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Full Length Research Paper

Design and development of an animal drawn farmyard manure spreader

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In India bullock-carts / tractor-trailers are used to transport the farm yard manure (FYM) from the compost pit to the field and manure is stack piled in the field. The spreading of stack piled manure is performed manually with spade, which involves human drudgery. Therefore the existing bullock carts used for transport of manure to the field could be modified for FYM spreading operation also. Keeping all these facts in mind an animal drawn FYM spreader has been developed for uniform spreading of manure and eliminate the human drudgery involved in spreading of manure in the field. The developed farmyard manure spreader of 480 kg capacity and gave manure application rate of 5 to 10 t/ha for the manure delivery rate of 0.38 to 0.74 kg/s at the operational speed of 2.4 km/h, respectively.

Key words: Bullocks, manure, carts, spreader.

INTRODUCTION

Total farmyard manure available in the country is approximately 1200 million ton including availability of 268 MT dung from livestock and 5 MT poultry droppings for bio- methanation to produce biogas and manure of high quality. 50% FYM is used to improve soil fertility and remaining quantity is used for fuel. FYM supplies organic materials to the soil together with plant available nutrients (relatively small concentration compared to inorganic fertilizers) 0.4 to 0.8% N, 0.3 to 0.9% P_2O_2 and 0.3 to 1.9% K_2O. It increases microbial biomass, carbon content and an enzyme compared to the inorganic fertilizer and improves the soil quality by improving the soil-plant-environmental system (Lague et al., 1994; Alam et al., 2002). Integrated use of organic wastes and chemical fertilizers is beneficial in improving crop yield, soil pH, organic carbon and available NPK in soil as compared to continuous use of only chemical fertilizer (Hansen et al., 2004). In India tractor-trailer/bullock carts are used to transport the FYM from the storage pit/bin to the field and manure is stacked piled in the field (Singh and Singh, 2013). Farmyard manure (FYM) is mainly being applied through manual broadcasting, resulting more labours and time per unit area with poor application uniformity and wide variation of the application rate. Solid stack piled manure losses about 21% of its nitrogen to the atmosphere. Proper spreading and incorporation in the soil would reduce the loss only 5%. The spreading of stack piled manure is performed manually with spades, which involves human drudgery (Lague et al., 1994). Therefore an animal drawn cart cum manure spreading system is required for proper application rate and uniform spreading of FYM for the consistent results from a crop

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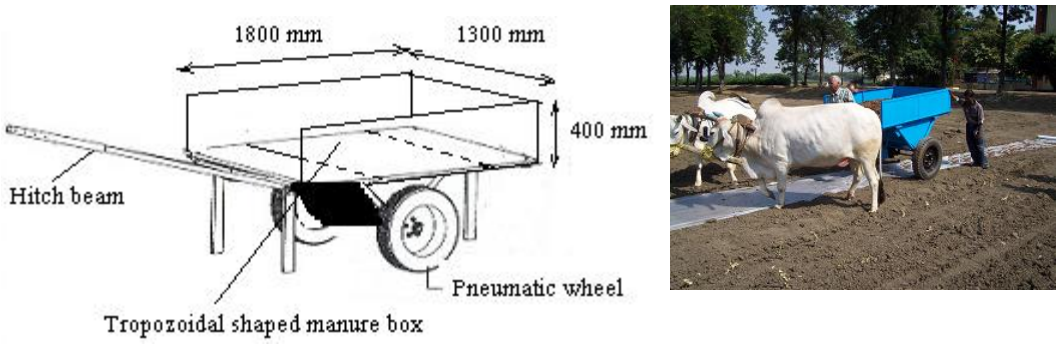

Figure 1. Schematic view of developed manure spreader.

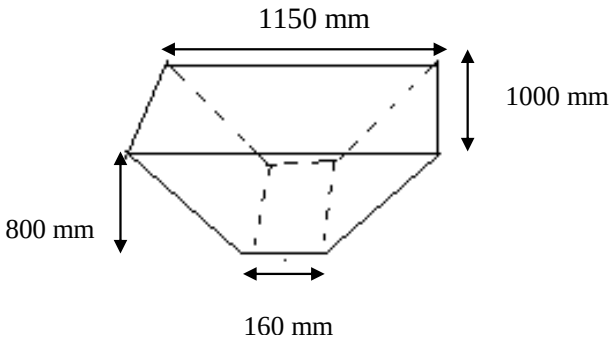

Figure 2. Trapezoidal shaped manure box,

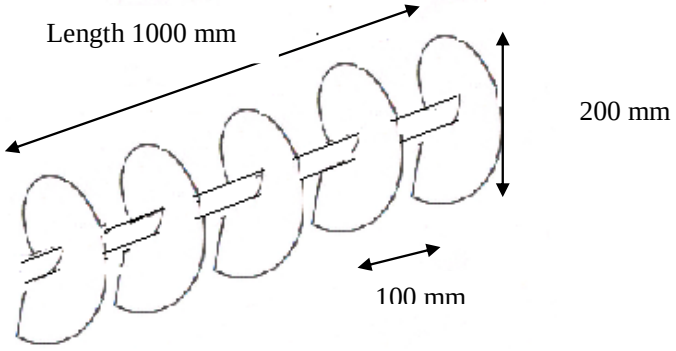

Figure 3. Details of spiral auger used for manure spreading. Spiral auger- disc size: Thickness = 2 mm; Pipe outside dia = 48 mm.

production system.

MATERIALS AND METHODS

A chassis for two-wheeled bullock cart was modified and adopted for animal drawn manure spreader (Figure 1). The chassis frame size (1800 × 1300 × 400 mm) was made of MS pipe of 40 mm inside and 48 mm outside diameter. The chassis frame was mounted on two sides frames, made of MS pipes of dia 48 mm and thickness 4 mm. The adopted axle of 300 mm diameter and 1500 mm was mounted on the both ends of side frames through the clamps. The distance between the side frames was kept 1000 mm. On the both ends of the body of chassis two MS sheet size of 400 × 1150 mm and 2 mm thickness were mounted and in the centre an open space of 1000 × 1150 mm was provided for manure box. A wooden platform of size 1000 × 1150 mm was used to cover the open space. Two pneumatic wheel size of 6 × 16 – 6 PR were provided at the both ends of axle. The track width of manure spreader was 1350 mm.

Manure box

The manure box was made of MS sheet of 2 mm thickness. The box has trapezoidal shaped body for storage and sliding the manure to the rotating auger for spreading (Figure 2). The side frames which gave the strength and support to the manure box made of 2 mm thickness MS sheet. Below the box a spiral auger was provided to spread the manure.

Spiral auger (spreading unit)

A manure-spreading auger made of mild steel spiral discs of 2 mm thickness was provided below the manure box (Figure 3). The diameter of disc is 200 mm. The discs are welded on MS pipe (of outside diameter 48 mm and inside diameter 40 mm) in such a way that the discs deliver the manure directly to the ground. The length of pipe was 1390 mm. At both ends of the pipe bearings have been provided for rotation of auger. It is used to crush the lumps and spreading the manure. Chain and sprocket arrangement have been provided for rotating the auger. To transmit the power from ground wheel to the auger shaft, a sprocket (17 teeth) on axle of cart has been connected with the sprocket (35 teeth) of auger shaft through the chain IS-10. The manure-spreading auger made 32 rpm (0.38 m/s) at cart speed of 2.5 km/h. A dog clutch was also provided to connect and disconnect the rotations of the rotating auger shaft from the ground wheel shaft.

Hitch beam

Two beams of MS pipe (V shaped) of inside diameter 40 mm and thickness 4 mm were used to pull the manure by a pair of bullocks. The length of beam was 2.4 m. Provisions were made for adjusting the angle of beam by lowering or raising the position of beams through changing the nut bolt on different holes on plates mounted on lower side of the body frame. The height of yoke from the

Figure 4. In field recording of pull and speed of operation with and without rotation of manure spreading auger

ground is 1.2 m. The yoke was made of babul wood of diameter 125 mm and length 1600 mm.

RESULTS AND DISCUSSION

The developed animal drawn cart cum manure spreading system was tested in the field for manure application rate and uniformity of manure distribution using a pair of Malvi breed of bullocks (Figure 4). The manure spreader was filled with the farmyard manure. The bulk density of manure was 492.5 kg m^{-3} at moisture content of 22% (d.b.) and manure clod size ranged from 10 to 110 mm with mean manure clod diameter of 46 mm. The manure spreader was operated in the field and time for 10 m travel was recorded.

After operation manure of 10 m length in the direction of line of travel was collected and weighed. Density of manure (kg/m^3) and the moisture content (%, dry basis) of farmyard manure were also determined. The manure application rate and coefficient of variation of uniformity of distribution were determined by using the Equations (1) and (2) respectively (Khurmi and Gupta, 2005).

$$AR = \frac{Q \times 10{,}000}{W \times V} \quad (1)$$

$$CV = 100\left[SD\left(\frac{\sum \times}{N}\right)\right] \quad (2)$$

Where, AR is the application rate in kg/ ha, Q is manure delivery rate in kg/s, W is width of application in m, V is the forward travel speed in m/s, CV is coefficient of variation of uniformity for manure distribution, %; SD is the standard deviation of a set of observations, Σ× sum of a set of observations, g.; and N is the total number of observations.

Measurement of draft and speed of bullocks

A pair of bullocks pulled the manure spreader, filled with the farmyard manure during the testing of manure spreader. The body weight of animal used to pull the manure spreader were determining by using an electronic weighing machine (capacity 0 to 1000 kg). The body size and weight of the experimental bullocks is given in Table 1.

The 21 X micrologger (Campbell Scientific, Inc. U. K.) with load cell (0-5000 N) were used to record the pull of animal. The angle of beam inclination (Ø) was measured by using an abeney level having marking for angles on its periphery. The speed of operation was determined by recording the time of travel of the bullocks for 20 m distance. The power out put of the bullock was determined by using the formula given below.

Draft = Pull × cos Ø
Power (kW) = Draft (kN) × Speed (km/h)/ 3.6

The power requirement for rotating auger and pulling the machine was calculated by using a pair of Malvi breed of bullock in the field. The draft required pulling the manure spreader without operation of auger (auger disengaged from the rotating axle through dog clutch) and with rotating auger for manure spreading (auger engaged with rotating axle through dog clutch) in the field was measured with the speed of operation.

Physical properties of FYM

Physical properties, that is, bulk density, dry matter content; moisture content and angle of friction of farmyard manure at different depth of manure pit of CIAE farm are shown in Table 2. The FYM is a heterogeneous

Table 1. Physical measurement and body dimensions of experimental bullocks.

S/N	Particulars	Malvi bread of bullocks	
		No. 1	No. 2
1	Average heart girth (mm)	1460	1570
2	Average body length (mm)	1480	1660
3	Average height at wither (mm)	1320	1380
4	Body weight (kg)	460	498

Table 2. Physical properties of farmyard manure at different moisture contents.

Moisture content [% (w. b.)]	Bulk density (kg/m^3)	Dry matter content (%)	Angle of repose (Degree)	Angle of friction, degree with	
				MS sheet	G I sheet
20.5	292	80.5	32	33	33
27.2	510	62.8	37	37	36
36.4	680	63.6	42	42	40

material and moisture content of its changes with the depth of storage pit. Hence angle of friction is more important for sliding the manure over a sheet as compared to angle of repose. On the basis of angle of friction slanting platforms were made from G I sheet. The platforms were fitted on the body of the trailer with an angle of 40° for sliding the manure from the top of the box to feeding auger. The moisture content of manure was determined on dry weight basis.

Design capacity

The design capacity of the developed spreader was calculated based on the density of FYM and volume of the developed spreader as given below. The volume of body frame of manure spreader was 0.96 m^2 and capacity was 253 kg considering the density of manure as 550 kg/m^3. The weight of manure in the box was calculated by using Equation (3).

$$V = \left[\left(\frac{l_1 + l_2}{2} \right) wh \right] \quad (3)$$

Where, V is spreader box volume, m^3; l_1 and l_2 are top and bottom length of manure box (l_1 = 1.0 m and l_2 0.20 m); w is width of manure box (1.15 m); h is height of manure box (0.6 m); Substitution of values in Equation (3) resulted in the capacity of manure spreader box as 227 kg. Total capacity of manure spreader was 480 kg. The user could load 4 to 5 bags filled with manure over the leveled manure of the box for transporting to field. Specifications of developed manure spreader are given below.

Specifications

Over all dimensions (l× w × h)mm: 4200 × 1300 × 1400
Type of manure box: Trapezoidal shaped, volume = 0.42 m^3
Type of spreading unit: Spiral auger of 200 mm diameter and pitch 200 mm, length 1000 mm
Power transmission: Speed ratio ground wheel to auger (1:2). IS: 10, chain and sprocket drive for spreading unit at 32 rpm.
Pneumatic wheel: Pneumatic wheel size 6 ×16 " – 6 ply
Ground clearance: 300 mm
Capacity: 480 kg
Width of spreading: 1.1 m
Unit Cost: Rs 25,000
Field capacity: 0.18 ha/h at 2.4 km/h speed
Cost of operation:Rs 70/h
Power source: A pair of bullocks
Suitable for:FYM spreading and transport of materials

Calibration of FYM spreader

The manure spreader was calibrated for manure delivery rate at forward speeds of 2.44, and 2.40 km/h. The manure delivery rate was varied by adjusting the opening width of cover of rotating auger. Manure delivery and application rate with respect to opening width adjusted by opening cover of rotating auger is given in Table 3.

Power requirement in the field

The power requirements for rotating auger and pulling the manure spreader filled with 500 kg were 0.18 and 0.27 kW at average bullock speeds of 0.76 and 0.68 m/s,

Table 3. Manure delivery and application rate with respect to opening width adjusted by opening cover of rotating auger.

Speed of operation (km/h)	Swath (m)	Manure delivery rate (kg/min)	Application rate (t/ha)	Coefficient of variation (%)
Opening area for discharge of manure (0.04 ×1) = 0.04 m^2				
2.38	1.1	8.76	2.22	22.2
2.55	1.1	9.34	1.99	22.4
Avg. 2.46	1.1	9.05	2.10	22.3
Opening area for discharge of manure (0.08 × 1) = 0.10 m^2				
2.36	1.1	22.68	5.13	20.3
2.52	1.1	23.20	5.02	19.9
Avg. 2.44	1.1	22.94	5.08	20.1
Opening area for discharge of manure (0.16 × 1) = 0.16 m^2				
2.26	1.1	42.20	10.12	18.3
2.55	1.1	46.65	9.96	18.1
Avg. 2.41	1.1	44.43	10.04	18.2

Moisture content of FYM = 21% (d b.)

Table 4. Draft and power requirement for operating of manure spreader in the field.

S/N	Draft (N)	Speed of operation (m/s)	Power (kW)	Power required for operation of auger, (kW)
Manure spreading, auger not working				
1	390	0.75	0.29	-
2	360	0.77	0.28	-
3	336	0.77	0.26	-
Avg.	362	0.76	0.27	-
Manure spreading, auger working				
1	650	0.70	0.46	0.17
2	682	0.68	0.46	0.18
3	695	0.66	0.45	0.20
Avg.	676	0.68	0.46	0.18

respectively. However power requirement for pulling the manure spreader in the field and spreading the manure was 0.46 kW at average speed of bullocks 0.68 m/s (Table 4).

Field performance of FYM spreader

The developed machine was tested for manure application rate by using a pair of Malvi breed of bullocks. The manure delivery rate, application rate and field capacity of machine are given in Table 5.

The variation of manure deliver rate at different levels of manure filled in the manure box is evident that the manure delivery rate has decreased from 24 to 21.8 kg/min as the level of manure has reduced from full level to $1/4^{th}$ level in the box. The manure delivery rate decreased 9.2% as the manure level decreased from full level to $¼^{th}$ level in the manure box of spreader.

The only limiting parameters for efficient operation of machine were moisture content of farmyard manure (range 10-30%) and the material would be free from plastic/cloth/wood/bricks and stone particles < 50 mm.

Conclusions

A bullock drawn farmyard manure spreader of 480 kg capacity was developed. The developed machine gave manure application rate of 5 to 10 t/ha for the manure delivery rate of 0.38 to 0.74 kg/s at the operational speed of 2.4 km/h, respectively. The coefficient of variation of

Table 5. Field performance of FYM spreader at different opening widths.

Manure delivery rate (kg/min)	Speed, (m/s)	Swath, (m)	Application rate (t/ha)	Field capacity (ha/h)	Field efficiency (%)
23.8	0.68	1.1	5.30	0.19	84
22.4	0.67	1.1	5.06	0.20	83
22.9	0.66	1.1	5.25	0.18	83
Avg. 23.0	0.67	1.1	5.18	019	83

Moisture content = 20% (db).

uniformity for manure distribution varied from 18 to 20%. The draft and power requirement of the manure spreader were 676 N and 0.46 kW within the draft ability of a pair of Malvi breed of bullocks (Body weight, 958 kg) (Srivastava, 2000; Upadhyay and Madan, 1985). The field capacity and the field efficiency of machine were 0.19 ha/h and 83% at operational speed of 4.1 km/h and the developed manure spreader reduced the drudgery involve in manure spreading over the conventional system.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of alkalinity on germination efficiency index, seedling growth, free proline and sugar during early seedling growth of rice

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Effect of varying level of alkalinity (pH 8.5, 8.8 and 9.2) induced by sodium carbonate on germination efficiency index, seedling growth, free proline and sugar content were recorded in embryo-axis of three rice genotype NDR-501, USAR-1, and IR-24 after 72, 96, 120 h of soaking. Germination efficiency index (GEI), seedling growth and sugar (reducing and non-reducing) decreased with increasing alkalinity levels as compared to control at all the stages of seedling growth. Free proline content of embryo-axis increased with increasing alkalinity levels at all stages of seedling growth. Since tolerant genotypes accumulated relatively more free proline, high sugar content and GEI than susceptible hence these parameter can be used as one of the indices for rapid screening of salt tolerant genotypes.

Key words: Alkalinity, genotype, free proline, sugar, germination.

INTRODUCTION

India has 12 million hectares of salt affected soils, out of which 7 million hectares (Gupta and Gupta, 1997) of land is lying barren due to alkalinity. The process of seed germination which is initiated with imbibitions of water is accompanied by increased metabolic activity (Abbasdokht et al., 2010). The first effective increment of sodicitity for a given crop retards the rate of germination with little or no effect on the ultimate number of seedling which emerges. At higher level, alkalinity further delays and also decreases the final germination percentage (Maghsoudhi and Maghsoudhi, 2008). Salt causes delay in germination because of reduced availability of water. However, certain salts or ions may also be toxic per se to the embryo or seedling if present in relatively higher amount and may thus limit germination (Gupta, 1997; Etesami and Galeshi, 2007). Sodicity also adversely affects seedling growth and different metabolic activities in plant (Begum et. al., 1997; Mostafavi, 2012). Genotypic variability for alkalinity responses during germination and early seedling growth in rice and their relationship between salt tolerance ability of their genotypes and quite meager and inconclusive. Therefore, the present work

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Table 1. Effect of alkalinity on Germination efficiency index in rice genotypes.

Genotype	Control	pH			
		8.5	8.8	9.2	Mean
IR-24	0.78	0.75	0.71	0.67	0.72
USAR-1	0.86	0.85	0.84	0.82	0.84
NDR-501	0.86	0.85	0.83	0.82	0.84
Mean	0.83	0.81	0.79	0.77	
CD at 5%	G=0.0037	A=0.0043	I=0.0074		

was conducted with three rice genotype having variable salt tolerance ability on seed germination, early seedling establishment, proline and sugar.

MATERIALS AND METHODS

The experiment was conducted in petridishes lined with Whatman™ No.1 filter paper under laboratory condition. Petridishes and seed were sterilized with absolute alcohol and 1% sodium hypochloride respectively. The solution of different level of alkalinity viz. pH 8.5, 8.8, and 9.2 were prepared by 50 mM of $NaHCO_3$ in distilled water. Ten healthy seed of three rice genotype IR-24 (salt susceptible), NDR.501 and USAR-1 (salt tolerant) were placed in each petridishes containing 10 ml solution of desired alkalinity level. The petridishes, containing seed were placed in seed germination cabinet at 30±1°C. The experiment was replicated three times in a completely randomized factorial design. Seed with radical emergence of 2 mm length were counted for germination percentage. It was recorded daily up to 6 days (144 h). Germination efficiency index was calculated according to the formula derived by Singh (1967).

$$GEI = \frac{N_0+N_1+N_2+\ldots\ldots\ldots\ldots N_n}{N \times 100}$$

Where N_0, N_1 ,N_2,N_3……N_n are the progressive total of N observation expressed in percentage. N is the number of days taken in complete germination.

A sample of five normal seedlings from each treatment was taken randomly and mean length of coleoptiles and radical daily up to 5 days. Five embryo-axis and endosperm from each replication was dried at 80±1°C for 24 h and the average dry weight was recorded. Sugar (reducing and non-reducing) and free proline was assayed according to method of Dubois et al. (1951) and Bates et al. (1973) respectively.

RESULTS AND DISCUSSION

The data showed that the germination efficiency index (GEI) was higher in genotype USAR-1 and NDR-501 than IR-24 under control condition (Table 1). Increasing pH from 8.5 to 9.2 decreased significantly the GEI in all the genotypes. However, the magnitude of reduction was greater in case of IR-24 than USAR-1 and NDR-501. Decresses in germination percent was recorded in many plant by alkalinity and salinity (Etesami and Galeshi, 2007; Khalessron and Aghaalikhani, 2006; Begum et al., 1997; Chakrabarty and Chattopadhyay, 2000). The delay as well as reduction in final germination and GEI due to alkalinity might be because of an adverse effect of salt on water imbibitions by seed and toxic effect of ions on the metabolism of seed (Gills and Singh, 1998; de Lacerda et al., 2005).

Plumule length decreased with increasing levels of alkalinity in all the genotype (Table 2). Maximum reduction in plumule length was recorded in IR-24 than USAR-1 and NDR-501. At 72 h, the reduction in plumule length in IR-24 was significant even at pH 8.5. But the reduction in USAR-1 and NDR-501 was non-significant except at pH 9.2 in case of NDR-501. However plumule length at 96 and 120 h stages decreased significantly at all level of alkalinity. Radical length was also decreased with increasing level of alkalinity in all the three genotypes (Table 3). Average radical length in USAR-1 was higher than NDR-501 and IR-24 respectively. Among the genotypes, IR-24 showed least reduction at 72 h but at later stages (92 and 120 h), it showed relatively greater reduction than USAR-1 and NDR-501 as compared to control. The reduction in growth of plumule and radical may be attributed to the reduction in the rates of cell division and expansion due to osmotic effect induced by salt stress (Ram, 1979; Singh 2003; Khalesro and Aghaalikhani, 2006; Hakim et al., 2010).

