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Full Length Research Paper

Analyses of constraints related to milk production in Liptako Gourma in Niger

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Livestock in Niger has great potentials. If well managed, this sector can significantly improve food security and boost poverty alleviation in the population. In order to encourage the development of local milk production in Liptako Gourma, the present study was undertaken to identify constraints related to milk production in the area. A questionnaire based survey was conducted to collect data from 360 dairy farmers. The study revealed that Liptako Gourma harbours a diversified cattle population comprised of 83.3% of Djelli in Niamey, 69.8% of Peul Zebus and 55.8% of Azawak in Tillaberi and 20.0% of Goudali in Dosso. The workhands is mainly constituted of Fulani farmers (77.3 ± 5.0% in Tillaberi) and Zarma agro- farmers (33.3 ± 16.8% in Niamey). They have traditional skills in milk production technology. The study also showed that animals' feeding is highly dependent on seasonality. The most prevalent animal diseases were fasciolosis (55.0, 36.6 and 51.7%, in Dosso, Tillaberi and Niamey, respectively), bezoars (15.0, 60.0 and 12.8% in Dosso, Tillaberi and Niamey, respectively) and respiratory problems (46.6, 40.0 and 35.8% in Dosso, Tillaberi and Niamey, respectively). The low level of education of farmers which is, for example, 16.2 ± 4.4% in Tillaberi, the low level of investment, the poor hygienic practices and difficulties associated with milk conservation and sale constitutes further constraints that thwart the development of the dairy sector. Findings of this study call for technical supports from veterinarians and hygiene services to dairy farmers so as to significantly control the aforementioned constraints.

Key words: Local milk, Liptako Gourma, Niger, milk valorisation, production constraints.

INTRODUCTION

Livestock plays an important role in the livelihood of populations around the world. It constitutes the main source of protein in human nutrition through its various products notably, meat, milk, cheese, eggs, etc (Laouali et al., 2014). In Sub-Sahara Africa countries, animal production contributes significantly to the improvement of food and nutritional security of households (Laouali, 2014). Niger is one of the major animal producing countries in sub- Saharan Africa, with a livestock size estimated at more than 42 million animals (all species combined) in 2015, which could worth over XOF 3451 billion with an annual milk production of about 1161926 tons (MEL, 2015). Animal production is practiced in Niger by 87.0% of the active population (FAO, 2011). The number of cattle is estimated at 12059951 heads in 2015 with an annual cow milk production of 596, 968 tons (DGPIA, 2015). Apart from the numerical importance of its cattle population, Niger hosts a wide diversity of breeds with many adaptation features to specific environments. There are five main cattle breeds in Niger: Azawak breed which means "sandysoil" in Tamajeq language (MRA CCN/GRGAD, 2003), Bororo, Kouri in kanouri language (Zafindrajaona et al., 1999), Djelli (Peul) and Goudali which means "short horns and legs" in Hausa language (Assani, 2013) and many crossbreeds offering interesting variants (MEL, 2013). Azawak and Kouri are among the best milk producing breeds in Africa (Geesing et al., 2001). Furthermore, Niger has an old culture of milk and other dairy products consumption (SOW, 2005). People usually consume the raw and fermented milk (FAO/SFW, 2010). In spite of the high number of cattle in the country, the produced milk does not satisfy the national needs (Moussa, 2016). The systematic presence of calves is one of the reasons of the low milk productivity from cows since a part of the milk is consumed by these calves (Corniaux et al., 2012). Moreover, the quantity of milk collected is usually low as compared to the actual potential of the animals. The point is also stressed by the rudimentary milk transportation means (bicycles, carts, motorcycles) and the defective or non-existent road infrastructures that decrease the capabilities of production (Corniaux et al., 2005). The national milk production covers only 50.0% of the needs of an increasing population, especially in urban areas. The population of Niger, estimated at nearly 15.2 million inhabitants in 2010, an increase of just under 30% compared to 2000 (11.02 million), has one of the higher growth rate in the world (FAO, 2012). At the same time, milk consumption for the same period is of 53 L per person per year to 63.8 L of milk per person per year (Duteutre et al., 2013). To satisfy the remaining demand, Niger usually imports massively dairy products (Vias et al., 2009). However, in case of overproduction during the rainy season, it is very common to see farmers discarding the unused milk especially when the herds are far from the market and this constitutes a serious loss (ONUDI / AFD-PSEAU, 2007). Previous studies conducted on the dairy sector in Niger were essentially focused on Niamey (Sioussaran, 2003; Marichatou et al., 2005; Vias et al., 2003, 2006, 2010; Vias, 2008, 2013; ONUDI /AFD-PSEAU 2007; Sow, 2005). However, the Liptako Gourma area is a big animal production zone in Niger and regroups 3 regions out of the 8 of the country (Dosso, Niamey and Tillabéri). As a cross-border area, together with its high transit position, Liptako Gourma constitutes a sort of mingling area for cattle from Burkina Faso, Mali, Niger and other bordering communities.

An improved use of the high cattle population of Liptako Gourma and its abundance in water resources and available large grazing spaces, could lead to a significant reduction of milk and dairy products importation. This area could serve as a zone of milk production susceptible to supply the local and regional dairy enterprises. The present study aimed to identify constraints related to milk production in Liptako Gourma in Niger in order to propose adequate solutions capable of improving the endogenous processes of milk processing and preservation.

MATERIALS AND METHODS

Study area

The study was carried out in Liptako Gourma, an area located astride the borders of Burkina Faso, Mali and Niger (Figure 1). It covers 9.7% of the total surface of Niger and covers 3 constituencies that are Dosso, Niamey and Tillabéri (Table 1). This area harbours important potentialities in energy, hydraulics and mines. However, it suffers from its hemmed-in position (Gado, 2007). The dominant economic activity is agriculture and animal production that are in serious difficulties due to the inaccessibility to water and the invasion of the rivers by water hyacinth. The dairy basins of the capital (Niamey) where the demand in dairy products is higher than the offer are located on both sides of this zone.

Selection of the investigated sites

The 3 regions of Liptako Gourma targeted by the study are constituted of 12 dairy basins. These basins are focal points of milk collection (high dairy production) for the supply of big cities and circumscribed in a radius of 100 km around the three regions located on a tarred road to facilitate a secured transportation of milk. A Dairy Basin (DB) is an area constituted of milk production units from which particular cities are supplied.

Sampling procedure

The study included individuals who produce and/or collect raw and fermented milk in the study area. This choice was made because of the non-existence of a database for dairy stakeholders in the area. Respondents were randomly selected based on their accessibility and availability to provide the required information. A total of 180 producers, 126 producer-

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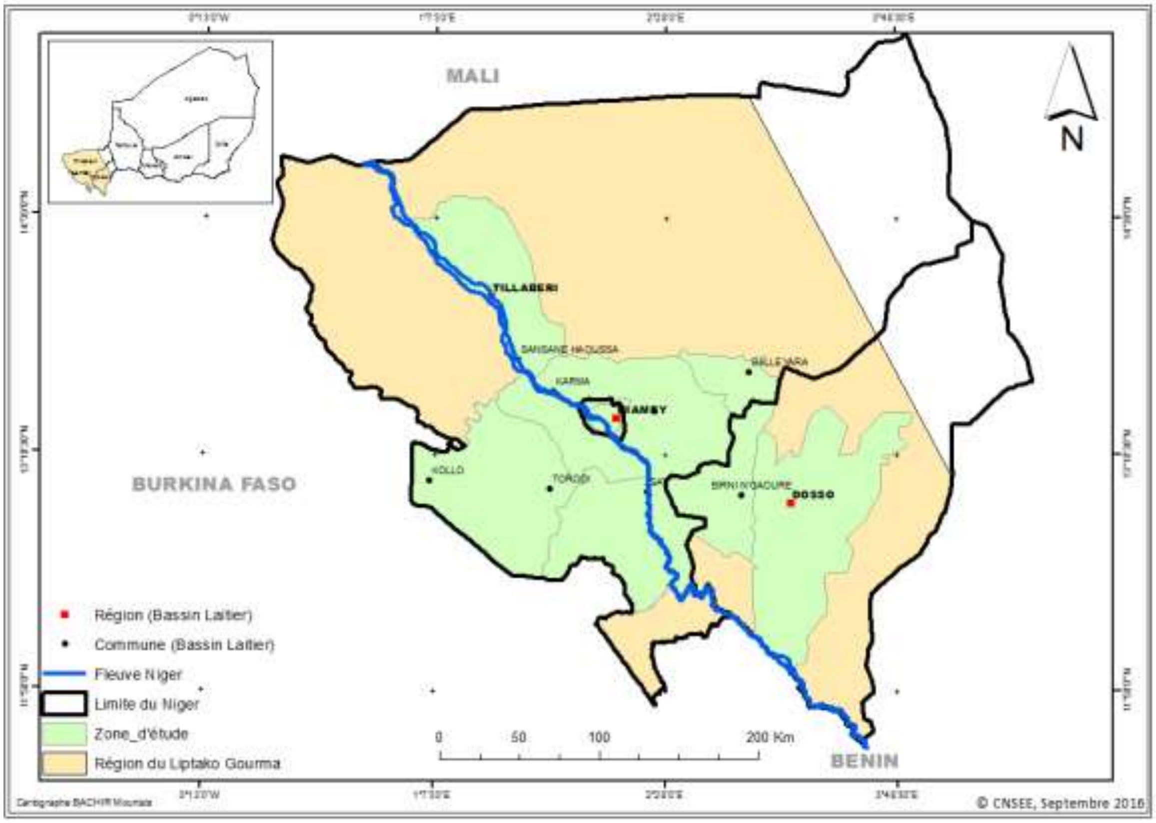

Figure 1. Map of Liptako Gourma.

Table 1. Respondents sampling in Liptako Gourma, Niger.

Region	Dairy basins (DB)	Producers	Producer-collectors	Collectors	Total
Dosso	DB of Dosso	15	11	4	30
	DB of BirninGaouré	11	15	4	30
Niamey	DB of Niamey	17	10	3	30
Tillabéri	DB of Hamdallaye	8	20	2	30
	DB of Kollo	16	8	6	30
	DB of Karma	15	12	3	30
	DB of Say	18	9	3	30
	DB of Torodi	17	6	7	30
	DB of Baleyara	16	10	4	30
	DB of Tillabéri	16	6	8	30
	DB of Dembou	17	11	2	30
	DB of Sansane haoussa	14	8	8	30
Total		**180**	**126**	**54**	**360**

collectors and 54 milk collectors distributed in the 12 retained dairy basins were interviewed (Table 1). Furthermore, key informants were interviewed from the Ministry of livestock, Departmental Directories of livestock, Local Directories of livestock, NGOs and farmers' associations, as well as, milk collection centers and dairy industries.

Data collection methods

This was across-sectional study in which investigations were based on face-to-face interviews with milk producers, producer-collectors and collectors. Structured pre-tested questionnaires were administered to respondents to probe information regarding

Table 2. Identification of dairy operators.

Variable		Dosso		Niamey		Tillaberi		Significance test
		%	CI	%	CI	%	CI	
Sex	Women	18.3[a]	9.7	10.0[ab]	10.7	3.0[b]	2.0	***
	Men	81.6b	9.7	90.0[ab]	10.7	96.9[a]	2.0	***
Ethnic groups	Haoussa	5.0[a]	5.5	-	-	4.9[a]	2.6	NS
	Kanouri	-	-	-	-	0.7[a]	1.0	NS
	Peul	68.3[a]	11.7	66.6[a]	16.8	77.3[a]	5.0	NS
	Zarma	26.6[a]	11.1	33.3[a]	16.8	16.9[a]	4.5	NS
Level of education		36.6[a]	12.1	53.3[a]	17.8	16.2b	4.4	***

%: Percentage; CI: Confidence interval; ***: significant at 1‰; NS: Not Significant; Percentages of the same raw followed by the same letters are not significantly different at 5%.

farms' management, the conduct of the dairy herds, the reproduction and milk production techniques, the quantitative and qualitative milk production, animal health and milk delivery.

Statistical analyses

The collected data were recorded in Microsoft Excel and analysed with SAS (2006) software. One way ANOVA was used and the parameter "region" was the only source of variation. The procedure Proc GLM was used for the analysis of variance. Proportions were computed by the procedure Procfreq of SAS and compared using Chi-Square and bilateral Z test. For each relative percentage, a confidence interval (CI) at 95% was calculated using the formula as follows:

$$ICP = 1.96\sqrt{\frac{[P(1-P)]}{N}}$$

Where P is the relative percentage and N the sample size.

RESULTS

Typology of the dairy operators

In this study, dairy operators are the milk producers, producer-collectors and collectors. Majority of them (96.9 ± 2.0% in Tillaberi) are men (Table 2). However, a number of women are also involved in this activity mainly in Dosso (18.3 ± 9.7%). Statistical analyses revealed a significant difference between the 3 regions based on the sex of the operators ($p < 0.001$). Most of them are from Peulh ethnic group (77.3 ± 5.0% in Tillaberi), followed by Zarma agriculturists natives of Liptako Gourma. More than half of the operators do not have school education except in Niamey where 53.3 ± 17.8% of them are educated. Included in schooling, any level of education combined. There was a significant difference between the 3 regions with respect to the education of the operators. Livestock production is the main activity at around 80.0 ± 14.3% in Niamey (Table 3) or secondary activity at over 50.0% in association with successively agriculture and trade. The development of a business network around milk production makes this activity more attractive than it has ever been. Civil servants and other socio-professional groups are also involved in cattle farming. About 10.0 and 6.7% of civil servants in Dosso and Tillaberi respectively; 14.2, 9.0 and 1.4% of craftsmen and other workers in Dosso, Niamey and Tillaberi respectively are also implicated in milk production.

Characteristics of the dairy farms

In all the investigated regions most of the dairy farms use domestic work hand (85.0%). Only selected operators notably retired or active civil servants (10.0% in Dosso) and traders (6.6% in Niamey) use salaried work hand. The main activities in these farms consist of feeding and serving water to the animals, milking and delivery of milk and treatments of sick animals. The required small material (feeding dish, drinking trough) common for all dairy operators is found in all farms. These materials are mostly constituted of recycled household containers like plastic or metallic buckets and bowls or barrels. Barrows are found in 16.6 ± 9.4, 20±14.3 and 26.7 ± 5.3% of the farms and carts were found in 16.6 ± 9.4, 13.3 ± 12.1 and 17.3 ± 4.5% of farms in Dosso, Niamey and Tillaberi, respectively. Animals are tied to a peg. Calves are kept away from cows at night. A very few operators (less than 2.0%) had cowsheds, feeds stores and cars.

Characteristics of the dairy herd

The most preferred cattle breeds used in the investigated dairy farms were Djelli breeds (83.3% in Niamey) and Peul Zebus (69.8% in Tillaberi) (Figure 2). Azawak breed occupied the 3rd position (55.8% in

Table 3. Activities of the dairy operators.

Variable		Dosso		Niamey		Tillaberi		Significance test
		%	CI	%	CI	%	CI	
Main activity	Agriculture	13.3[a]	8.6	13.3[a]	12.1	7.9[a]	3.2	NS
	Trade	3.3[a]	4.5	6.6[a]	8.9	6.4[a]	2.9	NS
	stock farming	73.3[a]	11.1	80.0[a]	14.3	78.1[a]	4.9	NS
	Teaching	5.0[a]	5.5	-	-	5.2[a]	2.6	NS
	Entrepreneur	-	-	-	-	0.7[a]	1.0	NS
	Soldier	3.3[a]	4.5	-	-	0.3[a]	0.7	NS
	Custom officer	1.6[a]	3.2	-	-	0.3[a]	0.7	NS
	Policeman	-	-	-	-	0.7[a]	1.0	NS
Secondary activity	Agriculture	21.4[a]	15.2	27.2[a]	26.3	16.9[a]	8.7	NS
	Trade	7.1[a]	9.5	9.0[a]	16.9	-	-	NS
	stock farming	57.1[a]	18.3	54.5[a]	29.4	81.6[a]	9.0	NS
	Guardian	7.1[a]	9.5	9.0[a]	16.9	1.4[a]	2.7	NS
	Tailor	3.5[a]	6.8	-	-	-	-	NS
	Carpenter	3.5[a]	6.8	-	-	-	-	NS

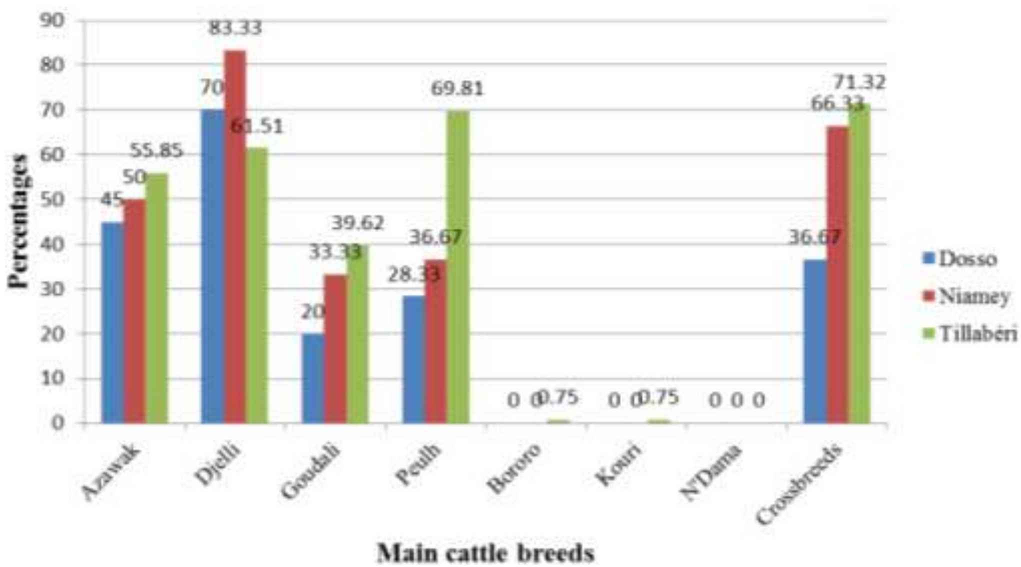

Figure 2. Main cattle breeds used in the study area.

Tillaberi) followed by Goudali (39.6% in Tillaberi). In the herds, more than half (57.8%) of the animals are cows with 14.7% of heifers. Furthermore, there were relatively more male calves (13.5%) than females (12.9%).

Management of dairy farms

Feeding

In peri-urban areas, feeding takes place on natural pastures within distances ranging between 3 and 5 km across the year. After a grazing of about 3 to 6 h, animals receive agricultural by-products (cereals' stems and haulms) and a complementation with brans (millet, wheat or rice bran). The distributed quantities depend on the season, the size of the cow and its milk production and also the operator's income: 0.9 to 1.8 Kg per cow per day in rainy seasons and 1.8 to 2.7 Kg in cold seasons. The distribution is made two times a day before milking.

In urban areas, there is almost no available grazing space and feeding in rainy seasons is based on a supply of green fodders that are sold along the roads in addition to some small natural pastures. Animals in this condition, receive an important extra-feed mainly brans like to 3.6 kg per cow per day. In dry seasons, the green fodder is replaced by hay, bush straw and bourgou (*Echinochloa stagnina*). In rainy seasons, animals drink water from dams, streams and rivers, while in dry seasons they basically use water from wells, borings and sometimes rivers. Animals drink water 2 times per day (85.0%) and *ad libitum*. In urban areas, operators buy water for their animals throughout the year. The different modes of conduct of the herds determine the different production systems that are of 2 types in the considered area (Dosso, Niamey and Tillaberi):

1. The intensive system (that was not investigated). It concerns dairy farms of big traders, high commissioners and dairy cooperatives; animals are maintained in free stalling and exclusively fed at the trough.
2. The semi intensive system within which different modes of herd managements are employed:

i. The peri-urban and urban livestock keeping practiced by 22.3% of the operators whereby settled keepers or small civil servants get settled around big cities in camps or small farms. The main objective is milk production that is delivered directly to the collection point or to retailers. Milk is generally sold raw or traditionally pasteurized. This mode is characterized by the proximity of the production zones and the consumption centers, the existence of processing units that influences the organization of the collection circuits.
ii. The agro-pastoral livestock keeping practiced by 28.9% of the operators; crop production constitutes the main activity of this system with a low amount of milk produced; this system is frequently found among autochthonous zarma producers of the study area.
iii. The pastoral, nomadic or transhumant livestock keeping is practiced by 48.8% of the operators: mostly Peulhs. It can be practised alone or combined with other activities. Milk production is the main objective and used for sale and to feed the family. This system is very efficient with regard to the use of space and pastoral resources.

Management of the reproductions

The management of reproduction within the dairy farms is based on various crossings between the existing local breeds (65.0% of the operators), aiming to get better crossbreeds for enhanced milk production. The mating is either free or directed. However, artificial insemination and the introduction of exotic breeds is considered expensive by the operators and thus not practiced.

Management of the production

Dairy production depends highly on a nutritional feed and therefore strongly subjected to seasonality. The periods of cereals and legumes harvesting correspond to the peak of lactation and availability of milk that decreases overtime across the year. The quantities collected per cow per day are more important in the rainy season with an average of 3.5 ± 0.8 L. On the other hand, in the dry season, there is a decrease of dairy production and consequently a decrease of the quantities of milk collected (1.5 ± 0.4 l per cow per day). Milking is carried out 2 times per day in the presence of the calf. The quantity milked very early in the morning is exclusively and directly sold (at collection point, to potential customers and collectors) by operators who are close to urban centers. The evening milk is sold by door to door or at a determined retail outlet and also serves for domestic consumption. The remoteness of urban centers and the lack of conservation methods are the reasons why such milk is usually consumed fresh or sometimes processed or given to people or completely discarded. The high heats of the region that can reach 45°C sometimes makes milk more perishable and the storage at the shade of trees is not sufficient for its preservation. Some operators try to prolong the shelf life of their milk by covering the containers with moistened bags. For morning milking, 98.0% of the operators deliver their products within 2 to 4 h. The delivery is made by the operators with the means that they have (motorcycles, bicycles) in 45.3% of the cases and by collectors (54.6%).

Production hygiene and health of the dairy cows

Utensils used for milking are calabashes (79.1%), followed by plastic (12.6%) and metallic (8.1%) utensils such as bowls, cups, buckets, sieves. For milk collection and transportation, the respondents use plastic cans of 5, 40 or 50 L. The used utensils are systematically washed except for the calabash in all the regions with soap by 90.9% of operators. However, hygiene practices are only limited to the washing of collection and milking utensils, whereas the preparation of cow's udder and personal hygiene are not respected among peri-urban operators. Hand washing is significantly ($p < 0.001$) practiced by all dairy operators in peri- urban areas of Niamey as compared to Dosso and Tillaberi (26.6 ± 11.1 and 16.2 ± 4.4%, respectively) (Table 4). Nevertheless, this hand washing is only done with plain water in all respondents of Niamey. Some few operators of Tillabéri and Dosso

Table 4. Hands washing and cow's teats cleaning.

Variable	Dosso		Niamey		Tillaberi		Significance test
	%	CI	%	CI	%	CI	
Hands washing	26.6[b]	11.1	100.0[a]	-	16.2[b]	4.4	***
Water	23.3[b]	10.7	100.0[a]	-	12.4c	3.9	***
Water + soap	1.6[a]	3.2	-	-	3.4[a]	2.1	NS
Cow's teats washing	5.0[a]	5.5	3.3[a]	6.4	4.9[a]	2.6	NS
Water	1.6[a]	3.2	3.3[a]	6.4	3.7[a]	2.2	NS
Water + soap	-	-	-	-	-	-	NS
Cloth	3.3[a]	4.5	-	-	1.1[a]	1.2	NS

%, Percentage; CI, confidence interval; ***,significant at 1‰; NS, Not significant; Percentages of the same raw followed by the same letters are not significantly different at 5%.

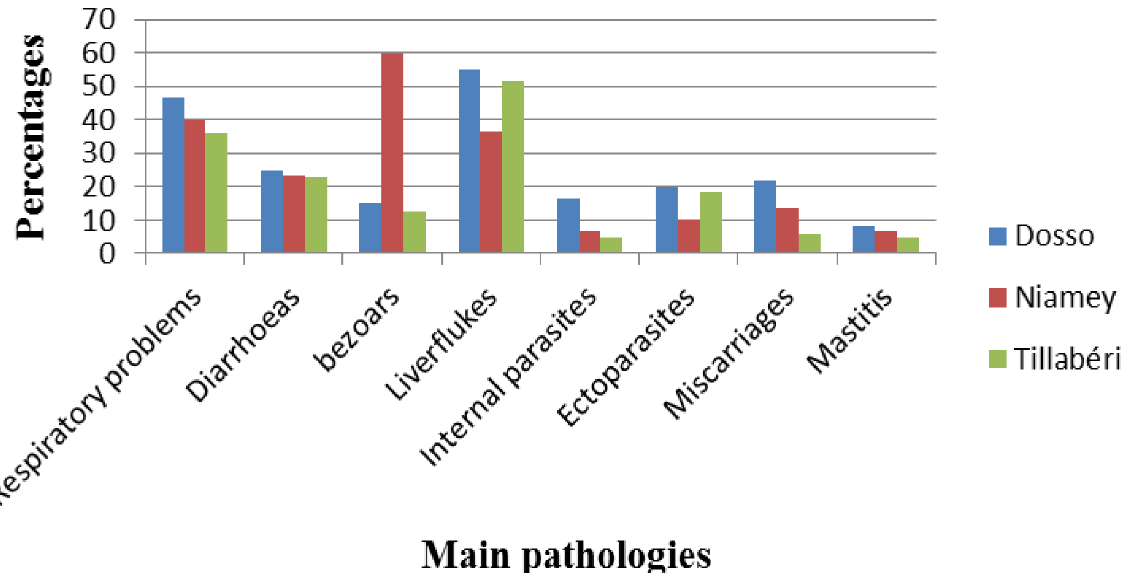

Figure 3. Major animal pathologies per region.

use soap. Farmers also consider Islamic prayer ablutions as hand washing regardless of the time that separates that act and the milking. Teats washing interests only 5.0% of the operators from all regions and this is made with plain water or by wiping the teats with a cloth. However, this washing cannot be effective if there is no clean water. The water supply, even though it is from a potable water source, is carried in dirty and sometimes unclosed cans that could compromise the quality of the water.

With regard to the health of the dairy cows, respiratory problems, diarrhoeas, bezoars (especially in Niamey) and liver flukes constitute the major handicaps to the health of the dairy cows (Figure 3). Despite these health situations, only government subsidized vaccines against contagious bovine peripneumonia (CBPP), pasteurellosis and anthrax are given to the animals. The use of veterinary services and laboratory analyses are often rejected for financial reasons (Table 5).

DISCUSSION

Dairy activities in Liptako Gourma in Niger are essentially practised by operators of Peulh ethnic group. The high level of involvement of this ethnic group in the diary sector was previously reported by many authors (FAO, 1995; Meyer et al., 1999, Vias et al., 2003, Marichatou et al., 2005, Assani, 2013). These populations have traditional skills in cattle management that are transmitted from one generation to the other (Allsopp et al., 2007), mainly regarding the virtues of medicinal plants (Ayantunde et al., 2009), the assessment of pastures' qualities (Soumana et al., 2010; Soumana, 2011), and the management of pastures and herds (Oba and Kaitira, 2005; Soumana,

Table 5. Animal health care.

Variable	Dosso		Niamey		Tillabéri		Significance test
	%	IC	%	IC	%	IC	
Vaccinations	95[a]	5.5	100[a]	0.0	98.1[a]	1.6	NS
Veterinary services	8.3[a]	6.9	13.7[a]	12.5	9.0[a]	3.4	NS
Lab analyses	8.3[a]	6.9	16.6[a]	13.3	8.3[a]	3.3	NS

%, Percentage; CI, confidence interval; NS, Not Significant; Percentages of the same raw followed by the same letters are not significantly different at 5%.

2011; Soumana et al., 2012).

Cattle production is the main activity of Peulh populations who have lately adopted other secondary activities because of a number of constraints that have affected the traditional dairy system (Schneider et al., 2007). Nevertheless, Zarma and Haoussa producers adopted livestock keeping as a secondary activity to enhance their incomes. When they settle, former nomadic keepers often develop crop production, wheres agriculturists opt for extensive animal production as a mean of diversifying their productions. In addition, the animals serve as workforce and provide manure for their crops. On the other hand, traders and civil servants use livestock activities as investment opportunities.

Technically, the integration crop-animal production is mutually profitable. In fact, the livestock provides workforce and manure to crop production and receives in return agricultural by-products as feed. Economically, such integrated production systems provide a favourable strategy of risks management according to Somda et al. (2004). The low enrollment rate of education of dairy farmers constituted in majority of Fulani was mentioned by Barry (2006).

Furthermore, the low income of most operators is the cause of their inability to appropriately equip their farms. Drinking troughs and feeding dishes constitute the sole equipment found in most of the farms because they are indispensable. The habitats of animals were just a small space around households and camps. Such housing condition can be a threat to animals' health because of the lack of hygiene and space capable of leading to various contaminations. In these dairy farms, money is not the key element for the business to keep running. Farmers share similar needs in terms of housing and small material resulting in low production cost. This low level of hardware investment confirms that production factors in the dairy exploitations are still traditional. Among the used cattle breeds, Djelli is the most preferred probably because of its resistance. Although Azawak is well reputed as a good dairy breed, it is not highly used certainly because of its feeding requirements for effective performances expression. Goudali is only used as a matter of breed heritage.

Dairy cows' feeding is based on fodders and agricultural by-products. Ngongoni et al. (2006) reported that fodder represents 90% of the available cattle feed in rainy seasons in Zimbabwe. However, the seasonal variability of fodder, the mobility of animals and the difficulties to obtain concentrated feeds constitute some of the constraints that hinder dairy cattle feeding (Duteutre, 2007). Feed complementation is only practiced in dry season while it would have been more beneficial on the zootechnic and economic parameters of the exploitations if it were also practised in rainy seasons where there are peaks of lactation through a well- balanced diet. Some elements of similarities do exist with respect to the quality of the feed given regardless of the financial status of the farmer as they basically rely on natural grazing and agricultural by-products. Differences are only found in the rationing where the distributed quantities are mostly lower than the required standards since the producers do not have any training in dairy cow feeding. Watering is an essential aspect of the breeding system and increases the production cost especially in farms that use public water supply (Boudon et al., 2013). However, any restriction due to this high cost can be fatal to the production. The recuperation of runoff water in pits and rain water from the roofs in appropriate recipients could somehow alleviate water scarcity challenges during dry seasons.

As the main dairy breeds are local breeds with some random crossbreeds, any nascent dairy development plan should consider genetic improvement with a compulsory resolution of feed related problems. This could significantly enhance the quantities of milk harvested. Araba (2006) confirmed that the production of milk that tripled in the last thirty years in Morocco was mainly due to genetic improvement of the breeds. Even when these production performances are improved, it will be necessary to reorganize the milk collection system in order to effectively obtain adequate profits from the dairy cows.

The quality and hygiene of milking utensils, personal hygiene during milking, hands washing and the safety of the milking environment constitute important factors in the reduction of health risks. With respect to hygienic practices, the milkmen are all at risk. During milking, farmers do not wash their hands; however, they lubricate their hands during the process by soaking their hands in the already harvested milk. Nevertheless, hand washing has become systematic in peri-urban dairy farms of Niamey because of APROLAN project (Project of support to the promotion of the peri-urban dairy sector in Niamey) that addresses issues of good hygienic practices. Moreover, milking utensils are not effectively washed. They are mostly rinsed without disinfectant and exposed to the open air with all the cross-contaminants (wind, dust, flies and other bugs). The use of small material that can be easily handled and washed such as bowls, cups and aluminium buckets should therefore be encouraged.

The predominance of bezoars can be attributed to the pollution by plastic garbage from urban centres. Most of the dairy cows of the study area suffer from worms. The high prevalence of these parasites is probably due to the presence of a river in the study area. This high vulnerability to diseases shows that the peri-urban dairy operators do not use veterinary inputs/services despite the presence of veterinary and para-veterinary services in the nearby cities. This situation exposes their animals to low milk production because the presence of diseases and absence of adequate prevention techniques reduce the milk production capabilities of cows (Coignard et al., 2014).

Possibilities for technological and organizational improvements of the dairy production exist. Technically, it is possible to improve the feed of dairy cows. Feeding practices have moved from transhumance to settlement and from exclusive grazing to grazing complementation. A thorough study on feed complementation practices could establish suitable production technologies. Since the raw material already contains many hazards, it would be healthy to prevent the initial contamination so as to increase the delay bacterial growth in the milk. A serious organization of the collection should also be considered to improve the economic performances of the dairy sector.

Conclusion

The study revealed a number of constraints that hamper the development of the dairy sector in Niger. Dairy operators are confronted with technical problems (health, feed, genetic, etc.), as well as political, institutional and environmental problems that limit the performances of the dairy production. These constraints are worsened by the impacts of climate change. Dairy herds often experience dramatic fodder and water scarcity in dry seasons. Various animal diseases continue to be a serious limitation for their productivity. The low level of valorisation of the genetic potential of local breeds limits the expression of their performances. The majority of investigated dairy operators does not have school education and lack professionalism resulting in low or no investment in their farms. Infrastructures and facilities are almost non-existent with a limited and old material. Dairy producers, who have links with milk collection centers are not many and are not specialized. The herds are not organized to produce only milk as priority but used for all possible options (meat, milk, traction). The production systems are seriously affected by land insecurity. In order to secure milk and dairy products supply, it will be necessary to have fodder stocks and effectively use agricultural by-products (especially stems). The genetic potential of local dairy breeds can be improved by the use of critical mass of genetically selected dairy animals. Future studies on this topic will have to consider all this dynamics so as to solve the constraints adequately.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Improved production systems for common bean on Ferralsol soil in south-central Uganda

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Common bean (*Phaseolus vulgaris* L.) is the most important source of dietary protein in Uganda but current grain yields are low. Production is particularly low on the degraded Ferralsol soils that dominate the landscape because these soils are generally weathered, acidic and infertile. A study was done on a Ferralsol soil in Masaka District, Uganda to compare productivity and economic return to labor and management (ERLM) for four bean cultivars grown under three management systems. The experimental design was a randomized complete block in a split-plot arrangement. Management system was the whole-plot factor and included the Conventional Farmer (CFS), Improved Farmer (IFS), and High Input systems (HIS). Management systems differed for seed fungicide treatment (no vs. yes), seeding density (10 vs. 20 seed m^{-2}), plant configuration (scatter vs. rows), fertilizer applications (P, K, Ca, Mg, Zn and S), rhizobium inoculation (no vs. yes), pesticide applications (no vs. yes), and frequency and timing of weeding. Subplots were four common bean cultivars that differed for resistance to foliar pathogens and the ability to tolerate low soil fertility. Increasing management level and planting bean cultivars tolerant to common bean diseases and low soil fertility improved bean grain yield. The only grain yield differences observed were between cultivars in the 2015A season; NABE 14 had greatest grain yield, 168% greater than that of NABE 15. The HIS with NABE 14 (1274 kg ha^{-1}), the HIS with NABE 4 (1225 kg ha^{-1}), and the IFS with NABE 14 (1025 kg ha^{-1}) had the greatest grain yield. The ERLM was only profitable for the CFS ($40 ha^{-1}); cultivars did not differ. Farmers planting bean on Ferralsol soil can improve yields with higher input systems but the tested systems were not profitable.

Key words: *Phaseolus vulgaris* L., soil fertility, crop management systems, improved cultivars, profitability.

INTRODUCTION

Low soil fertility and acidity are the most important common bean (*Phaseolus vulgaris* L.) productivity constraints in East Africa (Lunze et al., 2007). Bean is an important crop worldwide but it is especially important in East Africa where it is a staple crop for dietary protein (Kweka, 2001). Despite its importance, bean needs more attention as an alternative to expensive red and white meat protein to meet the dietary needs of the ever-growing population of Uganda. Uganda's population has increased very rapidly which has consequently increased the pressure on the land through continuous cultivation and reducing the frequency of traditional fallow periods (Ronner and Giller, 2012). Conventional management practices have resulted in infertile and degraded soils due to soil fertility mining (Nabhan et al., 1999).

Uganda's population is estimated to be 34.9 million (Uganda Bureau of Statistics, 2014), with 80% living in rural areas. Average farm size in south-central Uganda is 1.2 ha in south-central Uganda. Due to low opportunities of employment, many people are living on subsistence agriculture and therefore desire a crop that is productive, profitable and nutritious (Kilimo Trust, 2012). However, due to limited land, beans are often grown on highly weathered soils and are rarely productive under conventional management practices.

Beans are grown on many types of soils in Uganda but the strongly weathered soils, such as Ferralsols, form more than 70% of the soil on which most of the farming is practiced (Wortmann et al., 1998). Beans are preferentially grown on darker, more fertile soils but most of the land favorable for row crop agriculture is already in production. This leaves the highly weathered and nutrient depleted, acidic soils to be utilized for crop production (Ronner and Giller, 2012). These soils require substantial inputs to improve both soil chemical fertility and pH (Nabhan et al., 1999) because they are strongly leached and have lost most of their weatherable minerals (Jones et al., 2013). These soils are dominated by stable minerals such as aluminum oxides, iron oxides and kaolinite, giving this soil its red color (Jones et al., 2013). These Al and Fe oxides often bind with P, making it unavailable for plant uptake. It is therefore important to lime these soils to increase the cation exchange capacity (CEC), neutralize Al, and to increase the supply of essential minerals such as Ca, K and Mg (Lunze et al., 2012). A target pH of 5.8 to 6.5 is favorable for bean production (Lunze et al., 2012) and when the pH is in this range minerals become more soluble, microorganisms are more active, and plant nutrient uptake improves.

Bean production on these red soils is poor but little research has suggested methods to increase yields on these acidic weathered soils. Ronner and Giller (2012) showed considerable improvements in bean yield and profitability when adding fertilizer but other research suggests it may be unwise to invest in high input agriculture because the yield increases may not be sufficient to cover the input costs (Ojiem et al., 2014). Currently, inorganic fertilizer is applied in very low quantities in Uganda, despite many soils being nutrient depleted (Ronner and Giller, 2012).

Soil amendments are effective at improving soil health and productivity but smallholder farmers often cannot afford the amounts required to correct soil pH and nutrient deficiencies (Lunze et al., 2007). Alternatively, bean cultivars with tolerance to edaphic stresses can make it possible to improve bean yield and profitability on these low fertility and acidic soils by reducing the farmers' dependency on fertilizers and therefore reduce production costs (Lunze et al., 2007; Singh et al., 2003). Disease resistant cultivars have been developed to avoid the risk of yield losses but adoption is low (Broughton et al., 2003). To address the constraints limiting bean production on Ferralsol soil in south-central Uganda, this study aims at comparing grain yield and profitability of four bean cultivars grown under a conventional and two improved management systems.

MATERIALS AND METHODS

Experimental site

The experimental site was located approximately 13 km northeast of Masaka, Central Region, Uganda (latitude 0° 15' 49.2552" S; longitude 31° 48' 32.8752" E; altitude 1281 m). The climate is tropical and generally rainy with two dry seasons (Jones et al., 2013). Soil at the location was called Limyufumyufu (reddish) in the local language but is defined as a Ferralsol using the FAO-UNESCO soil legend and as a Eutrudox using USDA Soil Taxonomy (FAO, 1988; USDA NRCS, 1999). The soil at the experimental site was a sandy clay loam texture and formed from alluvial deposits. Prior to adding soil amendments, soil at the 0 to 15 cm depth had a pH range of 5.2 to 5.4, Mehlich-3 P ranged from 4 to 6 mg kg^{-1}, and organic matter (OM) ranged from 41 to 43 g kg^{-1}. Long term mean annual precipitation in Uganda is 1175 mm, with about 86% occurring during the crop growing seasons (World Bank Group, 2015). Precipitation data for the specific research site were not available before this project. According to the landowner, prior to the initiation of this study, the site had been in a maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogaea* L.), banana (*Musa* × *paradisiaca* L.) and cassava (*Manihot esculenta* Crantz) intercrop.

Experimental design

The experimental design and many of the materials and methodologies used in this study were similar or identical to those reported in a related study by Goettsch et al. (2016). The study was initiated in July 2014 and continued over two seasons, the second rainy season of 2014 (2014B), from the end of August through the beginning of December, and the first rainy season of 2015 (2015A), from the end of March through the middle of June. The experimental design was a randomized complete block in a split-plot arrangement. Management system was the whole-plot factor

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Table 1. Agricultural inputs and management methods for each management system in the 2014B season and the 2015A season. 2014B is 2014 second rainy season, 2015A is 2015 first rainy season, CFS is Conventional Farmer System, IFS is Improved Farmer System, HIS is High Input System.

Property	Units	2014B			2015A		
		CFS	IFS	HIS	CFS	IFS	HIS
Lime	kg ha^{-1}	0	15,900	15,900	0	0	0
P_20_5	kg ha^{-1}	0	84	84	0	67	67
K_2O	kg ha^{-1}	0	45	45	0	112	112
$ZnSO_4$	kg ha^{-1}	0	3.4	3.4	0	3.4	3.4
Vitavax	applied	No	No	Yes	No	No	Yes
Rhizobia	applied	No	Yes	Yes	No	Yes	Yes
Planting	seeds m^{-2}	10	20	20	10	20	20
Planting	method	Scattered	Rows	Rows	Scattered	Rows	Rows
Fungicide	g ha^{-1}	0	0	458	0	0	458
Insecticide	L ha^{-1}	0	0	2.5	0	0	2.5
Weeding*	frequency	Twice	Twice	Weekly	Twice	Twice	Weekly

*Weeding was done by hand between plants and with a hand hoe between rows.

and included Conventional Farmer System (CFS), Improved Farmer System (IFS), and High Input System (HIS) (Table 1). The subplots were four common bean cultivars. Two cultivars, NABE 14 and NABE 15, were new and improved, and the other two, K132 and NABE 4, were conventional cultivars. The new cultivars were released 7 to 16 years later (2006 and 2010) than the older cultivars (1994 and 1999) and have greater resistance to several bean diseases prevalent in the south-central region of Uganda. Individual plot size measured six meters by four meters. There were four replications of each subplot combination.

Crop management practices

Perennial crops and residual weeds from the previous rainy season were removed more than one month prior to planting in the 2014B season and agricultural lime with 38% Ca and 68.85% effective calcium carbonate equivalent (ECCE) was applied at 15,900 kg ha^{-1} to neutralize the soil pH. Results from analysis of pre-plant soil samples showed available K was low, therefore, muriate of potash was broadcast by hand prior to tillage both seasons in the IFS and HIS. Potassium was applied at 44.8 kg ha^{-1} in the 2014B season and 112 kg ha^{-1} in the 2015A season. One to two weeks prior to planting, tillage was conducted with a hand hoe to a depth of 15-20 cm over a period of several days. Beans planted in the CFS were scatter planted at a density of 10 seeds m^{-2} while beans in the IFS and HIS were planted in rows 50 cm wide with seeds planted every 10 cm, which resulted in the recommended planting density of 20 seeds m^{-2} for both the IFS and HIS (UEPB, 2005). Bean seeds were from Community Enterprises Development Organisation (CEDO) located in Rakai, Uganda. Seed for HIS were treated with VITAVAX® (Bayer CropScience, Research Triangle Park, NC.) fungicide (carboxin: (5,6-Dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide) by CEDO personnel. Seeds planted in the IFS and HIS were inoculated with Mak-Bio-Fixer rhizobia obtained from Makerere University prior to planting. Before planting the IFS and HIS, triple superphosphate (0-46-0) was banded at 84 kg P_2O_5 ha^{-1} in the IFS and the HIS in the 2014B season and at 67.3 kg P_2O_5 ha^{-1} in both improved management systems in the 2015A season. Bands were placed in hand dug furrows at a depth of 8-10 cm and covered with 2-4 cm of soil. Beans were then placed at the recommended depth of 3-5 cm (Amongi et al., 2014) before being covered with soil using a hand hoe. Planting dates were 17 and 18 August during the 2014B season and 23 March for the 2015A season.

Formulated azoxystrobin (methyl (E)-2-{2[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) was applied at identical rates and timing as described by Goettsch et al. (2016). Four days after applying the fungicide in the 2014B season, the insecticide cypermethrin ((±)α-cyano-(3-phenoxyphenyl)methyl(±)-*cis-trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) formulated as Dudu-Cyper® 5% EC (Bukoola Chemical Industries LTD, Kampala, Uganda) was foliar-applied to the HIS beans at a rate of 2.5 L ha^{-1} mixed with 3.36 kg ha^{-1} of $ZnSO_4$. This mixture was applied to the HIS with the hand-pumped backpack sprayer in approximately 625 L H_2O ha^{-1}. In the 2015A season, the fungicide, insecticide, and $ZnSO_4$ were foliar-applied with the backpack sprayer as a mix to the HIS at the same rates as the previous season. The IFS received the $ZnSO_4$ application both seasons at a rate of 3.36 kg ha^{-1}.

Weeding occurred twice per season for the CFS and IFS. The first weeding occurred about V3 in the 2014B season and between V3 and V4 in the 2015A season. The second weeding occurred between R7 and R8 both seasons. Weeding was done weekly for the HIS so that weeds were never competitive with beans.

Crop and soil data collection

The pre-amendment and post-harvest soil samples were collected at a depth of 0 to 15 cm from 12 subsamples collected from each replication of each whole plot. Soil samples were analyzed for pH and electrical conductivity (EC) using the potentiometric method. Extractable Al (1-N KCl), organic matter (Walkley-Black C/0.58) and N (Kjeldahl) concentrations were determined by colorimetry. The cation exchange capacity (CEC) was calculated according to Brady and Weil (2007). After extraction with Mehlich-3, inductively coupled plasma optical emission spectrometry (ICP-OES) was used for soil sample analysis of P, K, Mg, Ca, Na, Al, Mn, S, Cu, B, Zn and Fe following extraction with Mehlich-3.

Phenological development stages were recorded weekly in each plot using the standard system developed for common bean (Van Schoonhoven and Pastor-Corrales, 1987).

Briefly, V1 is emergence, V3 is first trifoliate leaf, V4 is third trifoliate leaf, R5 is preflowering, R6 is flowering, R7 is pod formation, R8 is pod filling and R9 is physiological maturity.

Table 2. Monthly precipitation during the course of the study, long-term precipitation and long-term temperature. LT is long term (1990-2012) mean for Uganda (World Bank Group, 2015).

