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Pandey R, Kalra A (2003). Root knot disease of ashwagandha Withania somnifera and its ecofriendly cost effective management. J. Mycol. Pl. Pathol. 33(2): 240-245.

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Full Length Research Paper

Effect of protected structures on yield and horticultural traits of bell pepper (*Capsicum annuum* L.) in Indian cold arids

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Under cold arid Ladakh condition, production of bell pepper is limited by climate and short growing season and people depend upon outside supply for consumption. Keeping this in view, seven promising hybrids popular in other parts of country were tried in six different protected structures. Design of the experiment was split plot. Results revealed that Mud wall greenhouse recorded highest yield per ha and number of fruits but par with Chinese and LEHO greenhouses. Bell pepper hybrid US-181 was highest yielder. Mud wall greenhouse in combination with hybrid Bharath recorded statistically highest yield per ha. Mud wall greenhouse and LEHO greenhouse may be recommended for small and marginal farmers while Chinese being costly for commercial bell pepper production to bring more economic prosperity.

Key words: Bell pepper, *Capsicum annuum*, cold arids, protected structure, yield.

INTRODUCTION

Bell pepper is one of the highly remunerative vegetables cultivated in most parts of the world. The bell pepper in India is grouped under non-traditional category of vegetables (Kalloo and Pandey, 2002). Nutritionally, bell peppers are rich in vitamins particularly vitamin A (180 IU) and vitamin C. Hundred gram of edible portion of bell pepper provides 24 Kcal of energy, 1.3 g of protein, 4.3 g of carbohydrate and 0.3 g of fat (Yellavva, 2008).

Due to successful protected cultivation of bell pepper, it is available throughout the year in India. Bell pepper is one of the emerging and remunerative crop vegetable in Ladakh region, the cold arid region of India. Under Ladakh condition, production of bell pepper is limited by climate and short growing season and people depend upon outside supply for consumption. Even during summers in most part of the region, bell pepper cultivation is not successful. Till few years back, bell pepper were exclusively imported from Indian plains during summer. Due to erratic behaviour of weather, the crops grown in open field are often exposed to fluctuating levels of temperature, humidity, wind flow etc. which ultimately affect the crop productivity adversely (Ochigbo and Harris, 1989). The concept of protected cultivation of bell pepper has slowly been mingling with the changing

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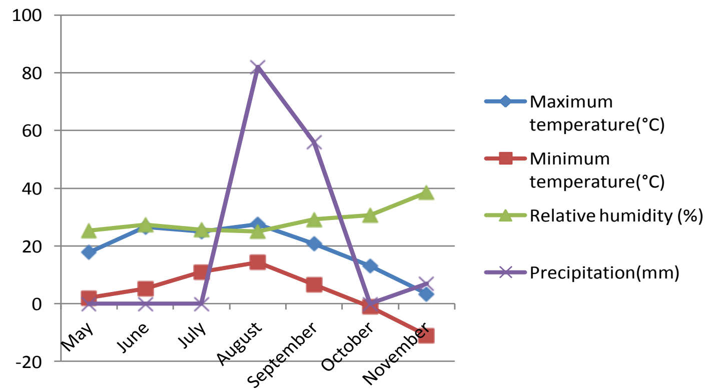

Figure 1. Weather parameters of experimental area.

trends in vegetable production owing to the increasing demand in variety, taste and quantity by Indian army, tourist, floating population as well as the local themselves. So, it becomes imperative to grow bell pepper under protected cultivations usually in green houses in these dry cold arid regions and its exact potential can well be exploited using greenhouse technology.

Bell pepper cultivation under protected structures in Ladakh has ample scope and is one of the most remunerative enterprises that would bring huge socioeconomic benefits to the farmers in specific and the region in general. Moreover, bell peppers are new to the region and they seem to find their way into the dietary habit of Ladakhi people. Information related to bell pepper production technology is scanty with respect to this region. Keeping this in view, promising hybrids from other parts of country were tried in various protected structures with the objectives of identification of hybrid for protected cultivation with suitable greenhouse for bell pepper cultivation.

MATERIALS AND METHODS

The present study was conducted under protected conditions at Experimental Farm, Stakna (Leh) of Precision Farming Development Centre which is situated at 3319 m amsl latitude 33°58.551' NS and longitude 77°41.995' EW. Climate of the area is typically dry temperate with extreme fluctuations in the temperature. Weather parameters during experiment period are represented in Figure 1, thereby showing necessity of growing bell peppers in greenhouses in cold arids. Performance of seven genotypes including Bharath as check was evaluated in six protected structures including Trench as check. Brief description of all the protected structures is given in Table A. Design of the experiment was split plot and material was replicated 3 times. Crop geometry was 45 × 30 cm.

Transplanting was done on 25 May, 2010 in all the protected structures. Standard package of practices followed to raise healthy crop. Data were recorded on 4 randomly taken plants/fruits per replication with respect to yield and horticultural characters and subjected to statistical analysis as per Snedecor and Cochran (1967). Main effects of protected structures as well as genotypes along with their interaction were studied to find out promising greenhouse and genotype for cold arid regions.

RESULTS AND DISCUSSION

Analysis of variance indicated that the greenhouses differed significantly for all the characters under study. Hybrids also exhibited significant differences for most of the characters except yield per plant, yield per hectare and days to harvest. However, greenhouses x hybrids could show significant effects for plant height, number of fruits per plant, fruit length and fruit weight only. Greenhouses can boost bell pepper production in the cold arids. Singh and Asrey (2005) found excellent quality and productivity of *Capsicum* in medium cost greenhouse. Results are appended and discussed under the following headings.

Plant characters

LEHO greenhouse recorded maximum plant height (127.4 cm) at par with Chinese greenhouse followed by Mud Wall greenhouse (Table 1). Stem diameter was at par in LEHO, Chinese and Mud Wall greenhouses (Table 1). Naturally ventilated greenhouses exhibited minimum plant height and stem diameter. Bell pepper hybrid SH-1 produced maximum plant height which was at par with SH-2. Maximum stem diameter is also shown by SH-1 which was at par with US-181. LEHO greenhouse x SH-1 recorded statistically highest plant height over all the other treatment combinations except Chinese greenhouse x SH-1.Perusal of the data in Table 2 indicated that highest numbers of fruits per plant was produced in Mud Wall greenhouse at par with Chinese greenhouse. Similarly, yield per plant was highest (580.35 g) in Mud wall greenhouse which was at par with Chinese and LEHO greenhouses. Numbers of fruits per plant were at par in Bharath, US-181, SH-1, SH-2; being

Table 1. Performance of capsicum hybrids under different polyhouses for plant characters.

Protected structure	Plant height (cm)								Stem diameter (mm)							
	Hybrids								Hybrids							
	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean
Chinese	119.5	99.58	139	136.4	98.5	126.7	108.1	118.2	13.97	15.48	15.26	13.59	13.47	13.78	14.44	14.28
Mud Wall	95.67	94.86	97.29	109.9	99	95.88	82.96	96.5	13.55	14.39	13.64	12.93	13.57	12.05	12.96	13.3
LEHO	122.7	122	154.2	109.2	117.5	123.2	127.4	125.2	14.86	14.29	16.65	12.62	12.81	14.36	14.37	14.28
Local	50.79	67.13	75.29	76.25	37.96	49.84	56.33	59.09	8.4	10.21	10.18	9.46	6.99	8.09	10.22	9.08
Trench	26.33	34.22	42.67	33.78	29.78	29.61	23.11	31.39	8.27	10.48	12.24	11.09	11.42	9.32	9.3	10.3
NV	25.04	26.92	31.33	31.83	26.58	30.92	25	28.23	8.09	9.83	8.27	7.86	10.13	8.62	7.67	8.64
Mean	73.35	74.12	89.96	82.89	68.22	76.02	70.48		11.19	12.45	12.7	11.26	11.4	11.04	11.49	
CD-$P_{0.05}$					13.99								1.62			
CD-$V_{0.05}$					7.76								0.94			
CD-$PV_{0.05}$					22.44								NS			

P: Protected structure; V: Variety; PV: Protected structure x Variety.

Table 2. Performance of capsicum hybrids under different polyhouses for plant characters.

Protected structure	Number of fruits per plant								Yield per plant (g)							
	Hybrids								Hybrids							
	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean
Chinese	8.67	7.08	9.00	5.5	6.92	7.00	5.08	7.04	463.1	430.0	494.5	271.7	381.9	498.7	416.9	422.4
Mud Wall	10.67	8.42	10.50	10.33	7.92	8.58	5.25	8.81	700.5	572.5	558.2	506.3	612.1	573.9	538.9	580.3
LEHO	8.42	4.75	6.97	7.42	5.17	5.92	6.00	6.38	489.7	298.8	358.7	295.0	336.3	449.1	541.6	395.6
Local	3.58	8.33	9.33	6.25	2.58	5.92	4.50	5.78	176.9	325.0	330.7	141.9	79.78	188.2	173.6	202.3
Trench	4.61	7.89	6.44	4.17	4.08	4.50	3.83	5.08	166.1	396.8	314.3	128.7	192.2	156.1	189.7	220.6
NV	3.25	2.83	2.75	4.25	2.58	2.75	2.53	2.99	209.4	299.1	121.7	210.0	265.9	204.7	182.2	213.3
Mean	6.53	6.55	7.50	6.32	4.87	5.78	4.53		367.6	387.0	363.0	258.9	311.4	345.1	340.5	339.1
CD-$P_{0.05}$					2.50								239.83			
CD-$V_{0.05}$					1.35								NS			
CD-$PV_{0.05}$					3.95								NS			

P: Protected structure; V: Variety; PV: Protected structure x Variety.

Table 3. Performance of capsicum hybrids under different polyhouses for fruit characters.

Protected structure	Fruit length (mm)								Fruit diameter (mm)							
	Hybrids								Hybrids							
	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean
Chinese	86.93	92.95	93.93	140.60	91.64	92.39	81.11	97.07	70.37	67.99	56.41	51.21	69.57	71.63	76.77	66.28
Mud Wall	86.48	86.23	88.42	141.20	84.69	85.00	76.88	92.71	72.22	77.64	65.19	44.50	77.48	71.81	79.32	69.74
Leho	84.77	91.80	90.16	129.50	86.46	89.10	77.36	92.74	71.79	72.66	64.49	51.41	70.77	75.14	76.54	68.97
Local	73.52	74.71	85.49	112.80	58.94	57.36	69.84	76.10	63.73	65.33	57.21	41.76	61.98	55.54	67.25	58.97
Trench	73.24	76.90	91.98	95.65	72.26	58.56	68.28	76.70	68.71	67.74	58.30	38.83	66.90	61.11	72.63	62.03
NV	80.27	83.16	77.47	120.50	82.18	74.50	72.39	84.35	64.60	68.56	55.73	45.13	69.46	69.81	68.92	63.17
Mean	80.87	84.29	87.91	123.40	79.39	76.15	74.31		68.57	69.99	59.56	45.47	69.36	67.50	73.57	
CD-$P_{0.05}$	11.26								3.63							
CD-$V_{0.05}$	4.52								2.91							
CD-$PV_{0.05}$	15.20								NS							

P: Protected structure; V: Variety; PV: Protected structure x Variety.

highest in SH-1. Mud Wall x Bharath produced highest number of fruits per plant closely followed by SH-1 and SH-2 in the same greenhouse. Hybrids as well as Greenhouse x Hybrid showed non-significant differences for yield per plant. Moreover, top hybrids with respect to yield were Bharath, US-181 and Spinx (Kanwar and Sharma, 2010) in cold arids. Zende (2008) recorded 23.44 fruits per plant under polyhouses and Yellavva (2008) observed significantly higher fruits per plant (11.66) in NV polyhouse. Variations in fruit number may be due to the differences in greenhouse types, location and season.

Fruit characters

Data presented in Table 3 showed that Chinese greenhouse produced longest fruit at par with LEHO and Mud Wall greenhouses. However, fruit diameter was maximum in Mud Wall greenhouse which was at par with fruit diameter in LEHO and Chinese greenhouses. Hybrid SH-2 exhibited statistically longest fruits. This is the varietal character of the SH-2 hybrid. Fruit diameter of Spinx was statistically highest among all the hybrids in consonance with the results of Kanwar and Sharma (2010). Mud Wall x SH-2, Chinese x SH-2 and LEHO x SH-2 produced fruit length at par with each other and recorded highest fruit length than other treatment combinations. Bell peppers were grown in greenhouse to produce good quality fruits during an extended growth (Jovicich et al., 2004). Zende (2008) observed fruit length and breadth to the tune 8.50 and 8.15 cm, respectively under polyhouse conditions.

Yield characters

Data on fruit weight (Table 4) revealed that Mud Wall greenhouse recorded statistically highest fruit weight. Fruits of hybrid Spinx were statistically heaviest. Mud Wall x Spinx also produced highest fruit weight at par with Mud Wall x US-181, Chinese x Spinx, LEHO x Spinx, Mud Wall x Super Gold and LEHO x Navratna. Yield per hectare was at par in Mud Wall (429.9 q), Chinese and LEHO, being maximum in Mud Wall greenhouse. Pandey et al. (2005) could get 2.50 kg/m^2 in *Capsicum* under glass house conditions. Brar et al. (2005) reported highest yield of 242.0 q per ha in *Capsicum* var. Bombay under polyhouse conditions in Pune region. Hybrids as well as interaction effects showed non-significant differences for yield per hectare. Kanwar and Sharma (2010) also obtained heaviest fruits in Spinx under NV greenhouse in similar climate. Zende (2008) recorded significantly higher fruit weight (147.74 g) and fruit yield (64.91 t/ha) under polyhouse. Moreover, Yellavva (2008) also got significantly higher fruit weight (160 g) and fruit yield (72.52 t/ha) under naturally ventilated poly

Table 4. Performance of capsicum hybrids under different polyhouses for yield characters.

Protected structure	Fruit weight (g)								Yield per ha (Q)							
	Hybrids								Hybrids							
	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean
Chinese	128.2	117.4	83.71	76.05	125.9	110.3	152.1	113.4	343.0	318.5	366.3	201.3	282.9	369.4	308.8	312.9
Mud Wall	131.8	152.3	105.8	97.96	142.5	123.1	165.9	131.3	518.9	424.0	413.5	375.0	453.4	425.1	399.2	429.9
Leho	124.2	127.0	95.59	74.42	130.7	140.9	146.8	119.9	362.7	221.3	265.7	218.5	249.1	332.7	401.2	293.0
Local	76.22	87.84	80.33	46.88	44.54	44.68	86.75	66.75	131.0	240.7	245.0	105.1	59.09	139.4	128.6	149.8
Trench	118.5	94.83	80.92	43.83	92.08	67.83	104.1	86.01	123.0	294.0	232.8	95.31	142.3	115.6	140.5	163.4
NV	103.3	123.3	78.50	64.58	132.3	113.3	111.8	103.9	155.1	221.5	90.16	155.6	197.0	151.6	135.0	158.0
Mean	113.7	117.1	87.47	67.29	111.3	100.0	127.9	103.6	272.3	286.7	268.9	191.8	230.6	255.6	252.2	
$CD\text{-}P_{0.05}$	8.75								177.65							
$CD\text{-}V_{0.05}$	8.93								NS							
$CD\text{-}PV_{0.05}$	28.21								NS							

P: Protected structure; V: Variety; PV: Protected structure x Variety.

Table 5. Performance of capsicum hybrids under different polyhouses for maturity.

Protected structure	Days to first harvest								Harvest duration (days)							
	Hybrids								Hybrids							
	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean	Bharath	US-181	SH-1	SH-2	Super Gold	Navratna	Spinx	Mean
Chinese	135.6	139.2	120.4	130.5	133.0	126.1	128.1	130.4	33.14	31.06	46.56	30.08	36.28	38.94	36.50	36.08
Mud Wall	121.4	124.6	123.7	117.3	123.4	128.7	122.4	123.1	38.83	37.11	36.39	42.45	30.39	24.95	22.99	33.30
Leho	123.0	121.4	126.4	129.9	139.4	126.6	122.3	127.0	34.91	22.78	31.94	29.05	17.09	31.50	31.97	28.46
Local	133.3	136.0	129.6	133.8	145.9	145.6	133.4	136.8	14.00	14.94	21.09	16.98	5.33	5.64	15.83	13.40
Trench	144.0	128.8	124.1	142.1	139.3	123.0	134.3	133.7	3.83	12.11	14.83	5.78	8.00	15.33	7.00	9.56
NV	112.4	101.3	110.8	103.4	111.7	115.7	108.6	109.2	33.75	37.67	34.92	34.67	25.17	14.08	31.75	30.29
Mean	128.3	125.2	122.5	126.2	132.1	127.6	124.9		26.41	25.94	30.95	26.50	20.38	21.74	24.34	
$CD\text{-}P_{0.05}$	10.51								14.71							
$CD\text{-}V_{0.05}$	NS								6.32							
$CD\text{-}PV_{0.05}$	NS								NS							

P: Protected structure; V: Variety; PV: Protected structure x Variety.

house than all other structures. Sharma et al. (2009) also recorded superior fruit yield in *Capsicum* in polytunnel.

Maturity characters

Naturally ventilated greenhouse was statistically earliest for first harvest (Table 5). Hybrids did not differ significantly for days to harvest (Kanwar and Sharma, 2010). Maximum harvest duration was recorded in Chinese greenhouse which was found at par with Mud Wall, Naturally Ventilated and LEHO greenhouses. SH-1 recorded harvest duration at par with SH-2, Bharath and US-181 Hybrids.

It may be deduced that Mud Wall greenhouse recorded highest yield per ha and number of fruits but par with Chinese and LEHO greenhouses. Mud wall greenhouse and LEHO greenhouse may be recommended for small and marginal farmers while Chinese being costly for commercial bell pepper production to bring more economic prosperity. Trenches did not prove to be a good structure for capsicum cultivation owing to its lesser depth disallowing proper plant growth. Protected structures having thick wall on north side proven beneficial (Appendix Table A). *Capsicum* yield and yield attributes were higher in environmental controlled polyhouse than in NV greenhouse (Dhandare et al., 2007) but operational cost would also be high increasing the cost of cultivation.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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APPENDIX

Table A. A short description of protected structures.

Protected structure	Brief description
Chinese greenhouse	Size: 100 ft. x 22 ft. excluding entry chamber, side walls 4 ft. thick and back wall 3 ft. thick. Made up of mud bricks.
Mud-wall greenhouse	Size: 34 ft x16 ft., side walls 3 ft. thick and back wall 2 ft. thick Made up of mud mortar
LEHO greenhouse	Size: 34 ft x16 ft. and double walled, single wall 1 ft.10 inch thick, insulated with straw in 4 inch gap between the two walls. Made up of mud bricks
Local greenhouse	Size: 34 ft x16 ft., wall 1 ft. thick, Made up mud bricks
Trench	Underground structure with unit size: 10 ft.x 6 ft. x 1.5 ft . Four such units will make a complete trench with path between 4 units.
NV greenhouse	Size: 50 ft. x 20 ft., gothic type, naturally ventilated with two side vents and entry chamber. Covered with 150 gsm rigidex polythene.

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Review

Dairy production and marketing in Uganda: Current status, constraints and way forward

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Dairy production in Uganda is mainly based on low-input traditional pasture production systems. This makes Uganda one of the few countries in the world that are low cost producers of milk. Dairy farming could play a greater role in the economy considering its strong potential to provide rural employment and regular income to the many resource-poor households. However, milk production is still largely subsistence. There is therefore a huge potential to increase dairy production and productivity. The aim of this article was to review the current status of the dairy sector in Uganda, to identify the major constraints to dairy production and productivity and to suggest possible areas of intervention so as to enable Uganda exploit its competitive advantage in dairy production for socio-economic development. The article identified and discussed the following as major constraints to dairy production in the country: breed factors, feed resources, climatic factors, particularly high ambient temperature, socio-cultural factors, and dominant informal sector in milk marketing. Selective crossbreed utilization, feed resource development, specific disease prevention and control strategies, support for pastoral production systems, and establishment and support for dairy co-operative societies were recommended for improvement of dairy production and marketing in the country.

Key words: Dairy production, Uganda, current status, constraints

INTRODUCTION

Uganda's economy is still dominated by agriculture. More than 80% of Uganda's workforce is engaged in agriculture based primarily on smallholder farms that are on average only 2 ha in area (Bahiigwa, 1999; RoU, 2004; FAO, 2010). The share of agriculture in the national gross domestic product (GDP) is about 14.6%. Although the contribution of the livestock sub-sector to the national GDP decreased from 1.5% in 2005 to 1.3% in 2010, the share of livestock in the agricultural GDP increased from 8.4 to 8.9% over the same period (MAAIF, 2010). The dairy industry is estimated to contribute more than 50% of the total output from the livestock sub-sector, making it the second major agricultural activity contributing to the national GDP after cereal products (RoU, 2004; Grimaud et al., 2007a; DDA, 2010; Balikowa, 2011). The livestock sub-sector in Uganda is evolving in response to rapidly increasing demand for livestock products that is largely driven by human population growth, income growth and urbanization (Delgado et al., 1999; Faye and Alary, 2001;

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Thornton, 2010).

Dairy production in Uganda is mainly based on the low-input traditional pasture production system. This makes Uganda to be one of the lowest cost producers of milk globally, an advantage that should be exploited (Hemme, 2007). Dairy farming could play a greater role in the economy, considering its strong potential to provide employment and regular income to the many resource-poor rural households. Uganda is one of the few African countries that has attained self-sufficiency in the production of milk (Hemme, 2007; FAO, 2010; Balikowa, 2011). However, milk production is still largely subsistence with the attendant inefficiencies and quality problems commonly associated with such production systems (Balikowa, 2011). Although total annual national milk production grew from 365 million litres in 1991 to over 1.5 billion in 2008 (Wozemba and Nsanja, 2008; DDA, 2010), the observed growth in milk production has been attributed mainly to growth in the cattle population rather than increased milk productivity per cow. Higher productivity per cow is still hindered by low adoption of improved technologies and management practices (Elepu, 2006). Therefore, there is considerable potential to increase dairy production and productivity in Uganda. The aim of this article is to identify the major constraints to increased dairy production in Uganda and to suggest possible areas of intervention so as to enable the country to exploit its competitive advantage in dairy production for socio-economic development.

CURRENT STATUS OF THE DAIRY SECTOR IN UGANDA

Annual total milk production was about 1.5 billion litres in 2008 (DDA, 2010). However, actual milk output in the country would be more than the recorded 1.5 billion litres if supplies were added from the Karamoja sub-region; the milk production from which is currently excluded from data collection due to hygiene consideration. About 85% of milk in Uganda is produced from indigenous cattle, mainly Ankole (Aliguma and Nyoro, 2004;Wozemba and Nsanja, 2008). The Ankole breed, a genetic intermediate between *Bos indicus* and *Bos taurus* and related to the Sanga cattle (Grimaud et al., 2007a), is not a dairy breed per se. Each Ankole cow produces on average 2 L of milk per day and graze over a wide expanse of land often in search of fresh forage and water. Approximately 70% of total milk production is marketed, with the balance consumed by producing households and their neighbours. The annual consumption of milk per person in Uganda was 54 L in 2009 (DDA, 2010) which is 73% less than the 200 L per capita consumption recommended by the FAO. However, the potential for expansion is high given the natural resources available for dairy production in Uganda. Seventy-five percent of the land (18 million square kilometers) could be used for crops or grazing. Currently only 5 million hectares is used as pasture and grazing land.

Dairy production in Uganda takes place under any of four systems (Wozemba and Nsanja, 2008):

(1) Communal grazing which involves pastoral grazing on communal land owned by clan. Although discouraged, it is still practiced in Northern and Eastern parts of Uganda. It is deeply rooted in the culture of these communities who are either pastoralists or agro-pastoralists historically.
(2) Free range grazing; where cattle are grazed by moving them all over the farm. It is a traditional practice in the extensive grasslands in the Southern part of Uganda. The farmland is often not paddocked, but the boundaries are fenced with local hedgerow plants.
(3) Fenced/paddock grazing which involves grazing cattle in paddocks, with supplemental feeding with feed concentrates, is a common farming practice in areas where the land holdings are fairly small. This type of grazing requires land clearing and improved pasture. It Is largely practiced by farmers of hybrid and cross-breed cattle.
(4) Zero grazing where animals are confined in a small enclosure and fodder, feed concentrates, and water are brought to the animals. According to a study by Mbabazi (2005), at least 20% of low income households in Ankole sub-region in Western Uganda have received a zero grazing cow from either government or from such non-governmental organizations as Send a Cow (UK) and Heifer International.

Uganda is divided into six milk-sheds or dairy regions that are defined by agro-ecological and milk production factors as well as their dairy market situation (Figure 1). Milk producers in the different agroecological areas use different means of dairy herd management, from pastoral and extensive systems to agro-pastoral and agricultural intensive zones (Grimaud et al., 2007a). In the intensive systems, herds often include exotic cows, with a predominance of the Friesian-Holstein breed (Grimaud et al., 2004).

The six dairy regions exhibit significant differences in terms of milk production, cattle numbers, market dynamics, and dairy infrastructure, among other factors (DDA, 2004; Balikowa, 2011). These have largely been influenced by the dominant dairy production systems, with the North and Eastern regions (including Karamoja) lagging behind in terms of milk production. A significant part of the national milk production is provided by the Mbarara area, located in the South-West milk-shed (DDA, 2004).

