops (Beltagi, 2008 in chickpea; Khan et al., 2010 in Mustard; Rafique et al., 2011 in Pumpkin).

Salt stress is extensively described to enhance production of H_2O_2, which can diminish essential cell components (Gill and Tuteja, 2010). Our observation also showed that salinity increased the H_2O_2 levels while both concentrations of exogenous AsA application in salt stress reduced the H_2O_2 concentration of all hybrids (Figure 2A). Similar finding were documented by Dolatabadian and Jouneghani (2009) and Ebrahimian and Bybordi (2012). On the other hand, malondialdehyde (MDA) is the end-product of lipid peroxidation. Under salt stress, the level of lipid peroxidation is used as a pointer of free radical damage to cell membranes. Thus, MDA has been commonly designated as assortment to measure the salt stress injury (Katsuhara et al., 2005; Jaleel et al., 2007). In the present study, application of AsA in maize seedlings decreased the salt induced MDA content (Figure 2B) suggesting that exogenous application of AsA is useful in reducing the cell damage.

Ascorbate plays manifold role related to plant growth, such as in cell division, cell wall expansion and other developmental progressions through molecular mechanisms (Asada, 1999; Pignocchi and Foyer, 2003). In our observation, salt stress significantly reduced AsA content which is improved due to AsA application in salt treatment (Figure 3A). Turan and Tripathy (2012) reported that salt stress reduced AsA contents in rice seedling and Sairam et al. (2005) also noted that AsA content in *Triticum aestivum* decreased in saline soil. In contrast, Yildiztugay et al. (2013) in *Sphaerophysa kotschyana* plant and Eltelib et al. (2012) in transgenic tobacco plant observed that endogenous AsA contents amplified at the saline condition. Augmented AsA concentrations in AsA treated plant could be as a result of its better biosynthesis and recovering through the Asada-Halliwell-Foyer pathway. Franceschi and Tarlyn (2002) documented that treated leaf cells improved 2 to 3 times of AsA in compares to the untreated sink cells/tissues of a plant. Endogenous AsA levels and Asada-Halliwell-Foyer pathway enzymes significantly increased due to the application of exogenous AsA in *Limonium stocksii* seedlings (Hameed et al., 2015) which support our present investigation.

In ascorbate-glutathione cycle, APX, DHAR and MDHAR are vital enzymes intricate in sustaining the AsA (Noctor and Foyer, 1998). In this cycle, APX reduced H_2O_2 to H_2O using ascorbate as the definite electron benefactor (Smirnoff, 2000). AsA is oxidized to DHA under oxidative stress (Noctor and Foyer, 1998). In our observation, DHA content significantly increased under saline condition over control and exogenous AsA application reduced the DHA stuffing (Figure 3B). Alternatively, salt-stress meaningfully decreased the APX activity of all maize hybrids in our observation and exogenous AsA positively improved APX activity (Figure 4A). Neto et al. (2006) noted that salinity reduces the activity of APX in roots organs of salt sensitive maize and upgraded the activity in salt endurance genotype. This result suggested that response APX fluctuates in different genotypes. MDHAR and DHAR are two crucial enzymes which are equally essential in regulating and regeneration of AsA level (Wang et al., 2010). In our study, MDHAR and DHAR activity showed fairly different response in different treatment and genotypes (Figure 4B and C). Sharp reduction was detected in MDHAR activity in Super gold and 900M gold under salt stress and PS-999 exposed significant increase over control. Super gold and 900M gold also displayed better response to AsA treatments. On the other hand, PS-999 almost did not show the response to AsA treatments. Rohman et al. (2016) demonstrated that MDHAR activity increased in tolerant maize inbreds while decreased in sensitive inbreds under salinity. However, AsA treatment increased the activity of MDHAR which assisted in a renewal of AsA in maize and detoxify the excesses H_2O_2 level.The result of the study agreed with observations of Ebrahimian and Bybordi (2012). In case of DHAR activity, Super gold exposed the increase of activity under salt stress and further promoted due to AsA treatment (Figure 4C). In contrast, 900M gold and PS-999 showed significant demotion in DHAR activity due to AsA application which may be responsible for lower regeneration of AsA and/or used other metabolic activities. Hossain et al. (2013) found their study that non-significance variation appearance in MDHAR activity and the noticeable increase in DHAR activity under saline condition.

The redox state maintained by GSH acts as a defensive role in salt tolerance (Shalata et al., 2001). Furthermore, the central action of GSH is to renew of ascorbate via reduction of DHA through DHAR activity in the antioxidant protection system (Noctor and Foyer, 1998). In our study, exogenous AsA noticeably increased GSH contents under salt stress (Figure 3C). Due to availability of exogenous AsA, GSH might not be used in AsA maintenance via DHAR activity. However, this higher GSH might be used by glutathione peroxidase (GPX, an important H_2O_2 decomposer). Nevertheless, amplified activity of DHAR in Super gold can use GSH. In the present study, GSSG contents meaningfully increased of all maize hybrids under salt stress and further decreased in the presence of AsA treatment (Figure 3D). The increased contents of GSSG under salt-stress in plant perhaps recognized to the reaction of GSH with oxyradicals formed by oxidative stress or diminished GR activity (Aravind and Prasad, 2005). Therefore, AsA application reduced the oxidation GSH. These findings were supported with earlier reports which exposed the intensification of the GSSG contents in salt treated plant (Rohman et al., 2016). However, APX conferred tolerance to all maize genotypes. The variation of MADAR and DHAR activity suggested that AsA mediated tolerance under salinity varies with maize genotypes.

Conclusion

In conclusion, it is clear that application of AsA as non-enzymatic antioxidant alleviated the adverse effect of salinity in maize hybrids and improved all growth and some biochemical attributes by regulating the antioxidative protection mechanisms. Therefore, AsA can performance efficiently in plants as medication when applied at the appropriate concentration during stress environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Identification of pulmonary lesions in slaughtered cattle and associated risk factors, North West Ethiopia

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A gross pathological examination of pulmonary lesions on 399 cattle slaughtered at Gondar ELFORA abattoir was conducted on in the period November, 2011 to March, 2012 with the objectives of estimating the frequency of various pulmonary lesions and the associated risk factors and gross lesions were screened and the most encountered lesions were emphysema, pale and dark red to dark gray color of the lung, hepatization, pleurisy, congested lung and abscess at different parts of the lung. The frequency of gross lesions identified were pneumonia (5.5%), hydatidosis (5.0%), emphysema (4.0%), calcified lung (1.5%), abscess (1.5%), congested lung (2.5%), pleurisy (1.3%) and hemorrhagic lung (1.5%) with an overall prevalence of 91(22.8%). The prevalence of gross lesions in adult and old animals was 17.8 and 26.3% respectively. A prevalence of 25.2, 22.4 and 20.4% were seen in poor, moderate and good body conditioned animals respectively. The prevalence of gross lesions in local breed was 23.4 and 20.0% in cross breeds. Therefore, in the current study, a significant number of gross pathological lesions were identified in cattle slaughtered in the abattoir and many organs were condemned as a result of the organ damage and a risk for public health.

Key words: Gross lesions, prevalence, cattle, abattoir.

INTRODUCTION

Ruminants represent an important segment of the Ethiopian livestock system. The national livestock population of Ethiopia is the largest in Africa and is estimated to be 49.02 million cattle, 26 million sheep, 21 million goats, 1.79 million horses, 5.42 million donkeys, 335 thousand mules, 760 thousand camels, 38.13 million chickens and 5.15 million beehives in the country (CSA, 2008). Diseases that occur in livestock have a major impact on large scale abattoirs where there is large number of animals' slaughtered and large number of workers present. In addition to the risks on them, abattoirs have high responsibility to provide risk free and wholesome products to the society (Marta, 2010). Meat inspection is commonly perceived as the sanitary control

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of slaughter animals and meat. The aim of meat inspection is to provide safe and wholesome meat for human consumption. The responsibility for achieving such goals lies primarily with the relevant public health authorities, who are represented by veterinarians and meat inspectors at the abattoir stage (FAO, 2007).

The purposes of meat inspection, comprising of ante mortem and postmortem examination are to remove gross abnormalities from meat and its products, prevention and distribution of contaminated meat that could result to disease risk in man and animals and assisting in detecting and eradication of certain diseases of livestock (Van Longtestijn, 1993). It is necessary to be aware of the extent to which the public is exposed to certain zoonotic diseases detected in abattoirs and the financial losses through condemnation of affected organs and carcass (Nfi and Alonge, 1987). Postmortem inspection is the center around which meat hygiene revolves since it provides information indispensable for the scientific evolution of clinical signs and pathological processes that affect the wholesomeness of meat (Libby, 1975; Gracey et al., 1999). Disease causes extensive financial wastes as a result of direct and indirect economic losses is the major concern to livestock industry.

Study conducted in different abattoirs of Ethiopia revealed that parasitic infection of livers, lungs (pneumonia), pericarditis and pyelonephritis are found to be the major causes of organ condemnation, with an approximate annual loss of 2.7 million ETB at Debre Zeit HELIMEX abattoir (Jibat, 2006); 106,788.18 ETB in Gondar municipal labattoir (Yimam, 2003); 180,942.4 ETB in Bahir Dar Municipality Abattoir (Yohannes, 1994). Cattle are affected by lung diseases that can be caused by different factors depending on the different causative agents. Of which, metabolic disturbances are the major causes which facilitate the lung diseases such as pulmonary calcification (Andrews et al., 2004), abscess (Radositis et al., 2007), pulmonary emphysema (Jubb et al., 2007), pulmonary congestion (Radostitis et al., 2007), pneumonia (Bradford, 1996), hydatidosis (Kassai, 1999). Therefore, this study was aimed at estimating the prevalence of pulmonary lesions on cattle slaughtered at Gondar ELFORA and associated factors associated to the major lesions encountered.

MATERIALS AND METHODS

Study area

The study was conducted at Gondar ELFORA abattoir, located in Northwest Ethiopia in the period November, 2011 to March, 2012. The animals slaughtered in Gondar ELFORA abattoir were brought from different areas such as; Fogera, Wogera, Chiliga, Dabat, Belesa, Gaint, and Wollo. The abattoir is located in the capital of North Gondar Zone of Amhara Regional State which is located at 748 km away from Addis Ababa (CSA, 2008).

Study animals and sampling methods

A total of 399 cattle were identified for ante mortem and postmortem inspections. The specific identification numbers, age, breed, body condition and any abnormalities were recorded. The study animals were selected by simple random sampling method. Determination of the sample size for the prevalence of lung lesion was considered by assuming an expected prevalence of 50% to get the maximum number required (Thrusfield, 1995), with 95% confidence interval and at 5% absolute precision. Therefore, at 50% expected prevalence, a sample size of 399 was included in the study.

Ante mortem examination

During ante mortem examination, detail records about breed, age, body condition of the animal and any abnormality on the animal particularly associated with the lungs were recorded. The age estimation was based on dentations and owner's information.

Post mortem examination

During postmortem examination, lungs were macroscopically inspected for the presence of any gross lesions by applying routine meat inspection procedures which consisted of primary examination by visualization of the organ followed by a secondary examination which involved further palpation and incisions.

Data analysis

Data generated from ante mortem examination, postmortem examination were entered in to Microsoft excel worksheet and was analyzed using Statistical Package for Social Sciences (SPSS version 17).

RESULTS

Frequency of lung lesions in slaughtered animals

From a total of 399 bovine lungs (n=133 adults and 266 older animals) examined, 91(22.8%) were found to have different lung lesions. The identified pulmonary lesions were found to fall into eight different pulmonary gross lesions with a respective prevalence rate of 5.5% (pneumonia), 5% (hydatid lungs), 4.0% (emphysema), 2.5% (congestion), 1.5% (calcification), 1.5% (abscess), 1.5% (hemorrhage) and 1.3% (pleurisy). Generally, the highest frequency of lung lesions examined grossly was pneumonia while the lowest rate was observed as pleurisy.

Prevalence of gross pulmonary lesions

Higher prevalence of lung lesion was recorded in old than in adult animals 26.3 vs. 15.8%. The difference in the prevalence rate between the two age groups was statistically significant ($P<0.05$). The prevalence of

Table 1. The prevalence rate of different lung lesions on the basis of age (P<0.05).

Age	N	No. of animals positive for each gross lesions and their percentage							
		Pneumonia	Emphysema	Abscess	Calcification	Hydatidosis	Congestion	Pleurisy	Hemorrhage
Adult	133	6 (4.5)	6 (4.5)	2 (1.5)	3 (1.1)	1 (0.8)	2 (1.5)	-	1 (0.8)
Old	266	16 (6.0)	10 (3.7)	4(1.5)	3 (2.3)	19 (7.1)	8 (3.0)	5 (1.8)	5 (1.8)
Total	399	22 (5.5)	16 (4.0)	6 (1.5)	6 (1.5)	20 (5.0)	10 (2.5)	5 (1.3)	6 (1.5)

Table 2. Prevalence of each gross lesion of the lung on body condition base (P<0.05).

BCS	N	No. of animals positive for each gross lesions and their percentage (%)							
		Pneumonia	Emphysema	Abscess	Calcification	Hydatidosis	Congestion	Pleurisy	Hemorrhage
Poor	135	6(4.44)	7(5.18)	3(2.22)	2(1.48)	7(5.18)	4(2.96)	3(2.22)	2(1.48)
Moderate	161	13(8.07)	5(3.12)	2(1.24)	-	9(5.59)	4(2.48)	-	3(1.86)
Good	103	3(2.9)	4(3.88)	1(0.97)	4(3.88)	4(3.88)	2(1.94)	2(1.94)	1(0.97)
Total	399	22(5.51)	16(4.01)	6(1.50)	6(150)	20(5.01)	10(2.50)	5(1.25)	6(1.50)

Table 3. Occurrence of gross lesions of the lung on breed base (P<0.05).

Breed	Examined animals	No of animals positive for each gross lesion and their percentage (%)							
		Pneumonia	Emphysema	Abscess	Calcification	Hydatidosis	Congestion	Pleurisy	Hemorrhage
Local	334	17 (5.1)	14 (4.2)	5 (1.5)	6 (1.8)	20 (6.0)	8 (2.4)	5(1.5)	3(0. 9)
Cross	65	5 (7.1)	2 (3.1)	1 (1.3)	0	0	2 (3.1)	0	3(4.6)
Total	399	22 (5.5)	16 (4.0)	6 (1.5)	6 (1.5)	20 (5.0)	10 (2.5)	5 (1.3)	6 (1.5)

different lung lesions falling into eight types was studied in relation to the age of animals. In adult animals, the prevalence rate of 4.5% pneumonia 4.5% emphysema, 1.5% abscess, 1.1% calcification, 0.8% hydatidosis, 1.5% congestion and 0.8% hemorrhage was recorded while the rate in old animals was 6.0% pneumonia, 3.7% emphysema, 1.5% abscess, 2.3% calcification, 7.1% hydatidosis, 3.0% congestion, 1.8% pleurisy and 1.8% hemorrhage (Table 1). The variation in the prevalence of the different types of gross lesions of the lungs in animals with both age group was statistically significant (P<0.05).

In addition, the occurrence of bovine lung gross lesions among the animals of different body conditions was studied and the highest rate was recorded in animals with poor body condition (25.18%) followed by those with moderate (35.44%) and good (20.38%) body conditions. The difference in the prevalence rates with different body condition was statistically significant (P<0.05). The occurrence of different gross lesions of the lung in animals with different body condition score was studied and provided in Table 2.

The occurrence rate of bovine lung gross lesions among animals of different breed was studied and the highest rate was recorded in animals of local breeds (23.35%) than cross (20%) breeds. The difference in prevalence rate of the two breed was statistically significant (P<0.05). And the prevalence of the different gross lesions was studied in relation to the breed of animals indicated in Table 3. The variation in the prevalence of the different gross lesions of bovine lung in animals with different breed was statistically significant (P<0.05) (Table 4).

DISCUSSION

In this study, the overall prevalence of gross lesion of the lung revealed 22.8%. An association was observed between the age of the animal and gross lesion findings with the highest rate in old 26.3% and lower in adult 15.8% animals (P<0.05). This might be associated reduction of developing immunity as the animals become old which makes the animal more susceptible to a variety of infection or favors for pulmonary lesion formation. From the older animals examined with gross lesions, slightly highest rate was recorded with hydatidosis 19(7.1%) which is lower than the previous findings who reported (27.2%) hydatidosis, (6.1%) pneumonia, (3.7%)

Table 4. Prevalence of bovine gross pulmonary lesions in relation to age, BCS and breed of animals.