Dry weight of embryo-axis decreased with increasing level of alkalinity (Table 4). The reduction was significant and relatively higher in the genotype IR-64 as compared to control even at lower alkalinity level (pH 8.5). The adverse effect of alkalinity was minimum in the genotype USAR-1 fallowed by NDR-501 and IR-24 respectively. A significant decline in embryo growth was noticed in IR-24 at 72 and 96 h. It was observed that dry weight of endosperm under all the salt treatment was higher than control in all the three genotype (Table 5). The adverse effect of alkalinity was more in IR-24 than USAR-1 and NDR-501which was evident from higher dry weight at 72, 96 and 120 h of germination period. More dry weight under salt stress was due to reduced mobilization of reserve food material from endosperm to the growing embryo-axis. Decrease in dry weight due to alkalinity was reported by a number of workers in different plant

Table 2. Effect of alkalinity on plumule length (cm) in rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	1.15	0.78	0.71	0.62	1.09
USAR-1	1.37	1.28	1.21	1.13	1.66
NDR-501	1.25	1.09	1.06	0.70	1.36
Mean	1.25	1.05	0.99	0.82	
CD at 5%	G=0.13	A=0.15	I=0.27		
		96 h			
IR-24	1.81	1.66	1.45	1.23	1.70
USAR-1	3.69	3.45	3.31	3.81	3.38
NDR-501	3.25	2.98	2.82	2.66	2.92
Mean	2.88	2.69	2.52	2.59	
CD at 5%	G=0.05	A=0.06	I=0.06		
		120 h			
IR-24	3.50	2.97	2.46	2.06	2.74
USAR-1	5.93	5.70	5.16	4.80	5.39
NDR-501	5.13	4.20	4.06	3.70	4.28
Mean	4.85	4.29	3.89	3.54	
CD at 5%	G=0.08	A=0.09	I=0.17		

Table 3. Effect of alkalinity on radical length (cm) in rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	2.19	2.13	2.06	2.12	2.12
USAR-1	4.08	3.66	3.88	3.03	3.53
NDR-501	3.42	3.19	2.88	2.73	3.05
Mean	3.23	2.99	2.77	2.62	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.07	0.09	0.15		
		96 h			
IR-24	4.70	4.06	3.37	3.06	3.79
USAR-1	7.00	6.86	6.81	6.73	6.85
NDR-501	6.88	6.74	6.56	6.03	6.55
Mean	6.19	5.88	5.58	5.27	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.38	0.43	0.76		
		120 h			
IR-24	6.71	6.09	5.80	4.66	5.81
USAR-1	8.73	8.43	7.83	7.50	8.12
NDR-501	8.84	7.99	7.49	6.03	7.58
Mean	8.09	7.50	7.04	6.06	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.15	0.17	0.30		

Table 4. Effect of alkalinity on dry weight (mg/5 axis) of embryo-axis in rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	13.30	11.93	10.90	9.10	11.30
USAR-1	22.60	21.73	20.80	19.40	21.13
NDR-501	20.60	19.93	19.23	18.30	19.51
Mean	18.83	17.86	16.97	15.60	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.63	0.73	1.26		
		96 h			
IR-24	19.59	17.60	16.70	15.80	17.40
USAR-1	25.80	24.89	24.20	23.20	24.52
NDR-501	24.60	23.69	22.60	21.53	23.09
Mean	23.33	22.03	21.16	20.17	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.46	0.53	0.93		
		120 h			
IR-24	25.70	25.80	24.10	21.80	24.35
USAR-1	27.30	26.86	25.33	23.76	25.81
NDR-501	28.00	27.20	26.60	25.36	26.79
Mean	27.00	26.62	25.34	23.64	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.54	0.62	1.08		

Table 5. Effect of alkalinity on dry weight of endosperm (mg/5 seed) in rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	73.93	76.36	77.13	76.63	76.01
USAR-1	73.96	76.03	76.35	76.30	75.66
NDR-501	73.63	75.87	77.20	76.65	75.83
Mean	73.84	76.08	76.89	76.52	
	Genotypes	Alkalinity	Interaction		
CD at 5%	1.38	1.59	4.03		
		96 h			
IR-24	70.01	75.53	76.10	76.08	74.43
USAR-1	69.63	75.90	74.50	74.43	73.61
NDR-501	68.90	75.83	73.29	75.01	73.00
Mean	69.51	75.42	74.63	75.17	
	Genotypes	Alkalinity	Interaction		
CD at 5%	1.38	1.59	4.03		
		120 h			
IR-24	68.06	74.56	71.50	75.09	72.30
USAR-1	66.77	73.10	72.45	72.09	71.10
NDR-501	67.23	70.06	70.19	70.70	69.54

Table 5. Contd.

Mean	67.35	72.57	71.38	72.62
	Genotypes	Alkalinity	Interaction	
CD at 5%	1.16	1.34	1.80	

Table 6. Effect of alkalinity on free proline content (mg/g fresh weight) in embryo-axis of rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	27.50	32.20	38.46	41.60	34.94
USAR-1	32.20	36.90	46.60	42.00	39.42
NDR-501	30.63	36.90	41.60	42.00	37.78
Mean	30.11	35.33	42.22	41.86	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.91	1.05	1.83		
		96 h			
IR-24	32.20	36.90	41.73	44.66	38.87
USAR-1	35.33	41.60	44.66	47.66	42.31
NDR-501	32.20	36.90	41.60	42.00	38.17
Mean	32.24	38.46	42.66	44.77	
	Genotypes	Alkalinity	Interaction		
CD at 5%	1.29	1.50	2.60		
		120 h			
IR-24	33.76	40.33	42.00	49.33	41.35
USAR-1	36.90	41.73	44.66	52.66	43.98
NDR-501	33.76	41.60	42.00	45.33	40.67
Mean	34.80	41.22	49.10	49.10	
	Genotypes	Alkalinity	Interaction		
CD at 5%	1.60	1.85	3.20		

(Narayana and Rao, 1987; Singh, 1989; Alebrahim et al., 2008). Gill and Singh (1998) also reported that the salinity levels reduced weight of endosperm was due to decreased mobilization in rice.

The free proline content of the embryo-axis increased significantly under the influence of increasing alkalinity as compared to control at all the stage of growth. Genotype USAR-1 accumulated more proline than NDR-501 and IR-24. Proline content at 120 h was higher than that of 96 and 72 h period. With increasing alkalinity levels, Proline accumulates depending on plant species, the genotypes and degree of stress to which the plants are exposed (Table 6). Similar views have been advocated by Khan et al. (2009). Increase in free proline content under stress could occur due to de novo synthesis of proline or break down of proline rich protein or a shift in metabolism. On the basis of these result, it could be deduced that proline accumulation is an index of severity of stress rather than resistance, though it does impart certain advantage to the plant under stress (Koca et al., 2007 and Ueda et al., 2007). Reducing sugar as well as non reducing sugar at 72, 96 and 120 h in embryo-axis decreased with increasing level of alkalinity more in IR-24 compare to USAR-1 and NDR-501 (Tables 7 and 8). It increased along with seedling growth in embryo-axis showing maximum content at 120 h in all the treatment and stage of growth. Singh et al. (1990) reported that lower reducing and non-reducing sugar in pea under PEG and salt induced stress. Salinity decreased the sugar content in wheat embryo-axis and endosperm (Singh, 2003; Bhagheri and Sadeghipour, 2009).

Lower sugar content in wheat embryo-axis and endosperm of rice genotype under salt stress as compared to control speaks of reduced hydrolysis of reserve activity of α-amylase under stress condition (Dhanapackiam and Illyas, 2010).

Table 7. Effect of alkalinity on reducing sugar (mg/g dry weight) in germination embryo axis of rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	19.85	17.73	15.53	14.40	16.88
USAR-1	21.06	19.43	18.30	16.06	18.71
NDR-501	20.53	17.16	15.33	13.86	16.77
Mean	20.48	18.10	16.45	14.77	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.91	1.05	1.83		
		96 h			
IR-24	21.26	20.60	17.73	16.60	19.04
USAR-1	23.30	20.50	18.30	17.73	19.95
NDR-501	21.06	19.96	18.30	17.16	19.12
Mean	21.87	20.35	18.11	17.16	
	Genotypes	Alkalinity	Interaction		
CD at 5%	NS	1.27	2.21		
		120 h			
IR-24	25.00	22.06	21.06	20.00	22.03
USAR-1	29.40	25.53	23.03	20.50	24.68
NDR-501	26.60	23.30	20.53	20.00	22.60
Mean	27.00	23.63	21.63	20.16	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.90	1.05	1.81		

Table 8. Effect of alkalinity on non- reducing sugar (mg/g dry weight) in germination embryo axis of rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	49.96	47.73	45.00	42.16	46.21
USAR-1	54.40	52.20	50.53	48.30	51.35
NDR-501	54.43	51.06	46.63	44.43	49.13
Mean	52.93	50.33	47.37	44.96	
	Genotypes	Alkalinity	Interaction		
CD at 5%	1.01	1.16	2.02		
		96 h			
IR-24	51.60	47.73	46.60	43.86	47.44
USAR-1	57.73	55.00	53.30	51.63	54.41
NDR-501	56.60	52.20	50.00	48.30	51.77
Mean	55.31	51.64	49.96	47.93	
	Genotypes	Alkalinity	Interaction		
CD at 5%	0.76	0.88	1.52		
		120 h			
IR-24	55.53	53.30	49.06	46.06	51.07
USAR-1	58.30	55.53	53.86	52.73	55.10
NDR-501	56.60	54.96	51.60	49.40	53.14

Table 8. Contd.

Mean	56.81	54.59	51.62	49.39
	Genotypes	Alkalinity	Interaction	
CD at 5%	1.10	1.27	2.20	

Conclusion

On the basis of the present investigation, it can be stated that USAR-1 genotype was relatively more tolerant to alkaline condition closely followed by NDR-501 as compared to IR-24 which proved to be more vulnerable against alkalinity. Genotypes USAR-1 and NDR-501 are probably better equipped to combat the adverse effect of alkalinity than IR-24. Differential proline accumulation in three rice genotype in response to alkalinity level is yet another index which can be exploited for screening salt tolerant rice genotype since it definitely play some role in stress sustenance.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Assessment of nicotine levels in Tanzanian honeys from tobacco growing and non-tobacco growing areas

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In Tanzania, tobacco is one of the major agricultural export crops, being the third largest foreign exchange earner after coffee and cashew nuts. Concurrently, in tobacco producing areas there are also honey production activities in which the quality of honey produced has been assumed to be affected by nicotine levels. The surveys were carried out in Tabora region as a major tobacco growing area and producer of honey, Dodoma and Kilimanjaro regions as non-tobacco growing areas but involved in honey production. Honey samples were collected at random from honey producers and analysed on nicotine levels at Sokoine University of Agriculture laboratories. The results showed that there is an existence of nicotine traces in Tanzanian honeys from both tobacco growing and non-tobacco growing areas. The factors contributing to this included the non-restriction movement of honeybees within the country, natural presence of nicotine in some flowering plants and airborne transmission of nicotine traces, especially through air pollution. Majority of farmers' perception on quality of honey showed they disagree that tobacco farming greatly influences honey quality through the presence of nicotine.

Key words: African honeybee (Apis mellifera scutellata), Tanzanian honeys, Miombo woodlands, tobacco growing and non-tobacco growing areas in Tanzania, nicotine levels.

INTRODUCTION

Tanzania is one of the ten-mega countries in the World with high potential in beekeeping for the production of bee products, mainly honey and beeswax. Beekeeping is also widely practiced in South Africa, Egypt and Kenya in the continent of Africa. Tanzania has been one of largest exporters of beeswax in the world with records dating back in 1973 where by 275 tonnes were exported (URT, 1998). Honey and beeswax is mainly exported to the

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Table 1. High and medium potential beekeeping areas and un-eploited beekeeping areas in the Miombo woodlands compared to non-Miombo woodland areas.

High producing areas (all in miombo woodlands)			Medium producing areas			Unexploited areas		
District	**Potential**	**Actual**	**District**	**Potential**	**Actual**	**District**	**Potential**	**Actual**
Manyoni	8000	1500	Kibondo	3000	150	Lindi	8000	50
Mlele	8000	600	Handeni	3000	150	Songea	6000	50
Chunya	6000	2000	Kondoa	3000	300	Iringa	5000	40
Sikonge	6000	1400	Kigoma	3000	100	Biharamulo	4000	15
Urambo	6000	400	Rufiji	2500	150	Kasulu	4000	5
Bukombe	5000	1200	Kiteto	2000	250	Newala	4000	15
Uyui	5000	800	Nkasi	1500	50	Tunduru	4000	15
Kahama	4000	500	Arumeru	1500	100	Singida	3000	5
Kilombero	400	400	Babati	1200	150	Hai	1500	5
Nzega	4000	50	Kilosa	1200	50	Kongwa	1500	5

Metric tonnes on potential and actual production is shown. Source: National Beekeeping Programme, 2001 (Mpuya, 2003).

United Kingdom, Netherlands, Germany, Italy, Belgium, and until recently to Far East (China and Japan) and Middle East countries. However, data on the size of local markets compared to total production of bee products are not well documented although consumption of honey is widespread in the country (MNRT, 1998).

Most of honey produced in Tanzania comes from Tabora region which is the main producer in the country. The remaining part comes from Singida, Mbeya, Dodoma, Arusha, Shinyanga, Rukwa, Kigoma and Tanga regions. The potentiality of beekeeping is especially dominant in Miombo woodlands, Acacia bushlands, Itigi thickets, Eastern Arc and Coastal forests, coconut and clove growing regions, which are Pemba and Unguja Islands (Riechart, 2000). Over 95% of beekeeping in Tanzania is practised in these Savannah forests within the Miombo woodlands ecoregion (WWF) (2002).

Miombo woodlands in Tanzania constitute the largest forest ecosystem in the country, covering more than two thirds of the total forested land (Mbuya et al., 1994; URT, 1998). Miombo woodlands are normally dominated by trees of the closely related genera such as *Brachystegia*, *Julbernardia* and *Isoberlinia* (subfamily Caesalpinioideae, family Fabaceae). Miombo woodland area covers about 425,000 km^2 out of 945,000 km^2 total forested area for Tanzania (Campbell, 1996). According to Monela et al., 2000, honey is an important non-wood product with a very significant contribution to cash incomes to the rural communities in the Miombo woodland area. It contributes to about 33% of the household income source compared to other sources like agricultural crops and other forest products. Most of the high production areas on bee products are concentrated in the Miombo woodland areas as shown in Table 1. There is also evidence of many unexploited areas in the Miombo woodland areas as compared to non-Miombo woodland (Riechart, 2000; Mpuya, 2003). This entails that beekeeping in Miombo woodlands plays a major role in creating employment to both men and women, old and the youth (Adams et al., 2004). It has been a common tradition in many tribes in the country; particularly the “Wanyamwezi” in Tabora region and “Wakimbu” in Singida Region, although it remains largely that beekeeping is a man’s activity.

Traditional beekeeping remains a very important mainstay for honey and beeswax produced in the country. Ninety nine percent (99%) of the total production comes from traditional hives (URT, 1998). Honey and beeswax production in Tanzania is dependent on small-holder beekeepers, using traditional hives for African honeybees, *Apis mellifera scutellata*. In the past, traditional beekeeping applied a “let alone method” practiced by hanging of log and bark hives using folks and associated with “folklores” that excluded women from beekeeping. Modern hives were first used under the Tanga Integrated Rural Development Programme in north-eastern Tanzania during the then German East Africa Protectorate (Morstatt, 1930). After independence, two hives were invented; the Tanzanian Transitional Hive (TTH) and Tanzanian Commercial Hive (TCH), the later been a modification of the Langstroth Hive and the two box hives were recommended for use for African honeybee beekeeping (Ntenga and Mugongo, 1978). The presence of both stinging and stingless honeybees coupled with existence of indigenous knowledge in beekeeping is also a great potential. According to Kihwele (1991), it is believed for example that, about 10% of honey available for the local market goes into factories such as bakeries and confectioneries, and pharmaceutical industries (Kihwele et al., 2001; Ngailo, 1997); the rest is consumed in various traditional ways as food and medicine. At present, the estimated number of bee colonies is 100 million, an increase of 320% in 30 years; 20% increases each year since 1978 (URT, 2005).

Honey is the natural food of honey bees, including the

African honeybee, *Apis mellifera scutellata.* Honeybees gather, modify and store the nectar and saccharine exudation of plants which forms the honey (Gidamis et al., 2004). Honey is a complex mixture composed of water, sugar (fructose, glucose, saccharose, maltose, high sugars, gluconic acid, lactone, nitrogenous compounds, minerals and some vitamins). The composition and properties of these complex mixtures making honey depends on floral origins utilized by the bees and climatic conditions of the area from which honey is harvested. Honey qualities is influenced by various factors including storage abuse (which leads to darkening and loss of aroma and flavour), overheating and adulteration with syrup. Good quality honey, that is, honey of value can be judged by five key factors, namely: water content, Hydroxymethylfurfural (HMF), inverted sugars, impurities and colour. The contents materials of a good quality honey are water (17.2%), sugar (80%), organic acids (0.6%), enzymes (2%), mineral materials (0.2%). For the case of impurities good quality honey is expected to be pure if it is visually free of defect, clean and clear (Gidamis et al., 2004).

Nipashe Newspaper, one of the major selling newspapers in Tanzania, published a report on September 12th, 2011 by the former Deputy Minister for Health and Social Welfare, Dr. Lucy Nkya; who was quoted stating that the government would soon loose its income from honey sales in China. The report claimed that there were complains from China that the honey produced from tobacco producing regions, particularly, Tabora had been found to contain nicotine traces. She continued to have advised the beekeepers to site hives away from tobacco producing areas. After the spread of the news, TAWIRI through Njiro Wildlife Research Centre (NWRC) received more complaints from customers who visited TAWIRI pavilion at the International Trade Fair in Mwalimu Nyerere Grounds at Kilwa Road in Dar es Salaam and Farmers Nane Nane Show Grounds in Dodoma and Arusha respectively. They also claimed that honey harvested in Tabora has traces of nicotine, although they had insufficient evidence of their complains. It is against these claims that this research project was initiated in the realms of policy makers, honey producers and consumers to need sufficient and reliable information through a thorough research by recognised organs, namely TAWIRI and TORITA.

This study is aimed to substantiate whether there is presence of nicotine traces in Tanzanian honeys produced in various tobacco producing districts of Tabora Region and from non-tobacco producing areas such as Dodoma and Kilimanjaro regions.

MATERIALS AND METHODS

Description of the study area

The study was conducted in three honey producing regions of Tabora, Dodoma and Kilimanjaro, which are regarded as amongst the most popular honey producing areas in the country. One district from each region was selected for nicotine assessment. A total of six villages were randomly selected to present the entire honey producing community of the regions. Amongst the selected regions, Tabora region was selected on the basis that it is mainly covered within Miombo woodland that favours both production of tobacco and honey. About 60% of farmers grow tobacco on average of 1.0 ha per farmer (Yanda, 2010). Kilimanjaro (Same District) and Dodoma (Kongwa District) were considered as regions producing honey with limited activities of tobacco farming. All study areas were described by the presence of a mixture of various natural plants making the honey to be mostly of polyflora in nature.

Honey sample collection and analysis

Fresh ripe honey samples were collected from bee apiaries in each district from October 2011 to February 2012. Eight (8) villages in Urambo District, five villages in Sikonge District, and one village from Uyui District, potential for honey and tobacco production were randomly selected where five honey producers were selected per village. One district in Kilimanjaro (Same) and Dodoma (Kongwa) regions were selected where two villages potential for honey production were randomly selected; honey samples were collected from five honey producers in each district. The honey samples collected were sent to Sokoine University of Agriculture (SUA), Department of Food Science laboratories for analysis. The samples were analysed for nicotine content and other parameters like Hydroxymethyl Furfural (HMF), *p*H, colour, moisture, fatty acid, and general acceptability.

Nicotine laboratory analysis

Nicotine was analysed based on method described by Carlos et al. (2011). HMF analysis was based on the determination of UV absorbance of HMF at 284 nm and the background at 336 nm (mg/kg). Both nicotine and HMF levels will be used to advise the honey consumers whether the honey they consume is harmless to their health.

Status on environmental degradation and beekeeping activities

The perception of honey producers, sellers and consumers on quality of honey harvested from tobacco growing areas and non-tobacco growing areas was assessed through interviews using structured questionnaires. Five producers and sellers from each district under study areas were interviewed. Questionnaires were analysed using Statistical Package for Social Sciences (SPSS). Both the structured questionnaires and checklists of probe questions were used to interview the District Beekeeping Officers (DBOs), District Agricultural Officers (DAOs), tobacco growers, and individual beekeepers in order to assess the status on environmental degradation, beekeeping activities, pesticide usage, etc.

RESULTS

Nicotine content

The laboratory results showed that levels of nicotine in honeys collected from Kongwa, Uyui, Urambo and Same Districts ranged from 0.0033 to 0.0051 mg/kg (Tables 2

Table 2. Chemical analysis of honey samples collected study areas.

Area/district/region	Sample size (ml)	Honey colour	Moisture content %	*pH*	HMF (mg/kg)	Nicotine (%)
Kongwa, Dodoma	301	Light brown	22.84	4.90	10.63	0.33
Makanyachini, Same	804	Dark amber	23.25	4.88	8.89	0.39
Itobela, Uyui	290	Dark brown	22.64	4.15	7.86	0.42
Kangeme, Urambo	230	Brown	23.81	4.30	23.52	0.46
Urambo Town	250	Dark amber	24.66	4.37	21.25	0.51
Kilometa 48, Urambo	270	Dark amber	23.25	4.08	12.03	0.44

Table 3. Chemical analysis of honey samples based on nicotine content (mg/kg) collected study areas.

Sample name	GPS readings	District	Village/area/ group/apiary	Distance from tobacco farms (km)	Nicotine (mg/kg)
TBR/UR/UG/F/RE	S 05º 47’ E 31º 97’	Urambo	Ugalla reserve	24	0.000553
TBR/UR/F/RE	S 05º 35’ E 31º 73’	Urambo	Sinyato village	10	0.004394
TBR/UR/ZU/NAA/VI	S 05º 32’ E 31º 65’	Urambo	Zugimlole	5	0.006568
TBR/UR/ZU/VI	S 05º 32’ E 31º 65’	Urambo	Zugimlole	4	0.005438
TBR/UR/IT/VI	S 05º 21’ E 32º 08’	Urambo	Itebulanda	10	0.007253
TBR/UR/ZU/V	S 05º 32’ E 31º 65’	Urambo	Zugimlole	3	0.004119
TBR/SI/KI/BU	S 05º 89’ E 32º 99’	Sikonge	Kiloleli Village	40	0.001667
TBR/SI/MK/FA	S 05º 89’ E 32º 99’	Sikonge	Mkola Group Igunawapina	10	0.001103
TBR/SI/KI/F/RE	S 05º 89’ E 32º 99’	Sikonge	Kinyato Group Igunawapina	95	0.003837
TBR/SI/F/RE	S 05º 92’ E 33º 25’	Sikonge	Nyahuwa F/R	38	0.003843
TBR/SI/KI/FA	S 05º 89’ E 32º 99’	Sikonge	Kikoleli	2	0.003655

and 3). These findings indicate that apart from tobacco plants, there are also other plant species with nicotine content that contaminate honey. Given that nicotine is an alkaloid substance commonly produced in the nightshade family of plants (Solanaceae), this implies that the source of nicotine in honey was also from other plant species found within the honey production areas. Tobacco and other members of Solanaceae were found growing in the

tobacco-growing areas within the Miombo woodlands and were also found in non-tobacco growing areas within the Miombo woodlands.