Month(s)	Precipitation* (mm)		Precipitation (number of rainy days)		Mean Precipitation (LT) (mm)	Mean Temperature (LT) (^oC)
	2014	2015	2014	2015	1990-2012 avg.	1990-2012 avg.
January	-	3	-	2	42	23.9
February	-	34	-	4	44	24.9
March	-	108	-	8	96	24.5
April	-	364	-	17	152	24.0
May	394	298	18	15	129	23.2
June	103	50	7	4	88	22.7
July	77	-	7	-	83	22.3
August	106	-	6	-	114	22.7
September	97	-	8	-	118	22.9
October	112	-	8	-	142	23.1
November	69	-	9	-	111	23.5
December	63	-	11	-	56	23.5
March-June	-	820	-	44	465	23.6
August-December	447	-	42	-	541	23.1
January - December	-	-	-	-	1175	23.4

*Precipitation values recorded at the experimental site; located 13 km NE of Masaka, Central Region, Uganda.

Between R8 and R9, aboveground crop biomass was determined by hand clipping five bean plants per plot. Biomass samples were oven-dried at 60°C for 7 days and then weighed. Yield, yield components, and extended plant height data were collected from all bean plants within a 2.0 m^2 area from each plot. Stand density of bean at R9 stage was determined at harvest by counting the number of plants within each harvested area. Extended plant height was measured on every plant harvested, up to a maximum of ten plants per subplot. At harvest, all pods were hand-picked, counted, placed in a paper bag, and weighed. Pods were placed in an oven at 60°C until dry and seed shelled, counted, and weighed. The pod harvest index (PHI; dry weight of seed at harvest/dry weight of pod at harvest × 100), pod number per area (pods m^{-2}), and seed number per pod (seeds pod^{-1}) were computed as described by Beebe et al. (2013). Grain yields are reported at 100% dry matter. Soil volumetric water content (VWC) was determined using a calibrated FIELDSCOUT® TDR 300 Soil Moisture Meter (Spectrum Technologies, Inc., Plainfield, IL). Sampling occurred weekly in each subplot at two points for each of two depths, 7.5 and 20 cm.

The costs of production and market prices of beans were based on local market prices for all agricultural inputs, except rhizobia, which was unavailable in the local market. It was assumed that inoculation will occur every four seasons. The economic return to labor and management (ERLM) was determined based on land rental costs collected from farmers in the Masaka District. The market price of bean used in the analysis assumed beans were sold immediately after harvest when farm gate prices ranged from 1500 to 1700 UGX kg^{-1}, depending on the cultivar. The UGX to USD conversion rate used for this study was 3400 UGX = 1 USD.

Statistical analysis

Data were analyzed as a randomized complete block in a split-plot arrangement with management system as the whole-plot factor and bean cultivar as the subplot factor. Statistical analyses for yield, yield components, height, biomass, PHI, VWC, phenological and economic data were performed with the GLIMMIX Procedure of SAS V9.4 (SAS Institute, 2013). Least squares means were generated for all variables when significant F values ($P < 0.05$) were observed and then separated using the LINES option at $P = 0.05$. Soil data were analyzed using PROC GLM, which enabled us to separate means using the multiple mean comparison of the protected least significant difference. Differences among treatments were reported as significant at $P = 0.05$ except for the phenological differences between treatments, which were reported as significant at $P = 0.01$. Management system, cultivar, rainy season and weeks after planting (WAP) were considered fixed effects. Replication, replication × management system, and cultivar × replication × management system were considered random effects.

RESULTS

Climate

Climate results are identical to those reported in a related study by Goettsch et al. (2016) (Table 2). Total precipitation during the study, July 2014 through June 2015, was 1381 mm, 18% greater than the long-term normal. Precipitation during the dry season months, July and again January through February, amounted to only 67% of the 22-yr average for these months. However, the precipitation in April 2015 was 139% greater than that of the long-term average and the precipitation in May 2015 was 131% greater than that of the long-term average.

Soil

The pre-amendment soil results differed among

Table 3. Pre-amendment and post-harvest soil (0 to 15-cm depth) nutrient concentrations, CEC, EC, organic matter and base saturation results from the three common bean management systems. Soil collected from Masaka District, Uganda with collection period for pre-amendment in July 2014 and post-harvest in December 2014. CFS is Conventional Farmer System, IFS is Improved Farmer System, and HIS is High Input System.

Property	Units	Pre-amendment			Post-harvest		
		CFS	IFS	HIS	CFS	IFS	HIS
pH		5.2^{b}	5.4^{b}	5.4^{b}	5.2^{b}	7.0^{a}	7.1^{a}
CEC	meq $100g^{-1}$	10^{b}	12^{b}	11^{b}	11^{b}	20^{a}	22^{a}
EC(S)	µS cm^{-1}	88^{b}	100^{b}	98^{b}	78^{b}	166^{a}	177^{a}
Extr. Al	meq $100g^{-1}$	0.273	0.174	0.166	0.425	0.150	0.125
P	mg kg^{-1}	4^{b}	6^{b}	4^{b}	4^{b}	15^{a}	19^{a}
K	mg kg^{-1}	55^{b}	56^{b}	47^{b}	49^{b}	79^{a}	87^{a}
Mg	mg kg^{-1}	200	253	246	195	256	267
Ca	mg kg^{-1}	710^{b}	926^{b}	911^{b}	785^{b}	3138^{a}	3603^{a}
Na	mg kg^{-1}	46^{ab}	58^{a}	48^{ab}	25^{c}	30^{bc}	60^{a}
Al	mg kg^{-1}	1228	1183	1180	-	-	-
Mn	mg kg^{-1}	163^{c}	201^{abc}	182^{bc}	221^{ab}	233^{a}	215^{ab}
S	mg kg^{-1}	6^{b}	6^{b}	6^{b}	9^{ab}	12^{a}	13^{a}
Cu	mg kg^{-1}	2.0^{d}	2.3^{c}	2.2^{cd}	2.9^{b}	3.2^{a}	3.1^{ab}
B	mg kg^{-1}	0.1^{c}	0.2^{bc}	0.2^{bc}	0.3^{ab}	0.4^{a}	0.4^{a}
Zn	mg kg^{-1}	1.0^{c}	1.5^{b}	1.2^{bc}	1.2^{bc}	3.1^{a}	3.3^{a}
Fe	mg kg^{-1}	99^{b}	110^{b}	99^{b}	129^{a}	132^{a}	131^{a}
N	%	0.13^{b}	0.13^{b}	0.13^{b}	0.17^{a}	0.19^{a}	0.19^{a}
OM	g kg^{-1}	41^{ab}	41^{ab}	43^{a}	38c	37^{c}	39^{bc}
C:N	ratio	18^{a}	18^{a}	18^{a}	13^{b}	12^{b}	12^{b}
Base saturation	%	55^{b}	61^{b}	61^{b}	54^{b}	94^{a}	95^{a}

Means within property followed by the same letter, or no letter, are not different at P=0.05. OM as Walkley-Black C/0.58; other elements determined with ICP-OES following extraction with Mehlich-3. Extraction of exchangeable Al done with 1N KCl.

management systems for Cu and Zn; all other physico-chemical parameters measured were similar among management systems (Table 3). Conversely, there were greater levels of the following properties in the two improved management systems in the post-harvest soil data as compared to the pre-amendment soil data: pH, CEC, EC(S), P, K, Ca, S, B, Cu, Zn, Fe, N and base saturation (Table 3). Additionally, post-harvest soil results differed for management systems for pH, CEC, EC(S), P, K, Ca, Na, Cu, Zn, and base saturation.

Volumetric water content

The VWC differed for management system, rainy season, and the interaction of rainy season × depth. No other main effects or interactions were significant. The VWC differed for depth during both rainy seasons. The mean VWC in the 2014B season was 0.19 and 0.21 cm^3 cm^{-3} for 7.5 and 20 cm depth, respectively while mean VWC in the 2015A season was 0.26 and 0.24 cm^3 cm^{-3} for 7.5 and 20 cm depth, respectively. The 2014B season was wetter at 20 cm depth as compared to 7.5 cm depth while the reverse was true for the 2015A season.

Bean development

The phenological development of beans varied for all main effects and their interactions, except management system (results not presented). The interaction of cultivar × rainy season × WAP was significant (Figure 1). In both seasons, there was a divergence of cultivars with NABE 15 reaching stage five of development sooner than other entries. However, in subsequent WAP, cultivars were once again at similar development stages. Then, around 10 WAP, both seasons, NABE 15 diverged for a week before converging at maturation (R9). In the 2014B season, maturity was reached in 13 weeks while in the 2015A season, maturity was reached in just 11 weeks (Figure 1).

Yield, yield components, height, biomass and pod harvest index (PHI)

At maturity (R9 stage), stand density of beans differed for management system, cultivar, rainy season, and interactions of management system × cultivar and cultivar × rainy season (Table 4). In both rainy seasons, NABE 15 had the lowest stand density (Table 5). Differences were

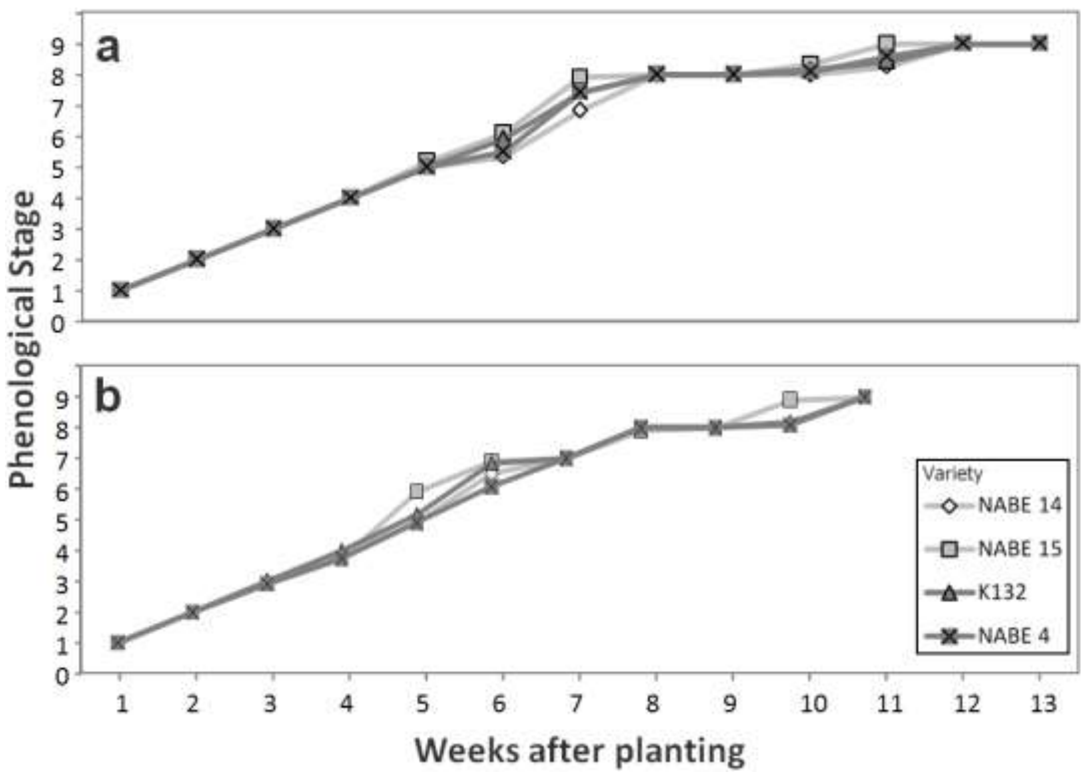

Figure 1. Weekly mean phenological stage of bean for four cultivars in (a) the 2014 Rainy Season B and (b) the 2015 Rainy Season A across three management systems, Masaka, Uganda.

observed in stand density each rainy season × management system, which is due to the differences in planting density. Plant stands did not differ between rainy seasons for the IFS or HIS but there was a decrease in plant stand for the CFS. The CFS resulted in eight plants m^{-2} in the 2014B season but only four plants m^{-2} in the 2015A season (results not presented). Height of beans at harvest varied for management system, cultivar, rainy season, and the interaction of cultivar × rainy season (Table 4). In both rainy seasons, bean cultivars differed in height (Table 5). NABE 4 was taller than NABE 15 and NABE 14 in the 2014B season while K132 was the tallest and NABE 15 was the shortest in the 2015A season.

Pod density of beans differed for management system, cultivar, rainy season, and the interaction of cultivar × rainy season (Table 4). NABE 14 produced the fewest pods m^{-2} in the 2014B season and NABE 15 produced the fewest pods m^{-2} in the 2015A season (Table 5).

Seed number pod^{-1} varied for management system, cultivar, rainy season, and the interactions of management system × cultivar, management system × rainy season, and cultivar × rainy season (Table 4). Seed number pod^{-1} varied for cultivar in the HIS but cultivar did not vary for CFS or IFS (Table 6). NABE 14 had the greatest seeds pod^{-1} in the HIS. In both rainy seasons, seed number pod^{-1} varied for cultivar (Table 5). NABE 14 frequently produced more seeds pod^{-1} than the other cultivars while K132 produced among the fewest seeds pod^{-1} both seasons. Seeds pod^{-1} varied for management system only in the 2015A season (Table 7). The IFS and HIS produced more seeds pod^{-1} than the CFS in the 2015A season. The 100-seed weight varied for management system and rainy season; however, the interactions were not significant (Table 4). Cultivar did not influence 100-seed weight but the seed weight in the 2014B season was 28% greater than for the 2015A season. K132 produced the heaviest seed, weighing 12% greater than NABE 14.

Aboveground biomass (g $plant^{-1}$) varied for rainy season and the three-way interaction of management system × cultivar × rainy season (Table 4). Biomass samples were not taken from nine CFS plots and one IFS plot in the 2015A season because plant stands were low. Collecting these plants for aboveground biomass would have compromised our ability to harvest grain yield and grain yield components. Consequently, the authors were unable to calculate protected least significant difference tests when including the 2015A season. Excluding the 2015A season and looking only at the 2014B season, aboveground biomass did not vary for any main effect or interaction.

Grain yield differed for management system, cultivar, rainy season, and the interactions of management system × cultivar and cultivar × rainy season (Table 4).

Table 4. Yield, yield components, height, biomass, pod harvest index (PHI), and net profit/loss for four bean cultivars in three management systems for two rainy seasons, Masaka, Uganda. CFS is Conventional Farmer System, IFS is Improved Farmer System, and HIS is High Input System. 2014B is 2014 second rainy season, 2015A is 2015 first rainy season.

Treatment	Plant stand (# m^{-2}) R9	Extended plant height(cm)	Pods (# m^{-2})	Seed (# pod^{-1})	Seed size (100 seed weight, g)	Biomass (g plant^{-1}) R8-R9*	Grain (kg ha^{-1})	Pod harvest index(PHI)	Economic Return to Labor and Management (USD)
Management System									
CFS	6^{b}	22^{b}	23^{b}	2.6^{b}	30.9^{c}	N/A	235^{b}	67^{b}	40^{a}
IFS	17^{a}	33^{a}	81^{a}	3.0^{a}	37.0^{b}	15	933^{a}	74^{a}	-812^{b}
HIS	16^{a}	34^{a}	83^{a}	3.1^{a}	41.1^{a}	15	1061^{a}	76^{a}	-1057^{c}
Cultivar									
NABE 14	14^{a}	29^{b}	62^{ab}	3.3^{a}	34.8^{b}	14	831^{a}	69^{b}	-583
NABE 15	11^{b}	23c	54^{b}	2.7^{b}	35.9^{ab}	N/A	613^{b}	72^{a}	-659
K132	13^{a}	35^{a}	65^{a}	2.7^{b}	38.9^{a}	13	746^{ab}	73^{a}	-605
NABE 4	14^{a}	32^{a}	68^{a}	2.9^{b}	35.8^{ab}	13	784^{a}	75^{a}	-592
Rainy season									
2014B	14^{a}	31^{a}	73^{a}	3.1^{a}	40.8^{a}	17	948^{a}	75^{a}	-491^{a}
2015A	12^{b}	28^{b}	52^{b}	2.7^{b}	31.9^{b}	N/A	539^{b}	69^{b}	-728^{b}
Significance						*P*>F			
System (S)	***	***	***	*	**	NS	***	*	***
Cultivar (C)	***	***	*	***	NS	NS	*	**	NS
S × C	NS	NS	NS	*	NS	NS	*	NS	NS
Rainy season (R)	***	*	***	***	***	***	***	***	***
S × R	***	NS	NS	**	NS	NS	NS	***	NS
C × R	**	***	**	*	NS	NS	***	NS	NS
S × C × R	NS	NS	NS	NS	NS	*	NS	NS	NS

Means within treatment and column followed by the same letter, or no letter, are not different at *P*=0.05. *, **, ***, and NS indicate statistical significance at *P* ≤ 0.05, 0.01, 0.001, and not significant, respectively. *Protected least significant difference tests not calculated on biomass (g plant^{-1}) because there were no enough samples available to be collected in the 2015A season.

Grain yield differed for cultivar in the HIS but cultivar did not influence yield in the CFS or IFS (Table 6). Under the HIS, NABE 14 (1274 kg ha^{-1}) and NABE 4 (1225 kg ha^{-1}) produced the greatest grain yields. Cultivars only varied for grain yield in the 2015A season (Table 5). NABE 14 produced the greatest grain yield (772 kg ha^{-1}) in the2015A season, recording 168% greater yield than NABE 15 (288 kg ha^{-1}).

The PHI varied for management system, cultivar, rainy season and the interaction of management system × rainy season; all other treatment factors and interactions were not significant (Table 4). The PHI only varied for management system in the 2015A season when the IFS and HIS had 16 and 25% greater PHI than CFS (Table 7).

Economic analysis

Management system and rainy season influenced

Table 5. Interaction of cultivar × rainy season for R9 plant stand density, height, pod density, seed number, and grain yield of bean for two seasons. 2014B is 2014 second rainy season, 2015A is 2015 first rainy season.

Parameter	2014B	2015A
Plant stand (# m^{-2}) R9		
NABE 14	14^{a}	13^{a}
NABE 15	12^{b}	10^{c}
K132	15^{a}	12^{b}
NABE 4	14^{a}	13^{a}
Height (cm)		
NABE 14	30^{b}	28^{b}
NABE 15	28^{b}	18^{c}
K132	32^{ab}	39^{a}
NABE 4	35^{a}	29^{b}
Pods (# m^{-2})		
NABE 14	64^{b}	61^{a}
NABE 15	74^{ab}	34^{b}
K132	72^{ab}	58^{a}
NABE 4	81^{a}	56^{a}
Seed (# pod^{-1})		
NABE 14	3.3^{a}	3.3^{a}
NABE 15	3.0^{ab}	2.3^{b}
K132	2.9^{b}	2.5^{b}
NABE 4	3.3^{a}	2.5^{b}
Grain (kg ha^{-1})		
NABE 14	889	772^{a}
NABE 15	937	288^{c}
K132	911	582^{b}
NABE 4	1054	514^{b}

Means within parameter and rainy season followed by the same letter, or no letter, are not different at *P*=0.05.

Table 6. Interaction of management system × cultivar for seed number and grain yield of bean over two seasons. CFS is Conventional Farmer System, IFS is Improved Farmer System, and HIS is High Input System.

Parameter	CFS	IFS	HIS
Seed (# pod^{-1})			
NABE 14	2.8	3.3	3.9^{a}
NABE 15	2.3	3.0	2.7^{b}
K132	2.6	2.8	2.7^{b}
NABE 4	2.8	2.9	2.9^{b}
Grain (kg ha^{-1})			
NABE 14	193	1025	1274^{a}
NABE 15	202	865	771^{b}
K132	261	1003	975^{b}
NABE 4	286	841	1225^{a}

Means within management system followed by the same letter, or no letter, are not different at *P*=0.05.

Table 7. Interaction of management system × rainy season for seed number and pod harvest index of bean for two seasons. 2014B is 2014 second rainy season, 2015B is 2015 rainy season. CFS is Conventional Farmer System, IFS is Improved Farmer System, and HIS is High Input System.

Parameter	2014B	2015A
Seed (# pod^{-1})		
CFS	3.1	2.1^{b}
IFS	3.1	2.9^{a}
HIS	3.1	3.0^{a}
Pod harvest index		
CFS	73	61^{b}
IFS	76	71^{a}
HIS	77	76^{a}

Means within parameter and rainy season followed by the same letter, or no letter, are not different at *P*=0.05.

the economic return to labor and management (ERLM) but the other main effects and interactions did not influence net profit or loss (Table 4). The ERLM in the 2014B season was greater than that of the 2015A season; however, both seasons resulted in a net loss. The CFS produced the only profitable ERLM as IFS and the HIS only produced net losses. Cultivar did not influence ERLM.

DISCUSSION

Climate, volumetric water content and bean development

Due to the close proximity of this research on Ferralsol soil and the research on Phaeozem soil, weather data were collected at one location and was previously reported by Goettsch et al. (2016). Precipitation was favorable for bean production during the 2014B season but the increased frequency and amount of rain in the 2015A season was unfavorable for bean production, and likely was a primary factor for decreased yield and increased VWC in the 7.5 cm depth as compared to the 20 cm depth in the 2015A season. The VWC results were nearly identical to the results reported for our Phaeozem results (Goettsch et al., 2016), which may be due to the close proximity of these two locations and the similarities in texture between the two soils. Elevated temperatures

(32/27°C) for only a few days can result in abscission of bean flowers and abortion of smaller, developing pods (Konsens et al., 1991). However, bean lines vary greatly in these responses (Gross and Kigel, 1991), allowing improvement of varieties. The differences in phenological development between cultivars at each date most likely were due to genetic differences in maturity among the four entries. This may be explained by the differences in maturity groups between the four cultivars, as reported in Goettsch et al. (2016). Interestingly, NABE 15 reached few developmental stages earlier than others cultivars which would suggest that this cultivar had more leaves earlier and therefore a potential for greater yields due to increased capture of light energy; however, this particular cultivar required fewer days to reach maturity as compared to the other three cultivars.

Pests and diseases

It is expected to see lower yields in the 2015A season as compared to the 2014B season because beans were planted on the same plots as the previous season. The bean-bean rotation could have been the cause of the greater occurrence of disease in the 2015A season; however, it could have also been due to the increased VWC in the soils, which is conducive to root rots and other diseases (Athanase et al., 2013). The increased amount of rain in the 2015A season as compared to the 2014B season could have caused the decreased prevalence of aphids (Weisser et al., 1997). Foliar diseases were less prevalent on the NABE 14 and NABE 4 cultivars in both seasons, which is likely due to their tolerance to foliar diseases. Surprisingly, NABE 15 had many disease symptoms even though it was selected for resistance to several common foliar and root diseases.

Cultivar selection and management of agricultural inputs and soil nutrients

Farmers prefer to plant bean on Phaeozem soil because they know that reddish soils (Ferralsols) are less fertile and poorer growing environment for bean. Even though Ferralsols are considered infertile, these soils rarely receive fertilizer applications, perhaps due to the greater amounts needed to increase production. Furthermore, it is questionable whether or not a return on investment is possible due to the great amount of expensive agricultural inputs required for increased production and the low market price of beans received at the farm gate. In most regions, beans are planted in rotation with cereals and therefore only benefit from the residual fertilizer applied in the previous season (Lunze et al., 2012).

Beans were replanted on the same plots in both seasons to develop a better understanding of lime carry-over effects on pH within each management system. The lime application increased pH to a level above the target range of 5.8 to 6.5 because there was no lime requirement functions developed before this project began. Lime requirements have since been developed for several soils in south-central Uganda by Tenywa et al. (personal communication, 2016) and are usually in the range of 0 to 4,000 kg ha^{-1} rather than the 15,000 kg ha^{-1} applied which was based on the best initial information. The dramatic increase in CEC from 11 to 22 cmol kg^{-1} clearly demonstrates that this soil was dominated by variable charge clays (Uehara and Gilman, 1982).

The main concern with agricultural lime was its cost and the great amount needed to reach the target pH range. There was similar economic concern with the amount and cost of fertilizer needed. A complement, or perhaps an alternative, to agricultural lime and fertilizer would be the adoption of improved cultivars with tolerance to low soil fertility, low soil pH, and extractable Al. New germplasm with tolerance to low soil pH and soluble Al was developed by Beebe et al. (personal communication, 2015) at CIAT and are a promising alternative to costly lime applications; however, incorporation of disease resistance is also necessary, so it is unknown when adapted cultivars with tolerance to low pH will be available for farmers. Additionally, it is unknown whether acid tolerant beans will have the characteristics that Ugandan consumers prefer, including taste and cooking time (Kilimo Trust, 2012).

A few bean cultivars have been documented to perform well under edaphic stresses; therefore, for comparison purposes, two cultivars that were tolerant to low soil fertility and two that were not tolerant were chose. When choosing the four cultivars, we ensured that each of them was accessible by smallholder farmers before testing for productivity under infertile soil and high acidity conditions. Three of the four cultivars (K132, NABE 4 and NABE 15) in this study were the most popular cultivars grown in Uganda. NABE 14 was included in this study because it was a newer cultivar with tolerance to low soil fertility and resistance to angular leaf spot (*Phaeoisariopsis griseola*) (ALS), bean common mosaic virus (*Potyvirus* spp.) (BCMV) and root rots (*Fusarium solani* f. sp. *phaseoli*). As predicted, this cultivar had greater yield on infertile soil with higher disease pressure.

Economic analysis

Ferralsols are widely reported to be infertile (Fungo et al., 2011; Musinguzi et al., 2015) with low productivity potential for bean (Nabhan et al., 1999), especially as compared to the Phaeozem soil described by Goettsch et al. (2016). The Phaeozem soil had more favorable pH, CEC, and better levels of macronutrients and micronutrients as compared to the Ferralsol soil. The greater level of infertility and need for higher rates of nutrients for enhanced bean production on this Ferralsol contributed to the poor economic returns to labor and management due to the added input costs. This is in agreement with Ronner and Giller (2012), who stated that it was profitable to fertilize fertile soils as fertilizing poor soils had only limited impact on yield and therefore limited return on investment. There are many tradeoffs to consider when selecting bean cultivars. Ugandan farmers choose which cultivars to grow based on soil fertility conditions, tolerance or resistance to heavy rainfall or drought, maturity, cooking time, taste, market prices, marketability locally and for export, and productivity (Kilimo Trust, 2012). Uganda currently imports many of its agricultural inputs such as fertilizers, lime, pesticides and herbicides (International Food Policy Research Institute, 2014), which is very costly since Uganda is a land-locked country. It currently takes more than 24 h of overland transportation to reach a major port to gain access to world markets, which not only increases the cost of agricultural inputs but also impacts the price of Ugandan beans at the farm gate (International Food Policy Research Institute, 2014). Due to the lack of quick and inexpensive transport to world markets, the demand for Ugandan beans is low and therefore grain prices remain low. This is one of the major reasons why only 20% of Uganda's bean production is exported while the rest is traded or consumed locally (Kilimo Trust, 2012).

Conclusions

The IFS and HIS increased common bean yield by 400 and 450%, respectively, over the CFS. However, lime and fertilizer prices need to be lower for high bean production systems to be profitable on the Ferralsol soils of south-central Uganda. The development of management systems that limit the use of expensive agricultural inputs and utilize improved cultivars with a tolerance to low soil fertility and to acidity is necessary to improve bean yield. Only the CFS was profitable on this Ferralsol in the 2014B season. This suggests that, if growing beans on Ferralsol soils is necessary, it is economically important to grow during the long rainy season (rainy season B) and to minimize the use of expensive agricultural inputs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of cultivation duration and mode on the microbial diversity of the *Amorphophallus konjac* rhizosphere

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***Amorphophallus konjac* cannot be cultivated on the same field consecutively for extended periods, but can be cultivated on the same grove. The underlying mechanism for this is unknown and may involve the rhizosphere microbial community. Therefore, the different duration and mode for konjac were researched. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) showed that microbial communities varied by cultivation duration and mode. Seven bacterial phyla were detected in the soil and Proteobacteria was the most abundant of these phyla. The microbial metabolic diversity following three years of continuous cultivation (sample QXFH3) was the highest, and this sample may have contained pathogenic and antagonistic microorganisms. There was almost no disease in the soil sample from grove conditions (QXN0), which was readily distinguishable from soils from field conditions (QXF0). Amino acids, carboxylic acids, and miscellaneous carbohydrates were the main carbon sources utilized by microbes in these soils. The microbial diversity index and multivariate analyses revealed that bacterial diversity increased with cultivation duration.**

Key words: Discontinuous cultivation, metabolic function, microbial composition, *Amorphophallus konjac*.

INTRODUCTION

Amorphophallus konjac has been used for food, medicine and fodder, as well as in wine production (Gao, 2004). China is the main producer of konjac, ~1.13×10^5 hectares being cultivated (Wu *et al*., 2014). Due to the increasing demand for konjac glucomannan, konjac is now regarded by the Chinese government as an agronomically important crop having significant potential in both domestic and international markets. However, disease occurrence during continuous cultivation is 35 to 50% higher than in non-continuous cropping fields. This is the major factor threatening konjac production (Zhang et al., 2012). Multiple lines of evidence indicate that four major factors can result in discontinuous cultivation: deterioration of soil physicochemical characteristics, soilborne diseases, imbalance of the soil microbial community, and autotoxicity (Ding et al., 2014). When crop monoculture is practiced, the microbial community is continuously exposed to the roots of the same crop that

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selects and enriches certain groups of microorganisms including yield-debilitating populations (that is, soilborne pathogens) of that crop (Cook, 2006). Several studies have determined differences of soil microbial communities between rotation/mixed culture and monoculture of cotton (Acosta et al., 2010), maize (Ceja et al., 2010), wheat (and maize) (Govaerts et al., 2007), rice (Xuan et al., 2010), soybean (Li et al., 2010), oilseed rape (Hilton et al., 2013) and potato (Larkin, 2003). Despite some recent investigations into soil improvement and sterilization, the underlying mechanisms driving the relationship between microbial diversity and discontinuous cultivation are still poorly understood. Increasing evidence indicates that microorganisms in the rhizosphere play a vital role in nutrient cycling, organic matter decomposition, and the maintenance of soil fertility (Larkin, 2003). The soil microbial community is also an important bio-indicator of soil function (Zuppinger et al., 2014). Therefore, many studies of discontinuous cultivation have been focused on evaluating soil quality and microbial communities. Several previous studies have demonstrated that continuous farming leads to an imbalance in soil ecology and alterations of rhizosphere microbial diversity (Wu et al., 2009; Urashima et al., 2012). Although a number of microbial strains (<1% of total organisms) have been isolated from successively cultivated soil (Sang et al., 2008; Hoang et al., 2014), the study of most microbial community members is still difficult. Modern microbial ecology tools have enabled the study of microbial communities relating to plant growth and development, *in situ* localization of important forms, and alterations in abundance of soil microbes (Johri et al., 2003). In this study, we used molecular culture-independent methods based on 16S rDNA and 18S rDNA gene diversity (Lv et al., 2012; Ma et al., 2005), polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), and random amplified polymorphic DNA to examine the microbial community and dynamics of the dominant microbial species in rhizosphere soil during growth (Li et al., 2012; Matsuyama et al., 2007).

Recently, a variety of culture-independent approaches, including random amplified polymorphic DNA, PCR-DGGE, and BIOLOG, have been used to investigate the diversity in bacterial structure and metabolic function of konjac soils (Bai et al., 2008). However, only a few trials have report the microbial community diversity of konjac soils with different cultivation duration using both PCR-DGGE and BIOLOG. The results of our study serve to elucidate the variations in soil microbial community and link those changes with continuous konjac farming; however, follow-up studies are required prior to modification of farming practices.

MATERIALS AND METHODS

Soil sampling and DNA extraction

The experiments were located in Quanxi village of Shiyan city in the Hubei province of China (B: 31°58.734′N, 109°40.213′E, and 1103 m elevation). This region has a typical subtropical monsoon climate with an average annual precipitation of 1000 mm and an average temperature of 14°C. The soils are loam. The grove was two years old kiwifruit orchards. The field was planted corn. The grove and the field contained 4 plots, respectively, and each plot was 5.0×10.0 m in size. Each year, starting from year 2013, konjac (*A. konjac* K. Koch ex N.E.Br.) was monoculture in field and interplant in grove. By 2014, all plots were used up. This experimental design provides opportunity to collect soil samples after konjac monoculture in field and interplant in two years old kiwifruit orchards from 0 to 3 years simultaneously.

Konjac was typically seeded in April 5 with a few days variation among years. Tuber pieces were buried on the top of raised paths (25 cm in height and 120 cm in bottom width) and plants were spaced 30 cm apart along the row. Two rows were planted on each raised path with 40 cm between the two rows. This resulted in a plant density of 225 plants per plot (equivalent to 45,000 plants ha^{-1}). Blended fertilizer (750 kg·ha^{-1}) were applied before seeding, with the ratio $(NH_4)_2SO_4$:P_2O_5:K_2O being 15:15:15, additionally supplemented with blended fertilizer (75 kg·ha^{-1}). All other field management activities were performed manually. Konjac was harvested in later October.

Soil samples were collected in July 20, 2015. In each plot, soil was collected at four diagonal points using a sterile plastic bag and combined into a single sample. Rhizosphere samples were collected by the root-shaking method (Kowalchuk et al., 2000). The bulk soil samples were obtained in depth 5 to 25 cm. The fresh soil samples were sieved through 2-mm meshes: The bulk soil samples were stored at 4°C for physical and chemical characterization, while the rhizosphere samples were stored at -70°C for DNA extraction (Ineceoglu et al., 2010). The physico-chemical properties of the soil are shown in Table 1 (Yu et al., 2014).

DGGE community fingerprints, DNA sequences, and phylogenetic analysis

PCR products were cleaned using the DNA Purification Kit [DP214, TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China]. The variable V3 region of 16S rDNA was amplified using GC-338F and 518R primers, which were designed to be specific for most bacteria (Inceoglu et al., 2010).

The PCR products were analyzed with DGGE using a BioRad DCode Universal Mutation Detection System (Bio-Rad, Richmond,CA, USA). Samples were run on 8% (w/v) polyacrylamide gels in 1×Tris-acetate-EDTA solution. Optimal separation of the bacterial community was achieved with a 35 to 55% urea-formamide denaturing gradient [100% denaturant corresponds to 7 M urea and 40% (v/v) formamide]. Bacterial gels were run for 4 h at 150 V and 60°C. DGGE banding patterns were analyzed using QuantityOne-1-D (version 4.5; Bio-Rad Laboratories). Images were normalized using markers and the patterns were compared by clustering methods in CANOCO for Windows (version 4.5; Microcomputer Power, Ithaca, NY, USA). Similarity matrices, consisting of defined numbers within each gel, were generated using Pearson’s correlation coefficient (*r*). Additionally, computer-assisted analyses of DGGE fingerprints, DNA sequences, and phylogenetics were employed according to previously described methods (Luo et al., 2010; Lyautey et al., 2005).

BIOLOG analysis

Functional diversity of the soil microbial community was characterized by community level physiological profiles using BIOLOG EcoPlates (BIOLOG, Hayward, CA, USA) (Schutter and Dick, 2001). All BIOLOG profiles were generated by a BIOLOG

Table 1. Soil samples used for analysis of diversity and main physicochemical characteristics.

Soil samples	Age (y)	Growth model	Health or ill	pH	Organic matter (g/kg)†	N	P	K
QXN0	0	Grove	-	6.20±0.01^{f}	2.56±0.02^{i}	106.63±0.95^{b}	5.19±0.51^{g}	81.00±3.06^{h}
QXNH1	1	Grove	Health	7.37±0.02^{a}	1.67±0.03^{k}	64.52±0.82^{h}	15.18±0.13^{d}	92.00±1.15^{g}
QXNI1	1	Grove	Ill	5.46±0.01^{i}	4.78±0.01^{a}	99.98±1.04^{c}	21.27±0.82^{c}	135.33±0.88^{d}
QXNH2	2	Grove	Health	5.44±0.02^{l}	3.38±0.03^{e}	56.35±0.81^{i}	16.50±0.13^{d}	59.67±0.88^{j}
QXNI2	2	Grove	Ill	5.47±0.01^{i}	2.84±0.02^{h}	76.30±1.13^{f}	22.01±2.36^{c}	117.67±0.88^{e}
QXNH3	3	Grove	Health	5.14±0.01^{c}	3.19±0.04^{f}	66.97±1.57^{gh}	17.75±0.48^{d}	53.33±0.67^{k}
QXNI3	3	Grove	Ill	4.96±0.04^{d}	3.37±0.02^{e}	85.17±0.42^{e}	10.63±0.53^{ef}	65.67±0.88^{i}
QXF0	0	Field	-	6.51±0.01^{e}	3.87±0.01^{b}	69.77±0.65^{g}	7.91±0.13^{f}	157.67±2.40^{c}
QXFH1	1	Field	Health	7.32±0.02^{b}	2.49±0.01^{j}	48.88±1.17^{j}	10.19±0.60^{ef}	79.67±1.20^{h}
QXFI1	1	Field	Ill	5.27±0.01^{j}	3.17±0.03^{f}	89.02±0.65^{d}	15.47±0.48^{d}	175.67±1.20^{b}
QXFH2	2	Field	Health	5.45±0.02^{i}	2.62±0.01^{i}	90.42±1.17^{d}	12.24±0.39^{e}	70.00±0.58^{i}
QXFI2	2	Field	Ill	6.00±0.01^{g}	2.98±0.02^{g}	165.20±0.73^{a}	23.77±0.34^{c}	109.67±3.53^{f}
QXFH3	3	Field	Health	5.74±0.02^{h}	3.78±0.02^{c}	67.78±2.30^{gh}	46.76±0.70^{b}	173.00±0.58^{b}
QXFI3	3	Field	Ill	5.22±0.01^{f}	3.66±0.01^{d}	107.68±0.62^{b}	91.26±1.98^{a}	194.33±2.67^{a}

†Letters indicate the Shortest Significant ranges (SSR) at P = 0.05 for different treatments. Different letters denote a significant difference at $p < 0.05$.

reader (ELx808BLG, BIO-TEK Instrument, Inc., Winooski, VT, USA) at 24 h intervals for 168 h (Li et al., 2012). The average well color development (AWCD), metabolic profile of microbial communities, and PCA were used to analyze the metabolic variance of rhizosphere soils. AWCD was calculated according to the procedure described by Garland et al. (1991). The total carbon substrate utilization ability of the microbial community was evaluated and the metabolic profiles of microbial communities were quantified via the Shannon (*H*) and evenness (*E*) indices. All community-level physiological profiles were calculated according to previously described methods (Li et al., 2012). The AWCD value at 120 h was used to calculate the Shannon index and IBM SPSS Statistics software (version 19.0; IBM, Armonk, NY, USA) was used for PCA analysis (Schutter and Dick, 2001).

RESULTS

Bacterial community structures in konjac rhizosphere soil as assessed by PCR-DGGE

Bacterial diversity of fourteen soil samples was evaluated using PCR-DGGE analysis of the amplified partial 16S rDNA genes (Figure 1). Overall, the bacterial community structures were relatively complex across different cultivation duration and modes. There were four bands (that is, band-1-1, band-1-4, band-3-4, and band-4-1) found in all samples (Figure 1A). All patterns derived from different planting configurations were generally similar, with an average similarity of 0.48 (using Unweighted Pair Group Method with Arithmetic Mean, a simple agglomerative [bottom up] hierarchical clustering method that is based on PCR-DGGE profiles). However, PCR-DGGE profiles from lower cultivation duration samples differed from higher cultivation duration samples, (average similarity rates of 0.56 to 0.74 and 0.65 to 0.76, respectively) (Figure 1B).

To evaluate the bacterial species, 17 bands common in the DGGE profiles were sequenced (Table 2). The similarity of all band sequences was ≥96% compared with those available in the GenBank database. Seven bacterial phyla (that is, Proteobacteria, Actinobacteria, Cellulomonadaceae, Acidobacteria, Firmicutes, Gemmatimonadetes, and uncultured bacteria) were detected; Proteobacteria was the most highly abundant. Alpha proteobacterium were most abundant and were found in all duration, modes, and healthly/disease sample. Firmicutes (that is, band-12-1 and band-12-2) were highly abundant in sample QXFI2; Gemmatimonadetes (that is, Band-14-1) were highly abundant in sample QXFI3; and Acinetobacter species (that is, Band-5-1) and uncultured proteobacterium (that is, Band-5-2) were highly abundant in sample QXNI2. Uncultured proteobacterium (Band-3-1) were not found in samples QXNI3 and QXNH3; this suggests that special groups, such as Gemmatimonadetes, Firmicutes, and uncultured proteobacterium were the main groups to change across samples.

Carbon substrate metabolic profiles of soil microbial communities

Functional diversity of the microbial community reflects the ecological function of the community. AWCD is one of the most important indices for determining the capacity for carbon utilization and is an important indicator microbial community activity (Zabinski and Gannon, 1997). The dynamics of AWCD were investigated with konjac soils cultivated for 24 h (Figure 2). In general, AWCD gradually

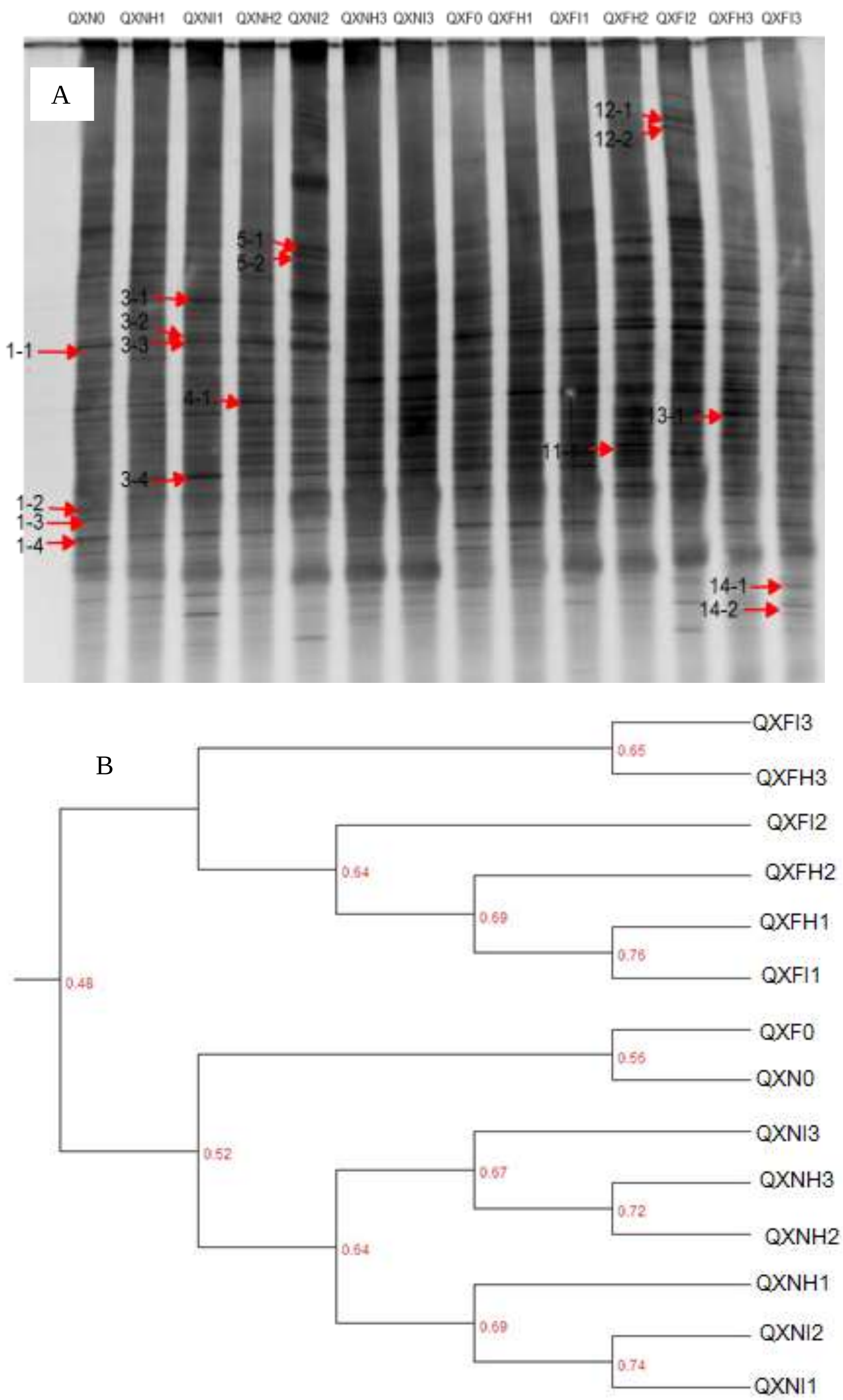

Figure 1. Denaturing gradient gel electrophoresis (DGGE) banding patterns of 16S rDNA fragments and the clustering of DGGE profiles in konjac soils of different cultivation duration and modes. Lanes corresponding to different soil samples are indicated by numbers at the top. The bands of DGGE profiles were detected, and some were excised, reamplified, and sequenced (Table 2). The arrow on the left indicates the direction of DGGE electrophoresis.

increased with the cultivation time. The carbon utilization was insignificant during the first 24 h. Soil microorganisms grew logarithmically from 24 to 120 h, and the AWCD of all soil samples increased to approximately 37. After 120

Table 2. Phylogenetic identification of selected denaturing gradient gel electrophoresis (DGGE) bands from the bacterial DGGE profiles in Figure 1[1)].