Parameter	Risk factor	N	No. of positive	Relative prevalence	P-value
Age	Adult	133	21	15. 8	0.01
	Old	266	70	26.7	
BCS	Poor	135	34	25.2	0.0
	Moderate	161	36	22.4	
	Good	103	21	20.4	
Bred	local	334	78	23.4	(0.04)
Total		399	91	26.5	

emphysema, (3.0%) congestion, (1.8%) pleurisy and hemorrhage, (1.5%) abscess (Yetnayet, 2010) and another study showed a prevalence of abscess (11.5%) and calcification (1.1%) (Kedir, 2010). This study has also shown significant (P<0.05) association among the three body condition scores on animals and gross lesion findings with highest rate recorded in poor body condition (25.2%), followed by moderate (22.4%) and good (20.4%) body conditioned animals. Such a slightly increased prevalence in poor body condition animals could be due to poor nourished animals would be less competent to fight against infections (Radostits et al., 2007). This study has shown statistically significant (P<0.05) association between the two breeds of animals which revealed a slightly higher prevalence in local breed animals (23.4%) and slightly lower in cross breeds (20.0%) and such variation in prevalence might be due to the difference in the micro environment of the animal origin, parasitic infections, difference in feed quality and the chance of exposing to the various risk factors is different. This study has shown moderate prevalence of pulmonary lesions in cattle slaughtered at Gondar ELFORA abattoir. Identified gross lesion during the study were emphysema, pneumonia, hydatidosis, congestion, abscess, hemorrhage, calcification and pleurisy. Therefore, taking this as base line information further histopathological examinations should be undertaken to characterize the root cause of the lesions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seasonality influence the nutrient content of litter fall in secondary forest in the Amazonian

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Secondary forest vegetation in the Bragantina area, Northeast of Pará State, is characterized by the abandonment of anthropized forests, locally known as capoeiras, in different successional stages. The current study aims to evaluate the nutrient concentrations in order to identify the treatment that have caused greater nutrient deficiency due to full and partial litter spacing well as to assess the capoeira with best nutrient cycling performance. The study area is located in 10-and-40-year-old secondary forests in Bragança County, Benjamin Constant community, Northeastern Pará State. Litter samples were collected during the dry and rainy seasons. The chemical analysis of the macro and micronutrients were further performed. The decreasing order of the herein observed nutrient concentration was N> Ca> Mg> In> K> P. By taking under consideration the 40-year-old capoeira and the treatments applied to the 10-year-old one (no thinning (NT), partial thinning (PT), and total thinning (TT)), it is possible to state that the highest concentrations in all analyzed elements showed the same descending order in micronutrient concentrations: Fe> Mn > Zn> Cu. The different-aged capoeiras within the Bragantina area showed that not all nutrients were influenced by seasonality, despite the applied thinning type, the vegetation age and the forest species.

Key words: Cycling, nutrients, amazon, Northeastern Pará, Capoeira, thinning.

INTRODUCTION

The increased land abandonment after agricultural use as well as the opening of new forest areas form a landscape mosaic in different successional stages. It is necessary to conduct nutrient cycling studies due to the

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importance and extent of this vegetation type, since the limited nutrient availability is one of the main factors influencing the recovery dynamics of the different successional stages, after agricultural use abandonment. Kauano et al. (2012) valuated that 83% of the total area of this conservation are dominated by forests, of which 68.5% was by the Dense Ombrophilous Forest physiognomy, and 9.5% refer to secondary forests.

Forty-three percent (43%) out of the total capoeira areas in Brazil belong to Pará State and 37% of them belong to Tocantins State. The other states in order of importance are Amazonas and Rondônia (both with 7%), Roraima (3%), Acre (2%) and Amapá (1%). The total capoeira area corresponds to scale 8% of the total lands and agriculturally and 17% of the lands used in agricultural activities and forestry in the Amazon region (Costa et al., 2006). Although secondary forests are considered to be a partially degraded vegetation, it does not mean that they are unsuitable for agricultural or forestry activities, since they have social, economic and environmental values.

Plant micronutrients, which include B, Cl, Cu, Fe, Mn, Mo, Ni and Zn are required by plants at very low concentrations for proper growth and reproduction. However, despite their concentrations within the tissues and organs of plants, micronutrients have the same importance of macronutrients for their nutrition. In these low concentrations, micronutrients are key to growth and the development of plants, acting as constituents of the walls (B) and cell membranes (B, Zn), as constituents of enzymes (Fe, Mn, Cu, Ni), as enzyme activators (Mn, Zn) and photosynthesis (Fe, Cu, Mn, Cl) (Kirkby et al., 2007)

Information on the distribution and flow of nutrients in different forest compartments are obtained by studies focused on nutrients cycling within the ecosystem (Laclau et al., 2010; Viera and Schumacher, 2010). Some works involving deposition of litter and cycling of nutrients in secondary succession have been developed in the various phytophysiognomies of the Dense Ombrophilous Forest.

The total transfer of macronutrients to the soil through forest litter deposition was studied by Schumacher et al. (2008) and they found the following values: 86.14 Ca; 81.81 N; 14.42 Mg; 13.71 K; 7.16 S and 5.54 P in kg ha^{-1} and the transfer of micronutrients showed 20069.10 Mn, 3278.83 Fe, 428.30 Zn, 180.12 B, and 49.98 Cu in g ha^{-1}. Ca (among the macronutrients) and Mn (among the micronutrients) showed the highest concentrations among the nutrients that have returned to the soil through litter deposition.

Nutrients concentration in plants changes according to vegetation age, sampling time and to the interaction between nutrients and nutrient availability in the soil (Schonau, 1983). The current study evaluated seasonality (rainy and dry seasons) and vegetation age (10-and-40-year-old capoeiras) as important variables affecting nutrients concentration in the litter.

Nutrient cycling studies are important to preserve the natural systems and their sustainability as well as to evaluate the impacts on the environment caused by nutrient losses in the litter. The ecosystem fragility may be understood as the balance between the nutrients and their quantity. It is worth highlighting the cycling efficiency. The current study aimed at evaluating the nutrients concentration, identifying the applied treatment that showed stronger nutrient deficiency due to full and partial litter removal as well as (delete) assessing the vegetation age with the best nutrient concentrations.

MATERIALS AND METHODS

The current study was conducted in Bragança County, Benjamin Constant community, east of Bragantina region and 25 km southeast from Bragança County (Rios et al., 2001) at 01°11'22" S and 46°40'41" W. Bragança County is located in a slightly wavy lowland area formed by sediments, with maximum slope of 26 m. Our area of interest was the family farmer properties, called Agricultural Units (AU), with total area 150 ha.

Two capoeiras aged 40 and 10 years were selected in the Agricultural Unit. They are approximately 400 m distant from each other and have the same history of use and the same physiographic conditions. They were derived from successive cutting and burning, planting and fallow cycles of cotton (*Gossypium hirsutum*), rice (*Oryza sativa*), beans (*Phaseolus vulgares*), tobacco (*Nicotiana tabacum*), cassava (*Manihot esculenta*) and maize (*Zea mays*) cultures.

The climate in the region is classified as Aw according to Köppen (Rocque, 1982). The mean annual temperature is 26°C, with relative humidity ranging from 80 to 91%. Annual rainfall ranges from approximately 2200 to 3000 mm. Insolation is between 2200 and 2400 h/year.

The soil in Bragantina region is predominantly dystrophic yellow latosol (DYL). It is medium textured and described as deep with advanced evolution, quite weathered, with relative concentrations of resistant clay minerals (EMBRAPA, 1999).

By considering the original cover, the predominant vegetation in Bragantina region is the Equatorial Rain Forest. Nowadays, the main original vegetation types in Bragantina region (primary dryland forest, flooded and igapó forest, dryland and mangrove fields) are very limited and sparse. The predominant landscape is featured by secondary vegetation at different ages, with different degrees of plant succession, agricultural crops and pasture areas (Vieira et al., 2007) derived from successive cutting and burning, planting and fallow cycles.

Experimental design

Six quadrangular-shaped Permanent Monitoring Plots (PMP) with 50 × 50 m (0.25 ha) dimension were systematically installed in a 40-year-old managed secondary forest. These plots were divided into twenty-five (25) 10 × 10 m subplots with no silvicultural treatment application. The experimental design in the 10-year-old capoeira was done in blocks and it was divided into plots measuring 20 × 40 m (800 m^2) each.

Thinning application in two intensities was considered to be the silvicultural treatment where treatment 0 (T0): control plot with no thinning and used as witness; treatment 1 (T1): moderate thinning intensity with partial vegetation cutting. Individuals under the canopy or those which canopy touched the selected plant were cut in order to completely release the plant; treatment 2 (T2): heavy

Table 1. Nutrient concentrations in the 10-year-old and in the 40-year-old capoeiras, Benjamin Constant community, Bragança – PA, 2009.

Capoeira	Period	Element					
		N	P	K	Ca	Mg	Na
CAP40	Rainy	16.55[a]	0.23[abc]	0.67[a]	6.57[a]	2.74[ab]	2.5[a]
	Dry	17.9[ab]	0.25[abc]	0.99[a]	6.07[a]	3.88[c]	5.29[b]
PT10	Rainy	17.64[ab]	0.25[abc]	0.48[a]	8.46[a]	1.86[a]	2.26[a]
	Dry	18.31[ab]	0.21[a]	1.47[a]	9.17[a]	2.78[ab]	5.87[b]
TT10	Rainy	16.81[a]	0.26[abc]	0.64[a]	8.39[a]	1.83[a]	2.21[a]
	Dry	19.52[b]	0.33[bc]	2.74[b]	15.75[b]	3.35[bc]	7.76[c]
NT10	Rainy	17.79[ab]	0.35[c]	0.68[a]	8.35[a]	2.15[a]	2.71[a]
	Dry	18.35[ab]	0.22[ab]	1.43[a]	10.23[a]	3.21 [bc]	5.86[b]

Means followed by lowercase letters on the rows do not statistically differ from each other according to the F and Tukey tests at 5% probability. CAP: capoeira; NT: no thinning; PT: partial thinning; TT: total thinning.

thinning intensity, the entire vegetation was cut in this plot. The entire woody vegetation (height ≥ 50 cm) was cut 30 cm above the ground.

The litter samples were collected during March (rainy season) and November (dry season) 2009, by using a square collector (0.25 × 0.25 m^2) placed directly on the ground and withdrawn after each collection. The litter samples were stored in paper bags while they were still in the field. Then, they were placed in the oven at 20°C for 72 h. After which they were dried and were separately ground in a Wiley mill. They were then placed in plastic vials and stored in a dry place.

Laboratory analysis

Samples were taken to the laboratory and divided into 12 groups. Each sampling group had three replications collected from each subplot. Regarding the nutrients analysis, 2 g of plant material were weighed and homogenized in order to be analyzed according to the methods by EMBRAPA (1999).

The classic Kjeldahl method was used to determine Nitrogen as described by Tedesco et al. (1985). The elements P, K, Ca, Mg, Na, Cu, Mn, Fe and Zn were determined by the samples' acid digestion, using nitric acid and perchloric acid at 180°C for 45 min in order to obtain limpid digestates. After the acid digestion and the distilled-water process, ammonium vanadate and ammonium molybdate solutions were used. Na and K readings were done by flame spectrophotometer. Ca, Mg, Cu, Mn, Fe and Zn readings were done by atomic absorption (SpectrAA 220, Varian, Palo Alto, CA, USA), according to the methodology by Embrapa (1997). These readings were carried out by directly determining the elements in the nitric-perchloric extract, using hollow-cathode lamp without interference or ionization problems.

Statistical analysis

The litter nutrient content values were subjected to the following statistical treatments in order to obtain greater representation in the result analyses: normality analysis by adhesion test (KS) at 5% significance level; variance analysis (ANOVA) with Tukey's test at 5% significance level and regression analyses were adopted to compare the means. Multivariate data analysis was conducted using the Principal Components (PCs) technique, which used the correlation matrix among variables to form the components. The first two components, that is, those with the biggest explained variance (Hair et al., 2009) were taken under consideration for interpretation purposes. The Minitab software version 17 was used.

RESULTS AND DISCUSSION

The mean macronutrient concentrations in the 40-year-old capoeira litter regarding the N, P, and Ca elements did not show significant variations (Tukey, $p < 0.05$) among each other in the rainy and dry periods (Table 1). However, they showed different concentrations among elements. The nutrients N and Ca showed higher macronutrient concentrations (Figure 1) than the other analyzed elements. The transfer magnitude of all macronutrients showed the following order: N>Ca>Na>Mg>K>P.

The macronutrient concentrations in the 10-year-old capoeira (NT) showed the following descending order in nutrient concentration in the non-thinned capoeira: N>Ca>Na>Mg>K>P. As for the rainy and dry peridos, P alone showed the highest concentrations during the rainy season and all other elements presented the highest concentrations during the dry season. The nutrients P, Mg and N showed statistically significant difference (Tukey, $p < 0.05$), as shown in Table 1.

As for the multivariate data analysis using the principal components technique, it is possible to see that the principal component 1 (PC1) showed eigenvalue equals to 3.1716 in the rainy season. This value corresponds to 31.7% explained proportion of the total variation. Principal component 2 (PC2) showed eigenvalue equals to 1.7382, thus corresponding to 17.4% of the total data variation. Much of the evaluated nutrients presented positive

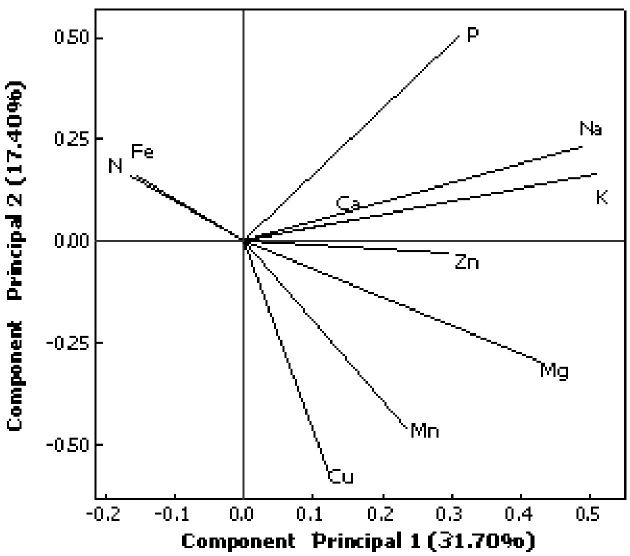

Figure 1. Variables' loads based on the principal components, in different system types. CAP: capoeira; PT: Partial thinning; TT: Total thinning; NT: no thinning, during the rainy season.

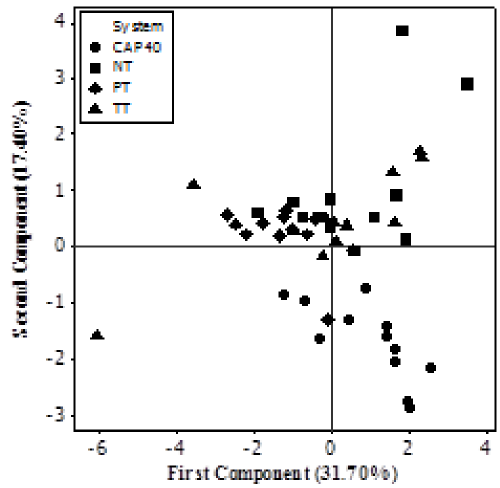

Figure 2. Variables' scores based on the principal components, in different system types. CAP: capoeira; DP: Partial thinning; DT: Total thinning; SD: no thinning, during the rainy season.

correlation with PC1, except for N and Fe (Figure 1).

Among these variables, K, Na, and Mg showed the most positive relationship with this component. Thus, by assessing the score graphic (Figure 2), one can see the association between the PC1 and the CAP40 system as well as the difference to other systems; fact that was not observed in N, K, Ca and N (rainy season) through the univariate analysis. PC2 showed bigger P representation (Figure 1). Therefore, its association with the NT10 system (Figure 2) was partially demonstrated by the univariate analysis (Tukey test), as shown in Table 1.

The principal components analysis showed different results in the dry and rainy seasons. PC1 presented eigenvalue equal to 3.2703 and it corresponded to 32.7% explained proportion of the total variation. PC2 presented eigenvalue equal to 1.4295, thus corresponding to 14.3% of the total data variation. PC1 is not represented by most of the nutrients in this analysis, but by Fe (Figure 3).