Tobacco is a night blooming plant producing nectar at night which might not attract bees, then nicotine might be assumed to be sourced through pollen collection. This is because nectar provides only the carbohydrate portion of a bee's diet. The protein portion comes from the pollen produced by various plants. Pollen is essential for drone and brood rearing and is particularly important for colony build up in preparation for honey flows and pollination work. Nicotine is an alkaloid found in the nightshade family of plants of Solanaceae family that constitutes approximately 0.6 to 3.0% of the dry weight of tobacco with biosynthesis taking place in the roots and accumulation occurring in the leaves. Nicotine is also found naturally in floral nectar mostly in all types of tobacco plants. This nectar is collected by bees to be converted into honey. It is estimated that tobacco (*Nicotiana glaura*) has about 6 to 8 mg/kg of nicotine per gram. Several studies showed that nicotine is essential for our health. As per research studies, a human being requires a certain amount of nicotine through the means of his diet or supplements, to manage the smooth functioning of her/his body. In case there is a nicotine deficiency, the body may not be able to fulfill the basic as well as complex mitochondrial functions. This in turn would leave us prone to several diseases that could have been avoided if our nicotine levels were kept up to the mark. But if nicotine level in human body is high several complications may occur (Castro and Monji, 1986).

There are possibilities of honey to contain nicotine as bees collect nectar of various plants. Different plants and common vegetables are reported to contain nicotine. As shown in several studies, eggplant, pepper as well as tomatoes have been known to contain high amounts of nicotine and are always consumed by human beings in large quantities (Carlos et al., 2011). The fresh ripe tomatoes are reported to contain 4.3 to 4.1 mg/g nicotine. In fact, Peanuts, Lettuce, Mushrooms, Tuna, Salmon and Mustard are known to contain high amounts of nicotinic acid as well (Edward et al., 1993). This acid is required to convert carbohydrates into sugar, so the body has enough fuel to carry out all its basic as well as advanced functions in an effective manner. Unless the body has enough of nicotine content, you would feel lazy, weak and can fall prey to several diseases that are directly or indirectly related to a weak immune system. The levels of nicotine found in the samples analysed trigger the idea of further investigation on nicotine content in other plants other than tobacco.

Moisture content percentage

The laboratory analysis results showed that moisture content of honey samples were from 22.6 to 24.7% (Tables 2 and 3). However moisture content of honey from Urambo and Same Districts were above the recommended amount for good quality honey. It was reported that the main parts of the honey are sugars and water. The standard water content of a ripe honey should be less than or equal to 22.0% (TBS, 2006). This implies that honey producers in Urambo and Same Districts rather than improving other aspects of good quality honey, water content has to be improved. Good quality honey essentially has low water content. Honey is likely to ferment and lose its freshness if the water content of honey is greater than 22.0%. The reason is that all unpasteurized honey contains wild yeasts. Due to the high sugar concentration, these yeasts will pose little risk in low moisture honey because osmosis will draw sufficient water from the yeast to force them into dormancy. The honey that has higher proportions of water favours the yeast to grow and survive and this may cause honey to begin fermentation during storage. Fermentation increases acidity, which then becomes an important quality criterion.

HMF (Hydroxymethylfurfural)

The results showed that all samples analysed were having the recommended standards of HMF which ranged from 7.9 to 23.5 mg / kg (Table 2). The recommended level of HMF has to be less than or equal to 40 mg/kg (TBS, 2006). HMF is a break-down product of fructose (one of the main sugars in honey) formed slowly during storage and very quickly when honey is heated. HMF's occurrence and accumulation in honey is variable depending on honey type. Honey that is traded in a bulk form is usually required to be below 10 or 15 mg/kg HMF to enable further processing and then give some shelf life before a level of 40 mg/kg is reached. It is not uncommon for honey sold in hot climates to be well over 100 mg/kg in HMF. This is mostly due to the ambient temperatures (over 35°C) that honey is exposed to in the distribution channel.

Colour and pH

The honey samples colour did not vary with nicotine or *p*H variability (Table 2). This implies that colour was not a factor of collected honey samples. The samples collected were having dark brown, light brown, brown and dark amber colours. Honey colour is measured on the Pfund Scale in millimetres.

Although it is not an indicator of honey quality and there are exceptions to the rule, generally speaking, the darker colour the honey, the higher its mineral contents, the *pH* readings, and the aroma/flavour levels. Minerals such as Potassium, Chlorine, Sulphur, Iron, Manganese, Magnesium and Sodium have been found to be much

Figure 1. Honey samples collected from study areas showing variation in colours (From right to left are samples from Urambo, Kongwa, Same and Uyui Districts).

Table 4. Socio-economic characteristics of respondents from the study areas.

Variable	Frequency	Percentage
Gender		
Males	20	66.7
Females	10	33.3
Total	**30**	**100**
Education level		
No education	7	23.3
Primary education	21	70.0
Secondary education	2	6.7
Higher education	0	0
Total	**30**	**100**

higher in darker honeys. The sample collected from Urambo and Same districts were found to have darker colour (dark amber colour) which is the best quality honey (Figure 1).

Socio-economic characteristics

The socio-economic profile of respondents examined were gender and education levels

Gender of respondents

The majority of respondents were male (66.7%) followed my female (33.3%) as shown in Table 4. This may be due to the fact that male are the ones who do most of the farm activities and are the ones who own land as compared to female.

Education of the respondents

The result showed that education level was similar across the study districts with over 70.0% of respondents having primary school (Table 4). Overall 70.0 and 6.7% of respondents had attained primary and secondary education respectively.

Producers/seller's awareness on effect of tobacco on honey quality

Consumers complains on the quality of honey

Information given from honey producers and sellers in all three regions under the study (Tabora, Kilimanjaro and Dodoma) revealed that there are some complains from the consumers/users of honey on its quality. The complains basis on water content of honey which is

Table 5. Issues that consumers complain on the quality of honey.

Variable	Frequency	Percentage
Complains		
Water content	9	30.0
Adulterated (heated) honey	7	23.3
No complains	14	46.7
Total	**30**	**100**

Table 6. Awareness of the effects of tobacco on honey quality.

Variable	Frequency	Percentage
Respondents awareness	11	36.7
Not aware	19	63.3
Total	**30**	**100**

Table 7. Factors that may affect the quality of honey in farm/storage room.

Variable	Frequency	Percentage
Pesticides and herbicides used in tobacco farming	9	66.7
Absorbents and flavours	3	10.0
Pesticides and herbicides used in vegetable farming	6	20.0
Acaricides	7	23.3
Total	**30**	**100**

above the standard level, that is, above 22.0% (TBS, 2006) of a good quality honey, this may be due to the fact that the honey sellers who sells honey as retailers tend to add some amount of water in a fresh harvested honey in order to increase the volume of honey for profitability. Furthermore they heat honey which results to increases of the HMF levels thus loosing the standards of a good honey like colour, flavour and other nutrients. As indicated in Table 5, 30.0% of respondents reported that consumers/users complained that the honey produced in all three regions have water content beyond the standard content. About 23.3% of respondents reported that consumers complain that the honey is heated and hence looses its good quality.

Effect of tobacco on the quality of honey

Awareness on the effects of tobacco on honey quality

Table 6 shows the results of respondents regarding the effects of tobacco to the quality of honey. Only 36.7% of respondents were aware that tobacco may affect the quality of honey harvested while over 63.3% of respondents were not aware that tobacco may have effects on the quality of honey. However, the results has also revealed that respondents from Dodoma and Kilimanjaro regions has low awareness that tobacco may affect the quality of honey as compared in Tabora Region.

Pesticides effects on the honey quality

Major factors that may affect the quality of honey on farm in the study area were identified. Results in Table 7, revealed that pesticides and herbicides used in tobacco crop (46.7%) may have effect on the quality of honey. This situation may be due to the fact that during pollination if tobacco crop were sprayed with pesticides or herbicide honey bees may carry the chemicals with the nectars or pollens to the beehives which will be used to produce honey. Furthermore, honey was found to have a tendency of absorbing smells (10.0%) of things stored close to it, for example if honey stored with tobacco will be smelling like tobacco. These were followed with the incidence of pesticides and herbicides used in vegetables (20.0%) and chemicals used in dipping animals (23.0%) especially cattle may have the effect on the quality of honey.

Field observations in tobacco farms

It was observed that tobacco farmers were spraying pesticides known as Hotshot 70 WG, cofidol and Yamaotea super EC (Tobacco desuckeride) to kill pests and cub the growth of shots. Other farmers were busy cutting off early flower stalks so as to strengthen the leaves. Some of tobacco plants were grown at the height of about 20 to 120 cm allowing 4 to 18 leaves. Some farmers especially in Sikonge West and Urambo East were harvesting leaves. Very few tobacco flowers were seen and farmers claimed that was a mistake to find flowering tobacco plants during this period.

Beekeeping situation

Very few beekeepers are siting their hives close to tobacco farms. Most of them claimed to understand that siting hives close to tobacco farms might contaminate honey and therefore affect its quality. Beekeepers reported that they have information of presence of nicotine in honey, as they were educated by beekeeping extension officers in several seminars and beekeeping workshops. Following the knowledge they got after these seminars, most beekeepers transferred their hives from 5 to 95 km away from tobacco farms. During the collection of information we were also told that some other beekeeping experts from Forestry and Beekeeping Division Headquarters visited in Tabora for honey sample collection to test for presence of heavy metals in honey e.g. Chroramphenical, polychrorinated byphenils, pyrethroids, chloropestsitides, carbamates, tylosine, tetracyclines, nitrofurans, supphonamides and streptomycine. Nothing was done to test for nicotine.

DISCUSSION

General results indicated that all the honey samples collected from all study areas contained nicotine traces; however the levels of nicotine were relatively higher in tobacco growing areas than non-tobacco growing areas. Furthermore, the traces were found in smaller concentrations making it harmless to human consumption. That said, these results are used to make a strong call to urge local and international consumers to continue their consumption of Tanzanian honeys as it is not detrimental to their health. The results of this study showed that all honey samples produced in tobacco growing areas and non-tobacco growing areas were found to contain nicotine levels. However the level of nicotine content differs from one sample to another. In all samples of honey collected even those very far away (95 km) from tobacco farms were found to have some amount of nicotine, this may mean that other sources of nicotine from other plants (from Solanaceae family) apart from tobacco may have contributed to the presence of nicotine in honeys. Honey harvested from tobacco growing areas had higher level of nicotine ranging from 0.0043 to 0.0051 mg/kg for Sikonge, Uyui and Urambo Districts followed by those in non-tobacco growing areas which had 0.0033 and 0.0039 mg/kg for Dodoma Rural and Same districts respectively.

The presence of nicotine as a natural product in a number of plant species apart from tobacco contributes the contamination of honey in bee colonies. The nicotine found in honey has been contributed by bees through collection of nectars and pollen grains from different plants thus making honey to be polyfloral in nature. Polyfloral honeys are composed of floral nectars and saccharine exudates from various plant trees to produce honey. This study therefore concludes that tobacco crop is not the only plant that contaminates honey with nicotine. In fact, the contribution of nicotine from tobacco plant is termed as minor in honey samples. One plausible explanation is fact that majority or all honeys produced in Tanzania are polyflora in nature. Another fact is that tobacco flowers are night blooming by nature which means they bloom at night because they do not have attractive colours which can attract pollinators during day time instead they attract pollinators by using their smell at night time and honeybees do not pollinate at night time. It was further found that honey has a characteristic of absorbing smell or flavour of chemicals stored close to it.

Few respondents were aware of the fact that tobacco crop may have effect on the quality of honey produced though their awareness was not on the presence of nicotine in honey but other factors, like pesticides and herbicides used in treating tobacco (46.7%), and vegetables (20.0%), and acaricides used in dipping animals (23.3%) (Table 6). The results also indicate that most of respondents were aware that pollination is another factor that causes or may affect the quality of harvested honey is pollination. During pollination the honeybees may come into contact with these chemicals and carry them to the beehives or some chemicals may even kill bees thus affecting both the quality and production of honey.

The study also showed that there is no direct relationship established between the producers and the consumers on the market of bee products; which has brought about mistrust in the supply of bee products. In some countries, for instance, in Europe and the Middle East, consumers of table honey have trust in the beekeepers that supply them with high quality honey year after year. In Tanzania, there is lack of marketing organisations indulged in marketing of bee products such as Beekeepers Co-operative Societies, some of which even in recent years have become less and less in its operations. The Cooperative Societies and private sector promise a bright feature in promoting the role of beekeeping industry in poverty reduction. Proper marketing approaches and strategies are needed to be

addressed. This study will explore marketing channels for proper advise and observance of quality products, including quality assurance mechanisms within the market.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Impact of an agricultural chronosequence in recharge areas of aquifers in the Brazilian savannah

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Current study was conducted, in 2010, on the Boa Vista Farm at the Rio Claro watershed of the southwestern region of Goiás, state, Brazil. The region features micro-reliefs with marshy fields and predominantly Haplic Plinthosols. Most areas were incorporated into the agricultural production systems without evaluating the impact on their hydro-physical characteristics. Current analysis evaluates the transformation impact of marshy lands into agricultural areas through an investigation of empirical methods of water infiltration into the soil. Three chronosequenced areas for agricultural use and a preservation area were selected. Water infiltration in the soil was analyzed by field data and by calculations following Kostiakov, Horton and Lewis-Kostiakov. Models overestimated infiltration rates at the beginning of the process and compared to those from field data. The model proposed by Horton was highly similar to rate of basic infiltration speed harvested from field data. Structural quality was reduced through infiltration velocity in the area with human interference with grown under no-tillage system.

Key words: Haplic plinthosol, mounds, hillocks, cylinder infiltrometers, no-tillage system.

INTRODUCTION

Micro-reliefs in marshy fields (murundus, covais, cocorutos and monchões, in Portuguese) of the savannah plateau of Goiás state, Brazil, form extensive areas predominantly featured as Haplic Plinthosols soil. The soil is highly relevant due to its role as a water recharge and supply for underground water and the maintenance of water levels in streams and rivers of one of the most important Brazilian hydraulic sources, or rather, the river Paranaíba basin.

During the last decades the above mentioned areas in

the Goiás state, Brazil, were incorporated to cash crop production systems. Landscape transformation in an agriculture-occupied process went beyond the loss of biodiversity and climate changes, with the modification of soil structure. The consequences, comprising soil compaction and erosion, accumulation of silt in waterways and liability in water resources, are perceptible, even though their evolution and economical and environmental consequences are still largely unknown. Impact caused by structural changes in the soil´s physical and hydraulic features has been reported by Alves et al. (2007) and Bonini and Alves (2012). According to Mota and Valladares (2011), human activities have produced environmental degradation, erosion, soil contamination and silt in the water courses. In fact, the impact model currently threatens the sustainability of the productive systems.

The incorporation of marshy areas, essential for the recharge of the region´s aquifers and characterized by soil saturation during most of the year, brought about the building of a great network of soil drainage. In other words, the underground water of the earth mound fields was lowered by a network of drainage canals in several municipalities of the region. The municipality of Jataí in the southwestern region of the Goiás state may be highlighted. Results quite often caused an excessive dryness of the soil and consequently the hardening of the plinthic ground surface by barring infiltration, hindering the natural drain of water, discharge decrease of streams with lower water flow towards the rivers of the watershed in which they lie.

Due to the region´s characteristics (high rates of organic materials and clay, land relief and possibility of mechanization), high productivity rates abounded throughout the last decades. Consequently, year by year producers have incorporated other areas to the production system without evaluating the effect of such incorporation on soil quality and environment. This is especially grave due to the importance of the region for the Brazilian hydrological system (Gomes Filho et al., 2011). According to Silva et al. (2012), the soil´s physical and hydraulic properties affect the hydrological processes which comprise infiltration, erosion, wetness redistribution and the transport of solved materials.

Models of water infiltration in the soil such as Kostiakov´s, Kostiakov-Lewis´s, Horton´s, among others, link the model´s parameters to soil characteristics without necessarily having any physical significance. In fact, they include some factors in the determination of their constants, such as soil heterogeneity, which are difficult to be assessed in theoretical models.

Infiltration velocity is affected by surface conditions, profile and soil´s initial water contents (Panachuki et al., 2006). Water erosion processes are greatly affected by surface materials, topography, rain seasonality and vegetation (Silva and Kato, 1998) which may be compounded by changes in texture, structure, porosity and organic matter caused by land usage and its respective management (Pereira and Teixeira Filho, 2009).

The objective of this work was current analysis evaluates the agricultural chronosequence impact on Haplic Plinthosol soil on the phytophysionomy of earth mounds in the savannah region close to the town of Jataí GO Brazil, by empiric models of water infiltration in the soil.

MATERIALS AND METHODS

Current assay was conducted on the Boa Vista Farm, in the micro-basin of the river Claro in the municipality of Jataí, GO, Brazil, in 2010. The region has been characterized by micro-reliefs of earth mounds (murundus, covais, cocorutos and monchões, in Portuguese) with a predominance of Haplic Plinthosol soils (Figure 1). The region´s climate is Aw, or rather, a savannah tropical mesothermal climate with well-defined dry and rainy seasons, according to Köppen´s classification. Mean annual temperature varies between 18 and 32°C. The rainy period ranges between November and May, with more than 80% of yearly rainfall. Mean annual rainfall varies between 1600 and 1700 mm (with gradual spatial variation without any differentiated rainy nucleus in the region under analysis).

Current analysis compared the areas during the period with agricultural usage (5, 10 and 15 years) and two areas without any agricultural usage, of which one was on the upper section of the mound and the other on the lower section (Figure 2). Since all areas were close to one another, with the same Haplic Plinthosol soil type, the environmental conditions were homogeneous. Five areas, which represent the different classes of soil use, were chosen (Figure 3).

Through the layout of chronosequence of human usage, the impact on soil infiltration speed was evaluated, according to the treatments below:

1. Treatment 1 (MURUNDUINF): Natural conditions exist in the lower section of the earth mound, with no human intervention in the area. The area lies on the lower section of the mounds, flooded most of the year, with no termite activity and covered by crawling graminoid vegetation.
2. Treatment 2 (MURUNDUSUP): Natural conditions on the higher section of the earth mound, approximately 2 m high, without human intervention, which remains dry most of the year, formed by termites. It has typically savannah vegetation, with a great diversity of shrubs and graminoids (Figure 2);
3. Treatment 3 (SPD5): Area occupied for 5 years by no-tillage system. Cash crops consisted of soybean in the harvest; maize (or millet or sorghum) in the winter harvest; fallow state in the interim harvest.
4. Treatment 4 (SPD10): Area occupied for 10 years by no-tillage system. Successive cash crops consisted of soybean in the harvest period; maize in the winter harvest; fallow land.
5. Treatment 5 (SPD15): Area occupied for 15 years by no-tillage system. Successive cash crops consisted of soybean in the harvest

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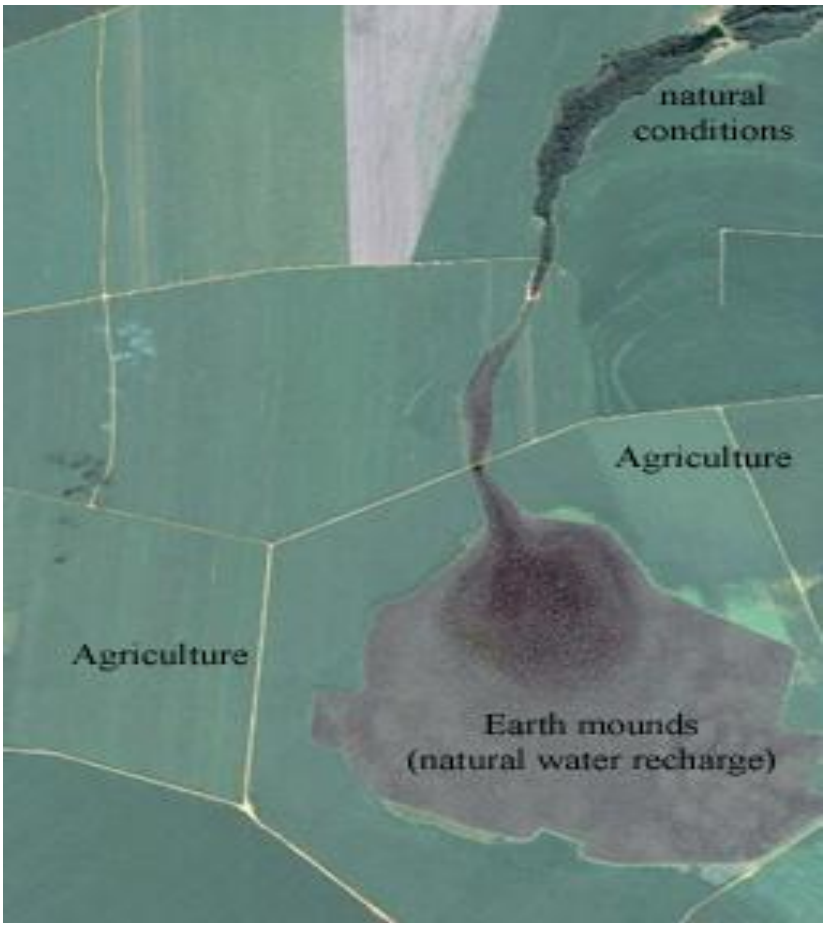

Figure 1. Satellite photo showing the natural composition of the micro-relief of earth mounds on the Boa Vista Farm under analysis.

Figure 2. Composition of preserved micro-relief of earth mounds, normally a flooded area. Black and white arrows show respectively the upper and lower section of the earth mounds.

period and maize in the winter harvest.

The process of the area´s incorporation to the agricultural production system required a systematization of areas which disturbed the approximately 2 m-high earth mounds. Systematization consisted of cuts and distribution of earth to the lower parts which were leveled. Since the area was plowed and harrowed, changes in the area occurred. It was formerly a rolling landscape and was transformed into a totally flat area with no traces of its natural form (Figure 4).

When the areas were incorporated to the production system, they

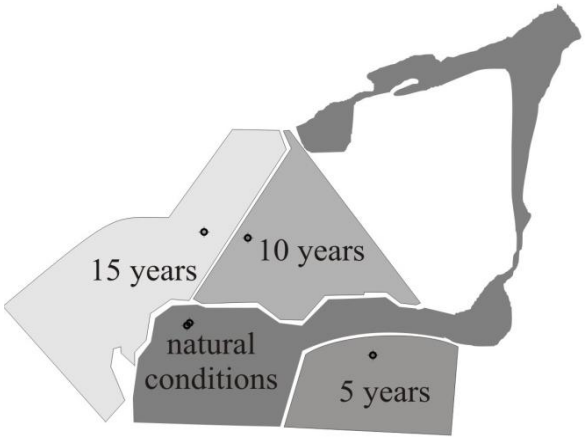

Figure 3. Site of the experiment following the chronsequence of human usage.

Figure 4. Agricultural area where formerly earth mounds abounded.

were first treated with limestone during the systemization process without any further treatment. This fact differentiates the treatments with regard to the management of each area under analysis.

Table 1 characterizes the clayey texture of the areas; the upper part of the earth mound, 5-, 10- and 15-year periods of incorporation to the no-tillage system (NTS) and the clayey-sandy texture of the lower part of the earth mound.