The infrastructure for rural milk collection is still limited. Where it exists, chilled milk is delivered to processing plants and raw milk markets in insulated milk transport tankers. Uganda has a very limited capacity to process milk into value-added products. There are over twenty

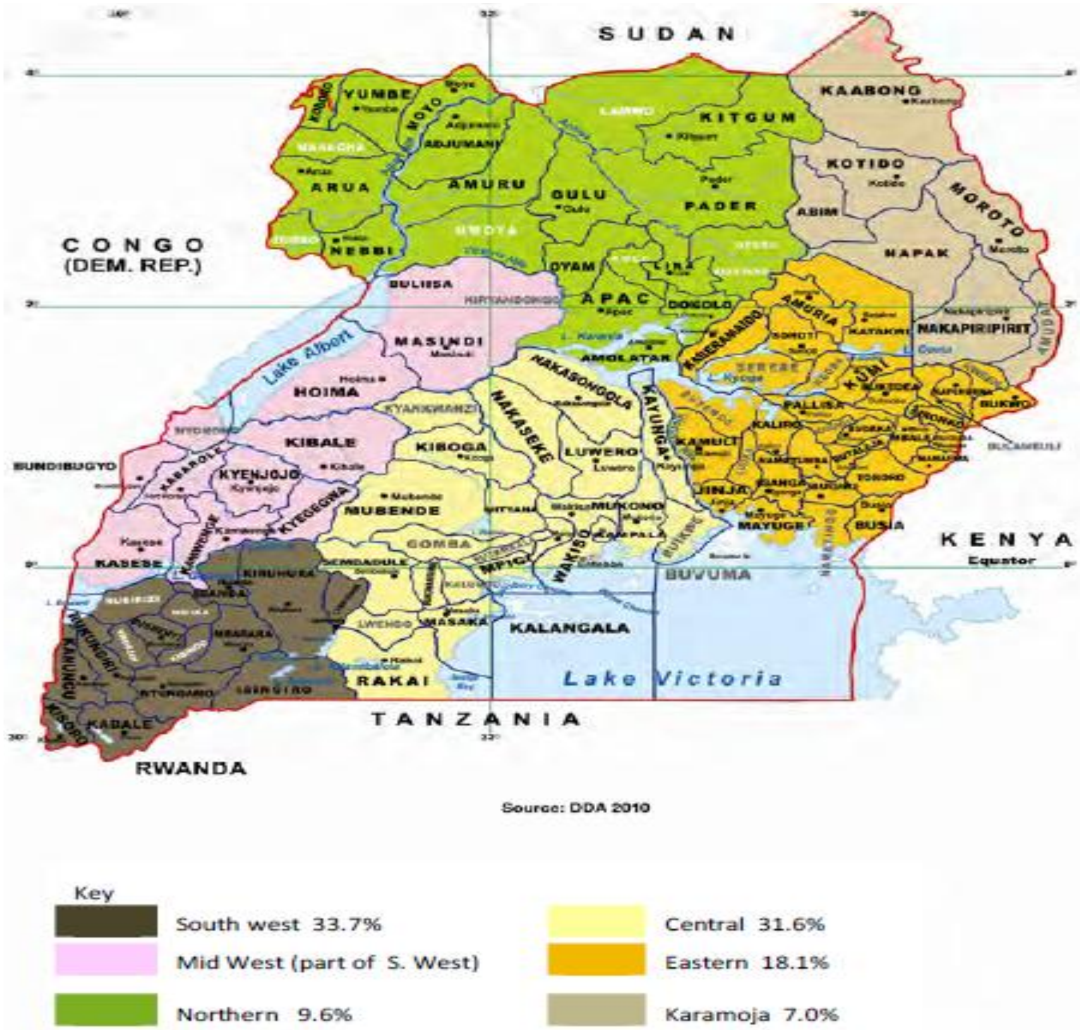

Figure 1. The dairy regions (milk sheds) of Uganda.

dairy processing companies, thirteen of which are milk processing plants and mini dairies. The combined installed capacity for processing plants was 463,200 L per day in 2008 (DDA, 2010). Only 10 to 20% of milk is currently marketed through the regulated formal market. The informal milk market takes up 80 to 90% of the marketable milk produced (Mpairwe, 2005; Grillet et al., 2005; DDA, 2010). The total quantity of milk and milk products imported into Uganda has been declining progressively from about 6,200 metric tons in 2003 to 800 metric tons in 2007. As a result of the decline in the imports, the amount of money spent on importing milk and milk products has experienced a steady decline from about $20 Million in 2001 to $1.6 Million in 2007. Between 2000 and 2008, Uganda exported an average of 380 metric tons of milk per year (DDA, 2010). UHT milk is the main dairy product exported to regional markets, including Rwanda, Kenya, Tanzania, DR Congo, Southern Sudan and Mauritius. Since May 2008 when one Ugandan milk processor began producing milk power, some milk powder has also been exported. Informal dairy trade however goes on across all of Uganda's borders, but the volume traded is generally not significant.

MAJOR CONSTRAINTS TO DAIRY PRODUCTION AND MARKETING IN UGANDA

Breed factors

Cattle are the major source of milk in Uganda. The population of dairy goats and other milk animals (buffalo and camel) is insignificant (Balikowa, 2011). However, most regions in Uganda have cattle breeds that are slow growing and have low feed conversion ratios with very low milk yield and market weights. Currently the average adult live weight in the predominantly Zebu herds of Teso sub-region, for example, is only 180 to 350 kg per head, and this requires about five years to attain even if adequate pasture is available (Jain and Muladno, 2009). Of the 11.4 million cattle in Uganda, the Ankole longhorn (Sanga) breed is the most common, comprising 50% of the population. The small East African Zebu breed follows with 30% of the total population (MAAIF and UBOS, 2009). The Nganda intermediate breed represents 16% of the total population. Exotic breeds and their crosses make up only 4% of the total cattle population. The indigenous breeds generally have limited genetic potential for milk production and remain mediocre producers (500 to 1500 kg per lactation) even when the best possible husbandry conditions are available to them (Pagot, 1992).

Feed resources

While the nutritional needs of dairy animals with respect to energy, protein, minerals and vitamins have long been known and refined over many years, dairy animals are highly sensitive to changes in feeding regimes, and production can fall dramatically with small variations (Thornton, 2010). As understanding of the science of animal nutrition continues to expand and develop, most of the world's livestock, particularly ruminants in pastoral and extensive mixed systems in many developing countries, suffer from permanent or seasonal nutritional stress (Bruinsma, 2003). Natural and planted pastures are the major components in the diet of both indigenous and improved dairy cattle in Uganda. Because Uganda has many agro-ecological zones, the common naturally occurring pasture species vary from one region to another (Grimaud et al., 2007b). In the traditional cattle corridor, common sources of forages include grasses such as *Hyparrhenia rufa*, *Chloris gayana, Brachiaria decumbens, Cynodon dactylon, Themeda triandra*, *Digitaria* spp., *Hyparrhenia filipendula*, *Panicum maximum*, *Paspalum dilatatum* (Balikowa, 2011).

Most of the milk in Uganda is produced by smallholder producers that rely almost entirely on rain-fed natural pastures. However, a severe decline in the quantity and quality of pastures occurs during the dry season. This is often accompanied by widespread invasion of unpalatable grasses (mainly *Cymbopogon afronardus* and *Sporobolus pyramidalis)* as well as bush encroachment, with subsequent overgrazing of the palatable species, mainly *Brachiaria brizantha and Themeda triandra* (Grimaud et al., 2007b; Balikowa, 2011). Only a small number of households keeping improved dairy cattle make the effort to plant improved pastures. Consequently, very few of farms with improved dairy cattle produce enough fodder to meet the needs of their herds throughout the year (Balikowa, 2011). In addition, shortage of grazing land is becoming a serious constraint to dairy farming in most regions of Uganda.

The human population has been increasing rapidly, resulting in increased demand and competition for arable land. Households give priority to production of food crops. Land available for grazing is steadily dwindling in most regions. Extensive grazing of cattle, which has always been the most common management system, and is steadily becoming less popular except in the traditional cattle corridor (Balikowa, 2011). Hence, most animals thrive on sub-optimal energy levels for most of the year. Poor nutrition is one of the major production constraints in smallholder cattle systems of Uganda. Research on improvement of quality and availability of feed resources, including work on sown forages, forage conservation, use of multi-purpose trees, fibrous crop residues and strategic supplementation is available (Thornton, 2010).

Livestock diseases

Efforts to increase milk production in Uganda started in the 1950s with the importation of temperate dairy cattle (*Bos taurus*). However, the susceptibility of improved dairy cattle to local diseases and parasites, particularly tick-borne diseases and trypanosomiasis, and the high management costs remain the biggest impediment to development of commercial dairy farming in Uganda. Improved dairy cattle are still very unpopular among the poor farmers. The total population of improved dairy cattle was estimated at 5.57% of the national herd (MAAIF and UBOS, 2009; Balikowa, 2011). Tick-borne diseases remain a major constraint to the improvement of dairy production in Uganda (Norval et al., 1992; Bell-Sakyil et al., 2004). The cost of controlling ticks and tick-borne diseases is estimated to constitute about 85.6% (pastoral) and 73.8% (ranches) of total disease control costs (Ocaido et al., 2009).

The major tick-borne diseases in Uganda are anaplasmosis, babesiosis, cowdriosis and East Coast fever (ECF). Together, these diseases constitute the most important constraint to livestock production in Uganda (Ekou, 2013). In 1984, the government of Uganda stopped importing and distributing subsidized veterinary drugs and chemicals to farmers. Since then, government only imports certain veterinary products for use in control programs of particular endemic diseases such as Foot and Mouth Disease, Contagious Bovine Pleural Pneumonia, Lumpy Skin Disease, rabies and vectors such as tsetse flies. Instead, from 1994, it has been encouraging veterinarians to leave public service and set up private veterinary practice. To date, Government is still the major provider of animal health services (Wozemba and Nsanja, 2008; Balikowa, 2011). It has been reported, however, that routine strategic vaccinations often are not carried out, targeted vaccinations during outbreaks are delayed, and that in instances where vaccines were availed for targeted vaccinations, the vaccines did not cover all the livestock population in affected areas resulting in livestock becoming susceptible to preventable livestock diseases (RoU, 2009).

Climatic factors

Popularity of the high yielding temperate stock among the local farmers has been curtailed by their inability to withstand the tropical conditions (Balikowa, 2011). Numerous experiments have shown that a prolonged period in which temperatures are more than 25°C, particularly in humid air conditions, leads to a reduction of dry matter intake by milking cows and, as a consequence, a drop in their production. Experiments have shown that a fall in appetite due to heat is the principal factor in the depression of production. High ambient temperatures have another depressive action on milk production by reducing the fertility of the cows, thus lengthening the interval between lactations (Bligh, 1976; Pagot, 1992; Igono and Aliu, 1982). Heat stress significantly impacts animal production and profitability in dairy cattle by lowering feed intake, milk production and reproduction (Chase, 2006). Climate change will likely worsen the situation. It is predicted that climate change will have severely deleterious impacts in many parts of the tropics and subtropics, even for small increases in the average temperature (IPCC, 2007). It will undoubtedly increase livestock production risks as well as reduce the ability of farmers to manage these risks (Thornton, 2010).

Socio-cultural factors

Exploitation of cattle for milk is one of the features of pastoral communities that have lived in symbiosis with their cattle for millennia. Herding people are often nomadic or transhumant and do not practice agriculture. Pastoralism, an economic and social system well adapted to dryland conditions and characterized by a complex set of practices and knowledge has permitted the maintenance of a sustainable equilibrium among pastures, livestock and people for generations (Koocheki and Gliessman, 2005). However, this livestock production system, which does not permit a place for intensive forage production, has limited possibilities for improvement (Pagot, 1992). Notably, milk hygiene practices among herding communities are usually poor. In Uganda, the Karamojong are a classic example. Due to hygienic consideration, milk supplies from the Karamoja sub-region are currently excluded from records on national milk production. As is the case in many countries, raw milk safety is a major public health concern in Uganda. Any improvement in the quality of milk contributes to the insurance of public health while at the same time having positive economic consequences (Grimaud et al., 2007b).

Dominant informal sector in milk marketing

The Dairy Development Authority (DDA), a statutory body under the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), is responsible for regulating and developing the dairy industry. DDA implements this mandate through enforcement of quality standards and implementation of regulations such as registration, inspection of premises and factories and issuing of licenses for milk collection centres, outlets, small-scale processors, importers, factories, coolers and freezers, Storage facility, milk road tankers, and input suppliers. However, Uganda’s dairy industry has too many players along the entire value chain (DDA, 2010). There are millions of small scale dairy farming households, tens of thousands of scattered middlemen trading in milk, and a handful of dairy processors. The informal sector handles about 80% of the traded milk. This informal segment, comprising mostly scattered middlemen and often beyond the reach of DDA operations, is driven by price and not by quality. This poses a big challenge regarding ensuring quality of the milk that reaches the low income segment of the consumers.

STRATEGIES FOR IMPROVING MILK PRODUCTION AND MARKETING

Developments in breeding, nutrition and animal health will continue to contribute to increasing dairy production (Thornton, 2010). Here the following factors are discussed.

Selective crossbreed utilisation

The use of conventional livestock breeding techniques has been largely responsible for the increases in yield of livestock products that have been observed over recent decades (Leakey, 2009). Of the conventional techniques, selection among breeds or crosses with the most appropriate breed or breed cross followed by selection within the resultant population has extensively been exploited to improve livestock production, especially in developed countries (Simm, 1998; Simm et al., 2004). While most of the gains from selective breeding have occurred in developed countries, there are considerable opportunities to do the same in developing countries. Crossbreeding can result in rapid productivity improvements, but new breeds and crosses need to be appropriate for the environment and to fit within production systems that may be characterized by limited resources and other constraints (Simm et al., 2004 Thornton, 2010).

The potential to produce milk in hotter climates, previously not given due attention, can no longer be ignored. Previous studies in the tropics have shown that at various heat intensities above 27°C, half Friesian-Zebu cattle produce more milk when compared to the three-quarter cross during the stage of maximum lactation, despite the higher genetic potential of the latter (Bligh, 1976; Igono and Aliu, 1982). Therefore crossing European breeds, such as the small sized Jersey, with local Zebu cattle that have proven to be more adaptable to local conditions is an appropriate dairy cattle breeding strategy for Uganda. This cross would produce relatively small animals with low feed requirements but high productivity. However, as pointed out by FAO (2007), institutional and policy frameworks that encourage the sustainable use of traditional breeds and *in situ* conservation need to be implemented so as to avoid losing the genetic merit of these local breeds.

Feed resources development

In tropical countries, modern agronomic practices, such as selection of forage species, fertilisation, and irrigation, levels of productivity comparable to the best obtained in temperate countries (Pagot, 1992). In Uganda, a thorough agronomic assessment of grasses and legumes could be conducted in the different agro-ecological zones. Multilocational trials would test various forage legumes, grasses and browses for their adaptability and performance on different soil types and in various environments. These trials would be supported by pot experiments to determine soil nutrient status and pinpoint any trace-element deficiencies (Reynolds, 1981; Kayastha, 1982). Forage grasses and legumes found suitable in multilocational trials could then be recommended for planting (Servoz, 1983). Dairy farmers should be supported to cultivate fodder species of high value on one to two acres of land per dairy cow through provision of planting materials. Examples of such fodder species include grasses such as *Pannisetum purpereum*, *P. maximum*, and *Bracharia mulato*; legumes such *Pueraria phaseolois, Chemaecrista rutondifolia*, *Arachis pintoi,* and *Centrosema arienarium*; and trees such as *Sesbania sesban*, *Calliandra calothyrsus*, *Leucaena leucocephala,* and *Leucaena diversfolia*. Trees can be interplanted with legumes.

Disease prevention and control

Through risk analysis, the Ministry of Agriculture, Animal Industry, and Fisheries (MAAIF) could identify areas prone to disease outbreaks and carry out routine strategic vaccinations in those locations. MAAIF also could also enter into contracts with vaccine manufactures to keep vaccine stocks readily available for delivery when disease outbreaks occur (RoU, 2009). Better still, government can establish a national veterinary drugs centre with sufficient resources to procure, store and avail existing vaccines and drugs of priority endemic diseases in the country. This would be the equivalent of the now largely successful strategy of the National Medical Stores for the health system in Uganda. Rehabilitation and construction of community dip tanks as a key intervention in the control of ticks and tick-borne diseases has been recommended, especially in areas where pastoral or communal grazing is still being practised (Ekou, 2013).

Support for pastoral production systems

Unhygienic handling practices in traditional milk production and in the informal milk trade represent serious obstacles for the introduction of modern dairy processing and marketing. The influence of pooling of different milk batches along the collection and marketing chain exacerbates the problem.

The successful adaptation of pastoral subsistence production to the needs of an improved milk production and marketing system will depend, to a large extent, on safeguarding the milk quality at production, during transport, processing and marketing. Optimising milk hygiene under pastoral conditions requires the availability of safe clean water, which is an unrealistic expectation in most situations.

However, the introduction of clean metal containers to producing herds has had a measurably positive effect on raw milk quality (Younan, 2004). Pastoral communities therefore ought to be supported to acquire simple metal containers in addition to continuous education on hygienic milk handling procedures and practices.

Establishment and support for dairy co-operative societies

Co-operative societies are an important forum for bringing together small holder farmers. Farmers should be mobilized to form cooperative groups in various milk producing areas. Government and development partners could support these farmers with startup capital and training.

Farmers can also be linked to the buyers through close and regular market interaction. These farmer groups would collect milk from various farmers to one collection centre where processors come and buy. This can bring many advantages. Because of the organised bulking (selling in large quantities), there is ready market for milk since processors come and take all the collections from one place.

It makes it easier for farmers to acquire milk coolers, generators and other equipment from processors at friendly terms to ensure quality standardisation and increased production of milk (EADD, 2013). Farmers can even receive other dairy services like farmer education

On improved breeding, disease control, pasture improvement and milk production enhancement from the dairy society.

Conflict of Interests

The author have not declared any conflict of interests.

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Full Length Research Paper

Effect of resistance and trichome inducers on attraction of *Euschistus heros* (Hemiptera: Pentatomidae) to soybeans

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Seed-sucking stink bugs are notable among the insect species that are economically relevant to Brazilian soybean crops. This study sought to evaluate how the application of resistance inducers affects the attraction of the neotropical brown stink bug, *Euschistus heros* (Hemiptera: Pentatomidae), to soybean plants, which is correlated with trichome density in these plants. Seeds from various soybean varieties, including IAC 17 and IAC 100 (resistant), BRS Conquista (moderately resistant), and BRS Jataí (susceptible), were sown in polyethylene pots with a mixture of soil and organic compost. Attraction, which was correlated with the number of trichomes on each variety, was assessed with or without the application of various resistance inducers, including acibenzolar-*S*-methyl (ASM), potassium silicate (K silicate), calcium and magnesium silicate (Ca+Mg silicate), and sodium silicate (Na silicate), and a control solution. The IAC 100 variety exhibited non-preference type resistance to *E. heros*. The IAC 100 and IAC 17 varieties exhibited non-preference-type resistance to *E. heros*. The application of Ca+Mg silicate, K silicate and Na silicate increased the resistance of soybean varieties to *E. heros*. In addition, increases in the number of trichomes of soybean plants were associated with reduced attractiveness of these plants to *E. heros*.

Key words: *Glycine max*, induction of resistance, silicon, plant resistance to insects.

INTRODUCTION

Various seed-sucking stink bugs damage the health of Brazilian soybean crops; notably, *Euschistus heros* (Hemiptera: Pentatomidae); the neotropical brown stink bug, is a particularly problematic species for these crops (Corrêa-Ferreira, 2005; Godoi and Pinheiro, 2009). These insects are found among soybeans from the vegetative phase to the reproductive phase of soybean growth and damage pod formation at the onset of ripening (Nunes and Corrêa-Ferreira, 2002). In particular, *E. heros* engages in the suction of material from soybean grains, which alters the protein and oil content of these grains and can cause the disease known as "crazy

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soybean" (Ni et al., 2010; Depieri and Panizzi, 2011).

Seed-sucking stink bugs are controlled in soybean crops in various ways, most notably through the use of chemical insecticides (Musser and Catchot, 2008; Brown et al., 2012). Because these insecticides may be inefficient, a large number of sprays and/or high doses of these chemicals may be necessary to control *E. heros* populations; these treatment characteristics may contribute to the emergence of resistant insect populations and cause damage to human health and the environment (Sosa-Gómez and Silva, 2010).

The disorganized sprays and undesirable effects, such as insect resistance to insecticides (Sosa-Gómez and Silva, 2010) have resulted in the development of alternative methods of insect control (Jackai et al., 1988; Arif et al., 2004) including silicon treatments to induce resistance in plants (Ferreira et al., 2011; Lemes et al., 2011; Cruz et al., 2012) and the use of plant varieties that exhibit insect resistance (Souza et al., 2012).

The resistance of soybean plants to insects is associated with volatile substances in soybean leaves (Li et al., 2004); variations in nutrient concentrations, food excitants or deterrents, and antibiotics (Fisher et al., 1990); and the morphological characteristics of plants (Valle et al., 2012). Among the various types of secondary plant metabolites, constitutive or induced flavonoids, including rutin and genistin, are found in different parts of soybean plants and are the deterrent substances that are most likely to account for the resistance of these plants to insects (Hoffmann-Campo et al., 2001; Piubelli et al., 2003, 2005).

The use of an integrated management approach in which the use of insect-resistant varieties is combined with other control methods may decrease and even eliminate the use of insecticides for insect control, increasing the sustainability of soybean production.

Silicon increases plant resistance to various pests, stimulates plant growth, and protects against biotic and abiotic stresses; these effects are mediated by not only the mechanical barriers formed by the deposition of silica in leaf tissues and trichomes but also the silicon-facilitated production of phenolic defense compounds (Epstein, 1999; Ferreira et al., 2011; Lemes et al., 2011; Cruz et al., 2012).

The induction of silicon-based resistance results from the accumulation of silicon and the polymerization of silicate in epidermal cells; these processes form a mechanical barrier known as the cuticle-silica layer (Savant et al., 1997). Epidermal silicification hardens plant cell walls, preventing stylet penetration and insect chewing (Datnoff et al., 1991).

Effects of interactions between silicon and resistant plant varieties on the attraction of stink bugs to soybeans plants have not been frequently studied. Therefore, this study sought to analyze the effects of resistance and trichome inducers on the attraction of *E. heros* (Hemiptera: Pentatomidae) to soybeans.

MATERIALS AND METHODS

Seeds of the IAC 100 and IAC 17 (resistant), BRS Conquista (moderately resistant), and BRS Jataí (susceptible) soybean varieties (Souza et al., 2012; Mcpherson et al., 2007; Belorte et al., 2003), were sown in polyethylene pots that each had a capacity of 5 kg, using a 3:1 mixture of soil and bovine manure.

These pots were incubated in a greenhouse; because the plants grown in these pots were not sprayed with insecticides, coverage with nylon screen netting was used to protect these plants from pests. Prior to the critical stages for stink bug attack, the pots were placed in a temperature-controlled room with a 12-h photoperiod at temperatures of 28±3°C and the cultivated soybean plants were submitted to the following treatments: T1 – spraying plants to the point of runoff with a 1% potassium silicate (K silicate) solution; T2 – spraying plants to the point of runoff with a 1% sodium silicate (Na silicate) solution; T3 – the application of calcium and magnesium silicate (Ca+Mg silicate), an inducer, to the soil; T4 – spraying plants to the point of runoff with a 0.3% acibenzolar-*S*-methyl (ASM) solution; and T5 – spraying plants to the point of runoff with distilled water (the control solution). Plants in V5 stage were subjected to testing, 10 days after the aforementioned treatments. *E. heros* stink bugs were obtained from insect breeding colonies using the methodology described by Depieri and Panizzi (2011).

Attraction of adults in the no-choice test

To select seed pods that were relatively resistant to damage by *E. heros*, attraction assays were performed using green pods from each variety that had been treated with inducers. Those pods were collected from plants at different phenological stages, beginning from V5 (Fehr and Caviness, 1977). Adult stink bugs were maintained under fasting conditions for 24 h prior to the start of the tests.

One pod from each treatment was inserted in a dish with a diameter of 6 cm, and one *E. heros* adult was added to the dish to assess the attraction of stink bugs to the treated pods. The number of insects attracted to pods that received each treatment was counted at 15, 30, 45, 60, 120, and 180 min after the release of the *E. heros* adults. The adults of the stink bugs used were at most 48 h of life and were fasted for 24 h before testing begins. Ten replicates were used per treatment in a completely randomized experimental design.

The preference index of *E. heros* for the examined soybean varieties were determined from the mean attraction results, which were calculated using the formula $C = 2A/(M + A)$ (Kogan and Goeden, 1970). In this equation, C represents the preference index; A represents the attractiveness of the tested cultivar; and M represents the attractiveness of the cultivar used as the baseline (in this case, IAC 17). The data were interpreted based on the calculated values of C. In particular, $C > 1$ indicated that the tested cultivar was more attractive to *E. heros* adults than the baseline cultivar (the tested cultivar was a stimulant for *E. heros*); $C = 1$ indicated that the tested cultivar was similar to the baseline cultivar with respect to attractiveness to *E. heros* adults (a neutral result); and $C < 1$ indicated that the tested cultivar was less attractive to *E. heros* adults than the baseline cultivar (the tested cultivar was a deterrent to *E. heros*).

Trichome density

The number of trichomes present in 0.25 cm^2 of each examined pod was counted, using the methodology employed by Paron and Lara (2005). These counts were used to relate the trichome

Table 1. The mean number of *Euschistus heros* (Hemiptera: Pentatomidae) adults attracted by green pods for four soybean varieties treated with resistance inducers.

Varieties (V)	Times reviewed [1,2]							
	15 min	30 min	45 min	1 h	2 h	3 h	6 h	Total
IAC 17	0.31^{bc}	0.33^{bc}	0.28^{b}	0.35^{ab}	0.33^{b}	0.40^{ab}	0.43^{ab}	0.34^{b}
IAC 100	0.21^{c}	0.19^{c}	0.25^{b}	0.29^{b}	0.35^{b}	0.36^{b}	0.30^{b}	0.27^{b}
BRS Conquista	0.51^{a}	0.51^{a}	0.51^{a}	0.51^{a}	0.56^{a}	0.52^{ab}	0.46^{ab}	0.51^{a}
BRS Jataí	0.41^{ab}	0.41^{ab}	0.47^{a}	0.52^{a}	0.53^{a}	0.57^{a}	0.55^{a}	0.49^{a}
F (V)	7.54**	8.27**	7.85**	5.67**	5.88**	4.09**	4.50**	29.81**

[1]Data transformed for analysis using (x + 0.5)1/2. [2]Means followed by the same letter in columns are not significantly different by Tukey's test at the 5% probability level. C.V. - Coefficient of variation. [ns]Not significant. **Significant at the 5% probability level.

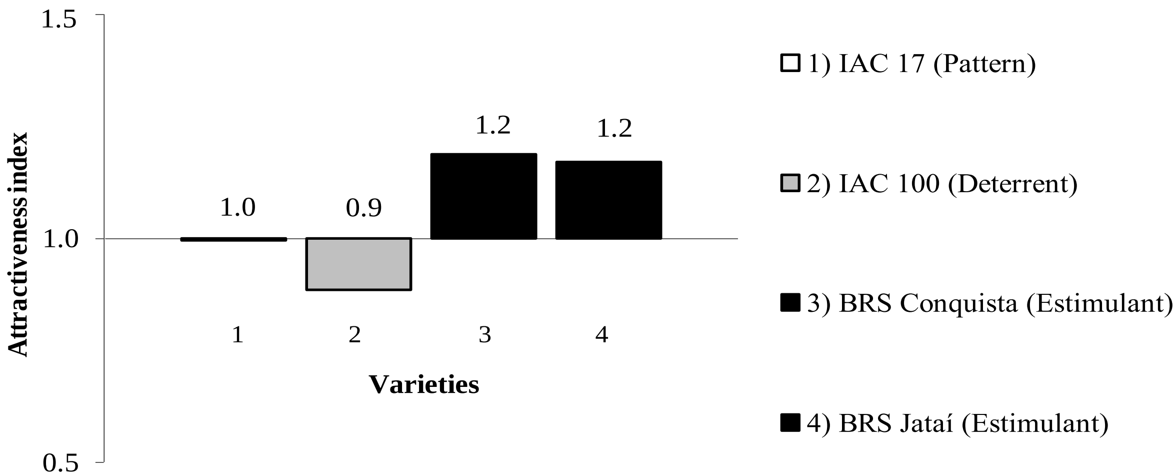

Figure 1. The attractiveness indices of soybean cultivars for *Euschistus heros* (Hemiptera: Pentatomidae) adults in the non-choice test and the corresponding classifications for these cultivars.

densities of pods from varieties treated with resistance inducers to the attractiveness of these pods for feeding by *E. heros*. A stereomicroscope and graph paper were used for determinations of trichome density. For each examined pod, the middle of the pod was standardized (on the second grain), and the total number of trichomes present in the delimited area was recorded. Trichomes were counted for 10 pods from each treatment. Each observation represented a replicate in the analysis, which utilized a completely randomized experimental design.