Thus, it is not possible to clearly see the distinction between systems when it comes to PC1, except for the TT10 system, which has negative correlation with this component. In fact, Fe concentration in the TT10 system litter is lower than that observed in the other systems, except for PT10, as shown by the Tukey's test (Table 2). In addition, the TT10 system also showed an overall greater nutrient availability than the other systems, thus justifying the scores disposition (Figure 4).

The 10-year-old capoeira, where the PT treatment was applied, showed the highest nutrient concentration values for N during the dry season, and the analyzed elements showed nutrient concentrations that met the following descending order: N> Ca> In> Mg> K> P. All of them showed the highest concentrations during the dry season,

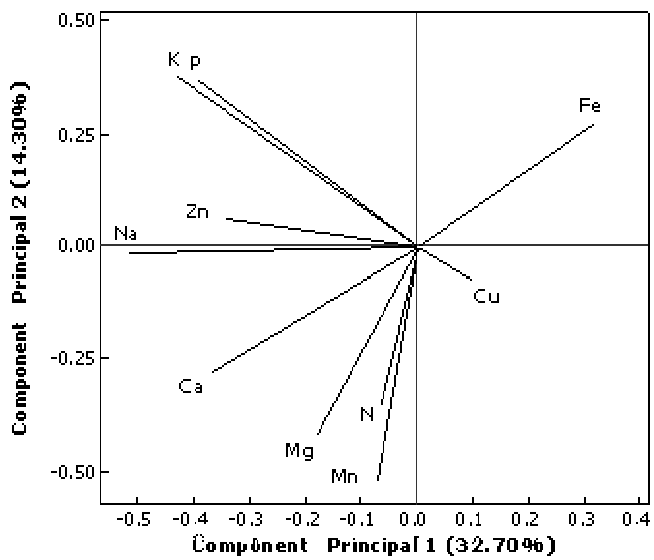

Figure 3. Variables' loads based on the principal components, in different treatment. CAP: capoeira; DP: Partial thinning; DT: Total thinning; SD: No thinning, during the dry season.

except for P, which showed higher concentrations during the rainy season.

The P nutrient showed the highest and the lowest nutrient concentrations in the (NT) area during the rainy and the dry seasons, respectively. Already, K nutrient showed the highest concentration in the (TT) area during the dry season and the lowest concentration in the (NT) area during the rainy season. The Ca nutrient showed the highest concentration in the (TT) area during the dry

Table 2. Micronutrient concentrations in the 10-year-old and in the 40-year-old capoeiras, Benjamin Constant community, Bragança – PA, 2009.

Capoeira	Period	Element			
		Cu	Mn	Fe	Zn
CAP40	Rainy	26.74[b]	368.49[bcd]	1015.77[abc]	26.01[abc]
	Dry	25.22[b]	339.01[abc]	1419.61[ab]	28.47[bc]
PT10	Rainy	15.57[a]	228.73[ab]	1644.82[b]	18.1[a]
	Dry	12.94[a]	291.58[abc]	899.89[ac]	21.13[ab]
TT10	Rainy	14.39[a]	153.02[a]	1146.11[abc]	33.16[c]
	Dry	14.28[a]	484.07[cd]	481.98[c]	32.13[c]
NT10	Rainy	12.71[a]	260.48[ab]	1456.52[ab]	24.38[abc]
	Dry	13.05[a]	558.18[d]	1471.46[ab]	21.1[ab]

Means followed by lowercase letters on the rows do not statistically differ from each other according to the F and Tukey tests at 5% probability. CAP: capoeira; NT: no thinning; PT: partial thinning; TT: total thinning.

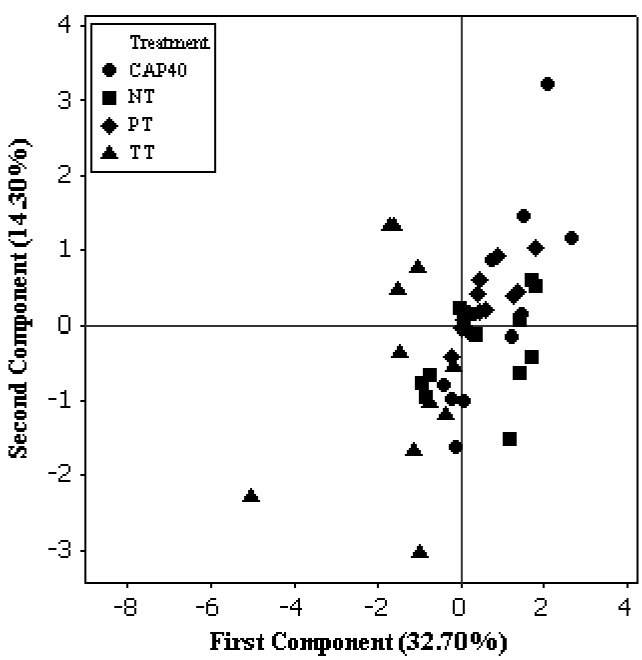

Figure 4. Variables' scores based on the principal components, in different treatment types. CAP: capoeira; DP: Partial thinning; DT: Total thinning; SD: No thinning, during the dry season.

season and the lowest concentration in the (NT) the rainy season. As for Mg, the highest and the lowest concentration values were found in the (TT) area during the dry and the rainy seasons, respectively. Na showed the highest and the lowest concentration values in the (TT) area during the dry and the rainy seasons, respectively.

Overall, the nutrient concentrations of each element in each treatment was presented in descending order as follows: N: TT>NT>PT>CAP40; P: NT>TT>CAP40>PT; K: TT>NT>PT>CAP40; Ca: TT>NT>PT>CAP40; Mg: CAP40>TT>NT>PT; Na: TT>NT>PT>CAP40; N, K, Ca and Na presented the same nutrient concentration order. The 40-year-old capoeira showed the lowest N, K, Na, and Ca concentrations, and the highest Mg concentration.

By considering the year the experiment was performed, the annual mean (2.323 mm) and the mean rainfall of the last 20 years, the mean annual rainfall (Figure 5) showed higher values in May (849.7 mm) and lower values in October and November months when there was no rain in the region. The rainfall variable showed influence on some nutrient concentrations. The highest rainfall rates in the region during the rainy season were observed in February, March, April and May. The high peak was in May, unlike the rainfall rates in the past 10 years. Since 1995, the highest rainfall value recorded in the region was 657.20 mm, and the mean recorded in May in the past 20 years was 347.6 mm.

Although rainfall favors nutrient absorption by plants, there was reduced concentration of these nutrients during the rainy season. Litter is characterized by showing bigger production during the dry season. Such period shows lower decomposition rates and nutrient concentrations. The monthly mean temperature was higher in December (27.7°C) and lower in April (25.2°C), with annual mean of 26.42°C. Thinnings change the forest microclimate by reducing the perspiration in the area and by increasing both the incidence of solar radiation inside the forest and the soil temperature; fact that favors the decomposing microorganisms.

The 10-year-old capoeira (NT) showed the following descending order in nutrient concentrations: Fe>Mn>Zn>Cu. Fe and Zn showed the highest concentrations during the rainy season. The nutrients Cu and Mn showed the highest concentrations during the dry season.

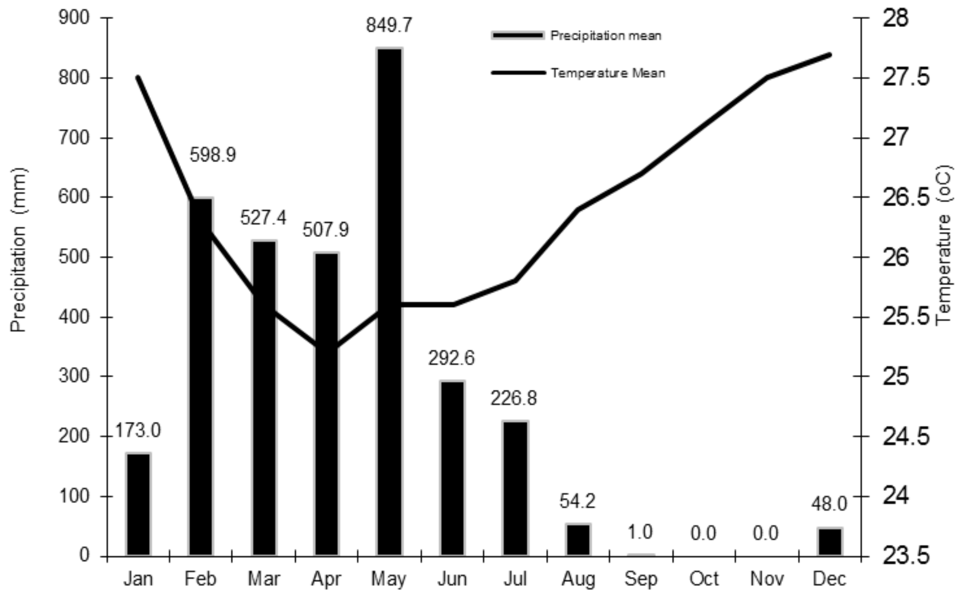

Figure 5. Annual rainfall and temperature range in Tracuateua County, 17 km distant from Bragança - PA during the experimental period, 2009.

Bianchin et al. (2016), the absence of a period with water deficit, together with the low occurrence of deciduous species in these environments, allows for greater deposition in higher precipitation and temperature, thus interfering with the concentration of nutrients.

The 10-year-old capoeira (NT) showed significant statistical difference (Tukey, p <0.05) in Fe and Mn, with the highest concentrations occurring during the rainy and the dry seasons, respectively. The other elements showed no significant differences. The 10-year-old capoeira (PT) showed the highest Fe concentration values during the rainy season, and the analyzed elements showed micronutrients concentration in the following descending order: Fe>Mn>Zn>Cu. The highest Fe and Cu concentrations occurred during the rainy season, and the highest Mn and Zn concentrations occurred during the dry season. The 10-year-old capoeira (PT) showed significant statistical difference (Tukey, p <0.05) in Fe, and the highest concentrations occurred during the rainy season (Table 2).

The 10-year-old capoeira (TT) showed the highest micronutrient concentration values in Fe during the dry season, and the analyzed elements showed nutrients concentration in the following decreasing order: Fe>Mn>Zn>Cu. The highest Fe and Mn concentrations occurred during the dry season and the highest Cu and Zn concentrations occurred during the rainy season. The 10-year-old capoeira (TT) showed significant statistical difference (Tukey, p <0.05) in Mn and in Zn. The highest concentrations occurred during the dry and the rainy seasons, respectively.

By taking under consideration the 40-year-old capoeira and the treatments applied to the 10-year-old one (NT, PT, TT), it is possible to state that the overall highest concentrations of all analyzed elements showed the same descending order in nutrient concentrations: Fe>Mn>Zn>Cu. The Fe concentration was higher than that of all other analyzed elements in all the applied treatments.

By taking under consideration just the treatments applied to the 10-year-old capoeira (NT, PT, TT), it is possible to see that the highest micronutrient concentrations in Fe occurred in the (NT) system during the rainy season. The Mn showed the highest nutrient concentrations in the (NT) area during the dry season and the lowest nutrient concentrations in the (TT) area during the rainy season. The Cu showed the highest concentration in the (PT) area during the rainy season and the lowest concentration in the (NT) the rainy season. The Zn showed the highest concentration in the (TT) area during the rainy season and the lowest concentration in the (PT) area during the rainy season. Scoriza and Piña-Rodrigues (2014) found positive correlation of litter contribution with precipitation and temperature, thus interfering with the concentration of nutrients.

Results showed that N and Mg concentrations were higher in the 40-year-old capoeira both in the rainy and in the dry periods. Similar results were found by Hayashi (2006) who compared young and old capoeiras (6, 10, 30 and 40 years old). The N loss during the change in land use was described by Davidson and Martinelli (2009). These authors associated these results to change the stoichiometric balance in N and P cycling processes.

The isotopically light N in mature forest ecosystems is lost due to fractioning during nitrification and denitrification, thus leaving the enriched N behind (Amundson et al., 2003). The lower nutrient concentrations found in the 40-year-old capoeira may probably due to the longer exposure time of this litter on the ground, which leads to an advanced decomposition state, thereby showing lower nutrient contents.

The results of the two studies on N fixation in the Amazonian secondary forests are mistaken, as stated by Davidson and Martinelli (2009) who found isotopic evidence for the significant symbiotic N fixation during the first 25 years of secondary vegetation regeneration in the central Amazon region, near Manaus. On the other hand, a recent study conducted in the eastern Amazon secondary forests, in Pará State, found no significant difference in leaf N between leguminous and non-leguminous species (Davidson and Martinelli, 2009).

It was found that P concentrations in the litter decreased due to age, but K, Ca and Mg concentrations did not significantly change because of it. The seasonal variation in the mineral elements concentration in the litter appears to be strongly related to these nutrients absorption and retranslocation mechanisms.

The high Ca concentration found in the current study may be caused by the big amount of collected branches. The highest Ca content is found in the branches, and the highest Mg content is found in the leaves (Vogel, 2005). Since it has little mobility in the plant, Ca is more concentrated in lignified parts such as the branches and the bark. The highest Mg content found in the leaves was probably due to the fact that this element is part of chlorophyll (2.7% of it), thus it is more abundant in these tissues.

Studies conducted in the Amazon region found that the nutrient concentrations in plants varied greatly according to the element, the plant tissue, the soil type, the vegetation and to the family of the monitored species. In addition, they were affected by factors such as the plants' age and the leaves' physiological stage.

The total thinning area showed higher nutrient concentrations than the control plot. It might have happened due to lesser species competition for nutrients, whereas most elements concentration was stable in the 40-year-old capoeira.

These studies also suggest that the reduced competition among the remaining trees would increase the nutrient availability per plant. It would enable species regeneration and would give rise to a more diverse, nutrient-richer and more easily decomposed litter.

Regarding the total thinning, the highest concentration of all nutrients occurred during the dry season, and it showed more than 50% significant difference during this period. It did not rain in the last three collection months. Since microorganisms show slower decomposing activity during the least rainy season, the nutrients concentration in the litter was expected to show lower values. The thinning effect may have affected the number of decomposing microorganisms. The areas showing higher concentrations were: TT>NT>PT. It was found that the high concentration found during the dry season may have also affected the number of decomposing micro-organisms in K, N, Ca and in Na. The highest nutrient concentrations found in all elements during the dry season was a statistically significant aspect in all treatments, and it showed seasonal variation.

The highest Fe concentration found in the accumulated litter may be justified by its mobility, which is low (Dechen and Nachtigall, 2006). This mobility is negatively affected by several factors such as high P content, K deficiency, high Mn amount and low light intensity (Dechen and Nachtigall, 2006).

The litter contamination with soil, that is, litter sample with soil, may be considered to be a justification. Clay and soil organic matter contents also influence Fe availability. Clayey soils tend to retain Fe, and the appropriate soil organic matter levels enable better Fe use by plants due to their acidifying and reducing features, and to the ability of certain humic substances in adverse pH conditions. The Fe content in the soil is influenced by pH, since Fe increases as the acidity increases. In addition, it reaches large contents in very acidic soils with pH lower than three, and in soils rich in humic and colloid acids capable of forming soluble complexes with Fe. The second bigger micronutrient content found in the accumulated litter was Mn. It might have happened due to contamination with soil, since the Mn found in the soil comes from oxides, carbonates, silicates and sulphides. Mn oxides and sulphides are the most often found forms in the soil, and its occurrence combined with Fe is very common (Dechen and Nachtigall, 2006). It is worth emphasizng that the larger Mn content in the accumulated litter may be explained by its higher content in some species leaves.

When there is good Mn supply, the leaves accumulate high concentrations of it, as the plant grows old. A small portion of this element is translocated from the old leaves to the new growing leaves, in which the element is found in lower concentrations. However, it should be taken under consideration that the Mn concentration in the plant varies greatly among plants and species parts (Caldeira et al., 2008; Dechen and Nachtigall, 2006).

It was observed that the nutrient concentrations deposited in the litter was influenced by seasonality in some elements, since the dry period showed the highest micronutrient concentrations in most of the analyzed elements. Studies conducted in forests by Silva and Almeida (2002) and Cunha Neto et al. (2013) show that litter production is higher during the dry season. Therefore, whenever there is higher litter production during this period, it is expected to find the highest nutrients concentration during the same period. A study conducted by Brun (2010) in 35-and-55-year-old secondary forests found the same micronutrient concentrations order in the litter that was found in the current study. However, according to Brun's (2010) study, the concentrations of all analyzed elements were higher in the oldest forest, whereas the current study found the highest micronutrient concentrations in the 10-year-old capoeira (the youngest one), except for Cu, which was higher in the 40-year-old capoeira (the oldest one). These studies differ from this study possibly possibly due to the higher accumulated biomass and also due to the significantly higher Fe and Mn contents. The quantitative storage order of nutrients in the biomass was Fe> Mn> Zn> B> Cu.

