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Full Length Research Paper

Evaluation of a different fertilisation in technology of corn for silage, sugar beet and meadow grasses production and their impact on the environment in Poland

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The most significant and irreversible is the overexploitation of the environment, which is constantly exposed to various forms of pollution. Soil pollution is the result of excessive use of fertilizer in a limited area for growing. Total of 30 technologies were analysed, specifying the pollution degree from fertilizers and natural manure. Technologies concerned three crops: corn for silage, sugar beet and meadow grasses. Informations were derived by surveys. During the analysis, it has been noted that some cultivation technologies exceeded the permissible level (170 kg N·ha^{-1}) of nitrogen fertilization. In analysis were noted the nitrogen excess delivered to the fields every year. Probably that was connected with the overproduction in surveyed farms. Overproduction (according to permissible level of nitrogen) averagely reached 98.29 kg·ha^{-1} of delivered nitrogen, giving 6 290 560 m^3 of biogas.

Key words: NPK fertilization, environmental protection, soil fertilization, technologies of agricultural production.

INTRODUCTION

Environmental degradation is a quick and adverse process to the surrounding nature. In case of agricultural land it can lead to disturbances in soil, irreversibly inhibiting the process of soil formation (Code of Good Agricultural Practice, 2004). Deteriorating physical, biological and chemical properties of the soil, effects of limiting production and greatly reduce soil fertility.

The consequences of environmental imbalances may be global, where the only way to preserve the balance is the policy of interdependency, which based on controls and sanctions, that keeps the highest standards (AgriLife, 2009). The European Union (EU) conducts policy, in which farmers must comply with standards and rules to maintain the liquidity of European subsidies. The cross compliance rule is consistent with the environmental standards of Good Agricultural and Environmental Conditions (GAEC) (2014), which are directly relevant to the protection of environment, public health, plant and animal welfare and the maintenance of agricultural land according to the principles of agricultural and environment respect (MRiRW, 2013). These standards are described in the regulation of the signature

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2009/73/EC, established within the EU (Directive 2009/73/EC). Directive regulates the political establishment and obliges all EU members for direct implementation of the provisions.

The rational agricultural land use in the main part lays on the responsibility of all agricultural entrepreneurs. Maintenance of land cultivation in proper condition, leads to the appropriate elemental soil saturation (Stachowicz, 2010), creating the crop yields of high quality (Smagacz et al., 2010). To apply appropriate doses of minerals for selected crops, the optimal mixture of synthetic fertilizers and natural manure should be prepared (Czuba, 2001; Kopiński, 2010; Fotyma et al., 2010; Tujaka, 2010). Fertilizers supply the soil with nitrogen (N), phosphorus and potassium in form of oxides P_2O_5 and K_2O (Konieczna and Roman, 2014). The most common way of selection of the mineral value of soil is the method "on the surface of the field" (Nowak, 2013), which is the procedure to monitor the balance of NPK in the soil. This tool is described in the Regulation of the Minister of Environment, 2002 (Dz.U. z 2003 r. nr 4, poz. 44). According to the regulations of Polish directives, the level of nitrogen supplied to the soil with fertilizers should not exceed 170 kg N/ha (The EU Nitrates Directive, 2010).

The aim of research was to determine the balance of NPK fertilization in technology of corn for silage, sugar beet and meadow grasses production. The following research hypothesis was formulated: Did the physicochemical properties of the soil and controlled growing conditions by optimal selection of technology result in cost reduction of the crop production allowing at the same to rational use of natural resources.

MATERIALS AND METHODS

The NPK balance method was used as described by Code of Good Agricultural Practice (2004), explaining how to determine the needs for individual elements of selected crops. The method relies on the balance between the volume of elements supplied to the soil contained in synthetic fertilizers as well as in the natural manure and the value taken up by the crop during the growing season. Survey data were grouped for each type of crop, for which individual technological cards prepared for each farm. Analysed crops were: corn for silage, sugar beet and meadow grasses. In surveyed farms, plants were cultivated on soil of the average bonitation class (IV-th class of soil), by the conventional crop production systems. The study was conducted on the 30 surveyed farms in Poland, in which the groups of 10 crops of corn for silage, 10 crops of sugar beet and 10 crops of meadow grasses (Table 1) were specified. The balance of the organic substance may be monitored, according to the Formula (1).

$$S = W + P + N_o \cdot W_o + N_g \cdot W_g + N_{gn} \cdot W_{gn} + N_s \cdot W \quad (1)$$

Where: S – balance of organic matter [$t \cdot ha^{-1}$], W – coefficient of soil reproduction or soil degradation of organic matter for crop [$t \cdot ha^{-1}$], P – coefficient of soil reproduction of organic matter for undersown plants, catch crops and mulch [$t \cdot ha^{-1}$], N_o – farmyard manure fertilization [t], W_o – coefficient of soil reproduction of organic matter for farmyard manure [$1 \cdot h^{-1}$], N_g – liquid manure fertilization [t], W_g – coefficient of soil reproduction of organic matter for liquid manure [$1 \cdot h^{-1}$], N_{gn} – slurry fertilization [t], W_{gn} – coefficient of soil reproduction of organic matter for slurry [$1 \cdot h^{-1}$], N_s – straw fertilization [t], W_s – coefficient of soil reproduction of organic matter for straw [$1 \cdot h^{-1}$],

The coefficient of soil reproduction and soil degradation of organic matter was derived from Code of Good Agricultural Practice (2004). To conduct the balance of fertilization needs in the technology it is necessary to use the Formula (2).

$$S_{NPK} = N_{NPK} - Z_{NPK} \quad (2)$$

Where: S_{NPK} – balance of NPK demand for crop [$kg \cdot ha^{-1}$], N_{NPK} – NPK applayed by fertilization [$kg \cdot ha^{-1}$], Z_{NPK} – NPK demanded by crop [$kg \cdot ha^{-1}$],

Discharged value of NPK (Z_{NPK}) that were demanded by the each particular crop has been derived from Code of Good Agricultural Practice (2004). The level of NPK values (N_{NPK}) that were supplied by fertilization were gathered from crop technology survey and presented in the Table 1. Fertilizers were applied by agricultural machines, which were located on the farm as a technical equipment. Natural manure were derived from livestock on the farm.

The analysis required the usage of dedicated computer software, which analytical module based on a mathematical algorithm. Algorithm allows to estimate the balance of NPK nutrients, humus content and level of nitrate nitrogen in the soil. The summary of data that was described in Table 1, shows the balance of NPK based on studies which were conducted on the surface of the field (Jończyk and Stalenga., 2006). The study used the "P.W. 3.3" computer program, which was created in the framework of the Multiannual Programme 2011-2015 titled "Standardization and monitoring of environmental projects, agricultural technology and infrastructure solutions for security and sustainable development of agriculture and rural areas" in Activity 3.3 titled: "Monitoring the effectiveness of the installation and agro energy efficiency of use of raw materials". Designed computer program, requires declare to variables for each crop. Input parameters in the analysis were type of soil, yield value and the level of synthetic fertilizers and natural manure. The analysis of particular crop was based on the phased specification of parameters characterizing the agricultural activities, under which the program calculated the balance of NPK, depending on factors specific to the technology.

Builded for the project realization (Multiannual Programme for 2011-2015, Activity 3.3) computer program and database, were created on the base of the principles of the Code of Good Agricultural Practice (2004). The program allows to perform calculations for the technology of the following crops: corn, beets, grains, legumes, meadow grasses and grain mixtures. The computer program database has also declared information concerning the content of chemical compounds (nitrogen, phosphorus oxide and potassium oxide) in particular synthetic fertilizers and natural manure. The contents of natural manures were presented in Table 2.

The computer program also prepares and declares database of synthetic fertilizers and natural manure, which can be modified according to the specific research needs. The coefficients of nutrients in fertilizers allow for calculation of the NPK balance using the data declared as an integral part of the used technology. The values of each chemical fertilizers are summarized in Table 3. The computer application was used as a tool for monitoring changes in the elemental content of the topsoil, influence of agrotechnical activities, which are contributing to the reduction of groundwater quality. Algorithm is also equipped with a module to estimate the value of nitrate nitrogen in the soil (N_{NO3}) depending on the crop. The value of nitrate nitrogen as an indicator of the potential environmental hazard (IUNG-PIB, 2008) was dependent on several weather stations in Poland, by analyzing the annual rainfall for that area.

Table 1. Characteristics of selected crop technology.

Technology	Yield [t·ha^{-1}]	Area [ha]	Nutrients [kg·ha^{-1}]					
			From natural manure			From synthetic fertilizers		
			N	P_2O_5	K_2O	N	P_2O_5	K_2O
SUGAR BEETS								
S1	62	3.24	199.50	157.50	182.00	122.52	0.00	92.59
S2	72	6	137.70	81.60	153.00	174.00	184.00	180.00
S3	57	2	239.40	189.00	218.40	50.20	196.00	30.00
S4	70	2.5	228.00	180.00	208.00	49.50	137.50	137.50
S5	75	2	228.00	180.00	208.00	121.00	49.50	108.00
S6	80	5	171.00	135.00	156.00	163.00	90.00	135.00
S7	55	2.5	228.00	180.00	208.00	55.76	92.00	120.00
S8	50	4	0.00	0.00	0.00	98.00	60.00	60.00
S9	40	6	216.00	128.00	240.00	81.00	60.00	60.00
S10	75	6.4	171.00	135.00	156.00	30.00	60.00	60.00
Corn for silage								
C1	80	13	156.68	61.85	193.78	197.16	92.25	180.38
C2	50	13	189.00	112.00	210.00	181.00	60.00	160.00
C3	80	4.5	84.00	46.00	74.00	148.40	96.00	144.00
C4	60	2	257.10	136.20	261.00	131.40	57.50	67.50
C5	50	2	0.00	0.00	0.00	138.00	60.00	114.00
C6	70	3.5	227.00	136.40	210.00	75.57	0.00	0.00
C7	50	2	216.00	128.00	240.00	115.00	28.00	25.20
C8	72	3.4	311.12	173.09	352.85	173.00	24.00	24.00
C9	65	3.25	122.98	62.35	143.34	114.65	0.00	0.00
C10	55	1	162.00	96.00	180.00	66.90	50.00	60.00
Meadow grasses								
M1	45	14.44	92.11	36.36	113.92	223.21	92.06	120.08
M2	50	3.25	77.54	42.46	68.31	46.03	0.00	0.00
M3	42	19	4.42	2.42	3.89	48.00	96.00	96.00
M4	30	15	126.00	74.67	140.00	84.36	30.00	40.00
M5	29	4	142.50	112.50	130.00	216.00	40.00	60.00
M6	60	7.9	146.20	80.06	128.80	123.04	0.00	0.00
M7	59	2.1	114.00	90.00	104.00	56.70	0.00	0.00
M8	35	10	39.90	31.50	36.40	129.20	0.00	0.00
M9	19	5	120.00	3.00	180.00	255.00	0.00	0.00
M10	53	8.24	77.03	29.99	94.25	96.85	0.00	0.00

S - Sugar beet, C - corn for silage, M - meadow grasses, Source: Own study.

Table 2. Contents of N,P,K in selected natural manure.

Fertilizer	Nitrogen (N) [%]	Phosphorus oxide (P_2O_5) [%]	Potassium oxide (K_2O) [%]
Farmyard manure	0.57	0.45	0.52
Liquid manure	0.42	0.23	0.37
Slurry	0.40	0.01	0.60
Straw	0.60	0.24	1.24

Source: Own study based on Grabowski 2009.

Table 3. Contents of N, P, K in selected synthetic fertilizers.

Name of fertilizer	Nitrogen (N) [%]	Phosphorus oxide (P_2O_5) [%]	Potassium oxide (K_2O) [%]
Can 27%N	27	0	0
Ammonium phosphate POLIDAP	18	46	0
Urea 46%	46	0	0
Polidap	18	46	0
Polifoska 4	4	12	32
Polifoska 6	6	20	30
Polifoska 8	8	24	24
Polifoska PK 20	0	20	18
Ammonium sulphate	27	0	0
Saletrzak	27	0	0
Potassium salt	0	0	60
Triple superphosphate	0	0	60
Dolomitic lime	0	46	0
Wuxal K (dolistny)	9	25	25

Source: Own study based on information published in manufacturer's website.

Table 4. Synthetic fertilizers and natural manure applied in the technology of sugar beet (S), corn for silage (C) and meadow grasses (M).

Technology	Natural manure			Synthetic fertilizers												
	Farm yard manure	Liquid manure	Slurry	Can 27%N	Ammonium phosphate POLIDAP	Urea 46%	Polifoska 4	Polifoska 6	Polifoska 8	Polifoska PK 20	Ammonium sulphate	Saletrzak	Potassium salt	Triple superph osphate	Dolomitic lime	Wuxal K (dolistny)
C1	N	U	N	N	U	U	N	N	N	N	N	N	U	N	N	U
C2	U	N	N	N	N	U	U	N	N	N	N	N	N	N	N	N
C3	N	U	N	N	N	U	N	U	N	N	N	N	N	N	N	N
C4	U	U	N	N	N	U	N	U	N	N	U	N	N	N	N	N
C5	N	N	N	N	N	U	N	N	N	U	N	N	U	N	N	N
C6	U	N	U	N	N	U	N	N	N	N	N	N	N	N	N	N
C7	U	N	N	N	N	U	N	N	N	U	N	N	N	N	N	N
C8	U	U	N	N	N	N	N	N	U	N	U	N	N	N	N	N
C9	U	U	N	N	N	U	N	N	N	N	N	N	N	N	N	N
C10	U	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N
S1	U	N	N	N	N	U	N	N	N	N	U	N	U	N	N	N
S2	U	N	N	N	U	N	N	N	N	N	U	N	U	N	U	N
S3	U	N	N	N	U	U	N	N	N	N	N	U	U	N	N	N

Table 4. Contd.

S4	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
S5	U	N	N	N	N	N	N	N	U	N	U	N	N	N	N	N
S6	U	N	N	N	N	N	N	U	N	N	U	N	N	N	N	N
S7	U	N	N	N	N	U	N	N	N	N	N	N	U	U	N	N
S8	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N
S9	U	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N
S10	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
M1	N	U	N	N	U	N	N	N	N	N	U	N	U	N	N	N
M2	N	U	N	N	N	N	N	N	N	N	U	N	N	N	N	N
M3	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N
M4	N	U	N	N	N	N	N	N	N	U	U	N	N	N	N	N
M5	N	U	N	N	N	N	N	U	N	N	U	N	N	N	N	N
M6	N	U	N	U	N	N	N	N	N	N	N	N	N	N	N	N
M7	U	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N
M8	U	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N
M9	N	N	U	N	N	N	N	N	N	N	U	N	N	N	N	N
M10	N	U	N	N	N	N	N	N	N	N	U	U	N	N	N	N

U – used, N – not used. Source: Own study.

All analyzed technologies have individual level of fertilization depending of quality and quantity. Calculations include the value of delivered nutrients from synthetic fertilizers, natural manure and aftercrops during the cultivation.The volume of NPK by which the technology has impoverished the soil, was determined in content of elements in the crop yields. Summary of applied synthetic fertilizers and natural manure in analyzed technology of sugar beet, corn for silage and meadow grasses were presented in Table 4.

RESULTS AND DISCUSSION

Tables 5 to 7 summarizes the results of NPK balance calculation from delivered and received nutrition for each crop. The calculations are characterized by maintaining the balance of nutrients in the soil during the cultivation of individual plants. The EU Nitrates Directive (2010) specifies the maximum value of nitrogen that can be delivered to the soil from the natural manure per year. The diagram (Figure 1) shows that the most of technologies were close to the upper level of allowed nitrogen fertilization value. The average content of nitrogen that was applied with natural manure amounted to 149.5 kg•ha^{-1}, and the average nitrogen value applied with the synthetic fertilizers amounted to 118.8 kg•ha^{-1}. Presented diagrams characterize the content of nitrogen, phosphorus oxide and potassium oxide in the soil for individual cultivation technologies (Figures 1 to 3). The positive and negative value of balance, were derived from calculation of NPK demand of crop (Formula 2).

The average content of phosphorus oxide that was applied with natural manure amounted to 94.0 kg•ha^{-1}, and for synthetic fertilizers amounted 55.2 kg•ha^{-1}. With respect to the potassium oxide values were respectively: for natural manure 153.1 kg•ha^{-1}, and for synthetic fertilizers 69.1 kg•ha^{-1}.

Conclusions

Analysis of the mineral substances balance allowed to state that several owners of surveyed farms exceeded the fertilization rate recommended by Code of Good Agricultural Practice (2010). Presented diagrams, show NPK balance in an extreme point with the value 408 kg·ha^{-1} (technology C8) and negative -492 kg·ha^{-1} (technology M8). Also the maximum volue 170 kg N·ha^{-1}, recomended by the Nitrote Directive was exceded in 46.6% of forms in the case of natural

Table 5. Balance of NPK in technology of corn for silage (C).

Symbol of technology	Main crop yield [t·ha^{-1}]	N [kg·ha^{-1}]	P_2O_5 [kg·ha^{-1}]	K_2O [kg·ha^{-1}]	NPK [kg·ha^{-1}]
C1	80	58	42	6	106
C2	50	53	115	-14	154
C3	80	-64	30	-150	-184
C4	60	174	163	30	368
C5	50	-47	-10	-116	-173
C6	70	-32	38	-112	-106
C7	50	158	138	3	299
C8	72	244	170	-6	408
C9	65	174	131	-27	278
C10	55	26	69	-13	82

Source: Own study.

Table 6. Balance of NPK in technology of sugar beet (S).

Symbol of technology	Main crop [t·ha^{-1}]	N [kg·ha^{-1}]	P_2O_5 [kg·ha^{-1}]	K_2O [kg·ha^{-1}]	NPK [kg·ha^{-1}]
S1	62	74	59	-128	5
S2	72	24	151	-135	40
S3	57	62	294	-123	233
S4	70	-3	206	-110	94
S5	75	49	110	-172	-13
S6	80	14	97	-229	-118
S7	55	64	184	-30	219
S8	50	-102	-20	-265	-387
S9	40	149	176	8	333
S10	75	-99	75	-293	-317

Source: Own study.

Table 7. Balance of NPK in technology of meadow grasses (M).

Symbol of technology	Main crop [t·ha^{-1}]	N [kg·ha^{-1}]	P_2O_5 [kg·ha^{-1}]	K_2O [kg·ha^{-1}]	NPK [kg·ha^{-1}]
M1	45	84	97	-56	124
M2	50	-130	-28	-227	-384
M3	42	-161	38	-145	-268
M4	30	57	63	3	123
M5	29	172	57	-20	209
M6	60	-37	-4	-225	-266
M7	59	-129	8	-243	-364
M8	35	-131	-51	-310	-492
M9	19	278	-24	67	321
M10	53	-90	-28	-239	-357

Source: Own study.

manure, and in 83.3% of forms in the case of total fertilisation. High volues of nitrogen may have negative impact on the environement. These excessive volues appeared on 90% of forms producing sugar beet (S)

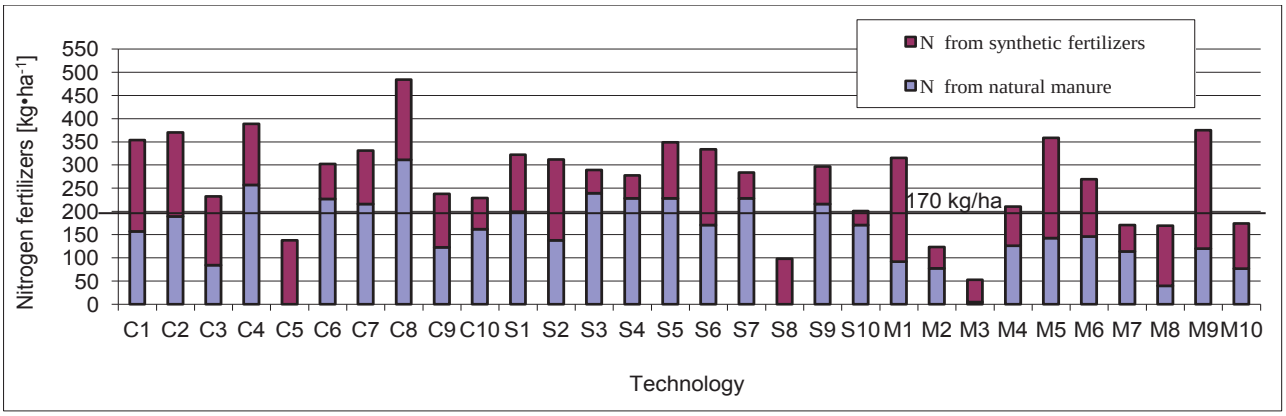

Figure 1. The level of nitrogen fertilization in technologies of corn for silage, sugar beet and meadow grasses. S - Sugar beet, C - corn for silage, M - meadow grasses.

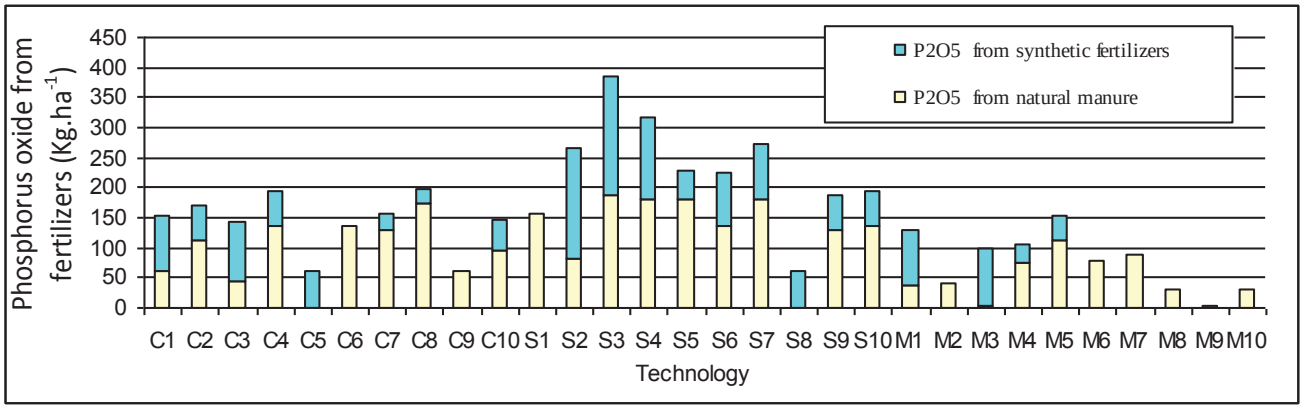

Figure 2. The level of phosphorus oxide fertilization in technologies of corn for silage, sugar beet and meadow grasses. S - Sugar beet, C - corn for silage, M - meadow grasses.

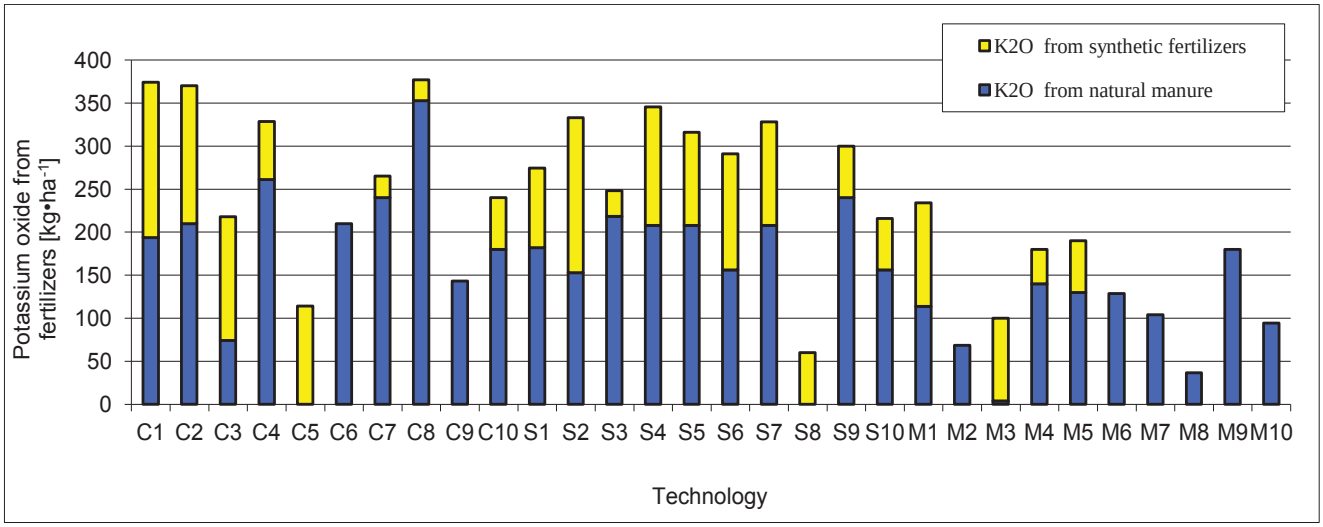

Figure 3. The level of potassium oxide fertilization in technologies of corn for silage, sugar beet and meadow grasses. *S* - Sugar beet, C - corn for silage, M - meadow grasses.

and corn for silage (C) and on 70% of forms cultivating meedow grasses (M). The average volue of this excess amounted to 98.29 kg·ha^{-1} over the recommended 170 kg N·ha^{-1}.

In the case of natural fertilization excess may be caused by animal waste overproduction in the survey farms overproduction of natural wastes could be better utilized for biogas production. If the natural manure content 0.5% (Table 2) of nitrogen. According to average excess of nitrogen is 98.29 kg·ha^{-1}, it can be calculated that 19 658 kg·ha^{-1} of natural manure is wasting every year on the field. Natural manure consists 60-80% of dry organic matter, that gives the capability of 300 to 700 m^3·t^{-1} of biogas production (Fugol and Szlachta., 2010). Overproduction of wasted natural manure could generate 6 290 560 m^3 of biogas, that gives 3 774 336 m^3 of pure methane (CH_4) every year.

Disclosure of Conflict of Interest

The authors have not declared any conflict of interest.

Conflict of Interest

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

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Review

Enhancing the growth and yield of pigeon pea through growth promoters and organic mulching- A review

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Pigeon pea, a widely spaced row crop having initial slow growth is sensitive to weed competition during early stages of its growth period. A large proportion of uncovered land during early stages is taken over by rank weed growth, which may cause drastic reduction in growth and yield of pigeon pea. Pigeon pea with additional canopy may suppress weeds, due to shade. To accomplish the same, foliar application of micro nutrient mixture and growth promoters as well as mulching with black gram crop resides were made to increase the growth and spread of pigeon pea and to find its effect on growth, yield and weed suppressing ability.

Key words: Pigeon pea, mulching micro nutrient.

INTRODUCTION

Organic mulches on crop growth

Wang and Li (1987) stated that dry matter production was increased in rapeseed due to paddy straw mulching. Application of coir pith either raw or composted as mulch gave significant increase in growth characters of maize Co 1 and finger millet Co 13 (Singaram and Pothiraj, 1991). Kulkarni et al. (1998) reported that dry matter production with paddy straw much was higher by 13% than the from control plot in maize. Stover mulch significantly enhanced vegetative growth of onion crop (Adetunji, 1999). Pramanik (1999) reported that the plant height and crop growth rate were improved considerably and significantly under paddy straw mulch as compared to saw dust coir dust, rise husk and no mulch in maize. Samui and Ambhore (2000) reported that in polythene mulched groundnut crop the shoot dry mass was significantly higher in mulched plots at 30 and 60 days after sowing. Root dry mass was also significantly higher in mulched plot than in non mulched plots at 60 days after sowing. Nagarajan and Wahab (2001) observed that paddy straw mulching in finger millet crop significantly influenced the growth components *viz.,* plant height, number of tillers hill^{-1}, leaf area index (LAI) and dry matter production. Sunil et al. (2008) reported that mulching produced significantly the highest plant height and nodules as compared to wheat straw and rice straw mulching.

Organic mulches on yield and yield attributes

Agarwal and Rajat (1977) had shown that straw application increased the production in barely. Mayalagu and Mahimairaja (1990) reported that coir pith application as mulch at 10 tha^{-1} increased the pod yield of groundnut.

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Gurcharan et al. (1994) opined that number of seeds pod^{-1} was significantly affected by mulching with daincha. The uses of mulches have been found to control weeds and increase the yield of different vegetable crops (Srivastava et al., 1994).

The data indicated that maximum seeds pod^{-1} (5.83) was recorded in hand weeding and in mulched field. However, it was statistically similar with newspaper and sawdust mulching respectively. Application of coir pith caused boosting of both pods and haulm yield of groundnut compared to press mud, Farmyard Manure and control plots (Mayalagu, 1997). Paddy straw mulches were helping in improving the soil physical aeration which ultimately resulted into better growth and yield of plant (Rao and Pathak, 1998). Kulkarni et al. (1998) reported that the mean cob yield and stover yield were significantly higher under paddy straw mulch than saw dust, coir dust, rice husk and control. Jayachandran et al. (2004) reported that sugarcane thrash mulching at 5 ha^{-1}, gave highest benefit cost ratio of 2.90 and weed control efficiency of 71.8% and produced higher number of tillers (3,69,200) ha^{-1} which enhanced the cane and sugar yield. James et al. (2006) reported that maximum number of seeds pod^{-1} were recorded under news paper mulch. Wheat straw 1 kg m^{-2} with FYM resulted in high pod yield per plot (4.55 kg), number of seeds per pod (5.43), pod length (7.98 cm) number of pods per plant (8.67), number of branches (3.00) and plant height (57.80 cm) in vegetable pea as reported by Singh et al. (2007).

Behara et al. (2007) reported that maize stover mulch at 5 t/ha gave statistically higher grain yield of piegonpea as compared to control. Sunil et al. (2008) reported that dust mulching significantly increased the crop dry weight, number of pods plant^{-1}, numbers of grains pod^{-1} and test weight in green gram. Idnani and Gautam (2008) reported that the maximum pod length and grain weight plant^{-1} were observed under dust mulching in summer green gram. Tamana et al. (2009) reported that number of pods plant^{-1} were also significantly higher in hand weeding and newspaper mulching, whereas, it was minimum in weedy check. These results showed that mulches like newspaper, hand weeding and polyethylene (black) controlled the weeds significantly as compared to weedy check and rest of the mulches. Raghupathi et al. (2009) reported that application of paddy straw at 5 tonnes ha^{-1} as mulch increased the head diameter and number of seeds per head in sunflower.

Organic mulches on nutrient uptake by crops

Coir pith application at 10 t.ha^{-1} with N and P fertilizers aided in building the soil N (Gothandaraman, 1985). Nagarajan et al. (1986) reported that application of coirpith inoculated with *Pleurotus* sp. in combination with N or P and K or N, P and K increased the nutrient uptake in groundnut. Nagarajan et al. (1986) observed that the continuous use of coirpith increased available P and K status on a sandy loam soil. Increased nutrient uptake was observed by Saucer et al. (1996) in corn due to mulching. Duraairaj (1996) found increased uptake of NPK in mulched plots when compared to unmulched plots in cotton. Paddy straw mulch had increased the available N, P, K and organic carbon content (Solaiappan, 1998) under adequate soil moisture in cotton. Virdia and Patel (2000) reported that paddy straw mulching increased the nutrient uptake in cotton.

Jat and Gautam (2000) reported that wheat straw mulching increased the nutrient uptake in pear millet. Singh et al. (2002) reported that total nutrient uptake of nitrogen, phosphorus and potassium was significantly higher under stover mulch than soil mulch in maize. Idnani and Gautam (2008) reported that higher quantity of nutrients were removed by weeds under no mulch treatment in green gram.

Growth promoters in red gram

Triacontanol on growth and yield

Triacontanol a long chain alcohol has been known as potent growth promoter in many plant species (Kumaraelu et al., 2002). It is a new group of photo hormones with significant growth promoting activity essential for many processes in plant growth and development (Rao et al., 2002). The growth regulator Triocontonal at 0.5 ppm has been reported to stimulate water uptake and growth in rice seedlings (Ries and Wert, 1987). Foliar application of Triacontanol has been reported to increase yield of rice (Knight and Mitchell et al., 1987).Kelalya et al. (1991) reported that application of Triacontanol at 200 ml ha^{-1} on 25 and 30 DAS improved the test weight and yield in groundnut.

Venkata et al. (2009) reported that Triacontanol at 0.2% increased harvest index in green gram. Varma et al. (2009) reported that foliar application of Triacontanol at two percent increased the number of branches plant^{-1} and harvest index in black gram.

Brassinolide on growth and yield

Brassinolide is the first steroidal hormone reported in plants, with significant growth promoting activity. In addition to growth promotion, it also plays important role in other development process like seed germination, flowering, abscission and maturation (Grove et al., 1979). Foliar application of 0.01 or 0.05 ppm Brassinolide as two or three sprays increased the photosynthesis and leaf chlorophyll content in tobacco (Han et al., 1988). Manibangsa et al. (1999) reported an increase in chlorophyll A with 0.05 ppm Brassinolide foliar spray in

rice. Mazorra et al. (2002) observed that the effect of Brassinolide an antioxidant, regulate enzyme activity there by imparting drought tolerance in tomato. Sivakumar et al. (2002) reported that Brassinolide at 0.25 ppm increased the sugar and starch amount in mature seeds in pear millet. Brassinolide was also reported to promote root growth in soybean (Sathyiyamoorthy and Nakamura, 1990) and in maize (Mussig et al., 2003). Brassinolide have the ability to confer resistance to plants against various abiotic stresses (Vardhini et al., 2006). Madhavi et al (2007) reported increased chlorophyll content in groundnut by Brassinolide application.

Micro nutrients on growth

Malewar et al. (1982) observed increased number of nodules and nodule dry weight due to application of zinc 15 kg ha^{-1} by enhancing the nitrogen fixation in black gram. Kalyani et al. (1993) reported that molybdenum, iron and boron increased the hormone synthesis and translocation, carbohydrate metabolism and DNA synthesis and improved the additional growth and yield in pigeon pea.

Gupta and Vyas (1994) observed that dry weight of soybean plant was increased due to application of zinc, iron and molybdenum. Iron, zinc and molybdenum are the metallic compounds of one or more enzymes which are involved in various physiological functions and there by increased the leaf area index, crop growth rate and relative growth rate leading to the development and productivity of plant. Increase in flower numbers, improved pod set and reduction in days to flowering are influenced by application of micronutrient *viz.,* zinc, molybdenum and boron in chickpea and pigeon pea were reported by Prasad et al. (1998).Nutrients limitations to legume production resulting from deficiency of micronutrients such as molybdenum, zinc, boron and iron are reported in crops like chickpea and pigeon pea (Bhuiyan et al., 1999). Jadhav et al. (2008) reported that spraying of Zinc as $ZnSo_4$ showed increased seed weight of soybean. Application of boron at 22 kg ha^{-1} at the time of sowing of soybean crop increased seed yield as reported Jadhav et al. (2008).

Micro nutrients on yield

Tomar et al. (1991) reported increased soybean yield by application of $MnSo_4$ at 2.5 kg ha^{-1} supplemented with 25 kg ha^{-1} zinc over control. Bhanavase et al. (1994) reported that Zn application at 25kg ha^{-1} had increased the seed quantity of soybean. Micronutrients in soybean is useful to improve productivity and seed quality parameters. Among the micro nutrients Mn, Zn, B, and Mb are important for increasing the productivity of soybean crop (Devarajan and Palaniappan, 1995).

Salicylic acid on growth

Salicylic acid acts as a potential non enzymatic antioxidant as well as a plant growth regulator, which plays an important role in regulating a number of plant physiological process including photosynthesis (Fariduddin et al., 2003; Singh and Usha, 2003; Waseem et al., 2006). Salicylic acid is also known to stimulate flowering in a range of plants and increased the flower life period and controls uptake of iron by roots and stomatal conductivity (Bhupinder and Usha, 2003). Boologasundar (2000) reported that foliar application of salicylic acid at 40 ppm at 30 and 45 DAS recorded increased growth and yield attributes in groundnut. Sugumar (2000) note d significant improvement in growth attributing characters with 100 ppm of salicylic acid spray in sesame. Radhamani et al. (2003) reported higher dry matter accumulation with salicylic acid spray at the rate of 100 ppm in green gram. Salicylic acid spray was also found to significantly increase the K content in leaf of green gram (Sujatha, 2001). Kalarani et al. (2002) stated that spraying salicylic acid at 100 ppm in tomato showed its distinct role in increasing chlorophyll content and nitrate reductase activity. The effect of salicylic acid was also observed in induction of flower, fruit set and yield in terms of fruit weight.