Infiltration tests were performed on each site under analysis by a double-ring cylinder infiltrometer, measuring 60 cm high and 10 and 20 cm diameter, respectively, for the inner and outer rings (Figure 5).

The two cylinders were placed at a depth of 30 cm and a constant water volume of approximately 19 cm to the soil surface was maintained during tests. Water flow was manually controlled by a register duly adjusted to a pipe linked to the inner cylinder, whilst the outer one was manually supplied. Reading was done at 0, 1, 2, 5, 10 15 and 30 min, starting from 0 min, with replications at every 30 min, up to total time limit of 210 min for each test.

Tests were performed till the infiltration rate, registered on the inner ring, became approximately constant with time. The criterion for constant infiltration rate was the repetition of the reading of the water flow in the inner ring for at least three times.

Water infiltrated into the soil was determined in situ by the ring infiltrometer method and empirically by methods proposed by Horton (1940), Kostiakov (1932) and Kostiakov-Lewis (1945), according to Equations (1), (2) and (3), respectively.

Horton´s model: $V = V_0 + (V_0 - V_f)\exp(-k_f.t)$ (1)

Kostiakov´s model: $V = V_0.t^b$ (2)

Kostiakov-Lewis´s model: $V = V_0.t^b + V_f.t$ (3)

Where: V (cm h^{-1}) is the velocity of water infiltration into the soil at a certain time (t) (h) after the formation of a water pool on the soil surface; V_0 and V_f are respectively the velocities of initial and final infiltration (cm.h^1); b and K_f are proportional constants which depend on the type of soil and rain intensity. K_f, V_0 and V_f may be obtained experimentally: V_f is the asymptote of graph V versus infiltration time; K_f is the slope of the straight line of the graph (V_0 - V_f) versus t; and V_0 - V_f is the intercept of the ordinate´s intercept when t = 0 (Dantas et al., 2011; Gomes Filho et al., 2011; Paixão et al., 2009; Alves et al., 2007; Alves Sobrinho et al., 2003; Castro and Souza, 1999; Silva and Kato, 1998).

Performance between infiltration field rates and the rates calculated by Kostiakov´s, Horton´s and Kostiakov-Lewis´s empirical methods for infiltration was evaluated by statistically comparative analysis of results by coefficient of determination R^2. After data collection, they were used in the laboratory to trace the curves according to the model used. The models´ adjustment

Table 1. Soil´s texture characteristics of the areas under analysis.

Usage	Treatment	Clay	Silt	Sand
		(g kg^{-1})		
Natural features of the earth mound	MURUNDUINF	434	110	456
	MURUNDUSUP	576	139	285
Chronosequence of agricultural usage	SPD (5 years)	482	193	325
	SPD (10 years)	501	185	314
	SPD (15 years)	398	213	389

Figure 5. Installation of the double-ring infiltrometer.

quality was evaluated by non-linear regressions between the estimated rates and mean rates registered in each treatment, coupled to the respective coefficients of determination. The following statistic indexes were in the evaluation: coefficient of residual mass (CRM), adjustment coefficient (AC) and efficiency (EF) (Alves Sobrinho et al., 2003), given by Equations (4), (5) and (6) respectively.

$$CRM = \frac{\left(\sum_{i=1}^{n} O_i - \sum_{i=1}^{n} P_i\right)}{\sum_{i=1}^{n} O_i} \quad (4)$$

$$AC = \frac{\sum_{i=1}^{n} \left(O_i - \overline{O_i}\right)^2}{\sum_{i=1}^{n} \left(P_i - \overline{O_i}\right)^2} \quad (5)$$

$$EF = \frac{\left[\sum_{i=1}^{n} \left(O_i - \overline{O}\right)^2 \Big/ \sum_{i=1}^{n} \left(O_i - \overline{P}\right)\right]}{\sum_{i=1}^{n} \left(O_i - \overline{O}\right)^2} \quad (6)$$

Where: O_i represents reported rates; P_i is the estimated rates; n is the number of observations; $\overline{O}$ is the arithmetical average of observations; and $\overline{P}$ is the arithmetical average of the estimated rates.

RESULTS AND DISCUSSION

Table 2 shows rates for Basic Infiltration Velocity (BIV) for each treatment in the area under analysis and the respective classification suggested by Bernardo et al. (2006). Further, BIV rates in soils without human interference were higher than those in soil with no-tillage system. Infiltration velocity was very high.

BIV´s low rates in cultivated areas may be due to changes in the soil structure caused by the destruction of the earth mounds for the preparation of the area for planting and by harvesting machines throughout the years. Sales et al. (1999) evaluated the association between BIV and the physical traits of surface and sub-surface layers of Dark Red Latosol and Red-Yellow Podzolic soils and reported that rates were extremely contrasting. Results may be associated to the distinct morphological characteristics related to the soils´ surface structure. The same authors registered 12.1 mm h^{-1} for BIV in the Red-Yellow Podzolic soil, with 422 g kg^{-1} clay and a 7.8% macropore volume; in the case of Dark Red Latisol they reported 653 g kg^{-1} and 16.8% macropores, with BIV at 56.6 mm h^{-1}.

Alves Sobrinho et al. (2003) studied water infiltration in soil cultivated under different management systems, crop rotation and acceptable analyses of equations by Horton and Kostiakov-Lewis to calculate water infiltration rate in the soil by aspersion infiltrometer. They reported the

Table 2. Rates of Basic Infiltration Velocity (BIV) of the areas under analysis, equations of infiltration velocity; coefficients of determination (R^2) of empirical models and statistic indexes for areas with or without human interference.

Treatment	BIV (mm.h^{-1})	Classification	Models	Equation	R²	CRM	AC	EF
			Kostiakov	$VI = 298.5t^{-0.06}$	0.978	-0.087	2.109	0.000002
MURUNDUINF	170.0	Very high	Kostiakov-Lewis	$VI = 467.43t^{-0.036}$	0.979	-0.840	0.055	0.000002
			Horton	$VI = 234.35t^{-0.072}$	0.835	0.180	0.881	0.000013
			Kostiakov	$VI = 451.86t^{-0.074}$	0.978	-0.097	1.958	0.000001
MURUNDUSUP	242.0	Very high	Kostiakov-Lewis	$VI = 691.44t^{-0.044}$	0.979	-0.844	0.072	0.000001
			Horton	$VI = 369.02t^{-0.095}$	0.839	0.411	1.080	0.000006
			Kostiakov	$VI = 37.1t^{-0.318}$	0.978	-0.123	2.770	0.000270
SPD5	2.0	Low	Kostiakov-Lewis	$VI = 38.105t^{-0.279}$	0.979	-0.277	2.418	0.000364
			Horton	$VI = 32.675t^{-0.606}$	0.899	0.411	1.726	0.000318
			Kostiakov	$VI = 23.992t^{-0.278}$	0.978	-0.117	3.163	0.000603
SPD10	2.0	Low	Kostiakov-Lewis	$VI = 25.345t^{-0.233}$	0.979	-0.331	2.427	0.000799
			Horton	$VI = 19.202t^{-0.495}$	0.890	0.418	1.848	0.000699
			Kostiakov	$VI = 27.999t^{-0.319}$	0.978	-0.123	2.885	0.000495
SPD15	2.0	Low	Kostiakov-Lewis	$VI = 29.054t^{-0.269}$	0.979	-0.327	2.372	0.000597
			Horton	$VI = 28.013t^{-0.574}$	0.896	0.293	1.603	0.000574

CRM, Coefficient of Residual Mass; AC, adjustment coefficient; EF, efficiency.

interference of some soil traits or BIV factors, mainly macro-porosity, management type and soil surface sealing. Further, the latter was the main factor for water infiltration overrate reported by Zonta et al. (2012) and Ali et al. (2010).

When the coefficient of residual mass (CRM) was investigated (Table 2), Horton´s equation used in the five treatments underestimated infiltration rate, whereas equations by Kostiakov and Kostiakov-Lewis overrated it. Behavior has been registered by positive rates of CRM index of Horton´s equations and by the negative ones of Kostiakov and Kostiakov-Lewis´s equation. Results corroborate rates by Alves Sobrinho et al. (2003) and Panachuki et al. (2006) in their studies on water filtration in the soil cultivated under different management systems and culture rotations by Horton´s and Kostiakov-Lewis´s models. Statistical index also confirmed the best adjustment for Horton´s equation with deviations close to zero. Adjustment coefficient and efficiency were also better for the five treatments by Horton´s equation. Indexes´ rates, close to one, confirmed the equation as the most adequate to estimate the infiltration rate in the type of soil under analysis.

Similar to results by Alves Sobrinho et al. (2003) and Panachuki et al. (2006), the Adjustment Coefficient (AC) was, as a rule, better in Horton´s equation since the mathematical model provided rates close to one for the five treatments analyzed. Since efficiency index (EF) was the same in the three equations with differences in treatments, it showed that infiltration models positively indicated the process of water infiltration in the soil and those treatments under natural conditions provided responses which were similar to agricultural chronosequence treatments.

Figure 6 shows the velocity curves of water infiltration and their respective adjustment equations in each soil of the areas under analysis. Equations showed coefficient of determination rates R^2 = 0.896 and R^2 = 0.872, respectively, in the area without human interference in the lower and upper sections of the earth mounds. Rates R^2 = 0.935, R^2 = 0.923 and R^2 = 0.976 were lower than those in areas, respectively, with 5, 10 and 15 years of human interference.

Figure 6 shows infiltration velocity rates related to time. Infiltration velocity rates in areas without human interference were higher than those in areas with no-tillage agricultural system. According to Silva et al. (2012), infiltration volume was greater when water infiltration rate in the soil at the basin´s bottom was smaller.

Figure 7 demonstrates comparative rates of infiltration velocities by cylinder infiltrometer and rates calculated by Kostiakov´s, Kostiakov-Lewis´s and Horton´s equations for areas without human interference in the upper and lower sections of the earth mound and for areas with 5, 10 and 15 years human interference by agricultural systems.

Figure 7 demonstrates that empirical models in all areas behaved similarly, or rather, Kostiakov-Lewis´s model had the highest velocity rates of water infiltration in the soil and Horton´s model provided velocity rates of water infiltration in the soil close to those from the

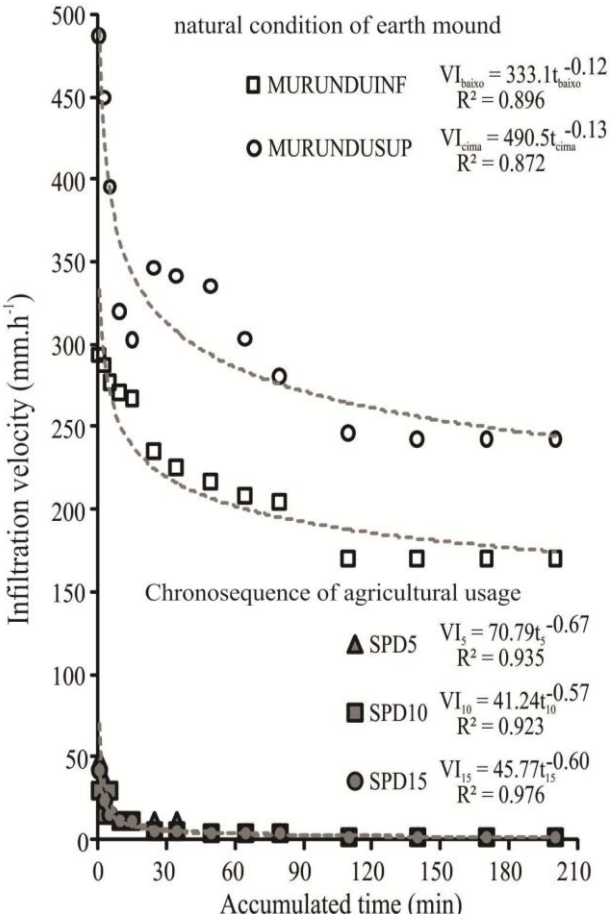

Figure 6. Velocity of water infiltration in soil of the areas under natural conditions and those with human interference, as a function of time.

cylinder infiltrometer field model. Rates by Kostiakov´s empirical model overrated rates by Horton´s model and those retrieved from the field model.

The behavior of empirical models in current analysis provided results similar to those reported by Paixão et al. (2009) who estimated water infiltration in soil through adjustments of non-linear functions and of empirical models proposed by Horton, Kostiokov and Kostiokov-Lewis and compared results with data retrieved from field ring infiltrometer in sandy texture soils in Lagoa Seca PB Brazil.

Table 2 shows the rates of empirical equations of models proposed by Kostiakov, Kostiakov-Lewis and Horton for all areas with and without human interference. Horton´s model presented basic infiltration velocity similar to rate from double-ring cylinder infiltrometer (Figure 7) (Montenegro and Montenegro, 2006). Horton´s process was predominant in some regions with a semi-arid climate. It was the main source for the production of discharge peaks in watersheds especially in areas with compacted soils or soils lacking any vegetal covering (Dalri et al., 2010; Tomasini et al., 2010; Thomaz, 2009). The model demonstrated better infiltration in soils with high infiltration capacity.

When compared with field data, the evaluation of empirical models revealed the model that better applied to local conditions which involved physical and water attributes and different phytophysionomies. According to Cavalcante et al. (2011), usage and management methods led, within a rising order, no-tillage system, conventional preparation and pastureland towards the deterioration of the soil´s physical attributes within the savannah context.

Water flow behavior in areas without any human interference evidenced the natural runoff conditions of rain through the soil. Due to the high velocity of basic infiltration, these areas naturally had a great capacity in draining rain water. Contrastingly, areas which underwent human interference but which formerly had the same infiltration velocity lost such a capacity and after the first five years revealed the low velocity of basic infiltration. The above characteristic is a great concern in events of

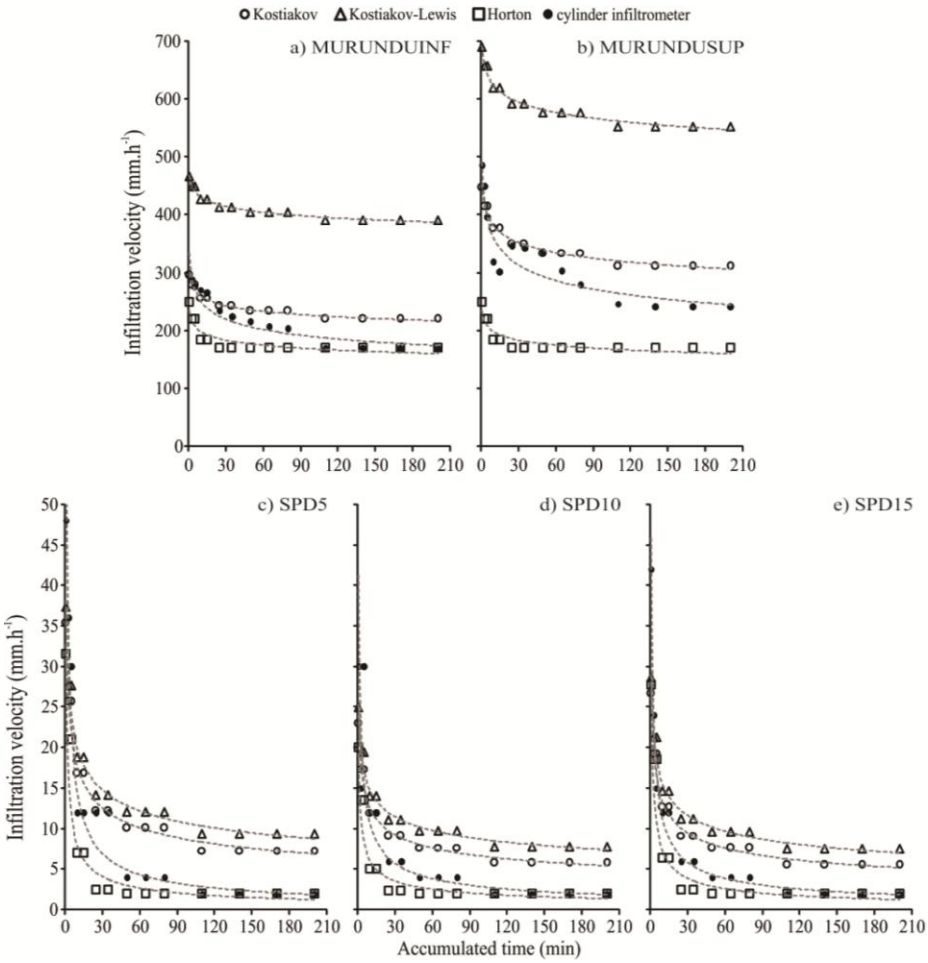

Figure 7. Field test of infiltration velocity of water in soil by the cylinder infiltrometer method and data by Kostiakov´s, Kostiakov-Lewis´s and Horton´s equations for the lower section of the earth mound.

prolonged or concentrated rains which will certainly cause surface runoff and erosion.

Conclusions

Basic Infiltration Velocity (BIV) rates in soils without any human interference were higher to those found in soils with no-tillage system. BIV was classified as very high. Areas with 5, 10 and 15 years under no-tillage production process had very low BIV rates. On average, empirical models overrated infiltration rates at the beginning of the process when compared to field data. Rates by Horton´s model were very close to those obtained from basic infiltration velocity in field data.

Conflict of Interest

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

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Abbreviations: AC, adjustment coefficient; **BIV,** Basic Infiltration Velocity (mm.h^{-1}); **CRM,** coefficient of residual mass; **EF,** efficiency; **MURUNDUINF,** natural conditions exist in the lower section of the earth mound; **MURUNDUSUP,** natural conditions on the higher section of the; earth mound; **SPD10,** Area occupied for 10 years by no-tillage system; **SPD15,** Area occupied for 15 years

by no-tillage system; **SPD5,** Area occupied for 5 years by no-tillage system.

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Full Length Research Paper

Compositional quality of Zalmati virgin olive oil: Effect of the aromatization process with rosemary essential oils (*Rosmarinus officinalis* L.)

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Zalmati olive oil was aromatized with essential oils obtained from rosemary (*Rosmarinus officinalis* L.), to improve its quality. Parameters such as fatty acids composition, pigments and quality parameters were characterized for various blends of virgin olive oils and essential oils (0.005, 0.01 and 0.02%). Results show that aromatized oils had an improved composition as compared to that of pure olive oils. An increasing in the oleic acid content and a decreasing in the palmitic and linoleic acids levels was observed with aromatization process. Moreover, this process improved the oxidative stability and the antiradical activity.

Key words: Essential oils, fatty acids, oxidative stability, virgin olive oils, volatile compounds.

INTRODUCTION

Olive oil is a fundamental component of the Mediterranean area diet, and it is widely used as a condiment, cooking medium, and in the storage of vegetable and animal food. Olive oil cultivation originated in Asia Minor and has spread to Greece, Italy, Spain, and North Africa. The Tunisian olive culture constitutes one of the principal economical and agricultural strategic sectors. About, 60 million trees as distributed and spread on 1.6 million hectares, representing a third of the cultivated area. The olive growing area spread from the northern to the southern regions. VOO is extensively consumed due to its nutritional value and its organoleptic characteristics. Besides, mention should also be mad of its use in medicine. It is unique among others vegetable oils due to its high levels of monounsaturated fatty acids (mainly oleic acid) and to the presence of minor component, such as phenol compound. Phenols are an important parameter for the evaluation of VOO quality as they contribute greatly to oil flavor and taste as long as the oil is protected from auto-oxidation. Moreover, it is widely known that the quality of VOO is influenced by various agronomic factors such as olive cultivar, climatic conditions, degree of maturation, and agronomic practices related to irrigation treatment (Morello et al., 2004). EVOO is highly appreciated by consumers due to their health benefits as well as their aromatic characteristics; this is the reason why is important to prevent off-flavours by different contamination processes (Gamazo-Vázquez et al., 2003). However, other studies have demonstrated that nutritional and organoleptic quality of VOO was ameliorated by some technological procedures as blending and malaxing (Ouni et al., 2011; Reboredo-Rodríguez et al., 2014a)..Zalmati variety which have similar characteristic with Chemlali variety, mainly

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cultivated in center and southern area of Tunisian country, is a productive variety, well adapted to severe environmental conditions. However, its oil is characterized by relatively low of oleic acid, high level of palmitic and linoleic acid, presents a high acidity and a low amount of phenols (Baccouri et al., 2007). These disadvantages could considerably limit the possibilities of exporting of the Tunisian OO production, especially in the presence of a very competition international market that demand high quality (Issaoui et al., 2009).

Hence, the improvement of oil quality by aromatization could to be a tool to provide a product with balanced fatty acid composition, optimal levels of antioxidants compounds and a good flavor and taste. The main goal of this work is to characterize Zalmati olive oils before and after aromatization with essential oil obtained from rosemary known for its high quality. In order to improve the quality of Zalmati fatty acid composition, phenols, oxidative stability and quality parameters (acidity, PV, K_{232}, K_{270} extinction coefficient, pigments contents) were attested at different percentage of aromatization with rosemary essential oils.

MATERIALS AND METHODS

Essential oil

Extraction

The aerial parts (stems and leaves) of *Rosmarinus officinalis* (L.) were collected from the South-East of Tunisia on March 2013 (Gabes, bioclimatic zone: lower arid, rainfull (mm/year): 100 to 200, Latitude: 33°27´34˝N, longitude: 10°8´17˝E, altitude 520 m). The plant material was dried at room temperature in the shadows, for 2 weeks until constant weight. The dried preparation was ground further to obtain a fine powder, and then stored at ambient temperature in a dry and dark place until being used. The dry matter was submitted to hydrodistillationf for 4 h, using a Clevenger-type apparatus. Essential oil was stored in sealed vials protected from light at -20°C until analysis.

Gas chromatography (GC)

An Agilent Technologies 6890N GC equipped with HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm; Hewlett-Packard) and connected to a FID was used. The column temperature was programmed at 50˚C for 1 min, then 7˚C/min to 250˚C, and finally left at 250˚C for 5 min. The injection port temperature was 240˚C; while that of the detector was 250˚C (split ratio: 1/60). The carrier gas was helium with a flow rate of 1.2 ml/min. The analyzed essential oil volume was 2 µl. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Mean per-centage of *R. officenalis* L. volatiles compounds represented the average calculated on three individuals. Retention indices (RI) were calculated for separate compounds relative to C9-C16 n-alkanes mixture (Aldrich Library of Chemicals Standards) (Kovàts, 1958).

Gas chromatography/mass spectrometry (GC/MS)

The volatile compounds isolated by HD were analysed by GC/MS, using an Agilent Technologies 6890N GC. The fused HP-5MS capillary column (the same as that used in the GC/FID analysis) was coupled to an Agilent Technologies 5973B MS (Hewlett-Packard, Palo Alto,CA, USA). The oven temperature was programmed as previously (50°C for 1 min, then 7°C/min to 250°C, andthen left at 250°C for 5 min). The injection port temperature was 250°C and that of the detector was 280°C (split ratio: 1/100). The carrier gas was helium (99.995%purity) with a flow rate of 1.2 ml/min. The MS conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50 to 550 m/z.