Plant physiology is another factor that may justify a particular element concentration. In this study, *Myrciaria tenella* was the predominant species in the area. This species does not need good fertile soil to develop (Carvalho et al., 2009). This is the fact that may benefit this species over other species that do not have this same feature, since each species needs a certain amount of nutrient.

Conclusions

The capoeiras with different vegetation age in the Bragantina area showed that not all nutrients were influenced by seasonality, despite the applied thinning, the vegetation age and the forest species. The oldest capoeira (40 years old) showed lower macro and micronutrient concentrations than the youngest one (10 years old). These differences may be justified by the longer abandonment time in the area and by the higher mean concentrations in the forest leaves in comparison to the other parts (branches, reproductive parts, bark) of the total litter. Besides, the vegetation management practices somehow change the balance achieved by forest ecosystems either by increasing the decomposition rate or by litter accumulation or even by its destruction.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of urea treatment and concentrate mix supplementations on feed intake and digestibility of Horro sheep fed cured maize husk (*Zea Mays*) at Bako, Western Ethiopia

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Feed intake and digestibility experiment were conducted using twenty yearling male Horro sheep weighing 20.42 ± 0.35 kg (mean ± SD). The objectives of the study were to evaluate the response of Horro sheep to feed intake and apparent digestibility when supplemented with different level of noug seed meal and wheat bran mix (1:1) on DM basis. For this study, randomized complete block design was employed. Experimental sheep were blocked into four blocks of five animals based on their initial body weight (BW) and randomly assigned to the four treatment diets within a block. The treatments allocated were sole UTMH (T1, control), UMH + 200 g concentrate mix (CM) (T2), UMH + 300 g CM (T3) and UMH + 400 g CM (T4) on DM basis per head/day for 90 days. Total DM intake over the experimental period was higher (P<0.001) for the supplemented groups as compared to the control group. The DM intake of control group showed a decreasing trend during the first 50 days of the experiment which later showed an increasing trend that brought the sheep to maintain their body weight. Digestible nutrient intake and apparent digestibility of nutrients were higher (P<0.001) for supplemented sheep than control group. It was concluded that supplementation with nougseed meal and wheat bran mix promoted feed intake and apparent digestibility of DM and CP in Horro sheep fed cured maize husk.

Key words: Apparent digestibility, concentrate mix, feed intake, Horro sheep, maize husk.

INTRODUCTION

The presence of high livestock population with limited grazing lands associated with severe land degradation is a critical problem in many tropical countries like Ethiopia. Moreover, farmers have limited opportunity to improve the feed of animals through cultivation of forage crops due to small size of land holdings, which is mainly used for crop production (Getahun, 2013; CSA, 2004). High demand for cereals and other food plants for direct

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human consumption in the future dictate animal industry and must adjusted to this scenario by making changes in feeding systems and management. To overcome the feed problem, matching livestock production with the available feed resources (crop residues) and increasing animal productivity through proper utilization of available feed resources is mandatory in mixed farming system (Solomon et al., 2008). In this regard, nonconventional feeds produced from cereals can be used as important feed resources for livestock.

Diriba and Lemma (2001) reported that maize is the major cereal crop in western Ethiopia. Maize husk is one of the widely known crop residues used as a feed for ruminants together with maize stalk after the harvest of the crop commonly during the dry season (Osafo et al., 1993). When observed under farmers' condition, it seems the husk has no palatability problem by ruminants.

But, it has not been fully exploited as feed for ruminants due to little or no knowledge of processing or level of incorporation of concentrate supplements (Melese et al., 2014). The stalk of maize residue is either left on the cultivated land for soil fertility conservation or it is used for construction of grain storage structure, fence and used as fuel wood (Adugna and Frik, 1999; FAO, 2001). Then, the husk is stripped, collected and fed to livestock.

However, studies were not conducted to verify the potential of untreated or treated maize husk as livestock feed under Ethiopian condition. Therefore, this study was initiated to investigate the effect of feeding urea treated maize husk (UTMH) and different levels of concentrate mix (CM) supplements on Horro sheep fed cured maize husk.

MATERIALS AND METHODS

Study area

The study was conducted at Bako Agricultural Research Station, Western Ethiopia which lies approximately at 09° 06' 56" N latitude and 37° 03' 30" E longitude at about 239 km from Addis Ababa capital of Ethiopia. Average elevation of the area is 1650 m above sea level. The area receives an average of 1242 mm rainfall annually and the mean annual minimum and maximum temperatures ranges from 13.3 to 27.9°C during the period of 1961-2010 (Meteorological Station; Bako Agricultural Research Center).

Experimental design and feeding management

Twenty yearling male Horro sheep with 20.42 ± 0.35 kg body weight were used in a feeding and digestibility trial. Experimental design employed was randomized as complete block design consisting of four treatments, namely, chopped UTMH (T1, control), untreated maize husk (UMH) supplemented with a mix of noug seed meal (NSM) and wheat bran (WB) (1:1) at 200 g (T2), 300 g (T3) and 400 g (T4) DM per day. The experimental sheep were housed in individual pens. They were vaccinated against infectious diseases, sprayed and dewormed against external and internal parasites, respectively. Maize husk (BH-660) was collected from production site after the harvest of the kernel (grain) and chopped to appropriate size using tractor mounted chopper. The husk was offered *ad libitum* allowing 30% refusal. Supplement feeds were offered in two equal proportions at 8:00 and 16:00 h each day after thorough mixing. Mineral salt and water were available. Daily feed offers and refusals were weighed and recorded for each animal to calculate daily feed intake.

Feces collection and samples preparation

Total feces collection was done for seven consecutive days by adapting the experimental sheep to carry feces collection bags during the digestibility trial. Feces were collected daily and mixed thoroughly and 20% was sub-sampled and pooled for seven days per animal. Feces samples were stored at -10°C pending chemical analysis. The composite fecal samples per animal were thawed at room temperature and dried at 60°C to a constant weight. Dried samples of feeds offered, refused and feces were ground to pass through a 1 mm sieve and preserved in airtight plastic containers pending chemical analysis. Apparent digestibility coefficients of nutrients were calculated as the proportion of nutrients consumed and not recovered in feces.

Laboratory analysis

Determination of DM, ash and organic matter (OM) contents were performed according to AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADF) were determined by the method of Van Soest and Robertson (1985).

Statistical analysis

Data collected during the experiment were subjected to the analysis of variance using the general linear model procedure of SAS (2004) version 9. Differences between treatment means were tested using least significant difference (LSD) test. The model used for the analysis of data on feed intake, digestible nutrient and coefficient of digestibility was:

$$Y_{ij} = \mu + T_i + B_i + E_{ij}$$

Where:
$Y_{ij} =$ the response variable, μ = overall mean, T_i= treatment effect, B_j = block and effect, E_{ij}= random error.

RESULTS AND DISCUSSION

Treatment feeds chemical composition

The result of the chemical analysis of treatment feeds is presented in Table 1. The supplement feed mixture contained high DM, OM and CP ingredients as compared to UMH and UTMH, where the former contained higher content of cell wall fibers.

Feed intake

The intake of untreated maize husk DM was high for low level supplemented sheep and significantly different (P<0.001) (Table 2). Total DM intake was higher (P<0.001) for the supplemented sheep than the control

Table 1. Chemical composition of the treatment feeds.

Feed types	DM (%)	Ash	OM	CP	NDF	ADF	ADL
		(%DM)					
Untreated maize husk (UMH)	92.24	6.44	93.56	5.76	75.8	43.13	13.04
Urea treated maize husk (UTMH)	87.57	5.95	94.05	10.42	71.5	38.84	12.67
Wheat bran (WB)	88.7	6.6	93.4	16.9	46.8	36.2	6.1
Nougseed meal (NSM)	94.2	8.2	91.8	31.4	37.9	33.6	6.1
WB and NSM mixture (1:1)	93.27	5.39	94.61	24.13	41.1	34.3	5.06

DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber. ADF = acid detergent fiber, ADL = acid detergent lignin.

Table 2. Daily nutrient intakes of Horro sheep fed sole urea treated maize husk and untreated maize husk supplemented with different levels of wheat bran and nougseed meal mix.

Parameter	T1	T2	T3	T4	SEM	SL
MHDMI (g/d)	514.5^{a}	471.1^{b}	429.5^{c}	446.5^{c}	8.16	***
CMDMI (g/d)	_	200.0^{c}	300.0^{b}	400.0^{a}	0.00	***
TDMI (g/d)	514.5^{d}	671.1^{c}	729.3^{b}	846.5^{a}	9.29	***
Ash intake (g/d)	34.5^{d}	40.1^{c}	44.1^{b}	49.6^{a}	0.74	***
OMI (g/d)	483.7^{d}	631.0^{c}	785.2^{b}	796.9^{a}	8.61	***
CPI (g/d)	60.5^{d}	70.2^{c}	91.6^{b}	114.6^{a}	0.57	***
NDFI (g/d)	344.1^{d}	472.7^{c}	499.1^{b}	571.8^{a}	6.84	***
ADFI (g/d)	194.7^{d}	278.8^{c}	296.8^{b}	351.5^{a}	4.06	***
ADLI (g/d)	57.5^{b}	68.4^{c}	68.2^{c}	81.5^{a}	1.13	***
EME (MJ/d**)**	5.9^{d}	7.6^{c}	8.2^{b}	9.4^{a}	0.13	***
DMI (%BW)	2.6^{c}	3.0^{b}	3.1^{b}	3.3^{a}	1.48	***
DMI (g/kgW$^{.75}$)	56.5^{d}	65.2^{c}	67.7^{b}	74.2^{a}	0.84	***
SR	-	-0.37^{b}	-0.04^{a}	-0.08^{a}	0.04	***

^{a,b,c,d} = Means with the same letter in the same row are not significantly different, ***= (P<0.001), ADF= acid detergent fiber; ADL= acid detergent lignin; Ash; CMDMI= concentrate mix dry matter intake, CP= crude protein; DMI (%BW)= DMI percent body weight; DMI (g/kgW$^{.75}$)= DMI g/kg metabolic body weight; MHDMI= maize husk dry matter intake, NDF=neutral detergent fiber; OM= organic matter; SEM= standard error mean, SL= significance level, SR= substitution rate, TDM= total dry matter.

group. Among the supplemented sheep, total DM increased with the level of supplementation, indicating it promotes the intake of the basal diet**.** Also, the trends in DMI of Horro sheep over the experimental period are given in Figure 1. The mean daily total DMI was higher in T4 and lower in T1 and different (P<0.001) in the order T4>T3>T2>T1, respectively. The reason for high mean daily DMI of UTMH (T1) might be attributed to relatively low fiber content of UTMH as compared to UMH of supplemented groups. This is in agreement with Melese et al. (2014) who fed urea treated straw to lambs of control group and found that intake increased as compared to supplemented one.

The mean daily DMI of UMH was higher (P<0.001) for supplemented sheep and lower for control group sheep, which resulted in negative substitution rate for the experimental period. In agreement with the present study, Ewnetu, (1999) reported that concentrate supplemented sheep had higher DMI as compared to those fed poor quality roughage alone. Total DM, OM, CP, NDF and ADF were also higher (P<0.001) in the order of T4>T3>T2>T1 (Table 2). The DMI as BW% in the current study was 2.7 to 3.3%, which is within the range of 2 to 4% of BW suggested by Susan (2003).

Variation in the amount of DMI recorded in the present experiment when compared with other similar studies might be due to differences in quality and coarse texture of the basal feed material, animal factors (breed, age and physiological status), rate of degradation of treatment feeds and the prevailing temperature and humidity (Van Soest, 1982; Nsahalai et al., 1991). Comparisons between UTMH and UMH showed an increase (P<0.001) in intake of maize husk and total DM, OM, CP and NDF for sheep fed UTMH alone and UMH supplemented with concentrate mix (Table 2). This indicated a useful effect of urea treatment and supplementation to enhance nutrient utilization.

In this study, negative substitution rates indicated

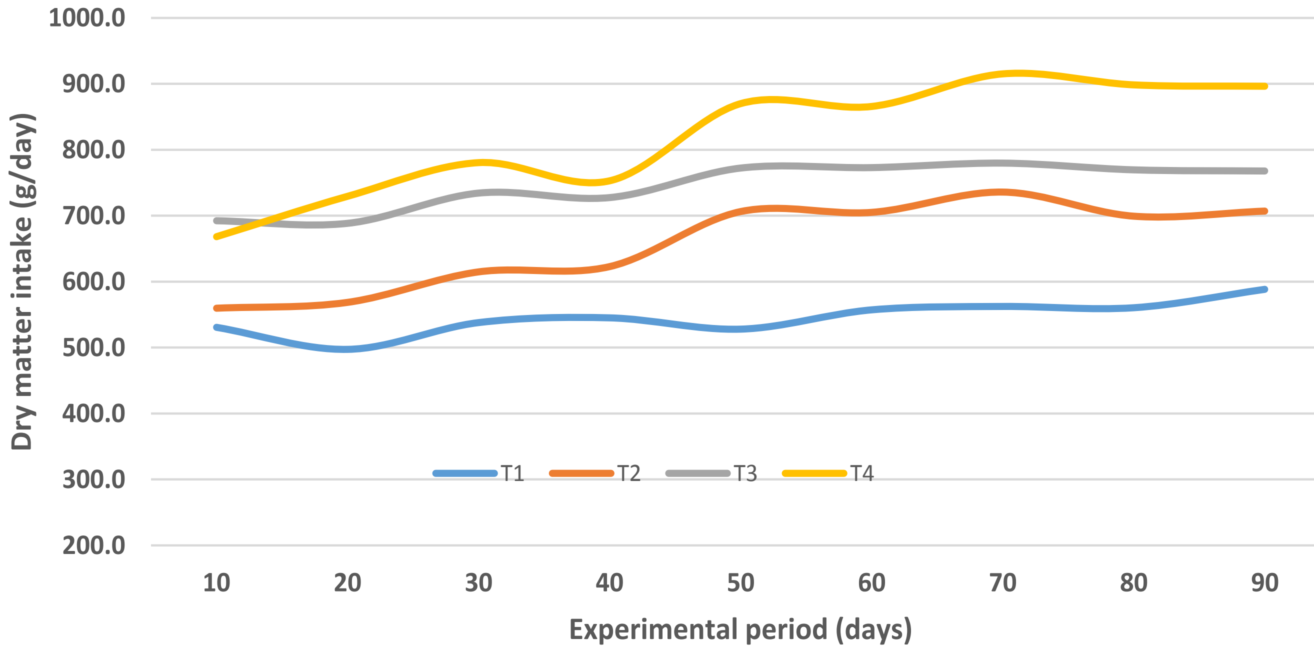

Figure 1. Trends in DMI of Horro sheep fed on sole urea treated maize husk and untreated maize husk supplemented with different levels of wheat bran and noug seed meal mix. T1 = UTMH, T2 = UMH + 200 g (WB + NSM), T3 = UMH + 300 g (WB + NSM), T4 = UMH + 400 g (WB + NSM).

supplementation promoted more basal diet intake as compared to the control group. Thus, it is possible to say supplemented group consumed concentrate in addition to UMH without replacing it. Dawit and Solomon (2009) reported supplementation with graded levels of vetch and alfalfa hay improved total DMI of Arsi-Bale sheep fed urea treated barley straw by 71%. Additionally, feed intake of experimental animals fed on a basal diet of urea treated sorghum residue was lower than the animals supplemented with concentrate mix (Ahmed et al., 2001). Figure 1 shows the change in daily DMI with time over the experiment period.

Moreover, the result of an experiment undertaken by Koralagama et al. (2008) to compare the effect of supplementing maize residue with cowpea (*Vigna unguiculata*) haulms or commercial concentrate on feed intake and nutrient digestibility of male Ethiopian Highland sheep showed that relative to the control group, cowpea increased maize residue intake. Estimated metabolisable energy (EME) indicated that the energy intake for all treatments were above the minimum maintenance requirement range (3.7 to 4.1 MJ/day) estimated for a 20 kg lamb (ARC, 1980). The calculated value was higher (P<0.001) in both UTMH and supplemented groups (5.9-9.4 MJ/day) (Table 2).