Salicylic acid on yield

Singh and Sharma (1989) reported that foliar application of 10 ppm salicylic acid to groundnut twice at 40 and 50 DAS increased the number of gynophores, pods $plant^{-1}$, dry pod yield and 100 seed weight. Foliar spray of 400 ppm aspirin (acetyl salicylic acid) recorded the highest dry pod yield of groundnut (Patel, 1993). Rathore (1995) revealed that application of salicylic acid at 40 ppm as foliar spray registered highest pod yield (47q ha^{-1}), 100 grain weight (68.7 g), oil (48.8%) and protein content (25.2%). Sugumar (2000) noted significant improvement in yield attributing characters with 100 ppm of salicylic acid spray in sesame. Negi and Prasad (2001) opined that the lower concentration of salicylic acid increased the soluble protein, free amino acid and free tissue ammonia contents and finally increased the yield of soybean. Thangaraj (2003) reported that foliage application of salicylic acid at 100 ppm increased the seed yield of sesame. Salicylic acid spray at 50 ppm recorded maximum grain yield followed by salicylic acid at 100 ppm and 200 ppm as reported by Jayavasuki et al. (2004) in rice.

Naphthalene acetic acid (NAA) on growth

Dani (1979) reported that foliar application of NAA at 20 ppm increased the grain yield and number of flowers and

inflorescence in pigeon pea.Nawalagatti et al. (1988) reported that planofix (NAA) at 10 to 20 ppm increased the leaf area index, dry matter production and crop growt rate in groundnut. Shinde and Jadhav (1995) reported that foliar application of NAA at 50 ppm increased the harvest index by seven per cent and dry matter production in red gram. Mahala et al. (1999) reported that NAA at 30 ppm increased the branches and number of leaf in black gram.

Prakash et al. (2003) stated that in black gram NAA at 30 ppm increased the branches and number of leaf. NAA at 40 ppm spray recorded in highest plant height 14.9 and 39.3 cm at vegetative and flowering stage as reported by Kumar et al. (2004) in green gram. Kadam et al. (2008) reported that NAA at 30 ppm concentrate was found to be more effective increasing the number of branches, total dry weight, number of pods per plant, 1000 grain weight and grain yield and chlorophyll content in black gram.

Naphthalene acetic acid (NAA) on yield

Gupta and Singh (1982) revealed that foliar application of NAA at 40 ppm to groundnut increased the shelling percentage, 100 seed weight and protein content. Kalita (1989) found significant increase in the number of pods in green gram by foliar application of NAA at 20 ppm. Application of NAA at 50 ppm significantly increased the cluster number in green gram (Kalita, 1989). Kalarani (1991) concluded that foliar spraying of 50 ppm significantly influenced the total N content in soybean. Application of one per cent urea with NAA at 40 ppm significantly increased the yield by 268 kg ha^{-1} in chillies (Katwala and Saraf, 1990).

Ghosh et al. (1991) showed that application of NAA at pre flowering stage significantly increased shelling percentage in groundnut. Foliar application at 40 ppm significantly increased the 100 seed weight in green gram (Ghosh et al., 1991). NAA was also found to increase the harvest index in pear millet (Rangacharya and Bawankar, 1991). Foliar application of 50 ppm NAA increased the amino nitrogen concentration in black gram. Singh et al. (1995) reported that application of NAA increased the umbel length and more umbel number in onion.

Kumar et al. (1996) found that the treatments NAA at 50 ppm reduced the number of days required to start the head formation in cabbage. Singh and Awasthi (1998) reported that protein content was increased by foliar spray of NAA at 40 ppm in green gram. According to Sujatha (2001) foliar application of NAA at 40 ppm significantly increased the number of seeds per pod in green gram. Foliar spray of NAA at 30 ppm at flowering increased the average pod weight, seed pod ratio and number of flowers in green gram as reported by (Sujatha, 2001). Radhamani et al. (2003) observed that increase in test weight was due to NAA at 10 ppm in green gram. Kumar and Kumar (2004) reported increased number of seeds per pod in the treatment given with NAA at 10 ppm in green gram. Foliar spray of NAA at 30 ppm concentrate was found to be more effective in increasing the number of branches, total dry weight, number of pods per plant, 1000 grain weight and grain yield, and chlorophyll content as reported by Ramanathan et al. (2004) in black gram. Foliar spray of NAA at 30 ppm was found to be more effective in increasing the number of branches, total dry weight, number of pods per plant, 1000 grain weight and grain yield, and chlorophyll content in black as reported by Sharma et al. (1999) in green gram. Karim et al. (2006) obtained that higher protein content (23.99%) in chickpea with 100 ppm of NAA. Naphthalene Acetic Acid is the organic substance which promotes the growth of plant and leads to more productivity, Varma et al. (2009) reported that NAA application increased seed yield in cowpea.

Weed spectrum in red gram

Tewari et al. (1983) reported that the weed flora of *Echinochloa colonum, Digitaria sanguinalis, Dactyloctenium aegyptium* and *Panicum* spp. among grasses, *Ageratum conyzoides, Commelina bengalensis, Euphorbia geniculata* and *Oxalis latifolia* among broad leaved weeds, *Cyperus rotundus* among sedges was found in weed free upto six weeks after sowing in Kanpur. In field trial conduced by Kolar et al. (1985) at Jabalpur in Madhya Pradesh on rain fed red gram, *Cynodon dactylon, Digitaria setigera, Dactyloctenium aegyptium, Eleusine indica, Cyperus rotundus, Cyperus iria, Commelina bengalensis* and *Sida acuta* were found to be the dominant weeds.

Ali (1991) reported that the weed flora composition in the experimental fields of sandy loam soils at Faizabad consisted of *Cyperus rotundus, Launea asplenifolia, Chenopodium album, Anagallis arvensis, Phyllanthus niruri, Melilotus indica, Ganaphalium pulvinatum, Polygonum plebejum, Trianthema monogyna* and *Euphorbia dracunculoids*. The weed flora of the experimental field at initial stage of crop growth was dominated by grassy weeds and broad leaved weeds. Bondarwad (1991) observed that *E. colonum, Echinochloa crusgalli, C. benghalensis, Digera arvensis, Trianthema portulacastrum* and *Phyllanthus niruri* were the dominant weeds in red gram field at Parbhani.

Upadhyay (2002) observed that most dominant weed flora in red gram grown under clay loam soil condition of Madhya Pradesh were *Cynodon dactylon* (21%) *E. colonum* (13%), *E. crusgalli* (12%), *Cyperus rotundus* (15%), *Agropyron repens* (11%), *Parthenium hysterophorus* (9%), *Commelina bengalensis* (2%), *Digitaria sanguinalis* (8%), *Eclipta alba* (5%) and

Euporbia hirta (4%).Common weed flora of the experimental plot in Dharwas as reported by Channappagoudar and Biradar (2007) were *Commelina benghalensis* (37.4%) *Parthenium* hysterophorus (22.5%), *Dinebra retroflexa* (14.0%) and *Oldenlandia* sp (12.98 %). The other weeds which were of minor importance were *Cyperus rotundus*, *Bracharia eruciformis*, *Hibiscus pondureformis* and *Ocimum cannum.*

Crop-weed competition

Red gram grows very slowly during early stage of its crop growth and hence, it is highly susceptible to weed competition during this period. The degree of crop-weed competition is determined by the weed species and their density, duration of infestation, associated crops in the field, growth habit of crop plants and environmental conditions. Weeds that grow with crops particularly during this period deplete considerable amount of costly fertilizer nutrients, limited moisture, light and space thereby resulting in poor growth and development and lower yield of crops reported by Channappagoudar and Biradar (2007) in red gram.

Critical period of crop-weed competition

Kasasian (1971) observed that dwarf strains of pigeon pea were more susceptible to weeds than the taller types. In Trinidad, the critical period of crop-weed competition was the first 5 to 7 weeks in tall pigeon peas. Pigeon pea has slow initial growth rate and is very sensitive to weed competition in the first 45 to 60 days of growth (Saxena and Yadav, 1975). Only when the plants have reached a height of about 30 cm, they can effectively compete with the weeds. Therefore, effective weed control at the early growth stages of the crop is one of the most important factors contributing to high yields. In many rainfed pigeon pea growing areas, optimum land preparation is seldom done, which results in heavy manifestation of weeds leading to severe yield losses, even up to 90% (Shetty and Rao, 1977).

Shetty and Rao (1977) stated that pigeon pea occupied only about 50% of the area due to the slow growing and poor competitive ability of the crop, thus allowing more weed growth. They stated that total weed growth was less in intercropping, and the weeding operations can be extended in order to obtain optimum yields of both the crops. Talnikar et al. (2008) reported that piegonpea gets heavily infested with weeds due to slow early growth of crop. The critical period is during the first eight weeks after sowing.

Organic mulches on weeds

Mulching is a good weed control method used in agriculture throughout the world (Gupta, 1991). Weeds remain one of the most significant agronomic problems especially in organic farming. As chemical methods of weed control is not advocated there is a strong interest in developing alternative methods of weed control in organic agriculture (Economou et al., 2002). Mulching reduced the weed population in wheat was reported by Radwan and Hussein (2001). Abmed et al. (2007) wheat straw mulch spreading had significant effect on weed suppression in wheat. Varma et al. (2009) reported that all organic mulch increased the weed control efficiency (90.94%) over no mulching in green gram.

Mulches on suppression of weed growth

Weed population of grasses, sedges and broad leaved weeds were very much reduced due to mulching with coir pith in groundnut (Rangaraj, 1991). In groundnut crop, coir pith mulching significantly decreased weed population and dry matter accumulation (Mayalagu, 1997). Ramesh (2003) reported that coir pith mulching significantly reduced weed intensity in cotton crop. The positive effect of mulches is particularly obvious in the period of intensive emergence of weeds in wheat (Jodaugiene et al., 2006). Bakhtl et al. (2009) reported from his field experiment that the data depicted a maximum weed density of 40.33 m^{-2} in the weedy check, while the minimum weed density was recorded with mulching by newspaper in pea.

Crop weeds competition for nutrient uptake, moisture and light

Okumara et al. (1986) opined that under weed condition, about 80% of the available nitrogen was utilized by weeds until the crop was approximately 50 cm height. Elakkad et al. (1992) observed that weeds reduced plant height of maize crop during early growth stage. Iwata et al. (1990) observed competition for light and nitrogen by weeds as a major factor for reduced yield of maize. Elakkad (1992) observed that natural weed infestation significantly reduced PAR available for the lower leaves and thereby reducing the yield of maize and soybean. Weed management in pigeon pea aims at manipulating the competitive equilibrium in favour of the crop and to keep undesired weed growth at manageable levels, rather than to totally eradicate weeds (Bond and Grundy, 2000).

Effect of canopy spread on weeds

Blessdal (1960) reported that increasing the density of crop canopy density through manipulation of seed rate and row spacing effectively reduced the weed growth in chick pea. Donald (1963) reported that light is another

environmental resources, which influences the weed growth canopy development. Better canopy development reduces light supply to the weeds and gives a better competition to the chickpea. Fisk et al. (2001) reported that cover crop reduced weed management cost by suppressing the weed emergence, growth and prevented weed seed production in annual legume.

A preliminary study by Brennan and Smith (2003) revealed that cereal and mustard cover crops were more suppressive of weed growth and weed seed production. Ghadiri and Bayat (2004) reported that decreasing plant spacing provided higher plant canopy within the rows, which reduced weed dry weight and biomass in pinto beans (*Phaseolus vulgaris* L.). Singh et al. (2004) reported that lateral canopy spread decreased the weed dry weight and weed bio mass in chick pea (*Cicer arietinum*). Olabode et al. (2007) opined that shaded leaves lower in the canopy had access to low levels of photo synthetically active radiation and a low-red to far-red photon ratio. Light also influenced flowering and fruit set. Dhiman (2007) reported that canopy spread reduced weed population and dry matter production, which had an impact in increasing grain yield in chick pea.

CONCLUSION

Organic mulching is one of the best methods for the weed control. It reduced the chemical input cost and avoids the health hazards in environmental condition. It also improve the cultivation practice with help of organic farming.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Characterization of bacterial strains and their impact on plant growth promotion and yield of wheat and microbial populations of soil

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The aim of this research work is to evaluate the potentiality of bacterial strains isolated from rhizosphere of various cropping systems on growth, yield and soil microbial populations at harvest of wheat under pot and field experiments. Bacterial isolates, isolated from rhizosphere of various cropping systems. Morphological, biochemical and molecular characterization of bacterial strains were done. All the bacterial strains were rod shaped and ammonia producers. Most of the strains were Gram's +ve and showed positive to catalase, VP, MR tests and HCN production. *Pseudomonas aeuroginosa* showed significantly lowest pH 4.42 of broth and solubilized 160.34 µgml^{-1} tri-calcium phosphates. *Enterobacter* sp. produced maximum 27.06 µgml^{-1} IAA at 100 µgml^{-1} tryptophan. All the bacterial strains showed >97% similarity with strains already submitted to NCBI Gene bank. *Arthrobacter chlorophenolicus* and *Enterobacter* sp. showed maximum and significantly 23.60 and 28.97% plant height in pot and field experiments at 90 days after sowing. *Bacillus megaterium* caused maximum and significantly 51.45 and 40.47% grain and straw yield, respectively under pot while *Serratia marcescens* showed 14.78 and 17.04% grain and straw yield in field as compared to control. Most of the strains showed significant effect on microbial populations of post harvest soil under pot and field. It was concluded that *A. chlorophenolicus*, *Enterobacter* sp., *B. megaterium* and *S. marcescens* are effective strains of plant growth promoting bacterial strains for wheat crop production under Indo- Gangetic plains of India.

Key words: Bacterial strains, biochemical, growth, microbial population, wheat, yield.

INTRODUCTION

During green revolution, better yielding varieties were introduced to feed the increasing population of world. Enhancing the crop production with the help of synthetic fertilizers and pesticides were used without caring environmental problems and soil health (Elkoca et al., 2010). Consequently, it is great challenge to search for sustainable strategies to alleviate detrimental effects ofintensive farming practices. Soil beneath our foot constitute rich source of different kind of microbes which were affected by both biotic and abiotic factors. The

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plethora of these microbes exists in rhizosphere zone and their size and activities are regulated by physico-chemical status of soil and promotion of plant growth by improving the soil health (Aira et al., 2007; Jolly et al., 2010). Plant growth promoting rhizobacteria (PGPR) are soil-borne bacteria that have ability to aggressively colonize the rhizosphere or plant roots or both when applied to seed or crops that enhance the growth and yield of plants (Ashrafuzzaman et al., 2009; Kaymak, 2011).

In India, soils are generally low in available phosphorus due to chemical sorption that renders the application of readily soluble P-fertilizers highly inefficient (Hegde et al., 1999). PGPR comprise different functional and taxonomic groups of bacteria like *Pseudomonas, Bacillus, Rhizobium, Azospirillum, Azotobacter, Enterobacter, Arthrobacter* and others (Ghosh et al., 2002; Esitken et al., 2010; Kumar et al., 2014). Their efficiency to mobilize either organically or minerally bound nutrients from pedosphere or to fix atmospheric nitrogen and make it available to the plants is an important feature of their application. The direct growth promotion may be due to synthesis of phytohormones (Xie et al., 1996; Ashrafuzzaman et al., 2009), N_2-fixation (Khan, 2005), synthesis of some enzymes such as ACC deaminase (Bal et al., 2013) that modulate the level of plant hormones (Glick et al., 1998) as well as the solubilization of inorganic phosphate and mineralization of organic phosphate which makes phosphorus available to the plants (Subba Rao, 1982). *Pseudomonas* sp. (Patten and Glick, 2002b), *Arthrobacter* sp. (Kumar et al., 2014), *Enterobacter* sp. (Slininger et al., 2004), *Bacillus* sp. (Idris et al., 2007) are examples of bacterial species which are capable of producing the indole-3-acetic acid (Marques et al., 2010).

Identification of bacteria in microbiological laboratory is traditionally performed for isolation of the organism and study of their phenotypic characteristics including Gram staining, morphology, culture requirements, and biochemical reactions. However, discovery of polymerase chain reaction (PCR) and Deoxyribonucleic acid (DNA) sequencing, comparison of gene sequences of bacterial species showed that the 16S rRNA is highly conserved within a species and among species of the same genus (Patrick et al., 2003). Hence, 16S ribosomal DNA (rDNA)-based molecular characterization will provide authenticity to identify universal distribution of bacteria (Weisburg et al., 1991) and the presence of species-specific variable regions. This molecular approach has been establishment of large public-domain databases (Maidack et al., 1996) and its application to identification of bacteria (Tang et al., 2000).

In the present study, attempt has been made for biochemical and molecular techniques for identification and characterization of bacteria and their effect on plant growth promotion, yield of wheat and status of microbial population in post harvest soil.

MATERIALS AND METHODS

Isolation and purification of bacteria

Soil samples (50 g soil for per samples) were collected from rice-wheat, vegetables (cabbage, spinach, ladyfinger, bottle guard), agro-forestry (mango, papaya, guava) and grassland rhizosphere (5 to 20 cm depth) from Indo-Gangetic plains of India (82°59' East, 25°15' North and 82°33' East, 25°8' North) during March to December, 2010. Nitrogen-free Ashby agar medium was used for isolation by serial dilution technique and purification on the same solid medium with repeated plating (Schmidt and Belser, 1982). Thirty-two pure cultures were procured and tested for their morphological and biochemical characteristics.

Biochemical characterization

Biochemical characters like gram's reactions, ammonia production (Dye, 1962), catalase activity, Methyl Red (MR), Voges Proskauer (VP) test (Aneja, 2003), IAA production (Sarwar et al., 1992), HCN production (Bakker and Schipper, 1987) and phosphate solubilization (Gaur, 1990) were determined by following the standard procedures.

Genomic DNA extraction

The bacterial strains were grown in nutrient broth at 28 ± 2°C in incubating shaker at 120 rpm for overnight. The method described by Sambrook and Russel (2001) was used with minor modification for Genomic DNA extraction. The 5 ml of overnight grown broth cultures were centrifuged at 10,000 rpm (C-24, Remi) for 10 min and supernatants were decanted. Pellet was resuspend in 0.4 ml TE (0.01 M Tris/HCl, pH 8.0; 0.05 M EDTA, pH 8.0) and added 40 µl of 10% SDS. Suspension was incubated for 30 min at 37°C in water bath with gentle shaking {In case of Gram +ve bacteria, the pellet was resuspend in 0.5 ml SET buffer (57 mM NaCl, 25 mM EDTA pH 8.0, 20 mM Tris-HCl pH 8.0) and added 10 µl lysozyme and incubated for 30 to 60 min at 37°C. After, this added 0.1 volumes of the 10% SDS and 10 µl of Proteinase K (10 mg/ml) and incubated at 55°C for 60 min}. After incubation, 0.4 ml phenol was added with proper mixing and centrifuged for 5 min at 8000 rpm. Then water phase was transferred to in new eppendorf tube and added 0.2 ml phenol and 0.2 ml chloroform with proper mixing. The solution was centrifuged at 8000 rpm for 5 min. Again, water phase was transferred to in new tube and added 0.4 ml chloroform with proper mixing. The solution mixture was centrifuged at 8000 rpm for 5 min. Water phase was transferred to in new tube and estimated volume of solution then added 0.1 volume of 3 M sodium acetate, pH 5.2 and 2 volume of 96% ethanol with proper mixing and kept it at -20°C for 1 h. After that, solution was centrifuged at 8000 rpm for 10 min. The supernatant was discarded and pellet was washed with 70% ethanol (-20°C). The pellet was dissolved in 40 µl of sterile distilled water and kept it at 4°C for further use.

Genomic DNA were checked on a 0.8% (w/v) agarose gel containing ethidium bromide at 100 V for 45 min in 1X TAE buffer (0.04 M Tris acetate, 0.001 M EDTA) along with EcoR1/ Hind III double digest λ DNA marker (Banglore Genei, Pvt., Ltd. Bangalore, India).

Amplification of 16S rDNA genes by polymerase chain reaction

Universal primer was used for the amplification of 16S rDNA gene in bacterial isolate. Primer was custom synthesized by Bangalore Genei, Bangalore, India. The 50 µl of reaction mixture consisted of 50 ng of genomic DNA, 2.5 U of Taq polymerase, 5 µl of 10 X buffer

(100 mM Tris-HCl, 500 mM KCl pH-8.3), 200 µM dNTP, 1.5 mM $MgCl_2$ and 10 pmoles of each primer. The forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-TACGGTTAC CTTGTTACGACTT-3') were used (Narde et al., 2004). Amplification was performed under following polymerase chain reaction (PCR) System 2720, Applied Biosystems, Singapore) conditions: initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1.5 min, extension at 72°C for 2 min and a final extension at 72°C for 7 min.

Amplified polymerase chain reaction (PCR) products (5 µl) were resolved on a 1.5% (w/v) agarose gel at 100 V for 45 min in 1X TAE buffer containing ethidium bromide (EtBr) along with 500 bp DNA ladder (Bangalore Genei Pvt., Ltd. Bangalore, India). polymerase chain reaction (PCR) product size was observed approximate 1500 bp. PCR product was purified using polymerase chain reaction (PCR) purification kit (Bangalore Genei, Bangalore, India) for the sequencing of 16S rDNA.

DNA sequencing

Sequencing of 16S rDNA was carried out at Bangalore Genei Pvt. Ltd., Bangalore, India. The 16S rDNA sequences were analyzed with nucleotide database available at the GenBank using BLAST tool at NCBI (www.ncbi.nlm.nih.gov) for identification of bacteria and were submitted at NCBI Gen Bank.

Seed bacterization

Wheat seeds var. HUW 234 was taken from Banaras Hindu University, Agriculture farm, Varanasi, Uttar Pradesh, India and were surface sterilized (0.1% $HgCl_2$ for 2 min and rinsed five times with sterilized water). Bacterial strains, *Bacillus megaterium*, *Paenibacillus polymyxa*, *Arthrobacter chlorophenolicus*, *Serratia marcescens, Enterobacter* sp., *Microbacterium arborescens* and *Pseudomonas aeuroginosa* were grown separately in nutrient broth at 120 rpm in shaking incubator at 30°C for 48 h. *P. aeuroginosa* BHUPSB02 was obtained from Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Science, Banaras Hindu University (Verma et al., 2013). Healthy wheat seeds were treated with 7 days old broth cultures of each of the bacterial isolate along with 1 ml of sticker solution (2.5 g gum acacia + 5 g sugar in 100 ml sterilized distilled water) such that a population in the range of 10^7 to 10^8 CFU $seed^{-1}$ could be obtained.

Pot and field experiments

Growth promotion effect of bacterial strains on wheat var. HUW 234 under pot and field experiments were studied during 2011 to 2012 and 2012 to 2013. Sandy loam soil collected from Agricultural Research Farm, BHU was sieved through 10 mesh sieve and filled in 5 kg of earthen pots lined with polythene. Recommended doses of fertilizers for N, P, K (120:60:60) were thoroughly mixed with required quantity of soil. Wheat seeds were treated with *B. megaterium, P. polymyxa, A. chlorophenolicus, S. marcescens, Enterobacter* sp., *M. arborescens* and *P. aeuroginosa in* four replications. Ten bacterial treated seeds were sown in each pot. Three plants after full emergence of the first leaf were maintained in each pot. Uninoculated seeds treated with nutrient broth without any bacterial strain were sown in pot as control. Pots were arranged in a complete randomized design. Necessary agronomical practices were followed. A field experiment with same bacterial strains as in pot culture experiment was conducted following the randomized block design at farmers' field of Bhadohi district, Uttar Pradesh, India with plot size 4 × 3 m (12 m^2). Each plot was basely dressed with N, P, K (120:60:60) ha^{-1}. Bacterial treated seed @ 100 kg ha^{-1} was sown to each plot in line. Plots were irrigated timely and all other agronomical intercultural processes were followed. Height of plant was measured at 30, 60 and 90 days of sowing. At maturity, crop was harvested, threshed and yield was recorded as described by Iswaran and Marwah (1980).

Enumeration of microbial population of post harvest soil

For enumerating the microbial population of post harvest soil was determined with Kenknight and Munaier's Medium for Actinomycetes, Czapek Dox Agar for Fungi, Pikovskaya agar for phosphate solubilizing bacteria and Soyabean Casein Digest Medium for bacteria via serial dilution techniques as described by Schmidt and Belser (1982).

Ten gram soil sample was taken in 250 ml conical flask containing 95 ml of sterile water and shaken on mechanical shaker to mix soil and water. With the help of sterile pipette 1 ml suspension from conical flask was transferred to culture tube containing 9 ml of sterile water. Culture tube was then shaken using stirrer. Desired level of serial dilution was obtained by adopting the similar procedure. Serial dilution of 10^{-5} was made for each microbe. 1 ml of suspension was transferred to sterilized petriplates containing about 20 ml of respective sterilized media (40°C). After solidifying the media, petriplates were inverted and kept in BOD at 28±2°C. Microbial counts were recorded when particular colonies developed. Data were analyzed by one-way analysis of variance (ANOVA). Significant differences between means were compared using Fisher's protected LSD test at $P \leq 0.05$. Statistical analysis was performed by using SPSS software version 16.0.

RESULTS AND DISCUSSION

Biochemical characteristics such as catalase, MR, VP test, NH_3, HCN production, gram's straining and shape have been given in Table 1. Bacterial strains; *S. marcescens, Enterobacter* sp. and *P. aeuroginosa* show Gram negative reaction. All strains are rod in shape. All bacterial strains show positive catalase test and ammonia production except *Enterobacter* sp which showed negative catalase test. Most of the strains show positive methyl red, Voges –Proskauer and HCN production. Biochemical characters were showed by bacterial strains play very crucial role in plant growth promotion activity (Aneja, 2003; Kumar et al., 2014). Various studies have indicated a disease protective effect to HCN as in the suppression of "root-knot" and black rot in tomato and tobacco root caused by the nematodes *Meloidogyne javanica* and *Thielaviopsis basicota*, respectively (Siddiqui et al., 2006). To date, different bacterial genera *Alcaligenes, Aeromonas, Bacillus, Pseudomonas* and *Rhizobium* have shown their capacity to produce HCN (Ahmad et al., 2008).

Plant growth promoting activity of bacterial strains *in vitro* condition

IAA concentration varied according to the efficiency of bacterial strains (Table 2). *Enterobacter* sp. showed maximum concentration of IAA (27.06 $\mu g ml^{-1}$) followed by

Table 1. Biochemical characteristics of plant growth promoting bacterial strains.

Bacterial strain	Gram's reaction	Shape	Catalase test	VP test	MR test	NH_3 production	HCN production
Bacillus megaterium	Gram+ve	Rod	+	+	-	+	–
Paenibacillus polymyxa	Gram+ve	Rod	+	+	+	+	-
Arthrobacter chlorophenolicus	Gram+ve	Rod	+	-	+	+	+
Serratia marcescens	Gram-ve	Rod	+	+	+	+	+
Enterobacter sp.	Gram-ve	Rod	-	+	+	+	+
Microbacterium arborescens	Gram+ve	Rod	+	+	-	+	+
Pseudomonas aeuroginosa	Gram-ve	Rod	+	-	-	+	+

VP', Voges –Proskauer; 'MR', Methyl Red; Data are average of four replicates ± SD. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Fisher's protected LSD).

Table 2. Plant growth promoting activities of bacterial strains *in vitro*.

Bacterial strain	pH of broth cultures	Phosphate solubilization in broth ($\mu g ml^{-1}$)	IAA production at 100 $\mu g ml^{-1}$ TP	Strains	Accession no	Maximum identity	Bacterial name of BLAST match
	5 day	5 day					
Control	7.00 ± 0.12^{d}	0.00 ± 0.00^{a}	00.00^{a}				
B. megaterium	4.83 ± 0.05^{b}	153.60 ± 1.70^{e}	19.41 ± 0.85^{bc}	BHU1	KC432646	99	*B. megaterium* strain GY-37 (KF982008)
P. polymyxa	5.00 ± 0.11^{c}	140.44 ± 2.10^{c}	25.29 ± 1.73^{de}	BHU2	KC453980	97	*P. polymyxa* strain B100 (FJ965336)
A. chlorophenolicus	5.12 ± 0.10^{c}	132.5 ± 00.92^{b}	16.12 ± 0.58^{b}	BHU3	KC453981	99	*A. chlorophenolicus* strain 19 (JQ958834)
S. marcescens	5.05 ± 0.09^{c}	143.58 ± 1.04^{cd}	21.29 ± 0.99^{c}	BHU4	KC453982	100	*S. marcescens* strain IARI-THW-5 (KF054974)
Enterobacter sp.	-	-	27.06 ± 1.70^{e}	BHU5	KC453983	99	*Enterobacter* sp. PXG11 (JQ396391)
M. arborescens	4.95 ± 0.10^{bc}	145.66 ± 1.98^{d}	26.82 ± 1.63^{e}	BHU6	KC453984	99	*M. arborescens* strain DSM-20754 (NR029265)
P. aeuroginosa	4.42 ± 0.06^{a}	160.34 ± 2.40^{f}	24.35 ± 0.38^{de}	BHUPSB01	-	-	-

'IAA', Indole acetic acid; 'TP', Tryptophan; Data are average of four replicates ± SD. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Fisher's protected LSD).

Table 3. Effect of bacterial strains on periodic growth of wheat under pot and field experiments.

Bacterial strain	Plant height (cm)					
	Pot			Field		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Control	27±1.24^{a}	58±0.29^{a}	65±1.13^{a}	12±1.50^{a}	33±0.03^{a}	38±1.25^{a}
B. megaterium	32±0.52^{c}	67±0.58^{de}	70±0.38^{b}	16±1.01^{bc}	37±0.10^{b}	43±1.64^{b}
P. polymyxa	33±0.64^{c}	64±0.48^{bcd}	75±0.58^{c}	15±0.38^{abc}	37±0.22^{b}	46±0.67^{bc}
A. chlorophenolicus	33±0.69^{c}	69±1.66^{e}	80±0.38^{d}	17±0.69^{c}	38±0.10^{c}	45±0.51^{bc}
S. marcescens	30±0.40^{abc}	61±0.29^{ab}	68±0.29^{a}	15±0.51^{abc}	40±0.19^{d}	47±0.59^{bc}
Enterobacter sp.	28±0.41^{ab}	66±1.15^{cde}	67±0.38^{a}	13±0.58^{ab}	40±0.42^{d}	49±1.41^{bc}
M. arborescens	32±1.45^{c}	63±0.29^{bcd}	71±0.10^{b}	17±1.04^{bc}	42±0.25^{e}	47±0.19^{bc}
P. aeuroginosa	31±1.10^{bc}	61±1.42^{abc}	68±0.77^{a}	13±1.39^{ab}	40±0.03^{d}	46±0.87^{bc}

'DAS', Days after sowing, 'cm', centimeter; Data are average of four replicates ± SD. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Fisher's protected LSD).

M. arborescens (26.82 μgml^{-1}), *P. polymyxa* (25.29 μgml^{-1}), *P. aeuroginosa* (24.35 μgml^{-1}), *B. megaterium* (19.41 μgml^{-1}), and *A. chlorophenolicus* (16.12 μgml^{-1}) at 100 μgml^{-1} tryptophan in broth after 24 h incubation. Reports of Asghar et al. (2004) showed that PGPR strains produced 24.6 μgml^{-1}of auxins in the presence of the precursor L-tryptophan in the medium which was 184-fold more than that without L-tryptophan. Bacteria belonging to the group of symbiotic as well as asymbiotic diazotrophs and P-solubilizers have been shown to produce auxins which help in stimulating plant growth (Idris et al., 2009; Ahmad et al., 2008).

The maximum and significant solubilization of tri-calcium phosphate was recorded by *P. aeuroginosa* (160.34 μgml^{-1}) followed by *B. megaterium* (153.60 μgml^{-1}) over control at 5 days after inoculation. Except *Enterobacter* sp. all strains changed pH of broth culture and also solubilized tri-calcium phosphate. Change in pH of broth culture varied from 4.42 to 5.12 *in vitro* conditions. *P. aeuroginosa* showed maximum and significant decrease in pH of broth followed by *B. megaterium* over most of the bacterial strains. The low availability of P to plants is because the conversion of soluble form of P to insoluble forms of P as plants can absorb P in forms of $H_2PO_4^-$ and HPO_4^{2-} (Glass, 1989). Most soil bacteria solubilize insoluble phosphates; particularly active are those that belong to the genera *Pseudomonas, Enterobacter* and *Bacillus* (Whitelaw, 2000). Bacterial strains name and their accession numbers were showed in Table 2 and also present BLAST search admirable agreement for such close 16S rDNA database similarity. The 16S rDNA gene characterization confirmed the identification of plant growth promoting rhizobacteria.

Effect of bacterial strains on periodic growth of wheat plant under pot and field experiments

Height of plant under pot culture experiment after seed inoculation with different strains increased with successive periods (Table 3). *A. chlorophenolicus* showed significant and maximum plant height at 30, 60 and 90 days after seed inoculation over uninoculated control. *P. polymyxa* gave same plant height as *A. chlorophenolicus* at 30 days but lesser height of plant at advanced period of wheat that might be due to delay acclimatization of *P. polymyxa* in soil than *A. chlorophenolicus.* Besides plant height, also other factors like efficiency and time taken in acclimatization of bacterial strains in soil. More plant heights were recorded at 60 days after sowing of bacterial strains. Increase in plant height from 4.70 to 22.20%, 4.30 to 19.50% and 3.60 to 23.60%, respectively as compared to uninoculated control at 30, 60 and 90 days after sowing due to various bacterial strains under pot experiment. Cakmakci et al. (2007) reported that 2.2 to 24.6% plant height was recorded in green house experiment with *Bacillus* sp., *Pseudomonas putida* and *P. polymyxa* as compared to the uninoculated control. Kumar et al (2014) reported almost similar finding in plant height of wheat with inoculation of different PGPR.

A. chlorophenolicus and *M. arborescens* showed significant and maximum 42% increase in plant height at 30 days while *M. arborescens* caused significantly 27% increase in plant height at 60 days after sowing over uninoculated control under field experiment (Table 3). However at 90 days, *Enterobacter* sp. could lead up to 29% height of plant as compared to control. The increase in plant height by various strains in filed at successive period was due to efficiency of strains to enhance the uptake of essential nutrients required to plants and efficacy of their acclimatization in soil. In general strains which performed better in pot experiment, also contributed significantly under field experiment. Higher plant height was observed under pot than field due to better management which helped in checking the loss of nutrients in pot culture experiment and consequently greater nutrient acquisition positively influenced the

Table 4. Effect of bacterial strains on yield of wheat under pot and field experiments.

Bacterial strain	Yield under pot (g pot^{-1})		Yield under field (kg ha^{-1})	
	Grain	Straw	Grain	Straw
Control	4.71±0.31^{a}	6.77±0.13^{a}	31.80±0.92^{a}	47.35±1.80^{a}
B. megaterium	7.13±0.24^{e}	9.51±0.05^{e}	34.23±0.75^{b}	50.58±1.59^{abc}
P polymyxa	5.48±0.37^{abc}	7.21±0.10^{ab}	35.30±1.24^{b}	54.10±1.64^{bc}
A. chlorophenolicus	6.68±0.10^{de}	9.34±0.31^{e}	35.80±0.12^{b}	52.29±1.07^{bc}
S. marcescens	5.34±0.08^{abc}	7.53±0.23^{b}	36.50±0.87^{bc}	55.42±1.46^{c}
Enterobacter sp.	6.43±0.17^{cde}	9.27±0.27^{de}	36.33±1.30^{b}	53.78±1.76^{bc}
M. arborescens	5.88±0.81^{bcd}	8.73±0.06^{d}	33.65±0.49^{a}	49.69±1.03^{ab}
P. aeuroginosa	5.17±0.03^{ab}	8.14±0.19^{c}	36.10±0.98^{b}	53.37±1.12^{bc}

Data are average of four replicates ± SD. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Fisher's protected LSD).

height of plant (Kumar et al., 2014). Ali et al. (2011) has reported that inoculation of wheat with thermotolerant *P. putida* significantly increased 1.12 fold more shoot height than uninoculated control. *Bacillus*, *Arthrobacter* and *Enterobacter* were evaluated for their effect on growth of wheat (Zhang et al., 2012; Kumar et al., 2014).

Effect of bacterial strains on yield of wheat plant under pot and field experiments

B. megaterium showed maximum and significant 51.45% grain and 40.47% straw yield followed by *A. chlorophenolicus* as compared to unninoculated control under pot (Table 4). All strains except *P. polymyxa*, *P. aeuroginosa* and *S. marcescens* caused significant enhancement in grain yield over uninoculated control. Bacterial strains increased from 9.77 to 51.45% and 6.50 to 40.47% grain and straw yield, respectively as over control pot. Almost, similar result was also recorded with inoculation of *B. polymyxa a*nd *E. cloacae* on winter wheat that increase grain yield by 17.56% and 4.65%, respectively over control under growth chamber (Renato de Freitas, 2000). Significant increase in yields due to seed bacterization of *P. aeruginosa*, *S. proteamaculans*, *Arthrobacter, Enterobacter* and *Bacillus* sp. also have been reported (Zahir et al., 2009; Kumar et al., 2014).