Statistical analysis

Using the Sisvar statistical software package, the study data were assessed by analyses of variance and F-tests, and Tukey's test was employed to compare means at the 5% probability level (Ferreira, 2011). In the statistical analyses of this study, linear regressions were utilized to correlate the mean attraction of *E. heros* with the mean number of trichomes for each examined soybean variety.

RESULTS

The attraction of *E. heros* adults differed for the examined soybean varieties at all examined time periods after the release of insects (Table 1).

Among the tested varieties, the IAC 100 and IAC 17 exhibited the least attractiveness to *E. heros* adults at all evaluated time periods and a lower attractiveness to *E. heros* adults than the other examined varieties, although the attractiveness of the IAC 100 and IAC 17 varieties did not significantly differ. In contrast, among the tested varieties, the BRS Conquista variety exhibited the highest attractiveness to *E. heros* adults at all times except for 3 and 6 h after the release of the stink bugs, although the attractiveness of the BRS Conquista and BRS Jataí varieties did not significantly differ.

Different attractiveness indices were calculated for the various examined varieties relative to the baseline variety of IAC 17 (Figure 1). *E. heros* was most attracted to the BRS Conquista and BRS Jataí varieties, which were classified as stimulants. IAC 100 exhibited the lowest attractiveness to *E. heros* among the tested varieties and was classified as a deterrent.

The application of various resistance inducers affected the attraction of *E. heros* adults to the tested varieties;

Table 2. The mean number of *Euschistus heros* (Hemiptera: Pentatomidae) adults attracted to green soybean pods treated with one of five different resistance inducers.

Inductors (I)	Times reviewed [1,2]							
	15 min	**30 min**	**45 min**	**1 h**	**2 h**	**3 h**	**6 h**	**Total**
ASM	0.34^{ab}	0.34	0.45^{ab}	0.47^{b}	0.46	0.49	0.56^{a}	0.44^{ab}
K Silicate	0.29^{ab}	0.32	0.34^{ab}	0.34^{ab}	0.39	0.36	0.40^{ab}	0.34^{c}
Ca+Mg Silicate	0.27^{b}	0.29	0.26^{b}	0.32^{b}	0.39	0.42	0.35^{b}	0.32^{c}
Na Silicate	0.41^{ab}	0.36	0.32^{ab}	0.42^{ab}	0.47	0.49	0.36^{ab}	0.40^{bc}
Untreated	0.49^{a}	0.49	0.51^{a}	0.52^{a}	0.50	0.55	0.50^{ab}	0.51^{a}
F (I)	2.89*	2.11^{ns}	3.73**	2.55*	0.89^{ns}	1.70^{ns}	2.88*	11.76**

[1]Data transformed for analysis using (x + 0.5)1/2. [2]Means followed by the same letter in columns are not significantly different by Tukey's test at the 5% probability level. C.V. - Coefficient of variation. ^{ns}Not significant. **Significant at the 5% probability level. *Significant at the 1% probability level.

Table 3. The mean number of trichomes on green pods from soybean varieties treated with different resistance inducers.

Varieties (V)	Inductors (I)					F (V)
	ASM	**K Silicate**	**Na Silicate**	**Ca+Mg Silicate**	**Untreated**	
IAC 17	147.00^{bB}	180.75^{aA}	171.75^{abB}	152.25^{abB}	173.00^{abAB}	3.75**
IAC 100	193.00^{abA}	205.00^{abA}	215.00^{aA}	177.75^{bAB}	193.50^{abA}	3.49*
BRS Conquista	107.00^{cC}	137.75^{abB}	156.75^{aB}	122.25^{bcC}	164.50^{aB}	10.06**
BRS Jataí	106.50^{cC}	135.50^{bcB}	163.50^{abB}	184.50^{aA}	127.25^{cC}	16.79**
F (I)	29.98**	20.51**	12.23**	14.21**	13.64**	-

[1]Data transformed into $(x + 0.5)^{1/2}$ for analysis. [2]Means followed by the same letter lowercase and uppercase in the column and line, do not differ according to Tukey's test at 5% probability. ^{ns}non-significant. **Significant at 5% probability.

notably, differences were observed at 15 and 45 min, 1 and 6 h after the release of insects (Table 2).

At each examined time point, *E. heros* adults were less attracted to pods treated with the resistance inducer Ca+Mg silicate, when compared untreated. However, at the tested time points, the levels of attractiveness to *E. heros* of pods treated with Ca+Mg silicate did not differ significantly from the corresponding levels of attractiveness to *E. heros* of pods treated with ASM, K silicate, or Na silicate. The only exception to this pattern occurred at 6 h after *E. heros* release; at this time point, attractiveness to *E. heros* was significantly lower for pods treated with Ca+Mg silicate than for pods treated with ASM. The control treatment consistently resulted in pods with the highest attractiveness to *E. heros*.

Among the examined varieties, the IAC 100 and BRS Jataí varieties exhibited the highest and lowest trichome densities, respectively. With respect to the examined resistance inducer treatments, the Na silicate and K silicate treatments resulted in greater trichome densities than either the Ca+Mg silicate treatment or the control treatment (Table 3).

Further analysis of the resistance inducer treatments for each examined variety demonstrated that Ca+Mg silicate treatment of the BRS Jataí variety resulted in markedly greater trichome densities than in the IAC 100 and IAC 17 varieties. Na silicate with association IAC 100 variety resulted in higher densities of trichome. This result suggested that silicon-based treatments may increase trichome densities of susceptible varieties, thereby contributing to the defenses of these varieties against insects.

Linear regression analyses correlating mean attractiveness to *E. heros* with mean number of trichomes demonstrated high and significant negative linear correlations ($y = -228.5x + 253.82$; $r^2 = 0.9337$**) between these quantities, indicating that a reduction in the first quantity is associated with an increase in the second quantity (Figure 2).

DISCUSSION

The IAC 100 and IAC 17 varieties were less attractive to *E. heros* than the other tested varieties. The stink bug-deterring effects and reduced *E. heros* attraction of these resistant varieties indicate that these varieties have indirect defenses against this insect, in particular, these defenses may involve the increased production of volatile compounds in response to *E. heros*-mediated herbivory (Li et al., 2004; Fisher et al., 1990; Piubelli et al., 2003; Piubelli et al., 2005). These induced secondary compounds,

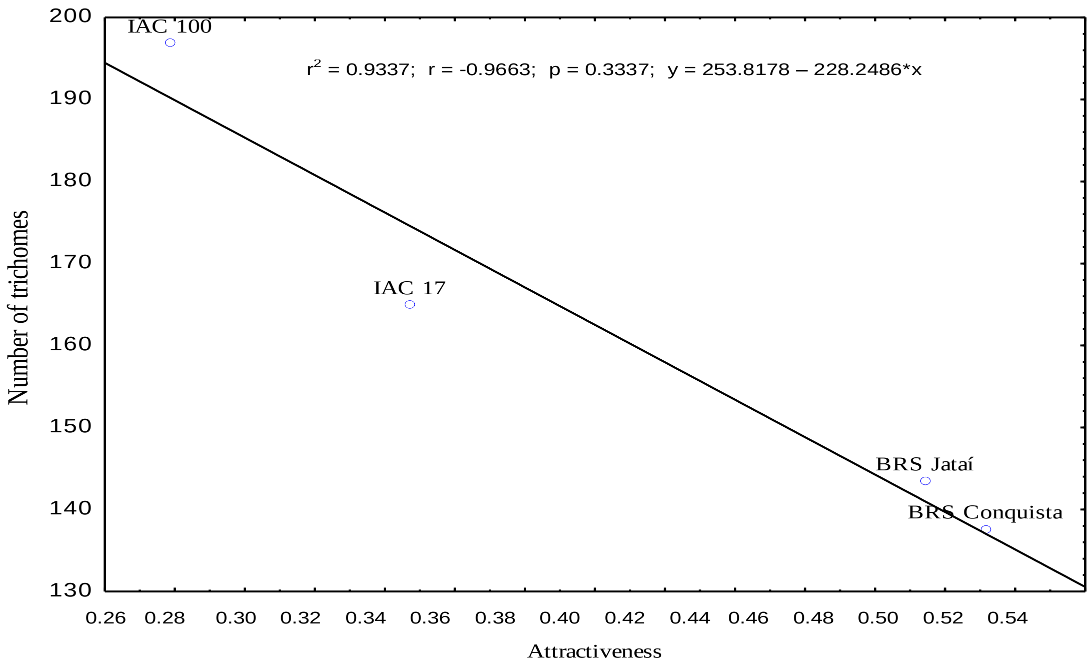

Figure 2. The correlation between mean number of trichomes and attractiveness to *Euschistus heros* (Hemiptera: Pentatomidae) for four soybean varieties.

which primarily consist of rutin flavonoids and the isoflavone genistein, are known to reduce the attraction and feeding preference of *E. heros* for these soybean varieties (Hoffmann-Campo et al., 2001).

The reduced attraction of *E. heros* to plants exposed to silicon-containing treatments relates to increases in plant resistance caused by not only the mechanical barriers formed by the deposition of silicon in leaf tissues and trichomes but also the production of phenolic defense compounds (Epstein, 1999; Ferreira et al., 2011; Lemes et al., 2011; Cruz et al., 2012).

This induction of resistance results from the accumulation of silicon and the polymerization of silicate in plant epidermal cells; these processes form a mechanical barrier known as the cuticle-silica layer (Savant et al., 1997), which hardens plant cell walls to prevent penetration and insect chewing (Datnoff et al., 1991).

Notably, with respect to the relationship between number of trichomes and stink bug attraction to green pods, the IAC 100 variety exhibited high trichome density and low attractiveness to *E. heros*, suggesting that trichomes are a key morphological feature of plants that contributes to insect resistance (Valle et al., 2012).

The IAC 100 and IAC 17 varieties exhibited non-preference-type resistance to *E. heros*. The application of Ca+Mg silicate, K silicate and Na silicate increased the resistance of soybean varieties to *E. heros*. In addition, increases in the number of trichomes of soybean plants were associated with reduced attractiveness of these plants to *E. heros*.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Analyzing the factors affecting the market participation of maize farmers: A case study of small-scale farmers in greater Giyani Local Municipality of the Mopani District, Limpopo Province

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The aim of the study was to analyze factors affecting the market participation of small-scale maize farmers in Greater Giyani Municipality. The study hypothesizes that there are no factors affecting market participation in small-scale maize farmers in Greater Giyani. The study used cross-sectional data collected from 92 small-scale maize farmers. The logistic regression model was used to analyze data. The logistic regression results reveals that out of twelve variables considered in the analysis as factors affecting market participation, seven of them were found to be significant, while five of them were insignificant. Gender, farmer's access to credit, marital status, market information and infrastructure were found to be positively significant, while distance to market and external source of income were negatively significant. Farmers' level of education and age of a farmer were positively insignificant. Distance to output market, experience in farming and external source of income were negatively related to market participation. The results from the study highlights that government can increase market participation of small-scale maize farmers through encouraging group market participation, upgrading of roads to enable smooth accessibility of farmers to output market and establishment of local point of sales in farming areas. Also government has to provide input subsidy to all farmers to enable them to produce more and to pay for all marketing cost. Finally it is recommended that government provides planned workshops to all farmers, to equip them with marketing knowledge.

Key words: Greater Giyani Municipality, market participation, logistic regression model, small-scale.

INTRODUCTION

For most small scale farmers in South Africa, maize serve as a form of capital that can be easily converted into cash through marketing of maize products. Maize product can be sold as green mealies (succulent), dry seeds or as processed maize meal to the local people, thus ensuring high level of food security to local households. The maize industry is important to the economy both as an employer and earner of foreign

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currency because of its multiplier effects (NDA, 2009).

The study is primarily focused on the market participation behavior of small-scale maize farmers in the Greater Giyani Local Municipality (GGLM). It attempts to determine the factors influencing the decision of the farmers, farming households to participate in the market, that is, the decision to sell or not to sell their produce. Commercialization of subsistence agriculture implies increased participation, or, an improved ability to participate, in output markets. In the developing areas of South Africa, like in other developing countries, small-scale farmers find it difficult to participate in outputs markets because of a range of constraints and barriers reducing the incentives for participation, these may be reflected in hidden costs that make access to markets and productive assets difficult (Makhura, 2001).

South African agriculture comprises mainly two categories of farmers, the subsistence farmers in the former homeland areas and the large-scale commercial farmers. This is in contrast with the situation in many other countries in the world where one would find a whole range of farm sizes, ranging from the very small or subsistence farmer to the very large agribusiness (Kirsten and Van Zyl, 1998).

Marketing of agricultural products is an important function of the agricultural industry as it plays a major role in transferring products from the farm to the final consumer. Marketing is a complex activity which commences at the farm, when the farmer plans his production to meet specific demands and market prospects, this may include farmer's decision on how to disperse his produce, and to the activities of intermediaries this may include product, assembling, transportation, storage, processing, packaging, wholesaling, retailing and ownership by the final consumer (Omiti et al., 2009).

According to Schuh (1987), agricultural marketing is a very important but rather neglected aspect of agricultural development; emphasis is usually placed on increasing food production, with the idea that this will both improve the material status of the population and act as a base for rural development. This is true due to the fact that, markets do not develop automatically and the lack of well-established or functioning markets can seriously hinder the production. Furthermore, if the surplus resulting from increase in production cannot be marketed neither, the producers in the area lose, they may even be hurt if costly resources must be used to produce output that is not sold. The fundamental objective of agricultural development should not be just to increase output but to increase net per capita and family income.

The contribution of smallholder agriculture to social (equitable distribution of income, food security, employment etc.) and national economy (e.g. provision of foreign currency), is recognized in various development strategies. However, smallholder farmers' participation in commercial agriculture is low despite the envisaged benefits of market-orientation, as well as favorable trends in drivers of commercialization. Access to agricultural output markets is seen to be directed in service of large-scale commercial farmers. Lack of market participation by small-scale maize farmers discourages or hampers the general growth in agriculture, thus the process of rural development could not be realized, and the level of food insecurity, inequitable distribution of income for majority of the rural households is increased.

Small-scale farmers find it increasingly difficult to penetrate the market channel and therefore need to develop and explore other market strategies. The inclusion of small-scale maize farmers in the area and then sustained participation in changing agro-food markets is of utmost importance for the development of the industry. It is generally accepted that farmers in traditional agriculture are poor but efficient (Ngqangweni, 2000). In this view, it is held that farmers remain poor because they have to connect with lack of technical and economic opportunities to which they could otherwise respond (Makhura, 2001).

Although previous studies (Azam et al. (2012); Rendela et al. (2010); Ramoroka (2012); Omiti et al. (2009) and DAFF (2011) attributed to the low market participation of different challenges even when various efforts to promote small-scale farming was made in the past decade, however, there is very little information on factors affecting market participation by small-scale maize farmers in the Greater Giyani Municipality.

Maize farming is one of the agricultural activities that most rural farmers practice in the area of Greater Giyani Municipality. Participation of small-scale farmers in commercial agriculture is important for unlocking suitable opportunities set necessary for providing better incomes and suitable livelihoods for small-scale farmers. It is through the coverage of this study, that major factors that determine full marketing participation by small-scale farmers will be outlined and focused at (DAFF, 2011).

The study raises issues which, when attended to, might increase the market participation of small-scale maize farmers in the Greater Giyani Municipality, particularly by enhancing access to information and providing incentives to farming households. Appropriate policy interventions and agricultural marketing development strategies require that policy makers have a good understanding of the factors affecting small-scale maize market participation. The results of this study could be used as input for policy and strategy formulation to alleviate constraints limiting market participation of small-scale maize farmers. They will further assist in generating knowledge in relation to the improvement of small-scale maize market participation for academics and evolving future professionals in agriculture.

MATERIALS AND METHODS

The study used secondary cross-sectional data which was collected in survey of small-scale farmers in the Greater Giyani Municipality

Table 1. Independent variables and their units of measurements.

Variables	Description	Unit
Dependent variable		
MKTP	1 if a farmer participate in the market,0 otherwise	Dummy
Independent variables		
FFE	1 if a farmer has formal education ,0 otherwise	Dummy
DTMK	Distance to the market	Kilometers
SLC	Size of land under cultivation	Hectares
MKIN	1 if a farmer has access to market information, 0 otherwise	Dummy
FGRNT	1 if a farmer has grants,0 otherwise	Dummy
FAC	1 if a farmer has access to credit,0 otherwise	Dummy
GEND	1 if a farmer is a male, 0 0therwise	Dummy

Table 2. Descriptive statistics.

Market participation	Participate	50.5%
	Do not participate	49.5%
Distance to the market	Less than 5 km	37.4%
	More than 5 km and less than 10 km	50.5%
	More than 10 km	12.1%
Farmer access to credit	Access to credit	40%
	No access to credit	60%
Area planted	Less than a hectare	44%
	Two to three hectares	30%
	More than four hectares	26%
Market information	Access to market information	33%
	No access to market information	67%

in Mopani district of the Limpopo province in 2009**,** with a sample size of 92 small-scale maize farmers. The Greater Giyani Municipality is characterized by various economic activities which include formal and/or informal activities; small-scale agriculture (Maize, vegetables, tomatoes, and beef), services, transport and retail development among others. All data collected were based on the factors affecting the market participation of small-scale maize farmers in the Municipality.

Data analysis

The logistic regression model was employed to analyze data in this study. This model was used to identify factors which affect the market participation of small-scale maize farmers in Greater Giyani Municipality. Like many forms, it makes use of several predictor variables that may be either numerical or categorical (Hilbe, 2008). It makes use of the independent variables as well as the categorical dependent variables (Agresti, 2002). The logistic regression model was necessary to estimate the probability that there are no factors affecting market participation of small-scale maize farmers in Greater Giyani Municipality. Furthermore, the model was employed to determine the percent of variance in the dependent variable explained by the independents and shows the impact of each independent variable on the dependent variable. General model

$$\text{Log}\,[p/(1-p)] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \ldots\ldots\ldots\ldots\ldots\ldots + \beta_K X_K$$

Where P = predicted probability that Y equals to one (dependent variables); $\beta_0 \ldots\ldots \beta_K$ = Estimated parameters; $X_1 \ldots\ldots X_k$ = Independent variables.

These independent variables and their units of measurements are described in Table 1.

RESULTS AND DISCUSSION

Table 2 shows the descriptive statistics of the variables included in this analysis. The results show that 50.5% of the respondents participate in the market for maize while 49.5% of them do not participate. The results of low market participation could be as a result of bias towards provision of marketing incentives among farmers and the distance to output market with no necessary transport to move farm produce to the market, which can then be reduced by bringing buyers closer to the small scale farmers. The results also revealed that access to market information by small-scale maize producers is still a

Table 3. Estimates of parameters of Logistic regression analysis

Variables	Co-efficient	Standard error	Wald statistics	Significance level	Exp (B)
FFE	0.110	0.295	0.139	0.709	1.116
DMTK	-0.775*	0.423	3.365	0.067	0.461
AOLC	0.556*	0.372	2.235	0.135	1.743
MKIN	1.497**	0.678	4.881	0.027	4.467
FGRNT	-1.665**	0.740	5.058	0.025	0.189
FAC	1.216**	0.583	4.343	0.037	3.372
GEND	1.630**	0.693	4.718	0.034	1.842

Chi-square = 6.25; Percentage correctly predicted = 76.9%.

challenge; with 67% of the sampled farmers having no access to market information. Findings from the descriptive statistics further indicated that majority of the farmers do not have access to credit, with 60% of the farmers not having access and 40% being able to access the credit. Perhaps this situation could be one of the factors that negatively influence the decision of farmers to participate in the market.

The availability of land by farmers seems to be also a concern in market participation, it is important that farmers have enough land to produce if they are to participate in the market. The statistics highlighted that 44% of the sampled farmers operating are producing on less than a hectare of land, 30% percent producing on less two hectares and 26% of the other farmers produce using more than four hectares. In addition to all this statistics it was revealed that 67% 0f the respondents have access to market information and 33% do not have access to market information.

As can be seen from Table 3 the cases which were correctly predicted were found to be 76.9%, which implies that 23.1% is as a result of exclusion of relevant variables or inclusion of irrelevant variables. This could have resulted from the fact that some respondents might have provided incomplete if not biased information.

The 'variable access to credit' is found to be positively significant to market participation of small-scale farmers in Greater Giyani Municipality of the Mopani District. This implies that the likelihood of farmers who have access to credit is high to participate in the market for maize as compared to the farmers that do not have access to credit. From the results it shows that a unit increase in access to credit by farmers might improve the likelihood of market participation of farmers by 1.216 times. The variable 'Gender' is also positively significant to market participation by small-scale farmers, implying that a one unit increase in the number of male small-scale famers is likely to increase the probability of market participation rate by 1.630. This as well means that male famers have some sort of preferences to market participation compared to female farmers. From the fact that most of the farms are distant from the place where goods and services are exchanged, it was expected that the variable distance to the market could play an important role in determining whether the farmer can participate in the market or not. The coefficient of the variable "distance to the market" was found to be negatively significant to market participation. This implies that a one kilometer increase in the distance travelled to the market could reduce the market participation by 0.775. This is as expected since an increase in the distance travelled might contributes to high transaction cost, which is consistent with Jagwe (2011)'s finding which says 'in most rural areas farmers are faced with a challenge of making a decision on whether to travel to market places to sell their produce or sell at the farm gate" due to costs attached.

The variable 'access to the information' is positively significant to market participation, implying that farmers who have access to market information are likely to participate in the market for maize. This is indicated by fact farmers who have access to information is likely to improve their participation by 1.497. Perhaps this might because access to information help in planning the marketing process of any farm business.

Conclusion

In conclusion the study revealed that, factors, such as credit, gender of the farmer and access to market information positively influence market participation by small-scale maize producers in Greater Giyani Municipality. The study further revealed that there is a negative relationship between the distance travelled to the market and market participation, implying that as the distance to the market increases farmers tend to participate less in the market. Access to land by small-scale farmers has been on debate for a long time through land reform policy and it seems as if little has been achieved. Therefore it is recommended that the process of allocating land to active and passionate small-scale farmers be reviewed, since the availability of land is still a major concern in agricultural production, which consequently affects the market participation. Furthermore, the study sees that there is a need for the development of appropriate marketing infrastructure, which should be able to address issues like the distance travelled to the market. Hence the study was conducted in small area it also recommends that further studies be conducted in other areas of the country.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Methods of extraction and bioavailability of zinc for the soybean culture fertilized with NPK+Zn

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This work aims to evaluate the extractors Mehlich-1 and diethylene triamine pentaacetic acid (DTPA) and their extraction capacity for zinc (Zn) correlating them with the bioavailability of this nutrient in the soybean culture [*Glycine max* (L.) Merril] cultivated in a Eutrophic Red oxisol, in order to find better correlation between fertilization doses and plant absorption of zinc in conditions of the Parana Western region. The experiment was performed in 2008, in the city of Marechal Candido Rondon – Brazil, in completely randomized design. The treatments were arranged in 3×3 factorial scheme, consisting of three fertilization doses ($N:P_2O_5:K_2O$ in kg ha^{-1}): without fertilization; recommended dose (4:40:40) and twice the recommended dose (8:80:80); and three Zn doses: 0.00; 1.00 and 2.00 kg ha^{-1}. Between the evaluated extractors, Mehlich-1 presented higher capacity of Zn extraction in the studied soil. Concerning fertilization with Zn, though the doses show increment in the soil, no increase was found in the Zn concentration in leaf tissue.

Key words: Micronutrients extraction, diethylene triamine pentaacetic acid (DTPA), Mehlich-1.

INTRODUCTION

Fertilization with micronutrients is of great importance in seeking for increase in crop productivities, and in this way, these nutrients are mostly used on a daily basis in fertilization in many regions of Brazil for the more varied crops. However, it is recommended that there should be a previous evaluation of the availability of these nutrients in soil before their application through the chemical analyses of the soil; because in soils with high concentrations of these micronutrients, the plants may not respond in productivity to the fertilization with these metals. In this way, the chemical analyses of soil enables the previous knowledge of the micronutrients availability in areas aimed for cultivation, allowing better information for decision-making in order to provide the necessary correction of the soil to the implantation of the crops.

The rational use of fertilizers in farms, allied to studies about bioavailability of nutrients by the crops are very important factors, due to the crescent shortage of raw-matters for the production of fertilizers and the high cost of these resources.

The studies about nutrients bioavailability, especially micronutrients, have presented contradictories results, due, in most of the cases, to the many relations and characteristics of each soil and the portion of nutrient taken as available to plants.

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Those results indicate the necessity of a sophistication of the results interpretations, seeking for a better expression of the availability of nutrients and the response by the plants (Gonçalves Jr. et al., 2006).

Silva and Menezes (2010), studying the availability of micronutrients in Paraiba State soils – Brazil, mentioned that there still does not exist a method which can be used as standard for the evaluation of micronutrients in the Brazilian soils, which occurs mainly as a function of the few number of studies evaluating extraction methods of micronutrients and the correlation with the plant absorption.

The most of the Brazilian soils are highly weathered, with high levels of iron and aluminum oxides which are predominant in the clay fraction (Bortolon et al., 2009), and the methods of extraction for micronutrients more used in the laboratories are those which use DTPA solution and Mehlich-1; however, there does was not found existing between them a clear definition as to which is more recommended for each soil (Abreu et al., 1996).

Researchers and technicians have alerted for the combined extraction of the available nutrients in the soil, because there are still lots of divergences and the methodologies of extraction are not well standardized, which may cause innumerous variations in the obtained results and consequent variations in the interpretation of the results.

According to Ribeiro and Saraiba (1984), Bataglia and Raij (1989) and Ferreira and Cruz (1992), despite many laboratories already performing micronutrient analysis in the soil samples, the lack of standardization due to the used extractor can compromise the reliability of the results. Thus, the standardization of the methodological procedures is undoubtedly necessary, ensuring in this way the reliability of the results of the micronutrients chemical analysis.

According to Ortiz et al. (2005), the comparison between the analytical results for the levels of zinc (Zn) and the other nutrients in soil samples, will only be possible and adequate, when the laboratories adopt the same procedures for the achievement of the extract.

Knowing the absorption of nutrients by plants and the forms of availability of these nutrients for the vegetables, the used reagents in the chemical analysis must extract from the soil quantities of nutrients with a good correlation with the quantities absorbed by the plants.

The Mehlich-1 extractor is the most used in the routine analysis in function of the operational facility, agronomy efficiency and because of the good correlations with the micronutrients absorbed by the plants (Da Silva and Menezes, 2010).