In the current experiment, differences (P<0.001) were observed among all treatments in daily DMI g/kg $W^{0.75}$ and as % BW. The difference observed might be due to variations in BW gain and efficiency of feed utilization of the experimental sheep. The values of DM intake (56.5 to 74.2 g/kg $W^{0.75}$) in the present study are relatively lower than that of Awet and Solomon (2009) that reported 90 to 108 g/kg $W^{0.75}$ in Afar rams fed urea treated tef straw supplemented with wheat bran.

Digestible nutrient intake

The positive effect of supplementation on feed intake might be a reflection of increase in the intake of essential nutrients such as energy, vitamin and minerals and particularly nitrogen. Similar to those previous results obtained from roughage basal diet supplementation by different authors (Melese et al., 2014; Michael and Yayneshet, 2014), treating maize husk with urea or supplementing it with protein and energy source feeds improved DM, CP and OM intake by sheep. Like that of DMI, digestible nutrient intake (g/kg DMI) were different (P<0.001) among the treatments in the order of T4>T3>T2>T1 since it is strongly correlated with total DMI (Tables 2 and 3).

In the present study, low nutrient intake because of high ambient temperature coupled with less nutrient content and digestibility made the control group to maintain their initial weight during the experimental period. Contrary to the present work, Gemeda et al. (2003) reported that all animals fed basal diet died before the termination of the study in an experiment that evaluated the growth performance of weaned Horro lambs fed a basal diet of barley straw and supplemented

Table 3. Daily digestible nutrient intakes of Horro sheep fed sole urea treated maize husk and untreated maize husk supplemented with different levels of wheat bran and noug seed meal mix.

Parameter	Digestible nutrient intake (g/kg DMI)/day					
	T1	T2	T3	T4	SEM	SL
DM	391.1[d]	517.2[c]	567.4[b]	652.2[a]	9.386	***
OM	375.6[d]	493.8[c]	543.0[b]	623.5[a]	8.575	***
CP	55.8[c]	58.1[c]	75.8[b]	95.8[a]	1.612	***
NDF	274.4[d]	363.6[c]	382.0[b]	436.1[a]	6.585	***
ADF	133.5[d]	212.3[c]	228.4[b]	272.3[a]	4.341	***

[a,b,c,d] = Means with the same letter in the same row are not significantly different, ***= (P<0.001), DDM= digestible dry matter; DOM= digestible organic matter; DCP= digestible crude protein; DNDF= digestible neutral detergent fiber; DADF= digestible acid detergent fiber.

Table 4. Digestion coefficients of nutrients by Horro sheep fed sole urea treated maize husk and untreated maize husk supplemented with different levels of wheat bran and nougseed meal mix

Parameters	T1	T2	T3	T4	SEM	SL
DM	0.71[b]	0.75[a]	0.75[a]	0.74[a]	0.017	***
OM	0.72[b]	0.77[a]	0.76[a]	0.76[ab]	0.017	***
CP	0.87	0.82	0.82	0.83	0.025	ns
NDF	0.71[b]	0.75[a]	0.74[a]	0.74[ab]	0.017	***
ADF	0.65[b]	0.76[a]	0.76[a]	0.77[a]	0.019	***

with different level of concentrate. The observation in the current study underlines the importance of supplementation with protein source feed when animals are fed on fibrous low quality diet.

Apparent digestibility of nutrient

The digestibility of DM, OM, CP, NDF and ADF in Horro sheep fed sole UTMH and UMH supplemented with WB and NSM mix are presented in Table 4. The apparent digestibility of DM, OM, NDF and ADF were lower (P<0.001) for control and higher (P<0.001) for the supplemented sheep. CP digestibility was similar between groups fed UTMH and those supplemented. Mulugeta and Gebrehiwot (2013) and Chenost (1995) suggested that urea treatment and concentrate supplementation tends to increase the digestibility of low quality roughages through its effect on plant cell walls. Additionally, FAO (2002) reported that as much as 20% improvement in digestibility would be expected upon ammoniating the poor quality roughages.

The similar digestibility in DM, OM, NDF and ADF among the supplemented sheep in the current study might be due to rapid digestibility of supplementary feed that might activate the micro-organisms in rumen for fiber digestion. The current result is supported by Solomon et al. (2003) in that feeding of protein diet to goat and sheep, respectively improved digestibility as compared to low protein diet. In agreement with the current result, Ferrill et al. (1999) and Getahun (2013) documented a significant improvement of DM, OM and CP digestibility in protein and energy supplemented feed as compared to sole roughage diet, which was attributed to the high digestibility of the supplements. Digestibility is much reduced when a ration contains too little protein in proportion to the amount of readily digestible carbohydrate (McDonald et al., 2002).

Various research reports support the fact that DM and nutrient digestibility of fibrous feeds could be improved due to supplementation of protein and energy source feeds (Getahun, 2013; Kaitho et al. 1998) and that the digestibility of DM, OM and nitrogen (CP) were increased with level of supplementation. In agreement with this, Getahun (2006) reported that urea treatment of straw and supplementation significantly increased apparent straw DM, OM and CP digestibility. However, contrary to the above reports and the present result, Fentie and Solomon (2009) reported that there was no significant difference in apparent digestibility of DM and OM between supplemented and non-supplemented treatments in Farta sheep fed a basal diet of grass hay supplemented with NSM, WB and their mixes which might be effect of variety of feed material and breed difference.

Conclusion

The chemical analysis result of the experimental feeds indicated that urea treatment improved the CP content of maize husk. Intake and digestibility of DM, OM, CP, NDF and ADF improved due to urea treatment and supplementation. Intake of DM was positively correlated with intake of OM, CP, NDF, ADF and digestibility of OM, CP, NDF and ADF. Similar CP digestion for T1 (control) and the supplemented group is as a result of urea treatment that enhances digestibility of roughage feed which is comparable to the supplemented ones that indicates effectiveness of the treatment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Towards attaining equity and satisfaction in water allocation mechanism for irrigated agriculture in Northern Nigeria

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Mechanism for water allocation in the irrigated agriculture has been subject of conflict and challenges in sub-Sahara Africa (SSA) due to higher input in rice farming and increasing water shortage. How satisfactory is the allocation mechanism by the stakeholders (the providers, policy-makers and the farmers) and how do farmers perceive equity objective on the existing water allocation mechanism in Nigeria? This paper examines constraints confronting water distribution, farmers' level of satisfaction and their perception on equity in the process of water distribution to farmlands in the Upper Niger River Basin Development Authority (UNRBDA) in Northern Nigeria. This study employs semi-structured interview and validated questionnaires survey approaches with stakeholders. Data collected were analysed using thematic approach based on themes generation and simple descriptive statistics. Findings show that weak institutions, legal framework incapacity and aging infrastructures majorly hinder equitable water allocation in the irrigated agriculture. Varied opinions were observed on the level of satisfaction among the stakeholders. While 77.7% of farmers reported that equity objective is observed, 22.2% declined that there is equity in the allocation policy. Institutional reform and adequate funding for maintenance of facilities is recommended for justice and equity in water allocation.

Key words: Constraint, equity, irrigated agriculture, satisfaction, stakeholders, water allocation.

INTRODUCTION

'Water is life' has become an incontestable slogan all over the world. That is why it is considered as a human right[1] regardless of users' status in the society (Gupta et al., 2010). Despite this fact, water resources management is complicated because it brings about tension(s) whenever one of its fundamentally embedded principles- *access* and *allocation* is tampered with. Whereas there is an established belief that over seventy percent (70%) of water allocation goes to irrigated agriculture in any River Basin Organisations (RBOs) world over (Gourbesville, 2008).

Meanwhile the existing body of literature on water

[1]UN Resolution 60/251, 2010

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sharing mechanisms show that there is no ground theory regarding water allocation practices especially for the irrigated agriculture in RBOs across the globe. However, the question of whether water is being distributed equitably within agricultural sector remains unclear. Nevertheless, the rise in water demand which outweighs supply in the dryer areas during the dry season is premised on rapid population growth coupled with increasing urbanization as a result of economic growth which many African countries are faced with.

Nigeria's water resources are under severe siege due to deteriorating water quantity and quality (Babatolu et al., 2014). Larger outputs in rice production and other agricultural produces in the Sub-Sahara Africa (SSA) come from the Northern region of Nigeria. Prominent location among the major farms in this context is situated in the Upper Niger River Basin Development Authority (UNRBDA). Although, the establishment of the up-to-date 12 River Basin Development Authorities (RBDAs) in the country was to serve the purpose of overall water management at the basin level. On one hand, water allocation technique to different categories of the irrigated rice farmers across the basins in the Northern part of Nigeria is considered lopsided and unsustainable. On the other hand, equity has been neglected within the irrigated agriculture. Presumably the body of literature on equity in water allocation focuses on either Transboundary Rivers or multi-purpose dams serving competing water users (households, industry, hydro-power, agriculture, etc.). Moreover, it has been suggested that "if there is one area where equity is crucial and essential, it should be on the issue of water distribution in the irrigated agriculture[2]. On this note, this paper sets out to examine constraints confronting water distribution in the irrigated agriculture, rice farmers' level of satisfaction and their perception on equity in the water allocation in the Northern Nigeria.

LITERATURE REVIEW AND THEORETICAL FRAMEWORK

The linkage between institutions and water allocation for agriculture cannot be over-emphasized. As a matter of fact, the need to assess how institutions shape the water allocation practices in any society is paramount to this study.

Institutions and water allocation

Institutions have been defined by many scholars as the rules of game which include laws, policies, regulations and social norms governing human behaviour and structuring the society (Bandaragoda, 2000; Hodgson, 2007). In relation to water resource, Saleth and Dinar (2005) define water institutions as rules which guide actions and provide incentives for individuals and collective decisions regarding water development and management. They submit that water institutions are hierarchical and subjective which are premised on a cultural, social, and political structure of a given society.

For clarity, institutions here refer to formal and informal ones. These two types of institutions have been viewed as 'formal' 'the organized routines of political and constitutional setup; regular elections, legal constraints on actors/stakeholders, and customary laws; while 'informal' institutions as 'the socially shared rules, usually customary which are communicated, and implemented outside the official realm. Both formal and informal institutions are persistent behavioural patterns which are pragmatic productive and advantageous to the society regarding natural resources management (Bandaragoda, 2000; Bratton, 2007; Hodgson, 2007; North, 1990). Moreover, institutions help to understand and analyse the operational responsibility of management functions in water allocation mechanisms (Bandaragoda, 2000; Hooper, 2010). And, physical and hydrological control of water should be completely replaced by "institutional arrangements" through the use of 'storage development' (Gupta and van der Zaag, 2008).

Arguably, both formal and informal institutions are required to tackle the issue of equity in water allocation, especially in the irrigated agriculture scheme. This is because informal institutions are synonymous with 'local rules' which have been confirmed that their application resolve equity issue in water allocation (Komakech et al., 2012). In establishing the importance of institutions in water resources management, some robust policies, laws, and mechanisms are central to water allocation as a means to manage the uncertainty of water resources' sustainability at the basin level. This implies that institutional arrangements require flexibility and periodic reforms regarding water allocation because of variability of water availability over time as a result of climate change, shifting hydrological conditions, and change in land use (OECD, 2015).

Having understood the concept of 'institutions' in water resources management, as defined in relation to this study, it is therefore pertinent to establish that institutions play a dual role in politics of water allocation by shaping human actions towards resources management. These roles of institutions, according to Bandaragoda (2000), could be either of 'constraint or liberation' of human actions in the society pertaining access to water and allocation. Five main factors that can constrain or aid water institutions at river basin level have been identified in extant literature as follows: (1) political system, (2) national economic policies, (3) legal framework, (4) socioeconomic environment, and (5) physical resource base (Bandaragoda, 2000; Heun and Van Cauwenbergh, 2015). According to these authors, these factors are useful because institutions

[2]Stated by Kofi Annan, Former United Nations Secretary-General.

always establish consistent orders in the society to control or checkmate human actions. Furthermore, two major institutional dimensions guiding water resources management at river basin level have been described as physical and non- physical dimensions. The former is related to the scale, quality, quantity, type and location of the water resources, while the latter includes the water users, affecting and affected stakeholders (Bandaragoda, 2000; Heun and Van Cauwenbergh, 2015). Apparently laws, policies and administration serve as the main 'pillars of institutions' which constitute water allocation principles. Institutions managing water resources have been grouped into three major categories. These categories are: water policies, water laws, and water administration (Bandaragoda, 2000; Saleth and Dinar, 2005; Solanes and Gonzalez-Villarreal, 1999).

Therefore institutional arrangements encompass the structure of stakeholders in a procedural and coordinated manner which aids equitable and sustainable use of water resources for agricultural development at the basin level (Bandaragoda, 2000).

Equity in water allocation

Defining equity is a difficult task in itself let alone in water allocation because there are no universally accepted mechanisms for water allocation which is premised on the concept (Wolf, 1998). Whereas, the question of 'equity' is the key objective to water allocation, but, what is equity is difficult to define, measure or determine in water allocation across the globe (van der Zaag, 2007). Despite this odd, the Articles 5 and 6 of the 1999 UN Watercourses Convention highlights its 'equity principles' as "equitable and reasonable utilization and participation". Therefore, Article 5 of the 1999 UN Watercourses Convention defines 'equity' in the context of Transboundary River as "The Watercourse States shall in their respective territories utilize an international watercourse in an equitable and reasonable manner. In particular, an international watercourse shall be used and developed by watercourse States to attain optimal and sustainable utilization thereof and benefits from that place, taking into account the interests of the watercourse States concerned, consistent with adequate protection of the watercourse" (McCaffrey and Sinjela, 1998; Nigeria, 2014). Article 6 of the Convention stresses that for the achievement of equitable and reasonable utilisation of water, therefore, 'equity' should encompass the following factors:

1. "Geographic, hydrographic, hydrological, climatic, ecological,
2. The social and economic needs of the watercourse states concerned;
3. The population dependent on the watercourse in each watercourse state;
4. The effects of the use or uses of the watercourses in one watercourse state on other watercourse States;
5. Existing and potential uses of the watercourse;
6. Conservation, protection, development and economy of use of the water resources of the watercourse and the costs of measures taken to that effect;
7. The availability of alternatives, of comparable value, to a particular planned or existing use" (Gupta, 2015; McCaffrey and Sinjela, 1998).

Despite these definitions by the United Nations, some authors have the opinion that these definitions are descriptive in nature because what constitutes 'equity' differ among the competing water users (Pieter van der Zaag, 2007; Wolf, 1998).The issue of equity requires some criteria to assess and realise water resources distribution among the competing users. Therefore, 'equity' has been defined as the activity of availing every user an equal opportunity for access to water according to one's needs. It is therefore seen that equity does not constitute 'equal quantity' but 'equal opportunity' access to water use (van der Zaag and Savenije, 2014).

Users' accessibility to water in term of affordability and equity have been recognized as a human right by the United Nations General Assembly and United Nations Human Right Council (UN, 2010). Speed et al. (2013) emphasize the concept of scarity, competition and avalaibility while describing the water allocation among the users. On water allocation principle in the river basin, Bernauer (2002) confirms that nearly all the existing studies on specific rivers' management practices are only descriptive in nature. He admits that there is no ground theory for water allocation practices in different river basin organisations. Whereas, water allocation at the river basin level is premised on productive (agriculture, industry, and energy) and social (health and domestic services) purposes and the protection of the environment.

Roa-García (2014) submits that weak legal institutions and transparency have been the major factors hindering equitable, efficient and sustainable water allocation among the users in the basin. She further opines that water allocation is always driven by various levels of power with different interests. She concludes that achieving efficiency for water allocation is more pronounced because is premised on neo-liberalization (full-cost recovery) while equity and sustainability seem unrealistic. In line with, Roa-García (2014), Komakech et al. (2011), and Hillman et al. (2012) argue that water allocation has always been characterized by unknown equity procedures. This is because agricultural growth takes a larger percentage of water at the expense of ecological integrity (environment/natural flow regime). However, they posit that the practice of allocating a larger percentage for agriculture has not proven economic efficiency. Supportively, Jaspers (2015) views that water allocation depends largely on the principles or systems being practiced in the different countries.