S. marcescens showed maximum and significant 14.78% grain and 17.04% straw yield followed by *Enterobacter* sp. and *P. polymyxa* as compared to uninoculated control in field experiment. All strains except *M. arborescens* showed significantly greater yield as compared to uninoculated control in field. However, yield depends on efficiency and performance of strains, how they can easily acclimate in soil environmental conditions. Turan et al. (2012) have reported that inoculation of *Bacillus* sp. and *Azospirillum brasilense* increased grain yield by 24 and 19% of wheat crop. Almost similar result was reported on wheat crop by inoculation of various PGPR strains (Rodríguez Cáceresa et al., 2009; Kumar et al., 2014).

Microbial population of post harvest soil under pot and field experiments

Microbial populations of bacteria, Actinomycetes, fungi and phosphate solubilizing bacteria (PSB) under pot have been depicted in Figure 1. As compared to uninoculated control, significant population of bacteria and PSB were recorded with inoculation of *P. aeuroginosa* while maximum and significant population of fungi and actinomycetes were caused by *M. arborescens* of post harvest soil. In general, post harvest soil having maximum population of bacteria followed actinomycetes, fungi and least by PSB. Analyzed post harvest soil increased population up to 58, 50, 87 and 114% of bacteria, Actinomycetes, fungi and PSB as compared to uninoculated control in pot experiment. Increases in soil microbial population due to seeds were treated with different bacterial strain which enhanced the microbial population in soil. Almost similar results have also been reported by Kalaigandhi et al. (2010) and Shinde and Latake (2009).

In field experiment, all the bacterial strains except *P. polymyxa* showed significantly greater bacterial population in soil as compared to uninoculated control (Figure 2). All inoculated strains gave significantly higher population of bacteria, fungi, actinomycetes and PSB those ranged from 4.17 to 58.33%, 7.14 to 85.71%, 10 to 50% and 14.29 to 114.29%, respectively over uninoculated control. In comparison to pot, higher population of various microorganisms recorded in field soil due to better acclimatized and natural environment to flourish the microflora. Maurya et al. (2012) also reported higher population of *Azotobacter* and *Azospirillum* in field condition.

Conclusions

These studies show that single inoculation of *S. marcescens*, *A. chlorophenolicus*, *Enterobacter* and *B. megaterium* enhance growth and yield of wheat. These

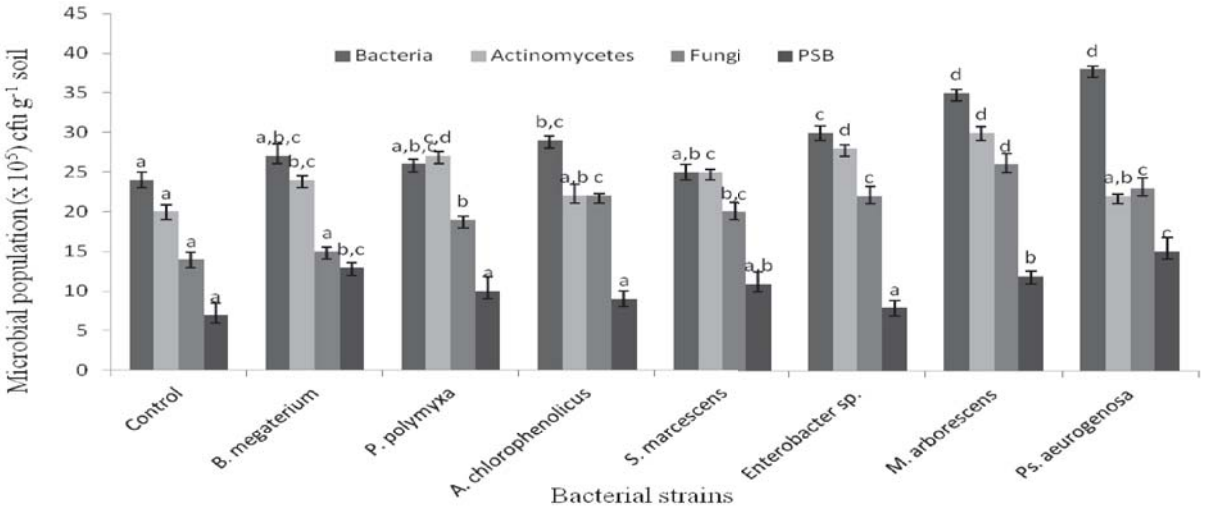

Figure 1. Microbial population of post harvest soil under pot experiment. *'B', Bacillus*; '*P. polymyxa*', '*Paenibacillus polymyxa*'; '*A*', '*Arthrobacter*'; '*S*', '*Serratia*'; '*M*', '*Microbacterium*'; '*Ps. aeuroginosa*', '*Pseudomonas aeuroginosa*'; Data are average of four replicates ± SD. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Fisher's protected LSD).

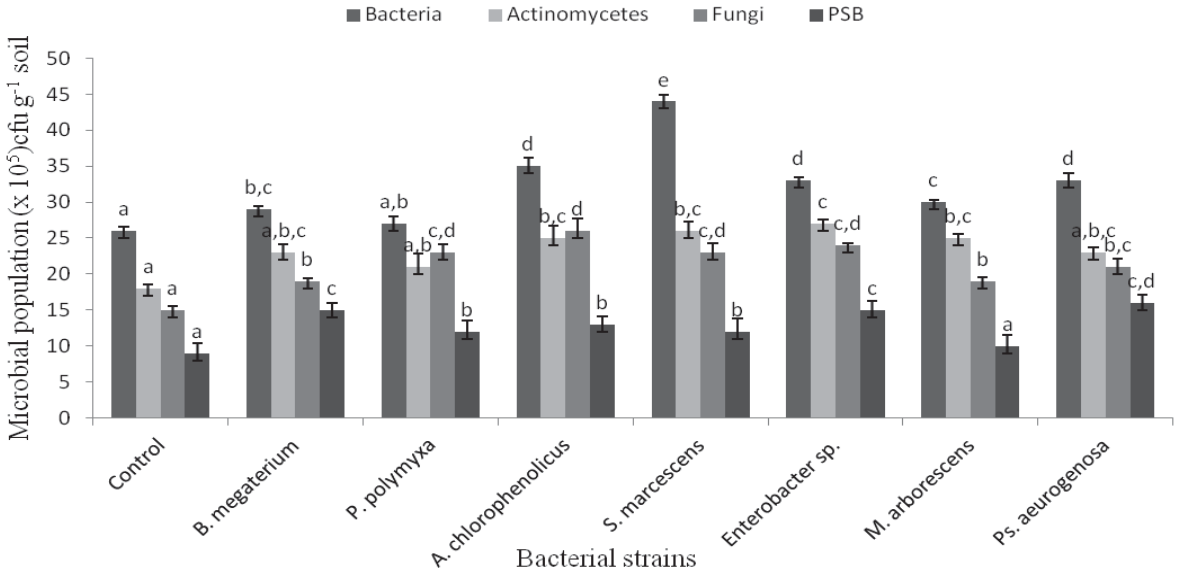

Figure 2. Microbial population of post harvest soil under field experiment. *'B', Bacillus*; '*P. polymyxa*', '*Paenibacillus polymyxa*'; '*A*', '*Arthrobacter*'; '*S*', '*Serratia*'; '*M*', '*Microbacterium*'; '*Ps. aeuroginosa*', '*Pseudomonas aeuroginosa*; Data are average of four replicates ± SD. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Fisher's protected LSD).

strains may be used as efficient PGPR for wheat production in farmer's fields. It is an environmentally friendly and cost effective technology.

Conflict of Interest

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

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Comparing stakeholder views for mutual acceptable food value chain upgrading strategies in Tanzania

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The number of rural poor has been reported to rise in Sub-Saharan Africa (SSA) while per capita food consumption in the region is on the decline and food insecurity has been much embedded. Thus, knowing upgrading strategies (UPS) to be used in making a living and would have great chance of benefiting majority hence provide solutions to poverty, food insecurity and malnutrition. This paper assesses and compares the views of local stakeholders and agricultural experts in terms of prioritizing food securing UPS along food value chains (FVC). Data and information have been collected in a highly participatory process so as to develop an approach and experience in Tanzania regions to support poor people in rural areas to upgrade their position in viable FVC. Local stakeholders' definition of food security rely on food availability component, hence this paper centers on two major FVC components such as natural resources and crop production for maize and millet subsectors in Morogoro and Dodoma regions of Tanzania, respectively. Given natural resources, agricultural experts favor soil improving upgrading strategies in Morogoro and water management in Dodoma, whereby, local stakeholders in both regions prefer farm inputs related UPS for improving soil fertility (seed varieties improvement and fertilizer use). There is no significant mismatch of views for production component apart from differences on ranks. Stakeholders in both regions prefer use of improved crop varieties, pests and diseases control and new livestock management including having village land use planning. It is recommended that satisfactory participation of local stakeholders should be considered during testing stage of FVC upgrading strategies, including packing these innovations to suit local conditions and finally empower all potential actors for successful dissemination and outreach.

Key words: Rural household, food security, upgrading strategy, food value chain, Tanzania.

INTRODUCTION

The world's population will be 34% higher than today by 2050 and 70% more food is needed especially in developing countries, thus, global food supply should increase significantly (FAO, 2013). Though developing

countries will demand more food, there is greatest production capacity potential (Haug and Hella, 2013). Tanzania like other developing countries is facing numerous challenges in the agriculture sector and food value chains (FVCs) requiring efforts towards poverty reduction and increasing food security (FS) (MAFAP, 2013). Therefore, an improvement in Tanzania's agriculture and rural areas is required particularly for farm level productions, yields and crop intensity, expansion of arable land and promotion of value addition. Thus, enhancing FVCs for increasing food security in this country is the best intervention point (Gómez et al., 2011). Also, reduction of food insecurity would require FVCs that links global beneficiaries to local actions in a highly participatory way such as a poor people-centered approach (Graef et al., 2014).

In this regard, an assessment has been done to verify if there is divergence of views and perceptions from local people and agricultural experts in terms of prioritizing food securing upgrading strategies (UPS) along FVCs. Whereby, FVCs comprise set of actors and activities required to bring the products to consumers including components like natural resources, crop production, processing, marketing and consumption (Gómez et al., 2011; Kaplinsky and Morris, 2000). Based on local definition of FS, this paper is restricted to natural resources and crop production hence adopts these two FVCs components only. And UPS means success stories, good practices and/or technological innovations (Graef et al., 2014). The central aim was to develop an approach and generate experience in Tanzania regions to support poor people in rural areas to upgrade their position in viable value chains.

Local FS definitions and main agricultural sub-sectors selected have been used to guide the assessment process and compare views emanated from village level key stakeholders and combinations of experts from Tanzania and German. The method adopted in the paper provides a replicable approach for involving both local stakeholders and agricultural experts. Their views and/or opinions of potential UPS along FVCs components can be used to design effective and efficient mechanism. In this paper, views from stakeholders show their prospects of different good practices or innovations which can increase efficiency of FVCs components. Thus, bringing multi-stakeholders views together is the promising way for agricultural development in countries like Tanzania (IFAD and UNEP, 2013).

OUTLINING STUDY, FIELDWORK AND METHODS

Context, level and themes overview

This work was carried out in the frame of a collaborative research project (Trans-SEC – Innovating Strategies to safeguard Food Security using Technology and Knowledge Transfer: A people-centred Approach). Trans-SEC has been designed to identify successful food securing UPS along local and regional FVCs, test and adjust them to site-specific, sustainable settings and tailor these concepts to be disseminated for national outreach. Before the next step of subjecting promising UPS with in-depth theoretical analysis, this paper attempts comparing UPS related views as they have been identified among main sub-sectors based on important FVC components in four case study sites (CSS).

In this light and as explained by Graef et al. (2014), the project scientists would specify and select a set of 3-5 UPS per FVC component, and subsequently the stakeholders would select only one most promising UPS per FVC component at each CSS for more in-depth analysis and tests. Also, various discussion and assessments would be done involving a wide range of partners and stakeholders to come up with suggestions for adaptations. Thus, the use of models simulations given environmental and socio-economic conditions, most successful UPS among FVC components will be disseminated though a German-Tanzania network of stakeholder organizations at policy, extension and farmer school levels (Figure 1).

Data collection overview

We used mainly focus group discussions (FGDs) to get required data and information from local stakeholders comprising men, women and youth. Checklist and structured questionnaire are main tools used for data collection. One FGD comprised 14-16 members with different professions and functions who were randomly selected from a respective village within Morogoro and Dodoma regions. Whereby, a total of 16 farmers, 10 traders, 6 processors and 4 millers were involved in the FGDs. Discussion which were held for 5-6 h per village were also guided by a checklist of key points to cover the major components of types of data required. The approach and the tools used were pre-tested for assuring their validity and reliability.

Agricultural expert views on UPS were collected based on main successful UPS brainstormed by 30 scientists and given under the guidance of associated information, for instance by (Kimenye and Bombom, 2009). Thus, this covered a wide range of not only criteria given under the Trans-SEC but also FS oriented ones (FAO, 2011). Expert views were obtained through structured questionnaire and a total of 32 Tanzanian and German members from the Trans-SEC consortium filled it. Given the list of targeted beneficiaries of the project in CSS and total number of the consortium members; only 10 and 32% of total number of targeted respondents contacted respectively. Thus, the sample of local stakeholders and Trans-SEC project scientists and the stratification method used results fairly better number of representatives of the study.

Case study sites overview

Local level data were collected from two villages in each district, that is, Kilosa district villages namely: Changarawe and Ilakala and for Chamwino district is Idifu and Ilolo in Morogoro and Dodoma regions respectively (Figure 2). These locations have been selected due to diversity of their food systems with both food-insecure and food-secure sensitivity (Table 1). Thus, an in-depth analysis to be done in the CSS as shown in Figure 1 and knowledge to be gained in these districts would be replicable and fungible (substitutable,

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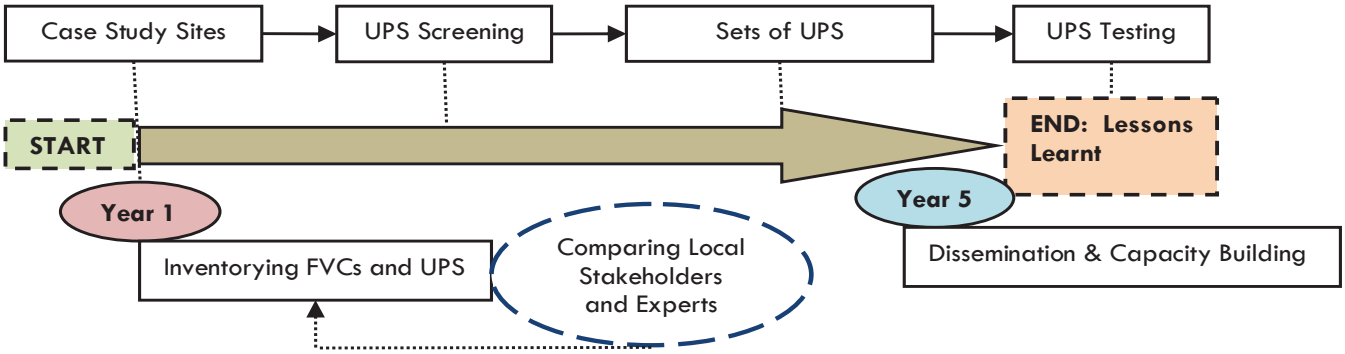

Figure 1. Simplified steps of food value chain spatio-temporal research design (modified from (Graef et al., 2014)).

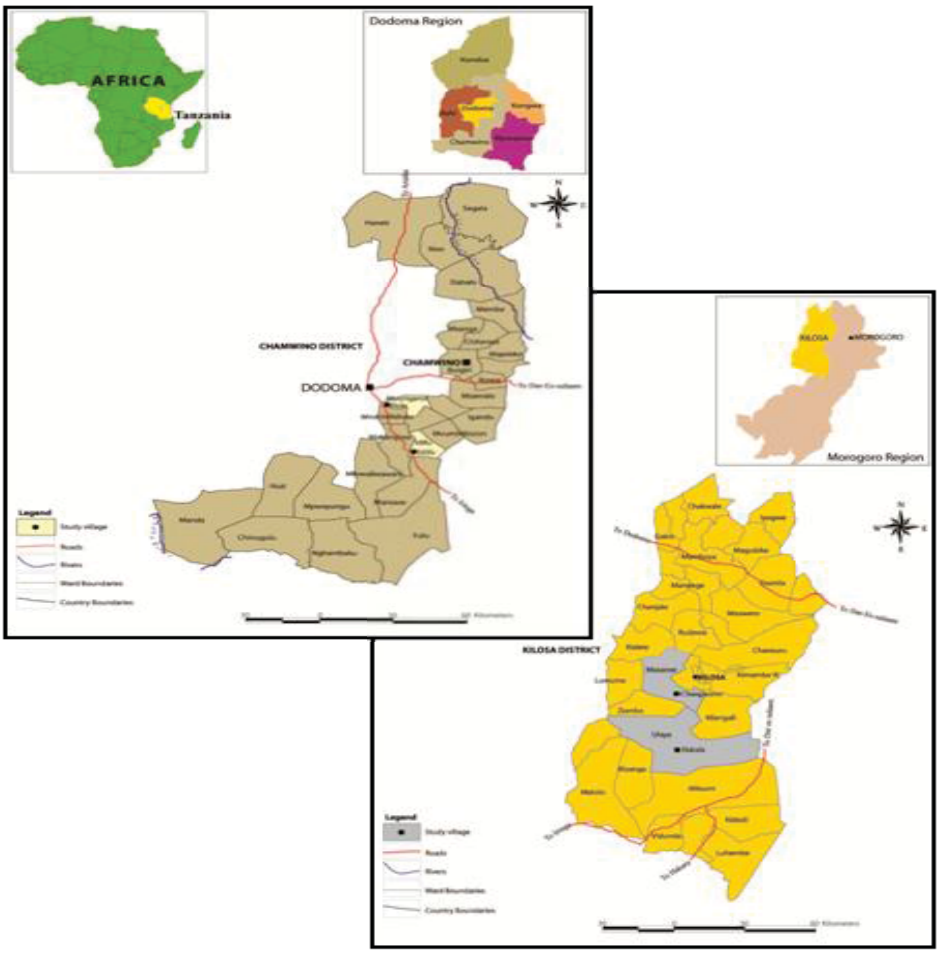

Figure 2. A Map of case study sites.

interchangeable, exchangeable or replaceable) from one region to other regions in Tanzania.

Stakeholders engagement overview

The UPS assessments originated from both local stakeholders (at the village level) and experts who are affiliated with different institutions (Figure 3; Graef et al., 2014). About 34% of total experts revealed to have expertise in both Morogoro and Dodoma regions. Whereby, local level stakeholders were dominated by smallholder farmers and millers, traders and processors occupy only 36%. We had the strong assumption that, the majority of stakeholders' have knowledge on all FVC components including natural resources (land and water) and production (seeds, planting, and crop husbandry).

Table 1. District food systems characteristics.

Feature	Kilosa district – Morogoro region	Chamwino district – Dodoma region
Food systems	Based on maize, sorghum, legumes, rice and horticulture	Based on sorghum and millet
Food security	Both food-insecure and food-secure areas	Sensitive to food insecurity
Highland	Flat plains, highlands and more diverse dry alluvial valleys	Flat plains and small hills
Livestock	Partly with livestock	Deep attachment to livestock
Climate	Predominantly sub-humid (600 to 800 mm)	Semi-arid (350 to 500 mm)
Markets	Weak and good market access	Weak and good market access
Productivity	Low to high	Low to medium
Land pressure	High	Medium and high

Source: Mutabazi (2013) and Graef et al. (2014).

Table 2. Local definitions of food security.

Morogoro		Dodoma	
Changarawe	**Ilakala**	**Ilolo**	**IDIFU**
Enough food	Reserving food for later use	Having reliable 3 meals (for current and future use)	Best storage of food and use insecticide in storage
Food storage/reserve	Making sure there is food whenever it is needed	Store food per annum	Enough food
Surplus production		Store and use food properly	Food reserve
Enough food for the week/month/year	Best storage of food for current and later use	Store food maintaining its quality i.e. free from pests and diseases	Good harvest cycles
			Best use of food year round
CSS stakeholder consensus			
Generally, enough food year round	A family should be assured to have enough food all year round and be best used	Assured of getting 3 meals on daily basis and food should be stored safely free from microorganisms	Enough food well stored (using insecticides) to be used all year-long

RESULTS AND DISCUSSION

This section presents results and discussion obtained from ranking exercise of UPS as views of local stakeholders from study villages and from experts. Ranks of these UPS have been grouped with respect to the main FVCs components which have significant contribution to food availability such as natural resources (soil and water) and production (seeds, planting, crop husbandry). Moreover, these two components have revealed to be very important among FVCs compared to others and supported by majority of stakeholders in Dodoma and Morogoro regions. As mentioned above, divergence or convergence of experts views are compared with local UPS ranks for maize and millet sub-sectors. These sub-sectors represent the main food crops in Morogoro and Dodoma regions, respectively, given local definitions of food security.

Local food security definition and sub-sector selection

Using highly participatory process, FVCs components have been quickly mapped in the CSS, an inventory of potential UPS have been prepared and finally prioritized at the local level. At this initial stage, given FVCs sub-sectors/crops were selected with CSS stakeholders. Criteria were used to give weight based assessments on the type of impact such as on food security, poverty and sustainability and impact on structure of the chain (Annex 1 and 2). As local definitions and criteria of FS to the great extent rely on food availability (Table 2), and given their weights attached to crop/sub-sectors, the discussion of the paper focuses much on Maize and Millet for Morogoro and Dodoma regions respectively (Annex 1 and 2). Food availability is probably key component of FS as far as Tanzanian government recently reported to struggle balancing food availability given food market prices (Haug and Hella, 2013). Thus, views which were collected from experts have been compared with local stakeholders as far as UPS assessments and ranking is concerned.

Local stakeholders main crops scores based on impacts

Local stakeholders in Morogoro and Dodoma regions

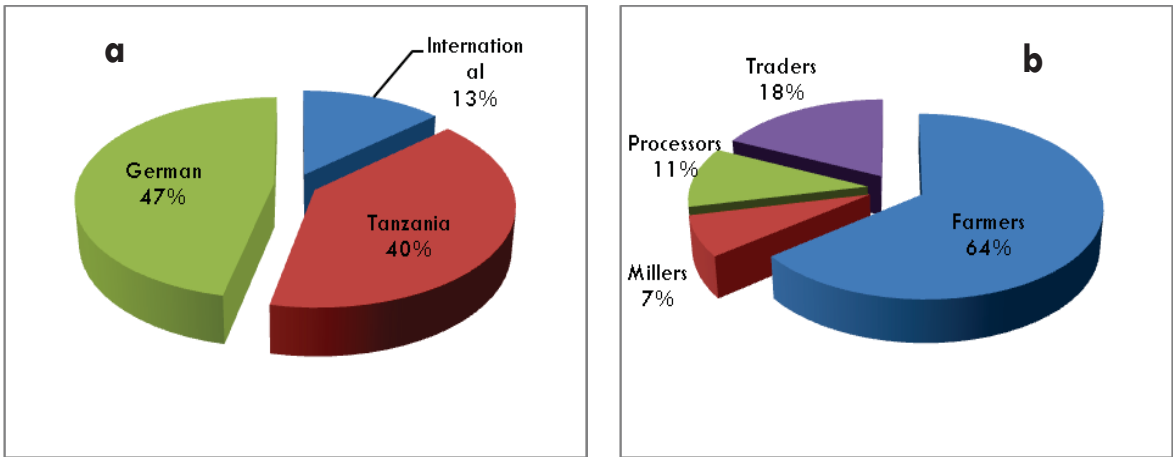

Figure 3. Nature of stakeholders engaged a. Institutional expert coverage b. Local stakeholders.

through their groups gather numeric scores for main crops which are grown in their villages then simple averages made (Figure 3). According to Sanogo (2010), this is very important step which used to check the way FVC conforms to the criteria developed. Thus, in the current paper we have used two types of impacts such as FS, poverty and sustainability and structure of the chain. Whereby, scores have been attached to a number of criteria under these main impacts. For instance, criteria which are under FS, poverty and sustainability are direct contribution to FS, future potential of the crop, number of poor household involved in the sector and availability of natural resources; and those which are under structure of the chain are extent of value adding potential (stability, profitability), number of different products produced, length of marketing chain (number of intermediaries), marketing potential and potential for lessons learnt/replication mechanism (Annex 1 and 2). These have been developed to add value on UPS selection process given main crops and/or sub-sectors.

The assessment was done to both consumption or market oriented FVCs (Figure 4). Based on local definitions of FS, the authors have to consider one crops/sub-sectors from each region with high possibility of increasing food availability hence observe highest score on FS, poverty and sustainability impact. In Dodoma region millet scored 5.0 out of 5.0 hence being selected (Figure 4b). While, in Morogoro region the highest score of 4.5 revealed on maize and beans crops (Figure 4a). In this regard, the authors have to consider an overall average after combining with other impact scores such as structure of the chain. Whereby, maize has the highest average score of 4.3 out of 5.0 followed by sesame which scored 3.8 out of 5.0 (Annex 2) hence beans were dropped. Thus, maize and millet represent main crops in Morogoro and Dodoma regions, respectively, with higher chance of securing food in rural areas of these regions.

Natural Resources (soil and water)

Agricultural experts favored soil improving UPS in Morogoro and water management in Dodoma as far as they are sub-humid and semi-arid regions, respectively. However, local stakeholders in both villages in Morogoro prefer much farm inputs related interventions for improving soil fertility such as through good seed varieties use and fertilizer application to increase their farm productivity. Whereby, views of stakeholders such as local and experts from both regions are more or less the same though the issues of farm inputs also emanated in Dodoma (Table 3). Farm inputs retailers reported to be located very far from households in all villages surveyed. This has also been reported by (Benson et al., 2013) that 4.8 km is likely to be the shortest average distance in Tanzania to the fertilizer retailer from the farm. Also, the main reported reasons for low rates use of improved seeds and fertilizers are costs and awareness (World Bank, 2012).

In Morogoro region, erosion as suggested by 42% of total experts interviewed is not considered a significant constraint to local stakeholders. Improvement of crop yields depends much on water availability in the soil (Makurira et al., 2011) in combination with proper use of fertilizers (Tesfaye et al., 2011) hence increase yield through crop intensification (Aune and Bationo, 2008). Also, local stakeholders in Morogoro have a preference on better land use planning as opposed to what suggested by experts (Table 3).

This is not surprising as documented by (Buck and Milder, 2012) in Southern Agricultural Growth Corridor of Tanzania (SAGCOT) green growth leaders' workshop reported that land use planning can improve land security, increase water flows and reduce human conflict if grazing pressure is a problem. For instance, in Kilombero and Kilosa which are districts in Morogoro region, land use planning has been implemented only in

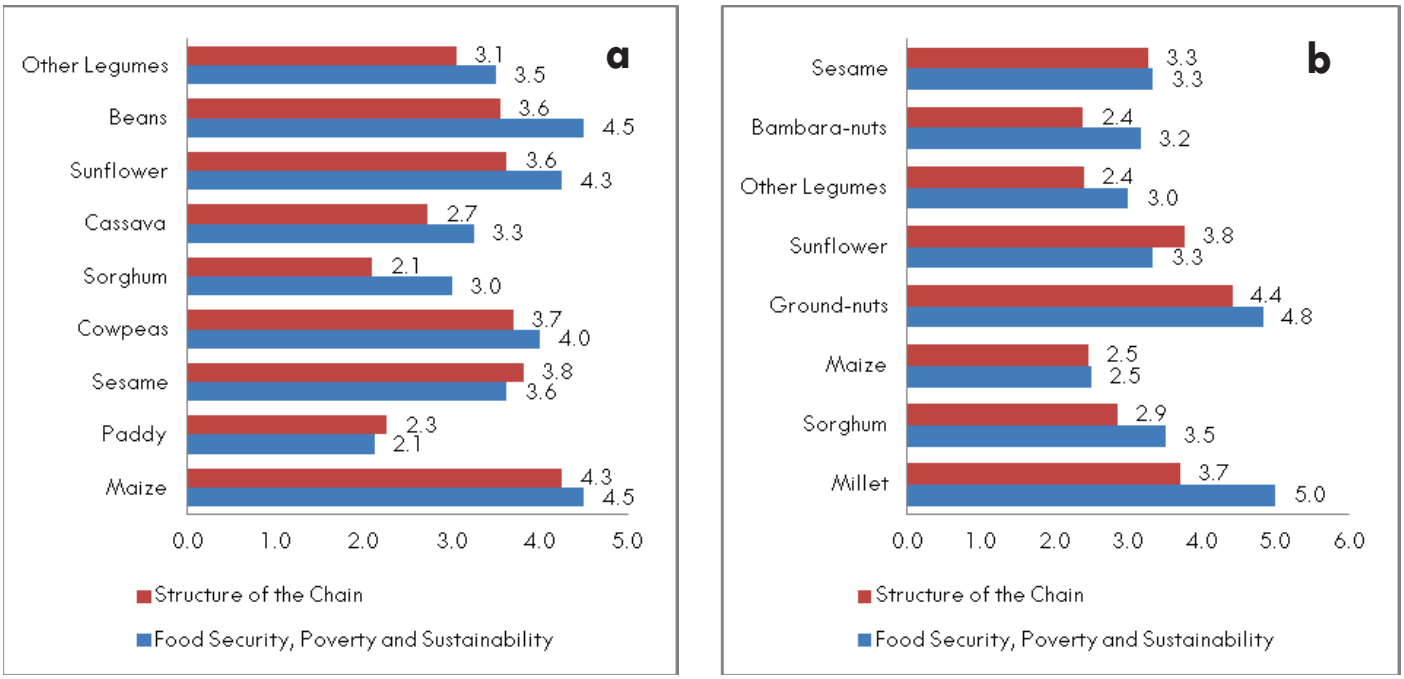

Figure 4. Average Local Stakeholders Crops Scores Based on Impacts a. Morogoro Region b. Dodoma Region. Note: A score of 1 meaning that the particular commodity did not meet that criteria (minimum compliance), and a score of 5 meaning that the commodity best met that criteria (maximum compliance).

35 out of 94 villages and 33 out of 118 villages, respectively. Whereby, grazing pressure is one of the main issues in the Kilosa district. Proper land use taking into consideration the size of the land and application of recommended rates of other farm inputs such as fertilizers and herbicides etc with proper management would increase farm productivity hence profit (Mwinuka, 2013; Vanlauwe et al., 2011). However, an indepth assessment of soils and perception of fertilizer use by smallholder farmers would add value before associated interventions take place in CSS (Oluwasegun Fasina, 2013; Marenya and Barrett, 2009; Aphunu, 2011) and land-use dynamics should be well analyzed because fertile land and freshwater is under pressure (Müller and Lotze-Campen, 2012).

Crop production (seeds, planting, and crop husbandry)

To a large extent, views from experts and local stakeholders are like counterparts though they differ the way they were ranked. However, cover crops and intercropping related UPS in Morogoro and Dodoma was not in the same direction of what suggested by local stakeholders in all villages (Table 3). As suggested by all stakeholders, availability of farm level inputs such as improved seeds varieties (according to 2010/11 National Panel Survey (NPS) only 16.8% of Tanzanian households used improved seeds), herbicides and knowing how to use them through availability of extension services would increase food availability. Availability of agricultural extension services is also a very important UPS as suggested by local stakeholders in both regions. Whereby, the related initiatives should be given a priority in rural areas of Tanzania so as to increase farm inputs uptake hence more food availability (Benson et al., 2013; Ricker-Gilbert et al., 2011; Xu et al., 2009). More importantly, the uptake of these inputs may be catalyzed by other UPS such as fertilizer micro-dosing (Camara at al., 2013; Twomlow et al., 2010). In this regard, something should be done in Tanzania since little uptake of existing improved soil, water and land management practices reported (Kristjanson et al., 2012).

In this regard, Kimenye and Bombom (2009) supported not only improving crop varieties and crop management practices but also finding the best way of working in partnership with all stakeholders, cluster UPS suiting farmer conditions and empower them to take charge of their UPS requirements. The same scenario has been insisted by (Verkuijl et al., 1998) that not only local stakeholders particularly farmers should participate in the process of developing UPS but also development of improved crop varieties should consider yield and other important features such as drought resistance/tolerance, resistance to storage pests, shelling quality, and taste of the produce for meeting consumers needs. As enlightened by (Liwenga et al., 2012) that local

Table 3. Local and expert UPS ranking for natural resources and crop production.

Morogoro Region – Natural Resources				Dodoma Region – Natural Resources		
Rank	**Ilakala local stakeholders**	**Changarawe local stakeholders**	**Experts**	**Idifu local stakeholders**	**Ilolo local stakeholders**	**Experts**
1	Short time seeds varieties	Land ownership and secure land tenure	Agroforestry	Water torerant varieties	Land use efficiency	Rainwater harvesting
2	Land use/planning	Irrigation	Consevation agriculture and ridges for erosion control	Short time varieties	Fertilizer application	Conservation agriculture and ridges as water catchments
3	Fertilizer use	Fertilization	Ridges as water catchments and rain water harvest	N/A	Extension services and ridges	Drip irrigation
Morogoro Region – Crop production				**Dodoma Region – Crop Production**		
Rank	**Ilakala Local Stakeholders**	**Changarawe Local Stakeholders**	**Experts**	**Idifu Local Stakeholders**	**Ilolo Local Stakeholders**	**Experts**
1	Education extension	Improved seeds	Intercropping	Apply best practice	Education on better waste/by products use as manure	Manure input and intercropping
2	Nearby stokist and follow best practice	Insectices and pesticides use	Improved crop varieties	Education on waste/by products use	Timely weeding	Cover crops
3	Herbicides use	Village land use planning	Cover crops and pest and disease control	Improved seeds	Extension officers	Pest and disease control and new livestock management

knowledge should be carefully considered when addressing different coping strategies related with food security such as flexibility on resource mobilization and use of labor for farm and off-farm activities hence manage food.

Conclusions

This paper presents the results of the research conducted to assess and compare the views of local stakeholders and agricultural experts for prioritizing food securing upgrading strategies. The research sought to develop the approach and experience in Tanzania on how to build up upgrading strategies and best practices of value chains activities through strong participatory process. These practices would be adapted to the local needs for impacting food insecure households.

Uncover complementarities during upgrading strategies development was necessary for having well branded good practices along food value chains. This bridge the knowledge gap between what is realistic and what is desirable given views from wider range of stakeholders. The approach and experience emanated through this paper which brings multi-stakeholder views together is the promising method for rural and agricultural development.

It is our strong believe that high stakeholder participation in the selection of upgrading strategies will strengthen their transferability and applicability to the other rural areas of Tanzanian regions and beyond. The authors found slight differences between expert and local stakeholders' views as expected. However, a consideration should be made on an improvement of crop varieties and crop management practices as suggested by local stakeholders to increase food availability and enhance food security. Thus, during the testing stage of different upgrading strategies local stakeholders should be involved fully, packing these upgrading strategies and/or innovations suiting their conditions and finally empower them for future successful dissemination and capacity building.

Conflict of Interest

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

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Appendix

Annex 1. Morogoro local stakeholders main crop/sub-sector scores based on impacts.

Type of Impact	Criteria	Sub-sector/crop								
		Maize	Paddy	Sesame	Cowpeas	Sorghum	Cassava	Sunflower	Beans	Other legumes
Food security, poverty and sustainability	Direct contribution to FS	5.0	1.5	3.5	3.5	2.0	2.5	4.0	3.0	3.0
	Future potential	5.0	2.5	3.5	4.0	3.0	2.5	5.0	5.0	3.0
	# of poor HH involved in the sector	5.0	2.5	4.0	4.0	2.0	3.0	3.0	5.0	3.0
	Availability of natural resources	3.0	2.0	3.5	4.5	5.0	5.0	5.0	5.0	5.0
	Average	4.5	2.1	3.6	4.0	3.0	3.3	4.3	4.5	3.5
Structure of the chain	Extent of value adding potential (stability, profitability)	3.0	2.0	4.5	2.5	1.0	2.0	2.0	5.0	4.0
	# of different products produced	3.0	2.5	2.5	1.5	1.0	2.0	2.0	2.0	2.0
	Lenght of marketing chain (# of intermediaries)	4.5	1.5	4.5	3.5	1.0	2.0	3.0	2.0	2.0
	Marketing potential	4.5	2.5	4.5	5.0	1.0	2.0	3.0	2.0	2.0
	Potential for lessons learnt/ replication mechanism	5.0	3.5	4.0	4.5	2.0	3.0	5.0	2.0	3.0
	Average	4.0	2.4	4.0	3.4	1.2	2.2	3.0	2.6	2.6
	Overall average	4.3	2.3	3.8	3.7	2.1	2.7	3.6	3.6	3.1

Note: A score of 1 meaning that the particular commodity did not meet that criteria (minimum compliance), and a score of 5 meaning that the commodity best met that criteria (maximum compliance).