According to Ortiz and Borker (2007), in the Parana State the extractor solution Mehlich-1 is the one which is more used for the extraction of iron (Fe), cupper (Cu) zinc (Zn) and manganese (Mn) in the soil samples. Adoption of this solution does not imply additional costs and dispenses adaptations in the physical structure of the laboratories, once it is already being used daily for the extraction of phosphorus (P) and potassium (K) in soil.

The micronutrients, between them, e.g. Zn, although demanded in small quantities by the plants, is essential for the complete cycle of the vegetal, being that, when provided in inferior quantities can cause a considered decrease of the productivity (Gonçalves Jr et al., 2007).

Determination of the available fraction of Zn, obtained by the correlation of the index of this micronutrient, is usually obtained by different methods of extraction. This indication of fertility is complicated for the micronutrients due to the small occurrence and the mechanisms which regulate the reactions of availability of this element in the soil, therefore, by the characteristics of the soil, as pH, organic matter, cation exchange capacity (CEC), effect of other ions, clime, species of plants and the interaction between the plants and the environment (Câmara, 2000).

Between the micronutrients extractors, which define critical levels and the necessity of nutrients fertilization, in the case of Zn, the Mehlich-1 have presented itself with a possibility of predicting the fertilization with this nutrient, however, complexing solutions, DTPA case, also present itself as capable of competition with the acid extractors and in many cases overcome them, presenting excellent correlation soil/plant (Bataglia and Raij, 1989).

The recommendation for micronutrient fertilization cannot be performed indiscriminately, because besides the money cost, excess fertilizer can cause phytotoxic effects to the culture. Functionally, knowledge of the availability of micronutrient in the soil, with emphasis in the annual crops, is essential for the adequate recommendation which can guarantee high productivities (Bataglia and Raij, 1989; Oliveira and Nascimento, 2006).

Seeking for the evolution of soil analysis and their correlation with many cultures, more studies on evolving methods of extraction of micronutrients and their correlation with the cultures, searching for ideal recommendation for the fertilizers application for the adequate supply of the micronutrients are necessary.

In this way, this work aimed to evaluate the extraction capacity of Zn using the extractors Mehlich-1 and DTPA, correlating them with the absorption capacity of Zn by the soybean culture with different doses of NPK + Zn in a Eutrophic Red oxisol.

MATERIALS AND METHODS

The experiment was performed in the city of Marechal Cândido Rondon – PR, in the Experimental Station of Prof. Dr. Antônio Carlos dos Santos Pessoa, belonging to the State University of Western Parana – UNIOESTE. The location coordinates are: 24º 31' SE 54º 01' W and with mean altitude of 420 m. The local clime is humid subtropical climate (Cfa) according to the Köppen classification, without defined dry season.

The soil used in the experiment was classified as Eutrophic Red oxisol (EMBRAPA, 2006a), clay texture, with 685.40, 254.10 and 60.50 g kg^{-1} compositions of clay, silt and sand, respectively.

For the chemical analysis, the soil was collected at 0.20 m deep, being the results presented in the Table 1. The soil chemical analysis was performed according to the Manual of Soil Chemical Analysis and Quality Control of the Agronomic Institute of Parana – IAPAR (Pavan et al., 1992).

The organic matter values obtained by the chemical analysis (Table 1) fit in level taken as “good” for organic matter levels in Brazilian soils (EMBRAPA, 2006a), which are included in the interval from 40 to 70 g dm^{-3}.

The treatments were disposed in a randomized complete block design (RCBD) in a factorial scheme 3×3, with four replications, three doses of fertilization $N:P_2O_5:K_2O$ in the sowing, and three doses of Zn. The three tested doses were: 0.00, 40.00 and 80.00 kg ha^{-1} of P_2O_5 and 0.00, 40.00 and 80.00 kg ha^{-1} of K_2O, characterizing, respectively, control, dose 1 (Bissani et al., 2004) and dose 2. Nitrogen was not used in the base fertilization. The three doses of Zn were 0.00, 1.00 and 2.00 kg ha^{-1} of Zn, applied with the fertilization $P_2O_5:K_2O$ at the point of sowing. The sources of P, K and Zn were dicalcium phosphate, potassium nitrate and

Table 1. Chemical analysis of the soil.

pH ($CaCl_2$)	K^+	Ca^{2+}	Mg^{2+}	H+Al	SB	CTC	Organic matter	P	Cu	Zn	Fe	Mn	V
	--------------------(cmol$_c$ dm^{-3})-------------------						(g dm^{-3})		-------------(mg dm^{-3})----------				(%)
5.16	0.39	4.99	2.56	6.21	7.89	14.1	23.69	62.1	8.1	1.7	37.1	132.7	56.0

H + Al – potential acidity, SB – sum of bases, CEC – Cation-exchange capacity; % - bases saturation.

Table 2. Variance analysis for pH, levels of P, organic matter (OM), K, Ca, Mg and levels of Zn in the soil extracted by Mehlich-1 and DTPA.

FV	DF	Mean squares							
		P	OM	pH	K^+	Ca^{2+}	Mg^{2+}	Zn Mehlich- 1	Zn DTPA
Blocks	3	47.01^{ns}	0.23^{ns}	0.08^{ns}	0.03**	0.34 ^{ns}	0.01 ^{ns}	2.92^{ns}	0.55 ^{ns}
Dose NPK	2	2116.79**	2.51^{ns}	0.07^{ns}	0.22**	0.44 ^{ns}	0.01 ^{ns}	8.38^{ns}	0.51 ^{ns}
Dose Zn	2	303.12^{ns}	1.46^{ns}	0.11^{ns}	0.03*	1.10 ^{ns}	0.14 ^{ns}	57.64**	14.87**
NPK X Dose Zn	4	76.75^{ns}	1.22^{ns}	0.24^{ns}	0.03*	0.31^{ns}	0.02 ^{ns}	4.14 ^{ns}	0.41^{ns}
Residue	24	373.42	1.42	0.16	0.01	0.34	0.06	3.81	0.79
C.V.(%)		63.82	4.48	7.19	14.74	11.41	9.76	51.23	53.92
MSD		16.85	1.21	0.4	0.08	0.59	0.26	1.99	0.91

**,* - significant at 1 and 5% of probability, respectively by the Fischer test; ns – not significant by F test.

zinc sulfate, respectively.

The zinc doses (0.00, 1.00 and 2.00 kg ha^{-1}) were chosen because in Brazilian soils, zinc deficiency causes one of the most limiting in terms of production, being a deficiency very common in the central region of Brazil, where there are predominant soils with low levels of this metal. In these cases, in terms of fertilization, the used doses vary from 1.0 to 4.0 kg ha^{-1} of zinc, in function of soil zinc deficiency and the plant requirement of this metal (EMBRAPA, 2006b).

In this case, the soil presented low levels of Zn available in Soil (Table 1), also justifying the choice for the tests of Zn applied doses (0.00, 1.00 and 2.00 kg ha^{-1}).

The results were submitted to variance analysis and Tukey test using the statistical program SISVAR (Ferreira, 2003), considering 5% of probability.

For execution of the experiment, each block (consisting of nine plots) had a width of 7.00 m by 27.00 m of length. The parcels corresponded to a length of 3.00 m by 7.00 width, consisting of 6 lines with spacing of 0.50 m and separated with hallways of 1.00 m. the two central lines were considered as useful area, ignoring the rest of the plot.

For implantation of the experiment, subsoiling and harrowing was performed. The soybean cultivar used in the experiment was EMBRAPA 48, with population of 15 seeds per linear meter, sowed in November 05 of 2010. The seeds were treated with the fungicides Difenoconazole®+ Thiram®, in the doses of 5.00 and 70.00 g of active ingredient, respectively for each 100.00 kg of seeds.

Weed control was conducted manually each 15 days, and the insecticide Monocrotofós® was used in the dose of 15 mL of active ingredient per ha for the pests control.

A leaf diagnose of the culture was also conducted; for this, soybean leaves were collected 53 days after emergence (DAE) in the phenological stage of R2 (full flowering with most racemes with open flowers). 20 leafs from the superior third of the plant, being the trefoil + petiole were collected and placed in each useful plot (EMBRAPA SOJA, 2006).

The petiole of leafs was removed, the tissue were washed and then dried at 65°C in an air circulation oven until constant mass was obtained; this was after milling.

For determinations of Ca, Mg, K and Zn, leaves were submitted to nitropercloric digestion (AOAC, 2005) followed by technics of Flame Atomic Absorption Spectrometry (Welz and Sperling, 1999).

The P determinations were conducted by sulfuric digestion of leaves (AOAC, 2005), being the quantifications of the levels of P determinated by Ultraviolet–Visible Spectroscopy (UV-VIS).

At the same time that the leaf tissue was collected, soil samples were also collected in each plot, which was constituted by three sub samples collected from the sowing lines, being soil samples dried in an air circulation oven until constant mass at 65ºC, and sifted resulting in a granulometry inferior to 0.20 mm. After that was performed, Zn with Mehlich-1 and DTPA were extracted (Raij et al., 2001).

RESULTS AND DISCUSSION

The analysis of soil and leaf tissue was performed for determination of the correlation between the fertilization of P, K and Zn with the bioavailability of soybean plants. Furthermore, a variance analysis was performed for the soil and leaf tissue.

In Table 2 the variance analysis for pH, levels of P, organic matter, K, Ca, Mg and levels of Zn in the soil extracted with Mehlich-1 and DTPA are presented.

In Table 3, the means are presented in relation to the content of P in soil. The results demonstrate expressive differences between the treatments in relation to the control ($P<0.05$), however no statistical difference was found between the treatments. Significant increase ($P<0.05$) in the levels of K in the soil was observed, with increasing doses of K added at the moment of the sowing.

As can be seen in Table 4, with the use of Mehlich-1 a higher extraction of the micronutrient Zn occurred when compared to

Table 3. Levels of P and K in the soil in function of the applied doses.

Doses of N:P_2O_5:K_2O (kg ha^{-1})	P (mg dm^{-3})	K ($cmol_c$ dm^{-3})
Control	10.73^B	0.42^C
00:40:40	35.43^A	0.50^B
00:80:80	31.53^A	0.68^A

Means followed by the same capital letter in the column do not differ by Tukey test at 5% probability.

Table 4. Levels of Zn in soil extracted by Mehlich-1and DTPA in function of the applied doses.

Doses of Zn (kg ha^{-1})	Zn Mehlich – 1 (mg dm^{-3})	Zn DTPA (mg dm^{-3})
Control	1.40^B	0.38^B
1.00	4.35^A	2.14^A
2.00	5.68^A	2.43^A

Means followed by the same capital letter in the column do not differ by Tukey test at 5% probability.

Table 5. Analysis of variance for the levels of P, K and Zn in the leaf tissue of soybean plants.

		Mean squares		
FV	**DF**	**P**	**K**	**Zn**
Block	3	0.95^{ns}	1.85 ns	7.84^{ns}
Dose NPK	2	2.25^{ns}	11.31*	0.78^{ns}
Dose Zn	2	0.88^{ns}	1.55 ns	20.28 ns
NPK X Dose Zn	4	0.65^{ns}	0.32 ns	12.12 ns
Residue	24	0.89	2.96	12.35
C.V.(%)		8.61	11.98	13.22
MSD		0.96	1.75	3.58

**,* - significant at 1 and 5% of probability, respectively by the Fischer test; ns – not significant by F test.

the DTPA. In relation to the level of Zn in the soil, in both extractors statistical differences were found between the applied doses and the control (P<0.05); however the same was not observed between the applied doses (P<0.05).

These results corroborate with those obtained by Ortiz et al. (2007) and Paula et al. (1991), however these differ from the results obtained by Oliveira et al. (1999), Bataglia and Raij (1994) and Abreu and Raij (1996), when higher correlations were found for the DTPA extractor.

Evaluating the availability of micronutrients and the use of different extractors, Abreu and Raij (1996) report that for the DTPA, higher levels of Zn were obtained in the treatments that presented higher levels of organic matter, indicating that the DTPA solution extract, rather than the micronutrient ligated to the organic matter. In this way the DTPA may have extracted lower quantities of Zn in function of the levels of organic matter in the soil (less than 2.5%) and also for being a soil with acid characteristics.

Many studies cited by Galrão (1995), report the search for reliable extractors for the various cultures, facing influence of the own extractor in the soil pH. In this way, acid extractors as the Mehlich-1 can extract more Zn from the soil, because they can solubilize levels of Zn superiors to those that are available to the plants, yet complexing extractors as DTPA extract less Zn from the soil, indicating higher efficiency of the Mehlich-1 in acid soils, and the DTPA in alkaline soils.

In Table 5, the analysis of variance for P, K and Zn in the leaf tissue are presented. According to the analysis of variance (Table 5) and the mean tests (Table 6), only for the K significant increase occurred in the levels of this element in the leaf tissue with the applied doses (P<0.05).

In the case of P, the non-statistical significance (P>0.05) can be explained because this soil had a higher concentration of this element (62.10 mg dm^{-3}), being that the optimal level of P for this culture is around 12.00 mg dm^{-3} (Gianelo et al., 1995).

In relation to K, the statistical difference between the treatments can be explained by the fact that this element was in a medium concentration in the soil (0.39 $cmol_c$ dm^{-3}) (Gianelo et al., 1995), allowing the soybean culture to respond to the applied fertilization.

The obtained results of this work for concentration of Zn in the leaf tissue of the soybean are different from that presented by Ortiz et al. (2007), which demonstrate an increase in zinc concentration in the leaf tissue of soybean and corn plants, respectively, for different doses of Zn. In this way, the bibliographic results are mostly contradictories because there are many factors that can influence extraction of Zn in the soil (principal factors related to the chemical and physical composition); so, the studies should be performed in a bid to standardize the adequate extractor for similar classes of soils,

Table 6. Mean levels of P and K in the soybean leaves in function to the applied doses.

$N - P_2O_5 - K_2O$ (kg ha^{-1})	P (mg dm^{-3})	K ($cmol_c dm^{-3}$)
00 - 00 - 00	10.50^{A}	13.39^{B}
00 - 40 - 40	11.01^{A}	14.38^{AB}
00 - 80 - 80	11.35^{A}	15.33^{A}

Means followed by the same capital letter in the column do not differ by Tukey test at 5% probability.

\always correlating to concentration of the nutrients absorbed by the cultures.

Conclusion

In relation to the extractors, it was possible to conclude that the Mehlich-1 presented higher capacity of extract Zn from the soil in relation to DTPA, due to this soil acid character.

However, the extractors did not demonstrate correlation with the absorption capacity of Zn by the soybean culture, because although there was increment from the applied doses of Zn, there was not found increase of the levels of this metal on the leaf tissue.It can be concluded that the Zn fertilization for the soybean culture is not justified in the studied soil as significant difference was not found in the concentration of this metal in the leaf tissue.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Short Communication

Incidence of heart rot at pomegranate fruits caused by *Alternaria* spp. in Cyprus

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During the growing season of 2012, pomegranate growers in Cyprus indicated that they face a troublesome problem causing fruit decays. This study conducted to identify the pathogen causing fruit decays and determine disease incidence in three different pomegranate cultivars in Cyprus. Disease defined as heart rot caused by fungi: *Alternaria* spp. Incidence of heart rot was determined as 20.31, 14.91 and 9.82% for the cultivars of Acco, Herskovitz and Wonderful, respectively. The considerable variation among susceptibility of cultivars was supposed to be because of differentiation of flower colour, pollen tastes and etc of pomegranate trees.

Key words: Heart rot, *Alternaria* spp., pomegranate, transmission.

INTRODUCTION

Pomegranate (*Punica granatum* L.) plant is known to be native to central Asia (Morton, 1987). It is reported by Bevan (1919) that pomegranate plant was grown for fresh consumption and exportation during early 1900s in Cyprus. However, there was a decrease in the area of pomegranate orchards from 1960s to 2007. New plantations started to be constituted since 2007 with the alternative crops projects of United States Agency for International Development (USAID) in Cyprus and total area of pomegranate orchards close to 100 ha at the end of 2013. Many pests are causing important damages on pomegranates. According to Ksentini et al. (2011), number of pests can reach up to 91 pests in India.

In 2012, an important problem raised at the pomegranate fields in Cyprus. Growers reported up to 20% damages on the fruits. They reported that inside of the fruits are becoming black and arils are decaying. It was a big challenge for the producers and packers where this disease has no obvious external symptoms. This disease is known to be heart rot or black heart and there are some fungi causing this damage: *Alternaria* spp., *Aspergillus* spp. (Barkai-Golan, 2001), *Penicillium glabrum* and *Pilidiella granati* (Michailides et al., 2010). Therefore, this research aimed to identify the pathogen causing heart rot disease and determine disease incidence in three different pomegranate cultivars in Cyprus.

MATERIALS AND METHODS

This research performed by collecting data from 13 pomegranate orchards during the growing season of 2013. Each orchard are covering at least 1 ha areas where 80% of the each orchard is demonstrated with Wonderful cultivar, 10% with Acco and 10% with Herskovitz. All orchards were established by 5 × 3 m distance and pruned as globe shape with one trunk in 2007. Totally 772.919

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Figure 1. Abnormal skin color and internal aril decays caused by heart rot.

fruits (Acco: 65.126; Herskovitz: 91.820 and Wonderful: 615.973) were controlled and packed at the packing house of Alnar Pomegranates Ltd. Number of damaged fruits by heart rot was firstly determined by professional workers and noted. Thus indefinable and/or speculative fruits from outside (by abnormal color or weight) were cut to determine if disease exists. Fruit samples from each cultivar were examined at the Biological Control Institute of Adana, Turkey. Fungi were scratched from fruits and growed under potato dextrose agar (PDA) medium. Thus the fungi examined under microscope by Dr. Ercan Canıhoş according to spores and determined as the *Alternaria* spp.

Data from the different orchards were used as replication to calculate means and standart deviation of the studied orchards and subjected to one-way ANOVA to determine any statistical differences among species. Mean separations were done by using the Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Examination of the fruit samples resulted that the fungi causing fruit decay in pomegranates is *Alternaria* spp. The disease is known as heart rot or black heart and is a major problem in California (Barkai-Golan, 2001). It is recognized as a postharvest quality problem but the infection begins in the orchard. It causes decay on pomegranate arils ranging from sections to all the arils without external symptoms except for slightly abnormal skin color and lesser weights than normal fruits (Figure 1). Spores of *Alternaria* are airborne and found in the soil, organic materials, weeds, fruit wastes and etc. and require a vector to bring them to the hosts. Barkai-Golan (2001) reported that *Alternaria* spp. enters the fruit during bloom and early fruit set. Additional research is needed to establish inoculum dissemination and time and type of infection in the case of pomegranate orchards.

Results about the incidence of heart rot were found to be as 20.31, 14.91 and 9.82% for the cultivars of Acco, Herskovitz and Wonderful, respectively (Table 1). Firstly it was thought that Acco is a sweet cultivar and this is why *Alternaria* is densly affecting it. However, the sweetness of this cultivar is not related with Total Soluble Solids (TSS) where TSS of Acco, Herskovitz and Wonderful is about 18, 17 and 20 Brix, respectively. And, on the other hand, *Alternaria* is being transmitted during flowering stage where there is no fruit and so no sugar. *Alternaria* may also be transmitted by pests after fruit set, but no pest damages determined on fruits. On the other hand, similar findings indicated by Michailides et al. (2011) where they reported that Alternaria is generally hosting pomegranates during flowering or early fruit set. Therefore, differences between damage degrees on cultivars can not be because of the diversity of TSS.

Acco and Herskovitz cultivars are early varieties, ripening about one month before Wonderful cultivar. Thus it was thought that incidence differentiation may be because of the earlier flowering of these cultivars. During flowering, daily observations were performed to determine if there is significant difference among the flowering dates of the cultivars. It was found that flowering starts about 6 to 7 days earlier at Acco and Herskovitz cultivars than the Wonderful cultivar. Flowering starts in February and continues until May for both cultivars. However, the full bloom (open flower) dates, where the most transmission takes place (Michailides et al., 2011), are approximately equal for all cultivars.

There are some transmission ways for the *Alternaria* spp. to the heart of the fruit; these are: wind (Bashen et al., 1991; Timmer et al., 2003), rain (Chen et al., 2003) and various pests (ex: pollen beetles [*Ceuthorrhynchus assimilis* Payk. (Coleoptera: Curculionoidea)] and seed pod weevils [*Meligethes aeneus* Fabricius (Coleoptera: Nitidulidae)]) (Köhl and van der Wolf, 2005). When considering present situation, environmental factors, such as: wind and rain are almost equal for all cultivars where they are planted together. However, pest occasions can be vary on different cultivars depending on the flower characteristics and etc. Some scientific studies reported that beneficial or pest insects may act as a vector and transmit fungal pathogens (Dillard et al., 1998; Köhl and

Table 1. Incidence of heart rot at different pomegranate cultivars.

Fields	Cultivars		
	Acco (%)	**Herskovitz (%)**	**Wonderful (%)**
F.1	19.75	9.10	9.60
F.2	13.87	6.00	7.86
F.3	12.11	14.60	8.82
F.4	16.85	8.60	9.96
F.5	22.50	20.20	7.78
F.6	20.06	15.90	10.50
F.7	16.09	8.80	8.96
F.8	27.93	12.60	9.64
F.9	20.23	17.00	12.52
F.10	22.02	14.40	11.76
F.11	39.98	27.30	13.84
F.12	19.79	19.40	7.71
F.13	12.85	19.95	8.65
Average*	20.31±7.34[a]	14.91±5.97[b]	9.82±1.90[c]

* Values followed by the same letter or letters are not significantly different at a 5% level (Duncan multiple range test).

van der Wolf, 2005). Such as, Dillard et al. (1998) reported that flea beetles (*Phsyllotreta cruciferae* Goeze [Coleoptera: Chrysomelidae] can play as a vector of *Alternaria brssicicola* in cabbage fields. Another research by Palou et al. (2013) reported that main causal agents of wound and latent infections were *Penicillium* spp. and *Botrytis cinerea* on pomegranates. They also indicated that, in contrast to pomegranate cv. Wonderful, infections by *Alternaria* spp. were not present in pomegranate cv. Mollar de Elche. It is clear from the results and other studies that the susceptibility of pomegranates to infection by *Alternaria* spp. is varying among cultivars. Since this disease is being transmitted by biotic and/or abiotic factors, the variation among cultivars' susceptibility may be because of flower colour, pollen tastes and etc. Further studies need to be undertaken to determine the reason of this choice.

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Conflict of Interests

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Full Length Research Paper

Application of wavelet transform in the classification of pollen grains

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This paper presents a proposal for the automatic classification of pollen grains, to assist in the analysis of the botanical origin of honey samples. For this task, techniques based on computer vision and machine learning were used. At first, the pollen grain image was segmented using a technique based on watershed. Then, a wavelet transform technique was used to extract texture features from the segmented image. Finally, a supervised machine learning technique was used to classify the pollen grain regarding its floral species. Other attributes based on shape, texture and color were taken for comparison with the proposed method. The technique has been assessed using an image dataset from 7 different pollen species and a 79% F-Score has been achieved.

Key words: Wavelet transform, texture descriptors, machine learning, pollen.

INTRODUCTION

Pollen grain is the male gamete of plants and it is present in anthers of flowers of angiosperms plants. The study of pollen is called palynology and has been used for identification of pollen grains in organic remains, fossils, coprolites, to determine the history of developments races on the planet, in addition to forensic palynology that uses pollen grains present at crime scenes and corpses for crime detecting. Melissopalynology deals with the study of pollen grains in bee products, which is of great importance for determining the botanical origin of bee products. The study of pollen has a wide field of applications and there is a growing interest in the development of computer programs to facilitate the identification of pollen types.

There are different methods of pollen identification but all of them need a human expert to analyze images captured using an optical microscope. Due to the subjective nature of this analysis, the result of the classification can be different than expected when different humans in different moods are involved.

The difficulties and delays in pollen identification led to the search for methods that facilitate the identification of

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pollen grains and its use by non-expert persons. Automating the identification of pollen grains makes the analysis process faster and less laborious, turning the identification of a large number of grains and the pollen identification more precise (Langford et al., 1990). There were several attempts to automate the identification of pollen grains in microscopic images by computer algorithms, yet this is not a cheap and fully automated process (Chica and Campoy, 2012).

This work aims at automating the classification of pollens through a computer vision software. This software takes microscopic images of pollen grains present in honey samples and classifies the pollen types according to their floral origin. At first, the pollen grain image is segmented by the software using a technique based on Watershed, then, a Wavelet Transform technique is used to extract texture features from the segmented image. Finally, a supervised machine learning technique is used to classify the pollen grain regarding its floral species.

The main contributions of this work are: (1) the use of the Wavelet Transform technique to extract texture features for the problem of automatic classification of pollen grain; (2) the analysis of the computational costs involved in the use of different feature extraction techniques and (3) the creation of an image dataset of pollen grains that can be used in other experiments.

This paper is organized as follows: First the materials and methods used are presented, as well as the description of the image dataset and evaluation metrics. After that, the results obtained from the execution of the exploratory tests are reported. Finally, we show the conclusions and future work.

MATERIALS AND METHODS

The proposed technique for pollen species classification is composed of three main modules. The first is for the pollen detection in the image and its segmentation, the second for the extraction of image attributes to be processed and, finally, the third is a classification module based on supervised machine learning. The following topics explain the main technique used for the description of pollen grain images, called Wavelets Transform, and each one of the modules of the software developed.

Wavelets Transform

The Wavelets Transforms are mathematical functions that cut a signal in different frequency components, and each component can be analyzed in different scales, which make them widely used in the analysis of textures (Arivazhagan and Ganesan, 2003; Randen and Husoy, 1999). The central idea of Wavelets Transforms is the use of a family of functions localized in time and frequency. The Wavelets Transforms decompose a signal through a series of functions created by translations and dilations (or contractions) of a transformation function, denoted Wavelet Mother in Equation 1, where *j* and *k* are real numbers different from 0. The *k* parameter controls the displacement while *j* controls the dilatation to the function. When *j* is greater than 1, the dilation of the function occurs; and when it is less than 1, contraction of the function occurs.

$$\psi j,k(x) = \frac{1}{\sqrt{j}}\psi\left(\frac{x-k}{j}\right) \quad (1)$$

There are several examples of specific Ψ functions that can be used in practical problems and three of the most cited in current literature are the Mexican Hat (or Laplacian of a Gaussian), Morlet (or Gabor) and the Haar functions presented in Equations 2, 3 and 4, where σ is the standard deviation of the Mexican Hat Gaussian, and k is the Morlet wave number.

$$\psi_{mexican\ hat}(x) = \frac{2}{\sqrt{3\sigma}}\pi^{-\frac{1}{4}}\left(\frac{x^2}{\sigma^2}-1\right)\ e^{-\frac{x^2}{\sigma^2}} \quad (2)$$

$$\psi_{morlet}\ (x) = \pi^{-\frac{1}{4}}\ \cos(kx)e^{-\frac{x^2}{2}} \quad (3)$$

$$\psi_{haar}(x) = \begin{cases} 1, & for\ x\ \in [0,\frac{1}{2}) \\ -1, & for\ x\ \in [\frac{1}{2},1) \\ 0, & otherwise \end{cases} \quad (4)$$

The functions presented are used for the analysis of continuous signals, however, in practice the signal analysis should be performed in discrete time or space intervals. Thus, it is more convenient to use the Discrete Wavelet Transform (DWT), in which both the signal and the parameters *j* and *k* are discretized. An efficient implementation of this type of processing for multiresolution analysis (Mallat, 1989) is the Fast Wavelet Transform (FWT).