Roa-García and Brown (2015) and van der Zaag (2007) indicate that allocation of water volumes among the farmers in agricultural sector has been found inequitable because the criteria for water allocation are always inconsistent. They concluded that the 'volumetric and administrative' mechanisms for water allocation always neglect ecological integrity. Therefore water allocation should be seen as a 'technical task' which requires transparency and accountability (Roa-García and Brown, 2015). In the same view, the consideration of complete hydrological units is crucial to water allocation which lies in the hands of lowest authorities. The need for flexible allocation mechanisms is required to reconcile "efficiency and equity" principles. In their submission, they identify four major segments of a water allocation system-(1) "water entitlements, (2) water allocation, (3) water delivery, and (4) water use." The most difficult segment among all the mentioned segments is "water allocation''.

Similarly, Speed et al. (2013) identify five major objectives of water allocation that should be in the minds farm operators. They are: (1) equity, (2) environmental protection, (3) priorities, (4) balancing demand and supply, and (5) promoting the efficiency use of water. They conclude that 'equity' is at the central of all but it extremely tough to achieve in water allocation. Arguably, Boelens et al. (1998) therefore, identify the following five levels of equity in irrigation and water management at the local levels:

1. Equitable water distribution and allocation among different water users and uses,
2. Equitable distribution of services involved in irrigation development,
3. Equitable distribution of the added agricultural production and other benefits under irrigation,
4. Equitable distribution of burdens and obligations related to functions and positions,
5. Equitable distribution of the rights to participate in decision-making processes, since this relates to the fundamental issue of whether or not every farmer has rights to speak, vote, claim an entitlement to irrigated land and enjoy equality of status (Boelens et al., 1998).

In order to ensure equity in water allocation on the part of UNRBDA authority, some of the mechanisms put in place are as follows:

1. Irrigation schedule: This is prepared by the UNRBDA and handed over to the farm operators, who are the schedule managers, to allocate and distribute water based on the needs of the Farmers' Association[3].
2. Farm reports: These are prepared by the Basin operators on a monthly basis detailing the achievements and difficulties encountered on irrigated sites and forwarded to the UNRBDA.
3. Use of overhead free boards: This is to take care of water balance to be supplied in case of excess demand by the farmers and water users associations.
4. Participatory irrigation management (PIM): This involves periodic meetings to resolve issues of conflict in water management in irrigation scheme and farm allocation as well as canal maintenance impacting on water equity distribution.

The mechanisms tend to prevent conflict in water management. According to Speed et al. (2013) water allocation planning always focuses on achieving equity as a part of policy objectives of water allocation mechanisms.

METHODOLOGY

This study adopted a pragmatic approach which involves nature of both qualitative and quantitative data, similar to; Ahmed et al. (2013), Mollinga and Gondhalekar (2012), Lorance et al. (2011), and Loucks et al (2005). Hence a hybrid-approach of qualitative and quantitative methodology was employed in this study for better presentation and understanding of the results. Apart from some sets of scientific journals database consulted for the literature review, this study also utilises semi- structured interview, questionnaire and government documents. On one hand, qualitative data was collected through the semi-structured interview administered on the policy-makers, UNRBDA staff (The Operators), and NGOs as well as academia. Some 'code names' were assigned to these three categories of interviewees such as: FGO represents 'policy-makers', UNRB represents 'the water allocators at the basin level' while the 'NGO' represents both the NGO and academia. On the other hand, the quantitative data was gathered through questionnaire on the rice farmers. Interview was conducted with 21 farmers, 18 policy-makers (Ministry of Water Resources, Ministry of Agriculture, and UNRBDA) and 11 Focus Group Discussions (FGD) with the farm operators, academia and Non-Governmental Organisations (NGOs) who are experts in the water resources in Kaduna and Niger states as well as Abuja that the study area covers. A pool of 50 population was sampled which consists of 8 females and 42 males in the study area.

50 Questionnaire (survey instruments) were administered on the farmers in order to gain an insight into wider perception and to ensure triangulation. The survey instruments were developed, subsequent to the interview and validated among 10 farmers. The validation result for internal consistency of the instrument yielded a Cronbach's α of 0.82 and a test-retest coefficient of .075 from a repeated administration of the pilot testing questionnaire during an interval of two weeks. While items relating to influencing factors (constraints) adopted five points Likert scale which ranges from 1 to 5, items that measured equity level from farmers' perspective are ranged from the scale of 1 to 10 from "very poor" to "very high". Concisely, the Table 1 shows the profiles of respondent stakeholders in this study.

Description of study area

Upper Niger River Basin Development Authority (UNRBDA) is the focus of this study. It is a parastatal of the Federal Ministry of Water Resources of Nigeria, with its administrative headquarters in Minna, Niger State (Nigeria, 2004). It was formally known as

[3] UNRB1,UNRB4

Table 1. Breakdown of the population employed in the study area.

S/N	Category	Stakeholders	Designation and gender	Organization
1	One	Policy makers at the federal level	Top officials and heads (4 males and 2 females)	Federal Ministry of Water Resources, Abuja
			Top Officials and Heads (6 Males and 2 Females)	Federal Ministry of Agriculture and Rural Development, Abuja.
2	Two	Decision makers and operators at the basin and state level	Executive Heads (3 Males)	Upper Niger River Basin Development Authority(UNRBDA)
			Head (Male) Agricultural Extension Officers (All Males)	Tungan-Kawo Dam and Irrigation Site, Tungan-Kawo Dam and Irrigation Site
3	Three	End users	Irrigated Rice Farmers (All Males)	Tungan-Kawo, Gurara and Agai/Lapai Dams and Irrigation Sites
			Head of Water Users' Association (Malefarmers)	
4	Four	NGOs and Academia	Units Heads (1 Male and 2 Females)	Maizumbe Farms International, Minna. Federal University of Technology, Minna, Niger State

Niger River Basin (NRB) before it was divided into Upper and Lower basins in 1982. This increased the number of River Basin Development Authorities established to twelve (12) under the Decree No. 25 of 1976 (as amended by River Basin Development Authority Act CAP 396 law of the Federal Republic of Nigeria). The basin covers an area of 158,100 km^2 which is located between latitude 7˚N and 12˚N and longitude 3˚E and 9˚E with tropical wet and dry seasons. The basin is drained by Niger River- transboundary river which flows from Mali as the upstream into Nigeria as the downstream country (Andersen and Golitzen, 2005).

Apart from the two major transboundary rivers flowing into Nigeria, the most important national streams drained to the Niger River include; River Kaduna, River Gurara, and River Kontagora. Figure 1 shows the River Niger, and the hydrological location of the study area.

RESULTS AND DISCUSSION

Constraints in water allocation for irrigated agriculture in Nigeria

The starting point in achieving this paper aim is the consideration of the major constraints encountered in the water allocation process from the perspective of the four categories of interviewees. Numbers of constraint are identified during the interpretive evaluative-interpretive strategy adopted in this study. The emergent themes from the data are categorised according to each category of respondents as follows: From policy-makers at the federal ministry level; maintenance problem and lack of institutional capacity building are found to be the major constraints. From the perspective of operational decision makers; inadequate funding, poorly designed canals and drainage system are the major constraints. From the end-users' (farmers) perspective, lack of water pressure/gravity during droughts, inadequate farming machinery, poor maintenance of infrastructure, and climate change are recognised as major constraints. From the NGOs and academia perspectives, climate change, seasonal change, institutional capacity building and mixed use of irrigated dams are recognized as major constraints.

Interestingly, three (3) *major* constraints seemed to come up more frequently based on the responses of the four (4) categories of stakeholders who are the interviewed respondents namely: Institutional capability building, funding and maintenance problems. Regarding institutional capacity building for instance, the existing but outdated Water Decree 101 which was promulgated by the military Government in 1993 needs to be repealed. As aptly mentioned by many of the policy-making respondents that the Integrated Water Resources Management Commission (IWRMC) established newly by the Federal Government solely as the apex body to allocate water licenses to end users is not derived from the Water Decree 101 and therefore does not have any legal backing4(FGO). This implied that its existence is not derived from any enabling law and hence cannot perform its extant functions. Perhaps with the draft of National Water Bill (2015) currently undergoing legislation with the National Assembly, the formulation of an enabling law for this contentious sector would soon be dispensed with. This is further reflected in some of the interviewees' response as quoted below:

I think in my own view, there is a lot to do with maintenance. Our maintenance culture of canal system is

[4]FGO1,FGO2, FGO3, FGO4, FGO5,FGO7

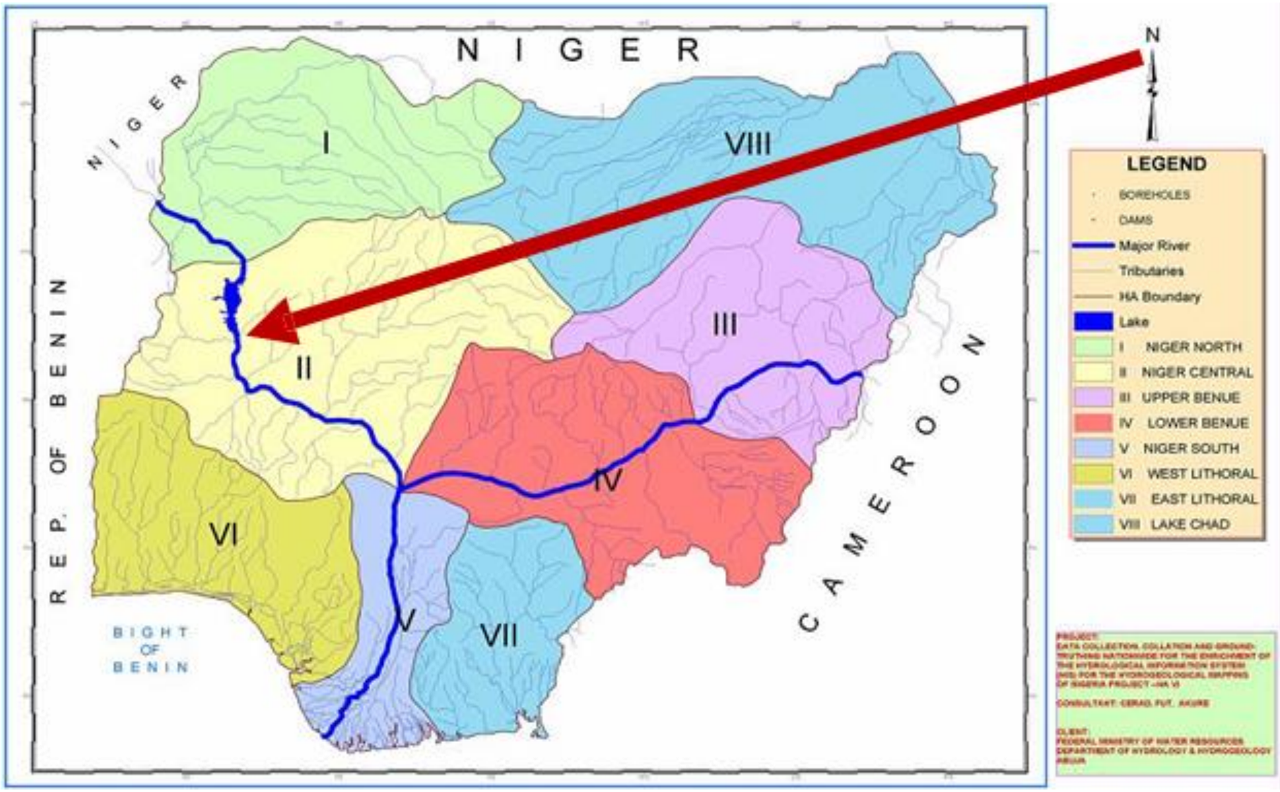

Figure 1. Map showing two transboundary rivers, hydrological areas and drainage systems in Nigeria. Source: FMWR (2015).

under siege, deterioration and on verge of collapse. If something is not done, there will always be problem of equitable water distribution to the users. I mean our farmers. ….If I should tell you now since what specific date these facilities have been replaced, there will be no answer. …though some repair works have been done but not enough to sustain adequate equitable allocation. This really has some effect on the input of the farmers. More still need to be done, I think [Directors; Ministry, UNRBDA, Academia]

Meanwhile, Machethe (2004) points out that insufficient irrigation infrastructure and maintenance contribute to low agricultural development and productivity in the African continent. Furthermore, Food and Agriculture Organisation (FAO, 2004) argues that insufficient irrigation infrastructure and poor operation and maintenance affect irrigation development in Nigeria. In the same vein, farmers in the case study basin also identified climate change as the major constraint affecting the water allocation mechanisms. For examples, many respondents expressed these:

…two years ago when there was not enough rainfall and later led to scarcity or shortage of water[5] (FGD). "There are no workshops organized by the government to enlighten us on this so that we (farmers) can have strategies to mitigate the climate change scenario (Policy-makers; and Farm operators).

Whereas Challinor et al. (2007) and Vermeulen et al. (2012) put forward in their different studies that agricultural production vulnerability to climate change cannot be over-emphasized as the global means temperatures predicts higher by the year 2100. Hence, they suggested two options for climate change mitigation-(1) accelerated adaptation through the use of integrated technology on the part of farmers and institutions managing water sector, and (2) more robust agricultural management risks through the use of awareness creation and 'safety net' (Challinor et al., 2007; Vermeulen et al., 2012).

It is indicated that insufficient funding constitutes one of the major constraints in the process of equitable water allocation to farmers. This is reported in script of the interview as follows:

Stringent budgetary allocation is one of the problems. Let me tell you, in the old time when things were working properly, maintaining and refurbishment of the pour irrigation system was never a problem. But today, funding is a big factor. We often read it in the national daily, the volume of allocation to the sector, but unfortunately, the money never got down to the base.

[5] FGD1,FGD2,FGD3

Table 2. Constraints identified by stakeholders on water allocation system for irrigated purpose in Nigeria.

S/N	Category of stakeholders	Constraints
1	Policy makers at the federal level	Maintenance problem, lack of Institutional capability building, Paucity of funds
2	Decision makers and operators at the basin and state level	Inadequate funding to access water and farm locations, poorly designed irrigated dams, poorly designed canals and drainage system, problem of desilting (accumulation of silt)
3	End users (Farmers)	Lack of water pressure/gravity during droughts, inadequate farming machinery, poor maintenance of infrastructure, climate change and greed of co-farmers.
4	NGOs and Academia	Climate change, seasonal change, institutional capacity building, mixed use of irrigated dams

So, what can one do without money? These facilities and infrastructure are capital intensive which implies that to sustain them, there is needs for adequate funding [Directors; Ministry, UNRBDA, Academia].

In buttressing this view, Komakech et al. (2011) opine that water allocation often faces different challenges such as variability of rainfall distribution, insufficient storage capacity and financial strength among others. They argue further that the inability of a basin to expand its storage capacity always hinders water allocation for irrigated agriculture sector, especially during dry seasons which is attributed to insufficient funding.

Against the aforementioned constraints; poor maintenance culture, climate change and insufficient funding, most of the respondents maintain that these are attributed to the problem of lack of institutional capacity building:

Based on my own years of experience, i can say that there is a need to define institutional roles. What I mean is that the situation of overlapping roles and responsibilities should be addressed….. Duties of parastatal and sectors should clearly be defined. And competent workforce who is trained in specific aspect of water resources management and services should be given chances to utilise their expertise….. Conflict should be avoided through a clear scope of services. You will agree with me that reform of our institutional structure will go a long way to resolve this recurrent problem. Because, if there is no appropriate or efficient legal instrument, there could not be efficient performance. So, I think I will suggest that our legal system on water should be reformed for improvement [Directors, farmers and Academia].

Roa-García and Brown (2015) indicate that allocation of water volumes among the farmers in the agricultural sector has been found inequitable because the criteria for water allocation are always inconsistent. However, Roa-García (2014) submits that weak legal institution and transparency have been the major factors hindering equitable, efficient and sustainable water allocation among the users in the basin. Differently viewed, Boelens et al. (1998) argues that equity should not be seen from the pervasive point of view dominated by the West. Rather, each society should be allowed to conceptualize 'equity' in line with their identity, values, economic and political sufficiency.

Table 2 presents the summary of the findings with the overlapping reoccurrence of institutional flaws and poor maintenance culture. Constraints are varied and can be summed together as representing stumbling-block to equitable water allocation process for irrigated agriculture in Nigeria.