Annex 2. Dodoma local stakeholders main crop/sub-sector scores based on impacts.

Type of impact	Criteria	Sub-sector/crop							
		Millet	Sorghum	Maize	Ground-nuts	Sunflower	Other Legumes	Bambara-nuts	Sesame
Food security, poverty and sustainability	Direct contribution to FS	5.0	2.5	2.0	4.5	4.0	3.0	3.0	3.0
	Future potential	5.0	3.5	3.0	5.0	4.0	3.0	3.0	4.0
	# of poor HH involved in the sector	5.0	4.5	2.5	5.0	2.0	3.0	3.5	3.0
	Average	5.0	3.5	2.5	4.8	3.3	3.0	3.2	3.3
Structure of the chain	Extent of value adding potential (stability, profitability)	2.5	1.5	2.0	2.5	5.0	2.0	1.0	1.0
	# of different products produced	2.0	2.0	1.5	3.0	5.0	3.0	1.0	1.0

Annex 2. Dodoma.

	Lenght of marketing chain (# of intermediaries)	2.5	2.5	1.5	5.0	3.0	1.0	1.5	4.0
	Marketing potential	1.0	1.5	3.0	4.5	3.0	1.0	2.0	5.0
	Potential for lessons learnt/ replication mechanism	4.0	3.5	4.0	5.0	5.0	2.0	2.5	5.0
	Average	2.4	2.2	2.4	4.0	4.2	1.8	1.6	3.2
	Overall average	3.7	2.9	2.5	4.4	3.8	2.4	2.4	3.3

Note: A score of 1 meaning that the particular commodity did not meet that criteria (minimum compliance), and a score of 5 meaning that the commodity best met that criteria (maximum compliance).

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African Journal of Agricultural Research

Full Length Research Paper

Rumen degradability of *Desmodium uncunatum*, *Mucuna pruriens* and *Vigna unguiculata* forage legumes using the *in vitro* Daisy^{II} technique

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An *in vitro* study was designed to evaluate rumen degradability of dry matter (DM), crude protein (CP), phosphorus (P) and calcium (Ca) of three legume forages; silverleaf (*Desmodium uncinatum*), velvet bean (*Mucuna pruriens*) and cowpea *(Vigna unguiculata)* (L.), Walp using the Daisy^{II} technique. Air dried samples were milled to pass through a 2 mm sieve and 0.70 g of sample was weighed into the Daisy^{II} bags. Rumen liquor was taken from 2 fistulated Friesian cows on dairy ration and the samples were incubated for 2, 4, 8, 12, 18, 24, 48, 72 and 96 h. Dry matter, CP, P and Ca disappearance of cowpea was highest at 96 h of incubation. Effective degradability (at 2, 4 and 6% out flow rates) of DM, CP and P of cowpea was the highest and was significantly different from that of velvet bean and silverleaf (P<0.05). Dry matter effective degradability of velvet bean was significantly higher than that of silverleaf. However, the CP effective degradability of silverleaf was significantly higher than that of velvet bean. Effective degradability of Ca was significantly the highest in velvet bean forage, followed by cowpea at all the assumed outflow rates. The disappearance profiles of these forage legumes indicated that they can be degraded in the rumen to produce microbial protein.

Key words: *In vitro*, rumen degradability, herbaceous legumes.

INTRODUCTION

Studies have revealed that the use of tree and shrub forages as supplements to different basal diets leads to improved ruminant livestock performance (Ondiek et al., 2000). Baloyi et al. (2008) reported that legume forages provide protein, vitamins and mineral elements, which are lacking in mature natural veld, particularly during the dry season. Thus, legumes which are also optional for soil fertility enhancement (Saha et al., 1997), due to their

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high nitrogen content, can be used as nitrogen supplements in ruminant diets. Castillo et al. (2001) postulated that supplementation of readily degraded energy will enhance the utilisation of available N (nitrogen) in the rumen and further improve the productivity through an increased efficiency of utilisation of ammonia-N for microbial protein synthesis. Calcium and phosphorus are accepted as important essential mineral elements in animal nutrition. Deficiencies of these minerals can limit animal performance and result in various health problems. Therefore, a snapshot on rumen disappearance of DM, CP, P and Ca is vital when legumes are used as supplements.

Mota et al. (2005) noted that the commonly used rumen *in situ* technique to determine crude protein rumen degradability may over-estimate its disappearance in dried and ground leaves because small particles pass through the bag pores without being degraded. Moreover, *in situ* technique is relatively expensive, time-consuming and tedious for regular evaluation and animal ethics are disputed (Stern et al., 1997). The *in vitro* Daisy^{II} technique mimics *in vivo* conditions and is widely adopted as a method for analyzing rumen degradability of forages. Studies done by Wilman and Adesogan (2000) evaluated the two techniques and established that the Daisy^{II} system presented a faintly less accurate estimation of ruminant *in vivo* degradability. However, Holden (1999) established that the *in vivo* and *in vitro* Daisy^{II} systems are comparable. This study was designed to aim at *in vitro* evaluation of rumen disappearance of dry matter (DM) crude protein (CP) phosphorus (P) and calcium (Ca) of three legume forages; silverleaf, velvet bean and cowpea using the Daisy^{II} technique.

MATERIALS AND METHODS

Forage materials from 3 species (*Mucuna pruriens, Vigna unguiculata* and *Desmodium uncinatum* were harvested from legume plantations grown in summer at the University of Venda farm (22° 56' 60S and 30° 28' 60E; altitude 709 m; 752 mm of rainfall per year with most rainfall occurring mainly during mid-summer; average minimum temperature 7.5°C and maximum 30.3°C). The forages were air dried under shade and the dried samples were then milled to pass through a 2 mm sieve. The *in vitro* Daisy^{II} experiment was carried out as done by De Figueiredo et al. (2000) at ARC Pretoria (Irene). Rumen liquor was taken from 2 rumen fistulated Friesian cows on dairy ration (19% crude protein dairy meal and maize silage) and kept in a flask bottle preheated with 39°C water. The rumen inoculum was blended at high speed for 30 s in a blender container flushed with CO_2. The blended inoculum was strained through four sheets of cheesecloth and constantly flushed with CO_2. The ratio 5:1 of buffer to rumen fluid was used for the Daisy^{II} procedure. An amount of 0.70±0.05 g of sample was weighed into the Daisy^{II} bags and 1600 ml buffer: rumen mixture was added to each digestion jar of Daisy^{II}. The digestion containers were purged for 30 s, the caps secured and the samples digested for 2, 4, 8, 12, 18, 24, 48, 72 and 96 h. After incubation, the jars were removed and the fluid drained. The bags were then immersed for 5 min in acetone and then rinsed thoroughly with cold water until the water was clear using a minimum agitation. The experiments were simulated 3 times.

Total CP, P and Ca were measured following procedures outlined by AOAC (2000). Disappearance of DM, CP, P and Ca of the three legume samples was calculated from their respective loss from the bags after washing (zero samples) and incubation into the rumen. The degradability characteristics were calculated according to the models:

$$P = a+b\ (1-e^{-ct}) \quad \text{(Ørskov and McDonald, 1979)} \quad (1)$$

$$P = a+b\ (1-e^{-c\ (t-tl)}) \quad \text{(McDonald, 1981)} \quad (2)$$

Time specific degradation and disappearance of DM, CP, P and Ca were calculated using equation (1) incorporating the modification done by Wulf and Südekum (2005) which presumes that there is zero degradation for the duration of the lag phase (Edmunds et al., 2012):

$$\text{Effective degradability (g/kg CP)} = a + (bc/\ (c + K_p)e^{-KpL} \quad (3)$$

Where: K_p is the rumen kinetics (outflow rate); a is the rapidly degradable fraction; b *is* the slowly rumen degradable fraction (estimated as d- a where d is the slowly rumen degradable part); c is the rate of disappearance of fraction b and L is the lag phase. Effective degradability was calculated at passage rates (K_p) of 0.02; 0.04 and 0.06/ K_p 2; 4; 6) to characterise extensive, semi-extensive and intensive feeding and representative rates of ruminal passage rates for forages. The degradation and disappearance values of the legumes were calculated using the model:

$$Y_{ijk} = \mu + A_i + I_j + (I \times A)_{ij} + e_{ijk}$$

Where; Y_{ijk} = DM, N, Ca or P disappearance constants; μ = mean; A_i = effect legume; I_j= incubation period; $(I \times A)_{ij}$ = interaction between incubation and legume; e_{ijk} = residual error.

Analysis of variance (ANOVA) was carried out on the disappearance values using GLM of (SAS 9.3 2009). The statistical significance of the different means was tested by means of the Duncan test.

RESULTS AND DISCUSSION

The nutritional composition of the three legumes is presented in Table 1. Silverleaf had a significantly higher DM than the other legumes (P<0.05). CP was highest in cowpea followed by silverleaf and velvet bean. Minerals as measured in ash were significantly higher in velvet bean contrasted to the other two legumes. Both CF and MADF were highest in the velvet bean and lowest in the cowpea. Silverleaf had significantly higher tannins level followed by velvet bean and cowpea.

Generally, DM, CP and P disappearance in cowpea were the highest (P<0.05) (Figures 1, 2, 3 and 4). Dry matter, CP and P effective degradability of cowpea were the highest and significantly different from velvet bean and silverleaf (P<0.05) (Tables 2 and 3). Dry matter effective degradability of velvet bean was significantly higher than that of silverleaf. However, the CP effective degradability of silverleaf was significantly higher than that of velvet bean. Effective disappearance of Ca was significantly highest in velvet bean forage followed by cowpea (P<0.05).

Table 1. Chemical composition g/kg dry matter (DM) of velvet bean, silverleaf and cowpeas.

Feed	DM	CP	P	Ca	ASH	CF	MADF	Tannins
Velvet bean	803.7^{c}	147.9^{c}	2.0^{c}	2.4^{c}	121.3^{a}	252.6^{a}	390^{a}	4.4^{b}
Silverleaf	963.6^{a}	163.4^{b}	3.0^{b}	4.8^{a}	46.1^{c}	227.5^{b}	270^{b}	11.2^{a}
Cowpeas	855.1^{b}	180.0^{a}	3.2^{a}	3.8^{b}	88.1^{b}	203.5^{c}	225^{c}	1.2^{c}
SEM	1.1	0.79	0.06	0.11	0.72	0.45	3.36	0.09

DM = Dry matter CF = Crude fibre MADF = Modified acid detergent fibre P = Phosphorus Ca = Calcium. ^{abc}Means within a column with different superscripts are significantly different ($P \leq 0.05$).

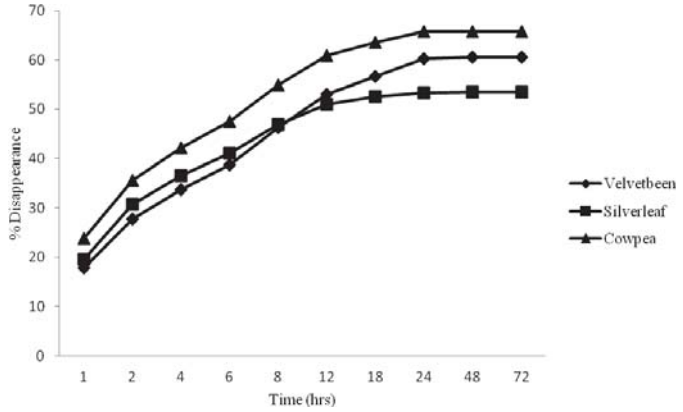

Figure 1. *In vitro* dry matter degradability (disappearance %) of velvet bean, silverleaf and cowpea.

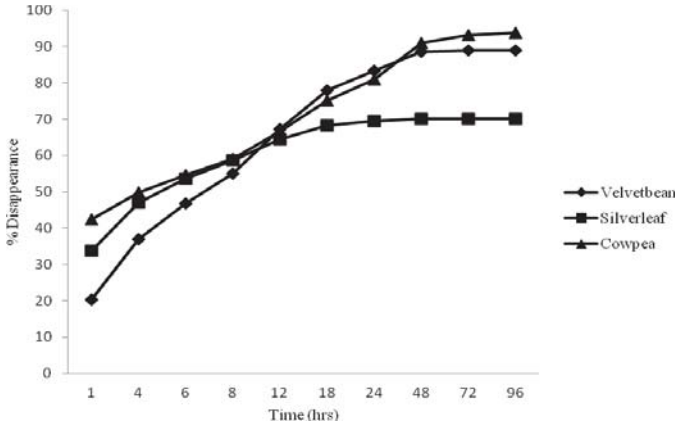

Figure 3. *In vitro* phosphorus disappearance (%) of velvet bean, silverleaf and cowpea.

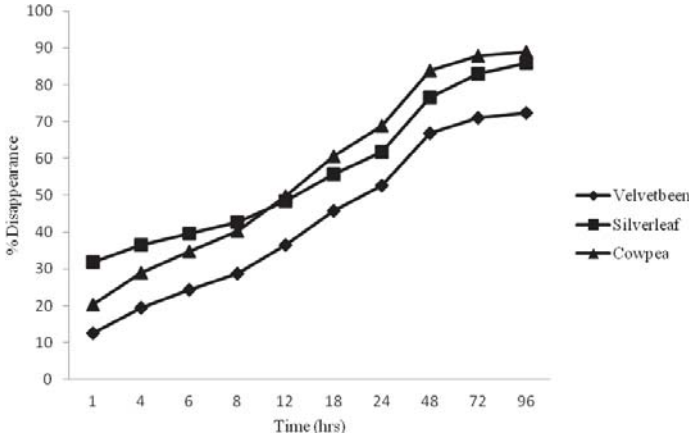

Figure 2. *In vitro* crude protein degradability (disappearance %) of velvet bean, silverleaf and cowpea.

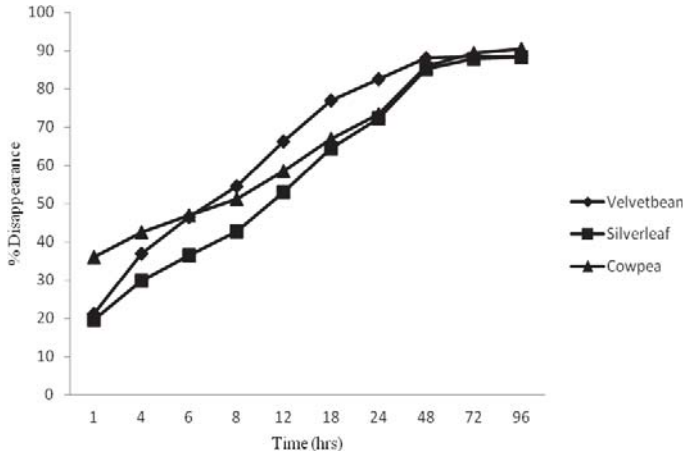

Figure 4. *In vitro* calcium disappearance (%) of silverleaf, velvet bean and cowpea.

The degradability of silverleaf is rather low and even much lower values have been recorded (Mtimuni, 1978). Silverleaf contains tannins that are responsible for the precipitation of protein to form complexes that negatively manipulates protein and fibre degradation in the reticulo-rumen (Baloyi et al., 2001). The low DM and CP values of degradability in this study indicate a possible significant tannin-induced depression for silverleaf. Moreover, tannins can decrease the fermentability of plant cell walls either by forming indigestible complexes with plant proteins or cell wall polysaccharides or by precipitating microbially-secreted cell wall degrading enzymes (Barry et al., 1986). Tannins may also have bactericidal or

Table 2. Mean dry matter (DM) and crude protein (CP) rumen degradability constants and calculated effective disappearance (ED) of velvet bean, silverleaf and cowpea at outflow rates (p = 0.02, 0.04 and 0.06).

Sample	Degradability constants			Effective degradability		
	a	b	c	0.02	0.04	0.06
Dry matter						
Velvet bean	12.88^{bc} (0.296)	47.66^{ab} (0.635)	0.113^{bc} (0.007)	53.38^{b} (0.61)	48.10^{b} (0.73)	44.03^{b} (0.78)
Silverleaf	13.31^{b} (1.053)	40.15^{b} (0.97)	0.160^{a} (0.016)	48.98^{c} (0.60)	45.10^{c} (0.95)	42.48^{c} (1.17)
Cowpea	17.69^{a} (0.965)	48.08^{a} (0.956)	0.132^{b} (0.0039)	59.46^{a} (1.73)	54.61^{a} (1.61)	50.78^{a} (1.52)
Crude protein						
Velvet bean	17.45^{b} (0.592)	67.17^{a} (7.082)	0.027^{c} (0.005)	55.62^{c} (5.95)	44.18^{c} (4.78)	38.03^{c} (3.94)
Silverleaf	17.33^{bc} (1.96)	53.58^{c} (2.986)	0.075^{a} (0.033)	58.6^{b} (6.74)	51.14^{b} (7.42)	46.05^{b} (7.48)
Cowpea	30.70^{a} (1.393)	59.98^{ab} (1.363)	0.052^{ab} (0.008)	73.98^{a} (2.18)	64.58^{a} (2.8)	58.55^{a} (2.66)

^{abc}Means within a column with different superscripts are significantly different (P ≤ 0.05) ( ) standard error.

Table 3. Mean phosphorus and calcium rumen disappearance constants and calculated effective disappearance (ED) of velvet bean, silverleaf and cowpea at outflow rates of p = 0.02, 0.04 and 0.06).

Sample	Degradability constants			Effective degradability		
	a	b	c	0.02	0.04	0.06
Phosphorus						
Velvet bean	12.86^{c} (1.13)	54.92^{b} (1.43)	0.12^{b} (0.035)	77.01^{ab} (2.79)	68.8^{b} (4.09)	62.5^{b} (4.75)
Silverleaf	24.02^{b} (2.07)	44.69^{c} (0.76)	0.19^{a} (0.019)	64.33^{c} (1.54)	60.74^{c} (1.51)	57.74^{c} (1.53)
Cowpea	37.65^{a} (1.86)	75.34^{a} (1.72)	0.062^{c} (0.0025)	79.12^{a} (1.48)	70.96^{a} (1.6)	65.49^{a} (1.65)
Calcium						
Velvet bean	14.77^{bc} (1.89)	73.26^{a} (2.47)	0.11^{a} (0.017)	77.06^{a} (1.23)	68.98^{a} (2.11)	62.76^{a} (2.63)
Silverleaf	16.61^{b} (1.85)	72.24^{ab} (1.89)	0.063^{b} (0.0036)	71.51^{c} (2.37)	60.89^{c} (2.27)	53.71^{c} (2.15)
Cowpea	30.56^{a} (2.45)	61.27^{c} (2.96)	0.051^{bc} (0.0024)	74.62^{b} (0.7)	64.95^{b} (1.03)	58.76^{b} (1.31)

^{abc}Means within a column with different superscripts are significantly different (P ≤ 0.05) ( ) standard error.

bacteriostatic effects on the rumen bacteria thus reduce the fermentation of feedstuffs (Longland et al., 1995).

The effective nitrogen degradability (at a passage rate of 0.02) is between the range of 32 and 80 per cent (Mgheni et al., 1993) for some tropical herbaceous legumes (*V. unguiculata* (*L.*) *Walp*, *D. uncinatum*, *Neanotonia wightii*, *Pueraria phaseoloides* and *Leucaena leucocephala*). The benefits of highly degradable forages as a supplement embrace improved energy and protein intake, improved feed efficiency, increased availability of minerals and vitamins and improved rumen function (Nyambati et al., 2003). In this study, the 3 legumes showed relatively high values of readily degradable crude protein with cowpea having the highest value. This is vital because readily degradable proteins are a major aspect in determining susceptibility to microbial proteases and thus, their degradability.

The extent of protein degradation in the rumen gives a measure of the available nitrogen to microorganisms and by-pass protein to the small intestine (Promkot and Wanapat, 2003). Protein quality can be determined through the use of rumen degradability characteristics of the protein, particularly the proportion of soluble degradable protein compared to undegradable rumen protein (Crawford et al., 1978). Concurrently, determination of Ca and P bioavailability is also important because the minerals are required for both host animal and rumen microbes. The high disappearance values of Ca and P in the legumes shows that the minerals are accessible by the rumen microbes. Therefore, optimum microbial protein yield may not be hindered by the availability of these macro-minerals.

Limitation of the *in vitro* degradability of feed in Daisy[II] is the late affect of low pH. While the optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0, degradation is reduced at the lower end of the pH environment (Cardozo et al., 2002). Moreover, the effect of pH and the substrate being fermented may affect the principal microbial inhabitants and alter protein degradation due to interactions among nutrients. Therefore, the reduction in cellulolytic bacteria as an outcome of low pH leads to or results in a decrease in fiber degradation plummeting access of proteolytic bacteria to proteins, ultimately reducing protein degradation (Stern et al., 2006). However, optimum amount of buffer was used in this study to simulate rumen degradability of the legume forages.

Conclusion

Generally, DM, CP, P and Ca disappearance in cowpea was highest at 96 h of incubation. Dry matter, CP and P effective degradability of cowpea were the highest and significantly different from velvet bean and silverleaf. The disappearance profiles of these forage legumes showed that they can be degraded in the rumen.

Conflict of Interest

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

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Full Length Research Paper

Influence of soil moisture levels and packaging on postharvest qualities of tomato (*Solanum lycopersicum*)

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Preharvest factors influence postharvest quality of tomatoes. Whereas water stress improves fruit total soluble solids; and polyethylene is used for packaging of fresh horticultural produce, little is known about their combined effects on quality and shelf life of tomatoes. The objective of this study was to investigate the independent and interactive effects of deficit irrigation and packaging on postharvest quality and shelf life of tomatoes. The experiment was a split plot arranged in a Randomized Complete Block Design with three replicates. Packaging was the main treatment and water levels the sub treatments. Water treatments were 20, 40, 60, 80 and 100% of pot capacity (PC). Packaging treatments were perforated, non-perforated and non-packaged (control). Fruits harvested at breaker stage were stored at 21±2ºC. Quality parameters assessed were fruit weight loss, colour change, firmness, total soluble solids, titratable acidity and shelf life. Polyethylene bags commonly used in the market (22 x 6.37 cm of size; 0.02 mm of thickness) were used as packaging material. At 16 days storage, unpackaged fruit had lost 34.23% of initial weight compared to 9.06% in perforated and 4.43% in non-perforated packaging. At 8 days of storage, 20% PC fruits were firmer than 80% PC fruits. At 10 days storage, 20% PC fruits were firmer compared to those from 40 and 80% PC. Total Soluble Solids (TSS) increased with decrease in moisture level. At 10 DAH, the lowest TSS was recorded in fruits subjected to 100% PC and highest in 40% PC. Deficit Irrigation effectively regulates tomato fruit quality; and combining it with packaging can enhance shelf life of tomato fruits.

Key words: Water stress, packaging, fruit firmness, total soluble solids (TSS).

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most popular fruit vegetables worldwide and plays a vital role in providing substantial quantities of vitamins C and A in human diet. The fruits are eaten either raw or cooked. Being a climacteric and perishable vegetable, tomatoes have a very short lifespan, usually 2 to 3

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weeks. Deterioration of tomato is brought about by several factors such as the harvesting stage, postharvest handling including packaging material (Mathooko, 2003). Tomato is sensitive to many environmental stresses, including extreme temperature, drought, salinity and inadequate moisture (Nahar et al., 2011). Water plays an important role in plant life; and is the major limiting factor in agricultural production. According to Nahar et al. (2011), since water is essential for plant growth, it is axiomatic that water stress will affect plant growth, yield and quality of yield. The degree to which they will be affected will depend on the severity and duration of the water stress. Judicious use of water in agriculture is a priority and adoption of irrigation strategies which use less water while maintaining satisfactory yields, thus improving water use efficiency may play a key role in preservation of this scarce resource (Patanè et al., 2011). Deficit irrigation (DI) is a water saving strategy whereby crops are subjected to a level of water stress either during a particular period or throughout the entire growing season.

Adequate soil moisture during preharvest periods is essential for the maintenance of postharvest quality. During the growing season, water stress can affect the size of the harvested plant organ, and lead to soft or dehydrated fruit that is more prone to damage and decay during storage (Shewfelt and Prussia, 1993). Postharvest qualities of tomatoes partly depend upon preharvest factors such as cultural practices, genetic and environmental conditions (Meaza et al., 2007). Although growth can be adversely affected due to water stress, fruit quality parameters such as total soluble solids usually improve (Birhanu and Tilahun, 2010). Birhanu and Tilahun (2010) found that total and marketable yield of tomato was lowest in the most stressed treatment (75% water deficit); but fruit soluble solid content increased with increase in water stress. In their study, Nahar et al. (2011) observed that water deficit stress did not cause physical and internal tissue damages in fruits; but instead improved the quality of fruits by increasing different solutes and organic acids.

Packaging of fresh horticultural produce is carried out to prevent kinds of degradation that might render it unsuitable for consumption. According to Kader and Rolle (2004), the principal purpose of packaging is to reduce damage during transport. As a feature of proper packaging in a sealed package, a fresh product will create a modified atmosphere by respiration and gas permeation through the packaging material (Sammi and Masud, 2009). Packaging has been reported to significantly reduce fruit weight loss (Sammi and Masud, 2009); and that tomatoes sealed in plastic films have an extended marketable life. Polyethylene is the most commonly used polymer film used for packaging of fresh horticultural products. Its advantages are that it is inert, permeable to gases and impermeable to water vapour. Consumers are interested in produce having long shelf life with minimal change in quality attributes during storage. An increase in the storage life and superior fruit quality is therefore desirable to the consumer (Sammi and Masud, 2009). Important tomato quality criteria to both traders and consumers are appearance, firmness, ripening behaviour and shelf life. In the developing countries, consumers are rarely concerned with the flavour and nutritive value.

Water deficit during production and packaging are important factors which have been found to influence the postharvest quality and shelf life of tomatoes. Few studies if any, have evaluated the combined effect of deficit irrigation and packaging on the quality and shelf life of tomatoes. The present study was therefore undertaken with the objective of investigating the independent and interactive effects of deficit irrigation and packaging on postharvest quality and shelf life of tomatoes.

MATERIALS AND METHODS

Field experiment

Tomato seeds (cv. 'Moneymaker') were sown in plastic pots in polythene covered greenhouse at the Horticulture Research and Demonstration Field, in Egerton University (Kenya). The plants were subjected to five different soil moisture levels (20, 40, 60, 80 and 100% of the pot capacity) until harvesting. Each water treatment had 6 plants and was replicated 4 times. Each plastic pot (20.32 x 35.56 cm in size) contained 10 kg of air dried soil (a mixture of sand, top soil and manure at the ratio of 1:2:0.5) and were arranged in a randomized complete block design (RCBD) (Appendix Figure 1). The top of the containers were covered with black plastic to prevent evaporation and were put on top of a plastic paper to avoid direct contact with the soil surface. The amount of water to be applied for each treatment was determined on the basis of the percentage of pot water capacity.

Laboratory experiment

Tomato fruits were harvested at the breaker stage from the field trial and stored in a temperature controlled chamber at 21±2ºC. The laboratory experiment was a split plot arranged in a Randomized Complete Block Design (RCBD) (Appendix Figure 2); with packaging as the main treatment and water levels being the sub treatments. The water treatments comprised of 5 levels (WS1: 100% of PC, WS2: 80% of PC, WS3: 60% of PC, WS4: 40 % of PC and WS5: 20% of PC) whereas packaging had 3 levels [Perforated (P), Non-Perforated (NP) and Non-Packaged or control (C)] and were replicated three times. Two trials were conducted, with the first trial running from June to July 2010, and the second trial from August to October 2010. Five fruits were used for each of the treatments. The quality parameters assessed during storage were fruit weight loss, colour change, firmness, total soluble solids (TSS), titratable acidity (TA) and fruit shelf life. The type of packaging used was the polyethylene bags, commonly used in the market - high density polyethylene bag - (HDPE: 22 x 6.37 cm of size; 0.02 mm of thickness). The bags were perforated with a punch (Model: Kangaroo Punch DP 520-8 cm of 2.5 mm punching probe).

Fruits were harvested at the breaker stage; and colour change determined on two fruits per treatment per replicate by use of a tomato colour chart according to Abdullah et al., (2004). Fruit firmness was analysed by a destructive procedure on 2 fruits per treatment per replication using a handheld penetrometer [Fruit

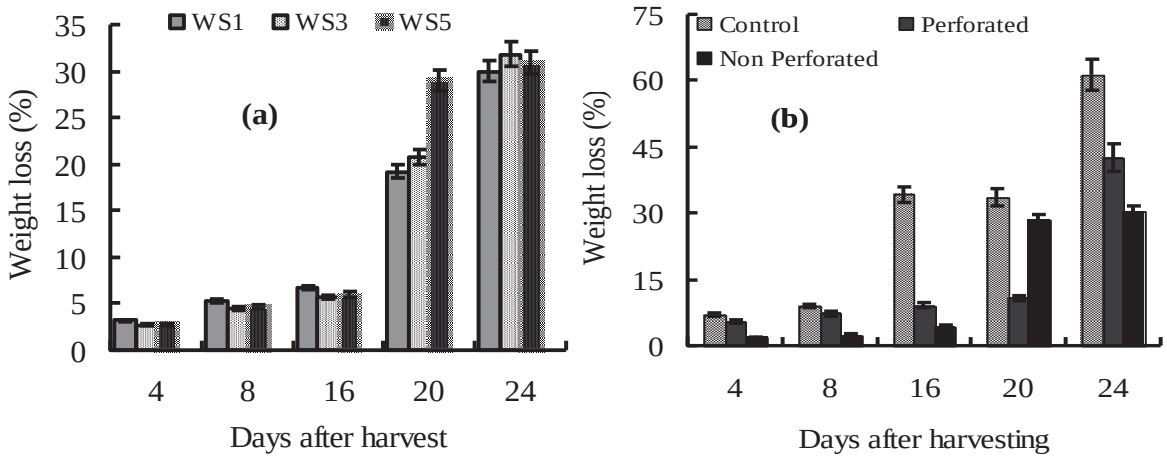

Figure 1. Effect of (a) Water stress and (b) Packaging on weight loss of harvested tomato (WS1 = 100% of PC; WS3 = 60% of PC; WS5 = 20% of PC).

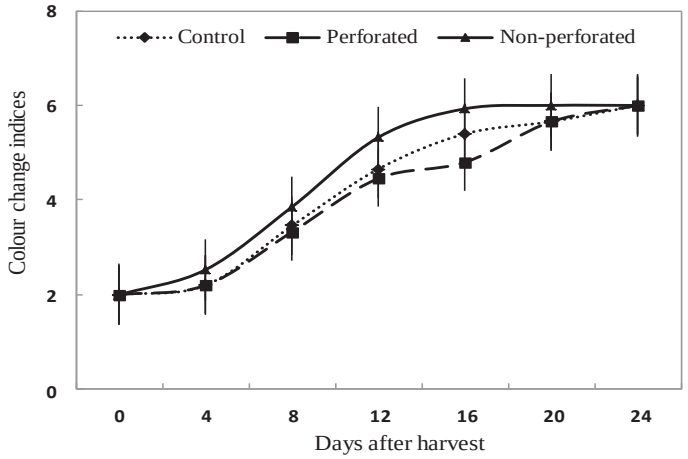

Figure 2. Influence of packaging on tomato fruit colour change.

pressure tester, Model: *FT 327* (1-12 kg), with 0.7 x 0.92 mm of probe size] and was recorded at the equatorial surface for each individual fruit. The firmness and colour readings were taken at harvest (day 0) and thereafter at 2-day intervals until termination of the experiment.

Total soluble solids (TSS) were determined on two fruits per treatment per replicate using a hand-held refractometer [Model SKU: MT- 032 (^{o}Brix, 0-32%)]. Determination was done by calculating the average TSS for the 2 fruits per treatment for each replicate. The final value was obtained by determining the average of the replicate for each treatment.

Fruit juice (5 ml) from 2 fruits per treatment per replicate was titrated with 0.1 N NaOH to pH 8.1 using phenolphthalein as an indicator and the percentage titratable acidity (TA) was calculated using the following formula by Monash Scientific (2003):

TA (g/l) = T x M x 0.75/ V x 10 x 0.1

Where,
M= Molarity (M) of 0.1 M NaOH
V= Volume (ml) of sample
T= Titre (ml) of 0.1 M NaOH

The fruit shelf life was considered to have elapsed when the fruits lost 75% of their initial weight (Marcos et al., 2005) and/or started showing signs of shrivelling and decay.

Data collected were subjected to Analysis of Variance (ANOVA) using SAS version 9.2 and mean separations were done using Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS

Fruit weight loss

Soil moisture levels had significant effects on the fruit weight. At harvest, fruit weight increased with increasing moisture levels. The highest fruit weight was recorded in fruits harvested from plants subjected to 100% Pot Capacity (PC); while those with the lowest weight were from plants which received 20% PC. All treatments were significantly different at harvest up to 16 days of storage or days after harvest (DAH). However, at 20 and 24 DAH, there was no significant difference in weight between fruits subjected to 80 and 100% PC. The differences in weight loss between all other treatments were significant (Figure 1a).

During the first 12 days of storage, packaging did not significantly influence fruit weight. Weight loss for fruits in non-perforated package was significantly lower compared to the control (non-packaged fruits) at 16 and 24 DAH (Figure 1b). Differences between perforated and non-perforated packaged fruits were not significant.

Colour change

Packaging had a significant effect on fruit colour change. Fruits packaged in non-perforated bags developed colour

Table 1. Effect of moisture levels on fruit firmness (Kgf).

Water stress (Pot capacity)	Days after harvest					
	0	2	4	6	8	10
WS_1	5.53[a]*	4.60[ab]	4.04[bc]	4.23[a]	4.69[ab]	3.88[ab]
WS_2	6.03[a]	3.98[b]	3.23[c]	4.30[a]	3.47[b]	3.24[b]
WS_3	5.94[a]	5.50[a]	4.90[ab]	4.69[a]	4.36[ab]	4.40[ab]
WS_4	5.30[a]	5.32[a]	5.46[a]	4.40[a]	4.29[ab]	3.71[b]
WS_5	5.52[a]	4.57[ab]	4.72[ab]	4.34[a]	5.12[a]	5.22[a]

*Means with the same letter(s) within a column are not significantly different at $P \leq 0.05$.

Table 2. Effect of packaging on tomato fruit firmness (Kgf).

Packaging	Days after harvest					
	0	2	4	6	8	10
Control	5.61[a]*	5.39[a]	4.63[a]	5.03[a]	4.63[a]	3.43[b]
Perforated	5.86[a]	3.84[b]	4.33[a]	4.25[b]	4.71[a]	4.86[a]
Non-perforated	5.53[a]	5.15[a]	4.45[a]	4.45[b]	3.81[a]	3.98[ab]

*Means with the same letter(s) within a column are not significantly different at $P \leq 0.05$

faster than those in perforated packages and the control (Figure 2). From 4 to 16 DAH, significant differences were observed between the perforated and non-perforated packaging. At 16 DAH, significant differences were observed between all treatments with the colour index being highest in fruits packaged in non-perforated bags.

The interaction between water levels and packaging treatments was significant. At 12 DAH, non-perforated packaged fruits from 40% PC had a significantly higher fruit colour development than control (unpackaged) fruits from 60% PC. The colour change in fruits from 100% PC in perforated bags was significantly slower compared to all other treatments in control and non-perforated packages and fruits from the 80 and 60% in the perforated bags at 16 DAH.

Fruit firmness

Fruit firmness was significantly influenced by soil moisture levels. At 2 days of storage, fruits from 80% PC were less firm compared to those from 60 and 40% PC. 4 days after storage, fruits from 80% PC were significantly softer compared to all other treatments except 100% PC; while at 8 days of storage, 20% PC fruits were firmer than 80% PC fruits; and at 10 days storage they (20% PC) were firmer compared to those from 40 and 80% PC (Table 1).

After 2 days storage, fruits in perforated packages were softer than those in non-perforated packages and control (unpackaged). After 6 days storage, control fruits were firmer than those packaged (perforated and non-perforated). At 10 days storage, fruits in perforated packages were firmer than the control fruits (Table 2).