Band no.[2)]	Similar strain (NCBI accession No.)	Ident (%)	Classification[3)]
Band-1-1	Uncultured alpha proteobacterium (GQ383865.1)	99	Proteobacteria; alpha proteobacterium
Band-1-2	Uncultured bacterium (KX239244.1)	100	Bacteria
Band-1-3	Uncultured actinobacterium (HQ397176.1)	97	Actinobacteria; Acidimicrobium
Band-1-4	Uncultured actinobacterium (HM756016.1)	99	Actinobacteria; Acidimicrobium
Band-3-1	Uncultured proteobacterium (EU298748.1)	99	Proteobacteria; proteobacterium
Band-3-2	Oryzihumus leptocrescens (NR113000.1)	100	Cellulomonadaceae, Oryzihumus
Band-3-3	Uncultured alpha proteobacterium (GQ383865.1)	100	Proteobacteria; alpha proteobacterium
Band-3-4	Uncultured delta proteobacterium (EU299843.1)	99	Proteobacteria; deltaproteobacterium
Band-4-1	Uncultured Acidobacteria (EF663316.1)	98	Acidobacteriales; Acidobacteriaceae
Band-5-1	Acinetobacter spp. (KT825794.1)	100	Acinetobacter
Band-5-2	Uncultured proteobacterium (JQ910786.1)	99	Proteobacteria; proteobacterium
Band-11-1	Uncultured alpha proteobacterium (JF319258.1)	99	Proteobacteria; alpha proteobacterium
Band-12-1	Uncultured Firmicutes bacterium (JF269153.1)	99	Firmicutes; unknown species
Band-12-2	Uncultured Firmicutes bacterium (JF269153.1)	99	Firmicutes; unknown species
Band-13-1	Uncultured bacterium (KU930809.1)	100	Bacteria
Band-14-1	Uncultured Gemmatimonadetes bacterium (HG325756.1)	99	Gemmatimonadetes
Band-14-2	Uncultured alpha proteobacterium (KF183247.1)	99	Proteobacteria; alpha proteobacterium

[1)]Only the highest homology matches are presented. [2)]Bands are numbered according to Fig. 1. [3)]Classification represents phylum, order, and family of each strain.

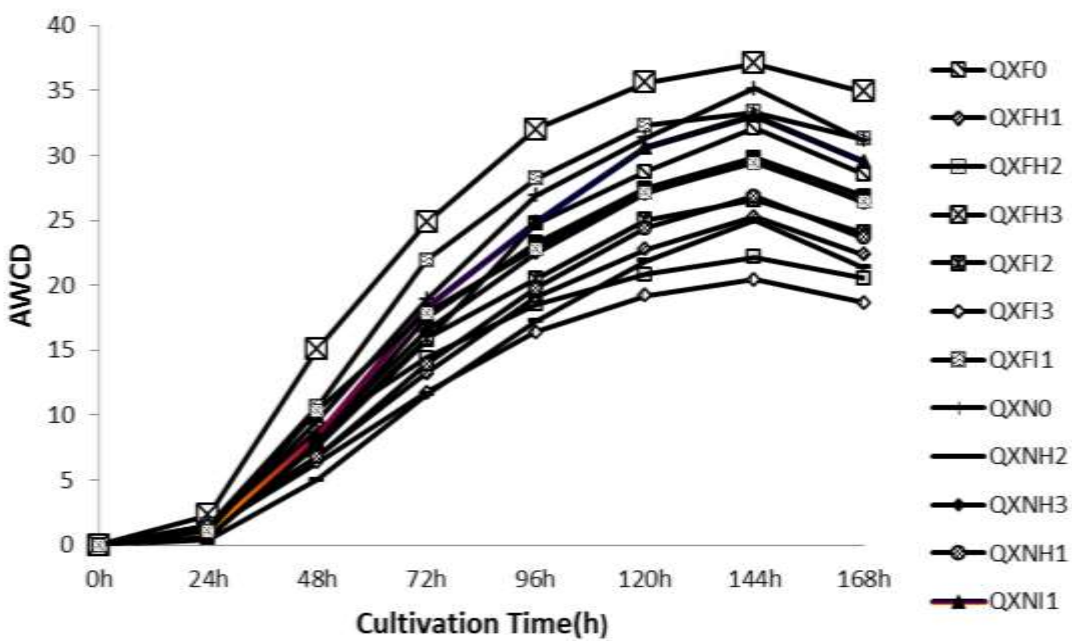

Figure 2. Average well color development (AWCD) development of soil microbial communities in konjac soil of cultivation duration and modes.

h, the rate of AWCD changed decreased because the soil microbes adapted to the environment of BIOLOG microplate. The rate of increase and final AWCD value depends on the abundance and activity of the microbial community (Garland and Mills, 1991). The AWCD values of konjac soils were between 19.18 and 35.6; these show that the utilization of a single carbon source by the soil microbial community significantly decreased in diseased

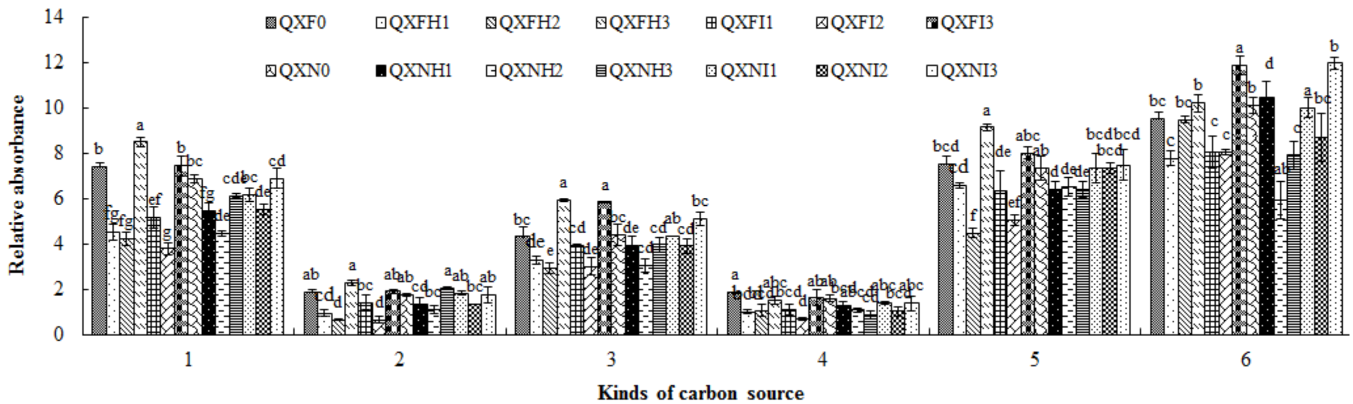

Figure 3. BIOLOG EcoPlate carbon utilization assay for soil microbial communities from different cultivation duration and modes: 1. amino acids, 2. amines/amides, 3. polymers, 4. phenolic acids, 5. carboxylic acids, 6. miscellaneous carbohydrates.

plants from field soils. From greatest to smallest, the AWCD in field samples was QXFH3>QXFI1>QXF0>QXFI2>QXFH1>QXFH2>QXFI3; in grove samples, the AWCD was QXN0>QXNI1>QXNI2>QXNI3>QXNH3>QXNH1>QXNH2. The metabolic activity of the soil microbial communities changed with cultivation duration, and samples from healthy plants showed significantly higher metabolic activity than samples from diseased plants.

Specific substrate utilization of soil microbial communities

There are 31 types of different carbon sources in the BIOLOG EcoPlate (12 carbohydrates, six amino acids, four polymers, five carboxylic acids, two phenolic acids, and two amines/amides). Amino acids, polymers, carboxylic acids, and carbohydrates were the main carbon sources utilized by samples from different cultivation modes and health conditions (Figure 3). The relative absorbance of amino acids, polymers, and carboxylic acids was from the highest to lowest: QXFH3 >QXFH1>QXFH2, QXFI3>QXFI1>QXFI2, QXNH3>QXNH1>QXNH2, QXNI3>QXNI1>QXNI2; this indicates that the metabolic function of soil microbial communities first decreased and then increased with increases in cultivation duration. And the QXFH3>QXFI3>QXF0, QXNI3>QXN0>QXNH3, which indicated that the metabolic function of healthy soil microbial communities was higher than diseased communities in field conditions; however, there was no difference in grove conditions.

Diversity index of soil microbial communities based on PCR-DGGE and BIOLOG profiles

The overall species richness and catabolic diversity of microbial communities in konjac soil were evaluated by the number of major bands present in the PCR-DGGE and the AWCD value in 120 h BIOLOG profiles (Table 3). It was clear that the metabolic diversity of planted konjac was lower than unplanted; the diversity increased with continuous cropping. The microbial diversity index increased in diseased konjac. In the field, the microbial diversity index was (from highest to lowest): QXNH2 > QXNH1 > QXNH3; in groves the microbial diversity index was: QXFH3 > QXFH2 > QXFH1. Healthy konjac soils had a higher microbial diversity (3.27 < H < 3.18) than diseased konjac soils (3.34< H< 3.03) in grove conditions; this trend was also seen in field conditions (3.18 < H < 3.13 and 3.15 < H< 3.08 for healthy and diseased soils, respectively). Bacterial diversity differed between field (E=0.991-0.97) and grove soil samples (E=0.947-0.992); this indicates the presence of more homogeneous and stable ecosystems in the first year of planting. Increasing cultivation duration resulted in disequilibrium in microbial diversity; meanwhile, microbial diversity increased with field and grove soils, crop rotation following three years of konjac cultivation, and continuous cropping in grove soils.

Multivariate analysis of DGGE fingerprints and BIOLOG data

PCA analyses of relative band intensity (DGGE gel profiles) and carbon substrate utilization (via BIOLOG EcoPlates) were used to investigate correlations between cultivation duration, cultivation modes, and health of the konjac (Figure 4). Multivariate comparisons showed that the complex microbial communities differed with cultivation duration, cultivation mode, and health of the konjac (Figure 4). The first two components of the PCA plot of relative band intensity account for 50.52 and 27.49% of the variance. The QXN and QXF samples were separated on the PCA plot; this suggests that the bacterial

Table 3. Microbial diversity index of konjac soil calculated from DGGE fingerprinting and BIOLOG analysis.

Soil samples	Analysis by PCR-DGGE method		Analysis by BIOLOG method	
	Shannon index (H)	Substrate evenness (E)	Shannon index (H)	Substrate evenness (E)
QXN0	3.24 ± 0.05^{abc}	0.991 ± 0.003^{a}	3.29 ± 0.004^{abc}	0.978 ± 0.005^{a}
QXNH1	3.18 ± 0.06^{bcd}	0.979 ± 0.002^{abcd}	3.24 ± 0.01^{cde}	0.973 ± 0.002^{a}
QXNI1	3.15 ± 0.02^{bcde}	0.969 ± 0.006^{cd}	3.19 ± 0.02^{ef}	0.990 ± 0.012^{a}
QXNH2	3.18 ± 0.04^{bcd}	0.967 ± 0.006^{cd}	3.35 ± 0.004^{a}	0.993 ± 0.002^{a}
QXNI2	3.08 ± 0.02^{def}	0.969 ± 0.008^{cd}	3.23 ± 0.02^{def}	0.983 ± 0.006^{a}
QXNH3	$3.13 \pm 0.05^{bcd}r^{f}$	0.963 ± 0.005^{d}	3.22 ± 0.03^{def}	0.981 ± 0.003^{a}
QXNI3	3.12 ± 0.05^{cdef}	0.970 ± 0.004^{cd}	3.33 ± 0.01^{ab}	0.992 ± 0.002^{a}
QXF0	3.00 ± 0.04^{f}	0.947 ± 0.008^{e}	3.30 ± 0.02^{abc}	0.978 ± 0.003^{a}
QXFH1	3.25 ± 0.04^{abc}	0.972 ± 0.008^{bcd}	3.18 ± 0.02^{f}	0.975 ± 0.003^{a}
QXFI1	3.03 ± 0.07^{ef}	0.977 ± 0.005^{abcd}	3.27 ± 0.02^{bcd}	0.979 ± 0.003^{a}
QXFH2	3.18 ± 0.04^{bcd}	0.983 ± 0.004^{abc}	3.20 ± 0.04^{ef}	0.979 ± 0.004^{a}
QXFI2	3.25 ± 0.05^{abc}	$0,989 \pm 0.004^{ab}$	3.29 ± 0.01^{bc}	0.983 ± 0.006^{a}
QXFH3	3.27 ± 0.04^{ab}	0.989 ± 0.004^{ab}	3.30 ± 0.01^{abc}	0.983 ± 0.005^{a}
QXFI3	3.34 ± 0.03^{a}	0.992 ± 0.003^{a}	3.27 ± 0.02^{bcd}	0.969 ± 0.005^{a}

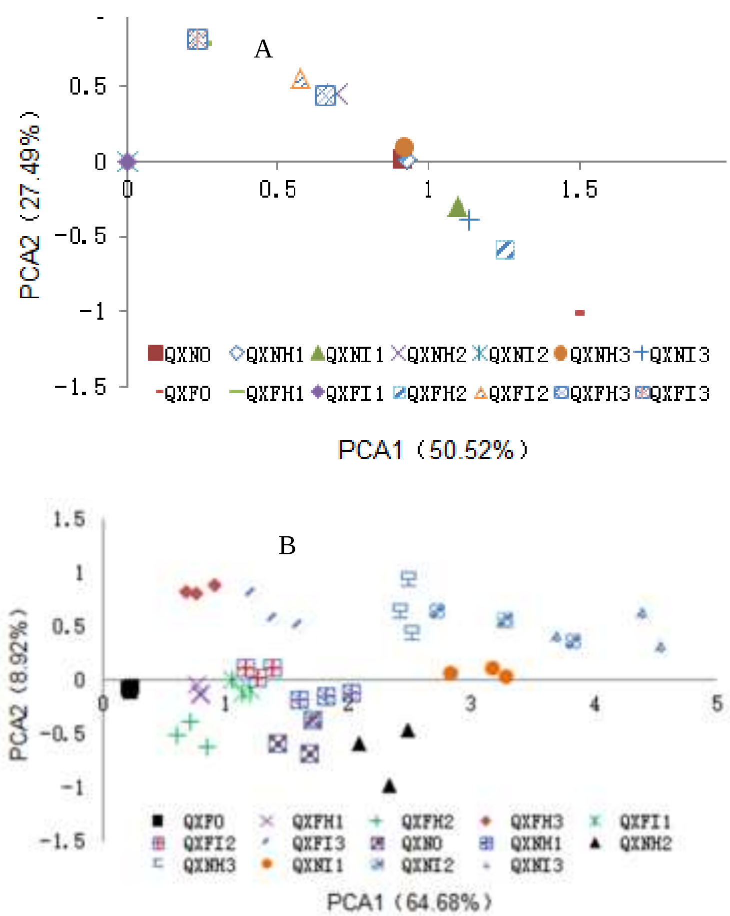

Figure 4. Principal component analysis (PCA) of the microbial composition of konjac soil samples from different cultivation duration and modes: (A) PCA of the bacterial composition of konjac soil samples by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), and; (B) PCA of the microbial composition of konjac soil samples by BIOLOG.

community structures of these soil samples were similar (Figure 4A). The first two components of the PCA plot of carbon utilization account for 64.68 and 8.92% of the variance. The PCA plot shows that the samples separated into three groups based on carbon utilization: Group 1 (QXFH3, QXFI3), Group 2 (QXNH3, QXNI1, QXNI2 and QXNI3), and Group 3 (QXF0, QXFH1, QXFH2, QXFI1, QXFI2, QXN0, QXNH1 and QXNH2) (Figure 4B). Collectively, the results of the PCA analyses indicate that functional metabolic diversity differed from metabolic bacterial community diversity. The primary drivers of differences in functional metabolic diversity between soil communities were konjac cultivation duration and mode.

DISCUSSION

The goal of this study was to investigate the effects of cultivation duration, cultivation mode, and plant health on the microbial genetic (via 16S rDNA gene profiles generated by PCR-DGGE) and functional diversity (via metabolic functional analyses generated with the BIOLOG EcoPlate) in konjac soils. The combination of these two methods was found to be useful for systematically understanding microbial communities in konjac soils. The combined approach is useful because soil functionality is thought to be dependent not only on the microbial species present, but also on the potential metabolic activities of the konjac soil microbiota. The BIOLOG profiles, which cannot separately represent the activity of bacterial and fungal communities, represent the total values of microbial communities. The main finding in this study was that the cultivation duration of konjac exerts the most profound influence on the genetic and functional diversity of konjac rhizosphere soils.

The bacterial community structure analyzed by PCR-DGGE was relatively complex, with significant diversity observed between different cultivation duration and modes. Seven bacterial phyla (that is, Proteobacteria, Actinobacteria, Cellulomonadaceae, Acidobacteria, Firmicutes, Gemmatimonadetes and uncultured bacteria) were detected. Proteobacteria dominated, suggesting that it is the major bacterial group in konjac soils. All members of Proteobacteria are gram negative (Berman, 2012); in the rhizosphere soil of plants, a selective effect favors Proteobacteria over Acidobacterium and gram-positive bacteria. This leads to the prevalence of the *Pseudomonas* group, which can cause disease or otherwise negatively impact plant development (Marilley and Aragno, 1999; Berggren et al., 2005). Therefore, we suspect that Proteobacteria play an important role during konjac growth. In this study, Actinobacteria represented an important component of the soil microbial population (Poltia et al., 2014) and is used as a biocontrolling agent for controlling soil- and seed-borne plant diseases (Priyadharsini and Dhanasekaran, 2015). Interestingly, some Actinobacteria species gradually appeared or disappeared with the konjac cultivation, suggesting that konjac soil significantly affects the Actinobacteria community.

To improve our understanding of the effect of cultivation duration and mode on microbial konjac soil communities, we applied the PCR-DGGE and BIOLOG methods to evaluate the metabolic activity and diversity indices. Wang et al. (2008, 2011) also used these two methods to investigate the effects of fertilization on bacterial community structure and function in black soils. The microbial metabolic activity in konjac soils was described by AWCD of substrates arranged on the BIOLOG EcoPlate. BIOLOG analysis indicated that the microbial activity of QXFH3 was the strongest; this could be due to the presence of pathogenic and antagonistic microorganisms following three years of continuous konjac cultivation. Konjac soft rot disease can cause losses of between 30 and 50% of total production; however, this can reach 80% or even complete destruction following three years of continuous cropping in field conditions (Xiu et al., 2006). However, there was almost no disease following continuous cropping years in grove conditions, and a grove soil sample (QXN0) was significantly stronger than the field soil sample (QXF0). Increase konjac cultivation duration in the same cultivation mode led to a decrease in microbial activity of diseased konjac soils (Figure 2). Amino acids, carboxylic acids, and miscellaneous carbohydrates were the main carbon sources utilized in konjac soils (Figure 3). Furthermore, the microbial diversity index and multivariate comparisons revealed that an increase in diseased konjac cultivation duration led to a larger increase in bacterial diversity than healthly konjac under field conditions. Furthermore, an increase in healthly konjac cultivation duration led to a larger increase in bacterial diversity than diseased konjac under grove conditions (Table 3 and Figure 4). Based on previous studies, cultivation duration was assumed to be an important factor that influenced microbial activity and diversity in the rhizosphere of plants (Yue et al., 2013; Ma et al., 2010). In addition, soil characteristics had a significant influence on soil microbial communities (Girvan et al., 2003), and soil pH was thought to be the primary driver of soil bacterial community composition (Landesman et al., 2014). However, in our study, soil pH decreased with increases in konjac cultivation (Table 1). Prior to our study, it was also thought that the rhizospheric microbial community and soil pH were influenced by the accumulation of root exudates (Wu et al., 2014). Therefore, root exudates could be a key factor that influences the microbial diversity of the konjac rhizosphere with increasing cultivation duration; however, the links between the microbial community composition and soil function were unclear (Anglet et al., 2014). Moreover, variations in the microbial community composition may not result in the alteration of soil function (Chapin et al., 1997). Thus, the underlying factors warranted further investigation.

The microbial structure of the rhizosphere was shown, particularly the outbreak of pathogenic bacteria, is driven by cultivation duration. Planting in groves effectively improved microbial diversity, and could potentially allow for continuous cultivation.

It was speculated that the key factors preventing continuous cultivation are changes in the microbial community structure and a microecological imbalance of the rhizosphere caused by the accumulation of root exudates. A more detailed examination of the correlation between a certain soilborne diseases and the relevant konjac root exudates is necessary; the elimination of konjac root exudates or addition of adsorption material would also be informative studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of dietary fat sources on growth performance, excreta microbiology and noxious gas emissions in Ross broilers

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An experiment was conducted to evaluate fat sources on growth performance, excreta microbiology and noxious gas emissions in broilers. Experimental birds were reared for 5 weeks and divided into two periods: Starter (0 to 3 weeks) and finisher (4 to 5 weeks). A total of 150 one day old Ross 308 broiler chicks were randomly allocated into five treatments with five replications (six birds per replicate) following a completely randomized design. Dietary treatments included FT1 (corn-soybean meal based basal diet with soybean oil), FT2 (corn-soybean meal based basal diet with chicken fat), FT3 (corn-soybean meal based basal diet with tallow), FT4 (corn-soybean meal based basal diet with tallow and lard) and FT5 (corn-soybean meal based basal diet with pork fat/lard). Overall body weight gain and feed intake did not differ due to addition of different dietary fat sources in broiler diet; however, feed conversion ratio was better in FT1 fed group in comparison to other fat groups ($P<0.10$). The result of the serum immunoglobulins data indicated that, a significant elevation of serum immunoglobulin M (IgM) was observed after dietary addition of FT1, FT2 and FT5 relative to FT4 ($P<0.05$) Excreta pH did not differ, however, yeast and mold count was highest in FT4 and FT5 relative to FT1 and FT3 ($P<0.05$). Excreta noxious gas emissions (NH_3, H_2S and SO_2) were lower in FT1 and FT2 in comparison to other fat groups ($P<0.05$). Overall, the results of the present study suggested that FT1 and FT2 can be prioritized in the diet of broilers with positive influence on body weight gain and feed efficiency, and substantial reduction of noxious gas emissions. Further detail study could be conducted to investigate the single and combination of different dietary fats (with different ratio) on performance and meat quality indices.

Key words: Broilers, growth performance, immunity, noxious gas emissions.

INTRODUCTION

The global population is currently 7.4 billion (World Population, 2016), and according to the United Nations reports, population will be 11.2 billion by 2100 (United Nations Report, 2015). The demand of food as well as meat is increasing all over the world (FAO report, 2009; Tilman et al., 2011). Among different meat red meat is under criticism due to different health aspects of human, while chicken meat is mostly favoured due to its lower price and risk of claimable diseases like cardiovascular diseases and colon cancer. However, many more factors influence the performance and production of chicken meat from the farm to the consumers table. Dietary sources are the concerns for the poultry nutritionist to test both the performance indices and the meat quality attributes. Most of the research are now-a-days focusing on the feed additives like prebiotics, probiotics, synbiotics, medicinal plants, fermented medicinal plant along with probiotics, extracts from natural plants to investigate the performance, microbiology and gaseous emissions (Bostami et al., 2015; Bostami et al., 2016; Kim et al., 2016; Sarker et al., 2016). However, the basal feed ingredients might also can affect the parameter indices and could be the basic research which can further helps to identify the proper amount and proportion of the feed additives. Most feed ingredients provided to animals are obtained from plant sources, which is generally accepted for the production purposes; however, there are some controversies regarding the use of genetically modified plants (GMP), because some plants are produced through animal gene transfer technology and they possess health and ethical issues (Kurien, 2002; Riaz and Chaudry, 2004).

To ensure different nutrients, several feed ingredients are provided to the poultry for growth performance, meat or egg production. Birds generally consume to fulfil their energy requirements; however, due to limitation of the gastrointestinal tract and parallel genetic improvement of the hybrid broilers, animal and plant fats are widely used as energy sources as well as source of essential fatty acids (Sanz et al., 2000). Deficiency of fatty acids in the diet cans results in metabolic disorders, depression of growth and immunity (Weiseman, 1984; Zollitsch et al., 1997). The amount and proportion of the fatty acids, especially the PUFA content varied between animal and plant fats and also within the similar source fats. However, replacement of animal fat by vegetable fat reported to be beneficial from the aspect of reducing abdominal fat pads in broilers (Newman et al., 2002). Moreover, better feed efficiency was reported for dietary plant oil than animal fat in broiler study (Newman et al., 2002). After ensuring the performance of broilers, it is also necessary to consider the effects of dietary fat sources on the animal excreta noxious gas emissions, which are the foremost research topics through dietary manipulation. Since the broiler industry is expanding tremendously, large gaseous emissions can greatly influence the health of both animals and humans (Myer and Bucklin, 2007). Research on feed additives (probiotics and natural plant materials) to minimize odorous gas emissions are under consideration and ongoing research all over the world (Bostami et al., 2015, 2016); where the basal feed ingredients can also influence the excreta microbial population and gas emissions. The gastrointestinal tract of poultry is the store house of microbiome (Pan and Yu, 2014). The type of fat source is likely to indirectly influence the intestinal microflora (Danicke et al., 1999), which can contribute to the gas emissions as well (Leek et al., 2004; Smith et al., 2004); because the microorganisms present in the gastrointestinal tract interact closely and densely with host and the ingested feed ingredients (Pan and Yu, 2014).

Although some researches has been conducted on fat sources to test the performance effect, however, the immunity, excreta microbiology and noxious gas emissions are the important task in the case of broiler industry. Due to the continuous genetic improvement in the broiler hybrids, the basic feed ingredients as well the feed additives research should be persistent. It was hypothesized that, different fat sources or their combinations could affect the growth performance, immunity, excreta microbiology and noxious gas emissions in broilers. Therefore, this study was conducted to investigate the dietary fat sources in broiler feed ingredients on the growth performance, immunity, excreta microbiology and noxious gas emissions as basic research for the further detail study on the other feed ingredients and additives.

MATERIALS AND METHODS

Experimental design, dietary treatments and birds husbandry

After collection of the broiler chicks from the local hatchery of Daejon, birds were reared at the experimental farm of Sunchon National University, Suncheon, Republic of Korea. A total of 150 one-day-old Ross 308 broiler chicks were randomly allocated into five treatments with six replications (six birds per replicate pen) in a completely randomized design. In Korea as well as other countries, different types of fats are being utilized based on the availability. Due to continuous genetic improvement of chicken, fats (energy and fatty acid sources) as major feed ingredients should be given paramount importance and to be tested for more efficacies. Based on availability and utilization of the fat sources in Korea, following fats were selected and divided into five treatments. Dietary treatments included FT1 (corn-soybean meal based basal diet with soybean oil), FT2 (corn-soybean meal based basal diet with chicken fat), FT3 (corn-soybean meal based basal diet with tallow), FT4 (corn-soybean meal based basal diet tallow and lard with 1:8 ratio) and FT5 (corn-soybean meal based basal diet with pork fat/lard). The basal diet was formulated to meet the Nutrient Requirements of Poultry (NRC, 1994; KFS, 2012). Feed was formulated and prepared for the period of 5 weeks dividing into two stages: Starter from 0 to 3 weeks, and finisher from 4 to 5 weeks. All feed ingredients were collected and were hand mixed based on feed formulation. The chemical composition of the experimental diet was analyzed in triplicate for moisture, ash, crude protein (CP), crude fiber (CF) and ether extract (EE), as described by AOAC (2000). The ingredients, chemical composition, and vitamin and mineral content of the basal diets are shown in Tables 1 and 2.

The plant oil and animal fat amended with the basal feed

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Table 1. Feed ingredients and chemical composition of the experiment diets (starter).

Ingredients (% DM as feed basis)	Starter diet (0 to 21 days)				
	FT1	FT2	FT3	FT4	FT5
Corn	50.00	50.00	50.00	50.00	50.00
Soybean meal	37.00	37.00	37.00	37.00	37.00
Corn gluten meal	0.50	0.50	0.50	0.50	0.50
Wheat-10%	6.00	6.00	6.00	6.00	6.00
Limestone-Small	2.03	2.03	2.03	2.03	2.03
Salt-Proc	0.25	0.25	0.25	0.25	0.25
DCP-18%	0.40	0.40	0.40	0.40	0.40
L-lys Sulfate 70%	0.30	0.30	0.30	0.30	0.30
Minemix	0.20	0.20	0.20	0.20	0.20
Vitamix	0.05	0.05	0.05	0.05	0.05
L-threonine-98%	-	-	-	-	-
MHA-Liquid	0.26	0.26	0.26	0.26	0.26
Sunphase5000FTU	0.01	0.01	0.01	0.01	0.01
Soybean oil	3.00	-	-	-	-
Chicken fat	-	3.00	-	-	-
Tallow		-	3.00	-	-
Tallow+Lard		-	-	3.00	-
Lard	-	-	-	-	3.00
Calculated composition					
ME (kcal/kg)	3,090.20	3,090.20	3,090.20	3,090.20	3,090.20
Crude protein (%)	22.04	22.04	22.04	22.04	22.04
Crude fat (%)	5.35	5.35	5.35	5.35	5.35
Crude ash (%)	5.71	5.71	5.71	5.71	5.71
Crude fiber (%)	2.59	2.59	2.59	2.59	2.59
Ca (%)	1.09	1.09	1.09	1.09	1.09
Phosphorus (%)	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.34	1.34	1.34	1.34	1.34
Methionine (%)	0.57	0.57	0.57	0.57	0.57

Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard. Provided the following amount of ingredients: Na 11.0%; Cl 0.19%; Cu 73.02 ppm (starter) and 72.07 ppm (finisher); Mn 77.92 ppm (starter) and 75.80 ppm (finisher); Zn 73.75 ppm (starter) and 71.57 ppm (finisher); I 0.94 ppm; Se 0.30 ppm; Fe 148.63 ppm (starter) and 4141.21 ppm (finisher); Vit A 12,061.00 IU/k and 12,122.00 IU/k (finisher); Vit D_3 3,000.00 IU/k; Vit E 28.06 ppm (starter) and 29.35 ppm (finisher); VIt K 2.10 ppm (starter) and 2.11 ppm (finisher); Choline 1,329.10 ppm (starter) and 1,146.20 ppm (finisher).

ingredients were purchased from local markets of Republic of Korea. Soybean oil and lard was purchased from the Daejon city; where chicken and tallow were collected from abattoirs of Suwon and Daejon city of Republic of Korea. Chicken fat and tallow was collected from a slaughterhouse in which chickens and cattle were slaughtered according to halal rules and in which birds and animals were inspected by a veterinarian to ensure that they were disease free. After collection of raw fat, it was chopped and sliced into smaller chunks of equal size (25 mm), then was placed in a fry pan with a lid. Water was added at a 1:1 ratio (W/W), after which it was covered with a lid and boiled for 30 min, followed by an additional 30 min of boiling without the lid. When the boiled fat showed a reddish color indicating that the fatty liquid had been extracted from the solid portions of the fat, the heater was switched off. Following fully boiling off the water, a wire mesh strainer was used to remove the remnants and particles. After straining three times, the fat was poured into a heat-proof ceramic container and covered tightly, then stored for further use. After collection of the lard from the pig abattoir, it was processed in a similar fashion as chicken fat and tallow, and then stored in a separate container and location until further use. The fatty acid composition of the experimental fat sources was determined by following the direct method for fatty acid methyl ester (FAME) synthesis using a gas chromatograph (GC), with slight modification as described by O'Fallon et al. (2007).

Broilers were reared in a closed, ventilated, wire-floor caged broiler house (100 cm long × 90 cm wide × 40 cm high) with a floor space of 1,125 cm^2/bird. During the experiment, all guidelines for the care and use of animals in research set by the Korean Ministry for Food, Agriculture, Forestry and Fisheries (2008) were followed. The cages had a linear feeder in the front and a nipple drinker in the back to provide *ad libitum* feed intake and free access to water. The internal temperature of the broiler house was set and maintained at 34°C for the first week, after which it was gradually reduced to 23°C with decreasing at the rate of 3°C per week, where it was maintained until the end of the experimental period. The internal house relative humidity was maintained at around 50% throughout the experimental period. Continuous lighting was provided for the entire experimental period.

Table 2. Feed ingredients and chemical composition of the experiment diets (finisher).

Ingredients (% DM as feed basis)	Finisher diet (22 to 35 days)				
	FT1	FT2	FT3	FT4	FT5
Corn	56.00	56.00	56.00	56.00	56.00
Soybean meal	28.84	28.84	28.84	28.84	28.84
Corn gluten meal	1.00	1.00	1.00	1.00	1.00
Wheat-10%	7.00	7.00	7.00	7.00	7.00
Limestone-Small	1.92	1.92	1.92	1.92	1.92
Salt-Proc	0.25	0.25	0.25	0.25	0.25
DCP-18%	0.46	0.46	0.46	0.46	0.46
L-lys Sulfate 70%	0.18	0.18	0.18	0.18	0.18
Minemix	0.20	0.20	0.20	0.20	0.20
Vitamix	0.05	0.05	0.05	0.05	0.05
L-threonine-98%	0.01	0.01	0.01	0.01	0.01
MHA-Liquid	0.29	0.29	0.29	0.29	0.29
Sunphase5000FTU	0.01	0.01	0.01	0.01	0.01
Soybean oil	3.80	-	-	-	-
Chicken fat	-	3.80	-	-	-
Tallow	-	-	3.80	-	-
Tallow+Lard	-	-		3.80	-
Lard	-	-	-	-	3.80
Calculated composition					
ME (kcal/kg)	3,210.90	3,210.90	3,210.90	3,210.90	3,210.90
Crude protein (%)	19.00	19.00	19.00	19.00	19.00
Crude fat (%)	6.23	6.23	6.23	6.23	6.23
Crude ash (%)	5.26	5.26	5.26	5.26	5.26
Crude fiber (%)	2.39	2.39	2.39	2.39	2.39
Ca (%)	1.04	1.04	1.04	1.04	1.04
Phosphorus (%)	0.43	0.43	0.43	0.43	0.43
Lysine (%)	1.07	1.07	1.07	1.07	1.07
Methionine (%)	0.55	0.55	0.55	0.55	0.55

Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard. Provided the following amount of ingredients: Na 11.0%; Cl 0.19%; Cu 73.02 ppm (starter) and 72.07 ppm (finisher); Mn 77.92 ppm (starter) and 75.80 ppm (finisher); Zn 73.75 ppm (starter) and 71.57 ppm (finisher); I 0.94 ppm; Se 0.30 ppm; Fe 148.63 ppm (starter) and 4141.21 ppm (finisher); Vit A 12,061.00 IU/k and 12,122.00 IU/k (finisher); Vit D_3 3,000.00 IU/k; Vit E 28.06 ppm (starter) and 29.35 ppm (finisher); VIt K 2.10 ppm (starter) and 2.11 ppm (finisher); Choline 1,329.10 ppm (starter) and 1,146.20 ppm (finisher).

Measurement of growth performance

Chicks were inspected daily and dead birds were removed following recording of the mortality (pen, date and body weight). No vaccination or medication program was followed during entire rearing period. Feed intake was measured based on residual feed deduction from the total feed provided to the birds. Body weight (BW) and feed intake were recorded weekly by replicate, and the feed intake (FI), body weight gain (BWG), and FCR (feed to gain ratio) per cage were then calculated by period and for the total experimental period.

Collection and analyses of blood samples for bird's immunity

At the termination of the experiment, two birds (based on mean body weight) were randomly selected from each replicated pen for blood sample collection. Blood samples were collected (10 mL) from the wing veins of the randomly selected birds into a 10-mL anticoagulant-free vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria). After collection of the blood samples, subsequently stored on ice during the period of collection and then immediately centrifuged to separate the serum (centrifugation for 15 min at 1,610 × *g* at 4°C). The serum samples were carefully transferred to plastic vials and stored at -20°C until immunoglobulin analysis was performed. The concentrations of serum IgG, IgM, and IgA were assayed using appropriately diluted samples by a sandwich ELISA with chicken-specific IgG (Cat. No. E30-104), IgM (Cat. No. E10-101), and IgA (Cat. No. E30-103) ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's instructions. For serum immunoglobulin test, each experiment was run in duplicate and the results represent the means of triplicate experiments. The absorbance of each well at 450 nm was measured within 30 min using a microplate autoreader (Thermo Lab Systems, Helsinki, Finland). The concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA) were determined using standard curves constructed from the respective immunoglobulin standards. The

Table 3. Effect of dietary fat sources on growth performance in broiler.

Item	FT1	FT2	FT3	FT4	FT5	SEM	P-value
BW (g/bird)							
0 day	39.53	39.80	39.60	39.53	39.73	0.62	0.997
22 day	576.67[b]	638.93[a]	574.10[b]	527.57[b]	547.53[b]	18.20	0.010
35 day	1,573.33	1,629.33	1,530.67	1,521.33	1478.00	45.09	0.272
0 to 3 weeks							
BWG (g/bird)	537.14[b]	599.13[a]	534.50[b]	488.03[b]	507.80[b]	17.97	0.009
FI (g/bird)	757.11[b]	860.43[a]	791.39[ab]	757.33[b]	783.53[ab]	24.76	0.063
FCR	1.42	1.44	1.48	1.55	1.54	0.03	0.111
4 to 5 weeks							
BWG (g/bird)	996.67	990.40	956.56	993.77	930.47	41.01	0.774
FI (g/bird)	1,573.25	1,644.20	1,582.00	1,573.97	1,500.63	47.88	0.414
FCR	1.59	1.66	1.66	1.58	1.62	0.03	0.198
0 to 5 weeks							
BWG (g/bird)	1,533.81	1,589.53	1,491.07	1,481.80	1,438.27	45.33	0.278
FI (g/bird)	2,330.36	2,504.63	2,373.39	2,331.29	2,284.16	58.86	0.168
FCR	1.52[b]	1.58[ab]	1.59[a]	1.57[ab]	1.59[a]	0.02	0.063

[a,b]Means with different superscripts within the same row are significantly different (P < 0.05). SEM: Standard error of the mean. BW: Body weight; ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio (feed to gain ratio); Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

result of the bird's serum immunoglobulins (IgG, IgM and IgA) were expressed as mg/ml of serum.

Collection and analyses of excreta microbiology and pH

Broiler excreta samples were carefully collected from the three replicated pens of each treatment for microbial analysis. Samples were diluted 1:10 in sterile saline solution (0.85% NaCl in distilled water). Next, 100 µl aliquots were plated in triplicate on MacConkey Sorbitol Agar, Salmonella Shigella Agar, Lactobacilli MRS (Mann, Rogosa and Sharpe) Agar, and Potato Dextrose Agar were used to screen for *E. coli*, *Salmonella*, *Lactobacillus*, and yeast and mold, respectively. Samples were incubated under anaerobic conditions at 37°C for 24 h (*E. coli* and *Salmonella)* and 48 h (*Lactobacillus*, and yeast and mold). Following enumeration of microbial colonies, microbial counts were expressed as log_{10}CFU/ml.

Excreta of broilers from each replicated pen were collected carefully by mixing well in falcon tubes after carefully removing any foreign materials. The pH of the excreta samples was determined by blending 2 g of excreta with 18 ml of distilled water for 1.5 min in a homogenizer. The pH values were measured using a standardized electrode attached to a digital pH meter (Docu-pH + meter, Sartorius, USA).

Collection of excreta sample and noxious gas measurements

At the end of experiment, 35th day, excreta samples (mixtures of feces and urine) were collected from each replicate pen of all treatments into plastic bags and stored immediately at -20°C until use. The total sampled manure from each pen was then thawed and homogenized, after which 500 g subsamples were placed in 2 L plastic boxes in triplicate to measure the NH_3, H_2S and SO_2 emissions. Each plastic box was equipped with a cover containing a hole to allow insertion of a gas measuring tube that was sealed inside with adhesive plaster. The samples were allowed to ferment for a period of 3 h at room temperature (24 to 28°C), after which the gas concentration was measured using a Gastec AP-20 gas sampling pump (Gastec Corp., Kitagawa, Japan) and Gastec detector tubes (No. 3M and 3LA for NH_3; 4LT and 4L for H_2S; and 5La and 5Lb for SO_2). For analysis, the adhesive plaster was punctured and 100 mL of headspace air was collected from approximately 2.0 cm above the sample surface. After sampling, the tubes were again sealed with adhesive plaster and incubated at room temperature. Additional gas samples were collected at 6, 12, 24 and 48 h. The concentration of NH_3, H_2S and SO_2 was expressed as ppm of excreta.

Statistical analyses

All data were subjected to ANOVA using the General Linear Models (GLM) function of the Statistical Analysis System (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA). Each cage was considered as the experimental unit for growth performance parameters (BW, BWG, FI and FCR), excreta microbiology, pH and gaseous emissions. A probability level of P<0.05 was considered statistically significant and a level of P<0.10 was considered as a statistical tendency.

RESULTS

Growth performance

As shown in Table 3, a higher final body weight was exhibited in the FT2 group during 22 day of experimental period (P<0.05); however, although a higher numerical body weight in case of FT2 during 0 and 35 day there

Table 4. Effect of dietary fat sources on serum immunoglobulins in broiler.

Serum immunoglobulins (mg/ml)	FT1	FT2	FT3	FT4	FT5	SEM	P-value
Immunoglobulin G (IgG)	3.62	3.73	3.89	3.96	4.20	0.16	0.124
Immunoglobulin M (IgM)	1.94[a]	1.99[a]	1.69[ab]	1.38[b]	1.78[a]	0.12	0.013
Immunoglobulin A (IgA)	5.01	5.01	4.68	5.10	5.21	0.41	0.924

[a,b]Means with different superscripts within the same row are significantly different (P < 0.05). SEM: Standard error of the mean. Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

Table 5. Effect of dietary fat sources on excreta microbiology and pH in broiler.

Excreta microbiology (log_{10}CFU/g)	FT1	FT2	FT3	FT4	FT5	SEM	P-value
Lactobacillus	8.68[b]	8.94[a]	8.77[ab]	8.96[a]	8.93[a]	0.07	0.053
Bacillus	8.18	8.11	8.50	8.48	8.74	0.22	0.296
Yeast and mold	8.20[b]	8.39[ab]	8.24[b]	8.49[a]	8.48[a]	0.06	0.013
E. coli	6.49	6.89	6.21	6.66	6.13	0.46	0.771
Salmonella	6.80	7.04	6.24	6.34	6.22	0.22	0.070
Excreta pH	7.18	7.10	7.38	7.00	6.96	0.28	0.846

[a,b]Means with different superscripts within the same row are significantly different (P < 0.05). SEM: Standard error of the mean. Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

was no differences among fat treatments. During 0 to 3 weeks, the body weight gain (BWG) was highest in FT2 fed birds followed by the FT1, FT3, FT5 and FT4 fed birds (P<0.05). While there was found no significant differences among fat treatments during 4 to 5 weeks and overall period. During 4 to 5 weeks, the feed intake (FI) was tended to be lower in FT1 followed by FT4, FT5, FT3 and FT2 fed birds. Moreover, during overall period, the feed conversion ratio (FCR) was tended to be lower in FT1 compared to FT3 and FT5 group which indicated the better efficiency (P<0.10).

Immunity of broilers

The result of the serum immunoglobulins data indicated that, a significant elevation of serum immunoglobulin M (IgM) was observed after the dietary addition of FT1, FT2 and FT5 relative to FT4 (P<0.05) (Table 4). However, no significant impact was found on immunoglobulin G (IgG) and immunoglobulin A (IgA) after different fat addition in the broiler diet.

Excreta microbiology and pH

Table 5 shows that, among the broiler excreta microfloral population, a tendency of lower *Lactobacillus* spp. was exhibited in FT1 group than that of FT2, FT4 and FT5 group (P<0.10). The yeast and mold count was substantially elevated in FT4 and FT5 in comparison to the FT1 and FT3 (P<0.05). In addition to that, excreta pH did not differ among the fat treatments groups (P>0.05).

Excreta noxious gas emissions

The result of excreta noxious gas emissions was shown in Figure 1, the average NH_3, H_2S and SO_2 gas emissions was found lowest in FT1 relative to the emission from other fat groups (P<0.05). The highest NH_3 and H_2S emission was exhibited in FT4 and lowest in FT1 group (P<0.05). The NH_3 emission was suppressed in FT1 and FT2 than FT3 and FT4 group (P<0.05). Where, the H_2S emission was depressed in FT1, FT2 and FT5 relative to FT3 and FT4 (P<0.05). The SO_2 emission was found highest in FT5 and lowest in FT1 group (P<0.05). The SO_2 emission was also diminished in FT1 than that of the FT2 group (P<0.05); while both in FT3 and FT4 it was lessen relative to FT5 (P<0.05).

DISCUSSION

Growth performance of birds

Appreciable quantities of dietary fat could be utilized by birds as an energy source, which can reduce the rate of passage of the digesta and ensure better utilization of nutrients (Peebles et al., 2000; Baião and Lara, 2005;

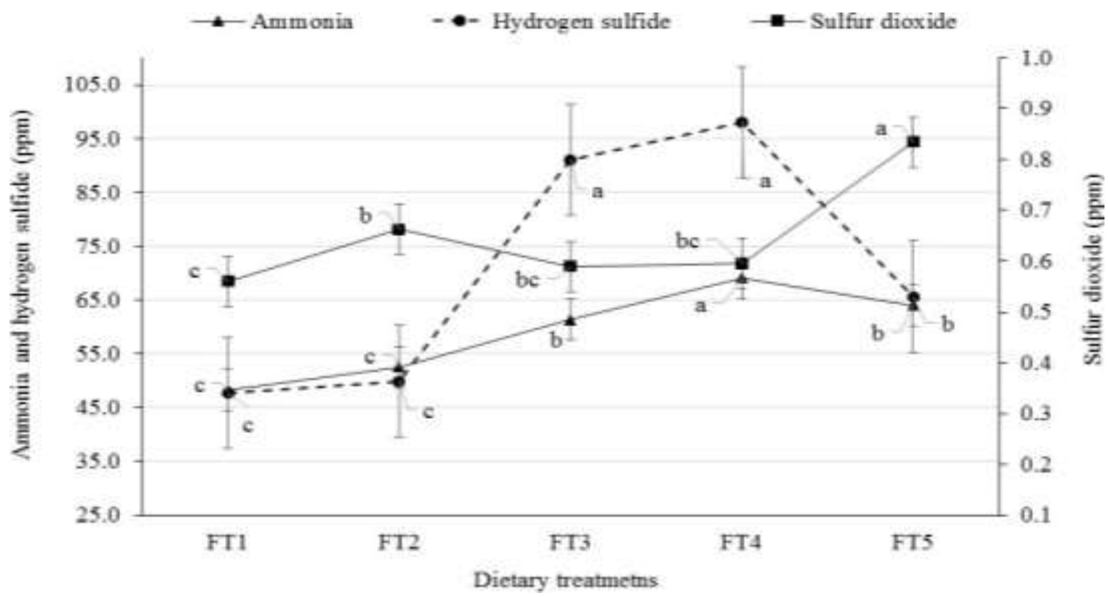

Figure 1. Effect of dietary fat sources on excreta noxious gas emissions in broiler. [a,b,c]Means with different superscripts within the same line are significantly different (P < 0.05). Error bar indicate standard error of the mean. Dietary treatments: FT1: Basal feed with soybean oil); FT2: Basal feed with chicken fat; FT3: Basal diet with tallow; FT4: Basal diet with tallow and lard (1:8): FT5: Basal feed with pork fat/lard.