The Wavelet Transform was originally formulated for one-dimensional signals analysis, for image analysis it must be expanded to the two-dimensional space. In most cases, this expansion can be obtained from one-dimensional transformations applied separately in the vertical and horizontal directions.

Detection

The aim of the detection and segmentation of pollens module is to reduce the unnecessary information of the image for later stages. For this purpose, preprocessing techniques, extraction of texture attributes and segmentation based on Watershed were used. The execution of this module consists of a blurring of the original image using Gaussian smoothing, the extraction of texture attributes and the application of a watershed based segmentation algorithm using texture information. An example of the results of this module can be seen in Figure 1.

Extraction of attributes

Three families of attributes have been extracted from each pollen image to be used in the classification step: shape, color and texture attributes. For the extraction of shape attributes the k-curvature (Rosenfeld and Johnson, 1973) and shape descriptors (Jain et al., 1995) algorithms have been used. For color attributes, the RGB and HSB models were used. Each color component was used separately.

For texture extraction the Fractional Splines Wavelets were used (Unser and Blu, 2000). With the application of a Wavelet Transform, the image is decomposed into four sub-bands, designated as LL, HH, LH, and HL. The sub-band LL has low frequency and it is an image approximation, the other sub-bands highlight frequency information in the vertical (HL), horizontal (LH) and diagonal (HH) directions (Figure 2).

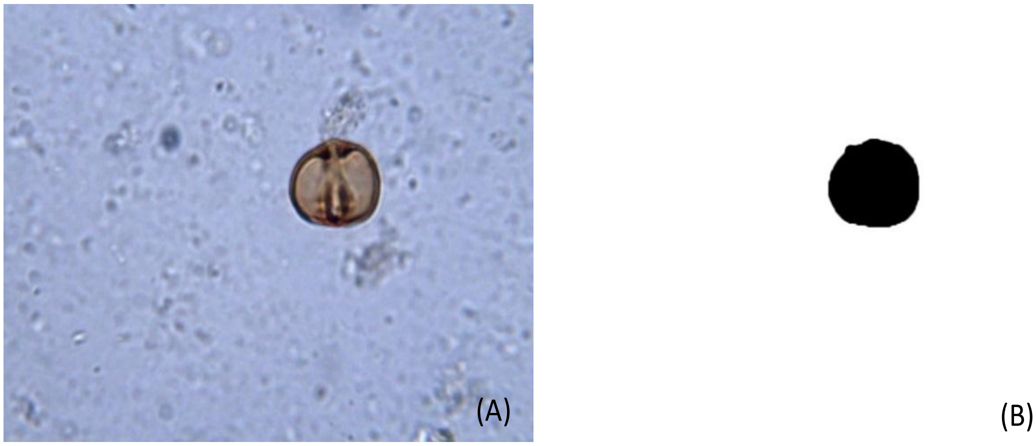

Figure 1. This image exemplifies the detection and the segmentation of a pollen grain. The Figure (A) shows the original image and (B) shows the same image after its segmentation.

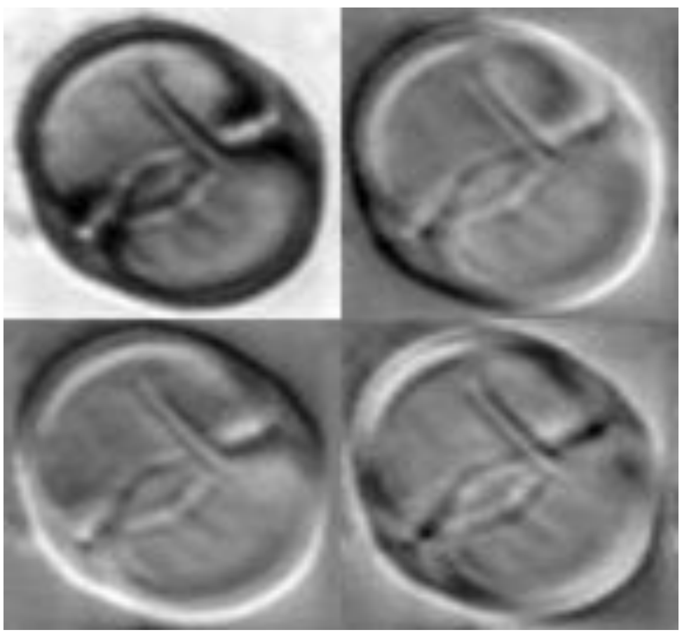

Figure 2. This image illustrates the decomposition generated by the Wavelet Transform.

A co-occurrence matrix (Haralick et al., 1973) was used in the directions of 0, 90, 180 and 270° for describing the textures for each sub-band generated by the Wavelet Transform. The attributes extracted from each co-occurrence matrix were Second Angular Momentum, Contrast, Correlation and Entropy.

Classification

After attributes extraction, a supervised learning machine module was used for training some classifiers. The implementation available through the software Weka 3.6 of the following algorithms have been tested: Decision Trees C4.5 (Utgoff, 1989), Support Vector Machines (Suykens and Vandewalle, 1999) and K-Nearest Neighbors (Guo et al., 2003).

Experiments

The main goal of the experiments conducted in this work was to determine the best arrangement of attributes and classifiers to be used in the pollen grain classification problem. All tests were performed in a set of images selected by a specialist in the field.

Dataset

To construct the image dataset used in these experiments seven classes of floral species were selected. These species were chosen due to their high prevalence in honeys collected in the Brazilian central region, where this research has been conducted. Some examples of pollens from each of the seven species used in the experiment can be seen in Figure 3; for each species 30 images were collected.

Sampling method

The cross-validation sampling method, with 10 folds, was used to select the training and testing set used for supervised machine learning. The cross-validation divides the samples into X sets, with the same size each, where X is the number of folds. After splitting the sets, X-1 sets are used to train the classifier, while the remaining set is used for the test, this process is repeated X times, considering a different set for the test in each iteration.

Metrics

The metric used to evaluate the performance of the different configurations of the proposed techniques was the F-Score, which is the harmonic mean of Precision and Recall values (Goutte and Gaussier, 2005). Its formula can be found in Equation 2. It is worth mentioning that, as there are more than two classes, the F-Score calculation is performed for each class separately, and the final result is the weighted average of the results obtained.

$$F = \frac{2*precision*recall}{precision+recall} \quad (2)$$

After the evaluation of the F-Score for each configuration the Friedman test (García and Herrera, 2008; Demšar, 2006), with a

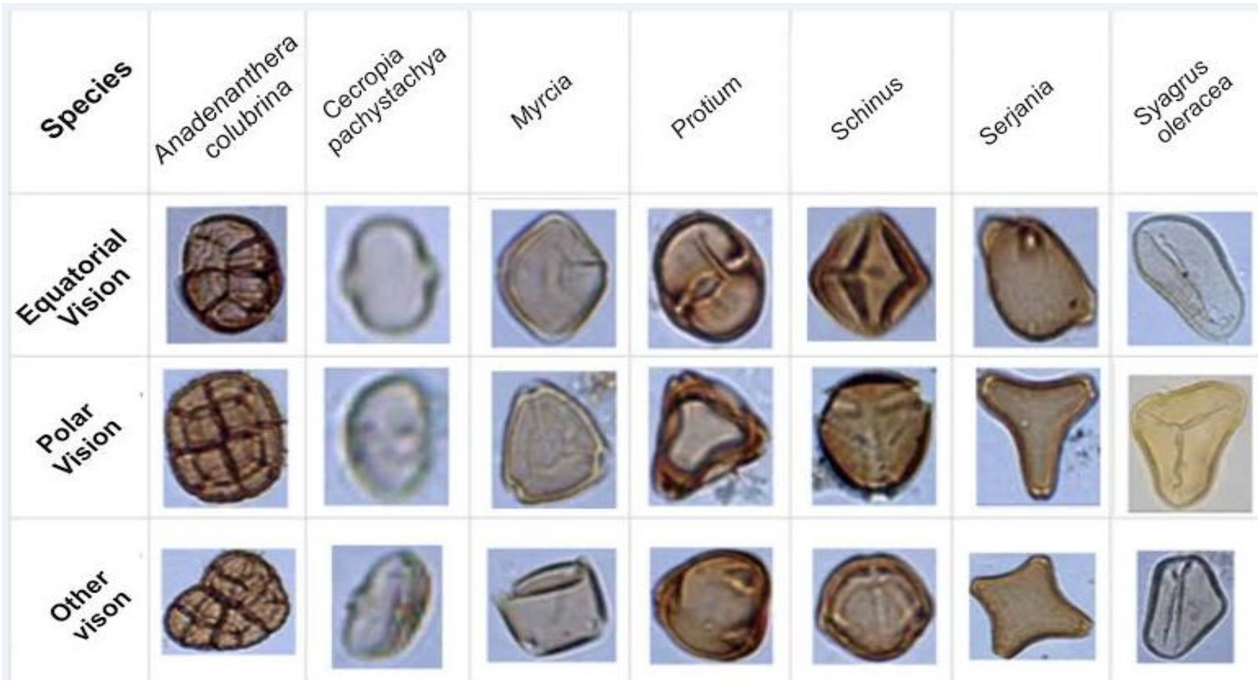

Figure 3. Pollen types used in the experiments separated in three classes.

Table 1. The final F-Score results obtained in the experiment.

Attribute	KNN	SVM	C4.5
S	0.57 (±0.10)	0.54 (±0.10)	0.57 (±0.10)
C	0.69 (±0.09)	0.74 (±0.10)	0.67 (±0.09)
W	0.50 (±0.10)	0.36 (±0.09)	0.61 (±0.10)
S+C	0.72 (±0.09)	0.79 (±0.08)	0.70 (±0.10)
S+W	0.59 (±0.11)	0.66 (±0.09)	0.66 (±0.10)
W+C	0.69 (±0.09)	0.74 (±0.10)	0.67 (±0.08)
S+W+C	0.72 (±0.09)	0.79 (±0.08)	0.70 (±0.10)

Each column represents one of the three classifiers tested. The value in parentheses is the standard deviation for each classifier (derived from the 10-Fold cross validation). Each line represents a different combination of the attributes extracted: S represents the use of only shape and colors, C represents the use of co-occurrence matrices and W are the attributes obtained by using the Wavelet Transform. S+C the combination of shape, color and co-occurrence attributes, S+W a combination of shape, color and Wavelets, and so on.

95% confidence interval (p-value < 0.05), was used to check whether there were statistical differences between the results obtained by the classifiers. The FWER (Family-wise Error Rate) based post hoc test implemented in R was used when the null hypothesis was rejected.

RESULTS AND DISCUSSION

As a large set of exploratory tests were carried out in order to determine the several parameters associated to each algorithm used in the proposed approach only the best results for each classifier are shown in Table 1. A p-value of 0.007017 resulted from the application of the Friedman test which, at a 5% level of significance (95% confidence interval), can be used to discard the null hypothesis.

The post hoc test indicated a statistical significant difference (p-value = 0.04489) between the texture attributes alone and the combination of texture and shape attributes. There is also a significant difference (p-value = 0.04502) between the texture attributes alone and the combinational of all attributes used (texture + shape + color).

Figure 4 presents the box-plots generated by the

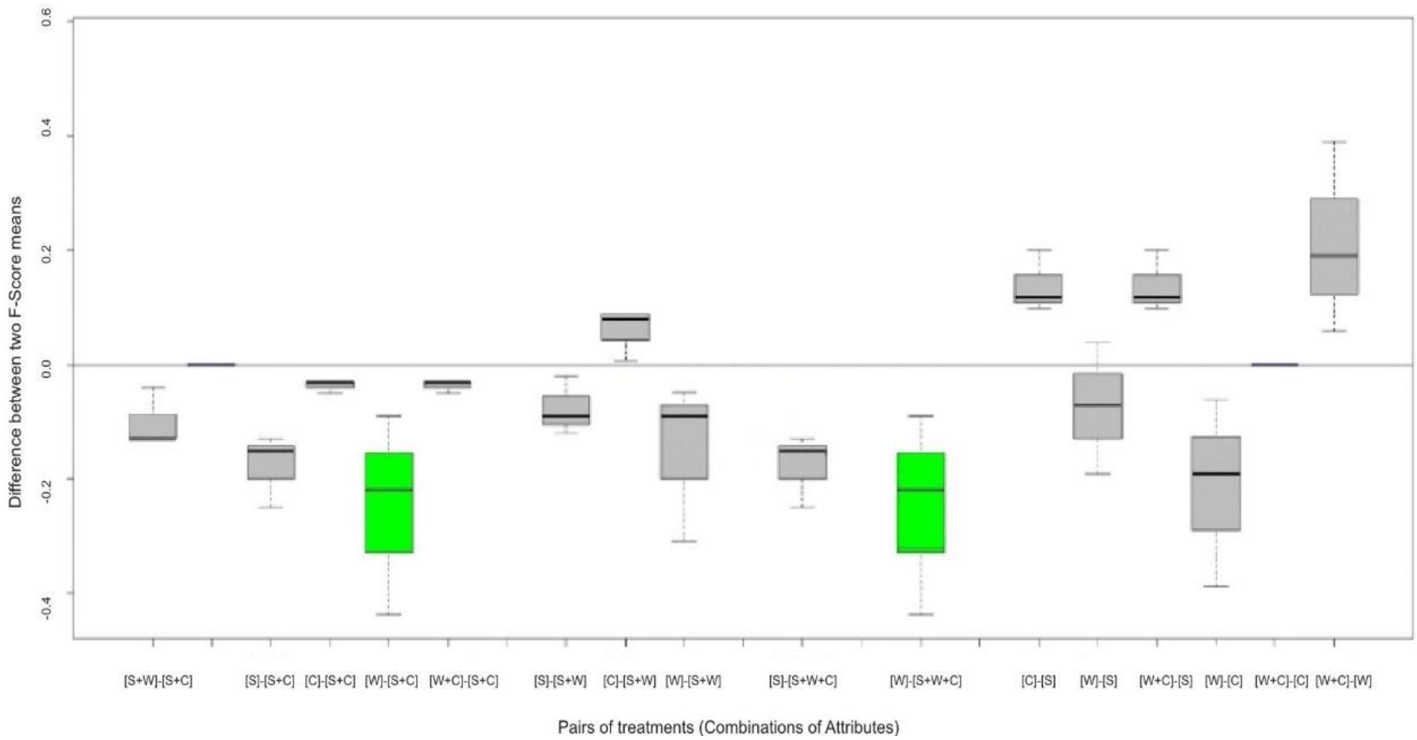

Figure 4. A box plot diagram for analysis extractor attributes.

execution of the post hoc test. It is clear by the box-plots that when the Wavelet Transform method (texture attribute) is applied without any other extractor it does not show a good performance in the classification. However, when combined with other attributes extractors, a good classification of the pollen grains can be achieved. The poor performance of the Wavelet Transform when applied alone could be explained by the fact that in the formulation used in this paper, no color or explicit shape information is used during wavelet calculations (only the gray level is used), and so, when color and shape attributes are added, the classification performance is enhanced.

In general, the combination S, C and W isolated, do not show good results, but when combined together, two by two, or all three, the results were improved. For example, the result of S, on the SVM technique, was 54% with 10% of standard deviation. Furthermore, using the combination S+C, the percentage of correct classification goes to 79 and 8% of standard deviation. With this combination, it had the best classification performance, with a lower standard deviation. Hence, the results tend to have a lower range of values and stay closer to the classification value presented, which increases the credibility of the classifier. When all three attributes are combined with the same supervised learning technique, S+W+C, the percentage and standard deviation are the same. In this case, the difference between them is only the processing time. This behavior is repeated for KNN and C4.5 techniques for the same combinations of attributes, that is, they have the highest percentage of correct classification and lower standard deviation using the combination S+C and S+W+C for each respective technique. Thus, as the approaches have the same percentage of correct answers for two different combinations, the most appropriate choice may be based on the processing time of the classification.

The processing times to train the classifiers with each different extraction methods were also analyzed in this paper. Table 2 presents the data obtained by the processing time of each method of attributes extraction. However, in the analysis of training time, the chi-square value in the Friedman test was 10.6154, with p-value of 0.101. So, the null hypothesis was accepted, once the level of significance of 5% was also adopted, demonstrating the similarity between the different types of extractions. Thus, considering the computational cost, they are statistically equals.

From the computational cost of extraction using Wavelet Transform and a combination of Wavelet Transform with some attributes, it is possible to observe that they were higher compared to other extractors. Thus, when all attributes were extracted, even with a larger number of attributes to be analyzed, the processing time was lower for those three classifiers. Therefore, it becomes feasible to use all attributes in the classification process, once the processing time is acceptable and the quantity of features to be analyzed allows a better

Table 2. Training time of each method of attributes extraction.

Attribute	KNN	SVM	C4.5
S	0.00 (±0.00)	0.08 (±0.02)	0.29 (±0.09)
C	0.00 (±0.00)	0.07 (±0.01)	0.20 (±0.06)
W	0.00 (±0.00)	0.12 (±0.02)	0.50 (±0.14)
S+C	0.00 (±0.00)	0.07 (±0.01)	0.21 (±0.06)
S+W	0.00 (±0.00)	0.12 (±0.02)	0.45 (±0.12)
W+C	0.00 (±0.00)	0.08 (±0.01)	0.20 (±0.05)
S+W+C	0.00 (±0.00)	0.08 (±0.01)	0.21 (±0.06)

classification of the pollen grains.

Conclusion

According to the results obtained, the use of Wavelet Transform for extracting texture attributes with shape attributes, do not show satisfactory results, in addition to requiring a lot of processing time. However, the application of all extractors together, showed better results in the test performed and had a relatively low processing time compared to other combinations of techniques, being feasible for application in the extraction of attributes for the pollen grain classification.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Applied multiple regression for autocorrelated sugarcane data

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The major concern with the substitution of fossil fuels is the air pollution which contributes to countries like Brazil to adopt new sources of clean and renewable energy as ethanol from sugarcane. It is extremely important to maximize the production of sugarcane mainly focused on the production of ethanol. This work aimed to establish a model of multiple regression that targets sugarcane traits, which could contribute to prediction of alcohol production. The data were obtained at São José dos Pinheiros distillery in Laranjeiras municipality, State of Sergipe. The sugarcane traits assessed were production of alcohol, Brix, percentage of sucrose (Pol) and fiber content. A descriptive analysis was carried out to verify the homogeneity of the traits and multiple regression analysis. To guarantee the consistency of results, the verification of the coefficient of multiple determinations, the significance of the model (ANOVA), the Student t-test and the mean absolute percentage error (MAPE) was performed. The software utilized was the Statistica. Through the regression by the method of ordinary least squares (OLS), the simulation results pointed an autocorrelated residue. Therefore, the application of a correction method, the iterative process Cochrane-Orcutt, to remove the autocorrelation of the variables is required. The new model allows found statistically significant coefficients at the 5% probability. With the new equation, it can be concluded that values of Pol and Fiber content in sugarcane may assist the prediction industrial sugarcane production.

Key words: Multivariable analysis, autocorrelation, *Saccharum* spp.

INTRODUCTION

The sugarcane (*Saccharum* ssp.) is a species from Asia which was brought to Brazil during the colonization in the first decade of the sixteenth century.

The social-economic importance of sugarcane has ancient roots in the national agribusiness due to potential production, which adapts easily in tropical climate with

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specific rainfall and solar lighting, besides be source for sugar and biomass energy.

Brazil is the world largest producer of sugarcane, with 604,513,600,000 tons in 2009/2010 crop year, in an area of 604,513.6 billion hectares, and with a yield of 81.585 kg/ha (CONAB, 2010). This performance is due taking into account, that the real agricultural productivity in many regions is only a small part of the real genetic potential of the crop (Taiz and Zeiger, 2004).

Interuniversity Network for the development of sucro-energetic sector (RIDESA) is an example of successful breeding networking in Brazil. For the logistic, each university creates clones in their respective states from seeds produced at “Serra do Ouro” Germplasm Bank. Annually, the best clones selected from each university are interchanged, which allow an increasing number of genotypes to be evaluated by each university in their respective edaphic and climatic conditions. The network is constituted by ten federal universities: UFPR, UFSCar, UFV, UFRRJ, UFS, UFAL UFRPE, UFG, UFPI and UFMT.

At the agro-industrial fields, new experimental plots are established for a period of 3 consecutive years. This phase is called Experimental Phase (EP). The promissory material is able to be registered as cultivars.

RIDESA was established in order to incorporate the activities of the extinct Planalsucar Program, and has the development continued for research aiming to improve the productivity of the sector. Its activities’ are based on thedevelopment of experimental research in 31 research stations strategically located in states where the sugarcane crop has higher expression.

In the state of Sergipe are six sugarcane agro-industry as São José dos Pinheiros in Laranjeiras municipality; the Taquari, UTE- Iolando Leite (Old distillery Carvão) and the Junco Novo, located in the municipality of Capela-SE; CBAA-Japoatã in Japoatã and the Campo Lindo, in the municipality of Nossa Senhora das Dores. Of these six distilleries, only São José dos Pinheiros is a RIDESA partnership and its data were used in this work.

Ethyl alcohol or ethanol is a flammable liquid obtained by the distillation of fermented organic products such as sugar, starch and cellulose. Ethanol from sugarcane is obtained from sucrose (sugar) broth. At the end of the distillation and rectification process, the product generated is an ethanol-water mixture that can be used directly as automotive fuel, or can undergo dehydration to obtain the anhydrous alcohol which is used in a mixture with gasoline as addictive.

According to information from National Electric Energy Agency (ANEEL), Brazil currently has 266 distilleries burning bagasse that produce electricity. These companies work with capacity of 3,682 MW, equivalent to 3.5% of Brazil's total generating facilities, or 16% of the energy produced by thermal sources in the country.

In recent decades, Brazil has doubled the area planted with sugarcane, primarily due to the production of alcohol, this expansion occurred mainly in regions with fertile soil and favorable climate (Takeshi, 2008).

For the production of alcohol (ethanol), Brazil is the second major producer, behind only the USA. Both countries account for 70% of all ethanol produced in the planet (Council for Biotechnology Information, 2009). However, the American product is exclusively derived from corn and supply the internal market. Thus, Brazil is also the world's largest ethanol exporter accounting for 54% of the market.

The production of ethanol from sugarcane contributes to environment preservation and sustainability in supply chains. Their systematic use can promote technical progress and the advent of positive changes in ecological impact and working conditions. In addition, the sugarcane is CO_2 sequester from atmosphere which is important to reduce the effect of global warming (Orlando Filho, 2007).

The prediction of alcohol production for sucro-energetic agro-industries, or even for small producer is extremely important aiming strategies to make decision on planting dimension, and reduce the risks of this farming activity. However, it is not just the knowledge that represents the characteristics of sugarcane production; we can estimate models to relate independent production traits aiming prediction of the possible commercial alcohol production. The method of multiple regression permits to create an equation showing the relationship between independent variables which influence on a dependent variable.

The objective of this work was to establish a model by multiple regression analysis, aiming insert variables of sugarcane, which could contribute to the prediction of ethanol production.

MATERIALS AND METHODS

The data of ethanol production were obtained at São José dos Pinheiros distillery and refers to period of 2009/2010. The traits used were Brix (total solid content, sugars and non-sugars), the percentage of sucrose (Pol), the Fiber content expressed in ton per day, and the alcohol yield expressed in liters per day. A descriptive analysis of the data was performed to verify the homogeneity of variables. The software Estatistica was used for all estimative.

Model of multiple regression

The multiple regression analysis according to Tabachnick and Fidell (1996) is defined as a set of statistical techniques that allows the evaluation of the relationship among a dependent variable, and several independent variables. The major objective of this analysis is to identify the equation that describes the relationship between these variables so that we can predict the value of dependent variable attributing values for the independent variables (Ragsdale, 2001; Subramanian et al., 2007).

According to Anderson et al. (2002), this model can be described as:

$$y = \beta_0 + \beta_1 . x_1 + \beta_2 . x_2 + \ldots + \beta_p . x_p + \varepsilon \quad (1)$$

Where $x_1, x_2, \ldots, x_p$ are constants and $\beta_0, \beta_1, \beta_2, \ldots, \beta_p$ are parameters called partial regression coefficients and ε the residues.

The following assumptions is assumed for the model:

(1) The error vector $\varepsilon = [\varepsilon_1, \varepsilon_2, ..., \varepsilon_n]$ is random, that is, the components $i = 1,2, ..., n$ are random variables;
(2) The hope of each component of ε is zero, that is, $E(\varepsilon) = 0$;
(3) The components of the vector ε are not correlated or better $(\varepsilon_i, \varepsilon_j)$ = 0, i≠j and have constant variance, ... Thus, the covariance matrix of ε is the diagonal matrix...In, where In is the identity matrix of order n, $V(\varepsilon) = \cdots In$. The distribution of ε_i $i = 1,2, ..., n$ is the Normal.

Given this assumption, we have the model:

$$y/_x \sim Normal\ (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k) \quad (2)$$

To assure the quality of the results, the coefficients of the multiple regression model, the significance test, the analysis of residues and the Mean absolute percentage error (MAPE) were calculated

The coefficient of multiple regression

To guarantee the quality of the results, the coefficients of the multiple regression, the significance test, and the analysis of residues were estimated.

The partial coefficient of regression

This coefficient measures the strength of the relationship between the dependent variable and an independent, when the effect of the other independent, are constant. This value is used to identify the independent variable with the high explanatory power of the dependent variable in addition to other variables in the model.

The beta coefficient

This is the coefficient of standardized, or that is used for a process in which the original data are processed into new variables with mean equal to zero. A standard deviation, and thus the term βo (intercept) assume the value of zero thereby enabling a direct comparison among the coefficients and relative powers of explanation of the dependent variable.

The coefficient of correlation (R)

This coefficient indicates the strength of association between any two metric variables. The value can range from -1 to +1 and the sign indicates the direction of the relationship.

The coefficient of multiple determinations (R^2)

R^2 measures the degree of the regression equation fits to, and it indicates the proportion of variance in the dependent variable according to its mean that is explained by the independent variables (Gujarati, 2000).

The significance test of the model

The significance test of the multiple regression model was performed, the F-test which is the average square divided by the average of the squared residuals (errors). According to Levine et al. (2000), this test is used to test whether there is a significant relation between the dependent variable and the entire set of independent variables.

Considering that there is more than one independent variable, you use the null hypothesis and the alternative hypothesis:

H_0: $\beta_1 = \beta_2 = ... = \beta_K = 0$ (There is no linear relation between the dependent variable and the independent variables.)
H_1: At least one non-zero B_j, j=1,2,…,k (There is a linear relationship between the dependent variable and at least one of the independent variables).