Fruit total soluble solids (TSS)

Fruit Total Soluble Solids (TSS) increased with decrease in moisture level (% PC). At the time of harvest, fruits from plants subjected to 20% PC had significantly higher TSS compared to all other treatments; while those subjected to 40 and 60% PC had higher TSS compared to 80 and 100% PC fruits. A similar trend was observed throughout the storage period (Table 3). At 10 DAH, the lowest TSS was recorded in fruits subjected to 100% PC and the highest in 40% PC. However, the differences in TSS between the 20, 40 and 60% PC fruits at 10 DAH were not significant. TSS generally increased with increase in storage time. Packaging had no significant influence on the fruit TSS. Significant effects on TSS were observed due to the interaction between water and packaging treatments. After 2 days storage, fruits from 20% PC plants in perforated packaging had significantly higher TSS than 100% PC perforated and non-perforated packaged fruits (Table 4). After 6 days of storage fruits subjected to 20% PC in non-perforated packaging had higher TSS compared to those from 100% PC in non-perforated packaging and unpackaged. At the end of the storage period (10 DAH), unpackaged 100% PC fruits had significantly lower TSS than unpackaged 80, 60, 40 and 20%; perforated 20 and 40% and 60 and 40% PC non-perforated packaged fruits (Table 4).

Fruit titratable acidity (TA)

Fruits from 20% PC exhibited the lowest TA while those

Table 3. Effect of moisture levels on tomato fruit TSS (° Brix).

Water stress	Days after harvest					
	0	2	4	6	8	10
WS_1	4.06^{c*}	4.46^{c}	4.37^{d}	4.28^{c}	4.71^{c}	4.28^{d}
WS_2	4.42^{c}	4.71^{bc}	4.94^{c}	5.03^{b}	5.15^{b}	5.16^{c}
WS_3	4.93^{b}	5.12^{ab}	5.68^{ab}	5.33^{ab}	5.31^{b}	5.43^{bc}
WS_4	5.38^{b}	5.43^{a}	5.52^{b}	5.28^{b}	5.78^{a}	5.82^{a}
WS_5	5.90^{a}	5.52^{a}	6.08^{a}	5.79^{a}	5.81^{a}	5.63^{ab}

*Means with the same letter(s) within a column are not significantly different at P ≤ 0.05.

Table 4. Interactive effects of water and packing on the TSS (° Brix) of tomato fruits.

WS	DAH packaging								
	2			6			10		
	C	P	NP	C	P	NP	C	P	NP
WS_1	5.0^{a}	4.1^{b}	4.3^{b}	4.0^{c}	4.5^{abc}	4.4^{bc}	4.2^{d}	4.2^{d}	4.5^{cd}
WS_2	4.6^{ab}	4.6^{ab}	4.9^{ab}	5.3^{abc}	4.7^{abc}	5.1^{abc}	5.5^{abc}	4.9^{bcd}	5.1^{abcd}
WS_3	5.3^{ab}	5.1^{ab}	5.0^{ab}	5.9^{ab}	5.1^{abc}	5.0^{abc}	5.6^{abc}	5.0^{bcd}	5.7^{abc}
WS_4	5.5^{ab}	5.4^{ab}	5.4^{ab}	4.9^{abc}	5.4^{abc}	5.6^{ab}	5.7^{abc}	5.5^{abc}	6.3^{a}
WS_5	4.9^{ab}	6.2^{a}	5.4^{ab}	5.7^{ab}	5.7^{ab}	6.0^{a}	6.0^{ab}	5.6^{abc}	5.3^{abcd}

Table 5. Effect of moisture levels on tomato fruit titratable acidity (%).

Water stress	Days after harvest					
	0	2	4	6	8	10
WS_1	7.90^{a*}	9.80^{b}	10.14^{ab}	10.82^{b}	11.08^{a}	11.07^{ab}
WS_2	6.36^{ab}	13.23^{a}	12.79^{a}	14.34^{a}	10.99^{a}	12.62^{a}
WS_3	5.06^{b}	8.49^{bc}	8.38^{b}	10.24^{bc}	12.70^{a}	9.74^{abc}
WS_4	5.65^{b}	8.17^{bc}	7.84^{b}	10.74^{bc}	7.32^{b}	8.96^{bc}
WS_5	5.57^{b}	6.41^{c}	8.42^{b}	7.44^{c}	8.69^{b}	7.24^{c}

*Means with the same letter(s) within a column are not significantly different at P ≤ 0.05.

from 80% PC had the highest TA (Table 5). At the start of storage, although TA tended to increase with increasing moisture levels, the differences between 20, 40, 60 and 80% PC were not significant; neither was the difference between 80 and 100% PC. At 2 and 6 DAH, fruits from plants subjected to 80% PC had the highest TA; while those from 100% PC had a significantly higher TA compared to 20% PC. No significant differences in TA were observed between fruits subjected to 100, 80 and 60% PC after 8 days storage. Likewise there was no significant difference between fruits subjected to 40 and 20% PC. In all treatments, TA tended to increase with storage time.

With respect to packaging, at 2 days storage, fruits in non-perforated packaging had lower TA compared to those in perforated packaging and control (Table 6). At 6 days storage, fruits in non-perforated packaging exhibited lower TA than the control. There were no significant differences between treatments after 8 days storage; however, after 10 days storage, fruits in non-perforated packaging had significantly lower TA compared to those in perforated packaging.

DISCUSSION

Weight loss

Most fresh fruits and vegetables contain so much water (80 to 90%), thus their quality suffer very quickly from water loss; including loss of saleable weight. Tomato quality changes continuously after harvesting. Fruit weight loss is normally due to senescence or desiccation of tomato fruits (Batu and Thompson, 1998). In this study,

Table 6. Effect of packaging on tomato fruit titratable acidity (%).

Packing	Days after harvest					
	0	2	4	6	8	10
Control	5.34[a]*	10.63[a]	10.40[a]	12.09[a]	10.82[a]	9.76[ab]
Perforated	6.94[a]	10.08[a]	9.83[a]	11.18[ab]	9.69[a]	11.55[a]
Non-perforated	6.04[a]	6.95[b]	8.31[a]	8.87[b]	9.96[a]	8.47[b]

as would be expected, fruit size and weight increased with increasing water levels. These observations are similar to those of Birhanu and Tilahun (2010) who reported that fruit size was reduced with reduction in the amount of irrigation water applied. According to Zegbe et al. (2006), reduction in fruit size is mainly due to reduced fruit water content. Larger fruit size can be the result of cell expansion or a larger number of cells and positive effect of water availability on cell division (Proietti and Antognozzi, 1996). Increase in fruit size due to higher irrigation has also been reported by Ehret et al. (2012). Since water stress results in lower fruit water content, the higher weight in well irrigated fruits is most likely due to the high fruit water content.

Plastic packaging for fresh fruits and vegetables has been in commercial use for decades. Respiring fresh fruits and vegetables sealed in plastic films will cause the surrounding atmosphere to change; in particular O_2 levels will be depleted and CO_2 levels increased. We observed that weight loss for fruits in non-perforated package was significantly lower compared to the control. According to Abdullah et al. (2004), packaging restricts the air movement around the produce, hence minimising fruit weight loss. This may be the reason why the highest percentage in fruit weight loss was observed in unpackaged fruits. MAP creates a water saturated or near saturated atmosphere around the fruit which lowers fruit transpiration rate; hence reducing water loss and shrinkage. Loss of moisture results in a reduction in fresh weight of harvested produce. This explains why unpackaged fruits lost more weight compared to fruits in non-perforated bags. Similar observations were made by Mathooko (2003), who reported that MAP reduced water loss in bell pepper by 40 to 50%. Weight loss in tomato fruits is primarily a result of water loss. According to Nyalala and Wainwright (1998), the rate of the water loss is a function of surface area and temperature; as they (surface area and temperature) increase, water loss also increases. The two authors further attribute water loss to polygalacturonase activity, which increases cell wall permeability, which results in increased transpiration.

Colour change

The influence of irrigation levels on fruit colour development did not give a very clear trend; though it (colour development) appeared to be higher at lower moisture levels. As ripening proceeds in tomato fruits, the colour changes from green to red. This, according to Li et al. (2015), is mainly due to increased lycopene and decreased chlorophyll content in fruit tissues. It has been reported that controlled or modified atmospheres delay fruit ripening at 12.8°C and that modified atmospheres resulting from the enclosure of mature green tomatoes in polyethylene or other forms of plastic packaging may also delay fruit ripening (Harold et al., 2007). According to Batu and Thompson (1998), tomato fruits sealed in plastic films change colour more slowly compared to those unwrapped. In contrast, we observed that colour change was fast in packaged fruits (especially in the non-perforated package). This variance could be due to stage of harvesting (breaker stage) and storage temperature (21±2°C). Another factor could be the package plastic type and thickness, which influences its permeability to gases (oxygen, carbon dioxide and ethylene). Similar findings that polythene packaging results in early ripening and better colour development in mature green tomatoes as well as better maintenance of the best physicochemical quality of fruit during storage to marketing was also reported by Moneruzzaman et al. (2009).

Fruit firmness

Our observations of fruit firmness decreasing with increasing moisture levels are similar to those of Proietti and Antognozzi (1996) who reported a slight decrease in firmness of irrigated olive fruits; and Ehret et al. (2012) who found that higher irrigation volumes reduced fruit firmness in blueberry. In well watered plants, (without stress), the water concentration in the fruits increase and tend to make the fruits softer during the period of storage. This might explain the higher levels of firmness that we observed in fruits from water stressed plants. Crookes and Grierson (1983) reported that as ripening progresses, the cell wall becomes increasingly hydrated and pectin in the middle lamella is modified and partially hydrolysed. The change in cohesion of the pectin gel governs the ease with which one cell can be separated from another, which in turn affects the final texture of the ripe fruit. This process occurs early at ripening stage in soft fruit such as tomato. According to Li et al. (2015),

tomato fruit hardness gradually decreased due to multiple coordinated processes, including the disassembly of polysaccharides in the primary cell wall and middle lamella and transpirational water/turgor loss. Packaged fruits were firmer than those unpackaged because MAP inhibits the synthesis and accumulation of cell wall degrading enzymes by slowing down their activities that cause fruit tissue softening. Low oxygen levels in modified or controlled atmospheres inhibit polygalacturonase activity, thus reducing the rate of fruit softening (Kapotis et al., 2004). Our findings that fruits in perforated packages were firmer than the control fruits corroborates observations by Batu and Thompson (1998) of softening of tomato fruits sealed in plastic film being significantly slower compared to those stored unwrapped.

Fruit total soluble solids (TSS)

This increased with decrease in moisture level (% PC). Similar observations were made by Birhanu and Tilahun (2010), Ehret et al. (2012). Water stress enhances sweetness of tomatoes by increasing their glucose, fructose and sucrose contents (Nahar et al., 2011). The increase in TSS with decreasing moisture level according to Patanè et al. (2011) is because water deficit induces a higher starch accumulation during the first stage of fruit growth, followed by conversion of starch into sugars during maturation. Decreased irrigation will induce greater TS and TSS contents because of a decrease in water accumulation by the fruit without any significant modification in the quantity of accumulated sugars (Patanè et al. 2011). It has been widely shown that reduced soil moisture and salt stress increase sugar content in tomatoes (Obreza et al., 2001; Hanson and May, 2003; Birhanu and Tilahun, 2010). Although water stress results in decreased yield in tomatoes, it increases brix values (Shinohara et al., 1995). The lowest TSS observed in fruits from the well and moderate water stressed plants (100, 80 and 60% PC) can be attributed to the higher water uptake by the plants which leads to the dilution of the concentration of TSS. Packaging had no significant influence on the fruit TSS. Similar observations have previously been reported. According to Mathooko (2003), MAP had no significant effect on TSS content in tomato fruit treated at the breaker stage of ripeness due to the inhibitory effect by MAP on respiration; since TSS has been reported to be closely related to the rate of respiration.

Fruit titratable acidity (TA)

Although TA tended to increase with increasing moisture levels, the differences between 20, 40, 60 and 80% PC were not significant; which was contrary to previous reports. Water stress has been reported to increase the synthesis of malic acid and increase citric acid content in tomatoes (Nahar et al., 2011). Patanè et al. (2011) found that deficit irrigation enhanced TA compared to full irrigation treatment. Fruits in non-perforated packaging exhibited lower TA than the control and perforated packaging. The relative humidity found under MAP is high; and according to Mathooko (2003), the low TA in fruits observed under such conditions is due to their higher retention of water.

Conclusion

Water levels and packaging independently and together significantly influenced the postharvest qualities of tomato. Moisture stress increased fruit total soluble solids (TSS) and preserving its firmness. In this study, the higher the water content, the faster the fruit lost its firmness. Packaging reduced loss in weight and firmness, and extended fruit shelf life. In light of the above, it can be concluded that deficit irrigation effectively regulates tomato fruit quality, and combining it with packaging, it can enhance the shelf life of tomato fruits.

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Conflict of Interest

The authors have not declared any conflict of interest.

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APPENDIX

EXPERIMENTAL LAYOUT

BLOCK 1	WS_1	WS_3	WS_4	WS_5	WS_2
BLOCK 2	WS_5	WS_4	WS_1	WS_2	WS_3
BLOCK 3	WS_2	WS_1	WS_5	WS_3	WS_4
BLOCK 4	WS_3	WS_5	WS_2	WS_4	WS_1

Figure 1. Field Layout: R**andomized Complete Block Design (RCBD).** WS_1: 100% of PC; WS_2: 80% of PC; WS_3: 60% of PC; WS_4: 40% of PC and WS_5: 20% of PC.

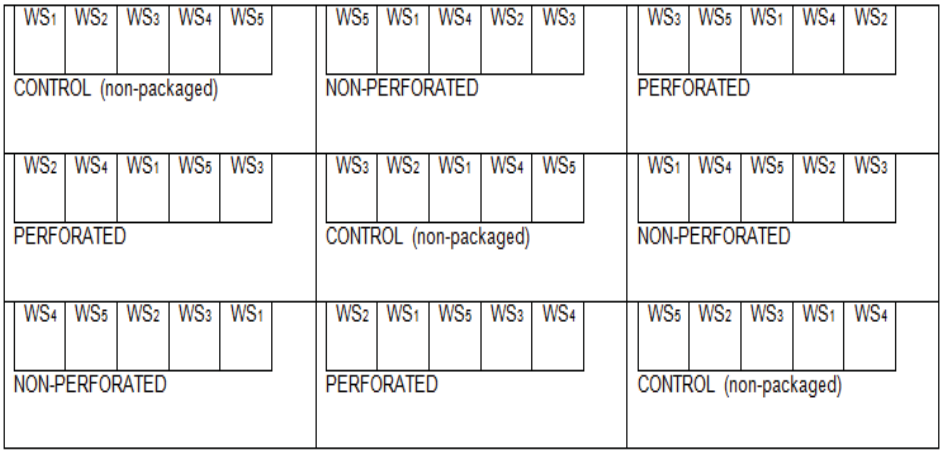

Figure 2. Laboratory Layout: Split-plot in a Randomized Complete Block Design (RCBD). WS_1: 100% of PC; WS_2: 80% of PC; WS_3: 60% of PC; WS_4: 40% of PC and WS_5: 20% of PC.

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Morphometric analysis of Sonar sub-basin using SRTM data and geographical information system (GIS)

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The study focused mainly on the geometry, more emphasis on the morphometric characteristics of the drainage basin. An attempt has been made to study drainage morphometry and its influence on hydrology of Sonar Sub-basin, M.P. India. For detailed study we used Shuttle Radar Topographic Mission (SRTM) data for preparing Digital Elevation Model (DEM), and slope maps, Geographical Information System (GIS) was used in evaluation of linear, areal and relief aspects of morphometric parameters. A total number of 196 streams were identified of which 146 are 1st order streams, 38 are 2nd order, 9 are 3rd order, 2 in 4th order and 1 is indicating 5th order stream. Drainage patterns of stream network from the basin have been observed as mainly dendritic type which indicates the homogeneity in texture and lack of structural control. The mean bifurcation ratio value is 3.65 for the study area falls within the standard range and shows that the basin conforms to the characteristics of a natural stream which indicates that the geological structures are less disturbing to the drainage pattern. The drainage density of the study area is 0.62 km/sq. km. This value indicates that for every square kilometer of the basin. This makes the study area fall into the group of low density basins which suggest that the low drainage density indicates that the basin is highly permeable subsoil and thick vegetative cover. These studies are very useful for planning rainwater harvesting and watershed management.

Key words: Morphometric analysis, shuttle radar topographic mission (SRTM) data, geographical information system (GIS), Sonar sub-basin.

INTRODUCTION

Morphometric analysis will help to quantify and understand the hydrological characters and the results will be useful input for a comprehensive water resource management plan (Jawahar raj et al., 1998; Kumaraswami and Sivagnanam, 1998; Sreedevi et al., 2001). A drainage basin is the part of the earth's surface that is drained by main stream and its tributaries. The drainage basin is fundamental geomorphic unit of land and all flow of surface is governed by its properties. It is an open system into which and from which energy flows. Drainage basin is a fundamental, precise and usually ambiguous unit that is recognized as a reliable and useful

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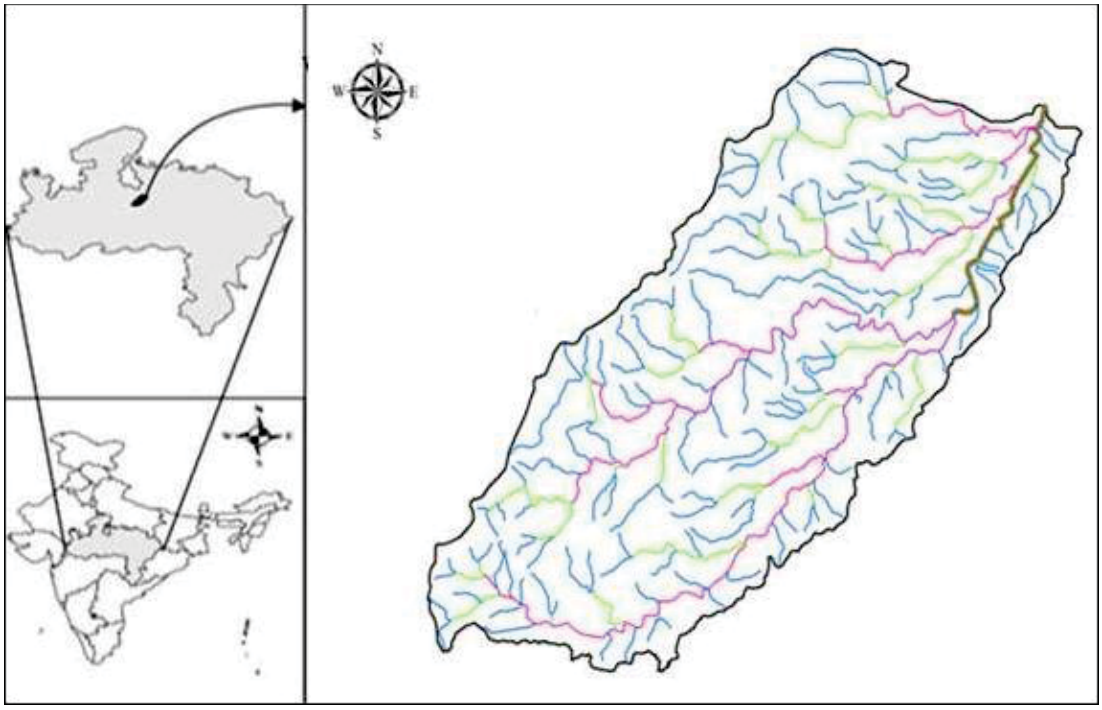

Figure 1. Location of the study area.

Planning unit. It has now formed a framework for human activities like agriculture and has guided river navigation towards sustainable agriculture (Ofomata and Umeuduji, 2000). The scientific approach to the hierarchical classification of streams and basin area was initiated by Horton (1945) who defined several drainage basin characteristics that were measurable on topographic maps. Today, these characteristics can be measured not only on topographic maps but also on satellite imageries. These characteristics include stream order, stream length, bifurcation ratio, basin area and length, perimeter, drainage density, stream frequency, elongation ratio, circularity ratio, texture ratio and form factor ratio (Shreve, 1966). The drainage characteristics of Sonar sub-basins were studied to describe and evaluate their hydrological characteristics by analyzing topographical map and SRTM data. The main occupation of the people in this area is agriculture. They depend on groundwater, because surface water resources are scarce. Due to erratic rainfall pattern and uncontrolled abstraction, groundwater levels have declined to deeper levels. Therefore watershed development schemes become important for developing the surface and groundwater resources in these areas. To prepare a comprehensive watershed development plan, it becomes necessary to understand the topography, erosion status and drainage pattern of the region. For the purpose of detailed morphometric analysis we used SRTM data for preparing DEM map slope and aspect maps. GIS was used in evaluation of Linear, Areal and Relief morphometric parameters. Using SRTM data and GIS techniques (Map Maker) is a speed, precision, fast and inexpensive way for calculating morphometric analysis (Farr and Kobrick, 2000; Smith and Sandwell, 2003; Grohmann, 2004; Grohmann et al., 2007). An attempt has been made to utilize SRTM data and the interpretative techniques of GIS to find out the relationships between the morphometric parameters and hydrological parameters.

MATERIALS AND METHODS

Study area

The Sonar sub-basin lies between 23°21′14″ to 23°50′05″ N and 78°35′48″ to 79°10′50″ E. The study area comes under the Sagar district of Madhya Pradesh, India (Figure 1). It is located in the north central part of the state of Madhya Pradesh or Bundelkhand and occupies an area of 1538 sq km (94% in Sagar and 6% in Raisen). The basin has a perimeter of 284 km and basin length of 72.12 km and an average width of about 40 km. Average annual rainfall of the region is about 1100 mm. The average numbers of rainy days are 45. The average annual potential evapotranspiration is 1852 mm. The mean annual temperature varies from 18 to 33°C.

Methodology

According to Clarke (1996), morphomerty is the measurement and mathematical analysis of the configuration of the earth surface, shape and dimensions of its landforms. The morphometric analysis carried out through measurement of linear areal and relief aspects of the basin and slop contribution (Nag and Chakraborty, 2003). SRTM has created an unparalleled data set of global elevations

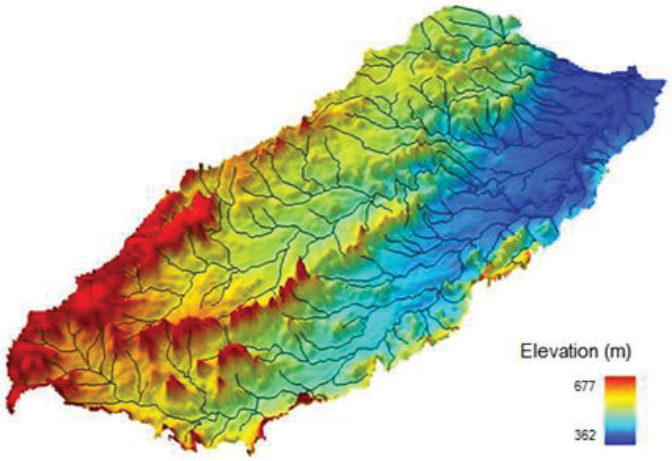

Figure 2. Digital elevation map of the Sonar sub-basin.

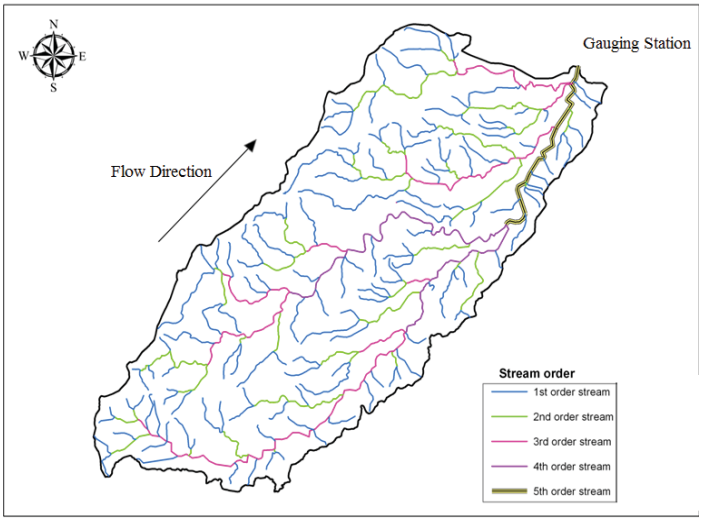

Figure 3. Drainage network of the study area.

that is freely available for modeling and environmental applications. The processed SRTM 90 m digital elevation model (DEM) for the entire globe was compiled by Consultative Group for International Agriculture Research Consortium for Spatial Information (CGIAR-CSI) and made available to the public at http://srtm.csi.cgiar.org. The SRTM DEM is a fast and inexpensive way for regional geomorphological analysis. Based on the data we prepared the digital elevation map, slope and drainage map (Figures 2, and 3) for the watershed. Stream network and micro watersheds were also prepared using ArcGIS. Based on the drainage order, the drainage channels were classified into different orders (Strahler, 1964). Basin parameters viz area, perimeter, length, stream length, stream order were also calculated which were later used to calculate other parameters like bifurcation ratio, stream length ratio, stream frequency, drainage density. Drainage texture, basin relief, relief ratio, elongation ratio, circularity ratio, and form factor were evaluated with the help of established mathematical equations (Strahler, 1964). The morphometric parameters were divided into three categories: linear, areal and relief aspects of the basin.

RESULTS AND DISCUSSION

The various morphometric parameters of the Sonar sub-basin using ArcGis 9.3 and are summarized in Tables 1 and 2

Linear aspects of the drainage basin

The linear aspects of drainage network such as stream order (N_u) bifurcation ratio (R_b), stream length (L_u) results have been, presented in Table 1.

Stream order (N_u)

The streams of the Sonar sub-basin have been ranked according to the method described by Strahler (1964), when two first order streams join, a stream segment of second order is formed, when two second order streams join, a segment of third order is formed, and so on. In the drainage basin analysis the first step is to determine the stream orders. The channel segment of the drainage basin has been ranked according to stream ordering system using ArcGIS 9.3. The study area is 5^{th} order drainage basin (Figures 3). The total numbers of 196 streams were identified of which 146 are 1^{st} order streams, 38 are 2^{nd} orders, 9 are 3^{rd} order, 2 in 4^{th} order and 1 is indicating 5^{th} order streams. Figure 4 show the results of the queries that retrieved the total number of streams for each of the order as shown in Table 1. Drainage patterns of stream network from the basin have been observed as mainly dendritic type which indicates the homogeneity in texture and lack of structural control. This pattern is characterized by a tree like or fernlike pattern with branches that intersect primarily at acute angles. The properties of the stream networks are very important to study the landform making process. The order wise total number of stream segment is known as the stream number. Laws of stream numbers states that the numbers of stream segments of each order form an inverse geometric sequence with plotted against order, most drainage networks show a linear relationship, with small deviation from a straight line. This means that the number of streams usually decreases in geometric progression as the stream order increases.

Bifurcation ratio (R_b)

Bifurcation ratio is the ratio of the number of streams of an order to the number streams of the next higher order (Horton, 1945; Strahler, 1964). Bifurcation ratios characteristically range between 3.0 and 5.0 for basins in which the geologic structures do not distort the drainage pattern (Horton, 1945; Strahler, 1964). Higher R_b indicates some sort of geological control (Agarwal et al., 2000). The mean bifurcation ratio value is 3.65 for the

Table 1. Linear aspect of the drainage network of the study area.

River basin	Stream order (U)	Stream number (N_u)	Stream length in Km (L_u)	Stream mean length in Km (L_u)	Cumulative stream length in Km (L_u)	Log N_u	Log L_u
Sonar Sub-basin	1	146	535.58	3.67	3.67	2.17	2.73
	2	38	187.82	4.95	8.62	1.58	2.28
	3	9	135.78	15.09	23.71	0.96	2.14
	4	2	60.15	30.08	53.79	0.31	1.78
	5	1	23.63	23.63	77.42	0.00	1.38
Total		196	942.96				
			Bifurcation ratio				
1st order/2nd order	**2nd order/3rd order**		**3rd order/4th order**		**4th order/5th order**	**Mean bifurcation ratio**	
3.85	4.23		4.50		2.00	3.65	

Table 2. Aerial aspects of the study area.

Morphometric parameter	Symbol/Formula	Result
Area (sq. km)	A	1538
Perimeter (km)	P	284
Drainage density (km/sq. km)	$D = \sum Lu/A$	0.62
Stream frequency	$F_s = \sum Nu/A$	0.13
Texture ratio	$T = \sum N_1 /P$	0.52
Basin Length (km)	L_b	72.12
Elongation ratio	$R_e = 2\sqrt{(A/\Pi)}/ L_b$	0.62
Circularity ratio	$R_c = 4\Pi A/P^2$	0.24
Form Factor ratio	$R_f = A/ L_b^2$	0.30

Where: $\sum Lu$ = Total stream length of all orders, $\sum Nu$ = Total number of all orders, $\sum N_1$ = Total number of 1 order streams 1st , $\Pi = 3.14$.

study area (Table 1) falls within the standard range and shows that the basin conforms to the characteristics of a natural stream which indicates that the geological structures are less disturbing to the drainage pattern.

Stream length (L_u)

Stream length is one of the most significant hydrological features of the basin as it reveals surface runoff characteristics streams of relatively smaller lengths are characteristics of areas with larger slopes and finer textures. Longer lengths of streams are generally indicative of flatter gradients. Generally, the total length of stream segments is maximum in first order streams and decreases as the stream order increases. The numbers of streams of various orders in the basin are counted and their lengths from mouth to drainage divide are measured with the help of GIS software. Plot of the logarithm of number of streams versus stream order, and logarithm of stream lengths versus stream order (Figure 4a and b) showed the linear pattern which indicates the homogenous rock material subjected to weathering erosion characteristics of the basin. Deviation from its general behavior indicates that the terrain is characterized by variation in lithology and topography.

Aerial aspects of drainage basin

Basin area (A_u)

The area of Sonar sub-basin is 1538 km^2. If the basin size is small, it is likely that rainwater will reach the main channel more rapidly than in a larger basin, where the water has much further to travel. Lag time will therefore be shorter in the smaller basin. The length of the Sonar sub-basin is 72.12 km. The shape of the basin is significant since it affects the stream discharge characteristics. It has long been accepted that a circular area is more likely to have a shorter lag time and a higher peak flow than an elongated basin. Three dimensionless ratios viz., form factor, circularity ratio and elongation ratio, reflect the basin shapes.

Form factor ratio (R_f)

It is the ratio of a basin area A_u (Horton, 1932) to the

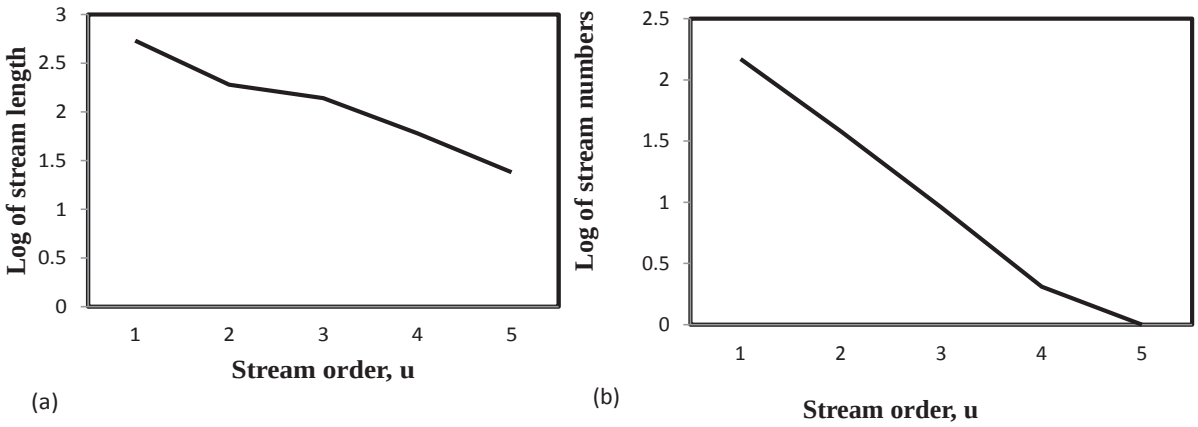

Figure 4. (a, b) Morphometry of streams.

square of the basin length L_b. The form factor value of the basin is 0.30 which indicate lower value of form factor and thus represents leaf shape tending towards elongation. The oval basin tending towards elongation with low form factor indicates that the basin will have a flatter peak of flow for longer duration. Flood flows in elongated basins are easier to manage than that of the circular basins (Nautiyal, 1994).

Circularity ratio (R_c)

Circularity ratio is defined as the ratio of basin area (Au) to the area of circle (A_c) having the same perimeter (P_r) as the basin (Miller, 1953). He described the basin of the circularity ratios less than 0.5 which indicates strongly elongated and highly permeable homogenous geologic materials. The circularity ratio value 0.24 of the basin does not corroborates the Miller's range which indicated that the basin is elongated in shape, low discharge of runoff and highly permeability of the subsoil condition but rather the basin of the study area is pear in shape with high level of integration.

Elongation ratio (R_e)

It is the ratio of the diameter of a circle of the same area as the basin to the maximum length of the basin (Schumm's, 1956). It is a very significant index in the analysis of basin shape which helps to give an idea about the hydrological character of a drainage basin. Values of elongation ratio ranging between 0 and 0.6 indicate rotundity and low degree of integration within a basin and values between 0.6 and 1.0 assumes pear shaped characteristics of a well integrated drainage basin (Strahler, 1964). The R_e value of the study area is 0.62, the basin in the study area assumes a leaf shaped characteristics indicating high degree of integration.

Drainage density (D)

The drainage density (Dd) is defined as the length of streams per unit area. It is obtained by dividing the cumulative stream length by the basin area (Horton, 1932). The drainage density (D) of the study area is 0.62 km/sq. km. According to Strahler (1964) values of drainage density under 12 are low density, those with values of between 12 and 16 are medium density basins while basins with values above 16 are high density basins. From this classification, Sonar sub-basin falls into the group of low density basins. It is suggested that the low drainage density indicates the basin is highly permeable subsoil and thick vegetative cover (Nag and Chakraborty, 2003). The type of rock also affects the drainage density. Generally, lower values of drainage density tend to occur on granite, gneiss and schist regions.

Stream frequency (F_s)

Stream frequency of the basin may be defined as the ratio of the total numbers of segments cumulated for all orders with a basin to the basin area (Horton, 1932). The stream frequency value of the basin is 0.13. The value of stream frequency (F_s) for the basin exhibit positive correlation with the drainage density value of the area indicating the increase in stream population with respect to increase in drainage density.

Texture ratio (T)

Texture ratio (T) is an important factor in the drainage morphometric analysis which is depending on the underlying lithology, infiltration capacity and relief aspect of the terrain. In the present study texture ratio of the basin is 0.52 and categorized as moderate in nature.

Conclusions

The quantitative analysis of morphometric parameters is found to be of immense utility in river basin evaluation watershed prioritization for soil and water conservation and natural resources management at micro level. The morphometric parameters evaluated using GIS helped us to understand various terrain parameters such as nature of the bedrock infiltration capacity, runoff, etc. Similar studies in conjunction with high resolution satellite data will help in better understanding the landforms and their processes and drainage pattern demarcations for basin area planning and management. Lower order streams mostly dominate the basin. The development of the stream segments in the basin area is more or less affected by rainfall. It is noticed that stream segments up to third order traverse part of the high altitudinal zones which are characterized by steep slopes while the fourth, and fifth stream segments occur in comparatively flat lands. The average bifurcation ratio of 3.65 reveal that drainage network in study area is well developed stage. The drainage basin size analysis reveals that the flooding may be lesser.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Agronomic performance of lettuce produced in trays with different cell number and field spacings

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The cultivation of lettuce is notable in the field of olericulture due to its worldwide economic and food importance. This study aimed to evaluate the agronomic performance of mimosa lettuce cv. Lavínia produced in a polystyrene tray system with different cell number and field spacings. Seedlings were produced in trays of 128, 200 and 288 cells and cultivated in a protected environment. Twenty-five days post-sowing, the seedlings were transplanted to beds with spacings of 30 × 40; 35 × 40; 40 × 40; and 45 × 40 cm. The experiment followed a split-plot randomized block design with four replicates. Harvesting was performed at 45 days after transplanting. Phytometric characteristics of the plants were evaluated after the crop cycle: number of leaves, plant height, stem length and diameter and fresh and dry weight of the shoot. Data were subjected to analysis of variance, and means were compared by Tukey's test at $p<0.05$. The agronomic performance of mimosa lettuce cv. Lavínia is favored when plants are produced in trays with 200 cells and is not influenced by field spacings.

Key words: *Lactuca sativa*, mimosa, cultivar Lavínia, seedling production, phytometric characteristics.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a crop of great economic and food importance for the majority of the population (Mota et al., 2003). It is a leaf vegetable cultivated in several regions of the world, with estimated global production of about 25 million t ano^{-1} (lettuce and chicory), and the China is the main producer (Faostat, 2013). Consumed in natura, lettuce is a source of vitamins and minerals; due to its low calorie content, it is recommended for diets rich in fiber (Filgueira, 2008).