Volatile compounds identification

The volatile compounds of *R. officinalis* L. leaves were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to RI data generated from a series of known standards of n-alkanes mixture (C8 to C26) and to those previously reported in the literature (Adams, 2001).

Olive oil

Extraction

Olive oil samples were obtained from fruits of Zalmati olive cultivar, which were picked by hand at the same stage of maturity from three trees during the crop season 2012/ 2013 (October) in an olive orchard located in Bengardene south of Tunisia. The olives were washed and deleafed and crushed; the same laboratory mill was used to prepare the olive oil samples. Only healthy fruits, without any kind of infection or physical damage, were processed. After harvesting, fresh olives (1.5-2.0 kg) were washed and deleafed, crushed with a hammer crusher, and the paste mixed at 25°C for 30 min, centrifuged without addition of warm water (oil produced from each extraction was 200-250 ml/kg). Samples were prepared by blending olive oils with essential oil in different pre-established proportions (0.005, 0.01 and 0.02%) and then transferred into dark glass bottles, and stored in the dark at 4°C until analysis.

Determination of oil quality parameters

Free acidity, expressed as percent of oleic acid (%18:1); peroxide value, given as milliequivalents of active oxygen per kilogram of oil ($meqO_2/kg$); and UV absorption characteristics (K_{232} and K_{270}) were determined according to the analytical methods described in the European Union Commission Regulations EEC/2568/91 and EEC/1429/92.

Determination of chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents were determined colorimetrically as previously described (Minguez-Mosquera et al., 1991).The maximum absorption at 670 nm is related to the chlorophyll fraction, while the maximum absorption at 470 nm is related to the carotenoid fraction. The values of the coefficients of specific extinction applied were E0 = 613 for pheophytin, a major component in the chlorophyll fraction, and E0 = 2,000 for lutein, a major component in the carotenoid fraction. Thus, the pigment contents were calculated as follows:

Chlorophyll (mg/ kg) = (A670× 106)/ (613 × 100 × d),
Carotenoid (mg/ kg) = (A470 × 106)/ (2,000 × 100 × d)

Where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

Fatty acid composition

The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g oil and 3 ml of hexane with 0.4 ml of 2-N methanolic potassium hydroxide, and analyzed using a Hewlett-Packard (HP 4890D; Hewlett-Packar Company,Wilmington, DE) chromatograph equipped with a capillary column (Supelcowax: 30 m × 0.53 mm; 0.25 mm), a split/splitless injector and an flame ionization detection (FID) detector. The carrier gas was nitrogen, with a flow rate of 1 ml/min. The temperatures of the injector, the detector and the oven were held at 220, 250 and 210°C, respectively. The injection volume was 1 µl.

Antiradical activity

The olive oil samples were examined for their capacity to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Kalantzakis et al., 2006). Oil solution 1 ml in ethyl acetate (10%, w/v) was added to 4 ml of a freshly prepared DPPH solution (10-4 M in ethyl acetate) in a screw-capped 10 ml test tube. The reaction mixture was then vigorously shaken for 10 s in a vortex apparatus and the tube was maintained in the dark for 30 min, until a steady state was reached. The absorbance of the mixture was measured at 515 nm against a blank solution. A control sample (no oil) was prepared and daily measured. A refined olive oil (Minerva S.A. edible Oils, Shimatari, Viotia, Greece), devoid of pro-oxidants/antioxidants, was used for comparison. The radical scavenging activity (RSA) toward [DPPH] was expressed as the percent reduction in DPPH concentration by the constituents of the oils:

% [DPPH] red = 100-(1 [DPPH]30/[DPPH]0)

Where, [DPPH]0 and [DPPH]30 were the concentrations of DPPH in the control sample (t = 0) and in the test mixture after the 30 min reaction, respectively.
Total phenol contents were quantified colorimetrically (Ranalli et al., 1999). Phenolic compounds were isolated by triple extraction of a solution of oil (10 g) in hexane (20 ml) with 30 ml of a methanol water mixture (60:40, v/v). The Folin–Ciocalteau reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts, and the absorption of the solution at 725 nm was measured. Values are given as milligrams of caffeic acid per kilogram of oil (Gutfinger, 1981).

Oil stability

Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 743 apparatus (Metrohm, Herisau Switzerland), using an oil sample of 3.6 g. The oil temperature was 101.6°C and the air flow was 10 L/h.

Statistical Analysis

Significant differences between means were determined by an analysis of variance, which applied a Duncan's test. Differences were considered statistically significant when the probability was greater than 99% ($P < 0.01$). The statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., 2004).

RESULTS AND DISCUSSION

Chemical essential oil composition

The identified compounds of the volatile constituents of the essential oils are shown in Table 1. Thirty seven different components were identified by GC-MS analyses representing 99.35% of the total oil. In the literature, several methods were used for the comparison between the essentials oils composition of many species (López Mahia et al., 1993). The analyzed oil contained a complex mixture consisting of mainly oxygenated mono and sesqueterpenes, and mono-and sesqueterpene hydrocarbons. The rosemary oil used in this study mostly consisted of monoterpenes: 1,8-cineole, camphor, and α-pinene, constituting 24.1, 19.88 and 18.48% of the essential oil, respectively. Flamini et al. (2002) classified rosemary oil into two chemotypes: The α-pinene chemotype with the main compounds being *α*-pinene (20.6%) and 1,8 cineole (6.6%) and the 1,8-cineole chemotype with the major components being 1,8 cineole (40.2%) and *α*-pinene (13.2%). The monotepenes hydrocarbons (42.01%), represented mainly by 1,8-cineole, *α*-pinene, camphene, formed the major group. Camphor was the major compound of Ketones class (Table 1). These volatile compounds should improve the aroma characteristics of the studied virgin olive oils. Recent study showed that sedimentation of oil samples plus racking for a minimum of 2 months was found to promote the formation of C6 alcohols in most samples (Reboredo-Rodríguez et al., 2013a). Different methods of analysis for many additives in olive oil; to control its aroma fingerprint and for the determination of phenolic compounds were also reported (Quinto-fernández et al., 2003; Reboredo-Rodríguez et al., 2012, 2014b). Others studies showed variation in the aroma profile of virgin olive for different cultivars and in the biogenesis and in the distribution between olive pulps and seeds (Reboredo-Rodríguez et al., 2013b,c)

Quality parameters

For all oil samples, values of the analytical parameters fell within the ranges established for the highest quality category 'extra virgin olive oil'. As shown in Table 2, these parameters are actually affected by the aromatization process. In all samples the free fatty acid content was much lower than the established upper limit of 0.8% for the best commercial quality olive oil, designated extra virgin (Regulation EEC, 2003) (Table 2). Peroxide value evaluates the hydroperoxides content and offers a measure of the degree of lipid oxidation. In all samples, this value was below the limit of 20 meq of oxygen kg^{-1} of oil, which is accepted as the limit for the extra quality virgin olive oils. To evaluate the oxidation level of the oil, the parameter K_{232} has been used (Table

Table 1. Chemical composition of *Rosmarinus officinalis* L. essential oils.

Peak number	Compound	Retention index (RI)	Percentage
1	Tricyclene	921	0.14
2	α-thujene	927	0.06
3	α-pinene	934	18.48
4	Camphene	949	8.63
5	Verbenene	954	0.12
6	Sabinene	974	-
7	β-Pinene	978	3.36
8	1-Octen-3-ol	981	0.11
9	3-Octanone	987	0.24
10	β-myrcene	992	2.18
11	β-terpinene	1005	0.08
12	α-phellandrene	1006	-
13	δ-3-Carene	1011	0.37
14	α-terpinene	1018	-
15	p-cymene	1027	3.78
16	Limonene	1030	3.42
17	1,8-cineole	1033	24.11
18	γ-terpinene	1060	0.08
19	trans-sabinene hydrate	1070	-
20	α-Terpinolene	1088	0.18
21	Terpinolene	1089	-
22	Linalool	1101	1.08
23	2,3-Dimethyl-2,3-Dihydropyridine	1107	0.14
24	Pinocarveol	1143	0.25
25	Camphor	1148	19.88
26	Isoborneol	1161	0.30
27	Borneol	1170	2.90
28	Isopinocamphone	**1177**	0.12
29	Terpinen-4-ol	1181	0.40
30	Cuminol	**1189**	0.17
31	α-terpineol	1194	1.85
32	Endo-isocamphonone	1198	0.16
33	α-terpinene	1200	0.20
34	Verbenone	1213	0.81
35	Thymol methyl ether	**1236**	-
36	Linalyl acetate	**1255**	0.11
37	Bornyl acetate	1289	1.56
38	Thymol	1299	-
39	p-Cymene-3-ol	1308	-
40	α-Terpinyl acetate	**1351**	0.16
41	β-caryophyllene	1427	1.25
42	Aromadendrene	1446	0.35
43	α-Humulene	**1461**	0.30
44	Lavandulyl acetate	1473	-
45	α-amorphene	1521	-
46	γ-cadinene	1529	-
47	Caryophyllene oxide	1594	0.71
48	Humulene epoxide	1621	0.08
49	T-Cadinol	1651	-
Total identified			99.35
Grouped components (%)			

Table 1. Contd.

Monoterpene hydrocarbons	42.01
Oxygenated monoterpenes	51.04
Sesquiterpene hydrocarbons	1.93
Oxygenated sesquiterpenes	0.78
Others	2.35

Table 2. Quality parameters of virgin olive oils and aromatized olive oils samples.

Parameter	Pure olive oils	Aromatized olive oils		
	100%	0.005%	0.01%	0.02%
Acidity	0.55±0.05[a]	0.55±0.05[a]	0.65±0.05[a]	0.65±0.05[a]
Peroxyde value	5.5±0.05[a]	9±1[a]	12.5±0.5[b]	14±1[c]
K_{232}	3.32±0.1[a]	3.26±0.05[a]	3.31±0.02[a]	3.32±0.11[a]
K_{270}	0.28±0.02[a]	0.27±0.005[a]	0.28±0.01[a]	0.29±0.03[a]
Chlorophylls (mg/kg)	1.63±0.2[c]	1.29±0.07[b]	1.52±0.4[b]	1.04±0.1[a]
Carotenoids (mg/kg)	2.66±0.6[b]	1.84±0.4[a]	2.06±0.3[a]	1.96±0.6[a]
Radical scavenging activity (%)	63.33±1.2[a]	65±0.88[b]	68±.0.99[c]	72±0.44[d]
Stabilité oxydative (h)	32±1.3[a]	37±1.1[b]	40±1.2[c]	45±1.3[d]

[a–b]Mean ± SD, significant differences within the same row are shown by different letters ($P < 0.001$). PV, peroxide value; K232 and K270, values of specific extinction given as absorbance at 232 and 270 nm, respectively.

2). Also the values of these parameters (K_{232} and K_{270}) were below the limits established for extra virgin olive oils (2.50 and 0.22, respectively). All samples exhibited very low values in the regulated physico-chemical indices assessed. The lower these values are, the higher is the quality one can expect from oil. These values are consistent with other studies (Reboredo-Rodríguez et al., 2014c). Summarising, all values of the analytical parameters fell within the ranges established for the highest quality category 'extra virgin' olive oil. As shown in Table 2, aromatization had no significant influence on these analytical parameters, which are basically affected by factors causing damage to the fruits, e.g., olive fly attacks or improper systems of harvesting, carriage, storage and processing of olives (Ranalli and Angerosa, 1996).

Fatty acid composition

Fatty acids identified in the oils were palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acids (Table 3). Palmitic, stearic, oleic and linoleic acids were the major ones. Oleic acid is always the main abundant compound in olive oils, ranging from 51 to 53% of total fatty acids. As reported Table 3, Zalmati olive oil exhibited a lower oleic acid percentage (51%) and a higher amount of palmitic and linoleic acids (19 and 18%, respectively). It has been observed that aromatization with rosemary essential oils could correct this problem (Table 3). At 0.01% aromatization, palmitic acid decreased from 19.1 to15.7% (Table 3). Using 0.02% essential oils, oleic acid underwent a significant increase to 53.49% and, at the same time, a decrease of palmitic acid to 14.35% was observed. In summary, aromatized oils at 0.005% showed an improved fatty acid composition, characterized by an increase of its oleic acid content and a concurrent reduction of the palmitic and linoleic acids levels with respect to those of pure Zalmati oil. In addition, the fatty acid distribution became within the range expected for high quality olive oils. The monounsaturated fatty acids (MUFA) content is very important because of its effect on nutritional value and oxidative stability of the oils. It has been observed that oils with a high content of saturated fatty acids (SFA) are more viscous and persistent on the mucous of the oral cavity. This gives rise to the defect known as 'fatty sensation' (Solinas, 1990). Aromatized oils at 0.005% exhibited a significant increase of MUFA; in contrast, the SFA decreased (Table 3). These results are in agreement with the findings of other authors when blending oils (Issaoui et al., 2009; Ouni et al., 2011).

Pigments

The total pigment content in olive oil is an important

Table 3. Fatty acid composition of virgin olive oil and aromatized olive oils samples.

Composition	Pure olive oil	Aromatized olive oil		
	100%	0.005%	0.01%	0.02%
$C_{16:0}$	19.11±0.16[d]	18.88±0.03[c]	15.74±0.2[b]	14.35±0.21[a]
$C_{16:1}$	1.99±0.17[a]	0.86±0.05[a]	1.09±0.12[a]	1.48±0.02[a]
$C_{18:0}$	6.39±0.3[c]	2.19±0.2[b]	1.1±0.31[a]	12.32±0.23[d]
$C_{18:1}$	51.61±0.05[a]	51.62±0.04[a]	52.02±0.03[b]	53.49±0.02[c]
$C_{18:2}$	18.89±0.05[c]	18.79±0.04[c]	16.19±0.1[b]	14.73±0.01[a]
SFA	25.5±0.20[c]	21.07±0.21[b]	16.84±0.22[a]	26.67±0.29[d]
MUFA	53.6±0.22[c]	52.48±0.23[d]	53.11±0.29[a]	54.97±0.26[b]
PUFA	18.89±0.27[c]	18.79±0.29[d]	16.19±0.29[a]	14.73±0.27[b]

[a] Mean ± S.D. (n = 6). Significant differences within the same row are shown by different letters (P < 0.01).

parameter for evaluating olive oil quality. Furthermore, pigments are involved in auto-oxidation and photo-oxidation mechanisms (Gutiérrez, 1989). The interest in the possible beneficial effects of chlorophyll pigments and related compounds has reemerged in the current decade. In this regard, some studies *in vivo* have appeared which seem to indicate that they may be antioxidants have appeared (Kamat et al., 2000; Lanfer-Marquez et al., 2005). More importantly, some authors have reported that they may also be beneficial in the prevention of cancer (Ferruzzi and Blakeslee, 2007). Zalmati olive oil has very low amounts of pigments (1.6 and 2.66 mg/ kg) of chlorophylls and carotenoids, respectively). At all percentage of aromatization with essential oils, chlorophylls and carotenoids in Zalmati olive oils underwent a slight decrease (Table 2). The production of highly pigmented oils should be of considerable interest for the industry and can influence the consumer usually prefer lighter oils.

Oxidative stability

Stability to oxidation is an important property of olive oil, which is improved by synergistic interactions between the various antioxidants present in the oil itself, and also depends on the lipid composition. As can be noted from Table 2, Zalmati olive oil has a low oxidative stability (32 h). The best influence of the process was clearly observed when aromatization was carried out at 0.02% (from 32 to 45 h) (Table 3).

Antiradical activity

Radical scavenging activity of the Zalmati olive oils varied according to the percentage of aromatization (63.33 to 72%) (Table). Zalmati oils produced at 0.02% showed the highest scavenging ability, with an average value of 72%. Conversely, at lower percentage of aromatization, the average value dropped down to 65%. This activity is necessarily linked to the much lower oxidative stability and oleic acid content. Our results show significant variation of the radical scavenging activity of the Zalmati oils due to the effect of the aromatization process. Overall, the correlation showed a positive linear relationship between radical scavenging capacity and oxidative stability measured by Rancimat (r = 0.66) (data not shown). These results are similar to those reported by several authors for other olive oil varieties (Usenik et al., 2008).

Conclusion

The aromatization process using different percentages of essantials oils improved the fatty acid composition by increasing the oleic acid content and decreasing the palmitic and linoleic acids levels, compared to those of pure Zalmati oil. The amount of chlorophylls, carotenoids increased quite slowly at the lower percentages of aromatization. Moreover, aromatization process improved the oxidative stability and the antiradical activity composition. In addition, these results clearly showed that aromatization application can play an important role in the changes of quality of monovarietal VOOs.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Fungicides phytotonic action on the development of soybean

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Some studies has shown that some fungicides may promote phytotonic effect on plants, resulting in gain on the growth and productivity. The objective here was to evaluate the phytotonic action of different fungicides, when applied on the seed and aerial part treatments on the soybean culture. Experimental design chosen was a randomized-block type with nine treatments consisting of eight groups of fungicides applied as seed treatment and aerial part of the plant with four replications. Pyraclostrobin + Methyl thiophanate favored root development on plantlets, plant height and grain yield, when it was applied on the soybean seeds. Fungicides applied on the plant aerial part showed positive effects on control of foliar diseases in soybean. The weight of one thousand grains was higher, when Pyraclostrobin + Methyl thiophanate was used for seeds treatment.

Key words: Pyraclostrobin, azoxystrobin, strobilurin, nutrients and productivity.

INTRODUCTION

The area destined to the grains harvest at Piauí state, Brazil, in crop year 2011/2012 was 1,173.9 thousands of hectares (ha), an increase of 2.4% compared to the harvest of grains at 2010/2011 and also it was higher than national average (2%). Plant species highlighted on this region are soybeans, corn, bean, rice and cotton which has the area for cultivation of 444.600, 351.600, 230.500, 117.400 and 21.300 ha, respectively (Conab, 2012). Moreover, preliminary surveys indicate that the Piauí state has the potential to expand its acreage.

Soybeans productive potential are determined by genetic factors, and withal, by extrinsic production factors as climate conditions, farm management, weeds, pests and diseases control. Huge losses are arising due to diseases incidence, mainly the fungal diseases. It can occur during all culture cycle or only on the later stages.

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The last one is known as late season diseases. It may causes losses that reach until 21% of the production, by decreases on the mass of 1000 grains (Fagan, 2007).

Beyond the effect on the disease control (Godoy and Henning, 2008), some studies show that some fungicide also can promote phytotonic effects on plants, resulting in gains on the growth and productivity (Glaab and Kaiser, 1999; Soares et al., 2011). Positive effects of fungicides are also shown by Fagan (2007) who noted that the use of some fungicides on the soybean culture resulted in increments on the carbon and nitrogen assimilation, photoassimilates partition and grains quality.

Dimmock and Gooding (2002) and Beck et al. (2002) emphasize that the strobilurin increases the period of photosynthetic activity in the leaves of wheat, providing quality and nitrogen content of the grain. Thus, improving the quality of grains is beneficial to the process of seed germination. Among the fungicides available and recommended to the soybean crop in Brazil, named sterol synthesis inhibitors, a systemic fungicide group popularly known as triazole and strobilurin group that act on the mitochondrial respiration process are highlighted. Both of them are used by singly or prefabricated mixtures (Koehle et al., 1997; Alessio, 2008). Because it is a very competitive market, there are several studies that have evaluated the effects of main fungicides registered for soybean culture. However, most existing studies are derived from tests conducted by the manufactures themselves.

Considering all information above, we have objective to evaluate the performance of nine fungicides groups applied on seed treatment and over aerial parts considering its phytotonic effect on the development and productivity of soybean crop.

MATERIALS AND METHODS

The experiment was performed in a Fazenda União, located at Serra da Laranjeira, municipality of Currais (Brazilian State of Piauí), latitude 16' 78"S, longitude 44° 44' 25"W and altitude of 628 m, during the crop year 2011/2012, from November 27 of 2011 (sowing) to April 6 of 2012 (harvest). The soil was classified as distrophic yellow Latossol. Textural analysis of soil on the layer of 0 to 20 cm had 190 g x kg^{-1} of clay, 50 g x kg^{-1} of silt and 760 g x kg^{-1} of sand. Chemical characteristics of the experimental area (0 to 20cm) were as follow: pH ($CaCl_2$): 4.7 mg/dm^{-3} (ppm), K: 47 mg/dm^{-3} (ppm), P (Mehlich): 37.4 mg/dm^{-3}, Ca: 1.9 $cmol.c/dm^{-3}$ (mE/100 ml), Mg: 0.6 $cmol.c/dm^{-3}$ (mE/100 ml), Al: 0.2 $cmol.c/dm^{-3}$ (mE/100 ml), H + Al: 2.2 $cmol.c/dm^{-3}$ (mE/100 ml), V: 54.42%, m: 7.09% and organic matter: 11 g x kg^{-1}.

Rainfall, temperature and monthly mean of relative humidity are described in Figure 1. Data were obtained on the meteorological station of Sabiá farm. It was installed 5 km far away from experimental area. Soybean was sown at November, 27 of 2012 using a pneumatic sowing. Row between lines were 0.5 m, inserted 12 seeds per meter. Pioneer® P98Y70 that have semi-indeterminate growth and phenological cycle around 125 days was chosen to performed the experiment. Fertilization at planting was performed applying 400 kg of 00-24-12 held in the row and topdressing with 100 Kg of KCl spread around.

The experimental design was randomized block, consisted by eight groups of fungicides plus one control and four replicates, as described in Table 1, totaling 36 treatments, each one are set to 4.00 m wide (8 rows) by 8.0 m long, with an area of 32.0 m^2. From each end were eliminated 0.5 m, considering the remaining area of 21.0 m^2.

Fungicides were sprayed on the aerial part as a preventive management, considering, local climate conditions, inoculum pressure and residual range of the fungicides. According to this features spraying it was performed with interval from 15 to 20 between applications. Backpack sprayer with CO_2 injection, tip XR 11002 and a flat fan with spray volume of 150 L x ha^{-1} was used. Pulverizations with Opera or Opera Ultra fungicides were performed with mineral oil assist at the dosage of 0.5 L x ha^{-1}. For PrioriXtra fungicide was used the Nimbus oil in a dosage of 0.6 L x ha^{-1} and for Fox fungicide was used Aureus mineral oil at the dosage of 0.3 L x ha^{-1}.

Plant height at flowering was measured with a measure tape taking nine plants per plot in the usable area by random choice. Plant population was evaluate as initial stand (7 DAE) and final stand (112 DAE) counting the plants along three meters considering three points per plot. Root length was measured at 14 DAE choosing randomly 3 plants per point considering 3 points per plot. Destructive method by soil excavation and removal of the plant was employed to assess the underground part. The same plants used to measure the root length was also used to count the number of branches. At the begin of flowering was collected the third leaf from the apex of plants for chemical analysis of N, P, K, Ca, Mg, Cu, Zn and Mn levels (30 per plot). Drying was carried out in the oven at 60°C during 72 h. The samples were ground and sent for laboratory analysis (Embrapa, 2009). Root and aerial part biomass were also dried in the oven at 60°C during 72 h. They were weighed after drying. It was represented by three growth phases: an initial phase with low accumulation of biomass (0 to 45 days), followed by a significant accumulation stage (45 to 90 days) and the stabilization phase.