The residues

To verify whether a model is appropriate, it is necessary to investigate if the assumptions made for the development of the model are satisfied.

For this purpose, the behavior of the model is studied using the set of observed data, notably the discrepancies between the observed values and the adjusted values by the model and this way proceeded to analyze the waste (Bussab, 2002).

Basically, this analysis provides evidence of possible violations of the model assumptions, such as normality and homoscedasticity, and when necessary it provides signs of lack of adjustment from the proposed model (Charnet et al., 1999; Subramanian et al., 2007). The residue for the observation *i* is the difference between the observed value of y_i and your estimated value:

$$\hat{u}_i = y_i - \hat{y}_i \quad (3)$$

According to Russo et al., (2010), we can evaluate the suitability of the adjusted regression model by plotting the residues on the vertical axis and the corresponding values to the values of X_i of the independent variable on the horizontal axis. If the adjusted model is appropriate for the data, there will be no apparent pattern of waste relative to X_i. However, if the adjusted model is not appropriate, there will be a relation between the values of X_i and the residues ε_i (Levine et al., 2000).

The best model will be the one in which is calculated the lowest MAPE:

$$MAPE = \frac{\Sigma\left|\frac{Xi - Yi}{X_i}\right|}{n} . 100\% \quad (4)$$

Where X_i are the observed and estimated values. Then, it was detected the existence of autocorrelation of the variables included in the model.

The autocorrelation

When the hypothesis of homoscedasticity (constant variance) is not kept, we say that the errors are heteroskedastic.

When $Var\,(u_t/X)$ depends on X, it often depends on the explanatory variables of the model t, x_t . When considering the hypothesis of the absence of serial correlation $Corr\,(u_t, s_t) = 0$ false, we say that the errors suffer serial correlation, or autocorrelation, because they are correlated over time (Wooldridge, 2006).

The autocorrelation function measures the correlation degree from a variable, in a certain instant, with itself, in an instant of time later. It allows analyzing the degree of irregularity of a variable.

The test to detect the autocorrelation was developed by the statisticians J. Durbin and G.S. Watson and it is commonly known as statistical d of Durbin-Watson and it is defined as the ratio between the sum of squared differences of successive residues and the residuals squared sum (RSS).

The Durbin-Watson statistic evaluates the existence of residuals independence, that is, it tests the null hypothesis that the covariance between the residuals variables is zero. The reference value of Durbin-Watson should be 2.00, so in that way the correlation does not occur.

Based on the results obtained through the regression analysis, it was necessary for the application of the Cochrane-Orcutt corrective method to remove the autocorrelation of the variables.

Cochrane-Orcutt corrective method

In the presence of serial correlation, the estimators of ordinary least squares (OLS) are inefficient and it is essential to seek corrective measures. The solution depends on the knowledge about the nature of interdependency of disturbances; one of the situations (addressed in this study) is when the structure of autocorrelation is unknown (Wooldridge, 2006; Gujarati, 2000).

Suggestion is used for the interactive process of Cochrane-Orcutt, which is a method based upon the statistic of Durbin-Watson that uses estimated residuals $\hat{u}_t$ to obtain information about the unknown (Gujarati, 2000).

Consider the model of two variables:

$$y_t = \beta_0 + \beta_1 . x_1 + \mu_t \quad (5)$$

And suppose that $\hat{u}_t$ is generated by the scheme AR(1), that is,

$$\mu_t = \rho\mu_{t-1} + \varepsilon_t \quad (6)$$

This way, Cochrane and Orcutt recommend the following steps to estimate:

(1) Estimate the model by the usual routine of the OLS and obtain the residues, $\hat{u}_t$.
(2) Using the estimated residuals, run the following regression:

$$\hat{u}_t = \hat{p}\hat{u}_{t-1} + V_t \quad (7)$$

(3) With the result of Equation 5, run the general difference equation:

$$(Y_t - \hat{\rho}Y_{t-1}) = \beta_1(1-\hat{\rho}) + \beta_2 X_t - \hat{\rho}\beta_2 X_{t-1} + (u_t - \hat{\rho}u_{t-1}) \quad (8)$$

or

$$Y_t^* = \beta_1^* + \beta_2^* X_t^* + \hat{e}_t \quad (9)$$

(4) At first, we are not sure whether the result of the Equation 9 is the "best" estimate. Replace the values of $\beta_2^* = \beta_1(1-p)$ and β_2^* obtained from the Equation 9 in the original regression of Equation 5 and obtain the new residuals, shall we say, like this:

$$\hat{u}_t^{**} = Y_t - \hat{\beta}_1^* - \hat{\beta}_2^* X_t \quad (10)$$

which can be easily calculated, because Y_t, X_t, β_1^* and β_2^* are all well-known.

(5) Now estimate this regression:

$$\hat{u}_t^{**} = \hat{\rho}\hat{u}_{t-1}^{**} + w_t \quad (11)$$

which is similar to Equation 5. Thus, $\hat{\rho}$ is the estimate of the second hound of ρ. As we do not know whether this estimate of the second hound of ρ, we may enter in the estimate of the third hound, and so forth. As suggested by the previous steps, the Cochrane-Orcutt method is interactive (repetitive). But how far should it proceed? Generally, to stop with the successive repetitions of ρ when this differs from values lower than 0.01 (Gujarati, 2000).

Then, it was carried out a regression variance analysis.

Regression variance analysis

The idea of the variance analysis is to compare the variation due to the treatments (varieties) with the variation due to the chance (residual), according to Medeiros (1999). In order to calculate the variance analysis, we should first calculate the degrees of freedom (G1), the correction factor (C), so that we calculate the sum of squares, the squared mean and the value of F.

In an experiment with K treatments and r repetitions, the average treatments indicated by $Y_1, Y_2, Y_3, \ldots, Y_k$ and the grand total which is given by the total sum of the treatments being = Σy, we should have:

The degrees of freedom for the treatments = k-1; for the total = n-1, with n = kr; for the residuals = (n-1)-(k-1) = n-k

The Correction factor

$$(C) = (\sum y)2/n \quad (12)$$

The sum of total squares

$$(SQT) = \sum Y^2 - C \quad (13)$$

The sum of squares of treatments

$$(SQTr) = \sum k^2/r - C \quad (14)$$

The sum of squares of residuals

$$(SQR) = SQT - SQTr \quad (15)$$

The mean square of treatments

$$(QMTr) = SQTr/k - 1 \quad (16)$$

The mean square of residual

$$(QMR) = SQR/n - k \quad (17)$$

The value

$$F = QMTr/QMR \quad (18)$$

For theoretical reasons, a variance analysis should only be applied to a set of data when it can admit certain assumptions, such as the independence, normality and homoscedasticity (Vieira and Hoffmann, 1989).

The F-value is linked to the number of freedom degrees of treatments (numerator) and to the number of freedom degrees of residual (denominator) and it is the value which measures the level of significance. When the calculated F is higher than the value of the critical F (tabulated), we can conclude that the regression is significant.

When the F calculated is greater than the F critical value (tabulated), it can be concluded that the regression is significant (LEVIN AND FOX, 2004).

Table 1. Summary of statistical descriptive analysis of the model of multiple regression for ethanol production of data obtained in São José dos Pinheiros distillery, Laranjeiras-SE, 2011.

Variable	Average	Median	Variance	Standard deviation	CV%
Ethanol production	101,746.0	113,619.0	921,080,129	30,349.30	0.30
Pol	17.2	17.1	1	0.84	0.05
Fiber	14.7	14.9	0	0.61	0.04

Table 2. Model of coefficients of multiple regression for ethanol production obtained from data of the São José dos Pinheiros distillery, Laranjeiras-SE, 2011.

Variable	Beta	Standard coefficient Beta	B	Standard error of B	t	p-value
Intercept			58,032.8	66,595.76	0.8714	0.3853
Pol	0.2938	0.0864	13,242.5	3,897.95	3.3972	0.0009
Fiber	-0.2218	0.0864	-15,168.3	5,914.80	-2.5644	0.0116

Dependent Variable: Alcohol production (Palcohol).

RESULTS

Descriptive analysis of the variables

The record of the descriptive statistics of the multiple regression for alcohol production is described in Table 1. The model was used to verify the homogeneity of variances.

Table 1 show the Pol and Fiber variables with coefficients of variation (CV) of 0.05 and 0.04%, respectively for the most homogeneous variables; while Alcohol production (Alcohol yield) with coefficient of variation 0.30% stands out as the most heterogeneous variable.

To create a multiple regression model in which the alcohol production is dependent feature, numerous equations with the sugarcane traits would eventually be adopted by the independent by the model in such a way that their coefficients were significant. The model of multiple regression chosen was that with Pol and Fiber production traits as predictors of alcohol production.

From this analysis, the coefficients of the model of multiple regression by the method of OLS for alcohol production carry the following equation which was estimated:

$$\text{Alcohol yield} = \frac{58032.8}{(66{,}595.76)} + \frac{0.2938}{(3{,}897.95)}\text{Pol} - \frac{0.2218}{(5{,}914.80)}\text{Fiber} \qquad (19)$$

R = 0.37, R^2 =14.13%, R^2 Adjust = 12.63%, Standard of estimate = 24955, DW = 1.18, MAPE = 26.78%.

According to linear correlation coefficient (R) found, the level of linear association between these variables is 37%, while 14.13% of the variability in the dependent measure (Alcohol production) is explained by Pol and Fiber, independent variables.

From the student's t-test and the respective p-values (Table 2), we observe that all coefficients are significant at 5% probability, thus, indicating that both Pol as fiber influence on the dependent variable alcohol yield.

DISCUSSION

Considering all observations (119) and the two independent explanatory variables (Pol and Fiber), the observed value for statistic of Durbin-Watson (which assesses whether independence of error, in other words, which test whether the null hypothesis that the covariance between variables residual is zero) was 1.18.

This value is inferior to the reference value of 2.00, indicating that the correlation between residues is present. Thus, it was necessary to apply the corrective method of Cochrane-Orcutt (CO) to remove residues autocorrelation.

Once performed the corrective measures that method of Cochrane-Orcutt proposes. The new data generated were used to perform another multiple regression analysis, with consequence determination of the coefficients and the explanatory equation, as well as the value of Durbin-Watson was calculated for the new model found with the purpose of verifying absence of autocorrelation between residues.

According to Table 3, from the student's t-test and the respective p-values, we can observe that all coefficients are highly significant at 5% probability, thus, indicating that both Pol and Fiber influence on the Alcohol production, dependent variable.

These coefficients contained in Table 3 are related to regression analysis of the model fixed, according to the following equation:

$$\text{Alcohol yield} = \frac{214{,}944.7}{(81766{,}93)} + \frac{0.2491}{(3{,}000.06)}\text{Pol} - \frac{0.3619}{(4{,}167.44)}\text{Fiber} \qquad (20)$$

Table 3. Model of coefficients of multiple regression after removal of autocorrelation for Ethanol production from data obtained in the São José dos Pinheiros distillery, Laranjeiras-SE, 2011.

Variable	Beta	Standard coefficient Beta	B	Standard error of B	T	p-value
Intercept			214,944.7	81,766.93	2.287	0.00973
Pol	0.2491	0.831	8,995.8	3,000.0	2.9985	0.00332
Fiber	-0.319	0.0831	-18,152.2	4,17.44	-4.3557	0.00002

Dependent variable: Alcohol production.

Table 4. Analysis of variance of the regression for the ethanol production obtained from data of the São José dos Pinheiros distillery, Laranjeiras-SE, 2011.

Sum of squares	GL	Mean	Square mean	F	p-value
Regression	2.1753	2	1.0876	14.5133	0.000002
Residue	8.933	116	7.4943		
Total	1.0868				

R = 0.44, R^2 = 20%, R^2 Adjust = 18.63%, Standard of estimate = 24955, DW = 1.95, MAPE = 19.85%.

Based on the estimation of the new linear correlation coefficient (R) for the prediction model of ethanol production, which was 44%, it can be observed that the level of linear association of these variables increased. The coefficient of multiple determination of the model indicates how much variability in the dependent measure (alcohol production) is explained by the independent variables and Pol Fiber also had an increasing (R2 = 20%).

The Durbin-Watson value calculated 1.95 for the new production model of alcohol proves the elimination of autocorrelation between the residues of the series.

The MAPE of the model of multiple regression for ethanol production was 26.78%, and the model generated after the removal of this error autocorrelation decreased to 19.85%, thereby improving the level of fitness of the model.

The analysis of variance contained in Table 4 provides the value for the F-test to verify the null hypothesis with all coefficients and are statistically equal to zero, against the alternative hypothesis that at least one of the coefficients is different from zero. Since the value of the F-test is 14.5133, and confirmed by statistical p-value, 0.000002, it is confirmed that at least one coefficient is statistically different from zero. As the calculated F is higher than the critical F-value (tabulated), it can be concluded that the regression is significant.

According to this study, the model of multiple regression generated from sugarcane data, which is aimed at predicting the future alcohol production presented a residue of autocorrelated variables except Brix. It is necessary to apply a corrective to the other variables method.

After removal of autocorrelations, the new equation generated by the model of multiple regression expresses how each independent variable is influencing the prediction of the dependent variable, which will facilitate the work of the farmer in planning the sugarcane planting and sizing alcohol production.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Resistance of grain of maize landrace varieties under breeding in Southern Piauí to attack by *Sitophilus zeamais*

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The objective of this study was to evaluate the resistance of grain of maize landrace varieties to attack by *Sitophilus zeamais* in new breeding program in Brazil. The experiments were performed in the Zoology and Animal Nutrition Laboratory, Federal University of Piauí/Professor Cinobelina Elvas Campus (UFPI/CPCE) in Brazil, using seed of two maize landrace varieties, one with two origins [purple straw maize from São Paulo (SP) and Espírito Santo (ES)] and Peruvian purple maize. The experimental design was completely randomized with four replications. The data for each response variable were subjected to analysis of variance, applying the F-test ($p \leq 0.05$), and when differences were significant, there was a comparison of means by Tukey test ($p \leq 0.05$). The statistical analyses were performed using SAS ® version 9.0 software and the analysis procedure was PROC GLM. It was observed that the maize varieties differed significantly by Tukey test in relation to the attack of *S. zeamais*, and the variety purple corn straw from SP stands out as the most resistant. The other varieties proved susceptible to insect attack.

Key words: Selection, genetic variability, grain resistance.

INTRODUCTION

Brazil is the third largest producer of maize, with an average contribution of 6% of the world supply (Duete et al., 2009). The crop is planted throughout the Brazilian territory, setting it apart from other crops by occupying the largest planted area in the country (Souza and Braga, 2004). In Brazil, it is the grain with the largest volume of production (76.07 million tons), occupying 15,410,000 ha and representing 41.45% of total grain production. In the state of Piauí, production of the cereal is of approximately 797,400 tons (CONAB, 2013).

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Although human consumption of maize and its derivatives is not as large as is animal feed, this cereal is a daily source of energy for a large part of the population of the semi-arid (Naves et al., 2004). Maize breeding practices evolved as knowledge accumulated about the cereal and requirements arose to satisfy nutritional needs in many countries whose populations have maize as a dietary staple (Poletine et al., 2004). In this context, the use of improved varieties, well adapted and with desirable agronomic attributes, in place of traditional varieties, will provide improvements in maize yields (Cardoso et al., 2003).

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidade), commonly known as the maize weevil, is considered one of the most important storage pests in tropical regions (Silveira et al., 2006). Thus, resistant varieties can be an alternative to control this pest due to ease of use, not burdening the producers, absence of contamination in grain, compatibility with other control methods, and offering less risk to human health and animals (Lara, 1991). To develop varieties resistant to insect pests, studies are needed to detect genotypes to serve as a source of resistance.

The heritage of resistance to arthropods has been extensively studied and documented (Smith, 2005). Initial studies dating from the early twentieth century, when Harlan (1916) demonstrated that resistance to the mite *Eriophyes gossypii* Banks is an inheritable character in cotton. Painter (1951) defined plant resistance to insects as the sum of the relative inherited these qualities, which affect the intensity of the damage caused by the insect.

For Morais and Pinheiro Neto in Fritsche and Borém (2012), the size of the infesting population influences the manifestation of resistance. Seno et al. (2009) found that some genotypes such as C 701 and AG3050, evaluated under laboratory conditions, showed an outstanding resistance to attack by *S. zeamais* in areas with high population pressure of infestation by these beetles. In addition, several other factors may influence the resistance of maize grain to *S. zeamais*. Singh and McCain (1963) and Veiga (1969) found that chemical composition and especially low sugar content are important factors for resistance. While Classen et al. (1990) found that the protein content is negatively correlated with the number of eggs laid by females in the grains. Arnason et al. (1993) found that the lipid content correlates negatively with susceptibility of genotypes to attack by *S. zeamais*.

According to Lara (1991), when a plant, under the same conditions, is less used by an insect for oviposition or feeding than is another, it presents an antixenosis type resistance, causing a behavioral response of the insect in relation to the plant. When a plant exerts an adverse effect on the biology of an insect under normal feeding conditions, it is said that it presents antibiosis type resistance. This is the resistance mechanism most sought after by plant breeders - it is tolerant and in relation to others and it suffers little damage when infested by an insect species, without influencing the behavior and biology of the same. Thus, with the existence of genetic variability, it is possible to accumulate factors of resistance in a new cultivar against a particular pest.

The objective of this study was to evaluate the resistance of grain of maize landrace varieties to attack by *S. zeamais* in new breeding program in Brazil.

MATERIALS AND METHODS

The experiments were performed in the Zoology Laboratory at the Federal University of Piauí/Professor Cinobelina Elvas Campus (UFPI/CPCE) in the town of Bom Jesus -PI , using seed of two landrace varieties of maize, one with two origins [purple straw maize from São Paulo (SP) and Espírito Santo (ES)] and Peruvian purple maize, in early breeding, being conducted by this University. The seeds received no treatment for the control of insect pests or fungi. The adults of *S. zeamais* used in this experiment were obtained from the laboratory stock population.

The nutritional analysis of grain was performed at the Animal Nutrition Laboratory at UFPI/CPCE. Grain samples (100 g) of each variety were ground separately and aliquots of these samples were subject to moisture determination by weight loss at 105°C until constant weight. Ashes were obtained by incineration of the material in an oven at 550°C. The crude protein (CP), neutral detergent fiber (FDN), acid detergent fiber (FDA), crude fiber (FB) and lipid/ether extract (EE) were determined using the methodology of Silva and Queiroz (2002). The experimental design was completely randomized with four replications. The results were expressed on a dry weight basis. Analysis of starch was performed using the methodology of Hendrix (1993), in the experimental scheme previously adopted.

The free choice test, used to determine the feeding preference of the insects, was performed using an arena consisting of a circular plastic tray of 15 cm diameter and 8 cm in height, filled with Styrofoam with a height of 7 cm. The same was provided with five cylindrical holes in the extremities, with transparent plastic vials 5 cm in diameter and 7 cm in height, equidistant from a central circle of 5 cm in diameter containing a cover with an orifice of 10 cm radius in the middle, closed with white 'voile' tissue. The central region received 50 adult insects, not sexed; the vials at the extremities received 50 g of grains of the two maize landrace varieties (3 sources), red maize and a control (commercial hybrid usually planted in the region, with semi-hard grain and orange color). The experiment was conducted with four replicates and assessment of the number of insects on each maize variety occurred 0.5, 2, 5, 12, 24 and 48 h after exposure.

The bioassay for assessing the nutritional index, where the rate of development of the insects was assessed, was completely randomized in design with ten replicates (no choice test). The experiment consisted of plastic jars of 150 ml capacity containing 10 g of maize grain and infested with 30 unsexed adult insects of *S. zeamais*. After 10 days, the insects were removed, the number of live and dead insects was counted and they subsequently were weighed. After removal of insects the final grain weight was measured on an analytical balance. The following nutritional indexes were calculated for each combination of deltamethrin-sprayed dose and maize weevil strain, as described elsewhere (Isman et al., 1990; Huang et al., 1997): relative growth rate (RGR) (daily weight gain per unit of insect weight), relative consumption rate (RCR) (daily consumption rate per unit of insect weight), conversion efficiency of ingested food (ECI) [(relative growth rate / relative consumption rate). 100]. Thereafter, the same material was used to determine the rate of population growth. After 90 days, the

Table 1. Analysis of variance for nutritional characteristics (starch (ST), ash, crude protein (CP), neutral detergent fiber (FDN), acid detergent fiber (FDA) and ether extract (EE)), for grains of maize landrace varieties and test mean. Bom Jesus, PI, 2012.

SV	DF	ST	ASH	CP	FDN	FDA	EE
		MS					
Varieties	3	15.84**	0.20**	19.59**	225.47**	6.70**	1.70**
Error	12	0.78	0.03	0.30	2.03	0.60	0.25
h^2		0,95	0.86	0.98	0.99	0.91	0.87
CV%		1.54	13.15	4.55	6.64	15.59	11.73
Mean		57.24	1.33	12.03	21.45	5.00	4.27
Varieties		Means					
Purple		58.82[a]	1.26[b]	14.32[a]	19.03[c]	4.32[b]	4.06[ab]
ES		57.74[a]	1.63[a]	10.60[b]	13.29[d]	4.04[b]	3.45[b]
SP		58.08[a]	1.36[b]	13.49[a]	22.26[b]	4.76[b]	4.70[a]
30F35		54.33[b]	1.09[b]	9.74[c]	31.25[a]	6.89[a]	4.48[a]

*,**, ns = significant at 5 and 1% probability level and not significant respectively by F test. Means followed by the same letter in the column do not differ by Tukey test at 5% probability. SV (Source of variation), DF (Degrees of freedom), MS (Medium Square), CV (Coefficient of variation), h^2 (heritability).

mass consumption (MC), emergence (EMERG), number of live insects (NIV) and number of dead insects (NIM) was recorded.

The data for each response variable were subjected to analysis of variance, applying the F-test ($p \leq 0.05$) and, when significant differences were observed, a comparison of means was done by Tukey test ($p \leq 0.05$). The statistical analyzes were performed using SAS ® version 9.0 software and the analysis procedure was PROC GLM. With the results of the analysis of variance heritability was estimated in the broad sense for all traits, using the formula described by Vencovsky and Barriga (1992).

RESULTS AND DISCUSSION

According to Oliveira et al. (2004), corn is widely used in food and feed as an energy source due to its high starch content, available in easily digestible and low cost manner. However, in terms of the protein source is weak as well as having low average levels (about 10%), its protein is of low quality, especially in relation to essential amino acids. Table 1 shows that there were significant differences for all variables analyzed and that the purple (Peruvian purple) maize had the highest result for CP (14.32). The variety ES showed the lowest values for all variables, except for ash content (1.63). The amounts of ash and EE are in agreement with those obtained by Gonçalves et al. (2003), who evaluated the yield and chemical composition of maize cultivars in dry milling and production of grits. The statistical difference for the variable ashes indicates that there are differences in the mineral content of the varieties. In relation to human food the oil content is important since it is a source of energy. In this study, it can be seen that the ES variety showed the lowest value (3.45). The values found here are lower than those found by Castro et al. (2009), which averaged 5.69%.

Despite these differences, the nutritional content of variables: starch, ash, CP, NDP and ADF did not affect the resistance of maize varieties to attack by *S zeamais* and, of nutritional parameters evaluated, the lipids (EE) were most influenced the resistance of varieties to attack by *S. zeamais*, as they showed a positive correlation with the biological cycle of the pest. The results corroborate those found by Marsaro et al. (2005), when working with nutritional components in maize hybrids and indicate that the increase in lipid content in the grains causes the increase of the biological cycle, which consequently provide fewer generations produced by *S. zeamais*. The CVs are low, indicating good experimental precision. The high heritability values indicate the possibility for gain via selection. These characteristics were not influenced by the environment reflected in high heritability values, so that recommending a particular variety or origin for cultivation would result, in future generations, and gain in grain quality.

In Table 2, it was found that up to 12 h there was no attractive effect on the insects of the materials tested; however, there were significant differences in the percentage of insects found on each material at 24 and 48 h.

It is noted that in the first hours there was a similar distribution among the arenas. There is movement to recognize feeding and oviposition sites. The insects were attracted, remained confined for a period and then sought more suitable feeding and oviposition sites. Beginning with the 12 h observation period it is seen that the 30F35 maize (control) is more attractive; this is maintained until the end at 48 h. It is noteworthy that this variety is widely used in the region. Already the lower susceptibility of the SP variety may be related to non-preference for feeding or oviposition as a form of resistance (Guzzo et al., 2002).

Table 2. Percentage of adults one of maize weevil, *S. zeamais* discriminating three varieties of maize (in new breeding program) and two controls maize. Bom Jesus, PI, 2012.

SV	DF	Time (h)					
		0.5	2	5	12	24	48
		MS					
Genotypes	4	148.24^{ns}	105.04^{ns}	139.70^{ns}	152.86^{ns}	505.84*	675.4*
Error	20	44.50	96.74	108.56	111.86	122.70	160.82
h^2		0.76	0.52	0.56	0.57	0.80	0.80
CV%		102.31	89.74	76.61	69.94	65.77	75.48
Mean		6.52	10.96	13.6	15.12	16.84	16.8
Genotypes		Means					
Purple		4.8^a	8.0^a	9.0^a	10.6^a	14.6^{ab}	13.8^b
ES		5.8^a	13.2^a	19.2^a	21.0^a	20.6^{ab}	16.8^b
SP		3.4^a	4.4^a	7.0^a	8.0^a	5.0^b	5.4^b
Red		10.0^a	15.2^a	16.4^a	18.0^a	12.2^{ab}	11.8^b
30F35		8.6^a	14.0^a	16.4^a	18.0^a	31.8^a	36.2^a

*,**, ns = significant at 5% and 1% probability level and not significant respectively by F test. Means followed by the same letter in the column do not differ by Tukey test at 5% probability. SV (Source of variation), DF (Degrees of freedom), MS (Medium Square), CV (Coefficient of variation), h^2 (heritability).

Considering the time 24 h as ideal for evaluation of genotypes, we note that the variety purple straw maize has great potential as a source of variability for breeding for resistance to insects in stored grain. Within this variable the Sao Paulo sourced material stands out for having, in all periods, lower incidence of insects. High heritability at the 24 and 48 h timeframes indicates high possibility of gains from selection for the trait, showing great genetic control in the antixenosis component of the grain, confirming that the ability to resist attack is genetically controlled and subject to selection.

Table 3 showed that there were significant differences for all parameters analyzed in relation to nutritional index with the exception of relative consumption in all varieties. For the characteristics RGR and ECI high values of heritability were found, which indicate it is possible to achieve advances from selection. The ECI is an important factor in the resistance of varieties to attack by insects, because the consumed food is little utilized, not reverted into population growth, thus maintaining a low incidence of insects and, consequently, small grain losses, due to a lower population density.