Latshaw, 2008). The polyunsaturated fatty acid (PUFA) content is generally higher in FT1 followed by FT2, FT5, FT4 and FT3. The major PUFA content in FT1 is linoleic acid (C18:2n-6), while others group contained low to moderate amount. The feedstuffs dominated with unsaturated fatty acids are competent to improve the digestion process; where digestion process can be promoted by the secretion of bile and formation of micelle, consequently intensify the utilization of saturated fatty acids, and finally can impact on the performance augmentation of birds (Fascina et al., 2009). The effects of dietary fat on weight gain are influenced by the age of the birds and the amount of fat added to the diet (Zelenka et al., 1997). It was reported that, addition of fat usually beneficial after the first week of age, because young birds are less able to digest the saturated fatty acids (Leeson and Summer, 1997). In the present study, however, the body weight gain was found higher in case of FT2 group compared to other group during 0 to 3 weeks of experimental period. According to Hulan et al. (1984) after addition of fat in the diet the body weight differences were significant during 28 day but not during 48 day of experimental period, which supports our study. Fatty acid pattern and composition is responsible for the growth performance of birds, where PUFA content reported to be more beneficial than the SFA. Although the PUFA content of FT1 group is higher than the other groups, the FT2 group exhibited higher growth, the reason is not clear to us. The possible explanation could be like this, FT2 might be utilized efficiently or there might be other factors responsible for the growth enhancement in case of dietary fats, warrants further detail study to discover the mechanism.

The degree of unsaturation of the dietary fat can influence the secretion and function of hormones and enzymes (Shall et al., 1989; Guimbaud et al., 1997), and digestibility (Zollitsch et al., 1997). Therefore, growth hormones might be linked with the dietary fat and fatty acids in case of broilers. Manilla et al. (1999) reported higher body weight in broilers those are fed vegetable oils relative to the birds fed animal fats. It has been reported that feed intake can be decreased after addition of dietary fat in broiler chickens (Firman et al., 2010). In the current study, feed consumption tended to be lower in the FT1 and FT4 group than the FT2 group. There was found no significant differences among the animal fat sources on body weight gain, feed intake and feed efficiency during 4 to 5 weeks of experimental period. Supporting to the present study, no significant effect of animal fat was reported by Crespo and Esteve-Garcia (2002) in broiler study. Other studies also reported no significant differences between various fat sources while added to the diet of young and finishing broilers (Pesti et al., 2002; Firman et al., 2008). In the present study, during overall period (0 to 5 weeks), weight gain and feed intake did not differ among fat treatments (although higher numerical value was exhibited in the FT1 and FT2), whereas a tendency of better feed efficiency was observed in the FT1 group than the FT3 and FT5 group, which might be attributable to the presence of more unsaturated fatty acids in the plant fats. Because fats from vegetable origin contain higher unsaturated fats relative to the fats from animal origin (Waldroup et al., 1995). It was reported that, plant fat such as corn oil, seed oil, or palm oil fed at the rate of 0.5 to 1.0% of the diet improved growth performance and feed efficiency in rats, mice and pigs (Dugan et al., 1997; Ostrowska et al., 1999). These findings are consistent with those of a previous study that showed feed efficiency could be improved in the birds fed diet with plant oil relative to those that received animal fat (Newman et al., 2002).

Although in the current study, the function of hormone and enzyme in the digestive physiology was not tested, however, it could be helpful explanation regarding the improvement feed efficiency. The secretion of the hormone cholecystokinin and prolongation of the gastrointestinal transit time of feed and therefore the presence of enzyme in the digestion and absorption canals may be another mechanism through which fats helps to improve feed efficiency (Hulan et al., 1984; Scheideier and Baughman, 1989). Fatty acids can stimulate the secretion of cholecystokinin hormone secretion (McLaughlin et al., 1998), which stimulates secretion of pancreatic enzymes into the small intestine for further feed utilization into the gastro-intestinal tract. Furthermore, unsaturated fatty acids are more influential to the stimulation of cholecystokinin than saturated fatty acids (Bradford et al., 2008). Since FT1 contains higher levels of unsaturated fatty acids than FT5, the FT1 group might potentially ameliorate the feed efficiency in birds in the current study. The interaction of saturated and unsaturated fatty acid is one of the most important factor which determines the function of combination of fatty acids (Ketels and DeGroote, 1989; Leeson and Summers, 2001). It was stated that interaction of saturated and unsaturated fatty acid is balanced while animal fat and vegetable oils are well mixed (Dvorin et al., 1998). However, in the current study, combination of two animal fat did not exhibit beneficial impact on the performance of broilers.

Immunity of birds

Now-a-days commercial broilers are marketed at 5 weeks of age due to fast growth, which resulting immunosuppression of birds and exposing to different stressors and pathogens under the farm environment (Cheema et al., 2003; Shira et al., 2005). Triggered by the present situation dietary intervention to modulate immunity of birds is important consideration to the animal scientists (Calder, 2001; Klasing, 2007). Immunity of birds in general can be governed by the nutrition, through modulation and regulation of the immune process, cellular activation and movement, anatomical development of lymphoid tissues, intracellular killing of pathogens, synthesis of immunologically active substances (Butcher and Miles, 2002). Immune response of chicken can be significantly affected by dietary fat sources with their fatty acid components (Weiseman, 1984; Fritsche et al., 1991). Different dietary fat sources can affect the immune response of chicken through influencing the lymphocyte proliferation and antibody production, where concanavalin, lipopolysaccharide and pokeweed mitogen is affected by the fatty acids, and consequently the immune system (Fritsche et al., 1991). The combination of animal fats (tallow and lard) in the present study indicated that, due to interaction of fatty acids there might negatively impacted on the serum immunity of the broilers, therefore, the IgM value was lower in FT4 than that of FT1, FT2 and FT5. Fatty acids act as immunomodulatory molecules which shaped on the mediation of cellular communication, elaboration of second messenger and membranal fluidity (Klasing, 1997; Watkins, 1991). Among fatty acids, the n-3 PUFA and n-6 PUFA acts as booster for the immune system (Butcher and Miles, 2002). Oleic acid has anti-inflammatory functions shaped on the activation of different pathways of immune competent cells (Carrillo et al., 2012); linoleic acid can enhance the antibody production in the broilers thereby can influence the immunity of birds (Friedman and Sklan, 1995). It was reported that, conjugated linoleic acid can induce the immune system in chicken and rat (Cook et al., 1993; Wong et al., 1996). After all, the differences in the fatty acids among fats might be attributable to the variation in immunity, however, further detail study could ensure the mechanism.

Excreta microbiology and pH

There is a close interaction between the gastrointestinal tract (GIT) of a host and ingested diet. The gut microbiota benefits the host through providing nutrients from the dietary substrates and thereby modulates the function of the digestive system. Dietary protein or fat can influence the gut microbiota (Knarreborg et al., 2002; Drew et al., 2004). Microbial population can be elevated in the birds those are fed animal fat compared to those received plant fat (Knarreborg et al., 2002). Fat originated from the plant has been reported to diminished the diversity of bacteria (De Wit et al., 2012) through its impact on lipid metabolism-related genes in the distal small intestine and changes in the composition of the bile acid of the host in response to the diet (Devkota et al., 2012; Huang et al., 2013). Consistent to that, in the current study, a tendency of lower *Lactobacillus* count was exhibited in the FT1 relative to FT2, FT4 and FT5 group. Latour et al. (1994) observed in their study that, addition of lard in the diet of broiler can increase the fat content in excreta, which can increase the chance of growing the microbes in the excreta, since the microbial diversity depends on the water activity, nutrient content, oxygen tension and pH of the matrix (Maciorowski et al., 2007). The tendency of difference in the bacterial population might also be affected by the type of fat and fatty acid content, which is supported by Knarreborg et al. (2002), who reported that, dietary fat sources can affect the ileal microbial population in the chicken. The lower transition time of the intestinal digesta can be influential on the microbial population into the gastrointestinal tract (Rinttilä and Apajalahti, 2013).

The rate of digesta passage is usually reduced by the addition of fat in the diet of chicken; however, due to mixing of two animal fats, the rate of passage might be affected (Peebles et al., 2000; Latshaw, 2008), opined to be influential on the microbial population of the current study. The type of fat indirectly can influence the intestinal microflora through its persuading on viscosity of the digesta, intestinal transit time, and digestion in the small intestine (Danicke et al., 1999). The yeast and mold count difference of FT1 with FT4 and FT5 might be due to the difference in the source of fat (plant and animal); however, difference of FT3 with FT4 and FT5 might be due to the type and compositional difference of the fats. The quantity and quality of fat shape the host physiology and lead to further downstream alterations in the intestinal microfloral count (Patterson et al., 2014). Fatty acids are a necessary waste product required to balance redox equivalent production in the gut environment (van Hoek and Merks, 2012). The variations in the fatty acid composition among fat sources observed in the present study might have swayed the intestinal and consequently the excreta microfloral population. The temperature, moisture, pH and environment can shape the growth of microorganism; especially, the yeast and mold growth is esteemed by the presence of moisture content of the materials (Maciorowski et al., 2007). In the current study, both the feed material as well as the excreta material could be the source of higher yeast and mold count in the fat groups (FT4 and FT5) due to moisture content.

Excreta noxious gas emissions

The emission of odor from the animal industry as well as from broiler industry is common phenomena which affecting the sustainability of the broiler industry. The emission of gases in and around the poultry production facilities can be a health and performance issue for birds and their caretakers (Patterson, 2005). The emission of the noxious gases are important factors and issues to be considered for broiler industry because higher emission of ammonia and sulfur-containing compounds causes poor performance, susceptibility to disease and mortality in broilers (Kristensen and Wathes, 2000; Wang et al., 2011).Therefore, along with performance parameters, microbial and gaseous concentrations were measured in the present study. Diets usually formulated to meet the nutritional requirements, however, undigested components excretion can govern gastro intestinal microbiology, excreta microbiology, pH and moisture content which all combinedly can have impact on the emission of odorous or noxious components (Sharma et al., 2017). Among nutrients, fat sources are provided in major to ensure energy requirements as well as fatty acid sources, however, utilization and excretion of undigested substances of all nutrients as well fats could be varied due to difference in composition. Increased excreta fat content after feeding animal fat (lard) in case of broiler was reported by Latour et al. (1994). Although we did not analyze the fat content of the broiler excreta in this study, however, it is postulated that, the phenomena of increment of fecal fat content might enhance the chance of elevation of ammonia gas in the animal fat group except the FT2 group in comparison to the FT1 group. Dietary addition of fatty acid can affect the energy loss through excreta and liver weight, where energy substrates can influence the microbial and gaseous emissions; and liver function can affect the utilization of nutrients, secretion of enzymes and hormones and consequently the microbial growth and gaseous emissions (Terpstra et al., 2002). An investigation of dietary oil inclusion reported that it can alter ammonia nitrogen emissions from the manure via the effects of fatty acid on microbial activity in the intestine (Leek et al., 2004). Present result apparently indicated that, mixing of two animal fats (FT4: tallow and lard) resulted elevation of both the NH_3 and H_2S gas which is the negative outcome. Such types of result might be due to inappropriate proportion due to degree of unsaturation of fatty acids into that fat.

The emission of noxious gases from the excreta of animals depends on the utilization of nutrients and the gastrointestinal microbial ecosystem (Ferket et al., 2002). Where the other factors are also associated, such as diet, pH and moisture content, chemical environment, and physical-chemical interactions and compositions (Elliott and Collins, 1982; Carr et al., 1990), presence of different microorganisms and numerous gaseous substances (Patterson, 2005). The utilization of the major and minor nutrients usually happens into the gastrointestinal tract (GIT) of the chicken. Fatty acids can influence the secretion of cholecystokinin hormone secretion and consequently secretion of pancreatic enzymes into the small intestine to utilize the nutrients (McLaughlin et al., 1998), where unsaturated fatty acids are more influential to the stimulation of cholecystokinin than saturated fatty acids (Bradford et al., 2008). Cholecystokinin hormone influence to secrete pancreatic enzymes and bile production, where pancreatic enzymes can affect protein, fat and carbohydrate utilization (Bender, 2004). After exhausting of the carbohydrate sources into the intestine, the protein sources materials are utilized as salvage energy (Macfarlane and Macfarlane, 1995). By the action of putrefactive bacteria, proteins and amino acids formed systemic toxins and carcinogens; where the common toxic end products are phenols, indoles, amines and ammonia generated by deamination, fermentation and decarboxylation into the GIT (Macfarlane and Macfarlane, 1995). The concentration of NH_3-N (associated with NH_3 emission) can be decreased by the vegetable oils (pulm oil, linseed oil and whole soybean) by reducing the deaminating bacteria; where microbial growth depends on the presence of energy substrates and fatty acids in the digestive tract (Russell et al., 1992; Doreau and Ferlay, 1995). Among fatty acids, UFA has the negative impact on the bacterial membrane (Maia et al., 2007). Therefore, in the present study, it could be anticipated that, the variant fatty acid content and available energy in the FT1, FT2, FT3 and FT5 than FT4 might causes reduction of NH_3 and H_2S emission with the association of microbes; because the end product of the metabolism and fermentation by the microbes in the lower intestinal part can consequently influence the pH and other associated factors as well (Rinttilä and Apajalahti, 2013).

Addition of fat to the diet can influence the secretion of enzymes which can swayed the metabolism and excretion of fibre, protein and lipid and finally the gaseous emissions (Canh et al., 1998a, b; Evans et al., 2002; Hiraoka et al., 2003). Among the fatty acids, especially the higher content of linoleic acid in the soybean oil and chicken fat (35 and 20%) than the tallow and lard (2% and 11%) can impact on the pancreatic enzyme which can shaped the utilization, fermentation in the GIT and excretion of nutrients, and finally the emission of gases (Evans et al., 2002; Hiraoka et al., 2003; Beccaccia et al., 2015). Hydrogen sulfide is the most prominent volatile sulfur generated by bacterial sulfate reduction and decomposition of sulfur-containing organic compounds under anaerobic conditions (Arogo et al., 2000). Lower efficiency of nutrients utilization causes excretion of unutilized nutrients through the urine and feces, which then undergoes anaerobic microbial decomposition, resulting in generation of odorous compounds (volatile amines and sulfurs, phenols, volatile fatty acids and indoles (Gilley et al., 2000). Volatile sulfur generation by the action of anaerobic bacteria involves reduction of dissimilatory sulfate and metabolism of sulfur containing amino acids (Kiene and Hines, 1995; Ushida et al., 2003). There was emitted higher SO_2 in FT5 of the current study which might be attributed to the excretion of higher fat and other substances in the excreta of broilers (although it was not measured) relative to other fat groups according to the findings of Latour et al. (1994). In addition, since fatty acids affects the secretion of cholecystokinin hormone and pancreatic enzymes and utilization of protein, fat and carbohydrate (Bender, 2004), which can influence the nutrient excretion and results variation in the emission of H_2S and SO_2 gases. Sulfur-containing gas emissions are influenced by the strong interaction between amino acids and carboxylic acids (e.g. methionine and benzoic acid) (Eriksen et al., 2010). The elevation of the sulfur gases might be attributable to the reflection of the excreta microbial population and substrates availability (Levine et al., 1998); where organic compounds like cysteines, mucin and taurocholate bestowed the readily utilizable substrates for the H_2S and SO_2 emission (Levine et al., 1998). As a whole, the variation of NH_3 and H_2S in FT1 and FT2 with FT3 and FT4; and variation of SO_2 in FT1, FT2, FT3 and FT4 relative to FT5 might be attributed to the fatty acid composition, secretion and function of pancreatic enzymes, differences in digestibility of nutrients, and excretion of indigestible nutrients in the feces and urine.

Conclusion

Effects of corn-soybean meal based basal diet with soybean oil (FT1), chicken fat (FT2), tallow (FT3), tallow plus lard (FT4), and pork fat/lard (FT5) on the performance, immunity, excreta microbiology and noxious gas emissions in broilers were investigated. The result of the present study revealed that overall body weight and feed intake did not differ among the fat treatments, however there was found a tendency of better feed conversion efficiency of broilers after feeding FT1 than the other fat groups. The result of the serum immunoglobulins data indicated that, a significant elevation of serum immunoglobulin M (IgM) was observed after dietary addition of FT1, FT2 and FT5 relative to FT4. In addition, for the excreta microbiology, higher yeast and mold count was displayed in FT4 and FT5 group relative to FT1 and FT2 group. Furthermore, excreta noxious gas emissions (NH_3, H_2S and SO_2) were lower in the FT1 and FT2 groups than that of other fat groups. Overall, the results of the present study suggested that FT1 and FT2 can be prioritized in the diet of broilers with positive influence on body weight gain and feed efficiency, and substantial reduction of noxious gas emissions. Further detail study could be conducted to investigate the single and combination of different dietary fats (with different ratio) on performance and meat quality indices.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Mass selection for enhancement fruit yield in Edkawy cultivar of tomato under different irrigation intervals in southern of Egypt

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Field experiments were carried out during the period from 2013 to 2017 in the winter season at the experimental farm, Faculty of Agriculture, South Valley University, Qena, Egypt. Improvement yield and its attributes in Edkawy local cultivar via mass selection were done for three cycles. The unselected base population (C_0) and selected populations (C_1, C_2, and C_3) evaluated under two different water availability, favorable normal condition, and drought. The combined analysis of variance showed that significant differences between unselected and selected populations as well as highly significant among three cycles populations were detected all studied traits. Average fruit weight, number of flowers per plant, fruit yield per plant, number of cluster per plant, number of fruit per plant, number of branches per plant, fruit set percentage, and plant height have significantly increased a response to mass selection under unfavorable conditions. These increasing values for average fruit weight were 3.24, 6.90, and 12.09% in C_1, C_2, and C_3, respectively. Furthermore, there was a significant increase in the number of fruits per plant in the first (2.45%), second (9.48%) and the third (13.72%) cycles under drought conditions. Fruits yield per plant was increased by 4.70, 12.44 and 21.40% for unselected and selected population, respectively. There was positive and highly significant correlation among all studied traits. In the respect to base populations under water stress treatments, results revealed that the use of mass selection for improvement yield and its components of tomato cv. Edkawy appeared to be increasing significantly for all studied traits.

Key words: Drought, yield attributes, Edkawy, mass selection, tomato.

INTRODUCTION

Nowadays, tomatoes are grown year around in Egypt, in addition to its daily needs as the main staple for rich and poor human. So, this requires the presence of many genotypes (cultivars, hybrids, and lines) that could be obtained on germplasm to give higher yields. This does not come only through one way of plant breeding

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(introduction, mass selection, pure-line selection, hybridization), and evaluated germplasm under different abiotic stress, viz., heat, drought, and salinity. Egypt is the fifth largest producer of tomato in the world after China, USA, India and Turkey. Tomato is also the second largest vegetable crop in the world after potatoes.

The information of genotypic and phenotypic coefficient of variety is being helpful in outlining choice criteria from variable populace. When all is said in done, it was noticed that the estimation of phenotypic coefficient of variety is higher than the genotypic coefficient of variation (Tiwari et al., 2013). Mass selection utilized for cultivar improvement in plants included tomato for quite a long while back and it was valuable in developing cultivars. The proficiency of mass selection relies on upon quality impacts of the chose attributes, their heritability, population size and G×E interaction. Mass selection is compelling for characters controlled by additive genes.

In Egypt, tomato is grown over an area of 214016 ha annually, which produces 8288043 tons/ha with 38.37 tons/ha (FAO, 2014). Qena is one of the leading tomato production governorate in Egypt, it is occupies the third rank in tomato production after Sharkia and Noubaria. On the other hand, Qena ranked third after Sohag and Ismailia in terms of productivity by 22.3 ton per acre. Lessening the measure of water which is subjected or decreasing the quantity of water irrigation times is assuming an incredible part through upgrading plant capacities for water utilizes effectiveness (Kirda, 2002).

Water is typically the most restricting component for plant development. If plants do not get satisfactory precipitation or water system, the subsequent drought stress can lessen development more than all other environmental stresses. A plant reacts to absence of water by ending development and diminishing photosynthesis and other plant forms with a specific end goal to decrease water utilize (Khan et al., 2015). Drought and salinity are now far reaching in numerous locales and are relied upon to bring about genuine salinization of more than 50% of every single arable land by the year 2050 (Ashraf, 1994).

Tomato (*Solanum lycopersicon* L.) is a standout amongst the most vital vegetable products and is a standout amongst the most requesting as far as water utilize (Peet, 2005). The extent of change in tomato is for the most part in view of the degree of genotypic and phenotypic variation display in the material more is the hereditary potential and there will be more prominent odds of delivering a coveted sort. Information in regard of the nature and size of relationship of yield with different segment characters is an essential to acquire change the coveted bearing. A harvest rearing project went for expanding the plant efficiency requires thought of yield as well as of its segments that have an immediate or circuitous bearing on yield.

Bodunde (2002), Metwally et al. (2003, 2004), Zakher (2005), Salib (2006), Bhnan (2008), Hidaytullah and Ghafoor (2008), Jitendra and Devendra (2011) and Rashwan (2015), the pervious researchers used different selection strategies for enhancing yield and its components of *S. lycopersicon* L, while Damarany (1994a) on cowpea and lettuce, Bakheit and Ali (2013) on Egyptian clover, and Gehan (2016) on sunflower. The review was done to improve the fruit weight and yield characters and to assess the performance of three cycles of mass selection in the drought stress of tomato cv. Edkawy.

MATERIALS AND METHODS

Experimental site

A field experiment was carried out at the Experimental Farm of Faculty of Agriculture, South Valley University, Qena, Egypt (26° 11 N and 32°44 E) during 2013 to 2017 winter season.

Seeds material

Seeds of Edkawy tomato local cultivar were obtained from Agricultural Research Centre, ARC, Giza, Egypt.

Mass selection procedure

In the first season, base population (C_0) seeds were sown in nursery at August, 1st 2013, without replication area, (50 rows 5.0 m long, 1 m apart and 30 cm between transplants). Agricultural practices were done and 50 plants with high yielding under drought stress (irrigation every 12 days) were selected according to 5% selection intensity. Seeds of selected plants were bulked together (C_1) and sown in nursery at August 4th, 5th 2014 and 2015, respectively. The same procedure of mass selection was done to produce C_2, and C_3. Mass selection populations (C_1, C_2, and C_3) and base population (C_0) were sown on August 1st 2016 and 2017 to evaluate selected plants to drought tolerance. All populations (C_0, C_1, C_2, and C_3) were assess under two irrigation intervals system which were: a. irrigation every 6 days (favorable), b. irrigation every 12 days (drought conditions). All agricultural practice was applied as recommend.

Data

Plant height cm (PH), number of branches per plant (NBP), number of cluster per plant (NCP), number of flowers per plant (NFP), number of fruits per plant (NFSP), average fruit weight (AFW), and fruit yield per plant g (FYP).

Statistical analysis

Data were statistically analyzed using analysis of variance for Randomized Complete Block Design (RCBD) for separate analysis. Combined analysis for the two years was analyzed using split plot analysis. Comparison among means was done using least significant differences (LSD) at 0.05% and simple correlation coefficient between traits were done according to Gomez and Gomez (1984). Realized gain% was estimated from combined means according to Gowda and Seetharam (2008) as follow:

Realized gain% for $C_1 = C_1 - C_0 / C_0 \times 100$, $C_2 = C_2 - C_0 / C_0 \times 100$,

Table 1. Mean square of separate and combined analysis of variance for all studied traits in selected and unselected populations after three cycle

SOV	d.f	PH	NBP	NCP	NFP	FS	NFSP	AFW	FYP
First season									
Replication	2	0.333	0.583	0.083	0.0001	0.799	0.250	1.750	158.333
Population	3	48.528**	2.972*	9.417*	10.528**	8.523 ^{NS}	13.000**	48.750**	18177.778**
Error	6	0.778	0.472	1.083	0.444	2.593	0.583	0.083	169.444
Second season									
Replication	2	0.250	0.333	0.083	0.333	1.006	0.083	0.333	558.333
Population	3	48.111**	3.194^{NS}	9.639*	10.889**	8.488^{NS}	12.972**	48.750**	19822.222**
Error	6	2.028	0.778	1.306	0.556	2.318	0.639	1.000	113.889
Combined									
Year	1	0.042	0.0001	0.167	1.042	2.024	2.042	0.667	1666.667
Error a	4	0.292	0.458	0.083	0.167	0.903	0.167	1.042	358.333
Population	3	96.486**	5.944**	19.000**	21.375**	16.767**	25.819**	97.500**	37977.778**
Y × P	3	0.153^{NS}	0.222^{NS}	0.056^{NS}	0.042^{NS}	0.244 ^{NS}	0.153^{NS}	0.0001^{NS}	22.222^{NS}
Error b	12	1.403	0.625	1.194	0.500	2.455	0.611	0.542	141.667

NS, *,**Not significant and significant at p = 0.05 and 0.01, respectively.

$C_3 = C_3 - C_0 / C_0 \times 100$.

RESULTS AND DISCUSSION

Population's performance

The separate and combined analysis for all studied traits in unselected (base population C_0) and selected population (C_1, C_2 and C_3 mass selection) are presented in Table 1. The mean square for all studied populations under investigation as well as the variance among populations were significant for all studied characters, except fruit set in both seasons, and number of branches per plant in the second season indicating the wide diversity among all population and selected populations and the presence of true differences among the populations. Interaction among populations and years were insignificant for all studied traits.

Means values of all studied traits in three cycles of selection increased gradually from C_1 to C_2 in both evaluations seasons as well as the combined analysis. The data are shown in Table 2. For plant height (cm), the means value ranged from 47.8 to 52.5 cm for C_1 and C_3, respectively, and 9.5 to 11.2 for number of branches per plant, 18.8 to 21.3, number of cluster per plant, 55.5 to 58.8 number of flower per plant, 33.5 to 37.2 fruit per plant, 60.4 to 63.2% fruit set, 79.7 to 86.5 g average weight of fruit and 856.7 to 993.3 g for fruit yield per plant. These findings indicating that fruit yield of tomato could be improved via mass selection cycles because, its efficiency by improving such trait through increasing the frequencies of desirable genes which result to specific irregular mating which could have helped in breaking closely linkage group, sequence complexes of genes or eliminates the recessive alleles (Gowda and Seetharam, 2008). These results are in agreement with the findings of Damarany (1994a, b), Ghosh et al. (2010), Bakheit et al. (2011), Bakheit and Ali (2013), Hassan and Abdel-Haleem (2014), Rashwan (2015) and Gehan (2016). In the present study, mass selection cycles had a significant role in enhancing the mean values of most studied traits; this may be due to the presence of genetics factors that have more effect on such traits (Gehan, 2016; Rashwan, 2015) indicating that the mass selection cycles could be more effective after three cycles in improving fruit yield of tomato cv. Super strain B, and observed a positive and highly significant correlation among the fruit yield per plant and the other studied traits, and this indicate that mass selection can be used as a tool to improve the fruit yield per plant.

Data are presented in Table 2 indicating that the realized gain % was gradually raised from the first cycle of mass selection to the third cycle in all the studied traits. For plant height, it was 10.65, 16.90 and 21.53 for C_1, C_2, and C_3, respectively; 7.95, 15.91 and 27.27 number of branches per plant; 8.67, 18.50 and 23.21 number of cluster per plant; 2.21, 4.97 and 8.29 number of flower per plant; 2.45, 9.48, 13.76 for fruit number per plant; 0.66, 4.33 and 5.33 for fruit set; 3.24, 6.87 and 12.05 for average weight of fruit, and 4.70, 12.44 and 21.90 for fruit yield per plant for C_1, C_2, and C_3, respectively, as compared to base population C_0. These results showed that significant increase was observed after application of mass selection cycles from the first cycle C_1 to the third C_3. Many researchers (Kansouh, 2002; Zanata, 2002; Bhnan, 2008; Ara et al., 2009; Zakher, 2010; Singh and Cheema, 2011; Meseret et al., 2012; Kashif et al., 2013;

Table 2. Mean performance of separate and combined for PH, NBP, NCP of the two seasons.

Traits	PH			Realized gain %	NBP			Realized gain %	NCP			Realized gain %
Season	2016	2017	Com.		2016	2017	Com.		2016	2017	Com.	
C_0	43.0	43.3	43.2	-	8.7	9.0	8.8	-	17.3	17.3	17.3	-
C_1	48.0	47.7	47.8	10.65	9.7	9.3	9.5	7.95	18.7	19.0	18.8	8.67
C_2	50.3	50.0	50.2	16.90	10.3	10.0	10.2	15.91	20.3	20.7	20.5	18.50
C_3	52.3	53.0	52.5	21.53	11.0	11.3	11.2	27.27	21.3	21.3	21.3	23.21
L.S.D 05	1.8	2.8	2.4	-	1.4	1.8	1.6	-	2.1	2.3	2.2	-
Average	48.4	48.5	48.4	-	9.9	9.9	9.9	-	19.4	19.6	19.5	-
CV %	-	-	-	-	-	-	-	-	-	-	-	-

Traits	NFP			Realized gain %	NFSP			Realized gain %	FS			Realized gain %
Season	2016	2017	Com.		2016	2017	Com.		2016	2017	Com.	
C_0	54.3	54.3	54.3	-	32.3	33.0	32.7	-	59.5	60.4	60.0	-
C_1	55.3	55.7	55.5	2.21	33.3	33.7	33.5	2.45	60.3	60.5	60.4	0.66
C_2	56.7	57.3	57.0	4.97	35.3	36.3	35.8	9.48	62.4	63.4	62.9	4.33
C_3	58.7	59.0	58.8	8.29	37.0	37.3	37.2	13.76	63.1	63.3	63.2	5.33
L.S.D 05	1.3	1.5	1.4	-	3.2	3.0	3.1	-	1.5	1.6	1.6	-
Average	56.3	56.6	56.4	-	34.5	35.1	34.8	-	61.3	61.9	61.6	-
CV %	-	-	-	-	-	-	-	-	-	-	-	-

Traits	AFW			Realized gain %	FYP			Realized gain %
Season	2016	2017	Com.		2016	2017	Com.	
C_0	77.0	77.3	77.2	-	803.0	833.0	818.16	-
C_1	79.3	80.0	79.7	3.24	850.0	863.3	856.7	4.70
C_2	82.3	82.7	82.5	6.87	910.0	930.0	920.0	12.44
C_3	86.3	86.7	86.5	12.05	983.3	1003.3	993.3	21.40
L.S.D 05	0.6	2.0	1.5	-	26.0	21.3	23.8	-
Average	81.2	81.7	81.5	-	886.6	907.4	897.0	-
CV %	-	-	-	-	-	-	-	-

Rashwan, 2015) haave studied the effect of selection on tomato yield and yield attributes traits as number of fruit, number of cluster, number of flowers per plant and fruit yield per plant. They observed that the selection improved tomato yield and yield components and suggested that the presences heritability and genetic advance for yield characters.

Correlation coefficient

Table 3 shows the correlation between fruit yield per plant and the other studied traits in the combined analysis for both evaluation seasons 2016 and 2017of the 3 cycles of mass selection (C_1, C_2, and C_3) and base population (C_0). Fruit yield per plant g showed highly significant and positive association with plant height (r = 0.932**), number of branches per plant (r = 0.970**), number of cluster per plant (r = 0.939**), number

Table 3. Correlation coefficient among studied traits.

Traits	PH	NB	NC	NF	NFT	FW	FY
PH	1.000						
NB	0.957**	1.000					
NC	0.958**	0.958**	1.000				
NF	0.948**	0.974**	0.953**	1.000			
NFT	0.874**	0.912**	0.890**	0.936**	1.000		
FW	0.926**	0.965**	0.931**	0.991**	0.936**	1.000	
FY	0.932**	0.970**	0.939**	0.992**	0.937**	0.997**	1.000

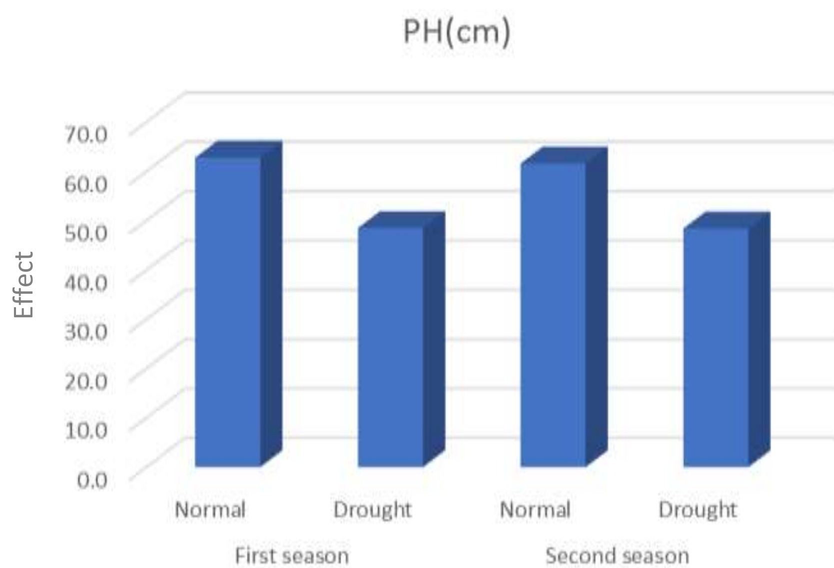

Figure 1. Effect of water stress on tomato cv. Edkawy on plant height (cm) in 2016 and 2017.

of flowers per plant (r = 0.992**), number of fruit per plant (0.937) and average fruit weight g (r = 0.997**).

Improvement of crops depends on multiple factors; one of these, is in understanding the magnitude of the correlations among different yield characters components and it is considered the primary interest for enhancing yield through yield components. These results are in line with findings by Bakheit and Ali (2013), Meseret et al. (2012), Kashif et al. (2013), Rashwan (2015) and Gehan (2016).

Performance of tomato cv. Edkawy under drought stress conditions:

Water stress could have the vital effect on vegetative growth, flowering, fruit set, and yield of plants. The main effect of drought on plants is wilt. As a result of water shortage and this led to reducing growth and yield of plants (Khan et al., 2015). It is observed from the mean values of Figures 1 to 8 that there is a decrease in plant height cm, number of branches, number of cluster per plant, number of flowers per plant, number of fruits per plant, fruit set %, average weight of fruit, and fruit yield per plant by 21.4 and 22.8% in both seasons, respectively for plant height, 38.1 and 39.6 for number of branches, 26.5 and 26.9% for number of cluster, as for number of flower the decrease was 19.1 and 18.8%, in both seasons, respectively. While, it was 14.1 and13.3% for number of fruit per plant, on the other hand, the reduction in average weight of fruit per plant after application of drought was 33.3 and 33.6% and the huge reduction was observed in fruit yield per plant for 69.3 and 49.0% in both seasons, respectively. These results indicate that water stress reduced the growth parameters due to their harmful effect on photosynthesis, which led to decrease in growth and development of plants, as a result of lake energy production in plant cell. Similar results were detected by Pervez et al. (2009), Celebi (2014), and Khan et al. (2015) on tomato; Abdel-Haleem

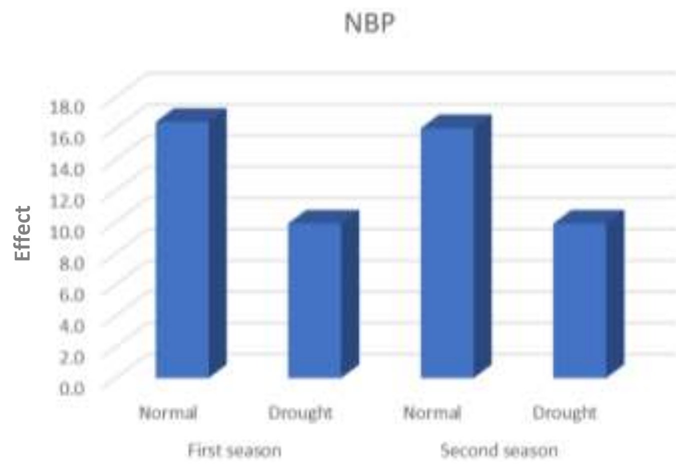

Figure 2. Effect of water stress on tomato cv. Edkawy on number of branches per plant in 2016 and 2017.

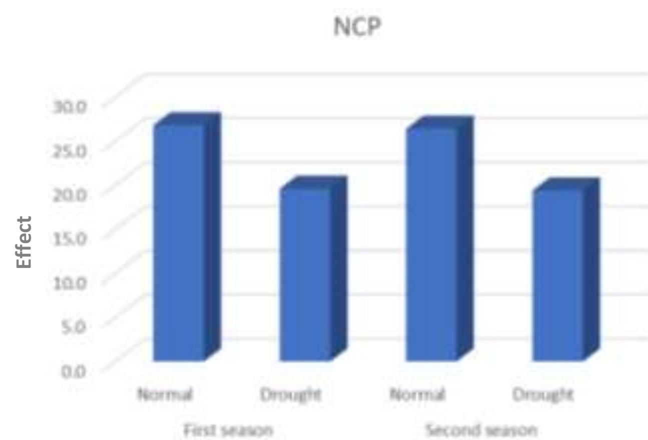

Figure 3. Effect of water stress on tomato cv. Edkawy on number of cluster per plant in 2016 and 2017.

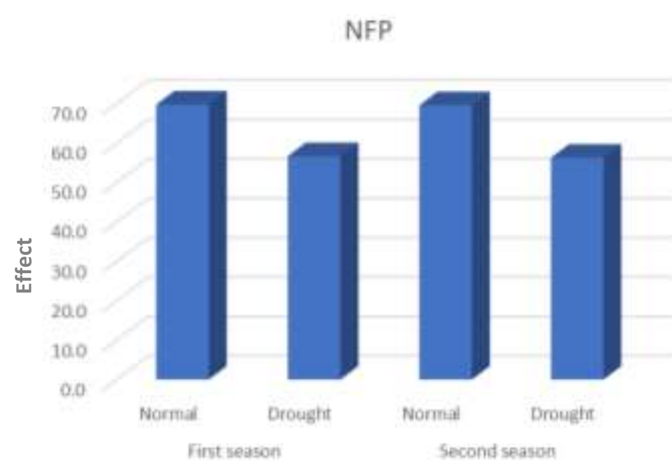

Figure 4. Effect of water stress on tomato cv. Edkawy on number of flower per plant in 2016 and 2017.

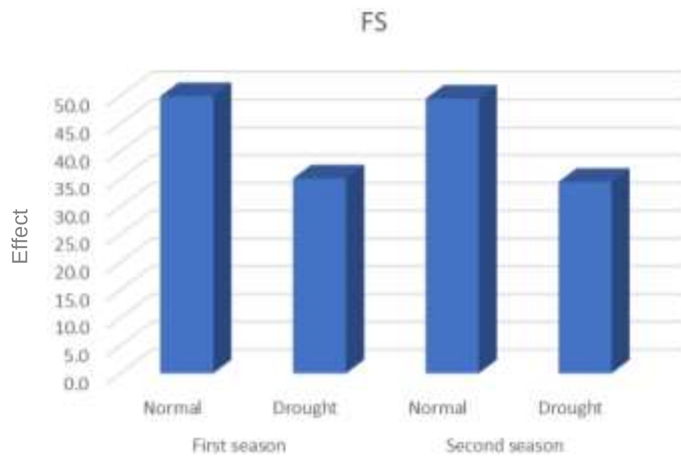

Figure 5. Effect of water stress on tomato cv. Edkawy on number of fruit set % in 2016 and 2017.

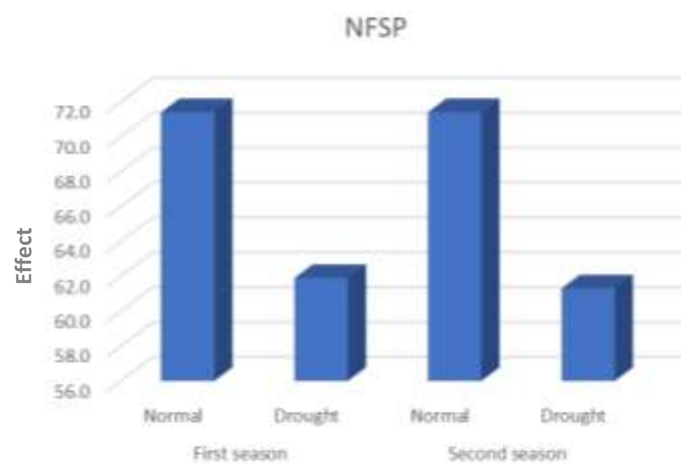

Figure 6. Effect of water stress on tomato cv. Edkawy on number of fruit per plant in 2016 and 2017.

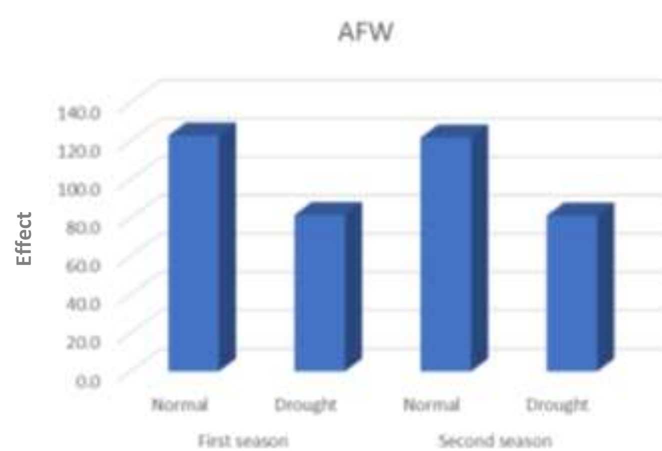

Figure 7. Effect of water stress on tomato cv. Edkawy on average weight of fruit per plant in 2016 and 2017.

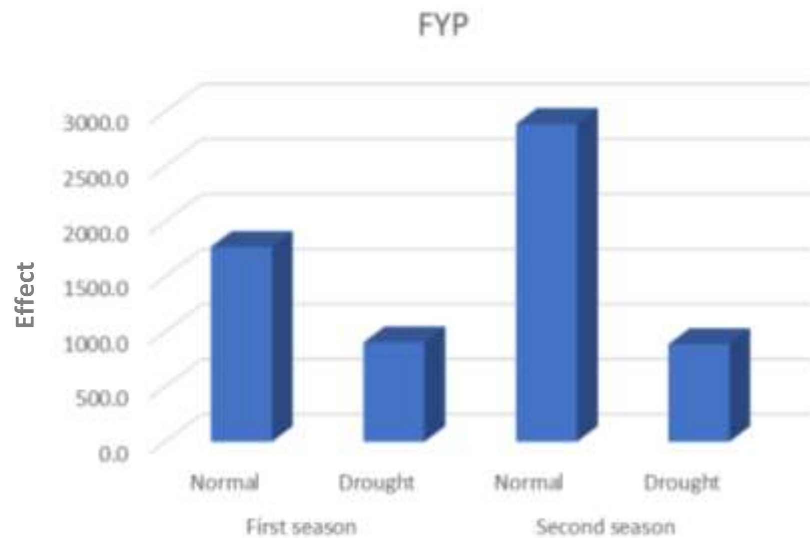

Figure 8. Effect of water stress on tomato cv. Edkawy on fruit yield per plant in 2016 and 2017.

(2017), Hussein and Abd El-Hady (2015), Hussein et al. (2014), and Farouk et al. (2011) on cowpea; Al Ameen (2012), Kheiralla et al. (1997) and Kheiralla and Ismail (1995) on wheat.

Conclusion

Data presented in this study indicated that mass selection for the three cycles has more effect for improving fruit yield per plant of tomato cv. Edkawy. In addition, the growth and yield parameters were reduced after exposure to water stress conditions.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

Abbreviations

PH, Plant height; **NBP,** Number of branches per plant; **NCP,** Number of cluster per plant; **NFP,** Number of flowers per plant; **FS,** Fruit set %; **NFSP,** number of fruit per plant; **AFW,** Average fruit weight; **FYP,** Fruit yield per plant.

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Full Length Research Paper

Agronomic characteristics and oil content of different genotypes of canola (*Brassica napus* L. var. *oleifera*)

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The aim of this study was to evaluate the agronomic performance and oil content of 13 canola genotypes in Western Paraná, Brazil. The experiment was carried out, during the year of 2015, in the municipality of Marechal Cândido Rôndon in the west of the State of Paraná. A Randomized Complete Block Design (RCBD) was utilized with four replications. The genotypes of canola evaluated were Hyola 401, Hyola 76, Hyola 61, Hyola 433, Hyola 50, Hyola 571CL, Hyola 575CL, Hyola 474CL, Hyola 555TT, Hyola 656TT, Hyola 559TT, W8006, and H92002. Sowing was done in plots with four rows, spaced at 0.45 and 5 m in length, on May 8, 2015. The evaluated parameter were phenology, plant height, number of pods per plant, grains per pod, weight of thousand grains, grain yield and oil content. Significant differences were observed where the genotype of canola Hyola 401 and Hyola 559TT reached the physiological maturation in 129 days. No significant differences were observed in the variables analyzed by the Tukey test at 5% probability except the mass of thousand grains in which the Hyola 76 genotype performed better, with 4.11 grams. The average grain yield of tested genotypes was 1518.14 kg ha^{-1} and the average of grain oil content was 42.45%.

Key words: Adaptability, *Brassica napus,* grain yield, Hyola.

INTRODUCTION

Canola (*Brassica napus* L. var. *oleifera*) is an herbaceous plant belonging to the genus *Brassica*. Its origin is linked to the cultivation of oilseeds known as colza. *B. napus*, however, has genotypes with erucic acid content lower than 2% in oil, and less than 30 µmol of glucosinolates per gram of oil-free dry matter (Santos et al., 2001). It is a plant that produces grains with 24 to 27% protein and 34 to 40% oil.