Number of string beans with 1, 2 and 3 grains were counted by random collection of 9 plants per plot. Trifoliate branches were also considered at previous sampling. The weight of one thousand seeds was performed with 8 sub-samples containing 100 seeds each separated in counter tray. Once we have these data was possible calculate the mean, variance, standard deviation and coefficient of variation to obtain the weight of 1000 seeds, according to the RAS-Rule Analysis of Seed (Brazil, 2009). The severity of phytopathogens *Corynespora cassiicola* and *Colletotrichum dematium* var. *Truncate* on soybean plants were evaluated according to the diagrammatic scale proposed by Soares et al. (2009) and Finoto et al. (2011), respectively.

Productivity was calculated as yield (kg/ha) by harvest the four square meter in each experimental plots. The moisture of seeds was measured with portable moisture meter (Multigran tool). Qualitative data were subjected to variance analysis. When the differences among means were significant they were compared by the Tukey and F tests at 5%. SISVAR 4.2. software was used to perform those analysis. Quantitative data were adjusted in equations with SIGMA PLOT 10.0. software.

RESULTS AND DISCUSSION

The initial and final stand does not show significant differences compared to the control treatment (Table 3). These results can be explained by the low incidence of pathogens which occur early in the development of soybean, since the number of seedlings observed were close to the number of seeds deposited in the sowing

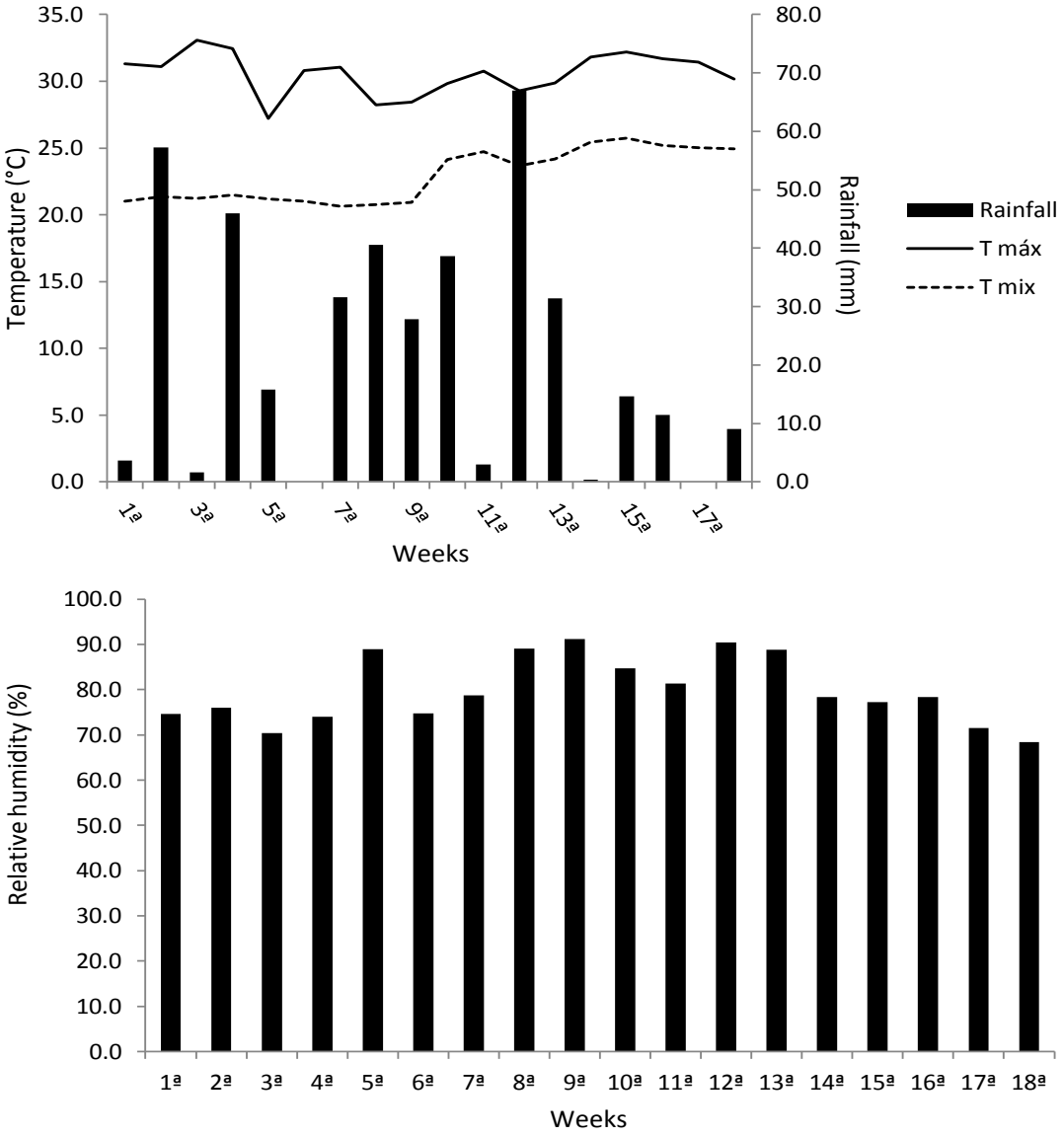

Figure 1. Precipitation, average temperatures (maximum and minimum) and relative humidity of air occurred during the experiment. Currais, PI.

furrows (germination and emergence above 80%). Moreover, climatic conditions may not have favored the occurrence of diseases that affect the initial stand. However, the stand can be severely affected under conditions of high disease incidence in soybean seedlings. As noted by Pereira (2002) the low percentage of germination due to high incidence of *Colletrotrichum truncatum* can be observed when seeds were not treated with fungicides, which reinforces the importance of fungicide to control diseases.

Pyraclostrobin + Methyl thiophanate on seed treatment (Treatments 1, 2 and 8) favored the root development of seedlings, once significant increases in the length and initial root biomass were observed compared to control treatment. Root development is important, because prolonged periods of water stress is common in the Brazilian savannah of Piauí. Practices with phytotonic effect can facilitate the rooting of the seedlings reducing the time of exposure to a soil, which has fungi that cause seed deterioration or seedlings death (Henning, 2005).

These results are reinforced by Rodrigues (2009), who observed that Pyraclostrobin in the treatment of soybean seeds can favor nitrogen assimilation. It happens due to increases in the activity of the enzyme nitrate reductase. Once nodulation is not efficient at this stage, the use of this fungicide may guarantee plantlet vitality.

All treatments which use fungicides at the soybean flowering (R1 and R2) showed increases in plant height

Table 1. Treatments with products, active ingredient, dose and time of spraying.

Treat.*	Commercial name	Active ingredient	C.p.dose (6)	Pulverization
			L or kg/ha	Stage
1	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.(1)
	Comet	Pyraclostrobin	0.3	V6(2)
	Opera	Pyraclostrobin + Epoxiconazole + assist	0.5	R1(3)
	Opera	Pyraclostrobin + Epoxiconazole + assist	0.5	R3(4)
	Opera	Pyraclostrobin + Epoxiconazole + assist	0.5	R5.3(5)
2	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R5.3
3	Standak	Fipronil	0.1	S.T.
	Protreat	Carbendazim + Thiram	0.1	S.T.
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R5.3
4	Standak	Fipronil	0.1	S.T.
	Maxin	Fludioxonil + Metalaxil-M	0.1L/100Kg	S.T.
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R1
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R3
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R5.3
5	Standak	Fipronil	0.1	S.T.
	Maxin	Fludioxonil + Metalaxil-M	0.1L/100Kg	S.T.
	Priori	Azoxystrobin	0.2	V6
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R1
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R3
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R5.3
6	Standak	Fipronil	0.1	S.T.
	Derosal Plus	Carbendazim + Thiram	0.2L/100kg	S.T.
	Fox	Prothioconazole + Trifloxystrobin	0.4	R1
	Fox	Prothioconazole + Trifloxystrobin	0.4	R3
	Sphere Max	Cyproconazole + Trifloxystrobin	0.15	R5.3
7	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera Ultra	Pyraclostrobin + Metconazole	0.5	R5.3
8	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.
	Comet	Pyraclostrobin	0.3	V6
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera Ultra	Pyraclostrobin + Metconazole	0.5	R5.3
9	Standak	Fipronil	0.1	S.T.

*Treat.: Treatments; (1) S.T.: Seed treatment; (2) V6: Fifth Trifoliate leaf fully developed; (3) R1.: Beginning of flowering stage; (4) R3.: Early formation of string beans; (5) R5.3.: 26 to 50% of the grain filling; (6) c.p.: Commercial product

Table 2. Initial and final stand, length and biomass root and plant height at flowering soybean under different fungicide treatments applied as seed treatment and over the aerial part. Brazilian savannah of Piauí. Crop year 2011/2012.

Treat.*	Stand		Initial root length	Initial root biomass	Height
	Initial(14DAE)	Final(112DAE)	14 DAE	14 DAE	56 DAE
	---plants x m^{-2}---		----cm----	----g x m^{-2}----	----cm----
1	19.92^{ns}	17.61^{ns}	18.41^{A}	4.80^{A}	80.42^{A}
2	19.15	17.34	17.71^{A}	4.83^{A}	81.66^{A}
3	18.15	17.11	15.29 ^{Bc}	4.39^{AB}	70.66^{B}
4	17.95	16.08	15.54^{BC}	4.25^{AB}	72.67^{B}
5	18.45	16.28	15.08^{C}	3.53^{B}	76.75^{AB}
6	18.55	16.22	15.71^{BC}	3.67^{AB}	75.58^{AB}
7	19.85	17.28	16.92^{AB}	4.42^{AB}	75.17^{AB}
8	19.10	15.72	18.33^{A}	4.79^{A}	79.50^{A}
9	18.10	15.89	15.83^{BC}	3.39^{B}	66.25^{C}
C.V.(%)	4.94	8.55	4.64	10.74	6.29

Means followed by the same lowercase letters in the column are not significant by Tukey test.*Treat: Treatments.

compared to control (Table 2), especially for Treatments 1, 2 and 8. The results showed that the increases in root growth attributed to the use of Pyraclostrobin + Methyl thiophanate in the seed treatment also have favored the growth of shoots of soybean plants. Similar results were observed by Koslowski et al. (2009), who shows that plant height was influenced by Pyraclostrobin via seed treatment.

The benefits of using Pyraclostrobin + Methyl thiophanate via seed treatment were also observed in dry matter accumulation of aerial part from 45 DAE, as can be seen in Figure 2. At the first phase of soybean growth (0 to 45 days) there were no significant differences among treatments. However, at the second phase of growth (45 to 90 days) Treatments 1, 2 and 8 (with use of Pyraclostrobin + Methyl thiophanate) were the most promising in promoting the biomass accumulation during soybean development.

According to our results the effects of the fungicides are most evident, when it was applied via seed treatment than the spraying over aerial part, since all treatments with the use of Pyraclostrobin + Methyl thiophanate had increases in plant growth-related variables, independent of fungicides applied over aerial part. These results corroborate with studies published by Beck et al. (2002), which observed the high photosynthetic activity in wheat cultivars treated with strobilurin when applied via seed treatments under field conditions. The vegetative stage had plants producing photoassimilates to use it at the growth. At the reproductive stage, plants had a reduction in breathing rate, which ensured greater amount of photoassimilates to be directed to the grain filling.

Once at the control treatment presented low infestation of plant diseases, it can be inferred that in the present study was observed the direct fungicides effects on the growth of soybean plants (Table 3). Rodrigues (2009) demonstrated that only a few fungicides have phytotonic effects on soybean plants, especially Pyraclostrobin on seed treatment. It is possible by providing enhancements in carbon assimilation (reduction of compensation point for CO_2) and nitrogen (activity of reductase enzyme over nitrate). Regarding nutritional status of soybean plants at the flowering stage (R2), we observed no statistical differences among treatments. The mean values of the nutrients found in the present study are within the ideal range: N, P, K, Ca, Mg, Cu, Fe, Mn and Zn with values of 4.70 dag x kg^{-1}; 0.25 dag x kg^{-1}; 1.70 dag x kg^{-1}; 1 dag x kg^{-1}; 0.40 dag x kg^{-1}; 10 mg x kg^{-1}; 50 mg x kg^{-1}; 20 mg x kg^{-1} and 20 mg x kg^{-1}, respectively. One exception must to be made for copper, iron and potassium that had values below the recommended. The ideal values was proposed by Ribeiro et al. (1999). According to our results the foliar nutritional analysis is not a tool for predicting the effect of fungicides on the growth of soybean plants. It may be explained due the soil fertility, soil water availability and cultivar, which can interfere on the nutrient levels present (Alcântara Neto et al., 2010). Wells (1993) and Board and Modali (2005), demonstrated that soybean foliar nutrition and its grains yield depends on the plants ability to maximize the radiation interception and/or accumulate a certain levels of dry matter depends of several factors as climatic conditions, date of sowing, plant genotype, population and spacing between plants. However, studies are needed in an attempt to evaluate the fungicides effects on the efficiency of absorption and nutrients redistribution and also photoassimilates produced via photosynthesis for soybeans plants.

Variables referring to the number of trifoliate leaves at flowering, number of branches and string beans at harvest did not differ among treatments (Table 4). These results are possible because these variables are related to the intrinsic characteristics of the cultivar used,

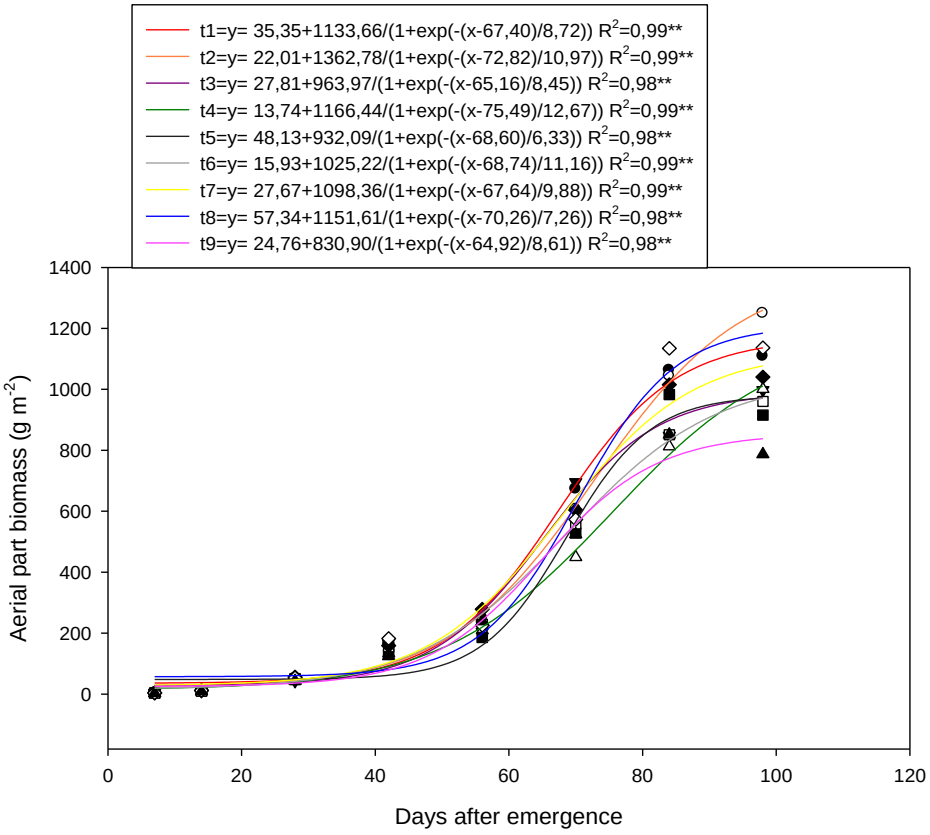

Figure 2. Aerial part biomass of soybean plants subjected to the fungicides via seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/2012.

Table 3. Number of branches, trifoliate leaves and string beans of soybean plants subjected to different treatments with fungicides applied via seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/12.

Treat.	Trifoliate leaves	Number of branches	Number of string beans			
			1 grain	2 grain	3 grain	Total
	--- m^{-2}--	No. of branches m^{-2}	---------- m^{-2}----------			
1	442^{ns}	273.89^{ns}	154.33^{ns}	719.21^{ns}	759.84^{ns}	1633.38^{ns}
2	413	279.07	192.34	705.88	629.90	1528.13
3	361	262.72	179.93	657.69	652.77	1490.39
4	373	248.80	202.91	611.45	607.80	1422.16
5	351	259.36	248.88	653.92	565.42	1468.22
6	372	257.15	253.94	623.45	515.22	1392.62
7	454	274.11	229.14	716.88	604.13	1550.15
8	430	260.65	127.15	739.40	736.06	1602.62
9	425	241.38	195.71	637.11	516.54	1349.37
C.V(%)	11.24	8.82	32.29	16.53	18.63	12.92

ns: not significant by F test ($p<0.05$).* Treat.: treatments

because of that; they are just a little influenced by fungicides.

The use of Pyraclostrobin + Methyl thiophanate in seed treatment also provided significant increments for grain yield. Effects on plant growth by the use of these fungicides are reflected in productivity gains up to 752 kg x ha^{-1} (Table 5). Increases at the productivity related to these treatments can be also observed at higher grain

Table 4. Severity of major diseases in soybean plants subjected to different treatments with fungicides applied via seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/12.

Treat.	*Colletotrichum dematium* var. *truncata*		*Corynespora cassiicola*	
	56 DAE	84 DAE	56 DAE	84 DAE
	----------%----------			
1	10^{A}	15^{A}	5^{A}	20^{A}
2	18^{A}	17^{A}	10^{A}	23^{A}
3	21^{A}	35^{A}	16^{A}	27^{A}
4	17^{A}	38^{A}	18^{A}	35^{A}
5	15^{A}	34^{A}	10^{A}	27^{A}
6	19^{A}	30^{A}	18^{A}	23^{A}
7	14^{A}	24^{A}	5^{A}	27^{A}
8	16^{A}	20^{A}	5^{A}	20^{A}
9	28^{B}	40^{B}	35^{B}	55^{B}
C.V(%)	37	38	39	37

Means followed by the same lowercase letters in the column are not significant by Tukey test.* Treat: Treatments. DAE: days after emergence of soybean.

Table 5. Weight of one thousand grains and soybean yield subjected to different fungicide treatments on seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/12.

Treat.	Weight of one thousand grains	Soybean yield*
	----g----	----kg x ha^{-1}---
1	192.50^{A}	3100^{A}
2	192.50^{A}	2990^{A}
3	177.50^{AB}	2739^{C}
4	177.50^{AB}	2851^{B}
5	182.50^{AB}	2979^{AB}
6	182.50^{AB}	2933^{AB}
7	185.00^{AB}	2967^{AB}
8	192.50^{A}	3169^{A}
9	170.00^{B}	2417^{D}
C.V (%)	3.48	4.24

Means followed by the same lowercase letters in the column are not significant by Tukey ($p<0.05$). *The seed moisture applied for this calculation was 13%.

weight, which reinforces the positive influence of fungicides on the production and redistribution of photoassimilates by plants. Fagan et al (2010) noted that the use of Pyraclostrobin via seed treatment and over aerial parts of soybeans delayed the leaves senescence and increased the photosynthesis rate by reducing the activity of the enzyme ACC synthase and ethylene synthesis. Another important change observed by the author was elevation of endogenous indole acetic acid level. Therefore, plants might accumulate more energy and increase the efficiency in the use of assimilates to produce substances against pathogens directing more energy for grains production.

Conclusions

1) Pyraclostrobin + Methyl thiophanate via seed treatment favored root development of seedlings, plant height and grain yield on the soybean crop.
2) The plant height and dry weight of aerial part had greater increase with the use of Pyraclostrobin + Methyl thiophanate via seed treatment.
3) Treatments with fungicides did not affect the stand of

plant under low pathogen infestation.
4) The nutritional status by leaf analysis is not a useful tool in predicting phytotonic effects of fungicides on the soybeans plants.
5) Fungicides applied over aerial part seem do not provide any phytotonic effect on soybean plants.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Spatial variation in soil moisture with subsurface drip irrigation in cane sugar

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The sugarcane agroecosystem has been the focus of much research. Mainly, because the sugarcane in Brazil has great economic importance and land occupancy. Prominent among this research is water replacement in the soil, which can be monitored by tensiometers. Therefore, this study aimed to monitor the matric potential at different positions in the soil to check the influence of the depth and horizontal distance from the tensiometer relative to the dripline in evaluating soil moisture in Dystrophic Red Latosol cultivated with sugarcane under subsurface drip irrigation. The experiment was conducted in the experimental area of the Federal Institute of Goias (IF Goiano), Campus Rio Verde, GO. The planting of sugar cane occurred in a double row, and the dripline was buried 0.20 m deep under the surface of the soil between the crop rows. The soil matric potential was recorded at four depths (0.20; 0.40; 0.60 and 0.80 m of the soil surface) and four horizontal distances from the dripline (0.15; 0.30; 0.45 and 0.60 m). The values of soil matric potential were evaluated through mathematical regressions and compared to each other using the homogeneity test for linear models. The depth of monitoring and the horizontal distance between the dripline and the tensiometric rods had strong and weak influences, respectively, in the soil's matric potential. The highest values of soil matric potential were observed in the deeper layers of the soil.

Key words: Matric potential, wetted bulb, water replacement, dystrophic red latosol, *Saccharum officinarum* L.

INTRODUCTION

Brazil is the largest producer of sugar and alcohol in the world (MAPA, 2013). Thus, the sugarcane agroecosystem has been the focus of much research by virtue of its great economic importance and land occupancy (Barros et al., 2010). Mainly, because the sugarcane in Brazil generates approximately 25 billion dollars per year, due with the production and sale of sugar and ethanol. Sugarcane is a crop with a high degree of technology applied to its production system (Dalri and Cruz, 2008). Among the technologies applied to the production of sugarcane, soil water replacement can be highlighted. Water replacement is water of

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irrigation applied to soil, mainly for replace the amount of water lost by evapotranspiration, that is, the sum of evaporation and plant transpiration. This practice is of great benefit to the crop, because it provides for better growth of plants, increased productivity and improved quality in the product (Porto et al., 2014). Water replacement in the soil by subsurface drip irrigation has been notable for presenting numerous advantages, such as reduced water evaporation, reduced mechanical damage to the system, less interference with cultural treatments, improved efficiency of fertilizer application dissolved in water for irrigation (fertigation) and increased canebrake longevity (Andrade Júnior et al., 2012).

Drip irrigation is performed with a high frequency of water application to maintain the soil water content at field capacity for appropriate root proliferation (Souza and Folegatti, 2010). Therefore, due to the low water volume applied to soil, the accuracy in the frequency of applications is essential for correct water replacement, making it a necessity to obtain data referencing soil water content. To obtain data on soil water content it is necessary to monitor the energy state of soil water. Several devices are used, to access the energy state of soil water in particular tensiometers, which are instruments that measure the tension under which water is retained by pore spaces in the soil with different diameters (macro and micropores) due to capillarity and adsorption phenomena at the particles' surfaces, called the conjunction matric potential (Bezerra et al., 2012).