The maize varieties differ significantly by Tukey test in relation to RGR and ECI. ES was the less consumed variety (1.07) and consequently with lower relative growth (1.02). However, the efficiency of conversion of ingested food was greater, which means that the chemical properties of the maize were more efficient in terms of insect metabolism. Already the purple maize showed the largest relative consumption (3.72) and a good efficiency of conversion of ingested food (2.32), not differing significantly from the Red control. Therefore, both were associated with good growth, suggesting susceptibility to the insect. The SP sourced material stood out for the three characteristics, having the lowest consumption efficiency and lower growth values. This variety showed no characteristic favorable to the insect, being ranked as the most resistant among those analyzed.

In relation to Table 4, it is observed that the means among the landrace varieties for MC, EMERG, NIM and NIV differ significantly as to the total number of insects, showing the presence of genetic variability.

The means of varieties for study variables indicate that the SP variety was most resistant when compared with the others, including the control, since it negatively influenced the development of the larvae, whereas the ES variety was more susceptible, being more favorable for insect emergence. This fact may be related to the lipid content and EE of the variety (Table 1), since the higher the lipid content the lower the level of degradation of the grain. The presence of *S. zeamais* leads to a reduction in CP levels and ether extract of the grain, because the insect directly attacks the grain embryo, and consumes these nutrients (Ferrari Filho, 2011). Larval death, which can be expressed by the low number of adults emerged is one of the most important indicators of antibiosis (Guzzo et al., 2002).

It is observed that the maize landrace varieties, with respect to *S. zeamais* attack, vary in terms of quality and resistance, and, in general, if maize planting is geared primarily to livestock (feed), the producer must make a choice as a function of variety. Evaluation of resistance to pests of stored grain should be made by more than one method, by choice and no choice test, so that we can assess the ability of the genotype both not attract the insect (antixenosis) and not to favor its growth (antibiosis).

Table 3. Nutritional index of adults one of maize weevil, *S. zeamais*: relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food (ECI) of *S. zeamais* on grains of maize landrace varieties. Bom Jesus, PI, 2012.

SV	DF	RGR	RCR	ECI
			MS	
Genotypes	4	0.048*	14.61^{ns}	13.12**
Error	45	0.02	7.31	0.43
h^2		0.58	0.49	0.97
CV%		13.28	120.88	24.66
Mean		1.07	2.23	2.66
Genotypes			Means	
Purple		1.16^{a}	3.72^{a}	2.32^{bc}
ES		1.02^{b}	1.07^{a}	4.78^{a}
SP		1.03^{b}	1.79^{a}	1.78^{c}
Red		1.12^{ab}	3.31^{a}	2.10^{bc}
30F35		1.01^{b}	1.27^{a}	2.53^{b}

*,**, ns = significant at 5% and 1% probability level and not significant respectively by F test. Means followed by the same letter in the column do not differ by Tukey test at 5% probability. SV (Source of variation), DF (Degrees of freedom), MS (Medium Square), CV (Coefficient of variation), h^2 (heritability).

Table 4. Population growth of one population of *S. zeamais*: Mass consumed (MC), emergence (EMERG), number of dead insects (NIM) and number of live insects (NIV). Bom Jesus, PI, 2012.

SV	DF	MC	EMERG	NIM	NIV
			MS		
Genotypes	3	224.51**	54912.39**	19132.42**	14571.75**
Error	31	22.04	2402.88	756.34	1099.77
h^2		0.91	0.95	0.96	0.92
CV%		33.14	29.04	34.52	28.89
Mean		14.16	168.74	79.65	114.77
Genotypes			Means		
Purple		16.37^{ab}	150.3^{b}	74.10^{b}	106.20^{b}
ES		14.81^{b}	259.7^{a}	140.30^{a}	149.40^{a}
SP		7.91^{c}	82.3^{c}	44.60^{c}	67.70^{c}
30F35		20.94^{a}	196.56^{b}	39.60^{c}	156.80^{a}

*,**, ns = significant at 5% and 1% probability level and not significant respectively by F test. Means followed by the same letter in the column do not differ by Tukey test at 5% probability. SV (Source of variation), DF (Degrees of freedom), MS (Medium Square), CV (Coefficient of variation), h^2 (heritability).

The ideal is for the genotype to present the two forms of resistance, but when a particular genotype has at least one source of resistance it facilitates better decision making for pest control.

The SP variety purple straw maize presents, based on the above, great potential for use in breeding programs for insect resistance, but inheritance of resistance over generations must first be confirmed. Future improvement of this landrace for agronomically important traits must be done with continued selection of resistance, so as not to lose the resistance. Likewise, studies need to be conducted to check for genetic variability within the varieties, based on progeny testing and including resistance in the indices of selection to maintain trait.

Regarding the nutrition data, population growth, nutritional index and food preference test, there were differences among the maize varieties in relation to the attack of *S. zeamais*, and the variety purple straw maize from SP stands out as the most resistant and other varieties proved susceptible to insect attack. The variety

SP purple straw maize is an important source of genetic variability in breeding for resistance to *S. zeamais* and can be indicated for family farming, with low use of technology in grain storage.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Initial development of crambe due to sowing in different depths

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The aim of this paper is to evaluate the initial development of crambe due to sowing in different depths. The experiments were carried out in conditions of protected cultivation in two agricultural seasons. It was used as delimitation completely randomized, with five treatments characterized by sowing depth (1, 2, 3, 4 and 5 cm), with five repetitions, totalizing 25 plots per experiment. The experiments were conducted in 3 L volume and 30 cm size plastic bags. The bags were previously filled with *Latossolo Vermelho distrófico údico*, a kind of oxisoil. The sowing was performed using ten crambe seeds per bag, and a pen was used to indicate the different depths in which seeds were sown for the treatments proposed. The plants were thinned 15 days after sowing, and only a plant was left per container. This plant was reference to the evaluations done. In the beginning of the period of blooming, three growing evaluations were started in which the plants were measured from the soil to the top of the plant. The dry matter of the plants was also calculated. It was verified that the ideal depth to sowing crambe is between 2 and 3 cm.

Key words: *Crambe abyssinica* Hochst, sowing depth, thinning.

INTRODUCTION

Crambe (*Crambe abyssinica* Hochst) is an oleaginous species belonging to the *brassicaceae* family, and it is originated in the Mediterranean region, being cultivated in large scale in Mexico and the United States for industrial oil production (Andrade et al., 2006). Crambe productivity is around 1000 to 1800 Kg ha^{-1} and its cultivation is an alternative to raw material for biodiesel production due to its high content of oil (it can reach 38%), which is easy to extract (Neves et al., 2007). This species has low production cost and is extremely tolerant to dry weather, water deficit, presenting rusticity, and allows for easy mechanization in planting and cropping, using the same equipment for traditional grains cultivation. The culture also presents a good production advantage, uniformed grains maturation, tolerance to frosts and precocity of more or less 90 days in the cycle (Moers et al., 2012).

Research on phenology of crambe are scant on a global level, which is important to define culture treats, sowing season, crop season estimation, species management, and sowing depth (Toeber et al., 2010).

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Table 1. Soil chemical characteristics from in experimental area at 0-20 cm depth (Umuarama, 2012/2013).

pH	P	O.M.	Ca	K	Mg	Al	C.C.C.	V
$CaCl_2$	mg dm^{-3}		cmolc dm^{-3}					%
4.32	5.20	7.79	1.50	0.41	0.75	0.60	8.01	33.21

O.M. = Organic Matter; C.C.C. = cationic change capacity; V = basis saturation.

According to Schmidt (1974) mentioned by Fonseca et al. (1994), the ideal sowing depth is the one that assure a homogeneous seed germination, fast seedlings emergence and vigorous young plants production. Excessively deep sowings interfere in the seedlings emergence and increase the period of susceptibility to pathogens (Napier, 1985). In other hand, shallow sowings can make easy the attack of predators or damages by irrigation, or exposition of radicle, causing destruction of the plants.

According to Toledo et al. (1993), and Brighenti et al. (2003), to know the depth in that the seedling can emerge can allow the use of related management practices such as the use of mechanical methods associated or not to chemical methods. Hartmann and Kester (1983) suggest that, practically, little seeds should be dispersed in the substratum surface; medium seeds should be covered by a layer of thickness approximate to its diameter. The emergence of seedlings depends not only on the energy of the endosperm or cotyledons, but also on the depth in which the seed is sown.

In the soil, the depth in which the seed can germinate and produce seedling changes between the species, and is ecologically and agronomically important (Guimarães et al., 2002). Sowing in improper depth can jeopardize the crop of any vegetal species in any kind of soil. Sowing depth depends on temperature, humidity and type of soil. The seed should be placed in a depth that makes possible a good contact with humidity. However, deeper sowing depths depend on texture. Heavy soils, without appropriate draining or with factors difficult hypocotyl extension, make seedlings emergence harder. In soils lighter or sandy, the seeds can be put deeper (Embrapa, 2010).

Crambe fits well to direct sowing, but no reports of the ideal sowing depth were found. In general, about 2 and 3 cm are used, but there are no specific research concerning this subject to prove if those are really the ideal depths – hence the need for studying the theme to standardize and to avoid eventual losses is needed (Moers et al., 2012). Thus, this paper aims to assess the initial development of crambe depending on the sowing depth.

MATERIALS AND METHODS

The experiments were carried out in protected cultivation in two agricultural seasons, at the Teaching and Research Farming of State University of Maringá [Fazenda de Ensino e Pesquisa da Universidade Estadual de Maringá], regional campus of Umuarama, state of Paraná, Brazil. The weather is subtropical mesothermal, with average yearly temperatures around 22.1°C (Caviglione et al., 2000). Table 1 shows information about the chemical characteristics of the soil in the experimental area. Based on values from the soil analysis, it was fertilized with 12 mg L^{-1} of nitrogen, 335 mg L^{-1} of phosphor, and 250 mg L^{-1} of potassium, and simple superphosfate and potassium chloride, respectively, were used as source of urea.

In order to conduct the experiments, delimitation fully randomized was applied, with five treatments characterized by different sowing depths (1, 2, 3, 4, and 5 cm), with five repetitions, totalizing 25 plots by experiment. The experiments used 3 L volume and 30 cm size plastic bags. They were previously filled with *Latossolo Vermelho distrófico údico* (USDA, 1998).

Sowing took place in 22 May, 2012 and 29 May, 2013. For that, ten seeds of crambe cultivar Fms Brilhante were used per bag. A pen was used to indicate each depth in the proposed treatments, and emergence occurred after seven days of sowing. Around 15 days after sowing, thinning was made; only a plant by container was left, which was used as benchmark for assessments. Also, 60 days after sowing, a covering fertilizing was applied using an organic-mineral fertilizer of 6% of N, 14% of P_2O_5, and 8% of K_2O.

In the beginning of the period of blooming, three growing evaluations were started, in which the plants were measured with measure tape in centimetres from the surface to the top of the plant. The dry matter of the plants was also calculated using an analytical scale. The data was submitted to variance analysis at 5% of probability and the averages compared through polynomial regression by Sisvar software.

RESULTS AND DISCUSSION

Figure 1 shows that in the first year of cultivation there was no germination when sowing was done in depths of 4 and 5 cm. In the second year, germination did not take place only in sowing at 5 cm depth. In deeper sowing, even those with appropriate humidity condition in the substratum, the seed germination can be affected by reduced availability of oxygen and high levels of CO_2. Furthermore, the emergence of smaller seedlings can be associated to the physical barrier posed by the soil to germination, as the embryo physical expansion is limited (Canossa et al., 2007).

In accordance with Gasparim et al. (2005), soil temperature is one of the most important factors to seeds germination, and temperatures near the soil surface are very similar, being significantly reduced only after 5 cm of depth, damaging germination. According to Carvalho and

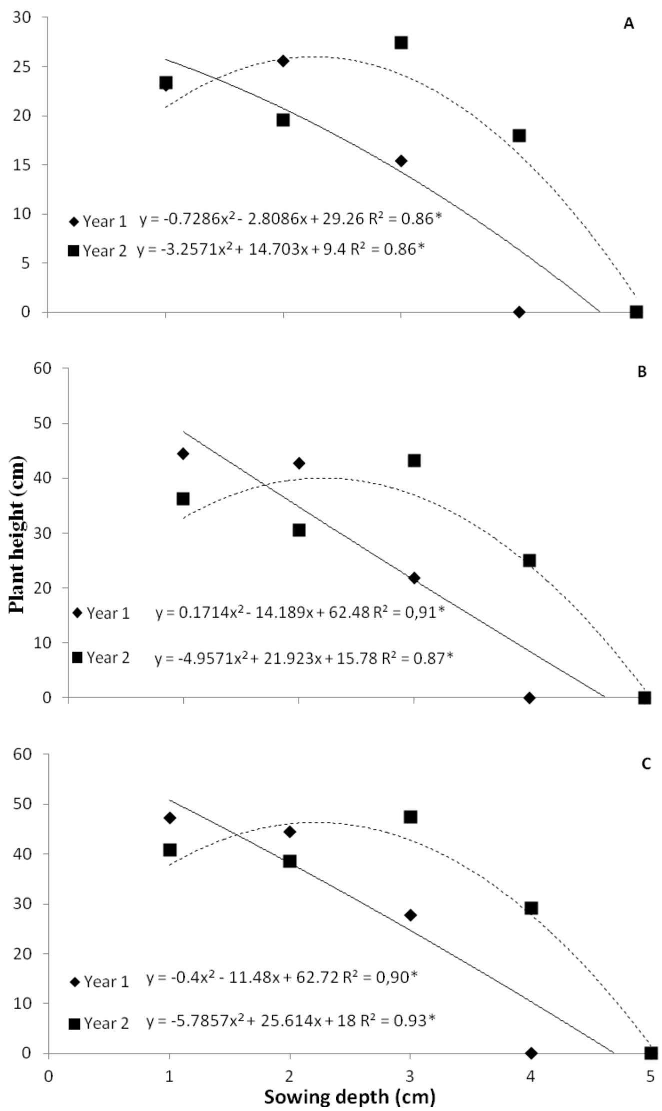

Figure 1. Plant height (cm) aos 40 (A), 47 (B) and 64 days after emergence (C) as a function of depth of sowing crambe in two seasons.

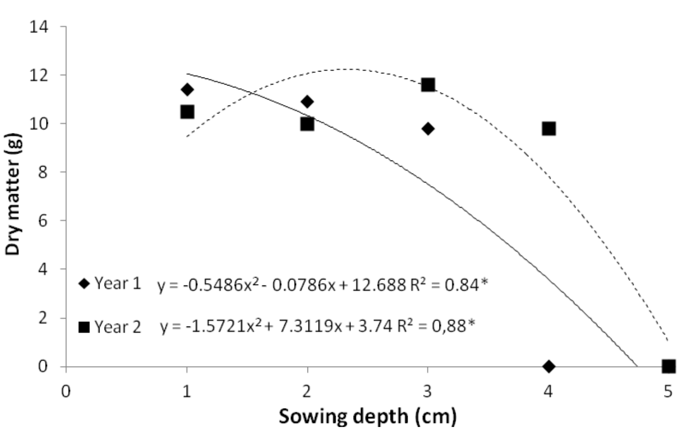

Figure 2. Dry matter (g) of crambe plant as a function of depth of sowing in both seasons.

Nakagawa (2000), the germination process in the seed is characterized by the reactivation of paralysed metabolism after physiological maturity, and can be affected by internal, external and environmental factors, such as water, oxygen, and temperature. It is a well known fact that the soil porosity reduces as depth increases, because the compression is higher (Beutler and Centurion, 2004) and therefore, there is less oxygen. Of course such reduction is small in the soil surface if compared to deeper layers, but it can be sufficient to affect the germination. Garcia et al. (2012) observed that depths larger than 1 cm limit germination of *Tabebuia caraíba* (*Ipê Amarelo*).

Yamashita et al. (2009), working with factors affecting seed germination and seedlings emergence of common rue (*Ruta graveolens*), concluded that, irrespective of the substratum used, higher percentages of seedlings emerged are obtained when the species is sown in the substratum surface. Figure 1A, B, and C show that the points of maximum height of plants occurred between 1.92 and 2.25 cm. In Figure 2, majority of dry mass in the overhead area was obtained in sowing at 2.32 cm. This led us to believe that the ideal depth to sow crambe is between 2 and 3 cm.

Species that has little seeds, such as crambe, have few reserves in cotyledons, in other words, insufficient reserves to emerge from high depths. They germinate when arranged in shallow depths in soil, because the majority of those seeds require luminous stimulus. Since the light is highly reduced as the soil depth increase, seeds of that species normally are not able to emerge from deeper sowing. However, there are species that do not require luminous stimulus to start the germination process and, therefore, can emerge from deeper sowing (Toledo and Marcos Filho, 1977).

In germination process, the seed uses its energy reserve (Carvalho and Nakagawa, 2000), so the deeper the sowing is, more energy will be used by the seed until its emergence. Higher reserve amounts increase the possibility of success in seedling formation, since it makes possible the survival for a larger period in environmental conditions that do not allow yet for taking advantage of nutritional and water soil reserves, as well as photosynthesis (Marcos, 2005).

Alves et al. (2005) observed that bigger seeds, that have more reserve amounts, show higher rate of initial seedlings growth. This increases the probability of success during their formation, since the fast root growth and overhead area would make possible for the plant to benefit from the nutritional and water soil reserves and make photosynthesis.

Conclusion

The ideal depth for seeding crambe is between 2 and 3 inches.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effects of intra-row spacing on plant growth and yield of onion varieties (*Allium cepa* L.) at Aksum, Northern Ethiopia

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Lack of improved varieties and production practices have been the major bottlenecks of onion production and productivity in Tigray, particularly at Aksum area. Since there have been no recommended intra-row spacing, farmers used to practice non-uniform plant spacing. Thus, a field experiment was conducted to investigate the influence of intra-row spacing, variety and their interactions on growth and yield of onion, thereby to recommend the optimum practices to farmers in the study area. The study was conducted between August 2010 and April 2011 at Aksum area (L/maichew district). Three different intra-row spacings (5, 7.5 and 10 cm) were evaluated using four varieties of onion ('Adama' Red, 'Bombay' Red, 'Melkam' and 'Nasik' Red) laid out in randomized complete block design replicated four times. Data on growth and yield parameters were recorded and subjected to analysis of variance (ANOVA). Results indicated that intra-row spacing of 10 cm was superior in plant height, leaf number per plant, leaf biomass yield, leaf dry matter content and percentage of bolters. Highest total bulb yield was recorded at the closest intra-row spacing (5 cm) followed by 7.5 cm. 'Melkam' variety was the highest yielder, while 'Adama' Red was the lowest yielder. Average bulb weight increased with increasing intra row spacing. 'Melkam' variety followed by 'Bombay' Red variety was superior in average bulb weight. 'Adama' Red recorded the highest unmarketable yield.

Key words: Intra-row spacing, variety, growth and yield.

INTRODUCTION

Onion (*Allium cepa* L.) belongs to the family Alliaceae (Hanelt, 1990). Onion is by far the most important of the bulb crops cultivated commercially in nearly most parts of the world. The crop is grown for consumption both in the green state as well as in mature bulbs. Onions exhibit particular diversity in the eastern Mediterranean countries, through Turkmenia, Tajikstan to Pakistan and India, which are the most important sources of genetic diversity and believed to be center of origin of onion (Astley et al., 1982 cited in Brewster, 2008). *Alliums* are typically plants of open, sunny, dry sites in fairly arid climates (Hanelt, 1990).

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Onion is considerably important in the daily Ethiopian diet. All the plant parts are edible, but the bulbs and the lower stem sections are the most popular as seasonings or as vegetables in stews (MoARD, 2009). It is one of the richest sources of flavonoids in the human diet which is relevant given that the flavonoid consumption has been associated with a reduced risk of cancer, heart disease and diabetes. Flavonoids are not only anti-cancer, but also are known to be anti bacterial, antiviral, anti-allergenic and anti-inflammatory.

Different cultural practices and growing environments are known to influence growth and yield of onion. So far, research in the country was mainly focused on the identification of superior cultivars of onions and adopting improved management practices. The control of plant spacing is a way of ruling bulb yield. Spacing has effect on different varieties as their root and leaf growth habits. Geremew et al. (2010) recommended intra row spacing of 4 cm for *'Nasik'* Red and *'Adama'* Red varieties, and 6 cm for *'Bombay'* Red variety, at which highest marketable yield was gained and reduced unmarketable bulb yield in central rift valley areas of Ethiopia. Higher yield could be obtained if plants are grown at optimum density. Varieties as well as planting densities significantly affected the onion bulb yield**.** Plant height decreased as population density increased. Total bulb yield increases significantly as population density increases. Number of marketable bulbs increases significantly with higher planting density (Kantona et al*.,* 2003).

To improve onion production, the agricultural research system of the country has made efforts to generate improved varieties. '*Bombay'* Red and *'Adama'* Red varieties are widely grown in Ethiopia (EARO, 2004). However, these varieties are not distributed to all or most growing areas of the country and are not tested in different agro-ecologies, particularly in the study area. Although some production technologies are developed in the country, it is very difficult to give general recommendation that can be applicable to the different agro-ecological zones. To optimize onion productivity, full package of information is required (Gupta et al., 1994; Lemma and Shimeles, 2003). Plant population needs to be optimized. The optimum use of spacing or plant population has dual advantages (Geremew et al., 2010). It avoids strong competition between plants for growth factors such as water, nutrient and light. In addition optimum plant population enables efficient use of available cropland without wastage.

Aksum-Adwa area is one of the potential areas for onion production in Ethiopia (EHDA, 2011). However, there are no packages of recommendation with regard to crop management practices. Market problem and poor cropping pattern are also major problems in the study areas. Onion is the first in area coverage, but the yield is 107 q/ha which is very less compared to the national average (187 q/ha) (Mintesnot et al*.,* 2005). Lack of proper agronomic practice used by farmers is one of the major problems in onion production (AxARC, 2009). This is because there had been no agronomic or varietal trial done for onion so far. The nationally recommended spacing between plants of onion has been 10 cm, which was based on the research done in central rift valley of the country some years back. Nevertheless, in the real situation, the practice, which is adopted by farmers, is a bit far (narrower or wider) from the recommendation. There is no recommendation made even in the region with regard to onion plant spacing. The present study was therefore, undertaken to investigate the effects of different intra-row spacing on the growth and yield of onion varieties with the following specific objectives: To determine the best plant spacing for optimum growth and yield of some onion varieties and to study the interaction effect of intra-row spacing and varieties on onion's growth and yield

MATERIALS AND METHODS

Description of the study site

The study was conducted in 2010/11 from August 2010 to April 2011 under irrigated condition at Aksum area (L/maichew district), Central Zone of Tigray National Regional State, 245 km away from Mekelle towards the North West. The experimental site lies between latitude of 14° 07' 00" and 14° 09' 20" N, and 38° 38' 00" and 38°49' 09" E longitude, and elevation of 2080 m above sea level. The soil is, classified as loamy clay vertisol. The rainy season of the area is monomial and receives 700 mm average rainfall per annum. The annual minimum and maximum monthly temperature ranges from 11- 15.1°C, respectively.

Experimental treatment and design

The experiment consisted of factorial combination of two factors viz; intra-row spacing (5 , 7.5 and 10 cm) and variety (Adama Red, Bombay Red, Melkam and Nasik Red) laid out in 3×4 factorial randomized complete block design (RCBD) replicated four times.

Experimental management

Cultural management practices other than intra-row spacing were done according to the national recommendations. Seeds were sown for nursery rising and transplanted after 50 days from sowing. DAP fertilizer was applied during the last land preparation and Nitrogen in the form of urea was split applied during planting and 6 weeks after transplanting. Weeds were controlled mechanically (by hand weeding). The plots were irrigated at intervals of 7-10 days until maturity. During maturity when 2/3 of the leaves become yellow in color, bulb was harvested and cured for 5 days (EIAR, 2007). Sample bulbs were taken from each plot for data collection.

Data collection and analysis

Data were collected on vegetative growth parameters (plant height, leaf length, leaf diameter, leaf number/plant, percentage of bolters and days to maturity), biomass parameters (leaf biomass, dry leaf

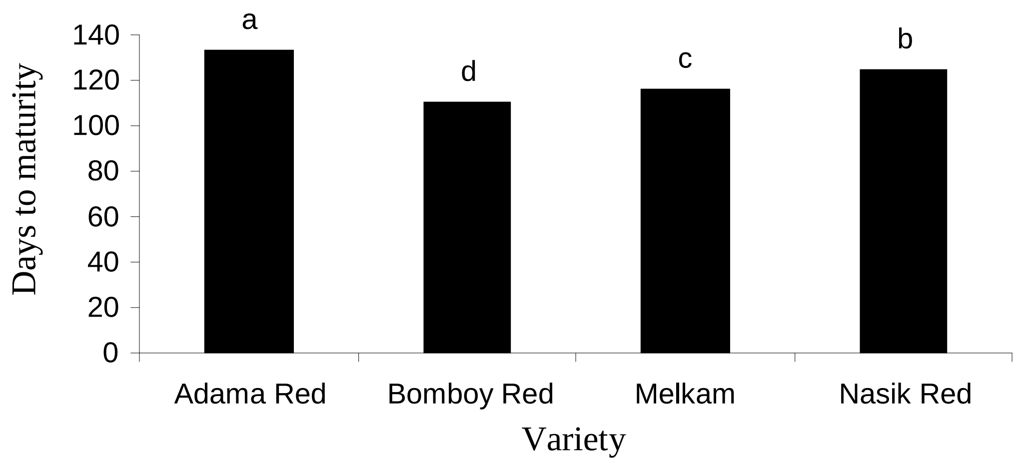

Figure 1. Average days to maturity of onion varieties, means followed by the same letter are not significantly different at p=0.05 as established by LSD-test (LSD=1.36), CV, Coefficient of variation; CV (%) = 1.35.

weight and harvest index) and yield parameters (total bulb yield, unmarketable bulb yield, marketable bulb yield and average bulb weight) using the standard procedures described by IPGRI (2001). The mean values of the above response parameters were subjected to the analysis of variance (ANOVA) using SAS version 9.2 Computer software (SAS Institute Inc., 2008). Whenever the treatment was significant, least significance differences (LSD) was used for mean separation at p=0.05.

RESULTS AND DISCUSSION

The average days to physiological maturity

The main effect of intra-row spacing and interaction effect of intra-row spacing and varieties did not show significant difference. A very highly significant difference (p<0.0001) was observed among the varieties on days to maturity. *'Bombay'* Red followed by *'Melkam'* varieties were the earliest, which matured 123 and 118 days, respectively, while *'Adama'* Red variety was late maturing (Figure 1). This result is consistent with the onion varieties characteristics defined in EARO (2004) that *'Bombay'* Red can mature in 110 days. Kimani et al*.* (1993) reported variations in days to maturity among onion cultivars. The varietal difference could be due to the inherent genetic variability of the crop in days to maturity.