The indication of the productive potential of a species depends on its genotype, on the environment, and on management practices such as spacing. The choice of a cultivar is crucial for the success of the cultivation system adopted (Echer et al., 2001).

Primary productivity is the most accessible and accurate means to evaluate development and to make inferences about the contribution of different physiological processes to plant behavior (Lopes et al., 2007). The accumulation of material resulting from photosynthesis is a physiological aspect of utmost importance for growth analysis (Benincasa, 2003).

In the production of lettuce seedlings, the most common method employed is the multi-cellular expanded polystyrene tray system and subsequent transplanting to the field, from which vigorous and productive plants can be obtained (Marques et al., 2003). This method

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contributes to save water, substrate and space within the nursery; uniformity of seedlings; a high rate of establishment after transplanting; a minimization of phytosanitary treatments; and low root damage during transplanting, enabling early harvests (Cañizares et al., 2002; Filgueira, 2008).

Some studies have shown that cell number influences the phytometric characteristics of plants after transplantation. For instance, Godoy and Cardoso (2005) recommend producing seedlings in trays of 128 cells for the cultivation of *Brassica oleracea* var. *Botrytis*. Similarly, Marques et al. (2003) concluded that trays with greater cell volume allow the formation of seedlings with greater strength and field potential for *L. sativa* cultivation. However, other authors such as Piovesan and Cardoso (2009), found no effect of tray cell number on the phytometric characteristics evaluated in *Cucurbita moschata*.

Still, the absence of recommendations for the spacing for each cultivar indicates the need for studies on this issue. Thus, the present study aimed to evaluate the agronomic performance of mimosa lettuce cv. Lavínia produced in a polystyrene tray system with different cell number and field spacings.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Umuarama, State of Paraná, Brazil, from April to June 2010 at the geographic coordinates 23° 47' 55" S and 53° 18' 48" W and an altitude of 430 m. According to the Köeppen system, the climate in the region is classified as Cfa, subtropical humid mesothermal. The mean temperature in the cold months remains lower than 18°C and in the warm months is above 22°C, with sporadic frosts during the winter. Rainfall varies between 1200 and 1600 mm, with a trend of rainfall concentration in summer months (Iapar, 2000).

The seedlings were produced in trays of 128, 200 and 288 cells, wherein each cell measures 0.014354; 0.012074 and 0.010897 m^3, respectively; filled with the commercial substrate Plantmax HT® (Eucatex), and were cultivated in a greenhouse with intermittent irrigation every 90 min. At 7 and 20 days after emergence, the seedlings received supplemental foliar fertilization consisting of calcium nitrate (0.3 g L^{-1}) and magnesium sulfate (0.2 g L^{-1}).

Twenty-five days post sowing, the seedlings had three leaves in addition to the cotyledonary leaves and were transplanted to beds formed of split plots with spacings of 30 × 40; 35 × 40; 40 × 40; and 45 × 40 cm, with 16 plants per plot. The plots consisted of four rows established according to the spacing being evaluated, with the two central lines being considered the useful area, totaling four plants. Plants on the edges were discarded. A split-plot randomized block experimental design was used, with four replicates.

The experiment was installed on soil classified as RED LATOSOL (Embrapa, 2013). The area was prepared by plowing and harrowing, and beds were built manually with the aid of a hoe. The amounts of NPK fertilizer applied followed the recommendations for lettuce (Trani et al., 1997). The fertilizers applied were simple superphosphate, potassium chloride and ammonium sulfate, incorporated at 5 days after transplanting (DAT) as the base fertilizer, and ammonium sulfate as top-dressing between rows, incorporated at 15 DAT. During crop growth, irrigation was performed through sprinkling, and the experimental area was monitored for pests, diseases and weeds.

The harvest was performed manually at 45 DAT in the useful area of the plot. The following phytometric characteristics were evaluated: Number of leaves (NL) – obtained by counting the leaves of each plant; Plant height (PH) – the distance measured with the aid of a graduated ruler from the plant collar to the top edge of the highest leaf, in centimeters; Stem length (SL) and mean diameter (SD) – measured with a caliper, the SD was obtained by averaging two perpendicular diameters and both are expressed in centimeters.

The shoot fresh weight (SFW) was determined by weighing the leaves, and the results are expressed in g $plant^{-1}$. The shoot dry weight (SDW) was measured by drying the fresh samples, which were stored in paper bags and dried to constant weight in a 65°C forced air oven. The samples were then weighed, and the results are expressed in g $plant^{-1}$.

Data underwent analysis of variance, and the means were compared by Tukey's test at $p<0.05$ (R Development Core Team, 2014).

RESULTS AND DISCUSSION

The tray × spacing interaction and the isolated factor spacing did not significantly affect the variables evaluated in this study. There were significant changes in the number of leaves and in stem length and diameter in response to the isolated tray effect, but no significant effect was detected on the variable plant height (Table 1).

The plants derived from seedlings produced in trays of 128 and 200 cells were superior to those from 288-cell trays, resulting in more leaves on the plants grown in the field. Regarding the variables stem length and diameter, the plants produced in trays of 128 cells were superior to those from 288-cell trays but did not differ significantly from those grown in trays of 200 cells (Table 1).

Godoy and Cardoso (2005) evaluated the yield of *B. oleracea* produced in trays with different cell number (128 and 288 cells) and noted that the variables analyzed, including the fresh weight of the head, number of leaves per plant, head diameter, total production and percent of commercial heads, also displayed better performance when using seedlings derived from trays with 128 cells compared to those of 288 cells, corroborating the results of the present study.

These authors stated that the superior development of seedlings produced in trays of 128 cells indicates that plant development is influenced by the substrate volume available to be exploited by the seedling root system at the time of production. This volume may be associated with the availability of nutrients to the plants, allowing increased seedling growth and manifesting as an increase in the production variables evaluated at the end of the crop cycle.

Marques et al. (2003) confirmed the relationship between the number of cells per tray used for seedling production and the potential of *L. sativa* cultivation in the field: plants from seedlings produced in trays with 128 and 200 cells displayed superior performance, with increased root length and plant fresh weight, compared to seedlings from trays with 288 cells. This implies that

Table 1. Number of leaves (NL), plant height (PH), stem length (SL) and stem diameter (SD) of mimosa lettuce cv. Lavínia produced in three trays with different cell number and four field spacings.

Causes of variation	Variables analyzed			
	NL	PH (cm)	SL (cm)	SD (cm)
Tray[1]				
128	27.30[a]	21.25	4.08[a]	2.15[a]
200	27.15[a]	21.41	3.77[ab]	2.09[ab]
288	23.29[b]	19.58	3.00[b]	1.61[b]
Spacing[2]				
0.30 × 0.40	25.00	19.60	3.65	1.95
0.35 × 0.40	24.65	20.40	3.49	1.74
0.40 × 0.40	26.57	21.10	3.55	2.05
0.45 × 0.40	27.41	21.90	3.76	2.07
F value				
Tray (T)	6.58*	1.36[ns]	6.28*	4.24*
Spacing (S)	1.11[ns]	0.98[ns]	0.41[ns]	1.25[ns]
T * S	0.57[ns]	0.44[ns]	0.36[ns]	0.53[ns]
CV[1] (%)	13.66	16.77	24.58	29.16
CV[2] (%)	16.51	16.54	17.91	23.97

Means followed by the same letter within a column do not differ by Tukey's test ($p < 0.05$). * Values significant at a 5% probability level; [ns] non-significant values.

seedlings with poor root development develop into inferior adult plants.

Thus, it again becomes clear that a smaller volume of substrate influences plant behavior and may affect plant architecture, development, weight and quality, thereby influencing production. These authors therefore recommended the use of polystyrene trays with 200 cells for the production of lettuce seedlings, which results in superior adult plants and has the advantages of using a smaller physical space in the greenhouse and saving substrate compared to trays with 128 cells.

For Seabra Júnior et al. (2004), seedlings produced under a higher substrate volume (121.2 cm^3) exhibited doubled leaf area compared to those produced under a smaller substrate volume (34.6 cm^3), regardless of the seedling age. These results are most likely due to the larger volume of substrate surrounding the root system, contributing to growth and the supply of nutrients, water and light and consequently to a higher production of leaves for the plant's growth and development (Menezes Júnior et al., 2000).

Studies with trays containing fewer cells have also been performed, such as that by Piovesan and Cardoso (2009), which evaluated phytometric characteristics in *C. moschata* plants derived from seedlings produced in polystyrene trays with 72 and 128 cells; however, the authors did not observe any effects of tray cell number on the fruit variables total and neck length, seed cavity and neck diameter, and yield, number and weight of fruits per plant.

Regarding the spacing effect, Gualberto et al. (1999) evaluated the competition of lettuce cultivars in three spacings (25 × 20; 25 × 25; and 25 × 30 cm) and did not find significant differences for the isolated effect of spacing, as observed in the present study (Table 1).

Nevertheless, a significant spacing effect was observed in Reghin et al. (2002), which evaluated the miniature lettuce cv. AF-469-Mini lisa and observed that plant height showed a decreasing linear response with increasing spacing among plants in the greenhouse and in the field. According to those authors, this type of response is associated with the density of plants because when there was a decrease in population density, there was lower height development, and when there was an increase in plant spacing (from 10 to 25 cm), there was an increase in the number of leaves, in contrast to the pattern shown in Table 1.

Silva et al. (2000) found that higher competition for light in denser spacings contributed to lettuce plants growing taller, but no significant differences in the number of leaves were observed.

The tray factor had a significant effect on the variables fresh and dry weight of the shoot (Table 2); plants derived from trays with 128 cells were superior to those from 288-cell trays but did not differ significantly from those grown in 200-cell trays.

However, Barbosa et al. (2010), when evaluating the development of *Calendula officinalis* seedlings in polystyrene trays of 128 and 288 cells, observed that the variables shoot dry weight, root dry weight and total dry weight of the seedlings were not significantly influenced by the cell volume, which had no effect on seedling quality. In that case, trays with a higher number of cells may be chosen, maximizing seedling production in a smaller space and saving on substrate.

Likewise, Piovesan and Cardoso (2009) observed that the variables fresh and dry weight of the shoot and root of *C. moschata* seedlings were also not influenced by different tray cell volumes when assessing expanded polystyrene trays with 72 and 128 cells. Moreover, Medeiros et al. (2010), when evaluating seedlings before transplanting, found no significant difference between trays of 128 and 200 cells for the variables percentage of seedling emergence, shoot height, number of leaves, root length, and shoot and root dry weight in *Cucumis melo* seedlings. It important to note that in the present study, the phytometric characteristics of plants were evaluated only after the cultivation cycle, with no data collected on seedlings before be transplanted in the field.

There was no effect of field spacing on the variables fresh and dry weight of the shoot of mimosa lettuce cv. Lavínia (Table 2). Likewise, Gualberto et al. (1999), when evaluating the competition of lettuce cultivars under three spacings (25 × 20; 25 × 25; and 25 × 30 cm), found no significant differences for the isolated effect of spacing on shoot dry weight.

Table 2. Shoot fresh weight (SFW) and shoot dry weight (SDW) of mimosa lettuce cv. Lavínia produced in three trays with different cell number and four field spacings.

Causes of variation	Variables analyzed	
	SFW (g.plant^{-1})	SDW (g.plant^{-1})
Tray[1]		
128	274.98^{a}	8.85^{a}
200	262.90^{ab}	8.42^{ab}
288	177.34^{b}	5.68^{b}
Spacing[2]		
0.30 × 0.40	201.89	6.50
0.35 × 0.40	234.73	7.50
0.40 × 0.40	253.58	8.12
0.45 × 0.40	263.42	8.48
F Value		
Tray (T)	4.35*	4.35*
Spacing (S)	1.08^{ns}	1.09^{ns}
T * S	0.10^{ns}	0.10^{ns}
CV[1] (%)	42.80	43.04
CV[2] (%)	37.76	37.78

Means followed by the same letter within a column do not differ by Tukey's test ($p < 0.05$). *Values significant at a 5% probability level; ^{ns} non-significant values.

However, Echer et al. (2001) found that as spacing decreases and population density increases, within certain limits, there is a trend toward increased total production per area, possibly resulting in higher profitability for the producer.

Lima et al. (2004), while working with crisp-leaf lettuce and spacings of 20 × 20 and 20 × 30 cm, observed that cv. Vera and Veronica exhibited greater shoot fresh weight in the larger spacing. For Echer et al. (2001), the cultivars Vera and Veronica did not differ significantly from each other in 20 × 20 or 25 × 25 cm spacings, although the cv. Vera was superior in regard to the shoot fresh weight. Conversely, Silva et al. (2000) found that smaller spacings (20 × 20 cm) result in superior shoot dry weight, diverging from the data obtained in the present study (Table 2).

When studying seedling production, assessment of the development after transplanting is of paramount importance because a seedling exhibiting satisfactory development at transplanting will not always favor greater productive potential in the field (Piovesan and Cardoso, 2009).

The results of the present study indicate that the polystyrene trays with 128 and 200 cells have the potential for use in the production of seedlings of mimosa lettuce cv. Lavínia. Thus, the use of trays with 200 cells is recommended, enabling the production of quality plants, in addition to the advantages of using a lower physical space in the greenhouse, obtaining a higher number of seedlings and saving substrate and water. This will result in a greater optimization and profitability for the producer.

Regarding the factor plant spacing, considering that it had no effect on the phytometric characteristics evaluated, using the lowest spacing evaluated (0.30 × 0.40 cm) is recommended, aiming to enhance the use of the planting area to increase yield and income for the producer of mimosa lettuce cv. Lavínia.

Nevertheless, further studies should be performed to clarify the performance of other cultivars and/or localities, in addition to evaluating variables associated with the seedlings when they are suitable for transplanting.

Conclusion

The agronomic performance of mimosa lettuce cv. Lavínia is favored when plants are produced in trays with 200 cells and is not influenced by field spacing.

Conflicts of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Difference in germination rate of Baobab (*Adansonia digitata* L.) provenances contrasting in their seed morphometrics when pretreated with concentrated sulfuric acid

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Baobab (*Adansonia digitata* L.) is considered an under-utilized species even though it is an economically useful tree often used daily in the diet of rural communities in West Africa. Baobab seeds do not germinate immediately after they are released from ripe fruits due to dormancy imposed by hard seed coats. A study was carried out to assess (1) best soaking duration for seed pretreatment in concentrated sulfuric acid (96%) to increase germination rate of baobab seeds; and (2) relationships between seed morphometric traits as varying with provenances and germination rate. Length, width, thickness and weight of baobab seeds collected from seven provenances spread across an agro-climatic gradient of 250 to 1100 mm in Senegal were first measured. Then, seeds were pretreated by soaking them in distilled water (control) and concentrated H_2SO_4 (96%) for 12 min, 30 min, 1, 3, 6, 8 and 12 h, respectively. Pretreated seeds were placed on moistened filter paper in 16 cm diameter Petri dishes in an incubator at 25°C for germination. Highest germination rates were obtained for seeds that had been soaked for 6, 8, and 12 h in concentrated sulfuric acid as evidenced by germination rates at 3, 7 and 10 days after sowing, respectively, although we did not find any significant differences among these three soaking durations. Significant variation among provenances was observed in seed morphology traits and seed germination rates. Highest seed trait and germination rate values were observed for Sudano-Sahel and Sudan agro-ecological provenances. This study has shown a significant variation in these characters mainly related to geographical origin. Overall, results could be useful for a further domestication and integration of baobab in the agro-productive system in Senegal as knowledge on seed germination requirements is a critical factor in seedling production for subsequent planting and reforestation.

Key words: Agro-ecology, baobab seed, dormancy, germination physiology, germination rate, non-timber forest product (NTFP), under-utilized species.

INTRODUCTION

Baobab (*Adansonia digitata* L.) is an African fruit tree species belonging to Malvaceae family. All eight species within genus *Adansonia* are distinctive trees growing in the tropics. *A. digitata* grows throughout the drier parts of Africa. A second species (*A. gibbosa* [A. Cunn.] D. Baum) is restricted to northwest Australia, whereas the remaining six species are endemic to Madagascar (Randall et al., 1998).

A. digitata is considered an under-utilized species even though it is a key economic species often used daily in the diet of numerous rural communities in West Africa (Codjia et al., 2001; De Caluwé et al., 2009; Assogbadjo et al., 2010) particularly in Senegal. Baobab has numerous medicinal and non-medicinal uses. Every part of the tree (bark, leaves, fruits, wood, roots, seeds, sap, etc.) is reported to be useful (Owen, 1970; Sidibé and Williams, 2002; Codjia et al., 2003).

For any specific species, germination responses vary according to site and environmental factors such as latitude, elevation, soil moisture and nutrient content, temperature, kind and density of plant cover and degree of habitat disturbance of the site where the seed matures (Ginwal et al., 2005; Assogbadjo et al., 2005b, 2006). Knowledge of seed germination dynamics is an important tool to increase reproduction success. In general, under adequate aeration and temperature conditions, non-dormant plant seeds need only access to water to be able to germinate after putting them in the soil. But even in such optimal conditions, baobab seeds often remain several weeks in the soil before germinating (Gebauer et al., 2002; ICUC, 2006, 2010).

Seeds may be dormant for a variety of reasons, including (1) seed immaturity at fruit harvest; (2) low seed coat permeability to water and/or oxygen; (3) seed coat resistance to embryo growth; (4) presence of metabolic blocks in the embryo; and (5) various combinations of the afore-mentioned (Kozlowski, 2002). According to Finch-Savage and Leubner-Metzger (2006), seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate. Sometimes, viable seeds which have no primary dormancy lapse into a state of secondary dormancy as an adaptation when exposed to unfavorable environmental regimes (Mayer and Poljakoff-Mayber, 1989).

In natural conditions, baobab seedlings do not emerge immediately after seeds are released from ripe fruits due to a physical dormancy imposed by hard seed coats which appear to be non-permeable (Esenowo, 1991; Sidibé and Williams, 2002). In natural conditions, baobab seed dormancy is broken by a passage through the digestive system of arge mammals (Gebauer et al., 2002). In nursery conditions, dormancy may be broken by immersing seeds in hot water for several minutes or chopping the seed coat (Esenowo, 1991). According to Danthu et al. (1995), acid scarification for 6 to 12 h is the optimal pretreatment method for breaking baobab seed coat inhibition. The conditions necessary for seeds to "break" dormancy and germinate can be highly variable among species, within a species, or among seed sources of the same species (Falemara et al., 2014).

Based on the annual rainfall gradient and local agricultural practices, there are four different agro-climatic zones in Senegal (Salack et al., 2011) where *A. digitata* occurs. *A. digitata* is slightly more represented of the humid zones (Sudano-Sahel and Sudan zones) particularly in areas with alkaline soils. Its morphology (fruits, leaves and stem) differs according to habitats (Diaité, 2005). Fagg and Barnes (1990) said that the tree exhibits considerable phenotypic variability across its diverse habitats, which could be indicative of its high genetic variability. Considering the vast distribution of wild *A. digitata* over different parts of Senegal, it is reasonable to expect genetic differentiation among *A. digitata* L. populations in a number of traits which could be exploited through selection of superior populations for seed collection and domestication needs. In addition, baobab is known to be mammal-pollinated (visited by bats) (Sidibé and Williams, 2002), which probably reduces gene flow and thus enhances the genetic differentiation between populations. In Senegal, there is insufficient knowledge about provenance and genetic variability of important indigenous species in general and *A. digitata* L. in particular. Genetic variation among and within baobab (*A. digitata* L.) provenances in seed germination and seedling growth has been reported in selected natural populations in Malawi (Munthali et al., 2012). Assogbadjo (2006) has reported variability in seed morphology traits and germination of different baobab provenances in Benin. Several studies have found genotypic variation in the physiology and morphology of tree species which can often be related to the habitat from which the plants originate. The genetic component of this variation among populations from different regions can, therefore, be identified by provenance testing (seed morphometric traits and germination studies).

For reforestation and enhancement of *A. digitata* seedling production, there is a need to understand the basic requirements for promotion of baobab seed germination in general and more specifically from different provenances. Therefore, we tested first how dormancy could be broken by applying concentrated sulfuric acid (H_2SO_4) to different Senegalese baobab seed accessions in order to increase their germination

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Table 1. Geographic coordinates of baobab seed provenances, their harvest dates, and climatic conditions (mean annual rainfall, mean temperature, and altitude) of sites where seeds were collected.

Agro-climatic zone	Site	Latitude (° ' ")	Longitude (° ' ")	Yearly rainfall (mm)**	Harvest dates (2008)	Mean annual temperature (°C)*	Mean annual rainfall (mm)*	Altitude (m)*
Sahel (250-500 mm)	Thielle	14°52'020"	14°58'468"	300-450	10th April	27.9	495	52
Sudano-Sahel (500-900 mm)	Gnibi	15° 38'237"	14° 26'159"	450-600	05th March	28.4	381	55
	Passi	13°52'994"	16°21'878"		14th February	27.4	685	14
	Balla	13°58'021"	13°24'859"		31th January	28.4	762	50
Sudan (900-1100 mm)	Wélingara	12°29'158"	12°05'655"	600-900	18th January	28.1	1211	178
	Diana Malary	12°51'214"	15°15'117"		29th January	27.2	1072	10
	Djimini	13°07'753"	14°06'469"		30th January	27.3	886	34

*Data obtained from the Worldclim database (Hijmans et al., 2005); ** Source: Agence Nationale de la Météorologie du Sénégal (2007).

rates and reduce germination duration. In this paper, we also report results on variations in seed morphometric characteristics and seed germination related parameters among seven provenances of *A. digitata* and their mutual relationships with climatic and environmental factors such as temperature, altitude, and humidity.

MATERIALS AND METHODS

Seed collection and sampling procedures

A. digitata seeds were obtained from trees collected during the dry season between January and April 2008 in three agro-climatic zones (Sahel: 250-500 mm; Sudano-Sahel: 500-900 mm; Sudan: 900-1100 mm) of Senegal (Salack et al., 2011) where baobab (*A. digitata*) is mostly represented (Table 1). Based on provenance sites, we extracted mean annual precipitation, mean annual temperature and altitudinal data using GIS maps of climatic variables of the Worldclim climate and altitude database (Hijmans et al., 2005). Worldclim maps represent interpolated surfaces at a spatial resolution of 30 arcseconds (~1 km) and are based on 50-year means calculated for the 1950 to 2000 period (De Smedt et al., 2011).

Sampling sites were situated along a decreasing rainfall gradient in order to reflect a climatic continuum. In each agro-climatic zone, three populations of baobab were randomly sampled (minimum distance between populations: 50 km). In each population, a random collection of trees separated by a minimum of 100 m was sampled with the distance chosen to reduce the probability of consanguinity between trees (Dawson et al., 2009). From 40 trees per population, 30 mature indehiscent fruits per tree were collected over the canopy. Each baobab population was geo-referenced with a GPS. Trees were marked with a metal tag to allow follow up measurements in subsequent years (on the tag: Population number-individual number).

In the laboratory, fruits were broken and seeds separated from pulp and fibers. Seeds extracted were soaked in distilled water for 6 HR (Sacande et al., 2006). Dead, empty or immature seeds float on the water while a fully healthy seed sinks (Sacande et al., 2006). Healthy seeds were collected and subsequently rinsed and dried under ambient temperatures for 2 days before storing in a cold chamber at 15°C.

Morphometric seed characterization

As seed germination rate may vary in relation to morphological seed traits (Loha et al., 2006), the following three morphometric parameters were measured on three lots of 20 seeds for each provenance, using a digital caliper (Titan 23175±0.02 mm): seed length, seed width and seed thickness. Three replicates of hundred (100) seeds of each provenance were weighed using an electronic balance (maximum = 400 g, d=0.01 g model Scout Pro Spu 402 s/n 7125050266).

Germination rate and germination parameters

The number of germinated seeds was counted every day to evaluate germination rates until seeds germination was over in each treatment. Germination parameters as defined by Evenari (1957) and Côme (1968) were calculated. Germination rate was calculated at 3, 7 and 10 days after sowing (DAS). Germination parameters monitored were germination speed (number of days to 50% of seeds germinated) and final germination rate (Gr) defined as the total number of seeds from the first to the last germinated seed. A seed was considered as germinated when the first radical pierced its integument (Redondo-Gómez et al., 2007).

Data analysis

At the end of the experiment, germination counts were calculated in percentages (%) and data subjected to an analysis of variance (ANOVA)) with Statistix version 8.1. Mean separation was performed using Tukey High Significant Difference (HSD) test at $P < 0.05$.

RESULTS

Morphological seed traits

Morphological seed trait values followed a wet to dry pattern with provenances from humid zones (Sudano-Sahel and Sudan zones) having the higher values. There were highly significant differences ($P<0.05$) among baobab provenances with regard to seed l ength, seed

Table 2. Morphometric characteristics of baobab seeds of different provenances and climatic zones of Senegal.

Agro-climatic zone	Provenance	Length (mm)	Width (mm)	Thickness (mm)	100-seed dry weight (g)
Sahel (250-500 mm)	Thielle	10.91^{ab}	8.27	6.36^{ab}	42.3^{cd}
Sudano-Sahel (500-900 mm)	Balla	10.41^{b}	7.46	5.89^{b}	34.19^{e}
	Gnibi	11.07^{a}	9.45	6.55^{a}	43.27^{bcd}
	Passi	10.49^{b}	7.81	6.33^{ab}	43.39^{bc}
Sudan (900-1100 mm)	Welingara	10.97^{ab}	7.98	6.29^{ab}	41.25^{d}
	Diana Malary	10.94^{ab}	8.27	6.62^{a}	44.99^{b}
	Djimini	11.43^{a}	8.67	6.63^{a}	50.59^{a}
	Probability (P > F)	0.0008***	0.1839^{ns}	0.0083**	0.0000***

Seed morphometric characteristics of baobab provenances measured across climatic zones of Senegal. Values are means of four replicates for each provenance. P > F probabilities are indicated by symbols: ns = no significant differences; ** significant differences at p<0.01; *** significant differences at p<0.001. For each column, values with the same letter indicate no-significant differences at 5%.

thickness and seed weight (Table 2). The average seed length, seed thickness, seed width and 100-seed weight are shown in Table 2. Overall, provenances from humid zones showed highest seed length, thickness and weight while one provenance (Balla) of the intermediate agro-climatic Sudano-Sahel zone had the lowest values for these morphological seed traits. High variation was observed in 100-seed weight for which almost all provenances differed significantly (P<0.001) among each other. Djimini and Diana Malary provenances both from the Sudan zone had the highest seed weight (50.59 and 44.99 g per 100 seeds, respectively), as compared to Balla which had the smallest value (34.19 g).

Seed germination rates associated with H_2SO_4 (96%) treatment

Baobab seeds from the seven provenances were subjected to concentrated sulfuric acid (96%) treatment in different soaking durations for increasing germination rate. Our results show highly significant differences among treatments. Seeds soaked in concentrated sulfuric acid for 12 h gave the best germination rates with 70, 96 and 97% of seeds germinated at 3, 7 and 10 DAS, respectively (Table 3). However, at 7 and 10 DAS, no significant differences were found between 6, 8 and 12 h soaking durations, even though germination rates increased with longer soaking durations for all treatments (Table 3). No germination was recorded for any seeds in the control treatments (To) for the duration of the experiment. Similar trends as for morphological seed traits were noticed in seed germination rates among the different provenances whereby highest values at 3, 7 and 10 DAS were recorded for provenances in the humid zone, particularly Balla and Passy from Sudano-Sahel zone which is characterized by small and intermediate seed sizes (Tables 2 and 3). Seed germination rate seems to be increased with increasing rainfall gradient.

For each provenance, results on germination parameters (Tables 4 and 5) show that soaking durations shorter than 30 min do not allow 50% of seeds to germinate, whereas 6 to 12 h soaking durations take 4 or less than 2 day to reach 50% of seed germination (Table 4). Baobab seeds of Thielle and Gnibi provenances from respectively Sahel and Sudano-Sahel zones never reach 50% of germination when they were soaked in concentrated sulfuric acid between 12 min and 3 h.

Furthermore, for each provenance, final seed percentage germination was very high when seeds were soaked for 6 to 12 h in concentrated sulfuric acid (96%), whereas the highest value was obtained for humid zone material (Sudan and Sudano-Sahel zones) particularly Balla and Passy from the Sudano-Sahel zone, which is characterized, respectively, by small and medium seed size (Tables 2 and 5).

Relationships of seed traits with climatic parameters and final germination rate of the baobab provenances

A close relationship of seed morphometric traits was observed with final germination rate of baobab provenances and climatic parameters. Seed size affects its ability to germinate, with smaller seed sizes (low values of seed length, thickness, and/or weight) yielding higher germination rates. The Principal Component Analysis (PCA) explains 71.89% of data variation in the two first dimensions (Figures 1 and 2). Dimension 1 (50.58%) indicates a tendency of high morphological seeds traits (length, width, thickness, 100-seed dry weight) and weak final germination rate. Dimension 2 seems to be the component presenting climate parameters. It was negatively associated with yearly

Table 3. Variation in seed germination rate (%) of baobab trees from different Senegalese provenances following various seed soaking durations and concentrated sulfuric acid (96%) treatment.

Agro-climatic zone		Days after sowing		
		3	7	10
Sahel (250-500 mm)	Thielle	20^{d}	46^{c}	84
Sudano-Sahel (500-900 mm)	Gnibi	32^{c}	54^{c}	60
	Passi	41^{abc}	73^{ab}	76
	Balla	46^{a}	76^{a}	79
Sudan (900-1100 mm)	Wélingara	44^{ab}	66^{ab}	70
	Djimini	34^{bc}	65^{b}	68
	Diana Malary	31^{cd}	64^{b}	66
	Provenances	**P > F= 0.0000*** **	**P > F= 0.0000*** **	**P > F= 0.8879^{ns}**
	12 min	5^{e}	28^{d}	32^{c}
	30 min	5^{e}	32^{cd}	37^{c}
	1 h	9^{e}	40^{c}	47^{bc}
	3 h	21^{d}	65^{b}	71^{bc}
	6 h	54^{c}	89^{a}	100^{a}
	8 h	85^{a}	94^{a}	95^{ab}
	12 h	70^{b}	96^{a}	97^{ab}
	Soaking duration	**P > F = 0.0000*** **	**P > F= 0.0000*** **	**P > F= 0.0000*** **

P > F probabilities are indicated by symbols: ns = no significant differences; *** significant differences at p < 0.001. For each column, values with the same letter indicate no-significant differences at 5%.

Table 4. Effects of soaking durations on time (days) to 50% germination for baobab seeds from the different agro-climatic zones.

Agro-climatic zones	Sahelian Zone	Sudano-sahel zone			Sudan zones		
Soaking durations	***Thielle***	***Gnibi***	***Passi***	***Balla***	***Welingara***	***Diana Malary***	***Djimini***
12 min							
30 min			9				
1 h			7	6			9
3 h			3	2	3	6	2
6 h	4	4	4	1	1	1	4
8 h	2	2	2	1	2	2	2
12 h	2	1	1	1	1	2	1

Table 5. Effects of soaking durations on final germination rate (%) of baobab seeds from the different agro-climatic zones.

Agro-climatic zones	Sahelian Zone	Sudano-Sahel zone			Sudan zones		
Soaking durations	***Thielle***	***Gnibi***	***Passi***	***Balla***	***Wélingara***	***Diana Malary***	***Djimini***
12 min	9	22	44	53	44	18	29
30 min	18	38	51	47	36	33	33
1 h	22	33	58	73	42	47	51
3 h	49	49	82	91	82	80	93
6 h	80	87	100	100	96	98	96
8 h	93	93	100	98	96	96	100
12 h	89	100	100	95	96	100	99

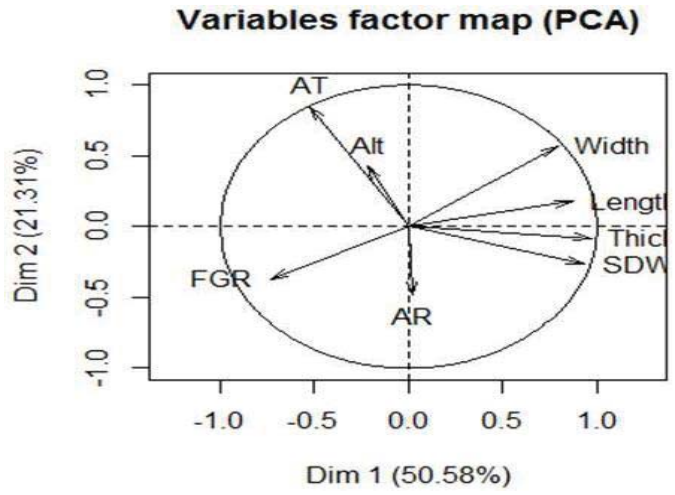

Figure 1. Loading plot of seed morphology traits, climate parameters and germination rates. AT= annual temperature; ALT= altitude; FGR= final germination rate; AR= annual rainfall; Thick= Thickness; SDW= 100-seed dry weight; Dim= dimension.

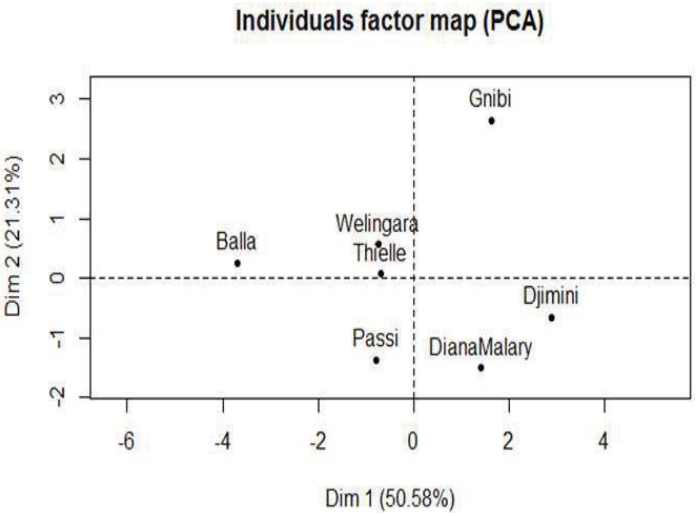

Figure 2. Score plot of seed morphology traits, climate parameters and germination rates for the different baobab provenances.

rainfall and positively correlated with mean annual temperature and altitude. As shown in Figures 1 and 2, PCA analysis revealed four groups of provenances: Djimini and Diana Malary accessions, with high rainfall, had larger and heavier seed sizes and consequently weak final germination rate. Balla from the Sudano-Sahel zone with small seed size and average rainfall had high final germination rate. Gnibi had heavier seed sizes with relatively high rainfall but low final germination rate. Passi, Thielle and Wélingara provenances (from respectively the Sudano-Sahel, Sahel and the Sudan zone), with intermediate seed sizes have average final germination rates.

DISCUSSION

The baobab (*A. digitata* L.) provenances exhibited considerable amount of variation in seed morphometric traits. Analysis of variance of the data on seed weight and seed size showed that the difference between baobab provenances were significant ($p<0.05$). The variation found in seed morphometric traits of the humid zones (Soudano-Sahel and Sudan zone) tended to follow provenance variation from the Sudan zone (high rainfall), particularly seeds from Djimini which had the heaviest seeds. Since seed weight and size are adaptive traits to varying edapho-climatic environments (Raddad, 2007), their smaller mean values in the Balla population might suggest a strong genetic selection for small seed size as an adaptation to the greater desiccation stress in this rather arid zone (Dangasuk et al., 1997; Assogbadjo et al., 2005b). Size and 100-seed weight in Thielle (Sahel zone) were not significantly different from those measured in both humid zones (Sudan and Sudano-Sahel zones). Baobab trees in Thielle are located in an edaphic environment where clayey soils help maintain soil water retention.

Variation in *A. digitata* provenances with respect to their seed morphometric traits could be due to the fact that these species grow over a wide range of climatic conditions. Several authors found a close relationship between environment (particularly rainfall) and seed weight, the latter reveals that plants growing in areas with lower rainfall tend to develop both lower fruit and seed weights (Raddad, 2007; De Smedt et al., 2011). High inter- and intra-population variation in seed weight which might influence seed germination might suggest that this parameter is quite the most sensitive to environmental factors (Marteen et al., 2002; Assogbadjo et al., 2005a) and could probably contribute to explain the relative distribution pattern of *A. digitata* over the different agro-climatological zones. In Senegal, baobab populations are slightly more represented in the humid areas. Since the seeds were collected from different locations and from trees approximately of the same age, differences observed in seed parameters, therefore, may be attributed to genetic in nature as a result of adaptation to diverse environmental condition prevailing throughout their distributional range (Mathur et al., 1984). Apart from age, vigour, crown exposure and genotype of mother tree, soil and climate of the place of seed origin are important factors affecting the seed traits (Salazar and Quesada, 1987).