Its bran contains 34 to 38% protein, similar to soy (Galdioli et al., 2002) and can be supplied to the animals, being an excellent protein supplement in the formulation of rations for sheep, cattle, pigs, birds and fish (Bell,

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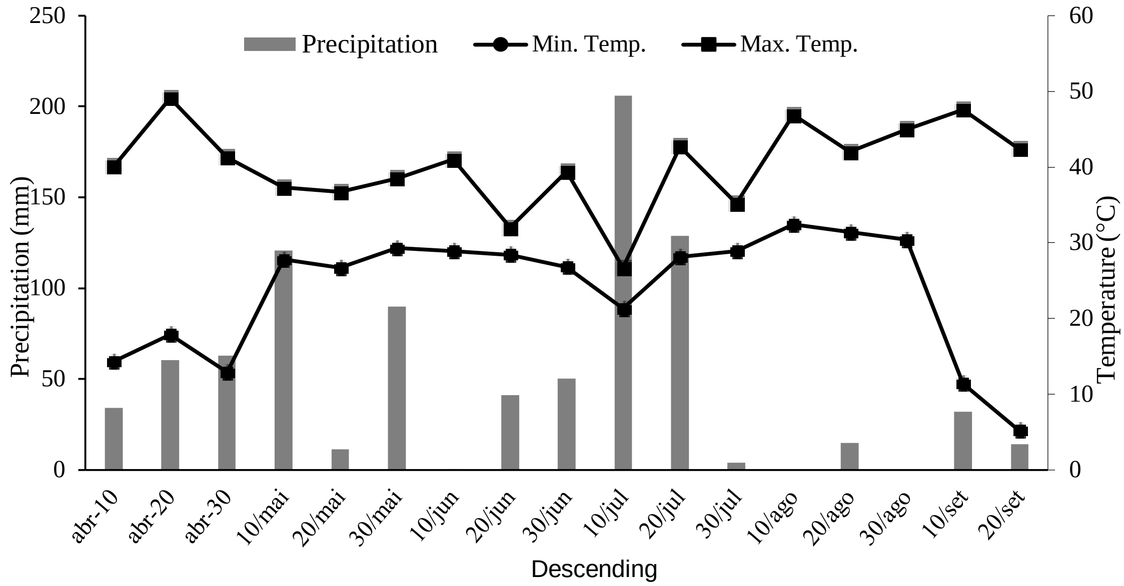

Figure 1. Descending meteorological data for the period from May to August 2015. Precipitation (mm), maximum temperature (Max Temp) and minimum temperature (Min Temp). Source: UNEMET climatological station / Marechal Candido Rondon / PR.

1993; Shurson and Sorrel, 1990). Worldwide, it is the third most produced oilseed and with adaptability characteristics to temperate climate. As for the soil, its best development occurs in frank, medium and high fertility soils and well drained (Tomm, 2000).

The cultivation of canola has great socio-economical value which gives the production of vegetable oils in winter, join the soybean production in summer, and thus, contributes to optimize the means of production (land, equipment and personnel) available. For being a crucifer contributes to the reduction of the occurrence of diseases in succeeding crops, as for example, the wheat sown in the following year, increasing the quality, productivity and minimizing costs (Tomm, 2000). This is due to the fact of not being hostess of the majority of diseases and pests that occur in species of grasses and legumes (Tomm et al., 2009).

In Brazil, the cultivation of canola are concentrated mainly in Rio Grande do Sul and Parana, totaling 44.7 thousand hectares with a production of 61.3 million tonnes (Conab, 2015). However, there is a great availability of land areas suitable for the cultivation of canola in Paraná state, with the possibility of significantly expands these numbers. The choice of more adapted genotypes could allow the increase of oil production for biodiesel formulation, human consumption and its bran destined to the formulation of animal rations.

For the selection of genotypes and/or cultivars, experiments have been conducted in different research centers, having as priority the selection of more productive genotypes in different environments (Coimbra et al., 2004). However, there is a need to broaden the research of the different genotypes, to evaluate the adaptation to new environments, in places where there is still no production tradition of this culture. With the development of the research, the crop recommendation has been particularized in each state, according to the characteristics of soil, climate, relief and altitude.

The objective of this work was to study the agronomic characteristics and oil content of 13 genotypes of canola in the environmental conditions of the West of Paraná.

MATERIALS AND METHODS

The study was conducted at the Experimental Farm "Professor Antonio Carlos dos Santos Person " (latitude 24° 33 ' 22' ' S and longitude 54° 03' 24 ' ' W , with an altitude of approximately 400 m) in the agricultural year 2015, at the Universidade Estadual do Oeste do Paraná - *Campus* Marechal Cândido Rondon in Eutrophic Red Latosol (LVe) (Bhering and Santos, 2008).

The local climate, classified according to Koppen, is Cfa, subtropical humid mesothermal dry winter with rainfall well distributed throughout the year and hot summers. For the average temperatures of the quarter, more cold vary between 17 and 18°C; the quarter more hot between 28 and 29°C, in its turn, the annual temperature ranged between 22 and 23°C. The total average annual precipitation normal pluvial for the region vary from 1600 to 1800 mm. For quarter, more humid presents the totals between 400 and 500 mm (IAPAR, 2002). The climate data of the experimental period were obtained in automatic climatological station of the University of Paraná, with approximately 100 m of the experimental area distance and are presented in Figure 1.

The soil of the experimental area was classified as LVe-1 Deep Eutrophic Red Latosol, with a clayey texture (Bhering and Santos, 2008). The soil was characterized by chemical and physical analysis, the layer of 0 to 10 and 11 to 20 cm of depth being vertically sampled, physically 6% sand, 4% silt and 89% clay by the

Boyoucos decimeter method.

The results of the soil chemical analysis showed mean values of: P = 32.32 mg dm^{-3}; K + = 0.51 $cmol_c.dm^{3}$; Al = 0.24 $cmol_c.dm^{3}$; pH in water = 5.43; M.W. = 23.06 g dm^{-3}; H + Al = 6.25 $cmol_c$ dm^{-3}; C.T.C. = 12.88 $cmol_c$ dm^{-3}; Al = 4.54%.

The experimental area was under the no-tillage system since 2009, under the succession oats/corn/oats/soybean. The soil acidity was corrected with application of 2.5 $tonne^{-1}$ of dolomitic limestone 30 days before sowing.

The experimental design was a randomized block design with four replications. The canola genotypes evaluated were Hyola 401, Hyola 76, Hyola 61, Hyola 433, Hyola 50CL, Hyola 571CL, Hyola 575CL, Hyola 474CL, Hyola 555TT, Hyola 656TT, Hyola 559TT, W8006 and H92002. The seeds were supplied by Embrapa Trigo.

Sowing of the genotypes was performed manually on April 8, 2015. The stand used was 33.3 seed per square meter. Weed control was performed with manual weeding. Fertilization was based on the interpretations of soil chemical analysis according to the recommendations suggested by EMBRAPA (Tomm et al., 2009).

At the sowing time, 200 kg ha^{-1} of the 10-20-20 formulation (N-P_2O_5-K_2O) applied in the planting line were used. 405 kg ha^{-1} of ammonium sulphate (21% N and 22% S) was applied in the B4 stage, when the plants had four true leaves, to meet the demands of nitrogen and sulfur.

The sowing periods were evaluated until the beginning of emergence, beginning of flowering, duration of flowering and period until harvest. Plant emergence was considered when plots consisted of 50% of emerged plants, the beginning of flowering when plots contained more than 50% of plants with at least one flower.

It was considered the end of flowering when no more flowers were observed in the plants. The date of maturation was considered when at least 50% of the silica had the seeds with the dark brown coloration.

Manual harvesting of canola plants from the experimental area was started on August 15 at the stage of physiological maturation. All plants of the area of each plot were harvested and counted when approximately 50% of the plants were in the G5 phenological stage, that is, they showed a change in the coloration of the grains from green to brown or black. The plants harvested were subjected to sun drying for 5 days.

Plant height was evaluated at the time of harvest, where it was measured from the soil surface to the apex of the plant using a graduated ruler and averaging ten plants of the useful area. The number of pods per plant was evaluated by removing randomly ten plants of the useful area in the phenological stage G5, where there was the count of pods in each plant. The number of grains per pod was determined randomly collecting four plants of useful area in the phenological stage G5, and randomly observed ten pods of each plant, removing three pods on top, four pods in the middle third and three pods from the bottom and quantifying the number of grains per pods. The determination of the weight of 1000 grains was performed according to methodology described in Rules for Seed Analysis (Brasil, 2009) where eight samples containing 100 seeds of each repetition were weighed with analytical balance precision and then calculate the average weight of thousand grains. To assess the productivity, the plants collected from the area of each plot were threshed manually and after removing the impurities. A profiler was used for moisture to standardize the moisture content of the grain and after certain productivity per hectare. The oil content of the seeds was determined in the Laboratory of Animal Nutrition of UNIOESTE. For such samples of uniform seeds were submitted to drying in an oven with forced air ventilation at 65°C for 48 h in order to standardize the moisture. After drying, the seeds were ground. Seed meal was packed in paper cartridges in the amount of 2 g per cartridge, in duplicate per experimental unit. The extraction adopted the methodology described by IUPAC (1979), using the Soxhlet system and petroleum ether extraction solvent, with extraction time of 6 h. After extraction the cartridges were kept in an oven at 60°C for 24 h for complete evaporation of the petroleum ether. The effects of treatments were compared by the F test and then analyzed through the comparison of means by the Scott Knott test (1974) at a nominal value of 5%. The analysis of variance and test of means were performed according to the usual techniques of SISVAR 5.3 software (Ferreira, 2011).

RESULTS AND DISCUSSION

In relation to sowing day until emergence (DAE) of canola, significant differences were observed ($p \leq 0.05$) where the genotype of canola Hyola 401 was the earliest (Table 1). The Hyola 401 is considered very premature in relation to its cycle. There were significant differences ($p \leq 0.05$), in relation to the beginning of flowering (IF) where Hyola 401 started flowering at 52 days after sowing (Table 1). Regarding the flowering days, significant differences were observed ($p \leq 0.05$), where the Hyola 401 genotype had 28 days to complete this cycle (Table 1). Genotypes Hyola 401, 50, 656TT and 559TT completed their cycle in 129 days.

Despite the high rates of flower abortion, flowering for long periods in canola is important for higher yields of grains (Iriarte and Valetti, 2008; Thomas, 2003; Tomm et al., 2009). The importance of the cycles of plant is rooted in that longer periods of flowering to compensate for any thermal conditions or adverse water fertilization of flowers or the formation of grains, when those conditions cease to affect the plant, so the knowledge of growth and development of cultures allows taking appropriate decisions of management.

Regarding plant height, Morceli (2014) evaluating canola genotypes in Campo Grande, MS, observed significant differences in plant height between the Hyola 61, 76 and 413 canola genotypes, in which Hyola 76 obtained higher plant height. However, in the present experiment, although no significant differences were observed, in absolute values, the genotype Hyola 76 obtained the highest plant height, with a mean of 151.76 cm (Table 2).

Ramos (2013), studying plant spacing and population with Hyola 61 hybrid canola, obtained the highest plant height of 119 cm. Turhan et al. (2011) observed that the sowing season was overdue and observed and plant height decreased, perhaps due to less light absorption.

The largest absolute number of pods per plant was observed in the cultivar Hyola 571 CL with an average of 228, and general average in the experiment of 194 pods (Table 2). The number of pods per plant is an important feature in the production components, being directly influenced by factors that affect the growth and branching of the plant, as well as by the climatic conditions during the flowering and beginning the formation of pods (Morcelli, 2014). According to Krüger et al. (2011), the number of pods per plant is a characteristic of quantitative inheritance, and in this way, it is governed by

Table 1. Duration of the subperiods, days until emergence (DUE), beginning of flowering (BOF), days of flowering (DOF) and harvest (HAR) of 13 canola genotypes in Marechal Candido Rondon in the 2015 harvest.

Genotype	DUE	BOF	DOF	HAR
Hyola 401	10[a]	52[a]	28[a]	129[a]
Hyola 76	12[b]	67[d]	40[d]	132[b]
Hyola 61	12[b]	58[c]	39[d]	132[b]
Hyola 433	12[b]	64[d]	43[e]	132[b]
Hyola 50	12[b]	60[c]	36[c]	129 [a]
Hyola 571CL	12[b]	61[c]	48[f]	132[b]
Hyola 575CL	12[b]	59[c]	42[e]	132[b]
Hyola 474CL	12[b]	59[c]	42[e]	136[d]
Hyola 555TT	12[b]	60[c]	37[d]	134[c]
Hyola 656TT	12[b]	58[c]	34[c]	129[a]
Hyola 559TT	12[b]	60[c]	29[b]	129[a]
W8006	16[d]	56[b]	53g	158[f]
H92002	13[c]	60[c]	49[f]	138[e]
Average	12.20	60.00	40.00	133.00
CV (%)	4.00	3.00	3.17	0.20

Means followed by at least one lowercase letter in the column do not differ from each other by the Scott Knott test ($p \leq 0.05$).

Table 2. Media for plant height (PH), number of pods per plant (NOP), number of grains per pods (NGP), weight of a thousand grains (WTG) of genotypes of canola in Marechal Cândido Rondon in the harvest of 2015.

Genotype	PH (cm)	NOP	NGP	WTG (g)
Hyola 401	140.85	216.42	18.55	3.46[b]
Hyola 76	151.67	166.71	18.85	4.11[a]
Hyola 61	148.27	193.49	19.40	3.55[b]
Hyola 433	144.92	201.72	16.75	3.26[b]
Hyola 50	146.40	183.54	20.57	3.50[b]
Hyola 571CL	141.35	227.71	19.47	4.01[a]
Hyola 575CL	145.67	186.05	16.80	3.76[a]
Hyola 474CL	137.75	182.77	17.07	3.70[a]
Hyola 555TT	135.02	172.38	16.85	2.97[b]
Hyola 656TT	138.07	207.47	16.02	3.52[b]
Hyola 559TT	149.62	193.15	19.22	3.89[a]
W8006	151.00	198.23	17.27	3.90[a]
H92002	137.20	196.22	18.52	3.80[a]
Average	143.67	194.13	17.90	3.63
F value	1.1^{ns}	0.59^{ns}	0.68^{ns}	3.21*
CV (%)	7.47	22.6	14.20	9.65

Means followed by at least one lowercase letter in the column do not differ from each other by the Scott Knott test ($p \leq 0.05$).

a large number of genes of small cumulative effect to the expression of the character and strongly responsive to changes in the environment.

Although canola presents high phenotypic plasticity, which is the ability of plants to alter their phenotypic expression, through morphological and physiological changes in response to environmental changes, there was no statistically significant difference in the number of grains per pods. In the present study the average number of grains per siliquas was 17.90.

Table 3. Media for productivity (PRO) and oil content (OC) of genotypes of canola in Marechal Cândido Rondon in the harvest of 2015.

Genotype	Productivity (kg ha^{-1})	Oil content (%)
Hyola 401	1755.00	41.89
Hyola 76	1531.87	45.94
Hyola 61	1413.12	42.27
Hyola 433	1358.12	43.59
Hyola 50	1511.87	42.46
Hyola 571CL	1565.62	40.03
Hyola 575CL	1456.62	42.70
Hyola 474CL	1600.00	41.91
Hyola 555TT	1664.37	40.80
Hyola 656TT	1697.50	41.35
Hyola 559TT	1483.12	44.54
W8006	1213.00	44.17
H92002	1485.62	40.25
Average	1518.14	42.45
F value	0.71^{ns}	1.64^{ns}
CV (%)	22.73	6.38

Means followed by at least one lowercase letter in the column do not differ from each other by the Scott Knott test ($p \leq 0.05$).

With respect to weight of 1000 grains, significant differences ($p \leq 0.05$) were observed between the evaluated genotypes (Table 2) where the Hyola 76, Hyola 571CL, Hyola 575CL, Hyola 474CL, Hyola 559TT, W8006 and H92002 obtained the largest mass in grams in relation to the other genotypes studied. Krüger et al. (2011) found that there may be variation in mass of grains, depending on the genotype used and depending on a lesser extent of the environmental conditions of the remaining components of production.

The genotype of canola Hyola 401 presented the highest grain yield among all evaluated with averages of 1755 kg ha^{-1} (Table 3). The average yield of different genotypes was 1518.14 kg ha^{-1}, lower when compared with Brazilian production values of 1728 kg ha^{-1} CONAB (2015). The grain yield in canola is the result of the following components of production: number of plants per square meter, number of pods per plant, number of seeds per pod and average mass of grain (Thomas, 2003). These components are dependent on the genotypes and the climatic conditions. Cycle of canola presented deficit in grain filling and end of cycle, causing losses in yield of grains in the genotypes of the long cycle, favoring the performance of genotypes of short cycle, as verified by Tomm et al. (2003) in the city of Maringa. Rathke et al. (2006) argue that the canola has a high phenotypic plasticity to a large number of variables, but that this plasticity has limited effect on productivity. Cheema et al. (2010) reported that the grain yield of canola is determined mainly by the components number of pods per plant and weight of 1000 grains.

The genotypes did not differ statistically among themselves regarding the content of oil ($p \geq 0.05$) ranging from 40 to 46%. All the evaluated genotypes obtained oil higher to those described in the literature for canola between 34 and 38% (Canola Council of Canada, 1999). Morcelli (2014), observed statistical differences for the oil content in grains; for canola oil, genotype Hyola 411 obtained 38% of the oil. The oil content in grains may vary as a function of climatic factors and nutritional disorders despite being a genetic characteristic of the genotype or species (Morcelli, 2014).

Conclusions

There were no significant differences of the genotypes for the agronomic characteristics and the oil content of the canola in the studied conditions, except for the mass of a thousand grains. Canola genotypes Hyola 401, Hyola 50, Hyola 656TT and Hyola 559TT showed the shortest cycle, with 129 days. The best yield was obtained with the Hyola 401 canola genotype, with an average of 1755 kg ha^{-1} of grains. Genotype Hyola 76 had the highest oil content, approximately 46% oil in the grains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Early growth and survival rates of crossbred lambs (*Dorper x indigenous*) under semi-intensive management at Areka, Southern Ethiopia: Effects of non-genetic factors

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Stagnant early growth and poor lamb survival are the major constraints of sheep production in Ethiopia. The aim of this study was to evaluate early growth and survival rate of crossbred lambs (Dorper × indigenous, *Adilo*) under semi-intensive conditions. Body weight (BW, kilograms) at 0-180 days, average daily gain (ADG, g/day), pre- (0-90), post (90-180) and overall (0-180 days), and pre-weaning survival rate (PSR) of crossbred (50%) lambs (n=305) were evaluated. The non-genetic factors (season, parity, sex of lambs, litter size) were also determined. The least squares means (±SE) of BW at birth, 30, 60, 90, 120, 150 and 180 days of age were 2.6± 0.63, 5.6± 0.12, 8.7± 0.18, 11.6±0.23, 15.0±0.46, 17.2± 0.31, and 18.4±0.26 kg, respectively. The crosses were higher by 11.9% and 9.3% at 90 and 150 days, respectively, than indigenous Adilo lambs. There was clearly evident effect of season on body weights at various ages, pre- and post-weaning and overall ADG. Parity influenced weight at 30, 60, and 90 days, and ADG (pre, post and overall). The litter size (1.68 ± 0.6) consistently affected BW at all ages, pre-weaning and overall ADG. Weight at birth, 30 and 90 days, and ADG from 30-60, 60-90 and 120-150 days were affected by parity-by-litter size interaction. The PSR rate (90.2%) was influenced by all non-genetic factors except sex. The improvement in litter size, body weight, and survival represents potentially significant economic advantage of crossbred over local sheep. Managing dam age through replacement ewes, and improving nutrition and litter size would improve lamb survival and growth that enhances total lamb output per ewe per year.

Key words: Growth rate, market weight, crossbred lamb, fixed factors.

INTRODUCTION

Small ruminant production is an integral part of mixed farming system of smallholder farmers in Southern Ethiopia. It plays a significant role in creating employment opportunities, income generation, capital reserve, and improving household nutrition (Kocho et al., 2011; Teklebrhan et al., 2014). Ethiopia's sheep population is estimated at 24 million heads categorized into 18 to 19 sheep breeds or populations (Gizaw et al., 2011).

Adilo sheep, named after routes to terminal market, is widely distributed in semi-arid to sub-humid areas of Central Southern Ethiopia. Generally, Adilo sheep is relatively productive than many other sheep populations or breeds of the region due to their higher prolific capacity (Deribe et al., 2014). In most parts of the country, the major objective of sheep rearing is for lamb production and is the main source of income for smallholder farmers (Kocho et al., 2011). As lamb marketing price depends mainly on the animal's body weight, improvements in lamb birth weight, pre-weaning and post-weaning growth rates are of interest (Gootwine et al., 2008). This could be achieved through optimization of growth (Deribe et al., 2014) and reproduction and survival traits (Gavojdian et al., 2013). To improve productivity of the indigenous breeds, Ethiopian Sheep and Goats Productivity Improvement Program (ESGPIP) imported tropically adapted sire breed (Dorper sheep). Dorper sheep breed is generally documented as one of the most popular mutton breeds in South Africa (Fourie et al., 2009). The Dorper rams can be used as terminal sire to produce lambs with high growth rates, better post-weaning feed efficiency, and superior carcass traits (Gavojdian et al., 2013). The advantage in growth and body composition of crossbred lambs over pure bred dam line lambs results from the additive contribution of the sire line and from the heterosis effect (Gootiwne et al., 2008). Evaluation of growth and survival performance of Dorper sheep and their crosses, under various and prevailing environment is essential before their wider use under smallholder management conditions. This study, therefore, was aimed at evaluating early growth performance and survival rate of crossbred (50%) lambs (Dorper ram × local, Adilo-ewes). It was hypothesized that non-genetic factors would not suppress growth and survival rate of crossbred lambs under semi-intensive management conditions.

MATERIALS AND METHODS

The study site

This study was conducted at Areka research sub-site, Boloso district of Southern Ethiopia, 309 km South of Addis Ababa. The area is situated at 7° 06'N latitude, 37° 47'E longitude, and altitude of 1772 m above sea level. It was characterized by semi-arid to sub-humid agro-ecological zones. The sub-site is a micro-environment suitable for production of diverse grass and legume species. Major cultivated grasses adapted in the area include *Chlorias gayana, Panicum maximum, Brachiaria brizantha* ('desho' grass) and *Pennisetum purpureum* while major legumes are *Medicago sativa, Vigna unguliguleta, Lablab purpureous* and *Sasbania sasban.*

Data collection

Four Pure Dorper rams were introduced to Adilo ewes flock in 2012, end of rainy season. 64 local Adilo ewes were categorized into 4 mating groups. The rams were 18 months old with a mean weight of 46 ± 3 kg. The rams were crossed with Adilo ewes (indigenous sheep) selected based on their reproductive traits, with initial body weight of 19.9 ± 2.30, reared under similar management conditions. The ewes were housed indoors during last trimester, early lactation period, and part of winter periods. Animals had *ad-libitum* access to a medium-quality *Rhodes* and *Brachiaria* hay, water, mineral and shade. Ewes were kept on a natural pasture with the average stocking rate of 6 to 8 heads ha^{-1}. During indoor housing, ewes received medium-quality Napier and Rhodes grass hays *ad libitum*, as well as 200 to 300 g concentrate per head, based on physiological status of ewes. Lambs were allowed to suckle their dams from birth until weaning (90 days). Supplementary concentrate feeds containing 120 g CP kg^{-1} DM on average was provided to all lambs. Within 24 h of parturition; date of birth, birth weight, litter size, sex of lamb and dam parity were recorded. All lambs were weighed at birth and then at weekly interval up to 180 days of age. Mortality of lambs at pre-weaning (birth until 90 days of age) was also recorded.

Data analysis

Data were subjected to the general linear model (GLM) procedure of SAS (SAS, 2008). In the analysis, all non-genetic factors were considered as fixed effects except the error term considered as random effect. Where F test declared significant, Tukey's test was used to compare least squared means (LSMs). The following linear model was employed to analyze the traits:

$$Y_{invmjo} = \mu + x_n + p_j + b_m + y_b + (px)\,jn + (pb)\,jm + \varepsilon_{njmbo}$$

where Y_{injmo} is the observation on production traits: birth, weight at various ages and average daily gain (ADG) (excludes birth weight category); pre-weaning survival rate (includes birth weight category):

μ = is the overall mean; x_n = is the fixed effect of n^{th} lamb sex (n=male, female); p_j = is the fixed effect of j^{th} ewe parity (j = 1, 2, 3, 4); b_m = is the fixed effect of m^{th} type of birth of lamb (m=1=single, 1=twin, 3=triple); y_b = the effect of m^{th} birth weight of lamb {b=1 (≤ 2 kg), 2(2 to 2.5 kg) and 3 (≥ 2.6 kg)}; $(px)_{jn}$ = the interaction effect of parity and sex on body weights and ADGs; $(pb)_{jm}$ = is interaction effect of parity and litter size on body weights and ADGs; ε_{njmbo} = effect of random error

RESULTS

Body weights at various ages

The non-genetic factors considered in this study were shown to affect body weights of crossbred lambs at birth and various ages (Table 1) and trends of weight changes (Figure 1). The least squares means and ±standard error

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Table 1. Least squares means ±SE analysis of variance for body weights (kilograms) from birth to 180 days of age of crossbred lambs (Dorper × indigenous) as affected by sex, parity and litter size in Areka.

Fixed effects	Body weight (kilograms) at various ages (days)						
	Birth weight	30 days	60 days	90 days	120 days	150 days	180 days
Overall	2.55 ± 0.63	5.57 ± 0.12	8.72 ± 0.18	11.6 ± 0.23	15.3 ± 0.46	17.2 ± 0.31	18.4 ± 0.26
Sex	*	NS	NS	NS	*	*	*
Male	2.62 ± 0.05[a]	5.56 ± 0.17	8.74 ± 0.26	11.79 ± 0.34	16.02 ± 0.89[a]	17.68 ± 0.42[a]	19.55 ± 0.39[a]
Female	2.48 ± 0.05[b]	5.57 ± 0.16	8.70 ± 0.24	11.55 ± 0.32	14.59 ± 0.34[b]	16.70 ± 0.36[b]	18.51 ± 0.35[b]
Parity	NS	***	***	**	NS	NS	NS
1	2.63 ± 0.06	5.37 ± 0.20[b]	9.08 ± 0.29[a]	12.00 ± 0.41[a]	16.10 ± 0.48	17.64 ± 0.49	19.15 ± 0.43
2	2.49 ± 0.07	6.30 ± 0.19[a]	9.34 ± 0.28[a]	11.63 ± 0.36[a]	15.34 ± 1.38	16.73 ± 0.44	18.84 ± 0.47
3	2.47 ± 0.10	5.02 ± 0.19[b]	7.58 ± 0.32[b]	10.14 ± 0.46[b]	13.14 ± 0.46	16.14 ± 0.46	18.75 ± 0.40
4	2.55 ± 0.08	4.75 ± 0.29[b]	6.88 ± 0.41[b]	11.69 ± 0.37[a]	14.30 ± 0.47	17.44 ± 0.41	-
Litter size	***	***	***	***	***	***	***
Single	2.93 ± 0.07[a]	6.51 ± 0.21[a]	10.34 ± 0.32[a]	13.79 ± 0.40[a]	18.47 ± 1.04[a]	19.61 ± 0.47[a]	21.04 ± 0.47[a]
Twin	2.36 ± 0.03[b]	5.07 ± 0.12[b]	7.65 ± 0.16[b]	10.24 ± 0.23[b]	13.27 ± 0.27[b]	15.58 ± 0.28[b]	17.68 ± 0.27[b]
Triplet	1.95 ± 0.02[c]	4.42 ± 0.49[c]	8.48 ± 0.91[b]	10.39 ± 0.81[b]	13.03 ± 0.65[b]	14.82 ± 1.21[b]	17.25 ± 1.09[b]
Parity×Sex	*	NS	NS	NS	NS	NS	NS
Parity×Litter size	***	**	NS	*	NS	NS	NS

Means with different letters (a, b) within a trait in a column are different at indicated P value: NS non-significant (*P*>0.05):**P*<0.05; ** *P*< 0.01; ****P*<0.001.

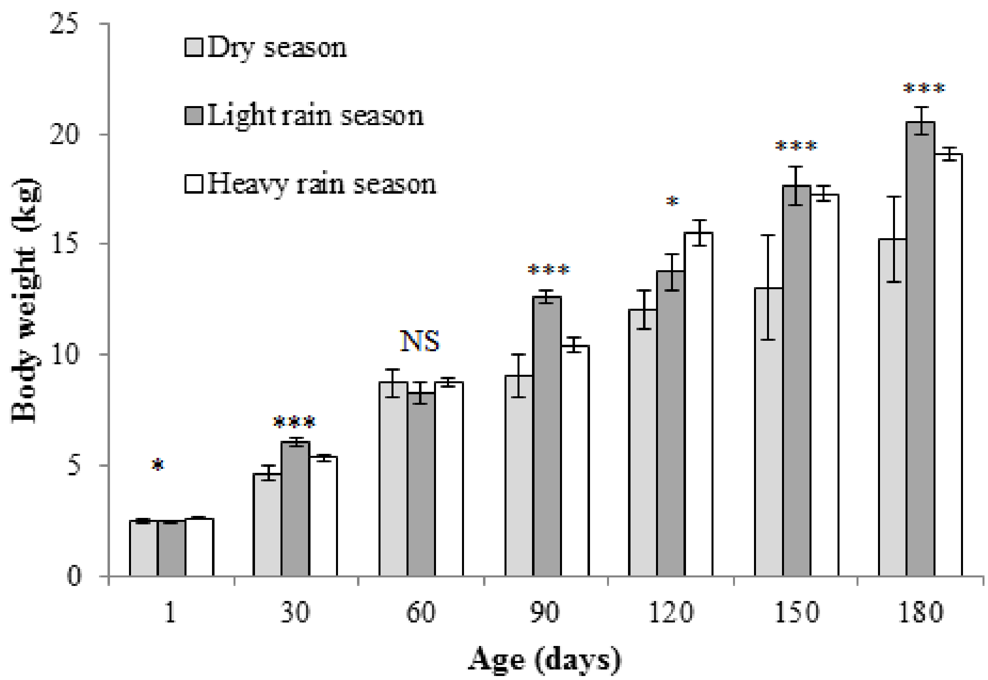

Figure 1. Body weight of crossbred lambs (Dorper x indigenous) from birth to 180 days of ages as affected by the season of birth in Areka. NS, non-significant (*P*>0.05); **P*<0.05; ***P*<0.01; *** *P*<0.001.

(SE) for birth weight (kilograms) of lambs was 2.6±0.63 (ranges from 1.3 to 4.2), while for weights at 30, 60, 90, 120, 150 and 180 days of age were 5.6±0.12, 8.7±0.18, 11.6±0.23, 15.0±0.47, 17.2±0.31, and 18.4±0.26 kg, respectively (Table 1).

Season is one of the most important sources of

Table 2. Least squares means ± SE analysis of variance for average daily weight gain (grams) from birth to 30,30-60, 60-90, 90-120, 120-150, and 150-180 days of age of crossbred lambs (Dorper x indigenous Adilo) by season, sex, parity and litter size in Areka.

Fixed effects	Average daily gain (g) at various ages (days)					
	Birth to 30 days	**30-60 days**	**60-90 days**	**90-120 days**	**120-150 days**	**150-180 days**
Overall	101 ± 3.20	109 ± 3.45	98.6 ±3.08	122 ± 13.73	80.3 ± 4.53	76.4 ± 4.14
Season	NS	**	**	*	NS	*
Dry season	95.9 ±12.5	77.3 ± 11.35^{b}	119 ± 9.85^{a}	103 ± 9.82^{b}	85.1 ±18.7	117 ±17.2^{a}
Light rain season	95.6 ± 9.00	88.3 ± 6.74^{b}	98.5 ± 6.92^{b}	92.5 ± 7.71^{b}	89.1 ± 9.62	105 ± 29.4^{b}
Heavy rain season	102 ± 3.56	115 ± 3.93^{a}	96.7 ± 3.53^{b}	129 ± 17.0^{a}	78.5 ± 69.7	72.0 ± 3.79^{b}
Sex	NS	NS	**	*	NS	NS
Male	98.1 ± 4.52	109 ± 5.00	104 ± 4.45^{a}	142 ± 27.4^{a}	83.9 ± 6.33	82.9 ± 5.05
Female	104 ± 4.52	109 ± 4.81	92.9 ± 4.22^{b}	103 ± 4.11^{b}	76.6 ± 6.49	70.0 ± 6.53
Parity (age of dam)	***	***	***	NS	**	**
1	126 ± 5.39^{a}	139 ± 5.62^{a}	122 ± 4.41$_{a}$	135 ± 3.86	61.0 ± 6.14^{c}	65.8 ±4.26^{b}
2	94.5 ± 4.64^{b}	101 ± 4.76^{b}	69.7 ± 5.01^{c}	132 ± 47.8	90.5 ± 5.64^{b}	89.4 ± 8.80^{a}
3	85.2 ± 4.88^{c}	78.1 ± 4.05^{c}	82.4 ± 4.89^{b}	80.4 ± 5.35	94.4 ± 4.06^{b}	103 ± 14.19^{a}
4	70.2 ± 8.45^{c}	65.5 ± 7.52^{c}	107 ± 7.12^{a}	94.1 ± 6.53	123 ± 25.17^{a}	-
Litter size	***	***	***	*	NS	NS
Single	123 ± 6.00^{a}	142 ± 6.16^{a}	118 ± 5.13^{a}	162 ± 34.82^{a}	77.1 ± 5.59	64.5 ± 7.54
Twin	89.4 ± 3.46^{b}	87.1 ± 3.17^{b}	86.7 ± 3.59^{b}	97.5 ± 3.45^{b}	83.8 ± 6.79	84.6 ±4.77
Triplet	83.0 ± 14.1^{b}	127 ± 18.8^{a}	63.8 ± 10.40^{c}	89.2 ± 16.4^{b}	52.2 ± 18.51	81.1 ± 16.7
Parity×Sex	NS	NS	NS	NS	*	NS
Parity×Litter size	NS	**	*	NS	**	NS

Means with different letters (a, b) within a trait in a column are different at indicated P value: NS non-significant (*P*>0.05); **P*<0.05; ***P*<0.01; ****P*<0.001

variation on lamb birth weight and weight at various ages (Figure 1). Lambs born during the heavy rain season demonstrated higher birth weight (*P*<0.01) and 120 (*P*<0.001), and those born during the light and heavy rain seasons attained higher body weights at 30 (*P*<0.001), 90 (*P*< 0.001), 150 and 180 days (*P*<0.001) compared to the dry season.

Litter size (1.68 ± 0.6) influenced body weights of crossbred lambs at all ages consistently (Table 1). Single births showed consistently higher body weights over their twin and triplet counterparts. Likewise, twin births were higher compared to triplets at birth (*P*<0.001) and 30 days (*P*<0.001) while non-significant (*P*>0.05) in the remaining ages. There was significant effects of sex on body weights at birth (*P*<0.05), non-significant from 30 to 90 days (*P*>0.05) and the influence resumed after 90 days (weaning) (Table 1). Male lambs were superior over their female counterparts at birth, 120, 150 and 180 days.

Parity (dam age) due to differences in ewe weight influenced body weights of lambs at 30, 60 and 90 days (Table 1). Lambs born from the 2nd parity attained significantly higher (*P*<0.001) body weights at 30 days compared to those from the 1st, 3rd and 4th parities. Likewise, lambs from the 1st and 2nd parity demonstrated higher (*P*<0.001) 60 day weight compared to 3rd and 4th parities. Similarly, lambs from 3rd and 4th parities attained significantly lower body weights at 90 day (*P*<0.01) and 120 days (*P*<0.05), respectively. There was parity-by-litter size interactions on birth weight (*P*<0.001), 30 (*P*<0.01) and 90 days (*P*<0.05). Single births from 3rd and 4th parities exhibited higher weights over single births from the 1st and 2nd parities and multiple births from the 3rd and 4th parities.

Average daily gain

All fixed factors considered in this study were shown to influence average daily gain at some stages of lamb ages (Table 2). Pre-weaning, post weaning and overall ADG were affected all fixed factors except sex at pre-weaning and litter size at post weaning (Table 3). The least squares means and SE for ADG from birth to 30, 60, 90, 120, 150, and 180 days of age were 101±3.2, 109±3.5, 98.6±3.1, 122±13.7, 80.3±4.5, and 76.4±4.1 kg, respectively. The pre-weaning, post-weaning and overall ADG were 99.8±2.4, 85.9±2.0 and 96.1±1.5 g/day, respectively. Lambs born during the heavy rain season had higher ADG at 30-60 days (*P*<0.01) and 90-120 days (*P*<0.05) compared to the dry season (Table 2). Conversely, lambs born during the dry season had higher ADG at 60 to 90 (*P*<0.01) compared to the heavy rain season. Likewise, lambs born during the dry and light rain seasons grew faster (*P*<0.05) from 150 to 180 days than

Table 3. Lease squared means ± SE of pre-weaning, post-weaning and overall ADG of crossbred lambs (Dorper-Adilo) as affected by season, sex, parity and litter size in Areka.

Effects	Pre-weaning ADG	Post-weaning ADG	Overall ADG
Overall	99.8 ± 2.43	85.9 ± 1.98	96.1 ± 1.52
Season of birth	**	*	**
Dry season	87.3 ± 10.8[b]	110 ±20.1a	85.0 ± 5.17[b]
Light rain season	105 ± 5.43[a]	89.2 ± 5.18[a]	109 ± 3.65[a]
Heavy rain season	101 ± 2.67[a]	84.9 ± 2.10[b]	94.4 ± 1.69[a]
Sex	NS	**	**
Male	101 ± 3.44	95.0 ± 2.48[a]	98.2 ± 2.06[a]
Female	98.9 ± 3.43	76.8 ± 2.76[b]	94.1 ± 2.23[b]
Parity (age of dam)	***	*	**
1	105 ± 4.25[a]	82.1 ± 2.56[b]	91.9 ± 2.28[b]
2	101 ± 3.49[a]	88.9 ± 4.86[b]	91.5 ± 3.19[b]
3	78.8 ± 4.46[b]	96.9 ± 2.89[a]	109 ± 3.04[a]
4	97.4 ± 5.63[a]	-	98.8 ± 2.80[a]
Litter size	***	NS	**
Single	120 ± 4.29[a]	86.1 ± 3.87	104 ± 2.73[a]
Twin	86.7 ± 2.49[b]	86.1 ± 2.12	91.9 ± 1.75[ab]
Triplet	93.2 ± 8.59[b]	80.4 ± 7.80	84.4 ± 7.09[b]
Parity×Sex	NS	*	NS
Parity×Litter size	NS	NS	NS

Within main effect and trait, means followed by different letters are different at indicated P level; NS, non-significant (*P*>0.05); **P*<0.05; ***P*<0.01.

those during the heavy rain season. The influence of season was also important on pre-weaning ADG (*P*<0.01), post-weaning ADG (*P*<0.05) and overall ADG (*P*<0.01). Litter size demonstrated variation on ADG at early ages of lambs: birth to 30, 30-60, 60-90 and 90-120 days (Table 2), pre-weaning, post weaning and overall ADG (Table 3). Single births were consistently heavier over their twin and triplet contemporaries from birth to 30 (*P*<0.001), 60-90 (*P*<0.001) and 90-120 days (*P*<0.05). Similarly, single and triple births grew faster (*P*<0.001) than twin from 30-60 days. Single births showed faster (*P*<0.001) pre-weaning ADG in comparison to twin and triplets, while single and twin births had heavier (*P*<0.01) overall ADG compared to triplets. Litter size by parity showed significances from 30-60 (*P*<0.01), 60-90 (*P*<0.05) and 120-150 days (*P*<0.01). Single births from 1st and 2nd parity were heavier over single birth of 3rd parity and twin and triplets of other combinations (data not presented). Single births showed faster pre-weaning growth (*P*<0.001) and overall ADG (*P*<0.01) over triplets. Triplet births from the 1st parity were found to be the least (1.91±0.11 kg) in birth weight compared with other combinations.

Except 30-60 (*P*<0.01), 60-90 (*P*<0.01) and 90-120 days (*P*<0.05), where male lambs were superior over their female contemporaries, sex effect was not significant (*P*>0.05) (Table 2). Sex effect was significant on post-weaning and overall ADG (Table 3). Highly significant, but variable, effect of parity was observed on ADG of lambs (Table 2). It was significant on ADG at all ages except from 90-120 days. Lambs born from the 1st parity demonstrated faster ADG compared to the higher parities at birth to 30 and 30-60 days (*P*<0.001). Similarly, lambs born from dams of 2nd parity grew faster (*P*<0.001) compared to the 4th parity from 0-30 and 30-60 days. The higher ADG of lambs from the 1st parity at early ages was reversed from 120-150 and 150-180 days. At 60-90 days, lambs from 1st and 4th parity were heavier (*P*<0.001) over 2nd and 3rd parities.

Lambs born from the 4th parity from 120-150 days and 2nd and 3rd parities from 150-180 days were heavier (*P*<0.01) over those born from the lower parities (Table 3). The pre and post weaning, and overall ADG were consistently and significantly affected by parity. Parity by sex interaction showed a marginal difference (*P*<0.05) at birth while non-significant (*P*>0.05) in the remaining ages while it was significant on ADG from 120-150 days (*P*<0.05) and post weaning ADG (*P*<0.05). Male lambs from 1st and 2nd parities where superior over males from 3rd and 4th parities and female lambs from other combinations (data not presented). Similarly parity by type of birth was significant on body weights at birth (*P*<0.001), 30 (*P*<0.01) and 90 days (*P*<0.05) while it was significant on ADG from 30-60 (*P*<0.01), 60-90 (*P*<0.05) and 120-150 days (*P*<0.01). Single births from 2nd parity were higher over single births of other parties and twin

Table 4. Maximum-likelihood analysis of variance of pre-weaning survival rate of crossbred lambs (Dorper x indigenous) in Areka.

Fixed effects	df	Chi-square	P-value
Season of birth	2	8.56*	0.036
Sex	1	1.44NS	0.223
Litter size	1	8.44**	0.012
Birth weight category	2	7.83*	0.020
Parity (age of dam)	4	10.45**	0.01

Table 5. Pre-weaning survival rate of crossbred lambs (Dorper x indigenous) as influenced by fixed factors and birth weight category in Areka.

Fixed effects	Total births	Pre-weaning survival		Died (%)
	Number	Number	Percent	Number
Overall births	305	275	90.2	30
Season of birth				
Dry	38	35	92.1	3
Light rain	31	25	80.6	6
Heavy rain	236	215	97.9	21
Sex				
Male	156	139	89.1	17
Female	149	136	91.3	13
Parity (age of dam)				
1	135	122	90.4	13
2	99	89	89.9	10
3	32	28	87.5	4
4	40	37	92.5	3
Litter size				
Single	112	108	96.4	4
Twin	181	165	91.2	16
Multiple	12	10	83.3	2
Birth weight category				
1 (<2 kg)	37	26	70.7	11
2 (2-2.5 kg)	137	125	91.2	12
3 (>2.5 kg)	131	128	97.7	3

Pre-weaning survival rate

Maximum likelihood analysis of variance of pre-weaning survival rate of crossbred lambs is indicated in Table 4. Effects of season, sex, litter size, parity and birth weight category on pre-weaning survival rate is shown in Table 5. The average pre-weaning survival rate of the crossbred lambs was 90.2%, excluding stillbirths and abortions. Sex ($P > 0.05$) did not affect pre-weaning survival rate while litter size ($P < 0.01$), influenced pre-survival rate of lambs (Table 4). The survival rate of lambs born during the dry and heavy rainy seasons is higher by 11.5 and 17.3%, respectively, compared with the light rain season. Lambs from 1st and 2nd parities had higher survival rate compared with 3rd and 4th parities. With average birth weight of 1.95 kg, the mortality rate of triplet births reached to16.7%, and was significantly ($P < 0.05$) higher at lower birth weight category. The maximum survival rate (98%) was found from lambs with >2.5 kg (2.6 to 4.2) of live weight at birth and triplets births of all other combinations.

DISCUSSION

Effect of season of birth

The influence of season of birth on birth weight, weight at various ages and ADG has been well documented at station (Gootwine et al., 2008; Teklebrhan et al., 2014) and under smallholder management systems (Hassen et al., 2002; Deribe et al., 2014). Generally, higher birth weight and subsequent weights found in our study, during the heavy rainy season, concurs with the previous reports (Taye et al., 2010; Teklebrhan et al., 2014). Except pre-weaning growth rate, there was a general faster post-weaning and overall ADG during the light and heavy rain season mainly due to feed availability both in quality and quantity. However, there were no clear cut trends of season on body weights and ADG's. This could be associated with seasonal nutrient fluctuations (Legesse, 2008) and feed selection behaviour of sheep (Deribe et al., 2014). The reported higher body weights and ADG of lambs during the heavy rain and part of light rain seasons is consistent with other reports (Teklebrhan et al., 2014). On the other hand, the higher body weight and faster pre-weaning gain during early dry season at some ages is partly due to better body reserves of dams at the end of heavy rain season, and the associated higher milk yield. It has been noted that during early dry season lambs born with higher weight and usually grew faster due to feed flushing of the dams during the heavy rain season (Deribe et al., 2014). The fluctuation of lamb weight (gain and loss) within and across seasons is a commonly reported event, and is attributed to heat stress during the dry season and disease and parasite infestation during the light and heavy rain seasons. Yilmaz et al. (2007) noted seasonal differences on weight of lambs due to differences in ambient temperature and maternal pre-natal effects during gestation. According to Hassen et al. (2002) and Gbangboche et al. (2006), effects of heat stress affects feed intake, and consequently body weights among indigenous lambs in sub-humid ecological zones. On the other hand, quality of feed and variations in feed composition as well as milk yield of dams are factors that have frequently been reported to influence lamb weight during early growth period (Legesse, 2008; Yilmaz et al., 2007; Deribe et al., 2014).

Effect of litter size

Litter size consistently affected lamb growth in our study due to the higher prolific capacity of the sire (Gavojdian et al., 2013) as well as dam breeds (Deribe et al., 2014). The higher litter size or percentage of multiple births, twins (59.3%) and triplets (3.9%) may have resulted in lower body weights of individual lambs at birth but have improved the overall lamb outputs. That could be a reason for decreased body weights and ADG as litter size increased. The overall ADG (0 to 180 days of age) and lamb output found in our study is comparable with previous reports of crossbred lambs under similar management condition (Teklebrhan et al., 2014). Generally, single births attained higher body weights (0 to 180 days) and ADG (pre, post and overall) than twin and triplets. At the same time, single births from 1st and 2nd parities were higher by 20.2 to 62.3% compared with single births of the 4th parity and twin and triplet births from other parity-by-birth combinations. The higher body weights and overall ADG of single births is due to the fact that they were the sole consumers of their dam's milk while twins and triplets compete for limited milk of their dams (Tibbo, 2006; Taye et al., 2010; Deribe et al., 2014).