Level of satisfaction on water allocation

Having examined the constraints that confront water allocation process in irrigated agriculture in Nigeria, the extent to which satisfaction on water allocation in the basin is considered. To examine the level of satisfaction on water allocation among the farmers, section of the items in the instrument involved in the computation of level of satisfaction on the distribution of waters to farmers are included. These items were scored in such a way that a "very high" response was scored 5, "high" response was scored 4, and "low" response was scored 3 while a "very low" response was scored 2 and a "neutral" response was scored 1. The resulting scores in each of the items were cumulated to build a mean measure of equitable water distribution in the context of three stages in the dry season of rice production in the study area. The resulting values are categorised into "high satisfaction level" for any score on the satisfaction measure that are greater than 3.14 (mean value of the computation, x), "moderate satisfaction" for any score around the range of 3.14 and "low satisfaction" for any score below 3.14. Table 3

Table 3. Assessment of farmers' satisfaction on equitable water allocation within the production year.

Dry season irrigation stages	1(%)	2 (%)	3(%)	4(%)	5(%)	Mean value (x)	Ranking
BOS	4(19)	3(14)	5(24)	5(24)	4(19)	3.09	3
MOS	4(19)	3(14)	5(24)	4(19)	5(24)		
EOS	0(0)	7(33)	6(29)	5(24)	3(14)	3.19	1

BOS, Beginning of season within a year; MOS, middle of season within a year; EOS, end of season within a year.

Table 4. The chi-square (x^2) test of level of satisfaction of the stakeholders.

Test	Value	df	Asymptotic Sig. (2-sided)
Pearson chi-square	10.000[a]	8	0.265
Likelihood ratio	9.503	8	0.302
N of valid cases		5	

a. 15 cells (100.0%) have expected count less than 5. The minimum expected count is 0.20.

presents the descriptive analysis. In the table, it is shown that at the beginning of the dry season, equitable distribution of water is low at 3.09. The reason for the low level of satisfaction might not be far from the fact that higher volume of water is usually needed by the farmers at the start of rice production season. Towards the middle stage of the production, the distribution is at the moderate satisfactory level, while at the end of the season, farmers' satisfaction with the distribution is high. The reason for the latter outcome might not be far from the fact that less quantity of water is usually needed at the tail end of the season.

Generally, the level of satisfaction with the present decision-making process for water allocation varies across the three (3) Focus Groups: UNRBDA, Academia and NGO(s) and the Policy Makers. When respondents were asked to rank their level of satisfaction on a 1-10 point rating scale, (with very poor assigned a score of 1-2; poor rated as 3- 4; fair as 5-6; high as 7-8 and very high rated as 9-10) the FGD1 ranked their level of satisfaction as 7 which equates to high; FGD2 on the other hand assigned a score of between 8 and 9 for their satisfaction level, which depicts very high ranking level. Interestingly, the FGD3 who are the farmers are also in consensus with the ranking by FGD1 and therefore ranked their level of satisfaction at 7 over 10 which implies a high level of satisfaction. It must be noted however that out of 18 farmers in this group, 55.70% (10) strongly agreed they are very highly satisfied, while 22.30% (4) respectively agreed that their level of satisfaction can be ranked high and fair respectively.

Apart from the FGD 3, most of the respondents from the Federal Ministry of Water Resources and Federal Ministry of Agriculture and Rural Development ranked their satisfaction level with the present decision-making for water allocation 7 out of 10 (high) while UNRBDA ranked it 9 from 10 (very high).

Given the varied responses across these respondents, chi-square (χ^2) test was employed to determine whether the level of satisfaction vary statistically across the categories of respondents or occurred mainly by mere chance. Table 4 presents the result of the Chi Square (χ^2) test for the level of satisfaction across the categories of the interviewees.

The result of the Pearson Chi-Square (χ^2) statistic = 10.000, df = 8, p> 0.05. This implies that there is no enough evidence to show that the level of satisfaction differs among the respondents. In other words, we can be confident that the stakeholders' level of satisfaction with water allocation decision-making process is either high or very high. This leads to the tested hypothesis below.

Null hypothesis (H0): There is no statistically significant difference in the level of satisfaction across the categories of stakeholders based on the existing water allocation practices.

Perception on equity of water allocation among the farmers

In view of the fact that water is a congestible and non-excludable public good that produces services for its users, the issue of equitable distribution within the water allocation process becomes increasingly important. Against the background that equity is central to the end users in the water allocation process; the responses of the farmers were given utmost consideration. In this regards, out of the 21 farmers surveyed, 44.40%(9) strongly agreed, 33.30% (7) agreed, while 11.10% (3) both disagree and strongly disagree respectively that water allocation based on the existing decision-making process is equitable. In addition, 83.3%(19) of the farmers opined that water allocation based on the existing decision-making process is based on their needs; 5.60% (1) responded that it is based on a proportion of their farm size while 11.10% (2) are largely undecided on this issue as they have no idea of the rationale for equitable water allocation in the study area. Hence, water allocation mechanism is based on the existing decision making process.

The existing allocation system originally intended to serve as check in order to prevent conflict in water allocation. It was also designed to ensure equity in water allocation process in relation to irrigation schedule, farm reports, use of overhead free boards, and participatory irrigation management.

1. Irrigation schedule: This is prepared by the UNRBDA and handed over to the Basin Operators, who are the schedule managers, to allocate and distribute water based on the needs of the Farmers' Association[6] (UNRB).
2. Farm reports: These are prepared by the Basin operators on a monthly basis detailing the achievements and difficulties encountered on irrigated sites and forwarded to the UNRBDA.
3. Use of overhead free boards: This is meant to take care of water balance to be supplied in case of excess demand by the farmers and water users associations.
4. Participatory irrigation management (PIM): This involves periodic meetings to resolve issues of conflict in water management in irrigation scheme and farm allocation as well as canal maintenance impacting on water equity distribution.

Supportively, Speed et al. (2013) argue that water allocation planning always focuses on achieving a set of policy objectives. These policy goals include; equity, environmental protection, economic priorities, balancing demand and supply, and efficiency in water use which results to sustainability.

Arguably, Roa-García and Brown (2015) indicate that allocation of water volumes among the farmers in the irrigated agriculture has been found inequitable because the criteria for water allocation are always inconsistent. However, Roa-García (2014) submits that weak legal institution and transparency have been the major factors hindering equitable, efficient and sustainable water allocation among the users in the basin. Differently viewed, Boelens et al. (1998) argue that equity should not be seen from the pervasive point of view dominated by the West. Rather, each society should be allowed to conceptualize 'equity' in line with their identity, values, economic and political sufficiency. Boelens et al. (1998) therefore, identify the following five levels of equity in irrigation and water management at the local levels:

1. Equitable water distribution and allocation among different water users and uses,
2. Equitable distribution of services involved in irrigation development,
3. Equitable distribution of the added agricultural production and other benefits under irrigation,
4. Equitable distribution of burdens and obligations related to functions and positions,
5. Equitable distribution of the rights to participate in decision-making processes, since this relates to the fundamental issue of whether or not every farmer has rights to speak, vote, claim an entitlement to irrigated land and enjoy equality of status (Boelens et al., 1998).

CONCLUSION AND RECOMMENDATIONS

In this study constraints that confront water allocation, farmers' level of satisfaction with water distribution to their farmlands, and achievement of equity as emphasized in the UN (2010) and Beail-Farkas (2012) reports were examined. Consequently, it was found that weak water institutions, legal framework incapacity, and aging infrastructures coupled with lack of maintenance, majorly hindered the equity for water allocation in the irrigated agriculture in the Northern part of Nigeria. However, varied opinions were observed on the level of satisfaction among the stakeholders with the highest mean value recorded at the end of rice production season in the area, while the lowest mean value was recorded at the beginning of the season. While 77.7% of farmers reported that equity objective is observed, 22.2% declined that there is equity in the allocation policy. This implies that there is seasonal variation Vis-a-Vis water requirement for farming and the value supplied by the basin authority. Thus, it calls for policy reform. Against the foregoing observations, the following recommendations need to be put in place for the irrigated agriculture development in Nigeria in order to address the issues of institutional challenges, which impacts deeply on decision making for water allocation as identified and the problem of maintenance especially on the aging infrastructure.

Starting from the policy-makers' perspective on improvements towards equitable water allocation, formidable institutional arrangements have been suggested to improve the influence of the disadvantaged stakeholders in decision-making process[7] (FGD1). Many of the policy-makers interviewed during the fieldwork in Nigeria submitted that institutional arrangement is a tool to get stakeholders involved which will in turn increase equity in water allocation achievable and make the smooth running of water resources management[8]. For instance, inadequate farming machinery mentioned as a constraint by all the farmers interviewed has alluded to weak

[6] UNRB1,UNRB4

[7] FGD1

[8] UNRB4,FGO1,FGO3

institutional arrangements. This is buttressed by this submission of some respondents:

In the country now we have Integrated Water Resource Management Commission (IWRMC), but up till now it is yet to be ratified by the law to be able to carry out its regulatory services. It only leans on the law establishing the Federal Ministry of Water Resources (FMWR) to carry out water allocation for various uses (Policy-makers, and Academia).

This view is supported by the FAO, which opined that inadequate maintenance has led to low capacity utilization and water logging in the irrigation system of Nigeria.

In addition, practical step towards institutional reforms and adequate funding for maintenance of facilities in order to optimally exercise justice and sustain equity in water allocation for the irrigated agriculture in Nigeria is imperative. Clear definitions of the powers of each of the institutions operating in the water sector, to remove situation whereby multiple agencies have authority and power over the same functions is required. Lastly, the idea of collaboration with private organization can be of help for efficient delivery.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Potential of antranilic diamides applied in seeds of soybeans with and without cry1ac protein for *Helicoverpa armigera* caterpillar control

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In Brazil, the caterpillar *Helicoverpa armigera* (Hübner) is a key economic pest of soybean, from seedling emergence to the reproductive stage. Control of this pest has relied on foliar insecticide sprays, and the use of insect-resistant cultivars. Characterization of the efficacy of insecticides that can be used for seed treatment, as a complement or alternative control for this pest, is needed for the production sector. The study objective was to evaluate the insecticidal potential of diamides for control of *H. armigera* larvae, when applied to soybean seeds. The effects of seed treatment with cyantraniliprole or chlorantraniliprole on 1st- and 3rd-instar *H. armigera* larvae, in both conventional and in "Bt soybean" (Cry1Ac), were evaluated under laboratory conditions. Two infestations were carried out at 8, 13 and 21 days after plant emergence, to determine the leaf area consumed, and mortality of larvae. The insecticides cyantraniliprole and chlorantraniliprole, used at rate of 60 and 62.5 g a.i./100 kg seeds, respectively, have potential for the control of 1st-instar *H. armigera* larvae up until at 13 days after the emergence of soybeans plants. The Cry1Ac protein controlled both 1st- and 3rd-instar *H. armigera* larvae at least until 21 days after plants emergence.

Key words: *Glycine max,* pest management, insecticides.

INTRODUCTION

The caterpillar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae) was recorded in the American continent in 2013, first in agricultural regions of Brazil (Czepak et al., 2013; Ávila et al., 2013), later in Paraguay (Senave, 2013), Argentina (Murúa et al., 2014) and, more recently, in the USA (North American Plant Protection Organization, 2015).

In Brazil, *H. armigera* is widely disseminated as a

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soybean pest and several other economically important crops, and feeding on weeds (Ávila et al., 2013; Czepak et al., 2013; Arnemann et al., 2014; Thomazoni et al., 2013). The management of this pest is hampered by its wide range of host plants, which includes non-monitored and fallow areas that serve as a refuge and source of multiplication of the pest (Tay et al., 2013).

In the soybean crop, this pest occurs from seedling emergence to the reproductive stage, when it feeds on flower buds and seed pods (Landim Filho et al., 2014). The occurrence as a pest at the beginning of the crop cycle can be due to two situations (Salvadori et al., 2013): caterpillars that are already present in the area, from the crop that precedes soybean, or infestations from eggs laid on the soybean plants, just after emergence.

The main tactic for control of *H. armigera* used by Brazilian soybean farmers has been foliar spraying of insecticides. The use of transgenic cultivars expressing the entomotoxic protein Cry1Ac from *Bacillus thuringiensis* (Fitt and Wilson, 2000), and seed treatment with insecticides are presented as alternatives to control *H. armigera*. Seed treatment products based on cyantraniliprole and chlorantraniliprole, from the diamide group, were recently introduced to the Brazilian market, and their efficacy in conventional and Bt soybean cultivars needs to be properly evaluated.

The objective of this study was to evaluate the insecticidal potential of cyantraniliprole and chlorantraniliprole for management of small *H. armigera* larvae, applied to soybean as seed treatments, with and without the presence of Cry1Ac protein.

MATERIALS AND METHODS

Experiments were conducted in the Entomology Laboratory of the Faculty of Agronomy and Veterinary Medicine (FAMV), University of Passo Fundo (UPF), Passo Fundo (RS), Brazil, in a controlled environment (25±2°C, 60±10% RH and 12-h photoperiod).

The effect of seed treatments with the insecticides on 1st- and 3rd-instar *H. armigera* larvae, in a conventional soybean cultivar (cv. BMX Potencia RR) and in a transgenic “Bt soybean” (cv. AS 3570 IPRO RR2, protein Cry1Ac) was recorded. Four chemical insecticides were applied in the treatment of seeds (Table 1), all are registered for this purpose in soybeans in Brazil (Agrofit, 2016).

The insecticides fipronil and imidacloprid+thiodicarb were used as positive controls, due to their established use in seed treatment of soybean. To obtain a uniform distribution, each insecticide was applied to 1.0 kg of seeds in a polyethylene bag with a small amount of water (0.3 L /100 kg seed). All the seeds were also treated with the carbendazim + thiram fungicides.

The insects used came from the rearing kept in artificial diet. Soybeans were grown in a greenhouse, in pots (5 plants per pot) with a capacity of 8 liters of soil and a surface area of 0.53 m^2. The pots were placed in trays, in which water was applied to irrigate the plants.

From plants in the V2 stage (second node on the main stem), leaf discs (1 or 4 cm², as required) were removed for bioassay with larvae placed individually in Petri dishes (9 cm diameter). Two infestations were performed for each larval stage (1st- and 3rd-instar), at 8, 13 and 21 days after plant emergence. Observations were performed daily, but it is considered for analysis the sum consumption, and the number of dead caterpillars five days after infestation. The caterpillars that did not show movement when touched with a brush was considered dead.

The experimental design was completely randomized, with 5 treatments (4 insecticides + negative control with only water), and 25 larvae/treatment. Data were subjected to analysis of variance, and means compared by Tukey's test (p≤0.05). For mortality analysis, the caterpillars were gotten together in 5 replicates of 5 individuals each.

RESULTS AND DISCUSSION

Seed treatment in conventional soybean

In 1st-instar caterpillars, at 8 days after plant emergence, all insecticides significantly suppressed foliar consumption by 1st instar larvae (Table 2).

However, the lowest foliar consumption was observed for cyantraniliprole and chlorantraniliprole, which also produced the greatest larval mortality (96.0 and 84.0%, respectively), demonstrating faster action than the other insecticides. Imidacloprid+thiodicarb caused less but still significant mortality, possibly explaining why it produced intermediate foliar consumption.

At 13 days after emergence, cyantraniliprole and chlorantraniliprole continued as the best treatments, both in terms of consumption and mortality, showing a persistent effect with comparable efficacy (Table 2). The other insecticides failed to significantly reduce consumption or kill larvae. At 21 days after the emergence, there was no residual effect (Table 2).

The rapid interruption of consumption in insects poisoned by cyantraniliprole or chlorantraniliprole applied to the seeds appears to provide protection to soybean against attack by small (1st and 3rd-instar) *H. armigera* larvae. Antranilic diamides, even before paralyzing the body of the insect leading to death, paralyzes the mandibles of larvae (Cordova et al., 2006; Lahm et al., 2009; Hannig et al., 2009; Álvarez and Abbate, 2013), so that only a small amount needs to be ingested for its effect to be observed (Table 2).

Regarding 3rd-instar larvae, the superiority of both diamides over the other insecticides was confirmed at 8 days after plant emergence, but only in terms of leaf consumption (Table 3). At 13 days after emergence, only ciantraniliprole reduced significantly leaf consumption, but offering only 59.6% protection. Due to this result, no evaluation was performed at 21 days after plants emergence, even though for the 1 st-instar larvae there was no residual effect after this time.

For the doses evaluated, the antranilic diamides proved to be a viable control option for neonate *H. armigera* larvae during the first week after emergence of soybean plants. The use of cyantraniliprole or chlorantraniliprole as seed treatments may be an important option for Brazilian producers, as the occurrence of infestations of this pest in the first days of the soybean cycle from eggs laid directly on the emergent plants is common (Salvadori

Table 1. Insecticides evaluated: active ingredient and respective trademark (name and rate).