To improve their germination performance, baobab seeds require physical or chemical pretreatment before they can germinate (Sidibé and Williams, 2002). According to Levitt (1974), immersion of seeds in high H_2SO_4 concentration disrupts the seed coat. Many researchers use pretreatment with H_2SO_4 with soaking durations differing between 30 min and 1 h (Sacande et al., 2006) and 3 h (Razanameharizaka et al., 2006) to

promote baobab seed germination. Our results show highly significant differences among baobab provenances in germination whereas seeds soaked in concentrated sulfuric acid for 6 to 12 h gave the best germination rates in our own experiment. This leads to germination rates of more than 90% (Danthu et al., 1995). In Mali, the World Agroforestry Center (ICRAF) used sulfuric acid for 90 min followed by rinsing in water for 24 h and obtained 92% germination for baobab seeds. Ibrahim and Otegbeye (2004) reported that soaking baobab seeds in a boiling solution of 4 ml of black potash/500 ml for 1 h consistently gave the best germination results, with 92.67% at 45 days after sowing (DAS). The same authors showed that the lowest germination result (4.33% at 45 DAS) was recorded for baobab seeds soaked in cold water for one hour and seeds soaked in boiling water for 24 h. However, Falemara et al. (2014) findings showed that seeds subjected to 98% acid concentration for 6 and 12 h gave a low germination percentage compared to average percentage germination on exposure to 10 and 50% acid concentrations at varying time intervals.

Our results evidence that provenances in the humid zone, particularly Balla and Passy from Sudano-Sahel zone which is characterized respectively by small and intermediate seed size gave the best performance in germination. Assogbadjo (2006) found that germination of freshly harvested baobab seed can start between 5 and 7 days after sowing without applying any scarification. He also reported that highest germination rates were recorded for more humid zone provenances, which is in agreement with our own results. In other studies, it was shown that germination of seeds was directly related to seed size and depth at which seeds had been buried upon planting (Bond et al., 1999; Ren et al., 2002). Small seeds have a better chance to enter into the soil than large seeds, and thus facilitate the build-up of a persistent soil seed bank, crucial for regeneration of the species (Ekta and Singh, 2000). However, the same authors reported that due to the availability of a large source of reserve material, larger seeds show higher germination percentages, greater seedling survival and better growth. Also, according to Milberg and Lamont (1997), large and heavy seeds contain larger amounts of reserves that promote germination. Subsequently, high seed reserves may enhance the abilities of larger and heavier seeds to persist by providing for metabolic requirements during the latent period, until suitable light or moisture conditions occur.

All baobab seeds from the various provenances yielded high final germination rates and rapidly reaching 50% of seed germination (1 to 4 days) when seeds were soaked in concentrated sulfuric acid for 6 to 12 h. Germination speed gives an idea of the vigour of the seed and of the seedling, which it produces (Willan, 1985). The interest in germination speed is based on the theory that only those seeds which germinate rapidly and vigorously under favourable conditions are likely to produce vigorous seedlings in field conditions, whereas weak or delayed germination is often fatal (Aldhous, 1972). Therefore, concentrated sulfuric acid (96%) for 6 to 12 h can be used to enhance baobab seed germination.

Results on correlations between parameters show that seed size affects its ability to germinate, with smaller seed sizes (low values of seed length, thickness, and/or weight) yielding higher germination rates. Climate parameters (particularly yearly rainfall) seemed to have a firm relation with our baobab morphological seed traits and germination rate. Close relationship within species between seed size and/or weight and percentage germination have been documented for many tropical tree seedlings (Ekta and Singh, 2000). These results are similar to those of Loha et al. (2006) who investigated on provenance variation in seed morphometric traits and germination of *Cordia africana* Lam. in Ethiopia.

Conclusion

The present study suggests that concentrated sulfuric acid (96%) disrupts the seed coat and facilitates rapid germination. We have shown that seed pretreatment with concentrated sulfuric acid (96%) significantly reduced time to full germination and increased percentage of germinated seeds for baobab seeds of all provenances tested. Seed germination increased with increasing soaking durations while the highest germination rates were recorded for provenances of the more humid zones (Sudano-Sahel and Sudan) and were obtained when baobab seeds were soaked in concentrated sulfuric acid for 6, 8 and 12 h. Results show that baobab germination can be stimulated by using concentrated sulfuric acid. However, optimum germination rate could also depend on seed size which might be influenced by environmental factors.

The present study revealed that considerable variation exists in baobab provenances with respect to seed morphology and seed germination characteristics. Based on our results, eventually, it may be concluded that baobab provenance with small seed size, rapid and high germination rates from Sudano-Sahel zone (Balla) seem to be easier to germinate and therefore could be used as a basis source material for domestication in Senegal. However, more efforts toward improving the germination rate of seeds from other provenances particularly Gnibi and Thielle need to be pursued.

In this study, we also noticed that the average values of morphological seed traits and germination rates are not always significantly different from one climate zone to another, even though climatic parameters, particularly yearly rainfall, that determine seed size were correlated with morphological seed traits and with germination rates. This may contribute to open a field of research to identify genotypic differences between the different baobab

provenances that could also explain differences in morphometric seed characteristics and germination behavior which are generally genetically controlled.

Conflict of Interest

The authors have not declared any conflict of interest.

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Educational and communication strategies used by extension workers in Onitsha Agricultural Zone of Anambra State, Nigeria

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The study examined the education and communication strategies used by extension workers in Onitsha Agricultural zone of Anambra State, Nigeria. A total of forty extension workers, comprising of thirty-five extension agents and five subject matter specialists were used. Structured questionnaires were used in collecting relevant information. Data were analyzed using frequency, percentage, mean and a Likert-type scale. The result revealed that Onitsha agricultural zone has young and highly educated extension personnel, which is an advantage for innovation adoption and transfer. The result showed that especially face to face, demonstration, group discussion, radio and formal lectures were effectively and efficiently used by the extension agents. The strategies had best performance in crops and livestock production by farmers. Majority (97.5%) of the respondents accepted incorporation of indigenous extension strategies such as meeting, town criers and group discussion because farmers learn easily through them and is used to them. It was concluded that incorporation of indigenous extension methods in the dissemination of information to farmers should be integrated into the extension delivery system in Anambra State.

Key words: Education, communication, indigenous, extension strategies.

INTRODUCTION

Education and communication are inseparable in that one cannot occur in isolation of the other but it is of note that they differ widely. Communication is the medium through which education achieves its goals and is an indispensable partner in development. Education and communication are the two most important tools in extension service delivery in rural settings. Extension education according to Adereti and Ajayi (2005) is not solely concerned with teaching and securing the adoption of a particular improved practice but with changing the outlook of the farmer and encouraging his initiative in improving his farm and living standard. Van Den Ban and Hawking (1996) hold the view that extension education deals with strategies and questions associated with

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extension process. It collects and integrates where possible existing knowledge about this process from other scientific disciplines and adds to the knowledge through extension research. They posit that extension education is committed to the transformation of the result of research to the farmers and equally to transmit the problems of the farmers to the research institutes. Education is a process that helps to develop an individual morally, mentally, socially and technologically. This implies that education provides a guided systematic sequence of experience that facilitates learning and change. For farmers to adopt and successfully use improved farm techniques, they must understand the scientific complex knowledge and this requires effective teaching by agricultural extension agents.

Communication is the process by which an individual transmits stimuli to modify the behaviour of the other individuals. (Havland and Lee, 2005). Effective communication is seen as an essential tool for the establishment and maintenance of good social and working relationship and it enables people to exercise control over their environment (Braimoh, 1998; Anyanwu, 1992). Also effective communication is a pre-requisite to every aspect of group functioning.

Education and communication are used to displace errors which hold sway as official truth and to replace false, erroneous beliefs with newly verified theories and truth (Onu and Anyanwu, 1990). They went further to say that most of the great revolutions that had taken place in the scientific, socio-economic and political sphere of human history would have been impossible without education and communication especially on these primitive rural dwellers who still revel in the might of a fetish god or culture and abhor modernism.

Extension in rural areas via effective communication involves getting information from those who know to those who need to know in such a way that they understand, accept and most importantly act (Mortion and Mathewman, 2005). This implies that it is the duty of extension agents to develop methods or ways of communication that will result in positive action.

The strategies adopted by extension workers in the optimum utilization and effectiveness of their methods depend upon the structures of the society or community, the pattern of behaviour of individuals forming the society, religious sentiments, tradition and custom, mental consciousness of the people and the standard of literacy.

Educational content of agricultural development programmes in Nigeria seem to be drawn up without thorough research on local condition. This may be a major shortcoming of the ADP extension system in Nigeria and it has resulted in lack of interest among farmers. This study therefore sought to find out the education and communication strategies used by extension workers in increasing agricultural productivity in

Onitsha agricultural zone of Anambra State. Specifically, the objectives of the study were to:

1) Describe the personal characteristics of extension workers in the zone.
2) Identify the education and communication strategies used by the extension workers.
3) Ascertain the local content of education and communication strategies currently in use by the Extension workers.

METHODOLOGY

This study was carried out in Onitsha Agricultural Zone of Anambra State, Nigeria in the first quarter of 2008. The zone is made up of 5 Local Government Areas (L.G.As) - Onitsha North and South L.G.As, Idemili North and South L.G.As and Ogbaru L.G.A. The target population for the study was extension agents (EAs) and subject matter specialists (S.M.S). A multi-stage random sample technique was used in selecting respondents for the study from the existing ADP structure in the state-zone, block and circle; three LGAs- Onitsha South, Idemili North and Ogbaru. From each LGA, 3 blocks were selected and from each block, 3 circles were randomly selected as well giving a total of 27 circles; from each, one EA was selected and one subject matter specialist (SMS) was randomly selected from each of the 3 LGAs. This gave a total of thirty (30) respondents used for the study.

Frequency counts, percentages, mean scores and a three-point Likert scale were involved in data analysis. The three-point Likert scale was weighted as follows: very high – 3, High – 2 and Low = 1. The following decision rule was used:

$\bar{X}$ 1.00 - 1.49 Low

$\bar{X}$ 2.00 - 2.49 High

$\bar{X}$ 3.00 - 3.49 Very high

This means that any mean score below 2.00 was rated as not a relevant strategy.

RESULT AND DISCUSSION

Personal characteristics of respondents

Table 1 showed that majority (40.0%) of the respondents were within 30 to 39 years; 37.5% fell within the age range of 40 to 49 years; 12.5% were within 50 to 59 years and those within 20 to 29 years accounted for only 10.0%. The mean age was 39.8. This implies that the respondents were relatively young. Age is considered as an important variable because of its influence on people's attitude, skill and aspiration (Obioha, 1995; Okolo, 2004). Because of their age, they could be highly responsive to education and communication strategies used in reaching farmers. This active age bracket will also favour variations in the use of strategies. The table also showed that majority (70.0%) were males while 30.0% were females. This is not surprising because field agricultural extension work in Nigeria had been dominated by male extension agents for the past years. This funding corroborates the work of Ogbanga (1998) that males were favoured more in extension activities than females.

Table 1. Personal characteristics of respondents (n=40).

Variables	Frequency	Percentage	Mean ($\bar{X}$)
Age			
20-29	4	10.0	
30-39	16	40.	39.8
40-49	15	37.5	
50-59	5	12.5	
Gender			
Male	28	70.0	
Female	12	30.0	
Marital status			
Single	6	15.0	
Married	34	85.0	
Educational attainment			
OND/NCE	7	17.5	
HND/BSc.	30	75.0	
M Sc and above	3	7.5	
Work/Job experience in (years)			
1-10	28	70.0	
11-20	8	20.0	9.5
21-30	4	10.0	

Table 2. Distribution of respondents on the education and communication strategies used (n=40).

Strategies Used	Mean Score	Interpretation
Face to face	3.0	Very high
Demonstration	2.7	High
Group discussion	2.7	High
Radio	2.4	High
Formal lecturers	2.3	High
Audiovisual aids	2.0	High
Extension newsletter	2.0	High
Television	1.8	Low
Handbills	1.7	Low
Bulletins	1.5	Low
Leaflets	1.5	Low
Magazine	1.4	Low
Newspaper	1.2	Low

Grand mean score = 2.02.

Most (85.0%) of the respondents were married while those who were single constituted only (15.0%). Because majority of the respondents were married, it enhances efficient dissemination of innovation through education and communication strategies. Entries in Table 1 further revealed that majority (75.0%) had HND/BSc, 17.5% had the minimum educational qualification of ordinary National Diploma/National Certificate of Education (OND/NCE) and only 7.5% had higher degrees (M.Sc and above). This implies that the agricultural zone had highly educated and competent men and woman, potentially capable of disseminating new innovations to farmers and need to be encouraged to put more efforts in extension delivery. This is also an advantage for innovation adoption and transfer. This finding agree with the work of Madukwe et al. (2000) and Agwu (2000), high educational qualification helps in achieving the objectives of agro technology transfer programmes. Majority (70.0%) of the extension workers had 1 to 10 years experience; 20% had 11 to 20 years and 10.0% had 21 to 30 years experience on the job. This implies that these field workers have acquired enough relevant field experience and expected to use effectively the available education and communication strategies. Also, over the years, they have benefited immensely from the training and visit (T and V) extension delivery system (Onu et al., 2005).

Education and communication strategies used

Table 2 showed that face to face ($\bar{X}$=3.0) was highly used by extension agents in the dissemination of information. This finding implies that discussing/chatting face to face with extension agents enables farmers to ask questions which enhance learning. The education and communication strategies that attracted high usage during the study include: Demonstration ($\bar{X}$=2.7), group discussion ($\bar{X}$=2.7), radio ($\bar{X}$=2.4), formal lectures ($\bar{X}$=2.3), audiovisual aids ($\bar{X}$=2.0) and extension newsletter ($\bar{X}$=2.0). Other strategies that received low utilization ranged between mean 1.2 to mean 1.8. The grand mean score was 2.02 indicating that the available education and communication strategies were efficiently and effectively utilized by the extension workers during the study. This finding is in agreement with Adebayo et al. (2003) who reported that the ultimate aim of an extension system is to effectively and efficiently deliver information to end users in a comprehensible and utilizable manner.

Conduct of meetings

Table 3 revealed that majority (65%) of the respondents organized meetings for the farmers every fortnight as a strategy of education and communication of information towards enhancing agricultural productivity and skills on the local farmers. Also 30.0% organized meeting for farmers every three weeks while (5.0%) had their meeting on monthly basis. This implies that there were regular interaction with the farmers through meetings

Table 3. Distribution of respondents on the basis of frequency of meetings (n =40).

Conduct of meetings	Frequency	Percentage
Every 2 weeks	26	65
Every 3 weeks	12	30
Monthly	2	5

Table 4. Areas of best performance of strategies by respondents (n = 40).

Strategy	Areas of best performance (%)				
Mass media	**Crop**	**Livestock**	**Fishery**	**Forestry**	**Processing**
Radio	80	60	60	40	50
Television	70	40	40	30	50
Newspaper	50	30	20	20	30
Magazine	40	40	30	10	20
Journal	40	20	20	10	20
Average	56	40	34	22	34
Interpersonal communication					
Home visit	70	50	40	20	40
Office visit	30	20	10	20	20
Farm visit	60	50	50	40	40
Meeting	50	40	20	30	50
Average	52.5	40	30	27.5	37.5
Small group communication					
Group discussion	60	70	50	40	50
Result demonstration	60	60	40	30	40
Workshop	50	40	30	30	30
Agric. Shows	70	60	30	30	20
Average	60	57.5	37.5	32.5	35

which seemed to be the most effective information dissemination and technology transfer strategy.

Effects of education and communication on some production variables

Table 4 showed the areas of best performance of strategies as perceived by the respondents. Mass media education and communication strategies on average for crop production (56%) and livestock (40%) were high while fishery (34%), forestry (22%) and processing (34.0%) were low. The implication of this result shows that mass media strategies used by the extension workers have helped farmers to perform well in crop and livestock production as well as income earning capacity. Also, a close scrutiny of the table revealed that radio performed better than all other variables considered. The above finding is in line with Chikwendu and Omenesa (1997) and Zubairu and Omenesa (1991) who reported that among all the tools of mass communication; radio emerged as the most effective. Also, Benneth (2003) and ADB (2003) observed that radio and television are powerful technologies for education and communication. This implies that the areas the farmers performed best were in crop and livestock production, applying the information technology received from extension workers.

Table 4 revealed that crop and livestock had the highest impact on the productive potentials and income of the rural farmers, even in small group discussions.

Levels of local content of education and communication strategies

Entries in Table 5 showed that majority (97.5%) accepted the incorporation of indigenous strategies in extension services while only 5% had the view that it should not be

Table 5. Distribution of respondents on the basis of local content of education and communication strategies (n = 40).

Variables	Frequency	Percentage
Incorporation of local strategy		
Yes	39	97.5
No	1	2.5
Reasons for accepting local content (Farmers understand them)		
Better	16	40
Farmers are used to them	19	47.5
Farmers learn easily with them	21	52.5
Farmers attitude towards you	-	-
Farmers manage their farm effectively	8	20

Table 6. Distribution of respondents on the effectiveness of indigenous education and communication strategies (n=40).

Variables	Level of effectiveness ($\bar{x}$ scores)	Interpretation
Town crier	2.8	High
Village drama	1.3	Low
Group discussion	2.7	High
Folktales	1.2	Low
Meeting	2.9	High
Market square	1.6	Low

Grand mean score = 2.1.

incorporated into the system. The implication of this result is that indigenous strategies when incorporated into extension services will help yield effective results and also more positive impact on the farmers' income. On their reasons for accepting indigenous strategies, majority (52.5%) were of the opinion that they learn easily with them while 47.5% said that they are used to them. This implies that it will yield good result on the farmers when applied in their agricultural training for production and income earning strategies.

Indigenous education and communication strategies

Table 6 showed that meeting ($\bar{x}$=2.9), town crier ($\bar{x}$=2.9) and group discussion ($\bar{x}$=2.7) were effective means of transferring new innovations to farmers by extension workers. The grand mean score was 2.1. The implication of this result is that indigenous education and communication strategies used were very effective for extension activities and has really helped the extension workers to reach the farmers easily and increase their standard of living. The findings gave credence to Conrey and Sutherland (2004) as well as Adebayo and Adedoyin (2005) who observed that it is necessary to identify who the potential adapters are and to design and implement a dissemination strategy for reaching them.

Conclusion

Information on personal characteristics indicated that extension division of Onitsha Agricultural Zone of Anambra State was dominated by young, married and highly educated workers, capable of effectively communicating with their clientele in the extension system and many of them had acquired enough relevant experience on the job.

The various education and communication strategies used showed that they contributed efficiently and effectively in achieving success in disseminated information and practices and had impact on the farmers especially on crop and livestock production and income earning capacities. The indigenous education and communication strategies incorporated into extension services yielded positive results on the income earning capabilities of farmers. In the light of the major findings of this study, the following recommendations were advanced. The state government should maintain high incentives on extension workers to enable them put

more efforts in extension services delivery.

Efforts in the use of education and communication strategies especially, face to face, radio, discussion groups and demonstration should be intensified by extension workers since this aid in increasing the income of the farmers, thus the possibility of better living standards. Finally, extension workers should incorporate indigenous education and communication strategies into extension service since it helped immensely in disseminating favourable information to farmers and raising their standard of living.

Conflict of Interest

The authors have not declared any conflict of interest.

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Process capacity index in drip irrigation with cassava wastewater processing

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The study aimed to evaluate the performance of drip irrigation systems with clean water application and cassava wastewater processing, by determining the distribution uniformity coefficient (CUD) and the process capability index (Cpl). The research was carried out in an agroindustrial area from western Paraná State, Brazil, where two irrigation systems were set and operated in different periods. After the essays implementation, the two irrigation systems were identified and stored in order to be retested after one year (2nd year of collection). So, the same conditions that were established in the first year had still been considered. Treatments with effluent showed flow averages near to those ones obtained with the use of clean water. Only in the T4 treatment, CUD was classified as good, while the others were excellent. The process was rated as efficient only in T1 with 80% LCL, with a 2.04 Cpl. Thus, based on this technique, it was possible to check that the system is able to keep suitable levels of uniformity.

Key words: Distribution uniformity, process capability, water quality.

INTRODUCTION

Due to water scarcity in many parts of the world, drip irrigation is becoming a popular system (Sahin et al., 2005), for its lower water consumption compared to other systems. This method of irrigation has a better efficiency of water use (Basso et al., 2008), minimizing the negative environmental impacts and becoming viable alternative for sustainable irrigated agriculture (Valipour, 2012; Bhattarai et al., 2008).

The use of wastewater for irrigation has many advantages. The most important of them concerns recovering a resource of great importance to agriculture: water; besides, its use works as an extra source of nutrients for plants. It helps on reducing development costs and with the addition of nutrients such as nitrogen, phosphorus and potassium from chemical fertilizers (Wang and Huang, 2008; Sandri et al., 2009; Juchen et al., 2013).

Paraná state is in the Southern Brazilian region and it is

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the largest producer of cassava starch in Brazil, 374.300 tons production in 2012 (CEPEA, 2013). The vegetable or cassava wastewater, a liquid effluent from starch extraction, it has high COD and presents toxic substances, hindering oxygen transportation into living beings organisms (Campos et al., 2006).

For the use of this effluent in irrigation does not become a problem for the environment, several factors related to its application need to be taken so that your exploration is properly managed. Among them, Alvarez et al. (2009) emphasize the study of water quality, the nutritional needs of plants, soil requirements and quality of effluent application throughout the area, represented by the distribution uniformity. This has directly influenced agricultural yield, which tends to increase as uniformity is improved (Brauer et al., 2011). Li et al. (2006) highlight the importance of managing a system with high uniformity, since low uniformity decreases the quality of irrigation as well as causes contamination and soil degradation. Many coefficients are used to express the distribution variability of water in irrigation, as the distribution uniformity coefficient (CUD) suggested by Keller and Karmeli (1974). Bralts (1986) has classified their values as: excellent when they were superior to 90%; good - between 80 and 90%; regular - between 70 and 80%, and bad inferior to 70%.

The techniques of statistical control are important to evaluate the quality of processes (Montgomery, 2009), since graphing to control and determine the index of process capability are options that can be used. These tools aim at evaluating the variability of a certain process, such as irrigation over time, to correct and eliminate possible wastes and failures, in order to increase yield (Justi et al., 2010).

Thus, this study aimed at evaluating the performance of drip irrigation systems by using clean water and cassava wastewater processing, with determining the CUD and establishment of process capability index.

MATERIALS AND METHODS

The study was carried out in a flat area without green cover crop, in a producing starch industry in Western Paraná, in Terra Roxa - PR municipality. Two systems of drip irrigation of approximately 66 m^2 (6 × 11 m) each was set in different periods. The systems consisted of one drip pipe with 1.49 L h^{-1} flow (85 kPa) Streamline 16080, every 0.30 m. The area had seven lateral rows with a total of almost 373 drippers, which were equivalent to an average total flow of about 555 h^{-1}.

The two used reservoirs of 1,000 L were 1.5 m above the floor, where clean water and cassava effluent processing were stored. Thirty trials were carried out for each of the treatments with 1 h difference between each one. It was a 4-min flow collection time for the sampled drippers (ISO 9261, 2006). Subsequently, this flow was measured in 100 ml graduated beaker with the same operating characteristics of the systems maintained and kept for all treatments.

Pressure was measured by two digital gauges, ITMPD Instrutemp-15 Model 8215, whose accuracy varied from ±0.3% to 25°C. This measure was taken in duplicate for each assay at the beginning and end of the system. After tests accomplishment, two irrigation systems were identified and stored to be retested after one year (2nd year of collection), and the same conditions were established in the first year. The data for the first year were collected from March to July 2012, while the one from the second year was obtained from March to July 2013.

Clean water was applied in the irrigation trials from an artesian well, installed near the studied area. The effluent was obtained from the last facultative pond, and this part of the treatment system was carried out by the agroindustry. Thus, the effluent was taken to the irrigation systems by dripping a CV 5 PPM 2900 pump AMP 15-85 220-330 VOLTS, installed near the facultative pond. Constitution of treatments can be seen on Table 1, while physicochemical effluents characterizations from cassava processing in the first and second collecting year are on Table 2.

The evaluation of irrigation systems was performed according to the methodology proposed by Keller and Karmeli (1974), using distribution uniformity coefficient (CUD), Equation 1.

$$CUD\ (\%) = 100 \cdot q_{25} / \bar{q} \quad (1)$$

Where,

q_{25} - average flow of 25% lowest values, L h^{-1};

$\bar{q}$ - average flow of all measurements in L h^{-1}.

The Shewhart chart was used to evaluate whether the tested irrigations were in accordance with the project specifications. The upper limits (UCL) and lower limits (LCL) of Shewhart charts for individual measures were calculated from Equations 2 and 3.

$$UCL = \mu + 3\sigma \quad (2)$$

$$LCL = \mu - 3\sigma \quad (3)$$

Where,
μ – average;
σ – standard deviation

Hence, only treatments that showed statistical control in Shewhart chart were considered, in order to determine process capability index (Cpl). The Cpl calculation was determined by Equations 4 and 5.

$$Cpl = UCL - \mu / 3\sigma \quad (4)$$

$$Cpl = \mu - LCL / 3\sigma \quad (5)$$

Where,
μ– average.

Montgomery (2009) describes that for new processes, the Cpl must be superior to 1.6, so that it can be classified as capable. The construction of control charts and calculating capability index were obtained by MINITAB 16 software.

RESULTS AND DISCUSSION

The lowest average flow (0.674 L h^{-1}) was obtained in treatment T4, whereas the largest one (0.735L h^{-1}) was obtained in treatment T3 (Table 3). Treatments that applied effluent showed flow averages very close to those ones obtained with clean water application. It is observed that there is a relationship between flow and pressure, because the treatment with the highest average

Table 1. Constitution of treatments.

Treatment	Water type	Year of collection
T1	Clear water	2012
T2	Cassava wastewater	2012
T3	Clear water	2013
T4	Cassava wastewater	2013

Table 2. Characterization of effluents used in treatments T2 and T4.

Parameter	1° Year of collection (T2)	2° Year of collection (T4)	Methodology
Total alkalinity (mg L^{-1})	607.26	580	APHA (2005)
Calcium (mg L^{-1})	0.23	23.20	APHA (2005)
Electrical conductivity (mg L^{-1})	3.25	1.44	APHA (2005)
COD (mg L^{-1})	154	469	APHA (2005)
Iron (mg L^{-1})	0.08	0.11	APHA (2005)
Magnesium (mg L^{-1})	0.03	18.90	APHA (2005)
Manganese (mg L^{-1})	0.33	0.32	APHA (2005)
pH	6.80	7.95	APHA (2005)
Total dissolved solids (mg L^{-1})	7.139	1.580	APHA (2005)
Total suspended solids (mg L^{-1})	466	380	APHA (2005)
Sulfide (mg L^{-1})	12.50	0.42	APHA (2005)
Turbidity (NTU)	43.70	428	APHA (2005)

Table 3. Descriptive statistics for the flow and pressure in the applied treatments.

Treatment	N	Pressure (kPa)	Average flow (L h^{-1})	SD*	CV**	Minimum	Maximum
T1	30	16.40	0.688	7.10	10.30	0.437	0.759
T2	30	16.70	0.708	4.40	6.27	0.543	0.800
T3	30	17.20	0.735	5.10	6.92	0.613	0.827
T4	30	16.10	0.674	6.20	9.26	0.497	0.792

*SD – standard deviation ** CV – coefficient of variation.

pressure also showed the highest average of flow, when compared to the other treatments. Based on linear regression, the following equation was determined, where: flow = 0.56 pressure - 0.234 with R^2 = 99.70%, and the dependent variable was the flow rate (L h^{-1}) and the independent variable was the system pressure (kPa).

It is observed that for all treatments there is a correlation between the variables pressure and flow (Figure 1), and the pressure increase (independent variable) results in increased flow (dependent variable). The highest coefficient of determination R^2 of 82.43% was obtained in the treatment T1 (clean water in first year of collecting). This result means that the fitted model explained 82.43% of the variation in the response variable Y (flow). That is, 82.43% of the variability of flow is explained by the regressor variable pressure. Ahmed et al. (2007) evaluated the emitter flow on irrigation tests using polluted water, obtained a coefficient of determination of 99.00%, much higher than reported here.

In general, the highest coefficients of determination between the flow and the pressure were obtained in the treatments that used clean water, which can be attributed to the better quality of the applied water. The use of water with high concentrations of suspended solids cause changes in the flow, a fact proven by Souza et al. (2005) that determined the pressure-flow equations for irrigation using clean water, wastewater from poultry and cattle.

For the CUD, only the treatment T4 was classified as good, while the other treatments showed excellent coefficients (Bralts, 1986) (Table 4). Borssoi et al. (2012) evaluated the water uniformity and fertilizer application with drip irrigation using the collecting methodologies of Keller Karmeli and Denículi. They also determined CUD values, classified as medium and excellent with averages ranging from 85. 8 to 91.7% fo r irrigation and 88.3 to

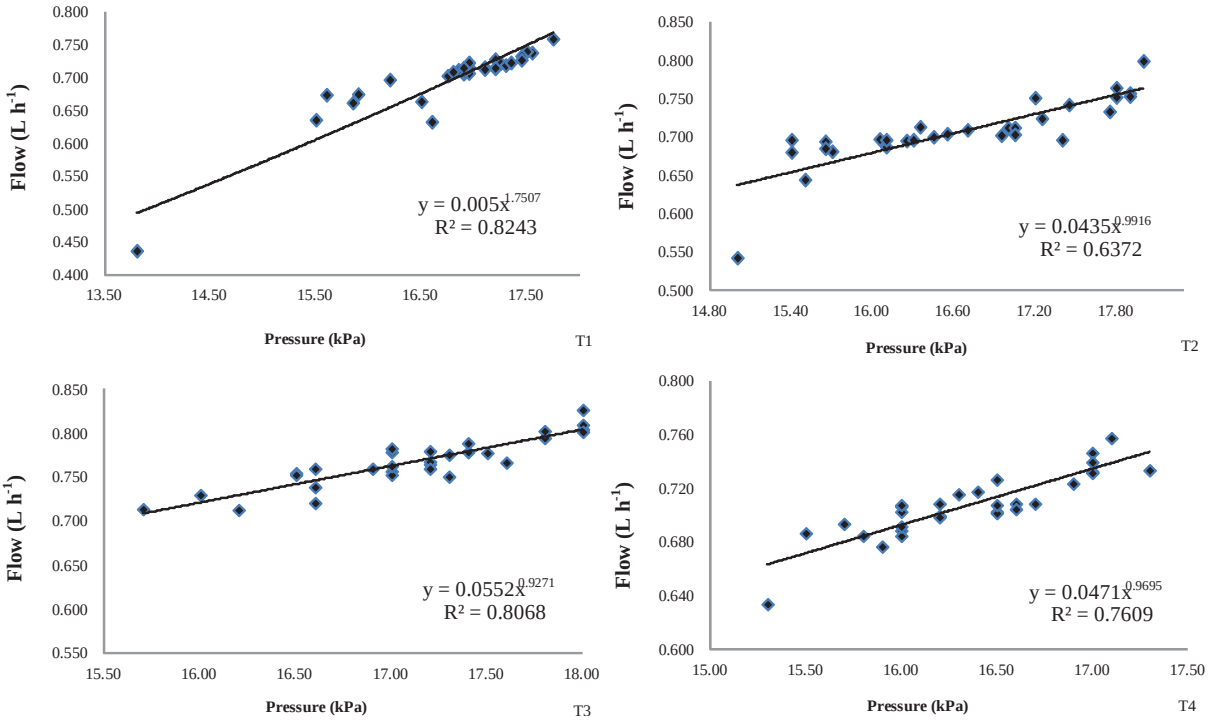

Figure 1. Relationship between flow (L h^{-1}) versus pressure (kPa) in treatments applied.

Table 4. Descriptive statistics for CUD values in the applied treatments.

Treatment	N	CUD (%)	SD*	CV** (%)	Minimum (%)	Maximum (%)
T1	30	91.93	2.46	2.68	87.20	95.26
T2	30	90.92	2.86	3.14	81.28	94.89
T3	30	90.40	4.05	4.48	81.44	95.66
T4	30	87.62	4.47	5.10	76.41	94.78

*SD – Standard deviation; ** CV – coefficient of variation.

91.0% for fertigation. There is some similarity with this trial concerning both values of pressure (12-18 kPa) and CUD.

Carvalho et al. (2006) obtained 68% CUD in drip irrigation with three-year use of conventional water supply. The authors ascribed this result to some clogging caused by bad storage conditions as well as system operation and physical damage of the equipment due to the time of use. In this study, there was a slight decrease in CUD from the first to the second cropping year.

Treatments T1, T3 and T4 are under statistical control (Figure 2); that is, they showed essays distribution next to their respective averages and there are no points outside the lower control limit (LCL) and upper control limit of control (UCL). The variability of essays in these treatments remained within the control, thus, indicating that there was no special factor that brought forth a different behavior when compared to the ordinary one or that could result in a shift regarding its expected quality (Juchen et al., 2013). Only treatment T2, which used cassava wastewater, did not appear under statistical control because the essay 28 is out of LCL (81.5%).

Hermes et al. (2013) compared the behavior of CUC on Shewhart control chart with clean water and diluted processing of cassava effluent. In both treatments, the values were out of control, with undesirable arrangements, plus an essay out of LCL to irrigate with clean water. Justi et al. (2010) used the Shewhart control chart for CUC in sprinkler irrigation and found out that one of the essays was above the UCL and none of the trials recorded CUC lower than LCL. The remaining values were within limits as well as under the control.

The values of process capability index (Table 5), which according to CUD, only LCL existence is considered, so that, 80 and 90% values were set. The first value is the minimum answer admitted in the irrigation system to a value classified as good (Bralts, 1986) and the second one was applied since the top most values were

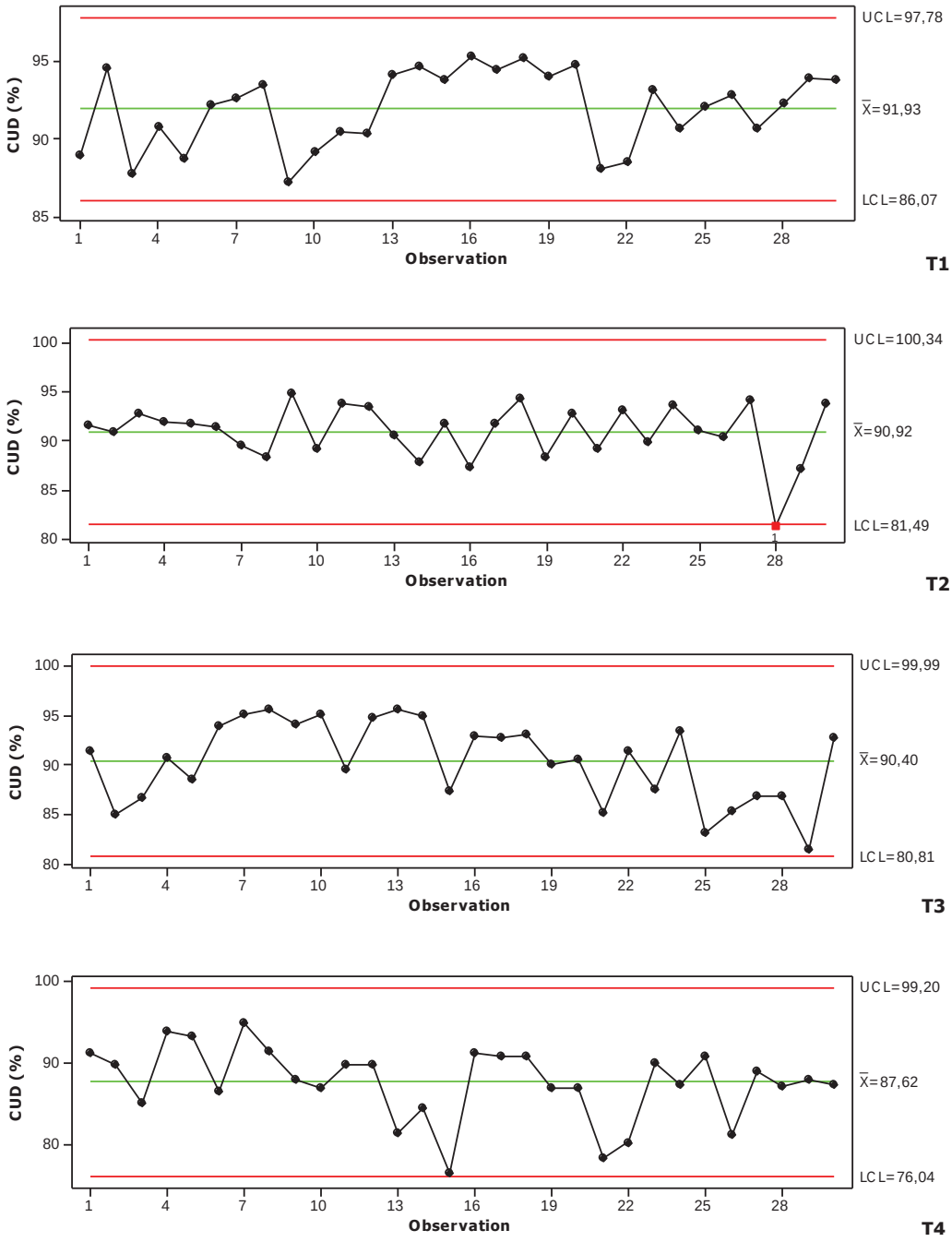

Figure 2. Shewhart control charts for CUD in the four applied treatments.