Effect of parity (dam age)

Differences in parity due to ewe age affected body weights of lambs mainly at early ages, pre-weaning, post weaning and Overall ADG. Generally higher parities (3rd and 4th) attained higher body weights and faster overall ADG compared with lower (1st and 2nd) parities. This results are consistent with reports of Teklebrhan et al. (2013) who have shown profound effect of parity on body weights of crossbred lambs (Dorper × Somali) in Eastern Ethiopia. The higher body weights and ADG (0 to 90 days) obtained from the lower parities, however, is that most ewes at lower parities gave birth of single lambs. Single births had advantage over their twin or triplet counterparts because they are the sole users of milk of their dams (Tibbo, 2006). This suggests that the individual weight of multiple births was lower compared to single although the total lamb output is higher from the prolific dams at higher parties. Dams from 3rd and 4th parities gave birth of large number of multiple births, which compete for limited milk of their dams, and it could be a possible reason for the reported lower body weights of lambs at some ages from 3rd and 4th parities.

Sex effects

The effect of sex on body weights has been variable. Majority of the findings indicated that male lambs had usually higher weight at birth and grew faster than females (Gardner et al., 2007; Deribe et al., 2014), while few have shown that there is less general effect of sex on body weights (Legesse, 2008). The higher weight and faster growth of male over female might be explained by the favour of sexual precocity articulated on body growth. This sexual dimorphism favours body growth in males than females. Hormonal differences between sexes and their resultant effects on growth may also be implicated. The difference in sex hormones, sexual dimorphism, affects feed intake, growth rate and feed efficiency (Mabrouk et al., 2010). In our study the general trend of sex variation was evident at later ages of lambs, which agrees with the findings of Hassen et al. (2002) who reported the importance of sex as lambs get older in the cool highlands of Ethiopia.

Survival up to weaning

In a smallholder farming system, where lambs are produced and kept for income generation, lamb survival is an important economic trait, influences overall productivity of a farm (Tibbo, 2006; Deribe et al., 2014). Except sex, all the fixed factors contributed to the survival or death of lambs. The survival rate obtained in our study concurs with previous reports under similar management condition (Gavojdian et al., 2013). This is due to the adaptive capacity of Dorper sheep under multi-environments (Brien et al., 2014; Teklebrhan et al., 2014). The higher survival rate observed during the rainy compared to the dry season may be due to feed availability. The higher deaths recorded during the light rainy season could mainly be attributed to disease and parasite infestations (Teklebrhan et al., 2014) who noted higher disease and parasite infestation in small rainy season. With average birth weight of 1.95 kg, the mortality rate of triplet births reached to16.7%, and was higher at lower birth weight category. The maximum survival rate (98%) was found from lambs with >2.5 kg (2.6 to 4.2) of live weight at birth. This higher survival rate among higher birth weights categories (2.6 to 4.2 kg) obtained in our study is consistent with previous reports (Taye et al., 2010; Teklebrhan et al., 2014). The crossbred lambs were heavier by 1.24 (11.9%) and 1.46 kg (9.3%) at 90 and 150 days, respectively, compared with indigenous Adilo lambs, which could partly be a reason for the higher survival rate. This agrees with reports of Teklebrhan et al. (2014) who found heavier lamb weights at 90 and 150 days of age from crossbred lambs (Dorper × Somali) in eastern Ethiopia. With the higher number of multiple births (63.2%), the overall survival rate attained in our study, is reasonably higher than other crossbred programs in the country under similar management conditions.

The results of this study indicated that the crossbred lambs (Dorper rams × Adilo ewes) performed well at Areka semi-intensive management conditions, and proved to be adaptable under this semi-arid environment. The non-genetic factors, particularly season, parity and litter size, were shown to influence body weight and daily gain considerably and need to be considered in the improvement plan. Higher prolific potential of the sire and dam breeds contributed to larger number of lamb crops, warrants improved husbandry practices under farmer's management condition. This may help smallholder farmers exploit the prolificacy advantage of the crossbred lambs by improving growth rate and lamb survival, and thus enhance total lamb output per ewe per year.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of supplementing pounded *Prosopis juliflora* pods on hematological profiles of Afar goats fed on *Panicum antidotale* hay

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A study was conducted on thirty two 15.5±1.4 kg (mean ± SD) male Afar goats so as to evaluate effects of increasing dietary levels of pounded *Prosopis juliflora (P. juliflora)* pod on their performance. Hematological values, individual feed intake and body weight gain were determined during a 90-day experimental period. Four dietary treatments were applied using complete randomized design (CRD). The treatments were the experimental units (goats). The experimental feeds were 0 g *P. juliflora* (T1 as control animals), 150 g (T2), 300 g (T3), and 450 g (T4) and *Panicum antidotale* hay was offered as a roughage source, that is, *ad libitum*. Results of total dry matter and nutrient intakes, growth and feed conversion rates were appreciable as the amount of pounded *P. juliflora* is increased from 0 to 300 g. However, these values declined sharply as the amount of pounded *P. juliflora* supplementation increased to 450 g. Hematological analysis showed that there is significant difference (P<0.01) among all treatments in all of the parameters taken. However, all of the values were under the normal range mentioned for healthy goat breeds. Overall, feed intake, growth rate and feed conversion were maximized at 150 g pounded *P. juliflora*. This result also indicated that pounded *P. juliflora* pod could be fed to Afar goats up to 450 g without compromising their health.

Key words: Afar goats, growth, health, hematology, pod, *Prosopis juliflora*.

INTRODUCTION

In Ethiopia, feed scarcity is the major limiting factor, which contributes a lot to the reduction of productivity of the livestock sector (Birhanu et al., 2013). This reduction is highly aggravated especially during the dry season and

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hence supplementation of concentrates is always a mandatory (Nurfeta, 2010). However, according to Mahmoud and Seyoum (2015) availability of such valuable concentrates is very limited due to scarcity and high cost. Therefore, looking for other substitutes as natural fodder trees and shrubs can be used as supplements to livestock, especially during long dry season and fodder stress periods (Abusuwar and Ahmed, 2010). Of these, *Prosopis juliflora (P. juliflora)* is among those fodder trees that can be used as supplements to livestock feed. *P. juliflora* is native to Colombia, Ecuador, Mexico, Peru, and Venezuela and then spread to Central and North America (Pasiecznik, 2002). It had been introduced to many arid zones of different countries, with rainfall of less than 200 mm/year (Mahgoub et al., 2005).

In Ethiopia, the tree is introduced in late 1970s as a biological soil and water conservation means (Sertse and Pasiecznik, 2005). However, it is invading the traditional agro-and silvo-pastoral lands making the rangelands inaccessible to livestock. Consequently, currently the species causes various socio-economic and ecological impacts particularly in Afar rangelands (Kassahun et al., 2005). Consequently, the tree is now affecting even the food security of the afar regional state (Dubale, 2008). This is because, the areas which have already been invaded by the tree have lost natural pasture and the grazing potential of these rangelands have been reduced, native trees have already been replaced or disappeared forever and the productivity of croplands have been reduced (Zeraye, 2008).

P. juliflora is a woody stemmed, thorned, evergreen shrub or small tree usually up to about five meters tall. The tree has green-brown twisted stem, flexible branches and produce flattened, multi-seeded curved pods with hardened pericarp (Getu et al., 2013). The pods of *P. juliflora,* based on the soil type and the process of extraction, have a crude protein level of 7 to 22% and a carbohydrate level of 30 to 75% (Choge et al., 2007). This makes the pod a good low cost alternative feed resource almost for all ruminant species (Abdullah et al., 2011). Since complete removal of the plant by mechanical cutting as well as burning has proven to be difficult, many studies have urged to use this tree as a feed supplement to livestock.

However, since the grinding process requires hammer mill and electrical power and mixing the pod with commercial concentrates is also very expensive and unavailable, almost all of the research out puts done in Ethiopia and outside turned to be non-recommendable to the pastoralists of Afar. Hence, direct feeding of the pod after being pounded with local handmade materials without mill grinding might serve as alternative recommendation to pastoral communities. However, the productivity of *P. juliflora* pod fed animals, for various products such as milk (Abedelnoor et al., 2009), egg (Meseret et al., 2011) and meat as well as their growth performance (Mahgoub et al., 2005) were impeded by the higher inclusion level of the pod. Tabosa et al. (2000) also reported that feeding the pod directly for prolonged time may cause mandibular tremors mainly during chewing, constipation, diarrhea, loss of appetite and other health related problems. These physiological changes and symptoms could be assessed using hematological parameters. Thus, blood is an important and reliable medium for assessing the physiological and health status of individual animals (NseAbasi et al., 2013). Therefore, the objective of this study was to assess the effect of supplementing pounded *P. juliflora* pod on feed intake, growth performance and health situation of Afar goats fed on *Panicum antidotale (P. antidotale)* hay.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Dubti Pastoral and Agro Pastoral Research Center (DPARC) which lies between latitude 11° 27' North, longitude 41° 20' East and an altitude of 382 m above sea level. The mean annual rainfall and temperature of the area are 400 mm and 34.1°C, respectively. The area has sandy loam soil with acacia species, such as *Acacia nilotica* and *P. juliflora*, dominated vegetation cover.

Research methodology

Chemical analysis

The chemical analysis of the feed samples was done as per the following protocols at Holeta Agricultural Research Centre (HARC). All feed samples were collected and dried at 55°C in a forced draft oven to a constant weight and ground to pass through 1 mm mesh screen size. Following this, the samples were subjected for the analysis of dry matter, organic matter, ash, and crude protein according to the procedures of AOAC (1990). Neutral detergent fiber and acid detergent fiber were analyzed according to the procedure of (Van Soest et al., 1991).

Experimental animals, design and management

Twenty four male goats of less than one-year age having 15.5 ±1.4 kg (mean ± SD) of body weight were purchased from the surrounding market. The goats were quarantined for seven days and during this period they were de-wormed against internal and external parasites and penned individually.

The basal diet, that is, *P. antidotale* hay was cultivated at DPARC by cutting at 35 days interval. It was at early blossom, leafy, light green and soft stage which was chopped immediately using a locally made stand chopper at the length of 20 to 25 cm. Similarly,

Table 1. Mean analysis of the treatment feeds during the feeding trial.

P. antidotale	***Prosopis* pod**	**Chemical components**
935.0	894.0	DM (g/kg)
102.0	147.1	CP (g/kg DM)
902.0	954.9	OM (g/kg DM)
737.0	430.4	NDF (g/kg DM)
425.0	270.0	ADF (g/kg DM)
98.0	42.1	Ash (g/kg DM)

the supplemental feed used for the study, that is, *P. juliflora* pods were collected from trees grown in the study area. Following this, pods were dried in the sun, pounded with traditional equipment (Mortar/*Mewqecha* (Amharic) and Pestle/*Zenezena (Amharic)*), mixed with salt and finally fed to animals.

Experimental design and treatments applied

The goats were arranged into four groups of eight animals each in a complete randomized design (CRD). Number of replication per treatment was determined using the general formula $8(CV)^2/d\%^2$; where CV is a coefficient of variation; d% is the expected difference among local control and treatment means. From previous studies, CV was estimated to be 9%, while d% was 10. Treatments were assigned to each group randomly. Four dietary treatments were applied using CRD. The experimental feeds were 0 g pounded *P. juliflora* (T1 as control animals), 150 g (T2), 300 g (T3), 450 g (T4) and *P. antidotal* hay was offered as a roughage source, that is, *ad libitum.*

Statistical analyses

Nutritional parameters were analyzed using generalized linear model (GLM) procedure of SAS (2003). The statistical significant difference among treatment means were checked by Tukey test. Weight gain and feed intake were analyzed using this model:

$$Yij = \mu + Ti + Ei$$

where Yi is the ith observation of the trait in question, μ is the the overall mean, Ti is the treatment effect, and Ei is the residual error. The effect of feed on hematological parameters was analyzed by the following model:

$$Y= a + bx+ei$$

where Y is the dependent variable, a is the intercept on y axis, b is the linear regression coefficient, X is the independent variable, E is the error term, $Y= a+b_1x+b_2x^2+e$, and b_2 is the quadratic regression coefficient.

Feed intake and body weight gain

Pounded *P. juliflora* supplemented for each animal were offered twice a day at 8:00 and 16:00 h in two equal portions. *P. antidotale* hay and water was made available to the goats at *ad libitum*.

To determine daily feed intake, daily feed offered and refusals were weighed and recorded for each goat. Representative samples of feed offered per batch and refusals per goat were collected and pooled on treatment for determination of chemical composition. The feed conversion ratio (FCR) was calculated as a proportion of daily intake (DM) to daily gain (BW). Initial BW of each goat was determined by taking the mean of two consecutive weights after overnight fasting, and BW was subsequently measured every 7 days after overnight fasting.

Hematological examination

Blood samples from all experimental animals were collected in vials containing ethylenediamine tetra acetic acid (EDTA), as anticoagulant, weekly from the day 0 to the end of the experiment (day 90). Blood was drawn from the animals at rest, that is, with minimum animal physiological disturbance or excitement by allowing animals to rest at least 5 min of an adaptation time before sampling. Rectal temperature, pulse, and respiratory rates were also checked for apparent normality. To avoid the effect of diurnal variation, the sampling time was adjusted for all goats at around 7:30 a.m. From each animal, 4 ml of blood was drawn from the jugular vein using a heparinized vacutainer tube following standard procedures. To dissolve the anticoagulants, the collected samples were immediately tipped back and forth a dozen times gentle enough to avoid hemolysis of red blood cells.

Within 1 h from sampling, hematological parameters such as total white blood cell (WBC) count, hemoglobin (Hb) and red blood cell (RBC) count, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean cell hemoglobin (MCHb) were measured using an automatic analyzer (Mindray BC, 2800).

RESULTS

Chemical composition

The chemical composition of feeds used in this experiment is shown in Table 1. The crude protein (CP) and organic matter (OM) content of *P. juliflora* pods were higher than *P. antidotale* grass hay. On the other hand, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of *P. antidotale* hay were higher than *P.*

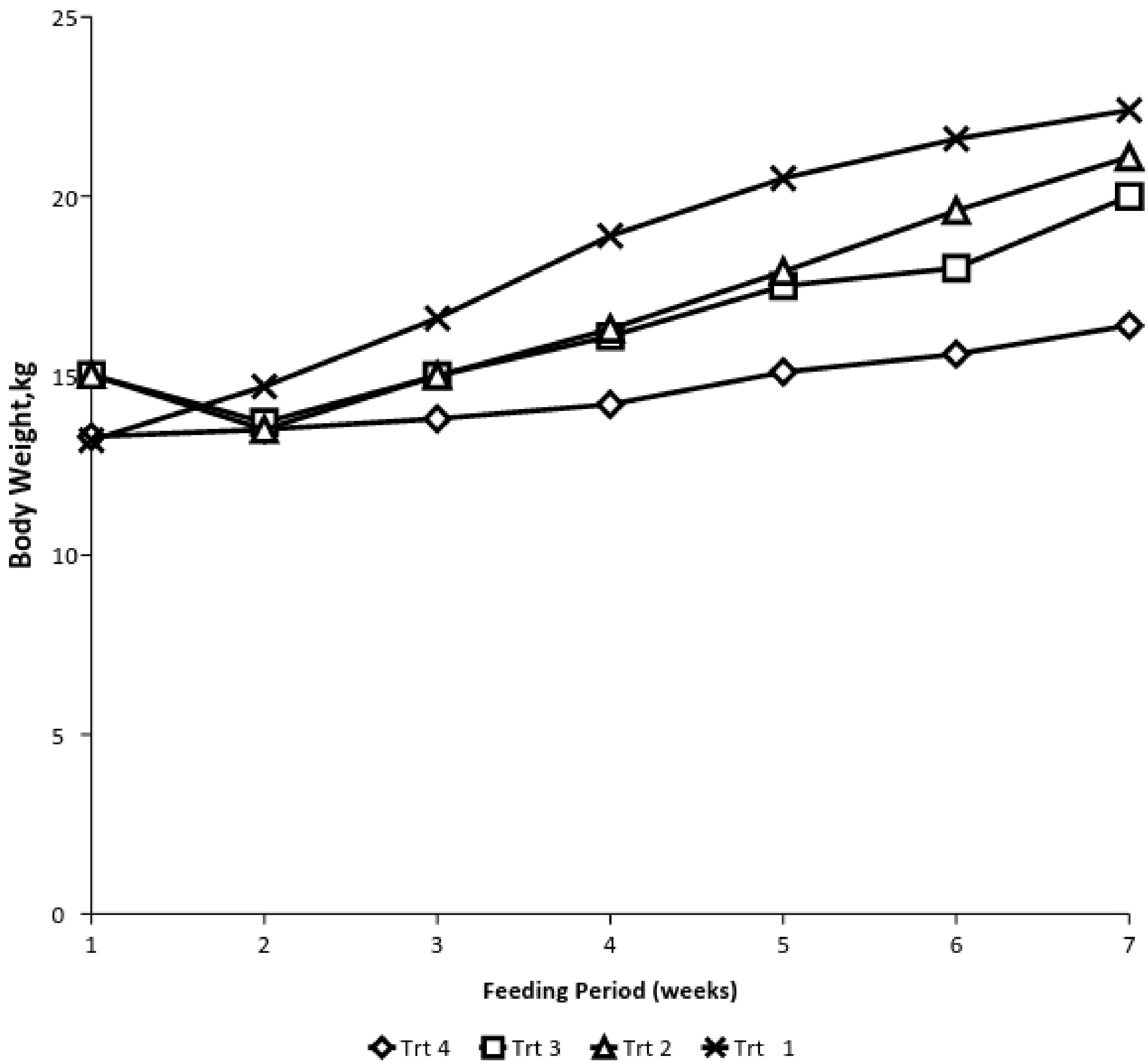

Figure 1. Growth curves of Afar goats fed various levels of pounded *P. juliflora* pod and *P. antidotale* grass hay.

juliflora pod.

Feed intake and body weight parameters

Goats which did not get *P. juliflora* and those fed 150 g of pounded *P. juliflora* pod had higher feed intake (P<0.05). In general, feed intake was found to decrease as the supplementation of pounded *P. juliflora* pod increased across the feeding regime (Figure 1). A similar pattern occurred when feed intake was expressed as a proportion of BW. Goats fed 450 g pounded *P. juliflora* pod had the lowest (P<0.05) final BW gain, body weight change and average daily gain than the other groups. The feed conversion ratio (FCR) was increased significantly (P<0.001) as the amount of pounded *P. juliflora* pod increased from 0 to 450 g (Table 2).

Hematological results

The least square means of erythrocyte series are presented in Table 3. The overall estimates for WBC, RBC, Hb, PCV, MCV, MCH and MCHC were within the

Table 2. Mean feed intake and body weight change of Afar goats fed pounded *Prosopis juliflora* pod and *P. antidotale* grass hay.

Linear	Quadratic	Treatment^{-1}				SEM Probability>F
		450	300	150	0	Variable
						Intake (g/day)
0.003	0.001	468.7±46.4	474.9	508.8	516.8	DM
0.002	0.001	3.1±0.68	3.2	3.4	3.5	DM (%BW)
						Growth performance
0.075	0.077	14.2±0.34	14.9	14.9	13.5	Initial weight (kg)
0.02	0.01	16.4±1.37	20.2	20.2	22.4	Final weight (kg)
0.0004	0.007	2.3±1.5	5.3	5.5	8.8	BW change (kg)
0.04	0.03	26.4±35.1	58.9	62.1	97.8	ADG (g/day)[2]
0.03	0.01	0.06±0.08	0.12	0.12	0.19	FCR[3] (kg gain/feed)

Table 3. Erythrocyte serious of Afar goats fed pounded *P. juliflora* pod during the feeding trial.

Linear	Quadratic		Mean values of all treatments	Treatment^{-1}				SEM Probability>F
				450	300	150	0	
0.15	0.15	2.39	8.0±0.11	7.2±0.1	8.0±0.1	8.2±0.1	8.6±0.1	WBC (10^9 g/L)
0.74	0.91	0.27	11.1±0.2	9.9±0.2	10.9±0.2	11.6±0.2	11.9±0.2	RBC (10^6)
0.18	0.26	0.30	8.0±0.1	7.2±0.1	8.0±0.1	8.2±0.1	8.6±0.1	Hob (g/dl)
0.66	0.84	0.71	24.3±0.2	23.8±0.2	23.8±0.2	24.2±0.2	25.2±0.2	PCV (%)
0.96	0.83	0.92	22.2±0.2	24.0±0.1	21.8±0.2	20.9±0.1	22.1±0.2	MCV (fl)
0.63	0.61	61.6	7.3±0.5	7.4±0.5	7.4±0.4	7.2±0.4	7.2±0.5	MCH (pg)
0.90	0.76	12.3	32.5±0.3	31.0±0.2	33.9±0.3	34.4±0.2	30.7±0.3	MCHC (g/dl)

normal range mentioned for healthy goats.

DISCUSSION

Chemical composition

In the current study, the chemical composition of *P. juliflora* pod was found lower than the results of Ahmed et al. (2012), although samples were collected from the same area. However, Mahmoud and Seyoum (2015) reported almost similar results for all the chemical compositions of *P. juliflora* pod collected from the same area. This similarity and/or difference might be attributed to genotype, stage of maturity at sampling, season of harvesting, pre and post-harvest management, soil type, climate, time and intensity of grazing and plant fraction as well as method of analysis used in the laboratories (Adesogan et al., 2012). *P. juliflora* pod, though its chemical composition differs among the studies, can serve as an alternative livestock feed when compared with other available feed resources especially to the commonly used *P. antidotale* hay (El Hag et al., 2000).

Feed intake and body weight parameters

Feed intake results indicated that *P. juliflora* pods may be included in goat diets up to 450 g. In the present study, reduced feed intakes were (Linear = 0.003, Quadratic = 0.001) observed due to the increased pounded *P. juliflora pod* supplementation which is similar to previous reports of Mahgoub et al. (2005). Such reduction in feed intake could be attributed to the presence of tannins and other phenolic compounds in the *P. juliflora* pods that suppress appetite (Koech et al., 2010). It seems that, due to this effect, goats fed on 450 g pounded *P. juliflora* pod had the least body weight gain.

As feed conversion ratio is an important economic factor, the objectives of many studies have been focused on increasing weight gain per unit of feed used and then a lower FCR (Sebsibe, 2006). The minimum FCR in the current study was observed in goats under treatment one (linear P= 0.03; Quadratic P = 0.01). This shows that as supplementation with pounded *P. juliflora pod* increased to 450 g, feed conversion efficiency decreased and thus the amount of feed used per unit of weight gained is increased. All the feed conversion values of this study, except for treatment four, were higher than (0.07) what was reported by Sebsibe (2006) for stalled Afar goats. Thus, in goat feed, as the inclusion levels of *P. juliflora pod* increases, the FCR increases and feed intake decreases.

Hematological result

As per our knowledge, hematological profiling of Afar goats on *P. juliflora* feed is a first report in its kind and hence related information on Afar goats was missing. The effect of feeding different levels of pounded *P. juliflora* pods on hematological parameters was not significant (linear and quadratic). In general, the hematological profiles of the goats in question were within the range for caprine species (8 - 18 × 10^6 μL^{-1}) mentioned by Latimer et al. (2003). However, the mean values for all hematological parameters of Afar goats were lower than the mean values reported for three Ethiopian goats Arsi-Bale, Central high land and long eared Somali (Tibbo et al., 2004).

This difference in hematological parameters could be due to the difference in altitude. It is already reported that, the shortage of oxygen in high altitudes, leads to an increased production and release of erythropoietin, thereby, stimulating erythropoiesis as a coping or adaptive mechanism to low oxygen level (Tibbo et al., 2004). Therefore, the lower RBC and Hb values exhibited in Afar goats in the present study (360 to 365 m above sea level) could provide evidence of adaptation of these breeds to high atmospheric oxygen.

The mean total WBC values observed in this study (8.0±0.11 × 10^9 g/L) were in close agreement with the result of tropical goats (8.0±0.6 × 10^3 μL^{-1}), for Sokoto Red, Kano Brown, Salla and Borono white goats in Nigeria (Tibbo et al., 2004) and were lower than the mean WBC values of Arsi-Bale goats (11.88 × 10^3 μL^{-1}), Central high land (11.05 × 10^3 μL^{-1}) and long eared Somali (11.09 × 10^3 μL^{-1}) (Tibbo et al., 2004). The observed difference in the mean total WBC might be attributed to the tanniniferous content level of *P. juliflora* which tends to suppress haemopoietic tissues with consequent production of low WBC count (Mahgoub et al., 2008; Olafadehan, 2011). Depressed leukocyte and lymphocyte counts were previously reported in sheep and goats fed tanniniferous diets (Mahgoub et al., 2008; Olafadehan, 2011).

In general, the present study reported that feeding pounded *P. juliflora* to Afar goats did not appear to affect their health condition. However, according to Tabosa et al. (2000) feeding *Prosopis* pod in high proportions and for longer periods can cause health problems in small ruminants. The author also reported that goats fed 600 and 900 g *P. juliflora* pods had mandibular tremors, mainly during chewing. Therefore, lack of manifestation of health problems in the current experimental goats may be due to either the short period of feeding or to the smaller proportions of pods in the diet (maximum 450 g as compared to 600 and 900 g/kg). Thus, further research on the health effects of afar goats fed on *P. juliflora* pods has to be conducted to rule out the benefits and risks associated with *P. juliflora.*

CONCLUSION AND RECOMMENDATIONS

In the current study, feed intake results indicated that *P. juliflora* pod may be included in goat diets up to 450 g without any negative side effect. The effect of feeding different levels of pounded *P. juliflora* was also found to be under normal range for tropical goats.

From these results, it can be concluded that, direct supplementation of pounded *P. juliflora* pod up to 450 g in Afar goats could serve as a means of maximizing feed intake, body weight gain and feed conversion efficiency without compromising any health abnormalities. However, further studies are needed to investigate the impact of direct supplementation of pounded *P. juliflora* pod on physiological behavior of goats and other feed intake and health related parameters.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Determinants of agroforestry adoption as an adaptation means to drought among smallholder farmers in Nakasongola District, Central Uganda

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Agroforestry adoption as a drought adaptation option has an omnibus of opportunities for smallholder farmers in semi-arid regions. This study assessed the severity and frequency of drought and the determinants of agroforestry adoption in Nakasongola District. The episodes were examined using the Standardised Precipitation Index (SPI) set at 3, 6 and 12 months timescales. A cross-sectional survey using semi-structured questionnaires, focus group discussions and key informants were adopted. A total of 200 farmers were randomly selected and studied. The adoption was determined using a binary logistic regression. The SPI results showed that the extreme drought years recorded were 1980, 1984, 1986, 1990, 1995, 1999 and 2000; while the wettest years were 2014, 2012, 2013, 2009 and 2010 as per the 3-time scales. The average return period of severe droughts was 4 years. The levels of agroforestry uptake were higher (85%) between July and June drought period. Agrisilviculture, agrosilvopastoral, silvopastoral and apiculture were the most adopted agroforestry systems by the farmers. The household age, level of education and income were the major significant determinants of agroforestry adoption (*p<0.05*) in adaptation to drought by the smallholder farmers. The potential benefits of agroforestry adoption included the provision of food, fodder, erosion control and soil fertility enrichment, however, the farmers were mainly constrained by inadequate funds, shortage of tree planting stock, limited extension services and information on agroforestry production. Thus, carrying out massive awareness campaigns on agroforestry practices is more likely to increase the uptake.

Key words: Drought, agroforestry, determinants, standardised precipitation index (SPI), adoption, smallholder farmers.

INTRODUCTION

Drought is a natural phenomenon that occurs when water availability is significantly below normal levels over a longer period; hence the supply cannot meet the existing demands (Wilhelmi and Wilhite, 2002; Zargar et al., 2011;

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Schwabe and Connor, 2012; Hepworth et al., 2015). This phenomenon can be classified into four major types: Meteorological, agricultural, hydrological and socio-economical drought (Mishra and Singh, 2010; Van Loon and Van Lanen, 2012). In particular, the meteorological drought is the most predominant of all (Wilhite and Glantz, 1985; Quiring, 2009; Wong et al., 2013; Stagge et al., 2015). The effects of these droughts may be estimated basing on the responses of different systems such as agriculture, water resources and forest ecosystems (Vicente-Serrano et al., 2013; Mosley, 2015). For example, in the semi-arid areas of East Africa, these reactions have negatively affected the sustainable agricultural production and thus hampering the food security status of farmers (Ntale and Gan, 2003; Kirkbride and Grahn, 2008; AghaKouchak, 2015). This is because most of the smallholder farmers are unwilling to implement sustainable soil and water measures as drought adaptation responses because drought is perceived as their most significant threat to agricultural productivity much as some have the capacity to adapt within their capacities (Slegers and Stroosnijder, 2008). Some of the implemented short-term adaptation responses by the farmers to the effects of drought are carrying out a holistic land-use planning to apportion the available land to farming and engage in off-farm employment aimed to reduce their vulnerability to future drought conditions (Liverman, 1999; Campbell et al., 2000; Palm et al., 2014). In addition, some farmers have adapted through applying mulches, planting of drought tolerant crop and pasture varieties, carrying out small-scale irrigation, application of organic and inorganic fertilisers and rainwater harvesting which have proved to be more expensive in both short and long-term (Kanyanjua and Ayaga, 2006; Deng et al., 2006; Valencia et al., 2015). However, some of these practices are not based on natural resources conservation and thus dependent on heavy inputs of chemicals which have accelerated the degradation of ecosystems (Victor and Reuben, 2000; Edmeades et al., 2003; Timilsena et al., 2015).

Agroforestry on the contrast puts forth many benefits because it integrates the concept of multifunctionality into practice including biodiversity, food safety, market-oriented production and rural development (Pattanayak and Mercer, 1998; Lasco et al., 2014; Fouladbash and Currie, 2015). Agroforestry is referred to as a management system that integrates trees in the agricultural and non-agricultural landscapes (Nair et al., 2009; Jose, 2012). Agroforestry systems such as agrisilviculture, agrosilvopastoral, silvopastoral are complex assemblages of ecosystem components, each of which benefits the farmers in various ways (Ojeniyi et al., 1980; Bijalwan et al., 2009; Luedeling et al., 2014). Thus, the importance of adopting agroforestry as a land-use system is receiving wider recognition not only in terms of agricultural sustainability but also on issues related to climate change (Chinnamani, 1993; Neupane et al., 2002; Albrecht and Kandji, 2003). The past and present evidence clearly indicates that the adoption of agroforestry, as part of a multifunctional working landscape, can be a viable land-use option that, in addition to alleviating poverty, offers a number of ecosystem services and environmental benefits (Jose, 2009; Buttoud et al., 2013; Alao and Shuaibu, 2013). In particular, the benefits may include but not limited to: First, agroforestry relies on indigenous farming knowledge and selected modern technologies to manage diversities, incorporate biological principles and resources into to farming systems and intensify agriculture production (Van Bael et al., 2008; Chen et al., 2016). Second, it offers the only practical way to restore agricultural lands that have been degraded by conventional agro-economic practices (Kho, 2000; Franzel et al., 2001; Jerneck and Olsson, 2014). Third, it provides environmental benefits: (i) Biodiversity conservation; (ii) Provision of goods and services to society; (iii) Augmentation of the carbon storage in agroecosystems; (iv) Enhancement of soil fertility, and (v) Provision of social and economic well-being to the farmers (Rao et al., 1998; Udawatta et al., 2010; Beetz, 2011).

Another important aspect to note in this study is the assessment of the determinants of agroforestry adoption amongst the smallholder farmers. This is because the determinants of agroforestry adoption by smallholder farmers differ from one region to another. For instance in the Southwest and Northwest parts of Cameroon, the social-economic factors such as the gender of farmer, household family size, level of education, farmer's experience, membership within farmers' associations, contact with research and extension workers, security of land tenure, agroecological zone, distance of the village from nearest town, village accessibility and income were the major factors that determined the adoption of agroforestry systems by the smallholder farmers (Nkamleu and Manyong, 2008). This is also in addition to the field characteristics (Bannister and Nair, 2003). Thus understanding the determinants of agroforestry adoption is vital for the uptake of agroforestry practices (Pattanayak and Mercer, 1998; Duguma, 2013).

In determining the adoption of agroforestry systems, it is very important for the farmers to track the occurrences and severity of drought given the fact that their livelihood is dependent on the sustainability of natural resources base (Do Pompeu et al., 2012; Jacobi et al., 2013). In drought assessment, drought indices have proved to meet the requirements of monitoring drought worldwide such as Standardized Precipitation Index (SPI), Palmer Drought Severity Index (PDSI), Crop Moisture Index (CMI), Surface Water Supply Index (SWSI), and Reclamation Drought Index (RDI) among others (Keyantash and Dracup, 2002; Jacobi et al., 2013). These indices have simplified the complex climatic functions and can quantify climatic anomalies as for their severity, duration and frequency (Hayes et al., 1999; Tigkas et al., 2014). From the existing modest and popular indices used for estimation of drought, the Standardised Precipitation Index, known as SPI, seems to win universal applicability (Tsakiris and Vangelis, 2004; Dai, 2011). The Standardised Precipitation Index (SPI) is commonly used to characterise droughts in different compartments of the hydro-meteorological system for any part of the world (Karavitis et al., 2011; Musuuza et al., 2016).

Therefore, assessing the severity and frequency of drought, determinants and environmental benefits of agroforestry adoption as an adaptation response by the smallholder farmers is important in establishing the values farmers attach to agroforestry practices and agricultural production. Besides, many agroforestry studies have only investigated tree-soil interactions (Wezel et al., 2000; Kinama et al., 2005), tree-water interactions (Abebe, 1994; Jones et al., 1998) and tree-crop interactions (Muthuri et al., 2005). However, a few studies (Bessems et al., 2008; Van Asten et al., 2011; Shukla et al., 2014) have documented the occurrences and severities of extreme drought episodes and determinants of agroforestry in tropical semiarid areas for the longer period of uptake such as the last 35 years (Kiptot et al., 2007). In addition, the knowledge, perceptions and attitudes of the potential farmers towards the agroforestry adoption plays a key role, but this has been less studied (Meijer et al., 2014).

This study, therefore, is a significant step forward towards assisting scientists and policymakers comprehend how and why the determinants of agroforestry adoption are important drivers that impact the farmers' adaptation to drought. The study aimed to score the prioritisation of agroforestry adoption in extension programmes tailored towards improving the smallholder farmers' agricultural productivity, especially in drought-prone regions. The specific objectives of this study were to; (i) determine the frequency and severity of drought episodes for the last 35 years (1979-2014) and; (ii) examine the determinants of agroforestry adoption as a drought adaptation response by the smallholder farmers in Nakasongola District, Central Uganda.

METHODS

Study area

Nakasongola district is one of the driest districts in Uganda, characterised with prolonged drought episodes, scattered woody biomass plant communities and savannah. The district is located in the north-western part of the central region of Uganda (Roothaert and Magado, 2011). The district has 8 sub-counties namely; Kalungi, Kakooge, Lwampanga, Nabisweera, Wabinyonyi, Nakitoma, Lwabyata, Kalongo and Nakasongola Town Council (Figure 1). The district experiences a bimodal type of rainfall with the first rain season occurring from March/April to June/July and second season occurring from August to October/November of each calendar year. The amount of rainfall received ranges between 500 to 1000 mm per annum. The maximum daytime temperature ranges between 25 to 35°C, while the minimum diurnal range is 18 to 25°C. The soil catena is composed of Buruli and Lwampanga; occurring in both undulating areas and valleys (Mugerwa et al., 2011). In terms of vegetation cover, the most predominant vegetation types occurring in the district include the open deciduous savannah woodlands with short grasses, tropical trees and plantations. For the survival of smallholder farmers, subsistence farming (crop and livestock rearing) is the main source of livelihood engaged by the smallholder farmers in the district. The major types of crops grown include cassava, sweet potatoes and bananas; while the livestock reared include cattle, goats, sheep and poultry (Mugabi et al., 2009). The next sources of livelihood include fishing, sand mining and charcoal burning among others.

Meteorological data

The studied area is one of the areas that are not well monitored in terms of dense meteorological data collection network in Uganda. The existing meteorological dataset had a series of gaps and could not be filled and used for drought assessment. The gaps were attributed to vandalism and subsequent system breakdowns. Hence given this inadequacy, this study downloaded and used the meteorological dataset from the Soil and Water Assessment Tool (SWAT) global weather database (http://globalweather.tamu.edu/). This dataset has been used to assess droughts in the East African region (Gies et al., 2014). The dataset was downloaded from four weather stations; the bounding box extent was: South Latitude 1.1590, North Latitude 1.7273, West Longitude 31.9482, East Longitude 32.6157 that encompassed the study area. The defined period of data collection was from 01/01/1979 to 07/31/2014. This period simplified strong assessment and characterizing drought occurrences and severities. The downloaded climatic parameters included temperature, precipitation, wind, relative humidity and solar; however, it was precipitation that was considered for drought assessment (frequency, duration and severity). The downloaded precipitation dataset was tested for homogeneity and inconsistencies before being used to run drought and wet period's assessment. The preliminary assessment of rainfall trend showed that the study area experienced the same pattern of rainfall distribution but with varying degrees of precipitation amounts over the studied period (Figure 2).

Drought assessment

The Standardised Precipitation Index (SPI) begins with building a frequency distribution from precipitation data at a location for a specified time period (Tigkas et al., 2013). The dataset should have at least a record of 30 years as a prerequisite (Hayes et al., 1999). SPI was developed by McKee et al. (1993). The calculations are based on long-term precipitation record for the desired period. This long-term record is fitted to a probability distribution, which is then transformed into a normal distribution (Hayes et al., 1999). The SPI was developed to detect drought and wet periods at different time scales, an important characteristic that is not accomplished with typical drought indices (Wu et al., 2001). The gamma distribution is defined by its frequency or probability density function:

$$g(x) = \frac{1}{\beta^{a}\Gamma(a)} x^{a-1} e^{-x/\beta}, \text{ for x>0}$$

In which α and β are the shape and scale parameters respectively,

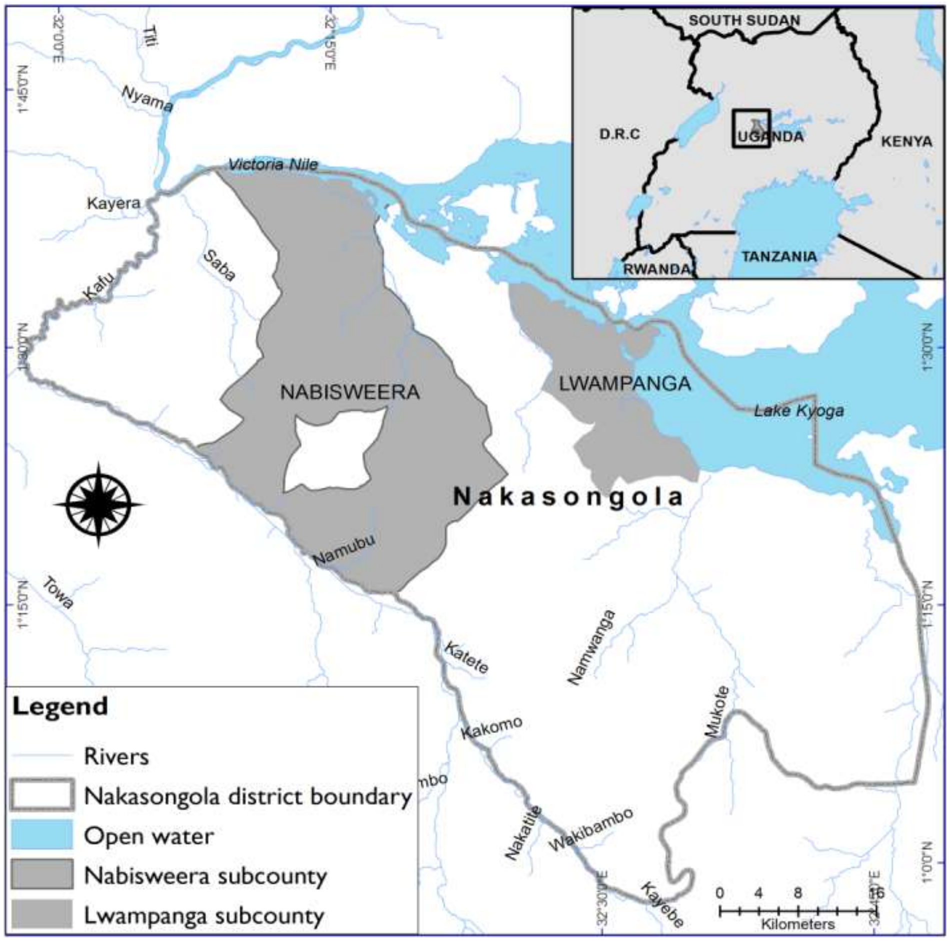

Figure 1. Location of study area.

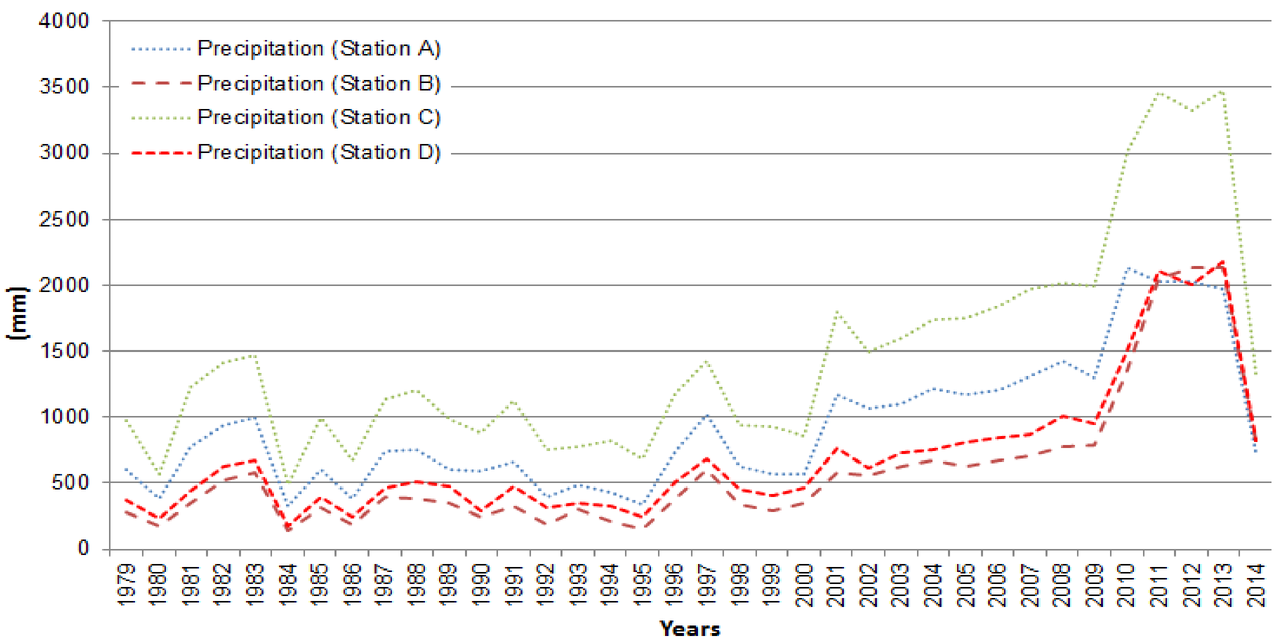

Figure 2. Annual precipitation between 1979 and 2014.

Table 1. SPI drought class classification (McKee et al., 1993).

Drought classes	SPI values	Time in category (%)
Non-drought	SPI ≥ 0	
Near normal	−1 < SPI < 0	34.1
Moderate	−1.5 < SPI ≤ −1	9.2
Severe	−2 < SPI ≤ −1.5	4.4
Extreme	SPI ≤ −2	2.3

x is the precipitation amount and $\Gamma(\alpha)$ is the gamma function. The maximum likelihood estimations of α and β are:

$$a = \frac{1}{4A}\left(1+\sqrt{1+\frac{4A}{3}}\right), \beta = \frac{\bar{x}}{a}, where A = ln(x) - \frac{\sum ln(x)}{n}$$

And n is the number of observations

The resulting parameters are then used to find the cumulative probability of an observed precipitation event for the given month and time scale for the location in question. Since the gamma function is undefined for $x = 0$ and a precipitation distribution may contain zeros, the cumulative probability becomes:

$$H(x) = q + (1-q)G(x)$$

In which q is the probability of zero precipitation and $G(x)$ is the cumulative probability of the incomplete gamma function. If m is the number of zeros in a precipitation time series, then q can be estimated by m/n. The cumulative probability $H(x)$, is then transformed to the standard normal random variable z with mean zero and variance of one which is the value of the SPI.

Because the annual rainfall amounts received in Nakasongola District ranges between 500 to 1000 mm per annum (Mugerwa et al., 2011), the rainfall dataset from Station A was selected and used for drought and wet period's detection assessment. The station data lies within the range of measured precipitation data for the study area. The selected timescales for the computation of SPI were: a 3, 6 and 12-month time scales from the 420 monthly precipitation timescales. The shorter timescales of less than 6 months are more useful for detecting agricultural droughts and, while longer ones, may be useful for considering drought impacts on ground water resources (Moreira et al., 2015). The 12-month timescale, as well as larger timescales, identifies anomalous of dry and wet periods of relatively longer duration and relates well with the impacts of drought on the hydrologic regimes and water resources of a region. The frequency and severity of drought were cross-validated with the Ministry of Disaster Preparedness and Management disaster database available for Uganda. The drought computations were grouped into classes as shown in Table 1.

Socio-economic data collection

A cross-sectional design was used to select the respondents. This strategy is easy but does not permit distinction between cause and effect (Mann, 2003; Powell et al., 2013). From the design, a total of 200 respondents were randomly selected from the village members list and visited for interviewing. With simple random sampling procedure, the sample means were unbiased estimators of the population means (Kirk, 2011). The procedures of carrying out simple random sampling were adopted from Kadilar and Cingi (2006). The sample size of selected farmers from each sub-county was 100 respondents. This size gave a moderate representation of the population in the selected sub-counties.