Tensiometers used to monitor soil water potential must be placed at strategic points for correct monitoring of soil water potential. Martins (2009) noted that there have not been many studies about the distances between the points of measurement in the soil, with the actual distances used typically ranging from 0.20 to 0.40 m (Cintra et al., 2000; Hutchinson and Bond, 2001). Tensiometers located at various depths and distances may indicate the actual size of the wet bulb, yielding more precise information about soil water conditions.

In this sense, the aim of this study was to monitor the matric potential at different points on the ground to assess the influence of the depth and horizontal distance from the tensiometer relative to the dripline in the management of soil moisture in Dystrophic Red Latosol cultivated with sugarcane under subsurface drip irrigation. Thus, it will be possible to determine the soil moisture afforded by the application of water in the soil near the dripline.

MATERIALS AND METHODS

The experiment was performed in the experimental area of the Federal Institute of Goiás, campus Rio Verde, Goiás State, Brazil, 17°48'28"S and 50°53'57"W, mean altitude 720 m. The climate is classified according to Köppen (CastroNeto, 1982) as Aw (tropical), with rain in the months of October to May and drought from June to September. The average annual temperature is 23°C and precipitation ranges from 1500 to 1800 mm annually. Table 1 presents the meteorological data and of the culture during the study period. The soil is Dystrophic Red Latosol. Latosols are tropical mineral soils, very weathered and small reserves of soil nutrients for plants. These are deep soils with over 2 m depth, B-horizons very thick (greater than 50 cm) with sequence horizons A, B and C little differed. Table 2 presents the physical-hydro characteristics of the soil in the experimental area.

The initial preparation of the ground consisted of prior disking to remove the existing vegetation, mechanical distribution of limestone at a dose of 2.0 t ha^{-1} and subsequent disking in order to incorporate the limestone at 0 to 20 cm depth and break up the clods of soil. Finally, it was made one ground leveling to build the planting furrows. Subsoiling was used, with subsequent removal of soil, raising the planting bed.

The planting of the sugarcane, cultivar RB85-5453, occurred on March 15, 2011, performed in a double row (W-shaped), 8 m long, with 1.80 m spacing between the double rows. The distance between the crops in the double row was 0.40 m, with a total area of 35.2 m^2 in each paddock. A subsurface drip irrigation system was used for irrigation, with the drip tube buried in the soil at a depth of 0.20 m among the furrows of the double row. The drip tube (DRIPNET PC 16150) comprised a thin wall, 1.0 bar pressure, nominal discharge 1.0 L h^{-1}, and 0.50 m spacing between drippers.

A 0.1 kPa puncture digital tensiometer was used to record daily readings of the soil matric potential (Ψm) in order to schedule irrigation. Four batteries of tensiometers were installed between the centerlines of the plots. The irrigation strategy was to irrigate to field capacity when the soil water tension exceeds -50 kPa, measured in the root-zone. The physical-hydro characteristics of the soil were determined by the water retention curve of the soil.

The van Genuchten model (van Genuchten, 1980) was adjusted to convert the measured matric potential (Ψm) to soil water content (θ), minimizing the sum of the squares of the deviations using the software SWRC (Dourado-Neto et al., 2000), thus, obtaining, the empirical parameters of adjustment used in the equation shown thus:

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{\left[1 + \left(\alpha \times |\psi_m|\right)^n\right]^m}$$

$$\theta = 0{,}3027 + \frac{0{,}59 - 0{,}3027}{\left[1 + \left(0{,}0447 \times |\psi_m|\right)^{1{,}7657}\right]^{0{,}43365238}}$$

Wherein: θ – soil water content, $cm^3.cm^{-3}$; Ψ_m – matric potential, kPa (in module); θ_s – soil moisture saturated, $cm^3.cm^{-3}$; θ_r – soil moisture residual, $cm^3.cm^{-3}$; α, n, m – empirical parameters of adjustment.

Each battery of tensiometers consisted of sixteen tensiometric rods, that were installed at four depths (0.20; 0.40; 0.60; and 0.80 m of soil surface) and four horizontal distances from the dripline (0.15; 0.30; 0.45; and 0.60 m) (Figure 1). The readings of the tensiometers for soil matric potential record were performed from October 26, 2011 to March 13, 2012 (35 observations of soil matric potential), using a digital tensiometer of puncture (mark: Tensimeter). Was subtracted the length of the water column in the tensiometer from the reading to get the soil matric potential.

The values of soil matric potential were evaluated through mathematical regressions according to their depths under the ground surface and the horizontal distances of the tensiometric rods in relation to the dripline. Construction of the graphics was

Table 1. Meteorological data and culture during the period of the experiment.

Year	2011										2012				Total
Month	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr*	
P (mm)	109.7	23.5	0.2	21.1	0	1.5	3.5	167.1	145	164.9	210.1	297.1	215.2	27.4	1386.3
ETc (mm)	74.4	63.5	48.6	28.9	39.5	95.8	145.6	142.1	139.1	137.9	130.3	94.5	63	41	1244.2
Irrigation (mm)	0	4	20	10	30	40	40	0	0	0	0	0	0	0	144.0

Source: Normal weather station INMET A025 – Rio Verde, GO, Brazil. P - precipitation; ETc - crop evapotranspiration (Penman-Monteith/FAO). * In April irrigation was cut for harvest.

Table 2. Physical-hydro characterization of Dystrophic Red Latosol in the experimental area.

Layer (m)	Granulometry (g kg^{-1})			θ_{FC}	θ_{PWP}	Bd	TP	Textural classification
	Sand	Silt	Clay	--- m^3 m^{-3} ---		g cm^{-3}	%	
0–0.20	458.30	150.20	391.50	51.83	30.50	1.27	0.55	Sand Clay
0.20–0.40	374.90	158.30	466.80	55.0	31.33	1.28	0.51	Clay

θ_{FC} - field capacity (10kPa); θ_{PWP} - permanent wilting point (1500 kPa); Bd - soil bulk density; TP - total porosity.

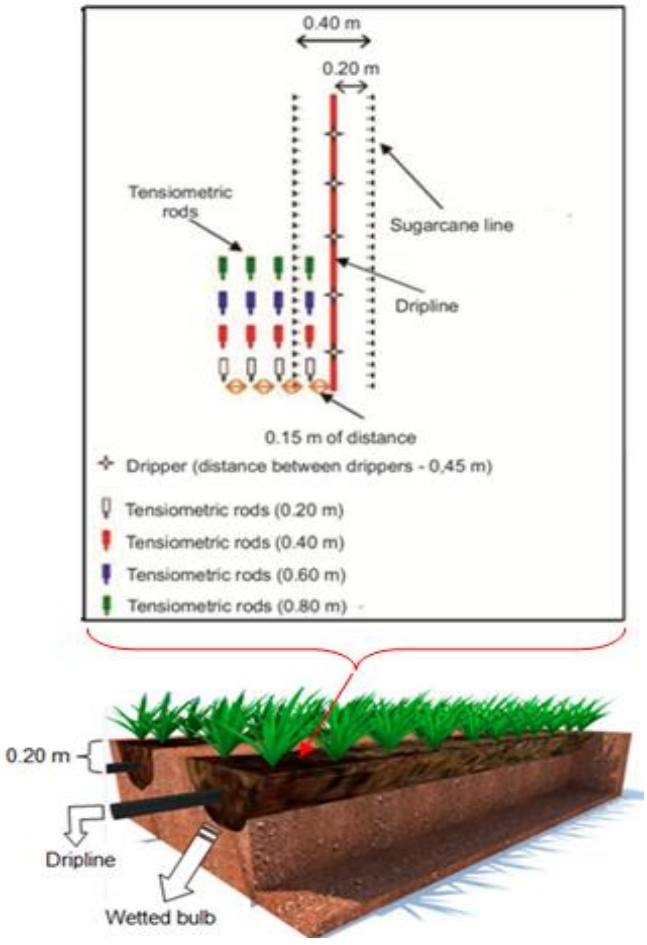

Figure 1. Outline of W-shaped planting and the laying of driplines, demonstrating the location of the tensiometric rods for monitoring soil matric potentialat different depths and horizontal distances from the dripline.

performed using the demo version of the app Sigma Plot 11.0 (Systat Software Inc®). A tridimensional graphic was constructed using the app Wolfram Mathematica versão 7.0. Linear regressions were compared to each other using the homogeneity test for linear models described by Snedecor and Cochran (1989). The homogeneity test for linear models considers two models that are compared by analysing the intercept "a", the angular coefficient "b" and the homogeneity of the data (Araujo-Junior et al., 2011). When two linear regressions presented homogeneous data and there were no significant differences between their coefficients ("a" and "b"), the data were combined and a new regression was built using all the data (Iori et al., 2012).

RESULTS

Table 3 presents the average values of 35 observations of soil matric potential (kPa), as well as the standard deviation and coefficient of variation for each depth and horizontal distance analysed. The values of the statistical parameters (average, standard deviation and coefficient of variation) for the data set of matric potential at depths of 0.40; 0.60; and 0.80 m were similar, causing low coefficients of variation. On the other hand, the highest coefficient of variation in the data on soil matric potential was observed at a depth of 0.20 m (average value of the coefficient of variation of 16.2%), whereas at the other depths a lower coefficient of variation (average value of the coefficient of variation of 4.6%) was seen (Table 3).

The values of soil matric potential as a function of depth for different horizontal distances from the dripline are presented in Figure 2. We observed increase in the matric potential with increasing depth, irrespective of the horizontal distance measured. The lowest values of the matric potential were obtained in readings from tensiometric rods located a distance of 0.60 m from the dripline. The proximity between the regressions obtained

Table 3. Values of soil matric potential (kPa) in Dystrophic Red Latosol cultivated with sugarcane and irrigated by subsurface drip at different depths below the ground surface and horizontal distances from the dripline.

Depth (m)	Horizontal distance (m)	Average	Standard deviation	CV (%)
0.20	0.15	-26.02	4.26	16.36
	0.30	-26.53	4.73	17.83
	0.45	-25.77	3.56	13.81
	0.60	-26.99	4.48	16.60
0.40	0.15	-22.86	1.80	7.87
	0.30	-22.40	0.79	3.54
	0.45	-22.03	1.70	7.70
	0.60	-22.25	1.10	4.94
0.60	0.15	-20.43	1.51	7.38
	0.30	-20.37	0.46	2.27
	0.45	-20.43	0.56	2.75
	0.60	-20.71	0.59	2.85
0.80	0.15	-21.00	0.71	3.37
	0.30	-21.37	1.15	5.36
	0.45	-21.39	0.92	4.28
	0.60	-21.43	0.66	3.09

Average of 35 observations. CV - Coefficient of variation.

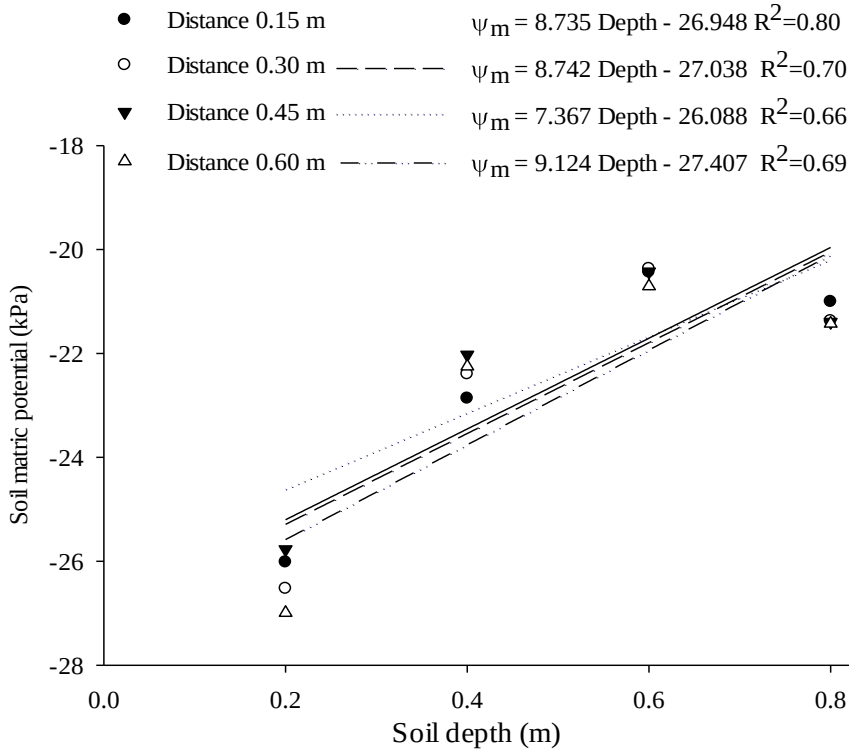

Figure 2. Linear regressions obtained from soil matric potential (kPa) as a function of depth below the ground surface (m) in Dystrophic Red Latosol cultivated with sugarcane and irrigated by subsurface drip, for four horizontal distances from the dripline.

Table 4. Significance test based on Snedecor and Cochran (1989) between the regressions obtained for soil matric potential as a function of depth below the ground surface at different horizontal distances from the dripline.

Horizontal distance (m)	Data homogeneity	Angular coefficient, b	Linear coefficient, a
0.15 x 0.30	0.56^{H}	0.00^{NS}	0.01^{NS}
0.15 and 0.30 x 0.45	0.62^{H}	0.13^{NS}	0.07^{NS}
0.15 and 0.30 x 0.45 x 0.60	0.44^{H}	0.05^{NS}	0.14^{NS}

^{H} - homogeneous; ^{NS} - not significant.

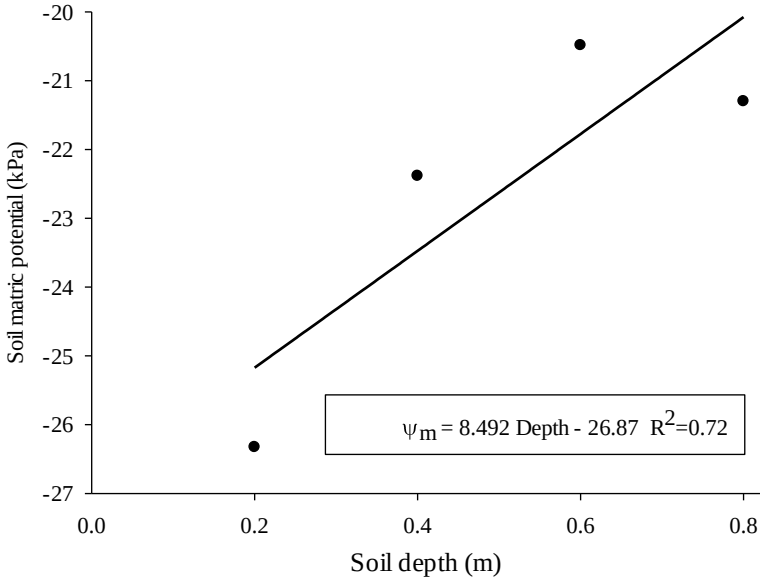

Figure 3. Linear regression obtained from soil matric potential (kPa) as a function of depth below the ground surface (m) in Dystrophic Red Latosol cultivated with sugarcane and irrigated by subsurface drip.

from the matric potential data as a function of depth at different distances is shown in Figure 2.

Firstly, we compared the regressions obtained at distances of 0.15 and 0.30 m. Looking at the test of significance values, the data in both regressions were homogeneous with no significant differences between the linear (a) and angular (b) coefficients (Table 4). It can therefore be stated that both regressions are similar and describe the same conduct. Thus, the data forming the regressions at distances of 0.15 and 0.30 m were joined to form a new regression and compared with linear regression at a distance of 0.45 m.

The similarity between these two regressions was verified in the same way (data homogeneity and similarities between the linear and angular coefficients). Subsequently, the regression data obtained at 0.15; 0.30; and 0.45 m was combined and used to compose yet another regression, which was compared with the regression at a distance of 0.60 m. It was again verified that there were no significant differences between the linear and angular coefficients and that the data were homogeneous. Thus, it could be argued that all the data obtained for all the regressions can comprise a single regression as presented in Figure 3. The behaviour of this new regression was identical to the previous behaviour of the less comprehensive regressions, that is, increasing the depth of the monitoring caused increase in the tendency of the soil matric potential (Figure 3). The evidence of similarity in the matric potential regressions as a function of the tensiometric rod's distance from the dripline up to 0.60 m demonstrates uniformity in the maintenance of soil moisture via distribution of water by a subsurface drip system. This result can be attributed mainly to the physical attributes of the soil type in the study (Dystrophic Red Latosol), allowing an expansion of the wetted zone that exceeded a radius of 0.60 m laterally, and underscoring that the moisture inside the bulb is relatively well distributed (Figure 2).

Figure 4 presents the regressions obtained from soil matric potential (kPa) as a function of horizontal distance from the dripline (m) for each depth evaluated. Among the four depths analysed, it was verified that the

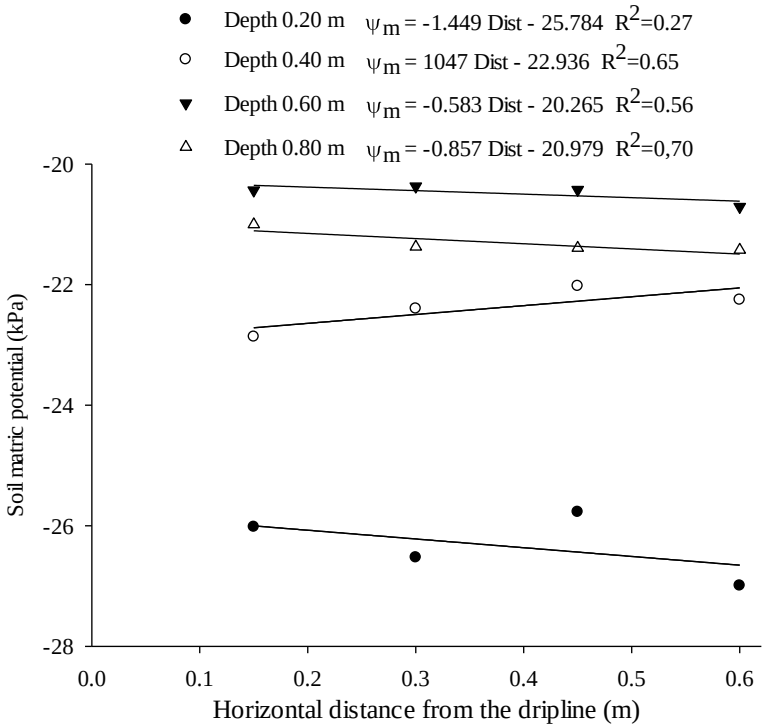

Figure 4. Linear regressions obtained from soil matric potential (kPa) as a function of horizontal distance from the dripline (m) in Dystrophic Red Latosol cultivated with sugarcane and irrigated by subsurface drip, at fourdepths below the ground surface.

Table 5. Second significance test per Snedecor and Cochran (1989) between the regressions obtained for soil matric potential as a function of horizontal distance from the dripline at different depths below the ground surface.

Depth (m)	Data homogeneity	Angular coefficient, b	Linear coefficient, a
0.20 x 0.40	4.88^{H}	2.44^{NS}	122.99^{**}
0.20 x 0.60	21.35^{NH}	0.25^{NS}	471.78^{**}
0.20 x 0.80	18.57^{NH}	0.11^{NS}	358.1^{**}
0.40 x 0.60	4.37^{H}	5.79^{NS}	90.08^{**}
0.40 x 0.80	3.8^{H}	7.24^{*}	24.88^{**}
0.60 x 0.80	0.87^{H}	0.26^{NS}	95.38^{**}

^{NH} - not homogeneous; ^{H} - homogeneous; ** - significant at 1%; * - significant at 5%; ^{NS} - not significant

regressions obtained for depths of 0.20, 0.60 and 0.80 m presented similar behaviour (slope), that is, increasing the horizontal distance from the tensiometer relative to the dripline provided little reductions in the soil matric potential. The only exception occurred at a depth of 0.40 m, where increasing the tensiometer distance from the dripline provided a little increase in soil matric potential.

The depth of tensiometers, presenting specific regression, enabled the creation of a tridimensional model to better express the soil matric potential along the soil profile (Table 5). Thus, the variables were subjected to statistical methodology to check the deviation occurring between the observed and predicted data, to obtain a better representation of the behaviour of the matric potential as a function of depth beneath the ground surface and horizontal distance from the dripline (Figure 5). The dependent variable (soil matric potential) presented a strong relationship as a function of depth below the ground surface and horizontal distance from the dripline in the tridimensional space ($R^2 = 0.99$);

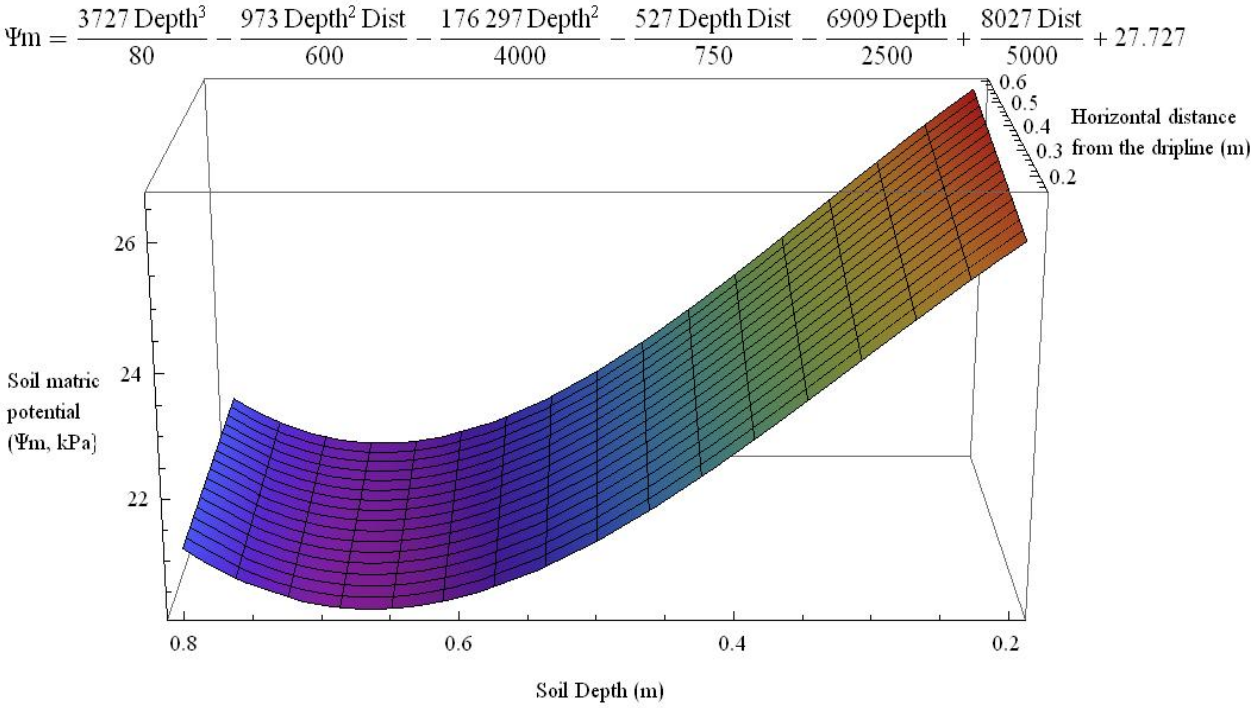

Figure 5. Tridimensional model representing the soil matric potential (kPa, in module) as a function of depth below the ground surface and horizontal distance from the dripline in Dystrophic Red Latosol cultivated with sugarcane and irrigated by subsurface drip.

consequently, adapting the variable of interest to a function of two independent variables, the behaviour of the matric potential can be predicted with great accuracy, as exhibited in Figure 5.