Active ingredient	Trademark	Rate (ml/100 kg seeds)
Cyantraniliprole	Fortenza 600 FS®	100
Chlorantraniliprole	Dermacor®	100
Fipronil	Belure®	200
Imidacloprid + thiodicarb	Cropstar®	500

Table 2. Mean foliar consumption and mortality of 1st-instar *H. armigera* larvae at 8, 13 and 21 days after emergence of soybean plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days				13 days				21 days	
	Consumption (cm²)		Nº dead		Consumption (cm²)		Nº dead		Consumption (cm²)	Nº dead
Cyantraniliprole (60)	0.20	c	4.80	ab	0.18	b	4.60	a	0.34ns	3.60ns
Chlorantraniliprole (62.5)	0.22	c	4.20	ab	0.22	b	4.20	a	0.28	4.00
Fipronil (50)	0.72	b	1.40	cd	0.70	a	2.80	ab	0.46	2.60
Imidacloprid+thiodicarb (75+225)	0.56	b	2.00	bc	0.71	a	1.20	b	0.46	3.40
Control (water)	0.97	a	0.20	d	0.87	a	1.60	b	0.46	2.00
C.V. (%)	8.14	-	18.19		14.31	-	14.86	-	35.05	17.80

Means followed by the same letter do not differ statistically (Tukey, p≤0.05).

Table 3. Mean foliar consumption and mortality of 3rd-instar *H. armigera* larvae at 8 and 13 days after emergence of soybean plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days				13 days		
	Consumption (cm²)		Nº dead		Consumption (cm²)		Nº dead
Cyantraniliprole (60)	5.46	b	1.40	ab	3.26	b	2.40ns
Chlorantraniliprole (62.5)	3.93	b	2.60	a	4.93	ab	1.80
Fipronil (50)	9.79	a	0.40	b	7.01	a	1.20
Imidacloprid+thiodicarb (75+225)	12.79	a	1.40	ab	7.65	a	1.40
Control (water)	12.76	a	0.60	ab	8.06	a	1.20
C.V. (%)	19.34	-	22.95		30.22	-	23.06

Means followed by the same letter do not differ statistically (Tukey, p≤0.05), ns = non-significant diferences.

et al., 2013).

The study results show that the effect of cyantraniliprole and chlorantraniliprole applied as soybean seed treatments depends on the age of the larvae, as it has been observed in cotton (Barbosa et al., 2014) and in soybean (Landim Filho et al., 2014), with chlorantraniliprole. On the other hand, confirm the efficiency of the active ingredient for the control of this pest, as already seen in spraying of plant leaves (Wakil et al., 2012; Carneiro et al., 2014; Misra, 2015).

H. armigera sensitivity tests for cyantraniliprole show that oral toxicity is on average 400 times greater than that for contact toxicity (Bird, 2016), demonstrating that ingestion is important for control of *H. armigera* by this active ingredient, supporting its importance as a seed treatment (Table 3).

The residual effect of chlorantraniliprole applied as a soybean seed treatment against *Anticarsia gemmatalis* larvae has been previously reported (Rodrigues et al., 2014). In the present study, in 1st- and 3rd-instar *H. armigera* larvae, the effect was observed beyond 7 days after emergence, consistent with the results of Filho et al. (2014).

The performance of fipronil and imidacloprid+thiodicarb was lower than that of the cyantraniliprole and chlorantraniliprole. In the case of the mixture imidacloprid+thiodicarb, the small effect shown on 1st-instar *H. armigera* larvae at 8 days after plant emergence

Table 4. Mean foliar consumption and mortality of 1st-instar *H. armigera* larvae at 8, 13 and 21 days after emergence of Bt soybean (Cry1Ac) plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days		13 days		21 days	
	Consumption (cm²)	Nº dead	Consumption (cm²)	Nº dead	Consumption (cm²)	Nº dead
Cyantraniliprole (60)	0.19^{ns}	4.80^{ns}	0.14^{ns}	5.00^{ns}	0.20	5.00
Chlorantraniliprole (62.5)	0.20	5.00	0.16	4.80	0.20	5.00
Fipronil (50)	0.20	5.00	0.18	4.80	0.20	5.00
Imidacloprid+thiodicarb (75+225)	0.20	5.00	0.14	5.00	0.20	5.00
Control (water)	0.20	4.60	0.21	4.60	0.20	5.00
C.V. (%)	0.78	2.78	4.29	3.31	-	-

Ns = non-significant differences (p≤0.05).

may have been due to the presence of thiodicarb (Bueno et al., 2010), but in an insufficient dose.

The inability of fipronil in prevent foliar consumption by *H. armigera* larvae indicates a limitation of this active ingredient, at the dose tested, when applied to soybean seeds. Its toxic effect on caterpillars (Colliot et al., 1992), including *H. armigera* (Carneiro et al., 2014) has been observed when sprayed in soybean foliage.

Seed treatment in Cry1Ac soybean

With 1st-instar larvae, there was no difference in leaf consumption or larval mortality between Bt soybean (Cry1Ac) treated or untreated with insecticides, either at 8, 13 or 21 days after plant emergence (Table 4). Consumption was not significantly affected in either evaluation and mortality was high in all treatments (including the negative control) reaching 100% or near (Table 4).

Although consumption was modest (limited to a few test bites), it was sufficient to kill 1st-instar larvae, regardless of the treatment and the time between plant emergence and infestation. The rapid and high mortality caused by the Cry1Ac protein was also observed for neonate larvae of *H. virescens* (Bortolotto et al., 2014).

In 3rd-instar larvae, all insecticides significantly reduced leaf consumption at 8 and 13 days after plant emergence, relative to the control (Table 5). This result indicates that for larger larvae, the use of seed treatments in Bt soybean can minimize caterpillar damage to foliage. The average reduction in consumption in insecticide treatments was 30.1% and 55.3%, at 8 and 13 days after plant emergence, respectively.

However, the number of dead larvae did not differ between treatments. At 8, 13 and 21 days, the mortality of 3rd-instar larvae fed discs from soybean leaves whose seeds were treated with insecticides did not exceed that observed in the cultivar expressing the entomotoxic protein Cry1Ac, which alone caused complete larval mortality (100%). These data suggests that there is no advantage to seed treatment with the tested insecticides in Bt soybeans for control of *H. armigera* small caterpillars, when the infestation comes from oviposition after plants emergence.

The toxic effect of the Cry1Ac protein on *H. armigera* larvae of different stages in soybean, showing a high mortality (Bortolotto et al., 2014), is confirmed. This insecticidal protein has also been shown to be efficacious in controlling *H. virescens* larvae in soybean and cotton (Terán-Vargas, 2005; Bortolotto et al., 2014). In soybean, even in more developed plants (withseed pods), the concentration of the insecticidal protein is sufficient to control *H. virescens* larvae (Bernardi et al., 2013; Bortolotto et al., 2014) (Table 5).

Foliar consumption by 1st- and 3rd-instar larvae was lower in Bt soybean leaves than in the conventional cultivar (Tables 2 to 5). Aside from the fact that they are different genotypes, this is still indicative of the action of Cry1Ac protein on *H. armigera*. This protein causes a reduction in feeding and, consequently, starvation of larvae by specific binding in the ciliated membranes of the midgut, leading to death (Yu et al., 2011).

The results of both experiments demonstrated the potential of cyanthraniliprole and chlorantraniliprole (in seed treatment), and entomotoxin Cry1Ac in the control of infestations of *H. armigera* caterpillars hatched in newly emerged soybean plants. However, these results require confirmation under field conditions and natural pest infestation.

CONCLUSIONS

(1) The insecticides cyantraniliprole and chlorantraniliprole, used at rate of 60 and 62.5 g a.i./100 kg seeds, respectively, have potential for the control of 1st-instar *H. armigera* larvae up until at 13 days after the emergence of soybeans plants.

(2) Constitutively, expressed Cry1Ac protein also controls 1st- and 3rd-instar *H. armigera* larvae at least until 21 days after the emergence of plants.

Table 5. Mean foliar consumption and mortality of 3rd-instar *H. armigera* larvae at 8, 13 and 21days after emergence of Bt soybean (Cry1Ac) plants, whose seeds were treated with insecticides (25 ± 2 ºC, 60 ± 10% RH, 12-h photoperiod).

Treatment (g a.i./100 kg seeds)	8 days			13 days			21 days	
	Consumption (cm²)		Nº dead	Consumption (cm²)		Nº dead	Consumption (cm²)	Nº dead
Cyantraniliprole (60)	0.80	b	5.00	0.86	b	5.00ns	1.056ns	5.00
Chlorantraniliprole (62.5)	0.80	b	5.00	0.83	b	5.00	0.832	5.00
Fipronil (50)	0.90	b	5.00	1.17	b	4.71	0.928	5.00
Imidacloprid+thiodicarb (75+225)	0.80	b	5.00	0.80	b	5.00	0.992	5.00
Control (water)	1.18	a	5.00	2.05	a	5.00	0.896	5.00
C.V. (%)	5.36	-	-	16.05	-	1.65	15.85	-

Means followed by the same letter did not differ statistically (Tukey, p≤0.05). ns: non-significant differences.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Short Communication

Two new wheat varieties for irrigated conditions of Afghanistan

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New high yielding and disease resistant wheat genotypes were introduced in 2008-09 crop season through 4th Elite Bread Wheat yield Trial (EBYT) as well as 2nd Stem Rust Resistance Screening Nursery (STEMRRSN). One genotype from EBYT performed 15% higher than commercial variety, Mazar 99 during five years of testing at over eight locations in Afghanistan and was therefore recommended for release for commercial cultivation. Another genotype, Wafer-15 from STEMRRSN showed superior performance (11 to 33% higher yield) than commercial varieties during three years of testing at six locations in Afghanistan and was therefore released in 2015 for commercial cultivation. Both varieties were found resistant to prevalent rust diseases as well as to Ug99 race of stem rust.

Key words: Wheat, Afghanistan, yield, variety, rust.

INTRODUCTION

Afghanistan is a land locked country with an agrarian economy with 23 million (over 70%) population living in rural areas (FAO, 2016). Wheat is the staple food of Afghans and occupies about 2.5 million hectares with an average production of about five million tonnes annually (Persaud, 2012; Akbar Waziri et al., 2013). Wheat yield in Afghanistan is relatively lower than other countries in the region. Lack of adequate number of resistant varieties for different production environments is one of the major reasons for this. International Maize and Wheat Improvement Centre (CIMMYT) in collaboration with Agricultural Research Institute of Afghanistan (ARIA) have been working to identify suitable high yielding wheat varieties for Afghanistan. CIMMYT, ARIA and other stake holders have successfully released about 34 new wheat varieties in the country before 2000. Introducing new germplasm and identifying new adapted varieties is a continuous activity since new races/pathotypes of diseases continually evolve rendering popular varieties susceptible (Zamarai et al., 2013). Yellow rust has been and is the major wheat disease of Afghanistan rendering varieties susceptible quite frequently. Therefore, breeding/selecting for new high yielding resistant varieties is crucial for Afghanistan's food security. The present paper reports identification and release of two new high yielding, disease resistant wheat varieties for

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Table 1. Genotypes introduced into Afghanistan for testing adaptation.

S/N	Genotype	Pedigree	Trial/nursery	Test locations*
1	Wahdat-15	KIRITATI/4/2*SERI.1B*2 /3/KAUZ*2/BOW//KAUZ	4th Elite Bread Wheat Yield Trial	KBL, NGR, BGL, TKR, HLM, KDZ, HRT, BLK
2	Wafer-15	BABAX/LR42//BABAX*2 /3/TUKURU	2nd Stem Rust Resistance Screening Nursery	KBL, NGR, BGL, KDZ, BLK, HRT

KBL: Kabul; NGR: Nangarhar; BGL: Baghlan; TKR: Takhar; HLM: Helmand; KDZ: Kunduz; HRT: Herat; BLK: Balkh.

Table 2. Average yield of test genotypes over years at several locations in Afghanistan.

Genotype	Yield						Superiority over check (%)
	2008-09	2009-10	2010-11	2011-12	2012-13	Average	
Wahdat-15	5129	4853	5802	5548	6453	5557	
Chonte #1		4521	5818	5569	5794	5425	104
Ariana 07		5256	5466		5789	5504	104
Mazar 99	4837	4129	4300		6044	4828	115
Wafer-15	7040	6563	4809	5427		5960	
Chonte#1		4930		5569		5249	114
Ariana 07	3907	6454	5567	5466		5348	111
Solh 02	4364		4576			4470	133

irrigated wheat areas of Afghanistan.

MATERIALS AND METHODS

One of the genotypes viz., Wahdat-15 was introduced into Afghanistan through 4th Elite Bread Wheat Yield Trial (EBYT) in the crop year 2008-09. EBYT trial was received from CIMMYT, Mexico and comprised of 30 new wheat genotypes. This trial was conducted at three locations viz., Kabul, Nangarhar and Baghlan during 2008-09. Over later years, Wahdat-15 was tested at several locations viz., Kabul, Nangarhar, Baghlan, Takhar, Balkh, Kunduz, Helmand and Takhar from 2008-09 to 2012-13 along with checks like Chonte#1, Ariana 07 and Mazar 99. The second variety Wafer 15 was introduced into Afghanistan through 2nd Stem Rust Resistance Screening Nursery (STEMRRSN) in the same year from CIMMYT, Mexico (Table 1). Wafer-15 was tested only for three years at six locations viz., Kabul, Nangarhar, Baghlan, Kunduz, Balkh and Herat. The trials were laid out in randomized complete block design (RCBD) with three replications. Plot size consisted of six rows of six meters each. Standard recommended agronomic practices were adopted to raise a successful crop in the irrigated conditions. Observations were recorded on days to 50% flowering, days to maturity, plant height, 1000 kernel weight and grain yield. Disease severity was also recorded as per modified Cobb's scale of Peterson et al. (1948). The genotypes were also sent to Ug99 stem rust screening facility at Njoro, Kenya for scoring for Ug99 resistance.

RESULTS AND DISCUSSION

The two genotypes tested across Afghanistan on an average had higher yield than the check varieties intended to be replaced. Wahadat 15 (Table 2) yielded an annual average from a low of 4853 to a highest of 6453 kg/ha during different years. The five years' average of 5557 kg/ha was found to be only numerically higher (4%) than Chonte#1 and Ariana 07, however it yielded whopping 15% higher than the popular variety Mazar 99. Similarly, Wafer 15 yielded an annual average ranging from 4809 to 7040 kg/ha with an overall average of 5960 kg/ha. Wafer-15 showed superior performance over the check varieties ranging from 11 (Ariana 07) to 33% (Solh 02). Wahadat 15 took about 141 days to mature and attained a height of 91 cm. This variety had a potential yield of 6453 kg/ha and an average 1000 kernel weight of 36 g. Wafer 15 on the other hand matured in 153 days with an average height of 92 cm. The highest yield recorded for Wafer 15 was 7040 kg/ha and its 1000 kernel weighed 39 g (Table 3).

Frequent detection of new virulent races of rust reduces availability of resistant varieties (Zamarai et al., 2013). Afghan wheat acreage of about 2.5 million hectares requires close to 300,000 tonne of seed. However, at a given time, not more than 10-15 resistant varieties are available in seed chain. A robust seed chain to produce even 10% of national requirement should ideally have 20 to 30 varieties, so that about 20 varieties can produce certified seed, and other 10 are either being inducted or are on their way out. This will ensure a much needed mosaic of resistance genes in farmer fields and will avoid monoculture which generally leads to break down of resistance when a single variety occupies large

Table 3. Salient features of two new varieties for Afghanistan.

Trait	Wahdat-15	Wafer-15
Target environment	Irrigated	Irrigated
Days to heading (no.)	113	115
Days to maturity (no.)	141	153
Plant height (cm)	91	92
1000 kernel weight (g)	36	39
Average yield (Kg/ha)	5557	5960
Potential yield (Kg/ha)	6453	6563
Yellow rust Afghanistan	10R	0
Yellow rust Kenya	1MR	5M
Stem rust Kenya	15MSS	10M

geographic area. Both these varieties were found resistant to yellow rust in Afghanistan under field conditions. The two varieties showed acceptable disease reaction under artificial infestation at Kenya for both yellow as well as stem rust including Ug99 race of stem rust. Both varieties were released by Afghanistan Government in 2015 for commercial cultivation by farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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