The selected respondents (both women and men) were interviewed using household questionnaires that apprehended information on the practised agroforestry systems, determinants and benefits of agroforestry adoption as a drought adaptation response in the district. Perceptions of farmer's on drought seasonality were also captured in the questionnaire. Interviewing is a more popular means of generating information (Holstein and Gubrium, 2004). The principle respondent was the household head and where the household head was absent, the spouse was interviewed. The respondents were interviewed from their homesteads with the aim to minimise the loss of production time. Field walks were also carried out to evaluate the performance of farmers in their gardens after adopting agroforestry. In addition, two focus group discussions were also conducted from each sub-county comprising of 7 to 10 participants. The focus group discussions were not sex-disaggregated, both men and women attended. The consultations were held at the sub-county headquarters. These discussions helped to assess the determinants of adopted agroforestry practices (Kitzinger, 1994). Furthermore, the key informant interviews were also steered. The interviewed key informants included the District Agricultural Officer, Production Officer and a representative from Nakasongola District Farmer's association. The collected socioeconomic dataset was validated for inconsistencies and coded in SPSS statistical software. The corresponding normality of data facilitated a statistical analysis to test the levels of significance of farmer's determinants of agroforestry adoption. A statistical binary logistic regression was performed in SPSS to examine the significant determinants of farmer's adoption of agroforestry as a response to drought in Nakasongola District. The logistic regression methodology and applications are well explained in detail by Agresti and Agresti (1970).

Determinants of agroforestry adoption by the smallholder farmers

Verifying the farmer's adoption of agroforestry practices requires an in-depth understanding of the household demographic and on-farm and off far characteristics. In addition, the intricate nature of the prevailing farming systems could be appropriately answered by carrying out a logistic regression in examining the determinants of agroforestry adoption. The regression can moderately quantify the relationship between one dependent binary variable and a set of independent variable. A binary logistic regression was implemented to assess the determinants of the farmer's adoption of agroforestry as a drought option using SPSS software and the relationship measured at 5% significance level.

As specified in Agresti and Finlay (1997), the simple logistic regression model has the form:

$$In\left(\frac{\pi}{1-\pi}\right) = \log(odds) = \log Y = \alpha + \beta X \quad (1)$$

When we take the antilog on both sides of Equation 1, we derive the equation to forecast the probability of the occurrence of the outcome of interest as shown in Equation 2:

$$\pi = P(Y) = \frac{e^{\alpha+\beta x}}{1+e^{a+\beta x}} \quad (2)$$

Where 'π' is the probability of the outcome of interest (Y=1); 'α' is the Y intercept (constant of the equation); *β*i represents the regression coefficients of the explanatory variables (that is, vector of coefficients to be estimated; e represents a set of predictors, and 'e' is the base of the system of the natural logarithms.

The dependent variable Y1i = { 0 if household has not adopted agroforestry practices; 1 if household has adopted agroforestry practices

Taking the log of Equation (2) we have the following logit model for estimating coefficients:

$$In\left(\frac{P(Y=1)}{P(1-P)}\right) = \alpha^* + \beta_1^*\chi_1 + \beta_2^*\chi_2 + ...\beta_n^*\chi_n, \quad (3)$$

Finally, an estimation Equation (3) was undertaken using SPSS statistical software to find the best linear combination of predictors to maximise the likelihood of obtaining the observed outcome frequencies. The predictors of the equation included the level of education, the age of the respondent, household size, environmental policies, land ownership and household income levels.

The interpretations are given in terms of odds ratios and not in terms of marginal effects. Marginal effects are suitable for linear probability models, whereas in the case of binary response models odds ratios give more intuitive meaning (Vittinghoff et al., 2011).

RESULTS

Severity and frequency of drought

Results are in conformity that shorter time scales (3-months and 6-months) had higher frequencies of change between the dry and wet periods (Figure 3). The 3-month interval showed higher displacement in the peaks periods of wet years. The increasing time scales presented lower time scales and longer durations. The recorded severe drought years were 1984, 1980, 1986, 1995, 1990, 1999 and 2000 for the assessed period, while the wettest years recorded included 2014, 2012, 2013, 2009 and 2010. The average severe drought return period was 4 years. Figure 4 shows a distinction between the anomalous dry and wet periods of moderately long duration of the drought episodes. The extreme drought events were experienced in the months of July followed by June, whereas the wettest month recorded was November across the studied period (Table 2).

Farmer's perceptions on drought seasonality

Table 3 shows the farmer's perceptions on drought seasonality for the last 10 years. During this period, the studied area experienced two rainy seasons with the first rains occurring during the months of April to June and the second rains received between August and November. The second rains are the lengthiest, while the dry spells were experienced in the months of December to March of each year.

Determinants of agroforestry adoption by the smallholder farmers

Table 4 summarises the results of a logistic regression highlighting the determinants of agroforestry adoption by the farmers as a major drought adaptation response. The study showed that the level of education, age and household income were the most significant determinants of agroforestry adoption ($P<0.05$); unlike the farmer household size, environmental policies and land ownership which did not significantly determine agroforestry adoption. The Omnibus test of the model coefficients was statistically significant while the exponential coefficient Exp(β) and the maximum likelihood estimate of the odds ratio showed that the level of education had a (0.201) negative coefficient which implied that having less education or being uneducated reduced the agroforestry adoption capacity by 0.201 units at 5% level of significance, holding other factors constant. Whereas, the age of the respondents (1.040) posted a positive coefficient which showed that the farmers who were in the 40 to 50 age group had a higher agroforestry adoption capability than those who were below or above the age-group at 5% level of significance, holding other factors constant. Lastly, the farmer income levels (2.103) also posted a positive coefficient which implied that the farmers who had higher levels of income had greater chances/willingness of adopting agroforestry systems as drought adaption responses.

Adopted agroforestry systems

The majority (95%) of interviewed respondents had adopted agroforestry as a drought adaptation response (Figure 4). Agrisilviculture was the utmost adopted agroforestry system undertaken by nearly all the farmers followed by those who practised agrosilvopastoral, silvopastoral and apiculture systems. The pastoral related agroforestry practices were the second most adopted practices implemented by the farmers given the nature of their locality in the semiarid region. Agrisilviculture was the most widely practised system because of its direct benefits it offered the smallholder farmers especially in terms of food and fuelwood provisions. As far as implementation duration was concerned, most of the

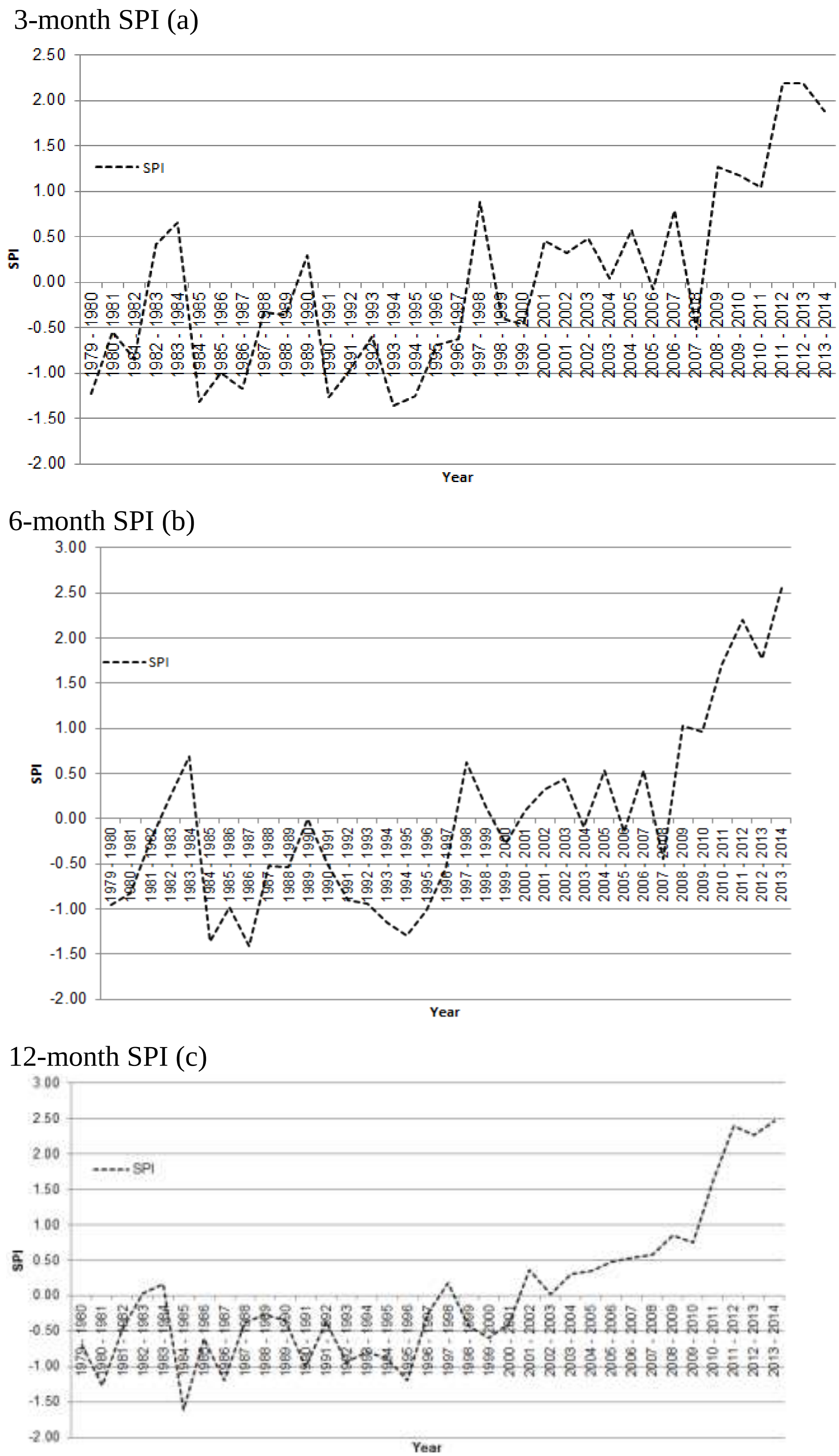

Figure 3. SPI values and the major dry and wet episodes recorded in Nakasongola District. Dry and wet periods (a = 3 month SPI; b = 6 month SPI; c = 12 month SPI).

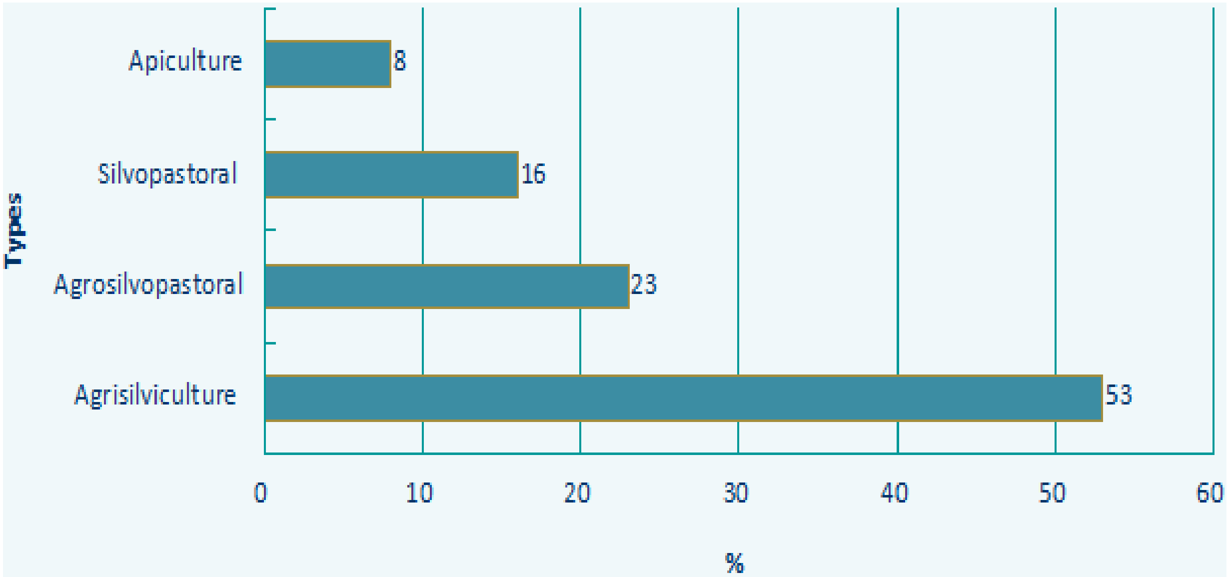

Figure 4. Adopted agroforestry systems across the sampled sub-counties.

Table 2. Extreme dry and wet periods.

Occurrences of extreme events	3-month period	6-month SPI	12-month SPI
Extremely drought month	March	March	March
Observed year	1984	1986	1984
SPI value	-1.3	-1.4	-1.6
Extremely wet month	November	November	November
Observed year	2011	2013	2013
SPI value	2.3	2.6	2.9

Table 3. Seasonal drought seasonality.

Events (months)	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Extremely dry periods	x									x	x	x
First rains		xxx	xxx	xxx								
Second rains						xxx	xxx	xxx	xxx			
Onset of dry season									xx	xx	Xx	

farmers (85%) explained to have planted their trees in the period of last five years (2012-2016) followed by those who planted earlier in the last 10 years (15%). Eighty-five percent of the farmers explained that the levels of agroforestry uptake were higher between July and June drought period.

Benefits of agroforestry adoption

Most of the smallholder farmers (80%) adopted agroforestry systems majorly for additional food provision to feed their families and for sale and harvesting of fodder for livestock feeding. These benefits were enjoyed at both on-farm and off-farm (sub-county) levels by the farmers in the studied sub-counties. The farmers benefited from agroforestry adoption through wind protection of their houses and crops from the destructive oscillating winds that were more prominent during both dry and wet seasons. Whereas the other farmers benefited from the systems through the fuel-wood provision, farmland boundary protection and soil fertility enrichment (Table 5). The adopted agroforestry systems were characterised by scattered tree planting, boundary planting, planting of fruit

Table 4. Determinants of agroforestry adoption by the farmers.

Variable in the equation						
Variable names	***B***	**Std.error**	**Wald**	**Df**	**Sig.**	**EXP(β)**
Level of education	-1.605	0.699	5.268	1	0.022*	.201
Age of respondent	0.039	0.024	2.717	1	0.059*	1.040
Household size	0.228	0.169	1.821	1	0.177^{ns}	1.256
Environmental policies	0.396	0.631	0.394	1	0.530^{ns}	1.486
Land ownership	-0.395	0.258	2.334	1	0.127^{ns}	0.674
Household income levels	0.743	0.446	2.777	1	0.046*	2.103
Model summary						
-2Log likelihood				49.3		
Cox and Snell R Square				0.27		
Nagelkerke R Square				0.36		
Hosmer and Lemeshow Test			Chi-square			
			14.2	7	0.049	
Omnibus tests of model coefficients			14.5	6	0.024	

*Significant at 5% level of significance (P<0.05); ^{ns}Non significant at 5% level of significance (P>0.05).

Table 5. Level of benefits for agroforestry adoption.

Benefits	Farm level	Sub-county level	Rank (%)
Boundary protection			3
Fuelwood			7
Wind protection			9
Soil fertility enrichment			12
Soil erosion control			15
Food (fruits)			24
Fodder			30

trees, tree plantations/woodlots, fodder planting and tree planting carried out in the backyard gardens, distant farmlands and rangelands. The most predominant tree species planted by the farmers to improve their food security status were oranges, mangoes, jackfruit and pawpaws; while for timber and fuel provision, the planted trees included *Pine, Maesopsis eminii, Eucalyptus, Grivellia, and Ficus.* For ecosystem restoration, the planted trees included *Caliandra callothaius, Acacia sp*., among others. A passel of these agroforestry practices was largely adopted at the onset and during the rainy seasons, though their pattern of implementation varied across the sampled respondents reliant on the availability of family labour, income, agro inputs and land tenure among others.

DISCUSSION

This study indicated that the smallholder farmers were disturbed by the severity and frequency of drought; where the quantities of water continued to reduce over time. In response, the adoption agroforestry offered them both direct and indirect benefits to cope with the effects of drought. This study also indicated that the average severe drought return period was 4 years (1979-2014). This finding was not anticipated given that the district lies in between two large water bodies (Lake Kyoga and Victoria) that have great influence on the local climate of the surrounding areas. The disastrous episodes were more common in the months of March and November. The catastrophic events suffocated the farmers by deteriorating the status of agriculture, water resources and forest ecosystems that are natural resources dependent (Mugabi et al., 2010; Mugisha et al. 2011; Roothaert and Magado, 2011). This condition devastated the farmer's food security status resulting from famine and loss of income (Nabalegwa et al., 2007). The droughts experienced over Africa, are normally triggered by the southward shift of the warmest sea surface temperatures in the Atlantic and warming in the Indian Ocean (Dai, 2011). Locally, the farmers attributed the increases in the frequency and severity of drought to anthropogenic factors such as deforestation, over-stocking, wetland degradation and bush burning. Similar observations were also made by Obua et al. (2006) that overgrazing, bush fires and deforestation caused occasional droughts in Nakasongola District. This was further emphasised by Laban et al. (2013) that the distribution of rainfall in eastern and southern Africa had declined by approximately 15% in the last 30 years.

The integration of forestry practices into the implemented farming systems offered the farmers anonymous benefits that helped them cope with drought. In addition to alleviating poverty, agroforestry offered a number of ecosystem services and environmental benefits to the smallholder farmers (Zziwa et al., 2012; Alao and Shuaibu, 2013; Mugerwa, 2015). The proven agroforestry practices implemented by the majority of the farmers included agroagrisilviculture, agrosilvopastoral, silvopastoral and apiculture to enhance their food security status. The adopted agroforestry practices were characterised by scattered trees planting, boundary planting, planting of fruit trees, the establishment of tree plantations and fodder planting. This finding was also reported by Scherr (1992) that farm trees are the main sources of current and future supplies of fuelwood, timber and other important tree products. The adoption rate for the implemented agroforestry systems was 70% for the crop-based systems, while livestock was 30%. The pastoral related agroforestry practices provided higher protein fodder to cattle during the prolonged droughts as was also witnessed by Franzel and Scherr (2002). This observation was in conformity with the findings of Tougiani et al. (2009) who also found out that food security and community resilience to drought enhanced farmer incomes for the farmers located in the semiarid areas.

The social-economic factors were the main determinants of agroforestry adoption by the smallholder farmers (Place and Otsuka, 2002; Bourne et al., 2015). The level of education, age and farmer income levels were the most significant determinants of agroforestry adoption ($P<0.05$) while the household size, environmental policies and land ownership did not. This was also not expected despite the fact that the Ugandan government has increased support in the agricultural sub-sector such as the provision of tree and coffee seedlings and extension services. This finding is also similar to that is made by Buyinza and Mukasa (2007) that young farmers (<50 years) highly adopted agroforestry practices than the older farmers in the cattle corridor. Elsewhere in India, Mahapatra (2002) also found out that the success of agroforestry programme, however, depended on the farmer perceptions, education, the age of the households and resource constraints such as land, labour and capital. Consequently, according to Siriri et al. (2010), the integration of trees on farms may exert complementary or competitive effects on crop yield. However, the constraints faced by the farmers in the adoption of agroforestry practices was a characteristic of smallholder farmers more dependent on the natural resources base for their survival and found in the hard to reach semiarid areas. In this respect, the most frightening constraints included inadequate funds, shortage of planting stock, pests/ parasites and diseases, limited extension services and information. This observation was also similar to that reported by Sonwa et al. (2005) that lack of funds and pests and diseases are one of the major constraints that constrained the farmers from adopting agroforestry practices in semiarid areas. Despite this assessment, further research is vital to determine the effectiveness of the most adopted agroforestry practices.

Conclusion

The Standardised Precipitation Index performed well in the characterization of drought and wet anomalies as collated with the secondary historical and present weather data records. The index distinctively separated longer durations of drought episodes. On average severe droughts were experienced after every 4 years in Uganda's semi-arid areas. The socio-economic factors were the major determinants of the smallholder farmer's adoption of agroforestry practices in the drylands. Agrisilviculture, agrosilvopastoral, silvopastoral and apiculture were the most outstanding agroforestry systems adopted by the farmers due to their multiple benefits. The adopted agroforestry systems were characterised by scattered tree planting, boundary planting, planting of fruit trees, tree plantations/woodlots, fodder planting and tree planting carried out in the backyard gardens, distant farmlands and rangelands. Thus, the adoption of agroforestry gifted the farmers with environmental benefits such as biodiversity conservation; provision of goods and services improved soil fertility and the social-economic well-being of the farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of irrigation intervals on growth, flowering and fruits quality of okra *Abelmoschus esculentus* (L.) Monech

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This study was done to investigate the influence of irrigation intervals and cultivars on growth, flowering and fruits qualities of okra (*Abelmoschus esculentus* L.). The experiment was conducted using Randomize Complete Block Design (RCBD) in Split-Plot Design at the vegetable field, Department of Horticulture and Gardening Landscape, College of Agriculture, University of Baghdad, Iraq. Two cultivars of okra were used: Local investigated (V1) and Copra cultivar (V2). The irrigation was done every 3 (T1), 5 (T2) and 7 days (T3) or irrigation depends on plants need (T4). The cultivars were used as main factor and irrigation intervals as subfactor. The local cultivar showed a significant increase in (V1) in the time of flowering initiation, no. of flowers/plant, and fruits total soluble solids (TSS), while copra cultivar gave a significant increase in the no. of branches/plant, no. of leaves/plant and the fruit length. There are no significant influences between cultivars in plant height, the fruit diameter, dry matter % and fruit firmness. Irrigation every 3 days (T1) positively affected the growth parameter and thus enhanced the flowering initiation, the flower initiation, and increased fruit diameter and fruit firmness. The irrigation every 5 days (T2) increased the number of flowers and the length of fruits, while the irrigation every 7 days (T3) influenced the fruit dry matter percentage and TSS. The interaction between the experimental factors was significant in all the studied parameters.

Key words: Okra, irrigation intervals, vegetative growth, flowering, total soluble solids (TSS), dry matter %.

INTRODUCTION

It is well known that okra (*Abelmoschus esculentum* L.) Malvaceae family is one of the most important summer vegetable crops and it is consumed in large scale in Iraq and other countries. It can be used fresh, frozen or dried especially when it is not available in the markets. It is common in different countries in Asia, Africa and areas

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around the Mediterranean Sea (Matlob et al., 1989). The largest cultivated area of okra under okra crop was in Ethiopia, India, Nigeria, Sudan and Iraq (FAO, 2010).

Okra needs a long warm season, so this crop needs to be watered and it differs from place to other according to the climatic conditions. The irrigation intervals requirements and the quantities of water, which have been supplied to the crops, must be adjusted to increase the productivity and reduce the washing of the mineral elements from the soil and prevent water stress (Boras et al., 2011; Oppongdanso, 2014).

The irrigation water allows the minerals element to be dissolved and transported to different parts of the plant and the water is very imported in the photosynthesis, so the water is an important factors influencing the growth of the plants (El-Sahookie et al., 2009). From their point of view, many research works were carried out to investigate the impacts of irrigation frequency for many crops and the okra is one of them and especially it a summer crop and the crop needs high quantity or water. Ghannad et al. (2014a) found that irrigating okra plants every seven days influenced the intervals to flowering initiation and gave the highest yield and no. of fruits/plant and seed/pod. Irrigating of okra plants every seven days significantly increased the plant height, the no. of branches and no. of leaves, leaf area, the length of fruit and no. of fruit per plant (Ghannad et al., 2014b).

The aim of this study was to investigate the effect of different irrigation intervals on growth, flowering and qualitative characters of okra plants cultivated at the middle of Iraq.

MATERIALS AND METHODS

This study was carried out to investigate the effect of the two okra (*A. esculentus* L.) cultivars and the irrigation intervals and their interaction on vegetative growth, flowering, quantitative, and qualitative characters of fruits. An experiment was carried out in vegetable fields, Department of Horticulture and Landscape Gardening, College of Agricultural, University of Baghdad, Iraq. Two okra cultivars were used: local cultivar (V1) with green medium pods, large leaves and early maturity and Copra cultivar (V2) with milky long pods, medium leaves and late maturity. Three irrigation intervals were used: 3, 5, and 7 days (T1, T2, and T3), respectively and the control (T4) was irrigation when needed.

The okra seeds were planted on the 20th of April in rows (4 m in length and 0.75 m in width), and the experimental unit with three rows and with the area (10 m^2) with three replicates and 24 experimental units. The soil texture was loamy clay and irrigation system was flood irrigation. The agronomic aspects were carried out as reported by Matlob et al. (1989). The experimental design used in this experiment was Randomize Complete Block (RCBD) with Split Plot Design. The cultivars were the main factor (less important) and were put in the main-plot, while the irrigation intervals were the secondary factor (most important) and were put in sub-plot. Data were recorded after 12 weeks from seeds planting. The study included the vegetative parameters such as plant hieght, number of branches/plant, number of leaves/plant, flowering parameters, time to flower initiation, number of flowers/plant, fruit quantities and qualities parameters such as fruit length and diameter by using vernier, percentage of dry matter, firmness by using penetrometer, total soluble solids (TSS) by hand refractometer (A.O.A.C., 1970). The results were analyzed using SAS (2004) program and the means were compared using least significant difference (LSD) at 0.05 probability level (El-Sahookie and Wahaib, 1990).

RESULTS AND DISCUSSION

The vegetative parameters

The results of vegetative parameters are shown in Table 1. It can noticed that there is no significant influence of the cultivars on the height of the plant while the cultivar (V2) gave the highest number of branch $plant^{-1}$ (12.16) and number of leaves (38.58) leaf $plant^{-1}$ as compared with 7.66 branch $plant^{-1}$ and 33.33 leaf $plant^{-1}$ in the local cultivar (V1). The irrigation every three days significantly gave the highest plant height reaching 287.7 cm and the highest number of branches (12.33 branch $plant^{-1}$) and number of leaves (40 leaf $plant^{-1}$) as compared with 133.5 cm and 6.833 branch $plant^{-1}$ and 32.00 leaf $plant^{-1}$ in the irrigation when needed (T4), respectively.

Table 1 shows the interaction of vegetative parameters between experimental factors. The local cultivar (V1) and irrigation every three days (V1T1) gave the highest value of plant height reached 197.5 cm as compared to 103.9 (cm) in plants from same cultivar and irrigation every seven days (V1T3). The plants from the cultivar copra (V2) and irrigation every three days (V2T1) gave the highest number of branches (16 branch $plant^{-1}$) and the highest number of leaves per plant (41.66 leaf $plant^{-1}$) as compared to the local cultivar and irrigated when needed (V1T4) which gave 5.667 branch $plant^{-1}$ and 29.33 leaf $plant^{-1}$, respectively.

The flowering parameters

The cultivars significantly influenced the flowering parameters studied in this experiment. Local cultivar (V1) initiated flowers significantly earlier than copra cultivar; local cultivar takes 63.50 days compared with 68.00 days in copra cultivar plants (V2). The local cultivar (V1) gave the highest number of flowers (82.24 flower $plant^{-1}$) as compared to 77.40 flower $plant^{-1}$ in copra cultivar plants (V2). Plants irrigated every three days (T1) initiated flowers earlier and took 59.00 days compared with plants which have been irrigated when needed (T4) which the plants take 70.66 days to flower. Table 2 also shows the influence of the interaction between the cultivars and irrigation intervals on flowering parameters of okra plants. The local cultivars plants irrigated every three days (V1T1) initiated flowers earlier and took 55.33 days to produce 85.98 flower $plant^{-1}$ compared with copra cultivar

Table 1. The influence of cultivars, irrigation intervals, and their interaction on vegetative parameters of okra plants local cultivar (V1) and cultivar (V2) grown in Iraq.

Parameter	Height of plant (cm)		Mean of irrigation intervals	Number of branches plant^{-1}		Mean of irrigation intervals	Number of Leaves plant^{-1}		Mean of irrigation intervals
Cultivars	V1	V2		V1	V2		V1	V2	
Irrigation intervals									
T1	197.5	178.5	**188.5**	8.667	16.00	12.33	38.33	41.66	40.00
T2	107.4	181.4	144.4	8.000	12.00	10.00	33.66	38.33	36.00
T3	103.9	175.6	139.7	8.333	12.66	10.50	32.00	39.66	35.83
T4	115.2	151.8	133.5	5.667	8.000	6.833	29.33	34.66	32.00
Mean of cultivars	131.0	171.0	-	7.66	12.16	-	33.33	38.58	-
LSD 5% cultivars	NS		-	1.961		-	2.647		-
LSD 5% Irrigation intervals	132.36		-	2.773		-	3.489		-
LSD 5% interaction	221.38		-	3.761		-	6.690		-

Table 2. The influence of cultivars and irrigation intervals and their interaction in flowering parameters of okra plants local cultivar (V1) and cultivar (V2) grown in Iraq.

Cultivars	Time to flower initiation (day)		Mean of irrigation intervals	Number of flowers per plant (flower.plant^{-1})		Mean of irrigation intervals
Irrigation intervals	V1	V2		V1	V2	
T1	55.33	62.66	59.00	85.98	75.53	80.76
T2	63.00	70.00	66.50	85.55	81.18	83.36
T3	64.00	69.66	66.83	81.56	81.61	81.59
T4	69.00	71.66	70.66	75.87	71.26	73.57
Mean of cultivar	63.50	68.00	-	82.24	77.40	-
L.S.D.5% cultivars	0.864		-	1.989		-
L.S.D.5% irrigation intervals	1.222		-	2.813		-
L.S.D.5% interaction	2.577		-	3.685		-

plants that irrigated as needed watering (V2T4) which took 71.66 days to produce 71.26 flower plant^{-1}.

The morphological parameters of pods

The cultivars copra (V2) gave the highest length of fruits (4.041 cm) compared with 3.191 cm in local cultivar (V1) (Table 3). While there were no significant differences between the cultivars in the diameter of the fruit. The irrigation intervals significantly influence the length and diameter of the fruit. Irrigation every five days (T2) increases the length of the fruit up to 4.216 cm compared with 2.5 cm in plants irrigated when the plants need irrigating (T4). The highest diameter of the fruit was 2.083 cm in plants irrigated every three days (T1) while the lowest fruit diameter 1.3 cm in plants irrigated as needed (T4). The influence of the interaction between the cultivars and irrigation intervals was significant when the plants irrigated every five days (V2T2) giving the highest fruit length (4.833 cm) compared with lower fruit length (2.166 cm) in local cultivar wateredas plants needed (V1T4). The highest diameter (2.166 cm) was in copra fruits when plants irrigated every three days (V2T1) compared with 1.1 cm the local cultivar plants that irrigated as plants needed.

The qualitative parameters of the yield

Table 4 shows that local cultivar (V1) okra fruits characterized by high TSS (5.758%) compared with 4.908% in copra cultivar (V2), while there was no significant difference between the two cultivars in influences of irrigation intervals in TSS and the percentage of dry matter and firmness of fruits. Also, there was a significant effect of the okra fruits TSS, the irrigation every seven days (T3) significantly increased TSS and the percentage of dry matter up to 6.5 and 19.35%, respectively compared with values in plants

Table 3. The influence of cultivars and irrigation intervals and their interaction in the morphology of okra fruits local cultivar (V1) and cultivar (V2) grown in Iraq.

Cultivars	Fruit length (cm)		Mean of irrigation intervals	Fruit diameter (cm)		Mean of irrigation intervals
Irrigation intervals	V1	V2		V1	V2	
T1	4.000	4.333	4.166	2.000	2.166	2.083
T2	3.600	4.833	4.216	1.500	1.833	1.666
T3	3.000	4.166	3.583	1.833	2.000	1.916
T4	2.166	2.833	2.500	1.100	1.500	1.300
Mean of cultivars	3.191	4.041	-	1.608	1.875	-
L.S.D.5% cultivars	0.639		-	NS		-
L.S.D.5% irrigation intervals	0.904		-	0.410		-
L.S.D.5% interaction	1.268		-	0.746		-

Table 4. The influence of cultivars and irrigation intervals and their interaction in qualitative of okra fruits local cultivar (V1) and cultivar (V2) grown in Iraq.

Cultivars	TSS (%)		Mean of irrigation intervals	Dry matter (%)		Mean of irrigation intervals	Fruit firmness (Kg.cm^{-2})		Mean of irrigation intervals
Irrigation intervals	V1	V2		V1	V2		V1	V2	
T1	6.067	4.800	5.433	17.50	17.33	17.41	6.200	6.133	6.166
T2	5.433	5.000	5.216	13.00	12.33	12.66	5.433	5.400	5.416
T3	7.267	5.733	6.500	19.03	19.66	19.35	5.700	5.700	5.700
T4	4.267	4.100	4.183	12.03	11.93	11.98	5.700	4.700	5.200
Mean of cultivars	5.758	4.908	-	15.39	15.31	-	5.758	5.483	-
LSD 5% cultivars	1.972		-	NS		-	NS		-
LSD 5% irrigation intervals	1.693		-	4.115		-	0.732		-
LSD 5% interaction	2.345		-	5.456		-	0.979		-

irrigated when the irrigation (T4) is needed 4.183 and 11.98%, respectively. Irrigating okra plants every three days (T1) significantly increased fruit firmness to 6.166 kg.cm^{-2} as compared to 5.2 kg.cm^{-2} in plants irrigated days need (T4). Table 4 shows that the influence of the interaction of cultivars and irrigation intervals was significant, local cultivar and irrigated every seven days (V1T3) gave the highest TSS (7.267%) as compared to 4.100% in copra cultivar irrigated when the plant need irrigation (V2T4). The copra cultivar irrigated every seven days (V2T3) gave the highest percentage of dry matter (19.35%) compared with the lowest value reached 11.98% in the same cultivar but when the plants irrigated as the plants needed (V2T4).The fruit firmness was higher in local cultivars plants irrigated every three days (V1T1) (6.2 kg.cm^{-2}) compared with 4.700 kg.cm^{-2} in fruits irrigated days needed (V2T4). These results are in agreement with those found by Ghannad et al. (2014a,b) who studied the impact of irrigation intervals and cultivation dates on vegetative parameters and flowering of okra palnts in Iran.

From the results of this experiment, the influence of the genetic factors can be noticed in the growth of the plants and their response to the ecological factors. The increases in the number of leaves may be due to the increase in the number of branches as shown in Table 1; this will increase the rate of photosynthesis activities, produce more biomass and early flowering, and increase the number of flowers in plants. The increase in the rate of growth will lead to increase in the hormones which increase the length of fruits (Hussein et al., 2011). The increase of total biomass production and the interaction with ecological factors will increase due to the differences in growth and flowering in plants (Jamala et al., 2011).

Irrigating okra plants at the right time will enhance the growth of vegetative parts, early flowering and fruit set as mentioned earlier, the okra crop need more water during growth and development (Matlob et al., 1989; Hassan, 1997). The influence of irrigation intervals of okra crop on different parameters may be due to stomata behavior of plants which affected plants by these intervals. The opening and closing of the stomata have been affected by the intervals between the irrigation, especially the stomata in the upper layer of the leaf (Anant et al., 2009) and saving the plant from the drought. Abd-El-Kader et al. (2010) noticed that morphological characters of okra plants were reduced by increasing the intervals between irrigation and the plant would face a drought and less humidity which the plants needed for growth, flowering, and yield.

From the experimental results, it is clear that all studied characters were improved by reducing the time between the irrigation, and hence increasing the humidity in the soil and increasing the mineral elements needed by the plants which dissolved by water inside the plant tissues (El-Sahookie et al., 2009). In addition, from this experiment, we can suggest that irrigation every three days characterized by best vegetative growth, earlier flowering initiation, and using new cultivars of okra while being characterized by resisting the drought especially nowadays that Iraq faces a rise in the temperature and lowering available irrigation water.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic diversity among papaya accessions

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Genetic diversity studies provide fundamental information for characterization, conservation and utilization of available genetic resources in plant genetic improvement programs. To evaluate the genetic divergence among papaya accessions, 17 morphoagronomic variables from 59 accessions of the active germplasm bank were evaluated in an experiment at Santa Teresinha Farm, belonging to Caliman Agrícola S/A, in Linhares-ES, Brazil. The experiment was conducted in a randomized block design with two replicates. The divergence between the accessions was estimated using the Tocher optimization method and the agglomerative hierarchical approach based on the Unweighted Pair-Group Method Using an Arithmetic Average. (UPGMA). There was significant difference for all the variables evaluated, showing variability among the accessions. The variables plant height, first fruit insertion height, greatest thickness of fruit pulp, fruit diameter, and fruit length had heritability above 80%, indicating expressive gains in the simple process of selection. Genetic variability was found among the accessions, and Americano, short-peduncle-STZ-03 and Califlora 209 were the most divergent. The optimization methods Tocher and hierarchical based on UPGMA were partially concordant for the formation of heterotic groups of papaya accessions. The variables fruit mass, fruit diameter and plant height contributed the most to the genetic diversity.

Keywords: *Carica papaya* L., genetic variability, multivariate analysis.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most cultivated fruits in tropical and subtropical regions of the world. Papaya fruits are widely used in food diets because they are excellent sources of calcium, pro-vitamin A and

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vitamin C (Serrano and Cattaneo, 2010). Brazil is one of the world's largest producers of papaya (FAO, 2014) and the states of Bahia and Espírito Santo account for more than 60% of the domestic production (IBGE, 2015).

Despite its economic importance for several countries, there are some drawbacks to papaya production such as the high prices of hybrid seeds, generally imported from Taiwan, and the narrow genetic base of the crop, which hampers the development of new hybrids because of the small number of cultivars planted in the main producing regions (Santos et al., 2009).

The low genetic variability makes the crop more vulnerable to economic damages (Oliveira et al., 2010). One solution is to develop new cultivars with characteristics of interest for producers and consumers, which begins with crop genetic improvement works.

Knowing the existing genetic variability makes it possible to develop breeding programs to obtain papaya hybrids with increased productivity and quality of fruits and meet the requirements of domestic and international markets. Studies like this can also provide import information to identify progenitors that in future crosses will enable greater segregation and recombination (Cruz et al., 2012).

Multivariate methods have been used for the quantification of genetic divergence, bringing together treatments into several groups through statistical analysis, such that there is homogeneity within each group and heterogeneity among groups (Bertan et al., 2006).

The hierarchical clustering methods and optimization methods stand out among the multivariate techniques. The hierarchical UPGMA method (Unweighted Pair-Group Method Using an Arithmetic Average) uses arithmetic averages of dissimilarity measures. The Tocher optimization method uses the criterion that the distances within each group are always smaller than those of the intergroup (Cruz et al., 2012).

Because of the importance for crop improvement, studies on the genetic diversity have been carried out in different crops such as guava (Pessanha et al., 2011), common bean (Cabral et al., 2011) and coffee (Martins et al., 2016). A number of genetic divergence studies have also been carried out with different accessions of papaya (*Carica papaya* L.) (Ramos et al., 2011; Quintal et al., 2012; Oliveira et al., 2011; Lucena and Dantas, 2015). Therefore, the objective of this study was to evaluate, by means of variables, the genetic variability among 59 accessions of papaya that have not been previously evaluated in studies of genetic diversity.

MATERIALS AND METHODS

The study was conducted at Santa Terezinha Farm (Caliman Agrícola S/A), 19º 23' 28" S latitude and 40º 04' 20" W longitude, 33 m altitude, average annual temperature of 23.4°C and average rainfall of 1.193 mm year^{-1}, between May 2011 and January 2012. Seventeen morphoagronomic variables were evaluated in 59 accessions of papaya belonging to the Germplasm Active Bank of Calimam Agrícola S.A (Table 1).

The experiment was arranged in a randomized block design with two replicates of five hermaphrodite plants per plot. The cultural practices followed the recommendation for the crop (Martins and Costa, 2003).

The first flower insertion height (FFLH, m) was evaluated at 120 days after planting (DAP). At 240 DAP, the following variables were evaluated: First fruit insertion height (FFRH, m); plant height (PLH, m); stem diameter (STD, m); peduncle length (PL, m); leaf length (LEL, m); maximum leaf width (MLEW, m); chlorophyll content (CHLC) - using a CFL-1030 ClorofiLOG® portable chlorophyllometer, according to the manufacturer's instructions (FALKER, 2008); number of commercial fruits (FRN); fruit mass (FRM, kg); fruit length (FRL, m); fruit equatorial diameter (FRD, m); smallest thickness of fruit pulp (STP, m); greatest thickness of the fruit pulp (GTP, m); soluble solids (SS) - measured in 1/4 ripe fruits by direct reading in bench refractometer (ATTAGO, model N1, Tokyo) and expressed as °Brix; internal firmness of the fruit (FRIF, kg cm^{-2}) measured by a penetrometer (Instrutherm, medol PTR-100) at three points equidistant along the thickness of the pulp of a cross-sectioned fruit, using a 7.9 mm diameter tip and expressed as kg cm^{-2}; and estimated first-year yield per plant (YIELD, kg.plt^{-1}) obtained by multiplying fruit mass by fruit number. The variables PL, LEL, MLEW and CHLC were evaluated on three fully developed leaves of each plant per access.

Data were examined by the analysis of variance. The multivariate analysis was performed using the Tocher's method and the hierarchical grouping techniques based on the UPGMA method using the generalized distance of Mahalanobis (D^2) as measure of dissimilarity (Cruz et al., 2012).

The number of groups formed by the UPGMA method was determined by the cut-off point in the dendogram as proposed by Mojena (1977), using the equation Pc = m + kdp, where m = the mean distances of the fusion levels corresponding to the stages; K = 1.25; dp = standard deviation. In order to test the efficiency of the hierarchical grouping method, the cophenetic correlation coefficient (CCC) proposed by Sokal and Rohlf (1962) was estimated. The data were analyzed using the computational resources of the Genes program (Cruz, 2016).

RESULTS AND DISCUSSION

There was significant difference at 1% probability level among the means of the papaya accessions for the 17 morphoagronomic variables (Table 2), showing that variability exists among the accessions, which is important for the study of genetic divergence.

The means obtained for FFLH at 120 DAP and FFRH and PLH at 240 DAP were 0.81, 0.96 and 1.58 m, respectively. Knowing the variability for such variables is of great importance to be explored in new breeding programs such as developing plants with lower PLH, FFRH and FFLH for cultivation in protected environment, besides the advantage of obtaining plants with lower FFRH, which favors a longer harvest (Lim and Hawa, 2007).

STD had an overall mean of 0.09 m. According to Silva et al. (2007), the selection of plants with greater STD can result in more productive plants, due to the high genetic correlation between these variables. Therefore, this descriptor must be used to compose selection indices in breeding programs.

Table 1. Accessions of Solo (S) and Formosa (F) groups papaya (*Carica papaya* L.) used in the study of genetic diversity, Linhares, Brazil, 2012.

Number - Name	Group	Number - Name	Group
1 - Caliman MS	S	31 - JS 12 (206)	F
2 - Sunrise Solo 783	S	32 - Califlora 209	S
3 - Costa Rica	F	33 - Golden Tipo Formosa	F
4 - Taiwan ET	F	34 - Short-peduncle-STZ-03	S
5 - Diva	S	35 - STA HEL III 12 A PLT 07	F
6 - Grampola	S	36 - STA HEL III 11 A PLT 08	F
7 - Sunrise Solo	S	37 - STA HEL III 50 A PLT 09	F
8 - Caliman AM	S	38 - STA HEL III 02 A PLT 01	F
9 - Caliman GB	S	39 - STA HEL III 14 A PLT 05	F
10 - Caliman SG	S	40 - STA HEL III 15 A PLT 04	F
11 - Caliman G	S	41 - STA HEL III 04 A PLT 02	F
12 - Sunrise Solo 72/12	S	42 - STA HEL III 12 A PLT 06	F
13 - Kapoho Solo Polpa Amarela	S	43 - Papaya 42 Formosa	F
14 - Baixinho de Santa Amália	S	44 - Papaya 45 Formosa Roxo	F
15 - Sunrise Solo TJ	S	45 - Papaya 46 Claro	F
16 - Tailandia	F	46 - Caliman Fruto Médio Verde	S
17 - São Mateus	S	47 - Golden Robusto	S
18 - Kapoho Solo Polpa Vermelha	S	48 - JS 11 (210)	F
19 - Sunrise solo (Progeny Tainung)	S	49 - Tainung 01 F_1	F
20 - Waimanalo	S	50 - STZ 23 Pedúnculo Longo	S
21 - Mamão Bené	F	51 - STZ 63	F
22 - Mamão Roxo	S	52 - Sekati Macuco	F
23 - Maradol (Source Mexico)	F	53 - Gran Golden	S
24 - Maradol (Grande Limão)	F	54 - Sunrise Solo Paulo Brunelle	S
25 - Sekati	F	55 - THB STZ-39	S
26 - Baixinho Super	S	56 - Incaper 39	F
27 - Americano	F	57 - B5 Geraldo	F
28 - STZ 51	S	58 - Formosa Golden	F
29 - 206/4	F	59 - Formosa Brilhoso	F
30 – Calimosa F_1	F		

CHLC had an overall mean of 52.65. Castro et al. (2014) investigated papaya genotypes with different leaf colors and found that the chlorophyll indexes (CHI) quantified are related to the total concentration of chlorophyll quantified by the destructive method. This result suggests that the higher the chlorophyll content, the greater the photosynthetic capacity and, consequently, the greater the increase in yield.

FRM had overall mean of 0.93 kg, ranging from 0.21 to 2.31 kg per fruit, for the accessions short-peduncle-STZ-03 of the Solo group and Maradol (Grande Limão) of the Formosa group, respectively. These results were close to those found by Quintal et al. (2012), who reported overall mean of 0.7017 kg for papaya genotypes also of the groups Solo and Formosa. Fruit mass is a useful variable for selection of plants that express good yield and is used in the separation of the heterotic groups Solo and Formosa.

The varieties of the Solo group are planted in many regions of the world, producing fruit with desirable characteristics for export, with red flesh, small size and weight between 300 and 650 g. Fruits of the Formosa group have red flesh and average size between 1000 and 1300 g. According to Oliveira et al. (2011), the Formosa group consists of commercial hybrids that have gained space in domestic and foreign markets, with strong sales growth in Europe, Canada and the United States.

Regarding fruit size, the variable FRD had mean of 0.10 m, varying from 0.07 m (Sunrise Solo 783) up to 0.15 m (Formosa Brilhoso) and the variable FRL had mean of 0.19 m, ranging from 0.11 m (short-peduncle-STZ-03) to 0.39 m (Americano), with the minimum values represented by the accession Solo group and maximum by the Formosa group, respectively. The fruit size in papaya has also been used to differentiate the heterotrophic groups Solo from Formosa. These

Table 2. Summary of the analysis of variance of the morphological variables of plants and the physical and chemical variables of fruits with the respective mean square of the treatment (MStrat), mean square of the residue (MSres), means, maximum (Max.), minimum (Min.), coefficient of experimental variation (CVe), estimate of coefficient of genetic variation (CVg), and coefficient of genotypic determination (H²) for the 59 accessions of *Carica papaya* L.