DISCUSSION

According to the statistical parameters (average, standard deviation and coefficient of variation), for the data set of matric potential at depths 0.40; 0.60; and 0.80 m below the ground surface presented similar values between one another. This similarity in the data was confirmed by the low coefficients of variation observed for these three depths (0.40; 0.60; and 0.80 m). The higher coefficient of variation in soil matric potential at a more superficial depth reflects a greater influence of external conditions on the soil that caused these oscillations; for example, the air temperature, as well as greater variation in the water content of this layer during the year. The soil surface is more susceptible to climate, as daily temperature and solar radiation variation, and to anthropic interference, as tillage and soil management. Coelho and Teixeira (2004) also attributed small oscillations in the curves describing the monitoring of soil matric potential to temperature fluctuations. Azooz and Arshard (1994) also noted a decline in the readings of matric potential in tensiometers with increasing soil temperature.

In this regard Melo Filho and Libardi (2005), studying the temporal stability of soil water content and soil matric potential in determining hydraulic conductivity in an instantaneous profile, underscored the importance of using the coefficient of variation as a descriptive parameter of the matric potential's variability. Thus, according to the criteria of Warrick and Nielsen (1980) for classifying the coefficient of variation, at a depth of 0.20 m the coefficient of variation was classified as average (coefficient of variation between 12 and 60%), while the other depths all presented coefficients of variation classified as low (coefficient of variation less than 12%). To determine if each such regressions was similar, i.e., if they each presented a unique behaviour, these linear equations were compared by the test of homogeneity according to Snedecor and Cochran (1989) (Table 4). This methodology is widely used in field studies of soil science to compare linear regressions (Araujo-Junior et al., 2011; Iori et al., 2012, 2013; Pais et al., 2013).

In this study, we obtained a linear relationship for soil matric potential as a function of the depth analysed. Martins (2009), studying the influence of the distance of installation of tensiometers in a field for calculating the

total potential gradient using the method of instantaneous profiling, verified that the total potential of the data as a function of soil depth adjusted to a quadratic equation. The regressions presented in Figure 4 were compared among themselves using the same methodology used previously, by the significance test according to Snedecor and Cochran (1989) (Table 5). Firstly, the regressions obtained at depths of 0.20 and 0.40 m were compared. This comparison showed that these regressions presented different linear coefficients ("a"); thus, these regressions were not similar, and each regression must be predicted by its own data. In the subsequent comparison, there were always significant differences, either in angular coefficient or linear coefficient, or insufficient data homogeneity. Therefore, each depth must be predicted using its respective data, that is, each depth presented must be specifically regressed. Consequently, the depths presented different and specific patterns. These results are similar to those obtained by Souza et al. (2007), who observed greater availability in water readings after an hour of irrigation at a depth of 0.65 m with a drip buried 0.25 m beneath the soil surface.

The soil matric potential is indirectly related to soil moisture, by having a, higher soil water content, at lower the matric potential (Bezerra et al., 2012). The regression shown in Figure 5 indicates that the lowest matric potential occurred at a depth of 0.68 m below the soil surface, with a value of 20.34 kPa (in module). The horizontal distance from the drip line did not have an effect on this result, due to better circulation of water in the soil, through increased permeability and proportionate suction, especially for the root system of the plants, which directly influences the flow behaviour of soil water (Bezerra et al., 2012). Moreover, Ajayi et al. (2009) confirmed that Latosols in the Cerrado region features a predominantly oxidic mineralogy and granular type structure, giving the latter soils high porosity.

With regards to the lateral movement of soil moisture from the buried drip system, the uniformity of the wetted diameter at different points on the soil surface was observed (Figure 4). According to Barros et al. (2009), subsurface drip irrigation systems allow the formation of a more dispersed wetted bulb, that is, despite having a smaller surface area of wetted soil in relation to the surface drip, the water applied reaches more distant points in the soil, both in depth as well as lateral distance from the dripline.

Conclusions

The depth of the tensiometers rods in the profile of Dystrophic Red Latosol contributed to the monitoring of soil matric potential via subsurface drip irrigation in sugarcane cultivation. Water storage in the soil was of low spatial variability in the horizontal direction, reflecting a high homogeneity in the level of moisture out to a lateral distance of 0.60 m from the dripline.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Evaluation of different treatment on the occurrences of seed borne fungi of Mungbean *Vigna radiata* (L.) Wilczek seed

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The efficacy of different treatment measures *viz*., corbendazim, Benomyle, Vitavax, Neem, Garlic, *Trichoderma harzianum* and *Trichoderma viride* were evaluated for the occurrences of seed borne fungi *viz*., *Aspergillus spp*, *Penicillium spp*, *Alternaria alternata*, *Rhizopus* and *Fusarium spp* which were isolated from two genotype of Mungbean *viz*., HUM-4 and HUM-12, from, 180 and 360 days period of storage. Both genotypes were taken from the Department of Genetics and Plant Breeding, Institute of Agricultural Science, B.H.U Varanasi. All treatments were found to significantly reduce the incidence of seed borne fungi but did not completely control it. The results showed that the incidence of seed borne fungi was found to be significantly declined when seeds were treated with different treatments but did not completely control it. Seed treated with mungbean seed showed least incidence. Due to an increase in storage period, efficacy of different treatment against seed borne fungi was found to be declined. After 180 days period of storage, effect of treatment was found to be intermediate. Least effect of treatment was shown at 360days period of storage due to an increase in the number of fungal population. Among all treatment, Benomyle, Corbendazim and Vitavax showed minimum occurrences against all fungi followed by Neem, Garlic and *T. harzianum.*

Key words: seed borne fungi, Pulses, mungbean

INTRODUCTION

Pulses are rich source of vegetative protein and they play an important role in nutritional security of the majority of vegetarian population in India. India is the largest producer and consumer of pulses occupying 33% of the world's area and 22% of the production (FAO, 2008). Pulse production in the country has fluctuated widely between 13 and 15 million tonnes (mt) with no significant growth trend between 1991 and 2010. The latest estimate indicates that the present production of pulses has reached 14.7 million tons (mt) with productivity of 637 kg/ha although, the projected pulse requirement by the year 2030 (32 mt) is estimated to be more than double

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the current production level (Anonymous, 2011). Mungbean (*Vigna radiate* L.) Wilczek) is a short duration; herbaceous, annual, self-pollinated legume pulse crop under the family. It also has the ability to fix atmospheric nitrogen in soil, which enriches the soil quality (Ather Nadeem et al., 2004). It is an excellent source of proteins considered as a "poor men's protein" (Mian, 1976). It contains 26% protein, 51% carbohydrate, 10% moisture, 4% minerals and 3% vitamins (Khan, 1981).

Areas for cereals and other pulses have decreased, however, that for mungbean has doubled in the last two decades with an annual rate of 2.5%. The area under pulses in India is around 24.38 million hectares with a production of 14.52 million tonnes. Nearly 8% of this area is occupied by mungbean which is the third most important pulse crop of India in terms of area cultivated and production next to gram and pigeon pea (Sathyamoorthi et al., 2008). To reduce the occurrences of seed borne fungi, seed treatment with fungicides plant extract and Bioagent plays an important role in prevention of seed borne fungi Erdey et al. (1997). Keeping these views in mind, the present research work has been undertaken to determine the efficacy of seed treating methods against the major seed borne pathogens of mungbean. Seed treatment with different botanicals including garlic extract has been tested and found effective alternative approaches to combat the seed-borne diseases of many crops including cucumber (Alice and Rao, 1987; Kurucheva and Padmavathi, 1997; Rahman et al., 1997; Islam et al., 2001; Anon., 2004). Hence, in the present study fungicides and chemicals were used as seed treatment to evaluate their efficacy in controlling seedborne fungi to enhance the seed quality.

MATERIALS AND METHODS

Corbendazim, Benomyle, Vitavax, Neem, Garlic and *T. harzianum* were tested for the occurrences of seed borne fungi. The required quantity of each seed dressing fungicide at 0.3%, plant extract 5% and bioagent 0.8% were weighed and treated separately as dry seed treatment. One hundred gram (100 g) each of unsterilized mungbean seeds were treated with 250 ml conical flasks by shaking, using a wrist action shaker to ensure uniform coating of the fungicide on the seed surface. The treated seeds were air dried overnight and were placed on half-strength Potato dextrose agar (PDA) medium and blotter plate at 16 seeds per Petri plate. The untreated seeds of respective crops served as control. The experiment was replicated thrice. The plated seeds were incubated at 25°C in a Biochemical Oxygen Demand (BOD) incubator for 5 days under 12 h alternate light and dark conditions. Incidences of different fungal pathogen was recorded as under:

$$\text{Incidence (\%)} = \frac{\text{No of infected seed}}{\text{Total number of treated seed assessed} \times 100}$$

RESULT

The percent reduction in seed-borne infection of target pathogenic fungi recorded in mungbean seeds were treated with five different treatments. Result found that, all treatments were found to significantly reduce the occurrences of seed borne fungi but did not completely control them. Efficacy of different treatment on occurrences of seed borne fungi is illustrated in details.

Testing of different treatment on seed borne fungi on fresh mungbean seed

Table 1 shows that Benomyle was found to be most effective against *Aspergillus spp*. it showed least incidence (1.00 and 2.90%) in genotype HUM-12 and HUM-4 through blotter method. The second best treatment was Corbendazim against Aspergillus spp., it reduced the occurrences (1.56%) in genotype HUM-12 through blotter method followed by (3.33%) in genotype HUM-4 through blotter method. Some treatment like *viz.* Vitavax, Neem, Garlic and Ginger were found to reduce the incidence (2.26, 4.80, 7.00 and 8.23%) in genotype HUM-12 through blotter method and low occurrences (6.64, 10.9, 11.0 and 13.9%) in genotype HUM-4 through Agar plate methods against *Aspergillus niger*. Among all the treatment against *Penicillium spp*, Corbendazim showed least incidence (0.67 and 1.18%) in genotype HUM-12 and HUM-4 through Blotter method and Benomyle showed the least incidence (1.58 and 1.67%) in genotype HUM-12 and HUM-4 through blotter paper methods. *Alternaria alternata* was found to be controlled most effectively when seed is treated with Vitavax and Corbendazim. It showed (0.67 and 1.00%) occurrences in genotype HUM-12 through blotter paper method. *Fusarium spp* was found to be controlled most effectively when seed is treated with Benomyle; it showed (0.50%) occurrences in genotype HUM-12 through Agar plate method. Benomyle and Vitavax were found to be most effective against *Rhizopus spp;* it reduced the (1.00 and 1.19%) occurrences in genotype HUM-4 through blotter methods. Neem, Garlic and *T. harzianum* were found to be most effective against *A. alternata.* It showed the least incidence (1.56, 2.00 and 3.00%).

Testing of different treatment on seed borne fungi on mungbean seed at the 180 days period of storage

Table 2 reveals that an increase in the incidence of seed borne fungi during storage and the efficacy of different treatments were found to be decreased against seed borne fungi of Mungbean seed. Present result showed that, Benomyle was found to be most effective against *Aspergillus spp*. it showed least incidence (6.98 and 7.45%) in genotype HUM-12 and HUM-4 through blotter method and Corbendazim reduced the maximum occurrences (8.85 and 9.98%) in genotype HUM-12 and HUM-4 through blotter method. Some treatment like *viz.* Vitavax, Neem, Garlic and Ginger were found to reduced

Table 1. Testing of different treatment on occurrences of seed borne fungi on fresh seeds.

Treatment	Genotype																							
	HUM-4												HUM-12											
	Agar plate method						Blotter plate method						Agar plate method						Blotter plate method					
	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th
Aspergillus spp	5.09	4.63	6.64	10.9	11.0	13.9	3.33	2.90	4.32	7.67	9.10	11.3	4.00	3.51	5.54	3.90	9.78	12.5	1.56	1.00	2.26	4.80	7.00	8.23
Penicillium spp	2.30	2.97	3.89	4.08	4.88	8.00	1.18	1.67	2.00	3.35	3.78	7.67	1.98	2.00	3.17	4.60	4.80	8.98	0.67	1.58	1.88	2.00	2.67	5.31
Alternaria alternata	2.47	2.00	1.35	3.80	3.90	5.89	1.33	1.56	0.94	2.00	2.56	3.90	1.80	1.98	1.20	2.20	3.00	4.32	1.00	1.28	0.67	1.56	2.00	3.00
Fusarium spp	1.34	1.32	1.21	3.00	3.21	5.32	3.89	2.09	2.00	4.98	5.06	7.89	1.00	0.50	1.00	1.56	2.67	4.21	2.00	1.08	1.55	3.52	3.98	6.62
Rhizopus spp	2.00	1.67	1.87	2.56	3.00	4.67	1.67	1.00	1.19	2.00	2.86	3.33	3.00	2.98	2.78	3.33	4.00	5.16	2.67	1.80	1.98	3.00	3.85	5.00
C.D. at 1%	1.58	0.56	2.00	1.73	0.75	1.69	2.00	2.33	1.50	1.21	0.50	0.44	1.32	2.00	2.74	0.98	2.58	1.83	1.55	2.79	1.84	2.50	1.75	1.55

Keys: Co = Corbendazim, Be = Benomyle, Vi = Vitavax, Ne = Neem, Ga = Garlic and Th = *Trichderma harzianum*.

Table 2. Testing of different treatment on occurrences of seed borne fungi on 180 days period of storage.

Treatment	Genotype																							
	HUM-4												HUM-12											
	Agar plate method						Blotter plate method						Agar plate method						Blotter plate method					
	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th
Aspergillus sp	9.19	12.8	13.6	16.9	18.0	22.6	9.98	7.45	10.6	13.9	14.6	18.8	10.8	8.98	11.8	14.8	16.9	20.8	8.85	6.98	8.90	11.8	12.8	16.6
Penicillium sp	3.70	4.15	4.96	5.58	7.83	11.8	2.31	3.00	3.36	4.56	6.67	9.84	3.24	3.98	4.56	5.00	7.09	10.5	2.00	2.56	3.08	3.27	6.00	8.84
Alternaria alternata	4.46	3.50	4.16	4.98	5.38	6.98	2.98	3.00	3.23	3.34	4.67	5.00	4.32	3.00	3.81	4.15	5.00	6.32	2.43	2.46	2.40	3.00	3.38	4.69
Fusarium sp	3.08	2.45	2.66	4.91	5.10	9.98	5.10	3.61	3.95	6.93	8.83	11.5	2.08	2.00	2.56	3.38	4.00	7.98	4.81	2.86	3.98	5.11	6.83	10.8
Rhizopus spp	3.32	3.56	3.32	3.89	4.56	6.67	3.80	2.56	2.50	3.78	3.82	5.50	5.85	5.00	4.56	5.00	6.67	7.00	4.00	4.56	4.18	3.50	4.00	7.60
CD (%)	1.70	0.82	1.98	1.70	0.73	1.65	2.34	1.03	2.20	0.82	1.00	0.34	1.02	0.34	1.74	0.92	1.58	0.83	1.50	0.79	0.84	2.00	0.75	1.50

Keys: Co = Corbendazim, Be = Benomyle, Vi = Vitavax, Ne = Neem, Ga = Garlic and Th = *Trichderma harzianum*.

the incidence (8.90, 11.8, 12.8 and 16.6%) in genotype HUM-12 through blotter method and lowest occurrences (13.6, 16.9, 18.0 and 22.6%) in genotype HUM-4 through Agar plate methods against *Aspergillus niger*. Among all treatment against *Penicillium spp*, Corbendazim showed least incidence (2.00 and 2.31%) in genotype HUM-12 and HUM-4 through Blotter method. *Alternaria alternata* was found to be controlled most effectively when seed is treated with Vitavax and Corbendazim, it showed (2.40 and 2.43%) occurrences in genotype HUM-12 through blotter paper method. *Fusarium spp* was found to be controlled most effectively when seeds were treated with Benomyle and Corbendazim it showed (2.00 and 2.06%) occurrences in genotype HUM-12 through Agar plate method. Benomyle and Vitavax were found to be most effective against *Rhizopus spp* it reduces the (2.56 and 2.50%) occurrences in genotype HUM-4 through blotter methods. Neem, Garlic and *T. harzianum* was found to be most effective against *A. alternata* it showed the least incidence (3.00, 3.38 and 4.69%).

Testing of different treatment on seed borne fungi on mungbean seed at 360 days period of storage

Table 3 shows that due to an increase in storage period, some great amount of seed borne fungi were found in substantial number and efficacy of

Table 3. Testing of different treatment on occurrences of seed borne fungi on 360 days period of storage.

Treatment	Genotype																							
	HUM-4												HUM-12											
	Agar plate method						Blotter plate method						Agar plate method						Blotter plate method					
	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th	Co	Be	Vi	Ne	Ga	Th
Aspergillus spp	12.6	14.9	16.9	18.8	20.8	24.9	10.0	10.6	12.9	15.8	16.9	20.9	10.4	12.8	14.4	17.7	17.9	22.4	9.98	9.95	10.7	13.3	15.5	18.9
Penicillium spp	4.59	5.00	6.79	8.56	10.6	13.9	3.56	3.78	4.00	6.78	8.00	11.7	4.23	4.35	4.78	8.00	9.98	12.5	2.98	3.33	3.09	5.78	7.80	9.80
Alternaria alternata	6.80	5.00	4.98	5.32	6.98	9.98	4.78	3.38	3.20	4.32	6.00	8.00	5.80	4.67	4.19	5.30	6.17	8.56	3.00	3.56	2.98	4.30	5.56	7.98
Fusarium spp	5.50	3.56	2.00	3.00	6.35	8.00	7.15	4.80	2.78	4.09	7.76	10.7	4.00	2.98	1.53	3.86	5.00	5.80	6.05	4.00	3.60	3.33	6.56	8.90
Rhizopus spp	4.89	2.55	4.51	7.45	8.43	9.00	4.00	2.26	3.43	4.09	5.13	6.00	7.00	4.42	6.98	3.81	3.93	7.00	6.56	3.00	5.51	2.18	3.94	6.67
CD (%)	1.86	1.21	2.50	1.98	0.56	1.68	3.00	1.55	2.56	0.85	1.42	0.50	1.27	0.31	1.70	0.82	1.60	0.85	1.51	0.80	0.87	2.13	0.85	1.51

Keys: Co = Corbendazim, Be = Benomyle, Vi = Vitavax, Ne = Neem, Ga = Garlic and Th = *Trichderma harzianum*.

different treatment *viz*., Benomyle, Corbendazim, Vitavax, *T. harzianum,* Neem and Garlic decreased against seed borne fungi of Mungbean seed. Benomyle showed least incidence (9.95 and 10.6%) in genotype HUM-12 and HUM-4 through blotter method, Corbendazim reduced the incidence (9.98%) in genotype HUM-12 through blotter method followed by (10.00%) in genotype HUM-4 through blotter method and some treatment like Vitavax, Neem, Garlic and Ginger reduced the incidence (10.7, 13.3, 15.5 and 18.9%) in genotype HUM-12 through blotter method against *Aspergillus spp.* Among all treatment against *Penicillium spp*, Corbendazim showed least incidence (2.98%) in genotype HUM-12 through Blotter method and Benomyle (3.33%) in genotype HUM-12 through blotter method. *A. alternata* was found to be controlled most effectively when seed is treated with Vitavax and Corbendazim.

It showed (2.98 and 3.00%) occurrences in genotype HUM-12 through blotter paper method. *Fusarium spp* was found to be controlled most effectively when seed is treated with Benomyle, it showed (2.98%) occurrences in genotype HUM-12 through Agar plate method. Benomyle was found to be most effective against *Rhizopus spp* as it reduces the (2.26%) occurrences in genotype HUM-12 through blotter methods. Neem, Garlic and *T. harzianum* were most effective against *A. alternata* as it showed the least incidence (4.30, 5.56 and 7.98%).

DISCUSSION

The obtained results showed that all treatment measures significantly, reduced the occurrences of seed borne fungi. Singh et al. (2002) has made a comparative *in vitro* study with some fungicides like Captan, Dithane M-45, Vitavax, and Bavistin for their efficacy in controlling the occurrence of seed borne disease of mungbean and found that all fungicide significantly reduced the occurrences of seed borne fungi. Vitavax, Thiram and Mancozeb were fungicides that performed better in reducing incidence of almost seedborne fungi (Sisterna and Ronco, 1994; Rahman et al., 2000; Continho et al., 2000; Parisi et al., 2001).

Plant extracts have played a significant role in the inhibition of seedborne pathogens such as *Fusarium oxysporum* and in the improvement of seed quality and emergence of seed embryo (Nwachukwu and Umechuruba, 2001). Reports of De and Chaudhary (1999) are also in confirmation with the present findings, who observed the minimization of wilt disease due to Bavistin, Mancozeb M-45 and Vitavax. Garlic extract was also found effective, in controlling seedborne pathogenic fungi such as *A. tenuis, B. sorokiniana, C. lunata* and *Fusarium spp* in wheat by Hossain et al. (1993). Different concentrations of fungicides against various pathogens Dithane M-45 and Bavistin were reported to be effective in reducing the incidence of seed-borne infection of maize seeds (Kumar and Agarwal, 1998). Raza et al. (1993) reported that seed treatment with Benomyle inhibited the growth and sporulation of *F. moniliforme in vitro,* while it enhanced the germination of seeds and reduced fungal infection effectively. Ouf (1993) also controlled seed–borne; *Aspergillus niger, A flavus, penicillium spp* and *Fusarium spp* when treated with the chemical. Narmada and Kang (1992) reported that laboratory evaluation of seed treatment of rice with Carbendazim controlled seed-rot, and significantly decreased seedling mortality.

Conclusion

The seed-borne disease infection can be effectively reduced if the seeds are treated before sowing as this is necessary for direct disease control. Work on Vitavax-200 has been done to control seed-borne pathogens of mungbean seeds by seed treatment which is in agreement with Jain and Kahare (1972), Shanmugan and Govindaswamy (1973), Rodriguez (1984), Singh and Singh (1986), Mortuza and Bhuiya (1988). Efficacy of Neem was also observed by Bhutta et al. (1999) against five seed borne fungi *viz.*, *A. alternata*, *Aspergillus niger*, *Fusarium solani*, *Macrophomina phaseolina* and *Stemphylium helianthi*. Bhutta et al. (2001) also reported the effectiveness of seed diffusates of neem in controlling several other fungi as *A. alternata* and *Aspergillus niger* and there was a significant increase in seed germination after elimination of fungi. Garlic tablet had better performance in controlling seed-borne infection of *A. tenuis* in Sherpur sample (94.7%) and *C. Lunata*. Zaman et al. (1997) reported that the efficacy of garlic, neem, ginger and onion extracts on seed borne fungi of mustard declined with increase dilution. Rathod (2004) reported that *T. harzianum* showed higher inhibition of mycelium growth of the *A. Alternata.*

Conflict of Interest

The authors have not declared any conflict of interest.

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