Leaf number per plant

The main effect of variety and interaction effect of variety and intra row spacing did not show any significant difference on leaf number per plant at maturity, while there was highly significant difference among the intra-row spacing levels at p=0.0001. Onion planted at 10 cm intra-row spacing produced significantly higher leaf number of leaves than bulbs planted at 5 and 7.5 cm, but there was no significant difference between spacing 5 and 7.5 cm. As intra-row spacing increased from 5 to 10 cm, the number of leaves per plant increased from 9.5 to 11.94 at maturity stage (Figure 2). It is apparent that when and intra-row spacing increases the number of plants per unit area; more mineral nutrients, light, moisture and space become available leading to vigorous growth possible that increase in planting density resulted in reduction in number of leaves because of shortage of more mineral nutrient, light, moisture and space. Jan et al. (2003), Akoun (2005), Aliyu, et al. (2008), Ahmed et al. (2010) and Jilani et al. (2010) reported that increase in planting density resulted in reduction in number of leaves. Karaye and Yakubu (2006) also reported that garlic planted at 15 and 20 cm intra-row spacing produced significantly higher number of leaves per plant than the 10 cm intra-row spacing. Ibrahim (1994) and Bodnar et al. (1998) also observed widely spaced garlic plants tend to grow more vegetatively and bear more leaves per plant.

Plant height (cm)

The interaction effect of intra-row spacing and variety on plant height at maturity was not significant. Intra-row spacing and variety significantly affected plant height at maturity at p=0.05 and p=0.0001, respectively). Intra-row spacing of 10 cm had significantly the tallest plants (Table 1). In agreement to current finding, Jan et al*.* (2003) reported that maximum plant height (61.4 cm) in plants having 22 x 9.5 cm spacing and minimum plant height (58.18 cm) was observed at 17 x 4.5 cm spacing. Aliyu et al. (2008) also found superior plant height at 25 cm intra-row spacing than at 15 cm intra-row spacing

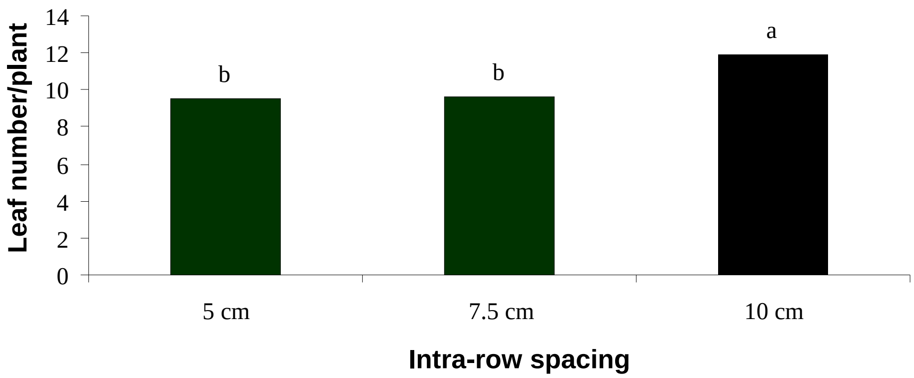

Figure 2. Effect of intra-row spacing on leaf number per plant, Means followed by the same letter are not significantly different at p=0.05 as established by LSD-test (LSD=0.77). CV, Coefficient of variation; CV (%) = 10.31.

Table 1. Effect of intra-row spacing and variety on plant height, leaf diameter and leaf length of onion.

Treatment	Plant height (cm)	Leaf diameter (cm)	Leaf length (cm)
Intra-row spacing (cm)			
5	48.58[b]	0.97[b]	37.56[b]
7.5	49.55[b]	1.08[a]	38.3[b]
10	51.31[a]	1.20[a]	40.44[a]
$LSD_{(0.05)}$	1.43	0.10	2.32
CV (%)	3.98	13.00	7.20
Variety			
'Adama' Red	51.90[a]	1.11[a]	40.75[a]
'Bombay' Red	46.80[c]	0.90[b]	35.17[c]
'Melkam'	49.17[b]	1.09[a]	37.83[b]
'Nasik' Red	51.58[a]	1.18[a]	41.25[a]
$LSD_{(0.05)}$	3.98	0.12	2
CV (%)	1.69	13	7.2

Means within a column followed by the same letter(s) are not significantly different at p=0.05at LSD-test.

(51.78 cm and 47.35 cm, respectively). The reduction in plant height at increased plant density might be attributed to the possible competition for soil moisture and nutrients as it was the case in Ibrahim (1994), Bodnar et al. (1998), Karaye and Yakubu (2006). Moreover, results are also in agreement with the findings of Zamir et al. (1999) on maize, Kantona et al. *(*2003) and Khan et al. (2003) on onion, Agele et al. (2007) on sunflower, and Woldemariam (2009) on ginger. *'Adama'* Red and *'Nasik'* Red varieties produced significantly higher plant height from the others (Table 1). In concurrent to the present findings, Jilani and Ghafoor (2003), Islam et al. (2007) and Jilani et al. (2010) found significant genotypic variation among onion varieties in plant height. Perez et al. (2004) recorded significantly different and highest plant height on radish of 72.2 cm at 15 cm spacing and 66.7 cm at 10 cm spacing.

Leaf diameter (cm)

The various intra-row spacing showed significant difference (p=0.01) in terms of leaf diameter. Among the intra-row spacing, the closest (5 cm) caused the lowest leaf diameter and significant difference to both treatments (7.5 and 10 cm) (Table 1). This result is supported by the findings of Broome (2009) who reported that leaf diameter of different *Allium* species plants grown at 20 cm was larger than plants grown at 15 cm which in turn were larger than plants grown at 10 cm. Palada and Crossman (1998) reported that leaf area of okra increased with increase in plant spacing linearly.

Nasik' Red variety recorded the highest leaf diameter (1.2 cm), but not significantly different from '*Adama*' Red and '*Melkam'* varieties (Table 1). *'Bombay'* Red variety showed significantly the lowest leaf diameter (0.9 cm).

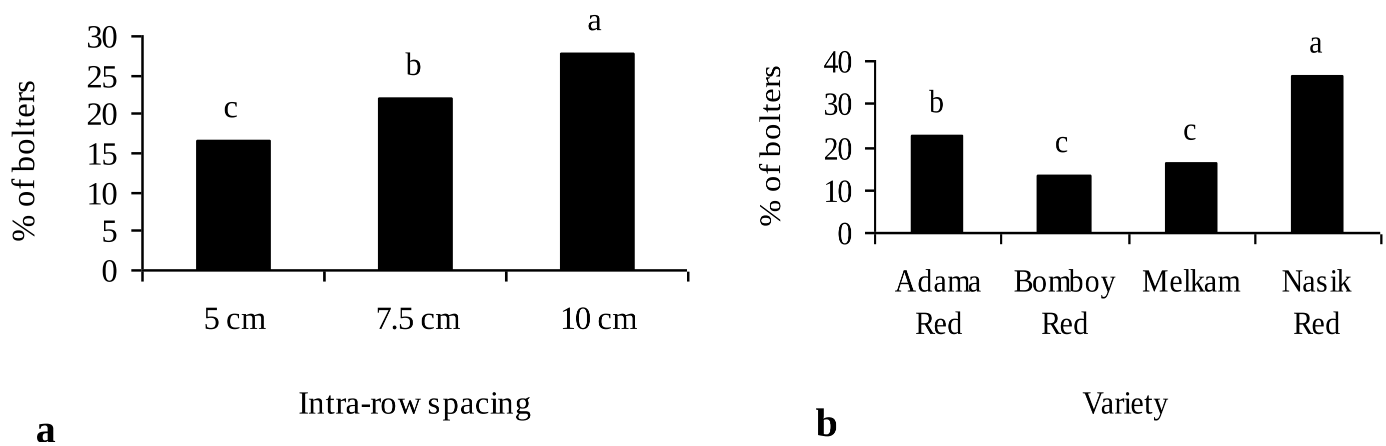

Figure 3. Effect of intra-row spacing (a) and variety (b) on percentage of bolters, Means followed by the same letter are not significantly different at p=0.05 as established by LSD-test (LSD=5.42 and 5.8, respectively), CV, Coefficient of variation. CV (%) =28.8.

Jilani et al. (2009) also reported that onion cultivars varied significantly with respect to leaf diameter due to genetic inheritance.

Leaf length (cm)

A significant variation (p=0.05) in the leaf length (cm) was observed at the different intra-row spacing treatments (Table 1). The effect of varieties on leaf length at maturity was also very significant (p=0.0001). Intra-row spacing of 10 cm showed higher leaf length and significant differences with both treatments (5 and 7.5 cm). Nevertheless, there was no significant difference between spacing of 5 and 7.5 cm. Onion bulbs planted at 10 cm intra-row spacing produced the tallest (40.4 cm) leaf length whereas onion bulbs planted at 5 cm produced the shortest (37.6 cm) leaf length. Jilani and Ghaffor (2003) suggested that plant densities could affect length of leaf. Lowest plant density can give highest leaf length. Jilani, et al. (2010) also reported the highest leaf length at wider spacing whereas shortest leaves correspond to the closest plant spacing. This is probably, attributed to increased competition for nutrients and moisture at higher plant density.

'Adama' Red and *'Nasik'* Red showed higher leaf length compared to other varieties. There was significant difference on *'Bombay'* Red from *'Melkam'* variety. The result is in agreement with Jilani and Ghaffor (2003), Jilani et al. (2009), and Smittle (1993) who reported that cultivars varied significantly from each other with respect to leaf length.

Percentage of bolters

The interaction effect failed to show any influence on percentage of bolted plants, but was very significantly (p=0.0001) affected by intra-row spacing and varieties. All intra-row spacing showed significant (p=0.05) difference among each other (Figure 3a). As intra-row spacing increased from 5 to 10 cm, the percentage of bolters increased from 16.49 to 27.8. This may be due to the availability of more nutrients, space and light in wider spacing, which contribute to luxurious growth leading to flower stalk development. In support of the current result Hassen (1978), Mohamodali (1988) and Khalid (2009) elucidated that at closer spacing, small bulbs are produced which are less susceptible to incidence of bolting.

'Nasik' Red variety showed higher percentage of bolters (36.36) compared to other varieties. While there was no significant difference between *'Bombay'* Red (15.98) and *'Melkam'* (13.19) they showed significant difference from *'Nasik'* Red and *'Adama'* Red (Figure 3b). Similarly, EARO (2004) reported differences among varieties in terms of bolting and seed setting. Khalid (2009) also observed variation in time of inflorescence in two onion cultivars. Bolting may be also a problem of physiological nature and is undesirable for better bulb production and onion varieties can differ in percentage of bolting. Jilani and Ghafoor (2003) also reported that bolting percentage was affected by varieties. Similarly Salunkhe (1998) also suggested that bolting may vary due to genetic factors.

Fresh leaf weight (g) per plant

The interaction effect of intra-row spacing and variety significantly (p<0.001) affected average fresh leaf biomass (Table 2). The highest fresh leaf biomass weight per plant (20.55 g) was recorded at intra-row spacing of 10 cm, followed by 7.5 cm (13.5 g) and 5 cm (11.79 g)

Table 2. Effect of intra-row spacing and variety on fresh leaf biomass, leaf dry weight and harvest index of onion.

Treatment	Fresh leaf biomass /plant (g)	Leaf dry weight/plant (g)	Harvest index
Intra-row spacing (cm)			
5	11.79^{b}	1.48^{b}	0.79^{b}
7.5	13.5^{b}	2.28^{a}	0.83^{a}
10	20.55^{a}	2.63^{a}	0.79^{b}
$LSD_{(0.05)}$	3.06	0.37	0.03
CV (%)	27.81	23.1	4.64
Variety			
'*Adama*' Red	17.42^{a}	2.28^{ab}	0.77^{c}
'*Bombay*' Red	11.82^{b}	1.9^{b}	0.83^{a}
'*Melkam*'	18.42^{a}	2.67^{a}	0.81^{ab}
'*Nasik'* Red	13.55^{b}	2.11^{b}	0.8^{bc}
$LSD_{(0.05)}$	3.54	0.43	0.03
CV (%)	27.81	23.1	4.64

Means within a column followed by the same letter(s) are not significantly different at p=0.05established by LSD-test.

intra-row spacing. This revealed that as intra-row spacing increased from 5 to 10 cm, average fresh leaf biomass per plant increased by 74%. The investigations of Jilani et al. (2009) support this result. In addition, Perez et al. (2004) reported that minimum total biomass per plant of Radish was obtained from 5 cm spacing while, maximum total biomass per plant was observed at 15 cm spacing followed by 10 cm spacing. Biomass is directly proportional to number of leaves, length of leaves, root length, root diameter, fresh root weight and weight of fresh leaves per plant. The positive correlation result of leaf biomass with plant height (r=0.55**), leaf number (r=0.55***), leaf diameter (r=0.41**) can supported the above conclusion. Varieties '*Melkam'* and '*Adama'* Red recorded the highest average fresh leaf biomass weight per plant (18.42 and 17.42 g, respectively) (Table 2). This might be because onion varieties may have different morphological and biochemical characteristics that affect the biomass accumulation among different storage and vegetative parts, as reported by Jilani and Ghaffoor (2003) and Jilani et al. (2009).

Leaf dry weight per plant (g)

Leaf dry weight per plant was significantly ($p<0.001$) affected by both intra-row spacing and variety. Significantly, the highest leaf dry weight (2.63 g) was recorded at intra-row spacing of 10 cm than at intra-row spacing of 5 cm (Table 2). However, it was not significantly different from that recorded at 7.5 cm intra-row spacing (2.28 g). Intra-row spacing of 5 cm showed significantly lower leaf dry weight (1.48 g). Results showed that dry weight of plant shoots decreased with increase in plant density. Differences in intra-row spacing enhanced plant-to-plant variation in terms of accumulated biomass and this phenomenon affected bulb yield and the stability of dry matter partitioning to bulbs. Large plants in wide in-row spacing have competitive advantage and this can be associated with high capacity for resource capture, which enhances vigorous vegetative growth. Shamsi and Kobraee (2009) reported an increase in dry matter accumulation of soybean parallel to increase of intra-row spacing.

Varieties *'Melkam'* and '*Adama'* Red showed significantly higher leaf dry weight (2.67 g and 2.28 g, respectively) (Table 2). The variety '*Bombay*' Red recorded the lowest leaf dry weight (1.9 g). Differences in onion varieties responses to different intra-row spacings and they are manifested in differential ability to transform accumulated biomass to bulb production under different intensities of interplant competition. In case of *'Bombay'* Red variety presence of shorter leaf diameter might have reduced the above ground biomass and resulted in higher harvest index. This result is elucidated by Izadkhan et al. (2010) who reported that varieties of onion differed in leaf dry weight.

Harvest index

The interaction effect of intra-row spacing and variety showed significant ($p<0.05$ and $p<0.01$, respectively) effect on harvest index. Intra-row spacing of 7.5 cm showed the highest harvest index (0.83), followed by 5 and 10 cm intra-row spacing, both of which recorded the same harvest index (0.79) (Table 2). The lower harvest index at the wider spacing might be due to the production of more vegetative parts including flower stalks, which might had diverted assimilate away from the economically important bulbs. Kabir and Sarkar (2008) also reported a significant interaction effect on harvest

Table 3. Effect of intra-row spacing and variety on total bulb yield (TBY), marketable bulb yield (MBY) and average bulb weight (ABW) of onion.

Treatment	TBY (T/ha)	MBY (T/ha)	ABW(g)
Intra-row spacing(cm)			
5	36.14[a]	34.49[a]	49.86[c]
7.5	33.82[a]	32.97[a]	69.69[b]
10	28.51[b]	28.10[b]	81.31[a]
$LSD_{(0.05)}$	3.1	3.1	4.76
CV (%)	12.9	13.63	9.9
Variety			
Adama Red	29.86[c]	28.45[c]	61.03[c]
Bombay Red	34.68[ab]	34.00[ab]	67.29[b]
Melkam	35.20[a]	34.36[a]	75.77[a]
NasikRed	31.57[bc]	30.60[bc]	63.74[bc]
$LSD_{(0.05)}$	3.53	3.6	5.5
CV (%)	12.9	13.63	9.9

Means within a column followed by the same letter(s) are not significantly different at p=0.05 according to LSD test.

index of mungbean and the highest value recorded from varieties at closer spacing probably due to the reduced vegetative biomass.

The highest harvest index (0.83) was recorded by *'Bombay'* Red variety. The lowest harvest index (0.77) was recorded by *'Adama'* Red variety (Table 2). Similarly, Naim et al. (2010) observed differences in harvest index on cowpea varieties. The authors further stated that some varieties gave the highest value of vegetative growth during growth stages and the highest seed and biological yield at the end of growth season. Fasika et al. (2008) also reported highly significant genetic differences among Ethiopian shallot genotypes for harvest index. This means that there can be variation in movement of food among the consumable parts and vegetative parts.

Total bulb yield (t/ha)

Results indicated that there was no significant interaction effect between the intra-row and variety, while main effects of intra-row spacing ($p<0.0001$) and varieties ($p<0.01$) significantly influenced total bulb yield of onion. As intra-row spacing increased from 5 to 10 cm, total bulb yield in tons/hectare decreased. The highest total bulb yield (36.14 t/ha) was recorded at 5 cm intra-row spacing. However, it was not significantly different from the total bulb yield obtained at intra-row spacing of 7 cm (33.82 t/ha). An intra-row spacing of 10 cm showed the lowest total bulb yield (28.51 t/ha) (Table 3). This is due to the reality that as intra-row spacing decreases total plant population increases and this in turn contributes to increase in total bulb yield, but the bulb dimension and weight decrease. The current result is in agreement with works of different authors. Jan et al. (2003) recorded the highest yield (40.44 t/ha) at spacing of 17 x 4.5 cm, while the lowest yield (19.95 t/ha) at 27 x 14.5 cm spacing. Hassan (1978), Mohamedali (1988) and Russo (2008) also found similar results. Rekowska and Skupien (2007) also reported higher yield of bulbs and green leaves of garlic in closer intra-row spacing.

Moreover, Kantona et al. (2003) noticed that onion yield increased from 17.4 to 39.5 t/ha as plant population per square meter increased from 50 to 150. Carlson et al. (2009) reported influence of plant density on the yield of two potato varieties, in which both varieties produced highest total yields at the closest plant spacing of 17.75 cm. Hemphill (1987) also reported that a fourfold increase in planting density doubled the yield of shallot. The author further stated that yield per unit area did not increase proportionally to the increase in planting density since bulb weight per plant decreased at higher densities, but low planting density and small planting stock size favored production of large bulbs required for some markets, but with greatly reduced total yield (Ademe et al., 2012).

Results also indicated that *'Melkam'* variety had the highest total bulb yield (35.20 t/ha).The least total bulb yield (29.86 t/ha) was recorded by *'Adama'* Red varieties. The present finding is supported by different investigations previously done. Jilani and Ghaffor (2003) and Jilani et al. (2009) suggested that varieties could have different yield potential in different agro-ecologies due to their genetic potential as well as genetic and environment interaction effect.

Marketable bulb yield

Marketable bulb was bulbs which are greater than 25 g in weight according the assessment of the local market. A highly significant ($p=0.05$) differences were observed among the levels of intra-row spacing and onion varieties on the marketable bulb yield (t/ha). As intra-row spacing increased from 5 to 10 cm, marketable bulb yield in tons per hectare decreased from 34.49 to 28.1 (Table 3). Among the intra-row spacing, a statistically similar result was obtained from 5 and 7.5 cm intra-row spacing (34.49 and 32.97, respectively) while intra-row spacing of 10 cm showed the lowest (28.1 t/ha). Generally, a trend of increasing gross marketable yield together with plant density was observed. Plant density has an impact on marketable bulb size and the higher the plant density the smaller the marketable bulb size (Seck and Baldeh, 2009). Kantona et al. (2003) also reported that as plant density can increase number of marketable bulbs significantly.

The highest marketable bulb yield (34.36 t/ha) was recorded in *'Melkam'* and the lowest (28.45 t/ha) in *'Adama'* Red variety (Table 3). In agreement to the present results, Jilani et al. (2009) reported similar observation. A cultivar performs differently under diverse agro-climatic conditions and various cultivars of the same species grown even at the same environment often yield differently. Thus, performance of a cultivar mainly depends on the interaction of genetic makeup and environment (Jilani and Ghaffoor, 2003).

Average bulb weight (g)

Main effects of intra-row spacing and variety highly significantly (p<0.0001) influenced average bulb weight, but the interaction was not statistically significant. As intra-row spacing increased from 5 to 10 cm, average bulb weight increased from 49.86 to 81.31 g (Table 3). The results are in line with the findings of Rashid and Rashid (1978) who noticed that onion bulb size and weight increases with increasing inter, and intra-row spacing, but recorded lower total bulb yield that increases with closer spacing. Densely populated plants produced lower bulb weight as compared to thinly populated plants. Increasing plant spacing resulted in heavier onion bulbs (Jilani et al., 2009). Mean bulb weight and plant height decreased as population density increased (Mohamedali, 1988). Jan et al. (2003) also found minimum bulb weight at narrower spacing (17 x 4.5 cm).

In the same way, Kantona et al. (2003) reported a decrease in bulb weight as the plant population per square meter increased from 50 to 200 plants likely due to competition associated with closely spaced plants that resulted in lower bulb weight per plant. Abubaker (2008) also reported that the highest yield per plant of bean was obtained from 20 x 30 cm and 30 x 30 cm planting densities as compared to higher planting densities of 10 x 30 cm. When onions are planted at wider spacing, the emerged shoots get a better microenvironment that resulted in healthy and larger bulbs and high bulb weight per plant. Moreover, better air circulation reduces disease occurrence, which contributes to higher yield per plant.

Palada and Crossman (1998) also reported that an increase in okra fresh weight per plant from 38 to 70 g with the increasing in plant spacing from 31 to 41 cm due to increasing in the number of stem and wider leaf area per plant at wider spacing (Ademe et al., 2012).

'Melkam' variety showed significantly high average bulb weight (75.77 g), followed by '*Bombay'* Red (67.29 g) and '*Nasik*' Red (63.74 g) (Table 3). The lowest average bulb weight (61.03 g) was recorded by *'Adama'* Red variety. Difference in average bulb weight within varieties was due to their genetic variability, which is consistent with the finding of Jilani and Ghaffoor (2003) and Jilani et al. (2009). Kimani et al. (1993) also reported significant bulb weight variation among eight onion cultivars. According the EARO (2004), *'Melkam'* variety is characterized by large bulb weight. The current result is also supported by reports of various workers (Mohamedali, 1988; Rumpel and Felcznski, 1997; Kantona, et al., 2003; Hyder et al., 2007; Russo, 2008) who found similar results.

Unmarketable bulb yield

The main effect of intra-row spacing, variety and their interaction on unmarketable bulb yield (t/ha) and percentage of unmarketable bulb yield from the total bulb yield showed highly significant (p<0.01) difference. The highest unmarketable bulb yield was produced, by the treatment combination of 5 cm intra-row spacing and *'Adama'* Red (2.26 t/ha) (Table 4). High unmarketable yield in closely spaced plants could be due to inter-plant competition resulting in a fewer large sized bulbs than wider spacing that negatively affected the marketable yield and favored the production of small sized bulbs. This finding is in agreement with related report of Seck and Baldeh (2009) who concluded that plant density had an impact on marketable bulb size. The result further revealed that '*Adama*' Red and '*Nasik*' Red varieties are relatively less tolerant to narrower intra-row spacing in the study area. In support of the present result, some authors (Rumpel and Felcznski, 1997; Russo, 2008; Jilani et al., 2009; Geremew et al., 2010) also reported similar results that marketable bulb yield and unmarketable bulb yield could be affected by both varietal differences and plant density.

CONCLUSION AND RECOMMENDATION

Onion is extremely popular and the most cultivated vegetables in Ethiopia, especially in Tigray region. Farmers in the study area produce onion as a cash crop using non-uniform plant spacing based on the existing indigenous knowledge. The study was conducted to investigate best plant spacing for highest growth, yield, better quality and shelf life of onion varieties and to recommend superior variety adaptable to the specific

Table 4. Effect of intra-row spacing and variety interactions on unmarketable yield of onion.

Intra-row spacing(cm)	Variety	Unmarketable bulb yield (t/ha)
5	Adama Red	2.67^{a}
	Bombay Red	1.16^{c}
	Melkam	1.07^{c}
	Nasik Red	1.68^{b}
7.5	Adama Red	1.01^{c}
	Bombay Red	0.63^{def}
	Melkam	0.93^{cd}
	Nasik Red	0.88^{cde}
10	Adama Red	0.54^{ef}
	Bombay Red	0.32^{f}
	Melkam	0.53^{ef}
	Nasik Red	0.29^{f}
LSD(0.05)		0.37
CV (%)		26.1

Means connected with the same letter(s) are not significantly different at p=0.05 as established by LSD test.

area and best plant spacing that give prime growth and yield. Results of the study showed that main effects of intra-row spacing, varieties as well as their interactions had considerable influence on different parameters. '*Bombay*' Red variety was found to be the earliest which matured 23 days earlier than the latest variety of all and followed by '*Melkam*' which is earlier in 18 days than the latest '*Adama*' Red. Leaf number was maximum (12) at the 10 cm spacing and minimum (9) at the 5 cm spacing. The highest (51.3 cm) plant was observed at the wider spacing 10 cm and the lowest (48.6 cm) was perceived at the narrow spacing 5 cm. As intra-row spacing increased from 5 to 10 cm the percentage of bolters increased from 16.5 to 27.86%. The percentage of bolters increased with increasing plant spacing especially in '*Nasik*' Red and '*Adama'* Red varieties.

The highest total bulb yield (36.14 t/ha) was recorded at intra-row spacing of 5 and 7.5 cm (33.82 t/ha). Highest total bulb yield (35.2 t/ha) was also recorded on *'Melkam'* variety, while the lowest yield (29.86 t/ha) was recorded on *'Adama'* Red variety. Intra-row spacings of 5 and 7.5 cm also had higher marketable yield than 10 cm. As intra-row spacing increased from 5 to 10 cm average bulb weight in grams increased from 49.86 to 81.31 g. The highest average bulb weight (75.77 g) was recorded on *'Melkam'* variety followed by *'Bombay'* Red (67.29), while the lowest average bulb weight (61.03 gm) was recorded by *'Adama'* Red variety. The highest unmarketable yield was produced, at the combination 5 cm intra-row spacing and '*Adama*' Red variety (2.67 ton/ha.8). The highest percentage of small size bulbs was produced by the treatment combination of *'Adama'* Red at 5 cm spacing and *'Bombay'* Red at 5 cm spacing (23.76 and 15.45%, respectively) while the minimum percentage of small size bulbs was found in the combination of *'Melkam'* at 10 cm and *'Adama'* Red at 10 cm spacing (4.4 and 6.9, respectively). The highest percentage of large size bulbs (20.71) was recorded in *'Melkam'* variety, while the lowest percentage of large bulbs (11.1) was obtained in *'Nasik'* Red variety. The finding suggested that it is better to use intra-row spacing greater than 5 cm to minimize small bulbs as this is not mostly preferred for market. Besides, the ultimate goal of onion production is profitability through yield enhancement; the result revealed that *'Melkam'* and *'Bombay'* Red varieties appeared to be superior for yield and earliness at the study area although it needs repeated research for complete recommendation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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