Variable	MStrat	MSres	Means	Max./1	Min./1	CVe (%)	CVg (%)	H²
FFLH	375.64**	24.33	0.81	1.10 (49)	0.52 (14)	6.07	16.31	87.83
FFRH	583.02**	74.21	0.96	1.31 (49)	0.58 (26)	8.84	16.56	77.41
PLH	1233.45**	161.2	1.58	2.07 (7)	0.98 (14)	8.04	14.67	76.88
STD	2.84**	0.84	0.09	0.14 (17)	0.07 (9)	10.31	11.2	54.09
PL	139.97**	20.68	0.72	0.89 (32)	0.37 (34)	6.33	10.74	74.25
LEL	61.23**	13.53	0.41	0.62 (32)	0.26 (34)	9.03	11.99	63.79
MLEW	104.71**	31.92	0.57	0.73 (41)	0.38 (34)	9.83	10.5	53.27
CHLC	26.29**	3.35	52.65	59.48 (22)	42.37 (8)	3.47	6.43	77.36
FRN	128.31**	21.2	15.58	41.75 (34)	2.50 (48)	29.55	46.96	71.63
FRM	585330.09**	32330.5	0.93	2.31 (24)	0.21 (34)	19.26	56.32	89.53
FRL	66.36**	11.39	0.19	0.39 (27)	0.11 (34)	17.76	27.59	89.53
FRD	6.09**	0.43	0.10	0.15 (59)	0.07 (2)	6.69	17.08	86.67
STP	56.25**	6.59	0.02	0.033 (37)	0.012 (58)	13.16	25.52	78.00
GTP	68.10**	5.72	0.03	0.039 (23)	0.018 (30)	8.76	20.46	84.48
FRIF	1.67**	0.45	10.09	12.27 (41)	8.69 (35)	6.67	7.73	57.30
SS	2.71**	0.74	9.66	12.06 (45)	7.39 (23)	8.91	10.26	56.99
YIELD	49.32**	18.44	11.46	28.57 (36)	3.14 (41)	37.46	34.26	45.55

** Significant at 1% probability by the test F. /1 Numbers in parentheses correspond to accession identification: 2, 'Sunrise Solo 783'; 7, 'Sunrise Solo'; 8, 'Caliman AM'; 9, 'Caliman GB'; 14, 'Baixinho de Santa Amália'; 17, 'São Mateus'; 23, 'Maradol (Source Mexico)'; 24, 'Maradol (Grande Limão)'; 26, 'Baixinho Super'; 27, 'Americano'; 30, 'Calimosa' F_1; 32, 'Califlora 209'; 34, 'short-peduncle-STZ-03'; 35, 'Santa Helena III Trat 12A PLT.07'; 36, 'Santa Helena III Trat 11A PLT.08'; 37, 'Santa Helena III Trat 50A PLT.09'; 41, 'Santa Helena III Trat 04A PLT.02'; 45, 'Papaya 46 Claro; 48, 'JS 11(210)'; 49, 'Tainung 01' F_1; 58, 'Formosa Golden'; 59, 'Formosa Brilhoso'.

variables are important in the process of transport and packaging of fruit.

The mean for FRIF was 10.09 kg cm^{-2}. This is important information, since this variable is considered an attribute of quality indicating the maturation stage of fruit or harvesting point, which has direct influence on commercialization. Morais et al. (2007) argue that fruit firmness establishes the post-harvest life, since low firmness results in fruit less resistant to transportation, storage and handling.

SS content had overall mean of 9.66°Brix. These results corroborate with those found by Ocampo et al. (2006) and Oliveira et al. (2010) in studies of papaya germplasm and those reported by Marin et al. (2006) in hybrid analysis. SS content may indicate the fruit harvesting point, since there is a relation between them and the maturation stage of the fruit. It is known that during the maturation phase, fruits increase the sugar content, which varies with the type of papaya, cultivar, climatic conditions, soil fertility, production time, and stage of development and maturation (Fontes et al., 2012).

The coefficients of experimental variation (CVe) ranged between 3.47 and 37.46% for the variables CHLC and YIELD, respectively. It was found that 87% of the variables had CVe below 20%, indicating a good experimental precision (Ferreira et al., 2016). These results corroborate with those found by Dias et al. (2011), who analyzed papaya descriptors and found coefficients of experimental variation ranging from 3.09 to 50.29% and that 80% of the variables evaluated had coefficients below 20%.

The genetic variation coefficients (CVg) were high for FRM (56.32%), FRN (46.96%), YIELD (34.26%) and FRL (27.59%) indicating that the variation occurring among the accessions are due to their genetic constitution. This genetic parameter is an important indicator of the relative magnitude of the possible changes that can be obtained in each variable through selection and has been used for peduncle length and number of fruits per plant (Oliveira et al., 2010), plant height and number fruits (Silva et al., 2008) in papaya.

The genotypic determination coefficients (H^2) were higher than 80% for the variables FFLH, FRM, FRL, FRD, and GTP. These results indicate that breeding programs can achieve genetic progress and increase the papaya yield through a simple selection process.

The variables YIELD, SS, MLEW and STD had lower H^2 values showing the influence of the environment on the expression of these variables. An alternative in such cases is the use of indirect selection, through genetic and/or phenotypic correlation.

Table 3. Clustering of the 59 accessions of *Carica papaya* L. by the Tocher method, using the generalized distance of Mahalanobis as measure of genetic distance.

Groups	Acessions[/1]
I	12; 15; 19; 7; 1; 5; 4; 28; 50; 11; 10; 58; 51; 6; 57; 18; 45; 8; 9; 54; 47; 33; 2; 13; 55; 3; 30; 22; 43; 49; 17; 21; 29; 20; 35; 31; 59
II	14; 26; 44; 36; 56; 23; 25; 52; 48; 40; 16; 24; 38; 41; 42; 39; 37
III	27
IV	32
V	34

Acessions[/1]: 1 - 'Caliman MS'; 2, 'Sunrise Solo 783'; 3, 'Costa Rica'; 4, 'Taiwan ET'; 5, 'Diva'; 6, 'Grampola'; 7, 'Sunrise Solo'; 8, 'Caliman AM'; 9, 'Caliman GB'; 10, 'Caliman SG'; 11, 'Caliman G'; 12, 'Sunrise Solo(72/12)'; 13, 'Kapoho Solo polpa amarela'; 14, 'Baixinho de Santa Amália'; 15, 'Surise Solo TJ'; 16, 'Tailândia'; 17 - 'São Mateus'; 18, 'Kapoho Solo polpa vermelha'; 19, 'Sunrise Solo (Progeny Tainung)'; 20, 'Waimalano'; 21, 'Mamão Bené'; 22, 'Mamão Roxo'; 23, 'Maradol (Source Mexico)'; 24, 'Maradol (Grande Limão)'; 25, 'Sekati'; 26, 'Baixinho Super'; 27,'Americano'; 28, 'STZ-51'; 29 - '206/4'; 30, 'Calimosa' F_1; 31, 'JS 12 (206)'; 32, 'Califlora 209'; 33, 'Golden Tipo Formosa'; 34, 'short-peduncle-STZ-03'; 35, 'Santa Helena III Trat 12A PLT.07'; 36, 'Santa Helena III Trat 11A PLT.08'; 37, 'Santa Helena III Trat 50A PLT.09'; 38, 'Santa Helena III Trat 02A PLT.01'; 39, 'Santa Helena III Trat 14A PLT.05'; 40, 'Santa Helena III Trat 15A PLT.04'; 41, 'Santa Helena III Trat 04A PLT.02'; 42, 'Santa Helena III Trat 12A PLT.06'; 43, 'Papaya 42 Formosa'; 44, 'Papaya 45 Formosa Roxo'; 45, 'Papaya 46 Claro'; 46, 'Caliman Fruto Médio Verde'; 47, 'Golden Robusto'; 48, 'JS 11(210)'; 49, 'Tainung 01' F_1; 50, 'STZ 23 Pedúnculo Longo'; 51 - 'STZ 63'; 52, 'Sekati Macuco'; 53, 'Gran Golden'; 54, 'Sunrise Solo Paulo Brunele'; 55, 'THB STZ 39'; 56, 'Incaper 39'; 57, 'B5 Geraldo'; 58, 'Formosa Golden'; 59, 'Formosa Brilhoso'.

The Tocher optimization clustering method using Mahalanobis distances separated the accessions into five groups, with similar behavior within the group and divergent among groups (Table 3). This is an optimization technique that groups individuals by maintaining the criterion that intra-group distances are always smaller than inter-group distances (Cruz et al., 2012).

Group I was formed by 62.71% of the accessions, with large part belonging to the Solo group and accessions of the Formosa group such as Tainung 01, Calimosa, Golden Tipo Formosa, and Formosa Brilhoso. This indicates that although they were of different heterotic group, the set of variables through the multivariate analyzes clustered them in the same group.

Group II consisted of 27% of accessions, including accessions from the groups Solo and Formosa (Baixinho de Santa Amália, Baixo Super, Papaya 45 Formosa Roxo, Santa Helena III Trat 11A PLT.08, Incaper 39, Maradol (Grande Limão), Sekati, Sekati Fruto Longo Macuco, JS 11 (210), Santa Helena III Trat 15A PLT.04, 16 - Tailandia, 24 - Maradol (Grande Limão), St. Helena III Trat 02A PLT.01, Santa Helena III Trat 04A PLT.02, Santa Helena III Trat 12A PLT.06, Santa Helena III Trat 14A PLT.05, Santa Helena III Trat 50A PLT.09). These results are similar to those found by Barbosa et al. (2011), who investigated the divergence among papaya accessions using artificial intelligence resources and found no separation of the accessions of the groups Solo and Formosa in the groups formed by the Tocher method.

Groups III, IV and V are formed by the single accessions Americano, Califlora 209 and short-peduncle-STZ 03 respectively, showing that these are the most divergent genotypes. This occurred because of a certain variable or set of variables that allowed the formation of isolated groups; supposedly, the variable of maximum value for FRM of the accession Americano, maximum value for PL and LEL for Califlora and the minimum values for PL, LEL, FRL, and FRM, and maximum for FRN of accession short-peduncle-STZ 03.

These results are promising for future crosses, since accessions clustered in different groups are more divergent and are an essential source for obtaining hybrids. According to Santos et al. (2012), crosses from genotypes grouped in divergent groups are considered more promising by possibly being more dissimilar and consequently providing greater heterotic effect on the following generations.

The Singh's method was used to demonstrate the relative importance of the 17 morphoagronomic variables for genetic diversity among the papaya accessions (Table 4). The variables FRM (16.88%), FRD (11.32%) and GTP (11.01%) showed the greatest contribution to genetic diversity. On the other hand, YIELD (2.26%), STD (1.17%) and FFRH (0.66%) showed the smallest relative contribution. These results corroborate with those found by Lucena and Dantas (2015), indicating that the variables with less importance express less variability.

Figure 1 shows the dissimilarity among the accessions by the UPGMA method. This method has the advantage of not working with extreme values, minimum and maximum values, but is based on the arithmetic average of the data.

The cut at the distance of 46.35% based on the Mojena's (1977) criterion allowed the formation of six groups of dissimilarity. Group I was formed by 67% of the total number of accessions evaluated, similar to that obtained by the Tocher's method (Table 3). This group is formed by a large part of the accessions of the Solo group.

The accessions 14 - Baixinho de Santa Amália, 26 – Baixo Super, 36 - STA HEL III 11A, and 44 – Papaya 45

Table 4. Relative contribution of 17 morphoagronomic variables to diversity, using the Singh's (1981) criterion based on D^2 of Mahalanobis.

Variable	S.j	Value in %
FFLH	41939.33	10.02
FFRH	2771.55	0.66
PLH	25808.41	6.17
STD	4934.47	1.17
PL	21913.04	5.23
LEL	10769.71	2.57
MLEW	25749.87	6.15
CHLC	26668.25	6.37
FRN	15111.40	3.61
FRM	70609.70	16.88
FRL	20588.29	4.92
FRD	47366.23	11.32
STP	14030.88	3.35
GTP	46055.21	11.01
FRIF	20395.91	4.87
SS	14077.19	3.36
YIELD	9475.13	2.26

Formosa Roxo form the Group II, which includes the accessions with the minimum value for the variables FFLH (0.52 m), FFRH (0.58 m) and PLH (0.97 m). Among these accessions, Baixinho de Santa Amália has short height and is early flowering; which are favorable variables for hybridization works aiming to improve the precocity of current cultivars (Lim and Hawa, 2007).

The Group III is formed by a single accession of the Formosa group (32 - Califlora 209), therefore one of the most divergent accessions, showing maximum values for PL (0.89 m) and LEL (0.62 m).

Group IV includes the accessions 25 - Sekati, 52 - Sekati Fruto Longo Macuco, 23 - Maradol (Origin Mexico), 56 - Incaper 39, 24 - Maradol (Grande Limão), 40 - STA HEL III 15 A, 37 - STA HEL III 50 A, 39 - STA HEL II 14 A, 41 - STA HEL III 4A, 42 - STA HEL III 12 A-06, 38 - STA HEL III 02A and 16 - Tailandia. It is likely that the combination of a set of variables as well as the same genetic base allowed to cluster in a single group the accessions of the Formosa group.

Groups V and VI were formed by the single accessions 27 - Americano and 34 – short-peduncle-STZ 03, respectively, indicating that these accessions as well as 32 - Califlora 209 of Group III are the most divergent in the germplasm bank and can be used as parents in future breeding work to obtain new hybrids.

In this study, the grouping of the accessions by the UPGMA method was similar to the group formation by the Tocher's optimization method. The similarity between these two techniques can be verified by the fact that accessions 32 - Califlora 209, 27 - Americano and 34 - short-peduncle-STZ-03 form single-accession groups using both clustering methods (Table 3 and Figure 1).

Silva et al. (2014) concluded the agreement between multivariate and clustering techniques is important in the study of genetic diversity, since it makes possible the recommendation of crosses between more divergent parents, aiming to broaden the genetic base and, consequently, increase variability. However, this recommendation should also take into account the behavior of each genotype, having greater importance those that show superior performance for the morphoagronomic variables of interest.

Based on the results of this study, it is expected that from the crossing between one of the three more divergent accessions with any other accession belonging to the other groups, which presents variables of agronomic importance, may contribute to improvement works and, therefore, increase papaya yield.

Conclusions

The variables plant height, first fruit insertion height, greatest thickness of fruit pulp, fruit diameter, and fruit length had high heritability indicating expressive gains in the simple process of selection.

There is genetic variability among the accessions of *Carica papaya* L., and the accessions Americano, short-peduncle-STZ-03 and Califlora 209 are the most divergent ones. The Tocher's optimization and hierarchical UPGMA methods were partially concordant in the formation of groups of the papaya accessions. The variables fruit mass, fruit diameter and plant height were

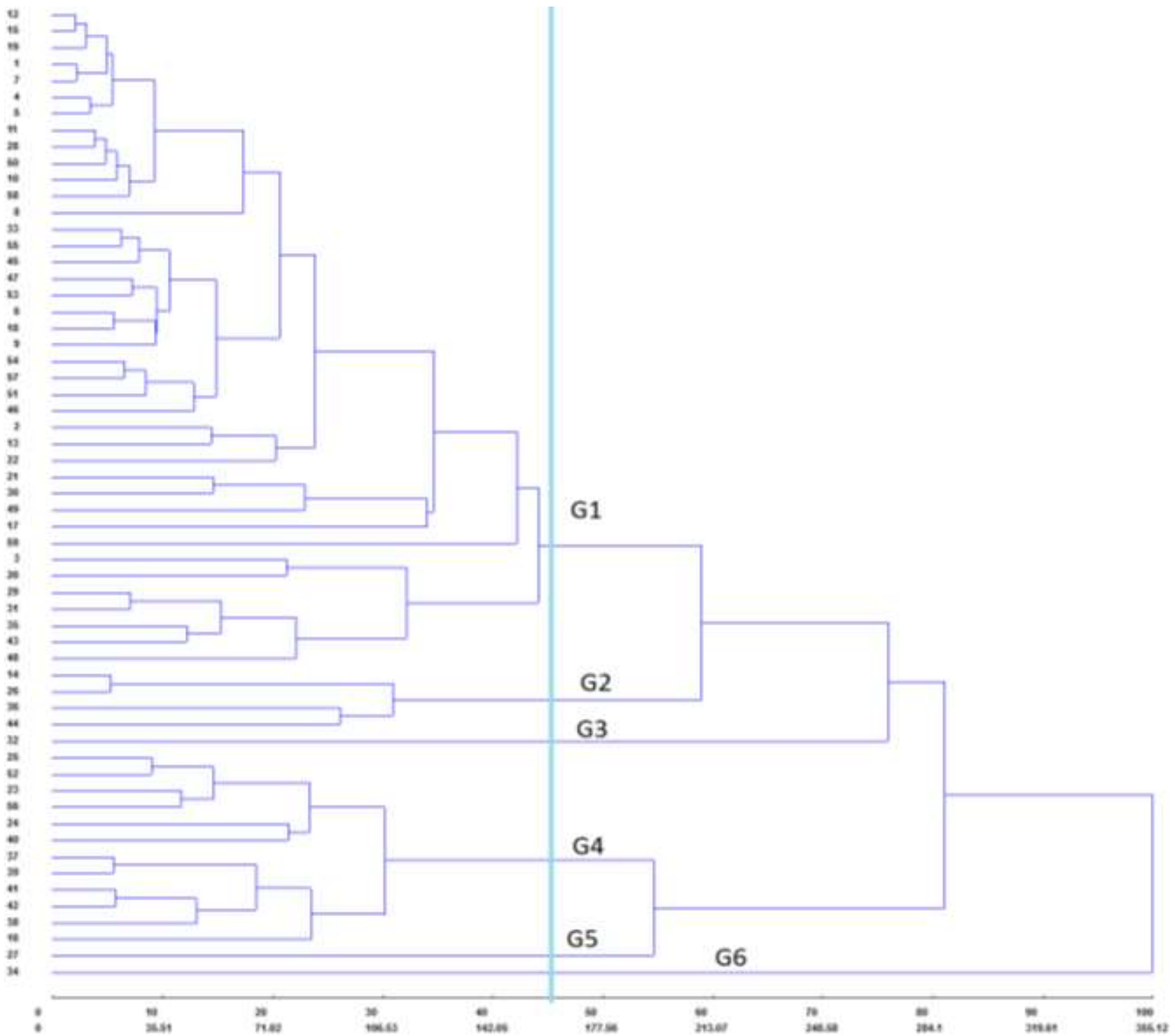

Figure 1. Dendrogram of the analysis of 59 accessions of papaya obtained with the UPGMA method and the Mahalanobis distance as a measure of genetic dissimilarity. The cophenetic correlation coefficient (r) is 0.74** (Acessions: 1 - 'Caliman MS'; 2, 'Sunrise Solo 783'; 3, 'Costa Rica'; 4, 'Taiwan ET'; 5, 'Diva'; 6, 'Grampola'; 7, 'Sunrise Solo'; 8, 'Caliman AM'; 9, 'Caliman GB'; 10, 'Caliman SG'; 11 - 'Caliman G'; 12, 'Sunrise Solo(72/12)'; 13, 'Kapoho Solo polpa amarela'; 14, 'Baixinho de Santa Amália'; 15, 'Surise Solo TJ'; 16, 'Tailândia'; 17 - 'São Mateus'; 18, 'Kapoho Solo polpa vermelha'; 19, 'Sunrise Solo (Progeny Tainung)'; 20, 'Waimalano'; 21, 'Mamão Bené'; 22, 'Mamão Roxo'; 23, 'Maradol (Source Mexico)'; 24, 'Maradol (Grande Limão)'; 25, 'Sekati'; 26, 'Baixinho Super'; 27,'Americano'; 28, 'STZ-51'; 29 - '206/4'; 30, 'Calimosa' F_1; 31, 'JS 12 (206)'; 32, 'Califlora 209'; 33, 'Golden Tipo Formosa'; 34, 'short-peduncle-STZ-03'; 35, 'Santa Helena III Trat 12A PLT.07'; 36, 'Santa Helena III Trat 11A PLT.08'; 37, 'Santa Helena III Trat 50A PLT.09'; 38, 'Santa Helena III Trat 02A PLT.01'; 39, 'Santa Helena III Trat 14A PLT.05'; 40, 'Santa Helena III Trat 15A PLT.04'; 41, 'Santa Helena III Trat 04A PLT.02'; 42, 'Santa Helena III Trat 12A PLT.06'; 43, 'Papaya 42 Formosa'; 44, 'Papaya 45 Formosa Roxo'; 45, 'Papaya 46 Claro'; 46, 'Caliman Fruto Médio Verde'; 47, 'Golden Robusto'; 48, 'JS 11(210)'; 49, 'Tainung 01' F_1; 50, 'STZ 23 Pedúnculo Longo'; 51 - 'STZ 63'; 52, 'Sekati Macuco'; 53, 'Gran Golden'; 54, 'Sunrise Solo Paulo Brunele'; 55, 'THB STZ 39'; 56, 'Incaper 39'; 57, 'B5 Geraldo'; 58, 'Formosa Golden'; 59, 'Formosa Brilhoso').

the major contributors to genetic diversity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

In vitro efficacy of certain botanicals against bacterial soft rot of tomato (*Solanum lycopersicum* L.)

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Tomato (*Solanum lycopersicum* L.) is a species of the family Solanaceae. It is herbaceous, annual to perennial, prostrate and sexually propagated plant with bisexual flower. Tomatoes are attacked by many kinds of plant pathogens such as fungi, bacteria, nematodes, viruses and viroid. Among bacterial diseases, bacterial soft rot devastates many important crops of the family Solanaceae particularly potato, eggplant and tomato, causing a huge decrease in yield and a greater loss in produce than any bacterial disease known. Yield losses due to post-harvest diseases of fruits and vegetables range from 20 to 30% but losses due to soft rot bacteria may reach up to 100% under insufficient conditions of storage facility, this have huge impacts on famers and vendors. *In vitro* efficacy of certain botanicals against bacterial soft rot of tomato were tested in the months of February to March, 2015 in the Department of Plant Pathology and Department of Biochemistry, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Deemed University)– Allahabad, UP, India. Eight botanicals were screened *in vitro,* out of these, four were selected based on their performances and evaluated against the bacterial soft rot of storage tomato at 2, 4, and 8 days after inoculation. Maximum zone of inhibition was obtained with treated Control (T_{0b}=17 mm), followed by Turmeric 30% (T_4=12.4 mm), Turmeric 20% (T_3=11 mm), then Neem 30% (T_6) while the least zone of inhibition was recorded with untreated Control/water (T_{0a}=0.4 mm) followed by Lemon 30% (T_{12}=1 mm). Turmeric 30% (T_4) proved to be best botanical under screening followed by Turmeric 20% (T_3=11mm). In case of mean disease intensity at eight days after inoculation on storage tomato, highest mean value was recorded in Ginger 30% (T_2=46.2) followed by Neem 20% (T_5=44.2) and lowest value in Streptomycin (T_{0b}=27), followed by Turmeric 20% (T_3=27.6) then Turmeric 30% (T_4=27.8). Among the botanicals, the lowest disease intensity was with T_3=27.6 followed by T_4= 27.8.

Key words: Tomato, *Pectobacterium carotovora* subsp c*arotovora*, botanicals, efficacy.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae (Taylor, 1986; Rashid and Singh, 2000). It is herbaceous, annual to perennial, prostrate and sexually propagated plant with bisexual flower. It is typically day

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neutral plant and self-pollinated vegetable crop. Scientific information indicates that the cultivated tomato originated in a wild form in the Peru-Ecuado-Bolivia area of the Andes, that is, South America (Vavilov, 1951 and Rick, 1969).

Tomatoes are attacked by many kinds of plant pathogens such as fungi, insects, nematodes, bacteria, viruses and viroid. Among bacterial diseases of tomato, bacterial soft rot devastates this important crop, causing a huge decrease in yield and a greater loss in produce than any bacterial disease known (Akbar et al., 2014). The disease is associated with infection by *Pectobacterium* species, formally known as *Erwinia* sp. (Czajkowski et al., 2011) such as *Pectobacterium chrysanthemi* (Pc), *Pectobacterium carotovora* subsp. *carotovora* (*P. carotovora subsp. carotovora)*, and *Pectobacterium carotovora* subsp. *atroseptica* (Pca). The latter is also the causal agent of blackleg of potato (Perombelon *et al.,* 1980). *Pectobacterium* species secrete different degenerative enzymes, including pectate lyases, pectin lyases, polygalacturonases, cellulases, proteases and phospholipases which can depolarize the plant cell wall and macerate tuber parenchymatous tissues (Kotoujansky, 1987).

P. carotovora subsp. *carotovora* is economically important because of its ability to cause severe soft rot on tomatoes (Perombelon and Kelman, 1980; Akbar et al., 2014). They cause wilting of whole plant, water soaking areas on stem and fruits, browning of vascular tissue and fruits, discoloration of fruits, hollowing of pith and soft rotting of stem and fruits. *P. carotovora* subsp. *carotovora* infects a much broader host range including many vegetables, for example, potato and tomato (Perombelon and Kelman, 1980; Bell et al., 2004).

In India, *P. carotovora* subsp. *carotovora* is identified as the major soft rot causing bacterium (MCC-Pune, Catalogue 2014**).** Although control of blackleg and bacterial soft rot with antibiotics have showed to be promising, large scale field studies are no longer encouraged because of the risks of introducing resistance to bacterial pathogens of man and animals. Chemical treatment also have the problem of reaching the pathogen which are well protected in vascular system, lenticels etc. and even systemic bactericide failed when applied postharvest, as there is no vascular activity in harvested fruit or tuber (Czajkowski et al., 2011).

The problems caused by synthetic pesticides and their residues have increased the need for the search of effective biodegradable pesticides with greater selectivity (Al-Samarrai et al., 2012; Slusarenko et al., 2008). The alternative strategies are focused on pesticides of plant origin, which are often effective against a limited number of specific target species, are biodegradable into non-toxic products and suitable for use in integrated pest management programs (Al-Samarrai et al., 2012).

Plant products effectively meet this criterion and have enormous potentials to influence modern agrochemical research. The use of botanicals is gaining popularity because they have been found to be non-toxic, more systemic with little mammalian toxicity (Bankole, 1996)**.** It degrades more rapidly than most chemicals pesticides, and therefore are considered to be eco-friendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention.

MATERIALS AND METHODS

A-Source of materials used

The bacterial culture MMC-2112 (T) used in the experiment was procured from Microbial Culture Collection Centre-Pune (ncc, 2015), National Centre for Cell Science, Maharashtra State, India.

B-Preparation of plant extracts (botanicals)

Aqueous extracts of easily available plants in Allahabad such as the ones listed below were prepared according to a method described by Obongoya *et* al. (2010), revised by Paradza et al. (2012) with minor modifications. For the experiment at two concentrations (20% and 30%) for each treatment were as follows**:**

T_1 -Ginger (*Zingiber officinale*) 20%, T_2 -Ginger (*Zingiber officinale*) 30%, T_3-Turmeric (*Curcuma longa*) 20%, T_4-Turmeric (*Curcuma longa*) 30%, T_5-Neem seed (*Azadirachta indica*) 20%, T_6-Neem seed (*Azadirachta indica*) 30%, T_7-Coriander (*Coriandrum sativum* L.) 20%, T_8-Coriander (*Coriandrum sativum* L.) 30%, T_9-Garlic *(Allium sativum* L.*)* 20%, T_{10}-Garlic *(Allium sativum* L.*) 30%,* T_{11}-Lemon peel (*Citrus aurantifolia*) 20%, T_{12}-Lemon peel (*Citrus aurantifolia*) 30%, T_{13}-Black Cumin (*Nigella sativa* L.) 20%, T_{14}-Black Cumin (*Nigella sativa* L.) 30%, T_{15} -Chilli (*Capsicum annuum*) 20%, T_{16} -Chilli (*Capsicum annuum*) 30%, T_{0a}-Sterile distilled water (Untreated Control), T_{0b}-Streptomycin sulphate (Treated control).

The plant materials were first oven dried (except black cumin, turmeric and neem seeds) and grinded into powder, using electric grinder (Mixer Grinder). Dried plant tissues (20 g/100ml and 30g/100ml) were measured and soaked for 24 h in distilled water. Then suspension of each plant extract was filtered using 4 layers muslin cloth, 2 times. Discs of 12.7 mm were soaked in these extracts for 24 h and used as botanical treatment on the bacterial lawns under *in vitro* screening. While for streptomycin sulphate, only 1 g of powder was used in 100 ml sterile distilled water after which discs were soaked and used as earlier described (positive control). In case of sterile distilled water (negative control), discs were just soaked and used as earlier mentioned.

C-*In vitro* screening of botanicals against the *Pectobacterium carotovora* sub sp. *carotovora*

In vitro screening of botanicals and its optimum concentration was carried out using bacterial zone of inhibition and disc diffusion method (Akbar et al., 2014) with little modification. A young culture of the bacterium (*Pectobacterium carotovora* subsp. *carotovora*) 24 to 48 h old was used for the preparation of bacterial lawn. Bacterial culture lawns were prepared by spreading the bacterial culture 10^7cfu/ml on the growth medium using sterile spreader. Each treatment (plant extracts and checks) was replicated five times and this was applied to the two concentrations. Each of the soaked disc was placed at the centre of a bacterial lawn. The inoculated plates were incubated at room temperature for 24 to 72 h, and the procedure earlier mentioned remained the same for all the extracts and checks. After incubation, data were taken as inhibition zones (mm) around the discs as effects of botanicals against the bacterium (bacterial lawn), promising botanicals were chosen for further experiment on storage tomato fruit.

D-Efficacy of selected botanicals against bacterial soft rot of tomato fruits

Method described by Rahman et al. (2012) was followed with little modifications. Based on the encouraging results of the bioassay as inhibition efficiency and efficacy, four botanical extracts with promising results were chosen to evaluate their efficacy against bacterial soft rot of tomato fruits.

The extracts were prepared as described earlier under screening. Each tomato fruit was drilled with 14 mm cork borer, then treated the cut section with the plant extract for 30 min. Inoculum of the soft rot bacterium was prepared at concentration of 10^7cfu/ml and 12.7 mm paper discs soaked for 30 min. The plant extract treated tomatoes were inoculated according to Opara et al. (2013) with some modifications where paper discs soaked in the inoculum suspensions10^7cfu/ml where a disc introduced per fruit's section, and then top tissues were replaced as cap. Inoculated tomatoes were then incubated in polyethene bags at room temperature along with controls. Visual observations were made at 4 and 8 days after inoculation and data on diameter of soft rotting site (mm) due to treatments were evaluated.

The number of fruits showing symptoms of the diseases in each treatment was counted and the percentage of disease incidence was computed using the following formula:

$$\textit{Disease incidences}\ (\%) = \frac{\textit{NUmber of Infected fruits}}{\textit{Total Number of fruits}} \times 100$$

Disease intensity assessment was carried out using a scale of 1 to 3 (Subrahmanyam et al., 1995; Saleem et al., 2011). Three fruits were selected at random, observed and scored. Based on the extent of observed disease damage on each, a scale number was assigned as follows:

1. "o" no visible symptoms (protected)
2. "1" a few minute lesions, approximately 10% of the total fruit surface (TFA) is rotted (moderately protected)
3. "2" approximately 50% TFA is rotted (unprotected)
4. "3" most fruits surface display symptoms, at least 75% of the TFA is rotted (severely unprotected).

Disease intensity was calculated as per cent using the following formula:

$$DI= \frac{\Sigma(No.of\ plts\ with\ rating\ X\ rating\ score)}{3\ X\ Total\ no.\ of\ plts} X100$$

Where;

Σ = Summation symbol
DI = Disease intensity
3 = Highest disease rating score
Plts = plants/fruits
No. = Number/sample size

E-Statistical analysis

The experiment was laid out in completely randomised design (CRD) and WASP-SOFTWARE of Web Agri. Stat. Package from ICAR Research Complex for Goa, India was used to analyse the data.

RESULTS

A-*In vitro* screening of botanicals against *Pectobacterium carotovora* sub sp. *carotovora*

In the *in vitro* screening of botanicals using disc zone of inhibition, it was observed that growth of *P. carotovora* sub sp. *carotovora* was inhibited by most of the tested botanicals when compared to untreated control. Maximum zone of inhibition was obtained with treated control (T_{0b}=17 mm), followed by turmeric 30% (T_4=12.4 mm), then turmeric 20% (T_3=11 mm), while the least zone of inhibition was recorded with untreated control/water (T_{0a}=0.4 mm) followed by lemon 30% (T_{12}=1 mm) (Table 1 and Figure 1).

B- Efficacy of selected botanicals against bacterial soft rot of tomato during storage

Four botanicals were selected based on their performance as inhibition efficiency and efficacy under screening and evaluated against the bacterial soft rot of tomato under storage condition. Disease incidence/infection (%) was determined according to Rahman et al. (2012).

Among the botanicals evaluated turmeric 20% (T_3), turmeric 30% (T_4), neem 30% (T_6), coriander 20% (T_7), and coriander 30% (T_8) had the lowest disease incidence of 40% at two days after inoculation compared to rest of botanicals. The least among all the treatments was found to be treated control (T_{0b}) with 0% (Table 2).

But incidences of the disease were observed in all the eighteen treatments (that is, including both controls) at eight days after inoculation where incidence of the soft rot disease was found to be 100% in all the treated fruits plus untreated control while in treated control it was found to be only 40% as depicted in Table 2.

Disease intensity was measured according to Nauvov (1924), Ahmed (1976) and Pangtey (1979). The highest disease intensity at four days after inoculation was recorded in coriander 30% (T_8=33.2) which means the disease aggravates early there while lowest appeared in Turmeric 20% (T_3=20.2) which is next to treated control/streptomycin (T_{0b}=19.8), (Table 3).

DISCUSSION

In vitro screening of botanicals against *Pectobacterium carotovora* sub sp. *carotovora*

Turmeric 30% (T_4=12.4 mm) was found to effectively

Table 1. *In vitro* screening of botanicals against *Pectobacterium carotovora* subsp. *Carotovora.*

Symbol	Treatments (%)	Mean bactrl. inhibition
T_{1}	Ginger 20	8.2^{cdef}
T_{2}	Ginger 30	7.8^{def}
T_{3}	Turmeric 20	11.0^{bc}
T_{4}	Turmeric 30	12.4^{b}
T_{5}	Neem 20	8.2^{cdef}
T_{6}	Neem 30	10.4^{bcd}
T_{7}	Coriander 20	7.4^{efg}
T_{8}	Coriander 30	8.6^{cde}
T_{9}	Garlic 20	4.6^{ghi}
T_{10}	Garlic 30	5.4^{fgh}
T_{11}	Lemon 20	1.8^{ijk}
T_{12}	Lemon 30	1.0^{jk}
T_{13}	Black cumin 20	2.8^{hijk}
T_{14}	Black cumin 30	2.4^{ijk}
T_{15}	Chilli 20	3.4^{hij}
T_{16}	Chilli 30	4.4^{hi}
T_{0a}	Sterile distilled Water	0.40^{k}
T_{0b}	Streptomycin sulphate	17.0^{a}

Means with same letter(s) in a column are statistically similar at 5% level of probability (CD (0.01) = 3.740, CD (0.05) = 2.815).

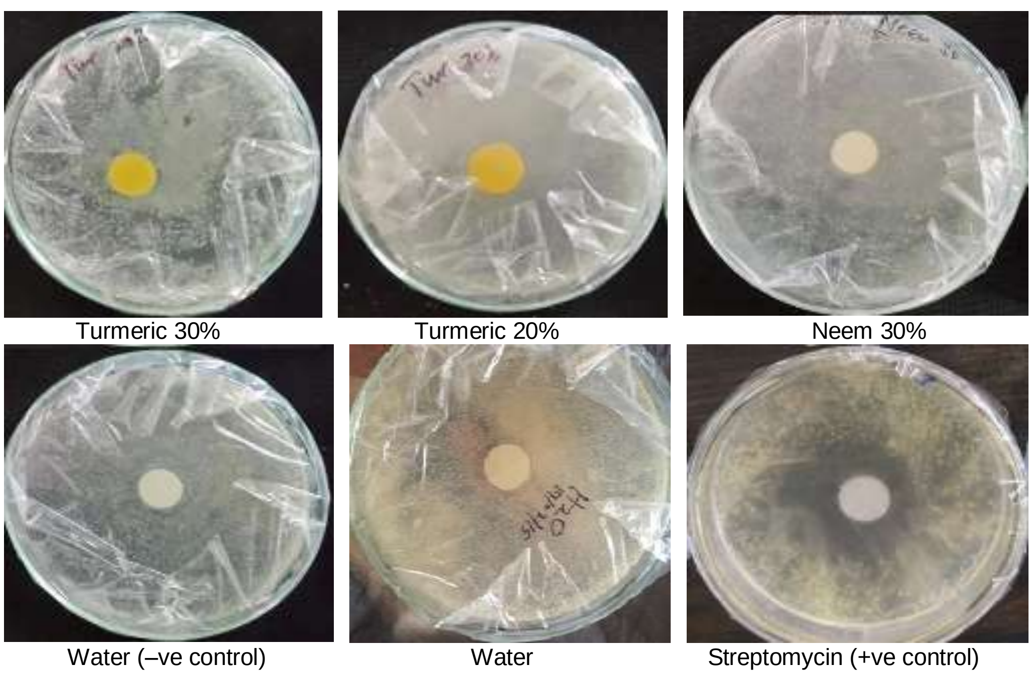

Figure 1. Bacterial inhibition zones as affected by different treatments under screening.

inhibit the bacterial growth as its second to treated control (T_{0b}=17 mm), and this value agrees with the findings of Akbar et al. (2014), who reported maximum zone of bacterial growth inhibition by turmeric (13.33 mm). Apisariyakul (1995) reported turmeric as potential antimicrobial, antioxidant, antiprotozoal and anti-allergic.

Table 2. Per cent disease incidence (soft rot of tomato) as affected by different treatments after two days and eight days of inoculation.

Treatments (%)	Disease Incidence after 2 days of inoculation (%)	Disease Incidence after 8 days of inoculation (%)
T_1 (Ginger 20)	60	100
T_2 (Ginger 30)	60	100
T_3 (Turmeric 20)	40	100
T_4 (Turmeric 30)	40	100
T_5 (Neem Seed 20)	80	100
T_6 (Neem Seed 30)	40	100
T_7 (Coriander 20)	40	100
T_8 (Coriander 30)	40	100
T_{0a}(Untreated Control)	100	100
T_{0b} (Treated control)	00	40

The active ingredient(s) in turmeric needs to be elucidated. The effect of neem (*Azadirachta indica)* as observed is in agreement with the findings of Opara et al. (2013) and Bhardwaj and Laura (2008), but not in accordance with Paradza et al. (2012).

The microbial activity shown by turmeric may be due to the action of its volatile oil constituent curcumin which has enolizable β-diketo group as chelating ligand (Rachana and Venugopalan, 2014) while Slusarenko et al. (2008) reported neem to have active substance Azadirachtin which is under subclass of compound limonoids, class triterpenes and is active against a wide range of microbes and/or pests with up to 90% efficacy in most cases (Akbar et al., 2014; Koul and Walia, 2009). Neem is reported to have fungicidal activity (Bankole, 1996; Govindachari et al., 1998) and bactericidal activity (Mahfuzul-Haque et al., 2007), while ginger (*Zingiber officinale*) was reported to have bactericidal effect on *Erwinia* sp due to its volatile essential oil (Opara et al., 2013).

Efficacy of selected botanicals against bacterial soft rot of tomato during storage

T_{0b} been synthetic antibiotic appeared to be highest as control agent, while the highest incidence was recorded with untreated control (T_{0a} =100%) (Table 2). Disease incidence was common throughout the treatments at 8 days after inoculation; this could be due to development of resistance in some of the inoculum or other microbial complex development (Nauvov, 1924; Pangtey, 1979). It might as well be due to resurfacing and proliferation of the resistant colonies of the pathogen after long period of inhibition. The slight difference in between this work mean values and the previous researchers' own might be due to difference in the inoculum concentration or atmospheric condition.

The antimicrobial activity shown by turmeric may be due to its chelating action as earlier stated (Rachana and Venugopalan, 2014) but coriander also proved to have antimicrobial activity which may be attributed to its essential oil, known to exhibited bactericidal activity against most gram negative and gram positive bacteria (Silva et al., 2011). Its mode of action is reported to be by membrane damage (Silva et al., 2011).

The highest intensity after four days of inoculation was recorded in coriander 30% which might have been due to effect of concentration which might possibly facilitated disease process, since concentrations were significant (Table 3 and Figure 2), while disease intensity was found to sharply increase at eight days after inoculation, with highest mean value recorded in ginger 30% (T_2=46.2) and lowest value with streptomycin(T_{0b}=27), followed by turmeric 20% (T_3=27.6) then turmeric 30% (T_4=27.8). Among the botanicals, the lowest disease intensity was with T_3=27.6, T_4=27.8, T_1=32.0 and T_6= 38.6 which did well when compared to both treated and untreated controls as they fall in between and more closer to the treated control (Table 3). This also agrees with the study of Akbar et al. (2014) findings.

Conclusion

Eight botanicals *viz: Zingiber officinale*, *Curcuma longa*, *Azadirachta indica*, *Coriandrum sativum* L., *Allium sativum* L., *Citrus aurantifolia*, *Nigella sativa* L. and *Capsicum annuum* each at two concentrations were screened *in vitro* along with treated (streptomycin sulphate) and untreated (sterile distilled water) controls, using disc inhibition zone. Out of these eight botanicals, four were selected based on their performance under the screening and used for evaluation of their efficacy on bacterial soft rot of storage tomato along with same controls. Significant results were obtained when eight botanicals were screened and the chosen four against the bacterial soft rot. In the present study, turmeric 30%

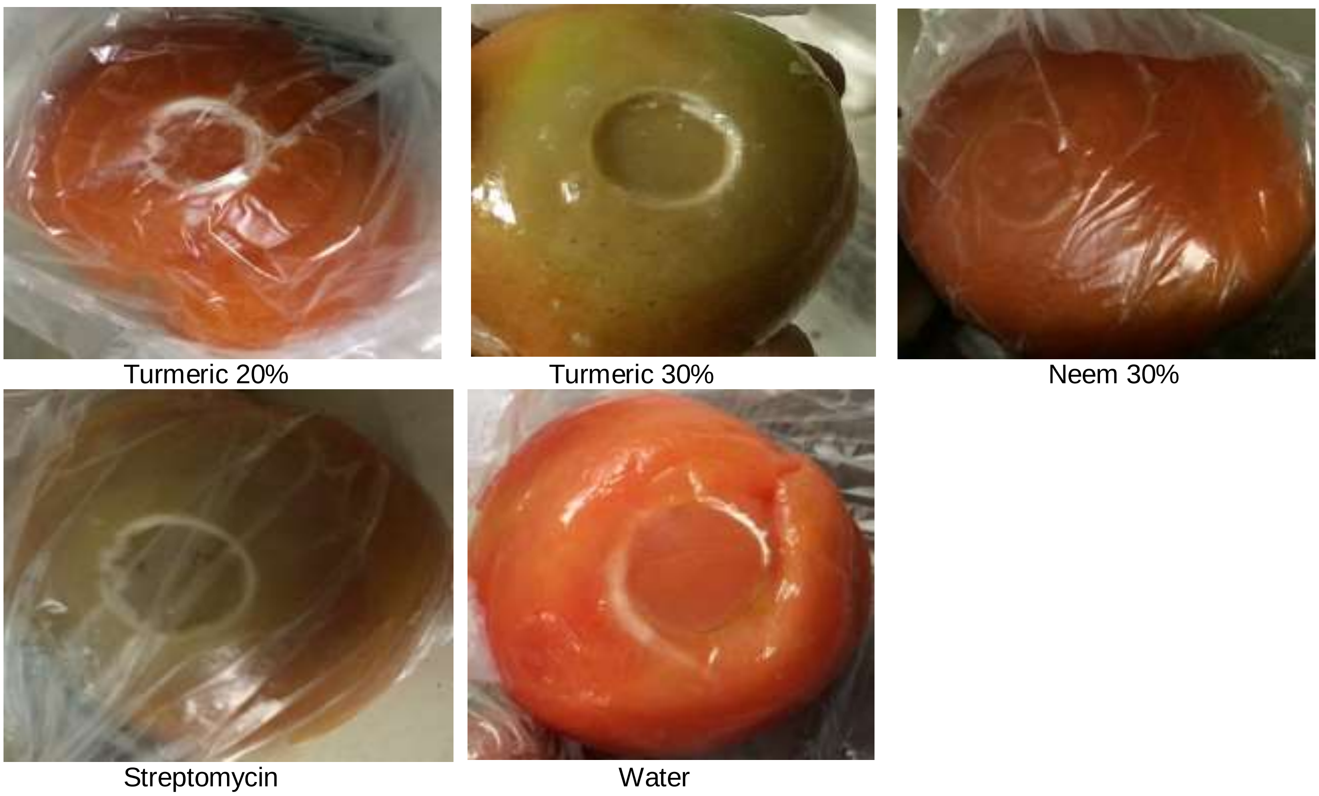

Figure 2. Disease intensity at eight days after inoculation.

Table 3. Percent disease intensity (soft rot of tomato) as affected by different treatments after four and eight days of inoculation.

S/N	Treatments (%)	Mean % disease intensity after 4 days of inoculation	Mean % disease intensity after 8 days of inoculation
T_1	Ginger 20	24.2^{bc}	32.0^{bc}
T_2	Ginger 30	23.2^{bc}	46.2^{a}
T_3	Turmeric 20	20.2^{c}	27.6^{bc}
T_4	Turmeric 30	21.2^{c}	27.8^{c}
T_5	Neem 20	28.0^{ab}	44.2^{ab}
T_6	Neem 30	31.6^{a}	38.6^{abc}
T_7	Coriander 20	32.8^{a}	41.0^{abc}
T_8	Coriander 30	33.2^{a}	43.6^{ab}
T_{0a}	Untreated control	32.8^{a}	42.2^{ab}
T_{0b}	Treated control	19.8^{c}	27.0^{c}

Means with same letter(s) in a column are statistically similar at 5% level of probability (CD (0.01) = 3.740, CD (0.05) = 2.815).

(T_4) proved to have highest potential to be used for the management of soft rot of tomato (*Pectobacterium carotovora* subsp. *carotovora*) disease compared to the rest botanicals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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