Table 5. Process capability index of drip irrigation system with application of clean water and cassava effluent.

Treatment	CUD medium (%)	Cpl (LCL = 90%)	Cpl (LCL = 80%)
T1	91.93	0.33	2.04
T2	90.92	-*	-*
T3	90.40	0.04	1.08
T4	87.62	0.00	0.66

* index that were not calculated due to lack of statistical control data.

superior to the first one.

It can be observed that for 80% UCL, the process has been reported as capable since it was superior to 1.6 only in T1 (Montgomery, 2009), while it was considered acceptable in treatment T3. On the other hand, all other indexes were classified as incapable. There was a behavior directly proportional among CUD and Cpl values, ie as CUD increased, there was an increase on Cpl. There was also a relation between such variables expressed by the Equation CUD (%) = 88.7 + 10.2 Cpl, whose coefficient of determination was R^2 = 70.10% for an LCL = 90%. For 80% LCL, the obtained equation was CUD (%) = 86.4 + 2.86 Cpl, whose coefficient of determination was R^2 = 85.7%. Capability indices determined in the second year of collection for the CUD (T3 and T4) were lower than those obtained in the first year of collection (T1 and T2) and the two treatments of cassava processing wastewater (T2 and T4) had lower rates when compared to the two treatments of clean water (T1 and T3). These results may indicate that both the wear of the irrigation system and water quality influenced the CUD values and consequently the process capability indices.

Justi et al. (2010) applied statistical techniques of control in a sprinkler irrigation system and determined values greater than 2.26 for Cpl, in such a way that as Christiansen's uniformity coefficient (CUC) increased, there was also an increase of capacity index. Thus, there was a relationship between these variables, which was expressed by CUC (%) = 46.07 + 10.55 Cpl, whose coefficient of determination was R^2 = 78%.

Hermes et al. (2013) monitored the uniformity of a drip irrigation system with diluted processing of cassava effluent. When CUC value ranged between 85 and 87.5%, the authors registered a 4.13 process capability index; when CUC values ranged between 87.5 and 90%, process capability index was 4.19; and finally, when CUC value was superior to 90%, the obtained a process capability index was 5.50. Thus, as well as this research, there was a directly proportional behavior between the CUC and Cpl values. This relation is expressed by: CUC (%) = 79.46 + 1.925 Cpl, with a coefficient of determination R^2= 61.0%, lower than those ones established in the present study. Juchen et al. (2013) have also worked with drip irrigation and applied effluents from agriculture, dairy and slaughtered industries and obtained a 2.87 Cpl, in order to indicate the process capability.

Conclusions

There were no significant changes in the flows of systems when clean water was applied or with cassava wastewater processing. Only one of the treatments with cassava wastewater processing showed a CUD classified as good, while the other treatments showed excellent CUD classification. Capability index that were determined in the second cropping year for CUD were inferior to those ones obtained in the first cropping year. Thus, in just one treatment with clean water, whose lower control limit was 80%, the process water was classified as capable.

Conflict of Interest

The authors have not declared any conflict of interest.

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Impact and simulation of soil organic carbon on soil water infiltration process

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Based on the data of soil organic carbon content and soil water infiltration rates on four kinds of forest land, features of the organic carbon content distribution and the infiltration process of water in the soil were analyzed. It is found that, from large to small, the sorting of the four kinds of forest land on average soil organic carbon content are respectively: broad leaved mixed forest, mixed coniferous and broad leaved forest, coniferous mixed forest, and mixed arbor and shrub forest. The organic carbon has an uneven distribution in soil vertical direction. The content in surface soil is comparatively high, and with the increase of depth the content decreases gradually. And it is shown that water infiltration rate has close relation with the soil organic carbon which can be expressed as the following formula: $i(t)=(i_f+(i_i-i_f)e^{-if\cdot t/10})\quad(1-t\cdot e^{SOC/10}/100)$**. Compared with the Horton soil water infiltration model simulation, the relative coefficients of the soil water infiltration rate simulation value and the practical measured value rise from 0.950, 0.951, 0.933, 0.921 to 0.968, 0.972, 0.961, 0.970. And the relative coefficients of the infiltration content simulation value and the practical measured value of a certain time period rise from 0.905, 0.628, 0.756, 0.898 to 0.941, 0.827, 0.905, 0.940. This model is much closer to the practical measured value and provides the estimation and simulation of the soil water infiltration process with a correct and effective approach.**

Key words: Soil organic carbon, soil water infiltration, infiltration rate, soil water infiltration model.

INTRODUCTION

Soil water infiltration is a vital part of the water circulation and one of the approaches for the ground water to be transformed to soil water that can be absorbed by the plants. It determines the speed, quantity, efficiency, and distribution etc of the transformation from groundwater to the soil water. Research on the infiltration issue is not only helpful for promoting the development of basic theoretical study of the soil water infiltration and migration, but also provides scientific basis for the comprehensive evaluation of the resources of the surface

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water and ground water, and the reasonable determination of the technical parameters for farmland irrigation. Some scholars research the soil texture, soil water content, and the soil water content and soil structure's impact on the features of the soil water infiltration (Gang and Xiangyun 2008; Suhua, 2005; Zhang, 2007; Franzluebbers, 2002; Helalia et al, 1988). The initial soil water infiltration rate increased with the moisture content increased, while the stability infiltration rate accompanied with the increase of the moisture content is reduced (Liu et al., 2009). Soil water infiltration capacity increases with the density of initial decline, infiltration capacity attenuation increases with increasing speed bulk density (Li and Fan, 2009). And it is considered that under the pressure water infiltration condition, soil water infiltration capacity is not only influenced by the basic physical properties of soil (soil texture, the dry density and soil moisture content, etc), but also influenced by the infiltration water head (Li et al., 2009). Only Zhao Yonggang refer to the soil structure factor except bulk density, porosity and the capillary porosity outside, but also the organic carbon content influence the soil stability infiltration rate (Zhao et al., 2008). However, there is few research about how much degree the soil organic carbon impact on the soil water infiltration speed and progress,.

The soil organic matter has colloid feature. It can absorb large amount of positive ion. Therefore, it not only has the function of maintaining the fertility and buffering performance of the soil (Francisco et al., 2007), but also has the functions of loosening the soil, promoting soil porosity (Al Haijian, 2002), and promoting the forming of the aggregated structure (Guan et al., 1991). Thus soil organic matter may affect the soil water infiltration more or less. Generally, soil organic carbon is the index of soil organic matter. So it will perform analysis on the relation of the soil organic carbon and the soil water infiltration speed, which is based on the data of measuring organic carbon content in the soil and the test for the soil water infiltration on four kinds of forest standard plots. Combining the Horton soil water infiltration model, it establishes the soil water infiltration model containing the initial infiltration speed, stable infiltration speed, and soil organic carbon content.

Sample plot overview

This institute sets the research sample plot in the middle part of Chongqing Simian Mountain of China. It is located at the end of the Three Gorges Reservoir Area, south part of Jiangjin District which is at the southwest of Chongqing City. It is of 1,150 m above sea level with the geographical location of E106°17′ to 106°30′, N28°31′ to 28°43′. This area has good natural secondary forest and planted forest with total coverage of 95.41%.

The soil of the sample plot is of slightly acid yellow soil. The base exchanging quantity of this forest area is comparatively low, so as the fertility. However, the raw organic matter accumulation is comparatively high and the nitrogen conversion rate is comparatively low; the soil has little clay content and is with good water infiltration performance, while the base can easily be eluviated. The soil is of bad water or fertilizer conservation performance. It has about 70 cm soil thickness.

SAMPLE PLOT SELECTION AND TEST METHODS

Sample plot selection

Comprehensively considering the factors as plant types, landform status, slope direction and sea level, four sample plots which cover an area of 20 m × 20 m each are set in Zhangjiashan forest area of Chongqing Simian Mountain, which are respectively the broad-leaved mixed forest (S1) taking *Lithocarpus glabra*, *Castanopsis fargesii*, and *Clethra fargesii* as the main plants, the mixed coniferous and broad-leaved forest (S2) taking the *Cunninghamia lanceolata* and *Pinus massoniana* as the main plants, the mixed coniferous and broad-leaved forest (S3) taking the liquidambar and *C. lanceolata* as the main plants, as well as the mixed arbor and shrub forest (YLMF3) (S4) taking the *C. fargesii* and *Eurya loquaiana* as the main plants. The soil matrix of the sample plots are as follows in Table 1.

Test equipment and methods

Soil section can be dug in all types of sample plots for research and the soil layers taken as the basis; the soil slope can be divided into 3 layers, with each layer being 20 cm. Three repeated soil samples shall be collected on each layer for analysis of the soil organic carbon.

Double-ring method is adopted for testing the soil water infiltration. The external ring diameter of the double-ring instrument is 22 cm while the internal ring is of 10.5 cm diameter. Both the external and the internal rings are of 25 cm height. The depths into the soil of the internal and external rings during the test process are both 10 cm, maintaining 5 cm internal and external ring infiltration head water supply till it reaches stable infiltration. The water temperature during the test process is maintained at around 20°C.

Soil saturation hydraulic conductivity of the soil samples of each are tested by ST-70A Soil Water Infiltration Instrument and constant-head method.

DATA ANALYSIS

Soil water infiltration process analysis

From the double-ring infiltration test for the water content of the 4 plots for 12 times, it is found that all the plots needs about 200 min to reach stable infiltration. Table 2 shows the soil water double-ring infiltration processes of the 4 plots under the same hydrological and meteorological conditions at the early phase.

From the test result of Table 3, it can be found that the infiltration processes of the four plots are different. For the initial infiltration speed, from large to small, the sorting is: broad-leaved mixed forest> coniferous mixed forest>

Table 1. Basic information of standard land of forest stands.

Sample plot No.	Plant group	Main plants	Arbor closure	Shrub coverage	Herbage coverage	Slope direction	Slope position	Surface slope
S1	Broad-leaved forest	Robur (*Lithocarpus glabra*), Mangrove tree (*Castanopsis fargesii*), Alder (*Clethra fargesii*)	0.6	40	30	SE29°	Downgrade	26°
S2	Coniferous forest	Chinese fir (*Cunninghamia lanceolata*), Masson pine (*Pinus massoniana*)	0.6	25	30	SW65°	Middle slope	19°
S3	Mixed coniferous and broad-leaved forest	Sweetgum (Liquidambar), Chinese fir (*Cunninghamia lanceolata*)	0.7	30	15	SE15°	Downgrade	27°
S4	mixed arbor and shrub forest	Mangrove tree (*Castanopsis fargesii*), Twigs Eurya (*Eurya loquaiana*) , ovatum Planch (*Rhododendron bachii*)	0.4	45	50	NW80°	Uprise	29°

mixed coniferous and broad leaved forest> mixed arbor and shrub forest. This is basically of the same trend as the change of the soil saturation hydraulic conductivity (except the mixed arbor and shrub forest) for the 0 to 20 cm surface layer forest soil. When it reaches stable infiltration speed, the speed ranking changes: Mixed arbor and shrub forest> broad-leaved mixed forest> coniferous mixed forest> mixed coniferous and broad leaved forest. Based on the analysis, the reason why the mixed arbor and shrub forest are having the fastest infiltration speed is that the soil of this land is of comparatively small soil bulk density but high total porosity so that the soil has rich pore space. However, at the front phase, the infiltration speed and the infiltration quantity are both small, therefore the soil still has space to contain more water content under certain soil water potential.

Analysis on the feature of organic carbon distribution

Through the testing of the organic carbon contents for 48 soil samples from 4 sample plots, it is found that the distribution of the organic carbon in different forests has obvious space aberrance/difference. First, generally from large to small, the organic carbon contents of various types of forest land are: broad-leaved mixed forest> mixed coniferous and broad leaved forest> coniferous mixed forest> mixed arbor and shrub forest. The broad-leaved mixed forest is of the highest soil organic carbon content with average value of 20.1 g/kg. The following is the mixed coniferous and broad leaved forest whose organic carbon content is of 1.4 g/kg lower than that of the broad-leaved mixed forest and is 0.9 g/kg higher than that of the coniferous mixed forest. This indicates that the plant group structure inside the forest has obvious function on the organic carbon content in the soil.

Secondly, through the testing for the soil organic carbon of the 4 sample plots, it can be found that there is obvious difference in surface soil organic carbon and first layer average soil organic carbon contents of different types of forest land. From the surface soil, the natural broad leaved mixed forest is of the largest organic carbon content. The followings are coniferous mixed forest and mixed coniferous and broad leaved forest, and the mixed arbor and shrub forest is of the minimum organic carbon content, namely the broad-leaved mixed forest> coniferous mixed forest> mixed coniferous and broad leaved forest>mixed arbor and shrub forest. Organic carbon contents of surface soil of the broad-leaved mixed forest, the coniferous mixed forest, and the mixed coniferous and broad leaved forest are respectively 2.47, 2.07, and 2.02 times of that for the mixed arbor and shrub forest. From large to small, the organic carbon contents of the first layer are: mixed coniferous and broad leaved forest> coniferous mixed forest> broad-leaved mixed forest> mixed arbor and shrub forest. Comparing the organic carbon contents of the surface layer and the first layer of soil, it can be found that the organic carbon content of the first layer of the broad-leaved mixed forest is 22% lower than the content in the surface layer; and the organic carbon content in the first layer soil of the mixed arbor and shrub forest is 29% higher than the content in the surface layer. This sufficiently indicates that the distribution of organic carbon in depth of 0 to 10 m is not even.

Third, the soil organic carbon contents of all the forest sample plots are of decrease status layer by layer, namely sharp decrease trend with the increase of soil depth. Sharp decrease of organic carbon content appears in soil layer with 0 to 20 cm depth and 20 to 40 cm depth. Maximum ratio of soil organic carbon contents in 0 to 20 cm soil depth and 40 to 60 cm soil depth reaches 24.77 while the minimum value is 6.59. That is because there is certain depth of dried up substances accumulated on the surface of the forest land which

Table 2. Selected soil properties of the experimental site (mean±SD).

Sample plot No.	Soil layer No.	Soil bulk density ($g.cm^{-3}$)	Total porosity (%)	Capillary porosity (%)	Non-capillary porosity (%)	Saturation water content (%)	Saturation hydraulic conductivity
S1	1	0.990±0.06	48.280±9.55	42.605±9.24	5.675±0.30	49.168±12.58	1.831±0.16
	2	1.074±0.03	42.320±1.07	32.125±4.91	10.195±3.84	39.397±0.23	1.133±0.13
	3	1.156±0.01	26.565±1.68	18.060±1.48	8.505±0.19	22.985±1.64	0.327±0.14
S2	1	1.008±0.04	44.895±4.56	31.935±4.63	12.960±0.07	44.496±2.65	0.659±0.13
	2	1.383±0.02	39.530±0.07	31.695±1.00	7.835±0.93	28.593±0.55	0.942±0.24
	3	1.309±0.08	35.560±1.74	32.880±1.34	2.680±0.59	27.521±1.45	0.325±0.23
S3	1	0.742±0.01	50.120±1.09	33.625±1.20	16.495±0.11	67.5472.64±	0.587±0.00
	2	1.011±0.06	51.580±1.71	48.875±0.28	2.705±1.44	51.166±4.48	0.669±0.12
	3	1.409±0.06	40.765±7.97	38.965±7.54	1.800±0.42	29.075±6.84	0.041±0.01
S4	1	0.951±0.03	54.310±0.37	48.595±0.22	5.715±0.59	57.158±1.93	0.926±0.16
	2	1.042±0.04	46.630±1.44	44.470±1.41	2.160±0.03	44.810±3.11	0.646±0.07
	3	1.106±0.00	49.675±2.31	46.105±3.10	3.570±0.79	44.900±2.18	0.633±0.24

Table 3. Soil water infiltration process.

Time (min)	S1		S2		S3		S4	
	I_i (cm)	i_i ($cm.min^{-1}$)	I_i (cm)	i_i ($cm.min^{-1}$)	I_i (cm)	i_i ($cm.min^{-1}$)	I_i (cm)	i_i ($cm.min^{-1}$)
2	9.8	4.900	5.6	2.800	4.6	2.300	3.9	1.950
5	13.2	4.400	6.2	2.067	5.8	1.933	4.6	1.533
10	18.8	3.760	9.2	1.840	8.5	1.700	6.9	1.380
15	11.3	2.260	8.2	1.640	8.2	1.640	6.3	1.260
20	11.5	2.300	6.8	1.360	7.2	1.440	6.2	1.240
25	10.2	2.040	6.2	1.240	5.8	1.160	5.8	1.160
30	9.6	1.920	6.1	1.220	4.9	0.980	5.3	1.060
35	8.4	1.680	5.8	1.160	3.8	0.760	5.2	1.040
40	8.1	1.620	5.2	1.040	3.6	0.720	5.1	1.020
45	7.5	1.500	5.1	1.020	3.2	0.640	5	1.000
50	6.8	1.360	4.7	0.940	3	0.600	4.9	0.980
55	5.5	1.100	4.1	0.820	2.6	0.520	4.6	0.920
60	5.3	1.060	3.2	0.640	2.6	0.520	4.5	0.900
70	9.6	0.960	6.0	0.600	4.4	0.440	9.3	0.930
80	8.3	0.830	5.3	0.530	4.8	0.480	9	0.900
90	7.8	0.780	4.9	0.490	4.4	0.440	8.1	0.810
100	7.5	0.750	4.3	0.430	3.8	0.380	7.2	0.720
110	5.5	0.550	4.1	0.410	3.3	0.330	7.1	0.710
120	4.9	0.490	3.8	0.380	3.2	0.320	7.3	0.730
130	3.6	0.360	3.6	0.360	2.8	0.280	6.9	0.690
140	3.9	0.390	3.4	0.340	2.5	0.250	6.6	0.660
150	3	0.300	3.1	0.310	2.5	0.250	6.3	0.630
160	3.6	0.360	2.8	0.280	2.2	0.220	6.1	0.610
170	3.6	0.360	2.4	0.240	1.9	0.190	5.5	0.550
180	2.5	0.250	2.2	0.220	1.8	0.180	5.3	0.530
200	5.2	0.260	4.1	0.205	3.4	0.170	10.5	0.525

I_i is infiltration quantity of certain time period. i_i is infiltration speed

provides large amount of organic matters. And the organic matter can be decompounded or transformed

Table 4. Soil organic carbon content of different sorts.

Soil layer	Depth (cm)	Sample plot			
		S1 (g.kg^{-1})	S2 (g.kg^{-1})	S3 (g.kg^{-1})	S4 (g.kg^{-1})
0	0	56.19±0.53	47.10±0.46	45.93±0.47	22.67±0.23
1	20	43.85±0.22	46.94±0.38	47.48±2.00	29.32±1.21
2	40	9.65±0.41	3.38±0.35	5.97±0.27	12.80±0.6
3	60	6.66±0.12	2.92±0.07	2.67±0.04	1.18±0.04

through physical and chemical effect in certain soil environment (Huang et al., 2002; Wang et al., 1999; Yang et al., 2005). If the decompounding speed is fast, the content of the soil organic carbon will be low. And if decompounding speed is slow, the content of the soil organic carbon will be high. Therefore, if the un-decompounded organic matter on the surface is of comparatively large amount, the organic carbon content will be high. However, the soil in depth layer has little organic matter sources and the organic matters are decompounded all the time, so that the organic carbon content is not much. Therefore, the organic carbon content distribution phenomenon of "much in upper layer and less in the lower layer" appears.

Response of the infiltration rate to the organic carbon

Response of the initial infiltration rate to the organic carbon

It is found through the test (Table 3) that, from large to small, the initial infiltration rates of different forest plots are broad-leaved mixed forest > coniferous mixed forest> mixed coniferous and broad leaved forest>mixed arbor and shrub forest. And the change trends of the surface soil layer organic carbon content and the initial infiltration rate are the same. The initial infiltration rate of the broad-leaved mixed forest which has the highest organic carbon content reaches 4.9 cm/min. Such rate is over 1.5 times of rates of other plots. The initial infiltration speed of the mixed arbor and shrub forest which is of the lowest organic carbon content is also the slowest, only 1.95 cm/min. This indicates that the initial infiltration speed is of positive correlation with the organic carbon content of the surface layer. It affects the initial infiltration speed together with other factors like the soil texture, structure, and pore space, etc. The initial infiltration speeds of different types of forest sample plots increase or decrease in the same direction as the changes of the organic carbon contents of the surface soil layer.

Response of the stable infiltration speed to the organic carbon

Through the regressive analysis on the soil infiltration speeds of different types of land use and the organic carbon content, it can be found that the stable infiltration speed has comparatively strong relativity with the organic carbon content of the lower layer. It can be deemed that the stable infiltration speed is obviously related with the organic carbon content of the lower soil layer (soil layer 30 cm under surface), which is also a major factor that determines the stable speed.

Response of the infiltration process to the soil organic carbon

During the soil water infiltration process, before the speed becoming stable, all the speeds of the 4 plots are decreased with the increasing of time. However, the infiltration speed is not evenly changed which results in different soil water infiltration speeds of the 4 plots.

The representation of the infiltration rate at the initial infiltration phase is: Broad-leaved mixed forest > coniferous mixed forest> mixed coniferous and broad leaved forest>mixed arbor and shrub forest. After 25 min of infiltration, the speed in soil of the mixed coniferous and broad leaved forest sample plot decreases suddenly. At this time, it replaces the mixed arbor and shrub forest to become the soil with minimum infiltration speed among the four sample plots. After 55 min, the infiltration speed becomes S1>S4>S2>S3, namely, broad-leaved mixed forest>mixed arbor and shrub forest> coniferous mixed forest> mixed coniferous and broad leaved forest. After 80 min, the mixed arbor and shrub forest becomes the plot with fastest infiltration speed among the four plots, leaving the sorting of speeds of other plots remain unchanged. From Table 4, the organic carbon content in the second soil layer of the mixed arbor and shrub forest is much higher than the organic carbon content in the second soil layer of other plots, which shows that the soil aggregates formed under the effect of the soil organic matter in the second soil layer of the mixed arbor and shrub forest are of comparatively large amount which help to form a certain amount of pore space groups. The change of the organic carbon content among soil layers is comparatively small; the amount of pore space among the soil layers is comparatively stable with good successive performance to thus guarantee relative stability of the infiltration speed of the mixed arbor and shrub forest. However, the organic carbon contents in the

Table 5. Regression analysis of soil infiltration rate.

Sample plot No.	Independent variable	R	Mean square	Residual mean square	F	Sig.	Constant term	Regression coefficient
S1	1	0.950	36.429	0.164	222.104	0.000	-0.327	0.878
	2	0.968	37.792	0.107	352.519	0.000	-0.106	0.857
S2	1	0.951	10.009	0.034	292.922	0.000	-0.185	0.834
	2	0.972	10.237	0.025	414.877	0.000	-0.064	0.807
S3	1	0.933	7.883	0.049	160.911	0.000	-0.354	0.932
	2	0.961	8.372	0.029	292.862	0.000	-0.133	0.947
S4	1	0.921	2.412	0.018	133.281	0.000	-0.597	1.042
	2	0.970	2.187	0.027	79.610	0.000	0.143	0.857

Independent variable 1 is the computed value of infiltration rate of Horton infiltration model while 2 is the modified infiltration rate value.

Table 6. Regression analysis of soil infiltration.

Sample plot No.	Independent variable	R	Mean square	Residual mean square	F	Sig.	Constant term	Regression coefficient
S1	1	0.905	280.817	2.597	108.140	0.000	-0.852	0.747
	2	0.941	303.968	1.632	186.238	0.000	0.476	0.730
S2	1	0.628	13127.662	840.854	15.612	0.001	148.841	-9.735
	2	0.827	17610.240	654.080	26.924	0.000	146.261	-10.578
S3	1	0.756	47.163	1.470	32.090	0.000	-1.135	0.728
	2	0.905	67.479	0.623	108.281	0.000	-0.473	0.871
S4	1	0.898	56.748	0.321	176.903	0.000	1.446	0.465
	2	0.940	42.502	0.914	46.484	0.000	3.316	0.456

Independent variable 1 is the computed value of infiltration rate of Horton infiltration model while 2 is the modified infiltration rate value.

second soil layers of other plots are obviously reduced by over 78% which greatly reduces the amounts of the soil aggregates and the pore space. Under the condition that the water content in soil improves greatly while the soil water potential is reduced, the infiltration speeds of other forest plots except the mixed arbor and shrub forest plot are greatly decreased.

Soil water infiltration model simulation and modification

Presently there are a number of models about soil water infiltration. Horton soil water infiltration model (Ma et al., 2005; Horton, 1940), Philip infiltration model (Wang et al., 2002; William Detar, 1989; Philip, 1957), Green-Ampt infiltration model (Juanjuan et al., 2010; John and Selker, 1999; Swartzendruber, 2000; Shlomo and Neuman, 1976) and so on are the most frequently seen. Though they can simulate progress of soil water infiltration, there exists difference between progress simulation and actual value, which is not accurate enough. Horton soil water infiltration model shall be calculated according to initial infiltration speed, stable infiltration speed, infiltration time and rated parameter c value. There exists difference between actual infiltration progress and simulated infiltration progress of models within the same period; especially accumulated infiltration content within period through calculation. Infiltration rate and infiltration content shall be respectively analyzed by means of SPSS software. The correlation coefficient of the actual and calculated infiltration content within period is respectively 0.905, 0.628, 0.756, 0.898.

Due to the fact that there are obvious correlativity between organic carbon and infiltration speed of different forest plots, it is considered that organic carbon shall be

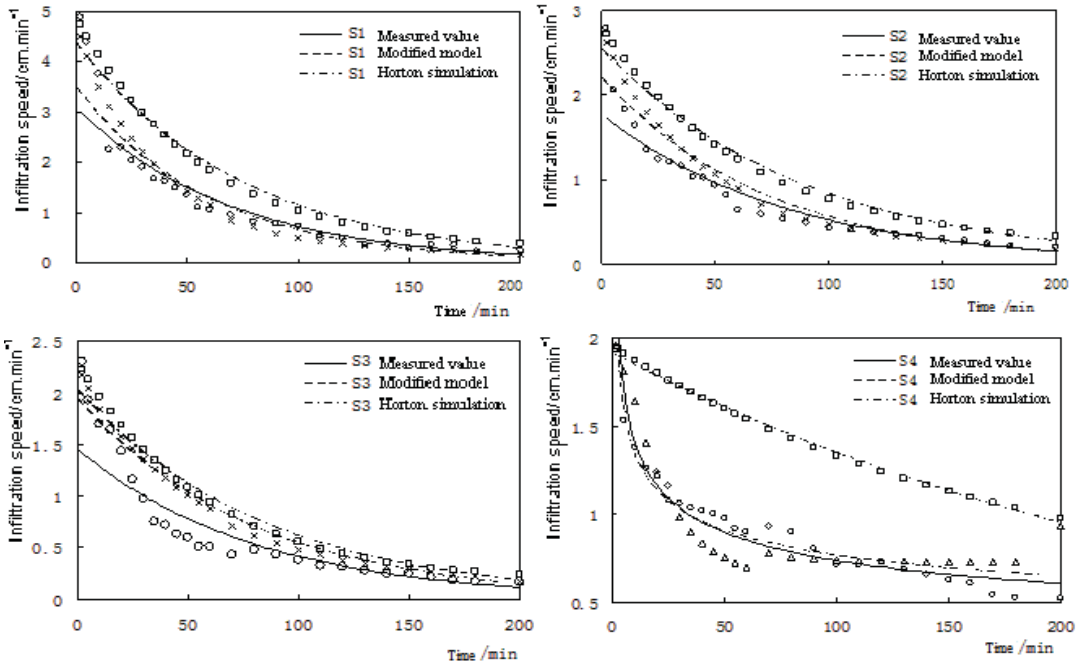

Figure 1. Soil infiltration process Simulation of 4 plots in the forest.

introduced into soil water infiltration model as an impact factor. By means of calculation of Matlab software, soil water infiltration model can be revised as,

$$i(t) = (i_f + (i_i - i_f)e^{-i_f \cdot t/10}) \quad (1 - t \cdot e^{SOC/10}/100) \qquad (1)$$

among which i_i is the initial infiltration rate, i_f is the stable infiltration rate, and SOC is organic carbon content of the lower layer of the soil.

The revised soil water infiltration model shall be adopted for simulation (Figure 1); related analysis shall be carried out in combination with actual data (Tables 5 and 6). Correlation coefficient of infiltration rate by means of actual measurement and simulated calculation of four forest plot have been improved from 0.950, 0.951, 0.933, 0.921 to 0.968, 0.972, 0.961, 0.970. Correlation coefficient of infiltration content within period by means of actual measurement and simulated calculation of four forest plots have been improved from 0.905, 0.628, 0.756, 0.898 to 0.941, 0.827, 0.905, 0.940.

Conclusions

The research conducts determination test on four forest plots on all-round hills by means of observation on the spot. By analysis of organic carbon and soil water infiltration progress, it is discovered that there is obvious correlation between soil organic carbon and soil water infiltration progress. The calculation result will be more accurate by means of certain model description. The detailed conclusions are as follows.

(1) Surface soil organic carbon contents are variable according to different types of forest plots. The soil organic carbon contents, in proper order, are broad leaved mixed forest, coniferous broad mixed forest, coniferous mixed forest, mixed arbor and shrub forest in which are mostly shrubs. Organic carbon is not evenly distributed in soil for the same land. Soil organic carbon contents are high for general surface and will reduce with the increasing soil depth.

(2) In tests, due to the fact that there exist obvious differences among organic carbon contents of different type of forest plots, it is concluded by analysis that initial infiltration speed of forest plots with high content of organic carbon is generally above that of forest plots with low reserve of surface soil organic carbon. At the same time, infiltration rate in the process of soil water infiltration for different forest plots will reduce with time increase. However, digression rates vary according to different forest plot soils, the above which is closely related to organic carbon content in the soil.

(3) Correlation coefficient between simulated value and actual value of soil water infiltration rate have been improved from 0.950, 0.951, 0.933, 0.921 to 0.968, 0.972, 0.961, 0.970 after soil organic carbon content indicator of soil water infiltration model is increased. Correlation coefficient between simulated value and actual value of water soil infiltration content within period have been improved from 0.905, 0.628, 0.756, 0.898 to

0.941, 0.827, 0.905, 0.940. This model does not require rated parameter and is nearer to actual value, which provides an accurate and effective method for prediction and simulation of soil water infiltration process.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Phytotoxicity of *Solanum aculeatissimum* Jacq. leaves extract

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The objective of this study was to evaluate the phytotoxicity of *Solanum aculeatissimum* Jacq. leaves ethanolic extract in seeds germination, development and fixation of *Lactuca sativa* seedlings. The same study also aimed to assess the mitotic index of lettuce roots meristematic cells, quantification of phenols and total flavonoids and triage by mean of phytochemical testing of the main secondary metabolites classes. Bioassays of germination, development of root and hypocotyl were carried out in Petri dishes using achenes of *Lactuca sativa* L. cv. 'Grand Rapids' (lettuce). Concomitantly, were evaluated the physico-chemical characteristics (pH, osmotic potential and electrical conductivity), mitotic index, quantification of total phenols and flavonoids and determination of phytochemical profile of the treatments extract. The results obtained in the bioassays demonstrate that the ethanol extract of *S. aculeatissimuma* presents phytotoxic potential in the development of lettuce seedlings, given that the concentration of 20 mg/ml showed greater inhibition (41% of germination). The extract contains significant amounts of antioxidants, total flavonoid and phenols, where the concentration 1000µg/mL showed higher values (86.50%). Furthermore, it was possible to observe the presence of compounds with allelopathic activity in the phytochemical screening test as coumarins, tannins, terpenes, flavonoids and alkaloids. Given the above it is clear that the ethanolic extract of *S. aculeatissimum* presents allelopathic substances with phytotoxic activity that can affect the germination and development of other plant species in their natural environment.

Key words: Allelopathy, antioxidants, 1,1-diphenyl-2-picrylhydrazyl (DPPH) test, *Lactuca sativa*.

INTRODUCTION

The term allelopathy can be described as the interference of a plant in the growth and establishment of another (including microorganisms) by mean of the liberation of chemical compounds in the environment (Rice, 1984). These interactions can proportionate positive or negative responses in the target organism, and these substances

are denominated allelochemicals, which in their vast majority are originated from the secondary metabolism of plants (Blanco, 2007). Generally, allelochemicals act by promoting cellular and metabolic changes, including modifications in membranes functionality, the absorbance of nutrients and water, the photosynthetic and respiratory activities, protein synthesis and enzyme activity, and in the genetic material promoting RNA and DNA alterations (Inderjit, 2006).

Many studies aimed to better understand and identify plants with allelophatic potential in the environment, researchers believe that in the future allelochemicals can become herbicides, insecticides and even alternative nematicides, aiming to lower the presence of chemical defensives in the environment (Dias and Dias, 2007). In this context, many agronomic studies have been applied to the discovery of new substances with phytotoxic action originated from plants with allelophatic potential, aiming the control of weeds and plant pathogens (Ferreira and Aquila, 2000; Souza filho et al., 2006; Santana et al., 2006; Moreira et al., 2008; Pelegrini and Cruz-silva, 2012). Among angiosperms, the Solanaceae family is considered one of the most important because it presents a vast diversity of active secondary metabolites, which many of these metabolites present elevated antioxidant capacity (Ribeiro et al., 2007). Having as main identified component the espirostano and solasodine glycoalkaloids, very common in the *Solanun* genus, considered the most important in the family (Kohara et al., 2005), also by the allelopathic activity discovered in glycolyse alkaloids of the green fruits of *Solanum crinitum* (Alves, 2003; Lu et al., 2011; Ohyama et al., 2013; Muruhan et al., 2013).

Inside the *Solanum* genus, the *Solanum aculeatissimum* species, popularly known in Brazil as "Joá do mato" stands out for being a plant with invasive characteristics for many crops cultivated in Brazil, such as pastures, citrus orchards, gardens and grass, coffee plantations, also found in natural open fields, cerrado and Atlantic rainforest (Lorenzi, 2006). It is a semi woody weed that can reach 50 cm in height, easily characterized by the excessive number of prickles all over the plant's end (Mentz and Oliveira, 2004). Its fruit is used directly in edema and skin conditions such as boils (Rodrigues and Carvalho, 2001). Studies point out that the methanolic extract of the leaves present a potential source of substances with antibacterial properties, among them the rutin flavonoid (Pereira, 2006).

Given the above information, this study aimed to evaluate the phytotoxic potential of the ethanolic extract of *S. aculeatissimum*, in the germination of seeds and in the growth and development of seedlings of *Lactuca sativa*. The same project also aimed to evaluate the mitotic index of lettuce roots meristematic cells, quantification of phenols and total flavonoids and triage by mean of phytochemical testing of the main secondary metabolites classes.

MATERIALS AND METHODS

Experimental procedures

The bioassays were carried out in the Plant Physiology and Phytotherapics Laboratory of the Biological Sciences Department, College of Science and Letters of Assis, Universidade Estadual Paulista (UNESP), Assis-SP, Brazil.

Sampling and preparation of the plant material

The leaves of *S. aculeatissimum* were sampled from specimens present in the College of Science and Letters, UNESP-Assis/SP (22°32'20''S and 50°22'60''W). The taxonomic identity was carried out with the collaboration of Dr. Renata Giassi Udulutsch, professor of State University of São Paulo "Júlio de MesquitaFilho", Assis campus. After the samples collection, the leaves were selected and dried in forced-air oven at a temperature of 40°C for 24 h, right after, they were grinded and the resulting powder was stored in dark plastic flasks.

Preparation of the ethanolic extract

The ethanolic extract was prepared by mechanic maceration of the plant's powder, with ethanol in PA (IMPEX, Brazil) (at a concentration of 1:10 [p/v]) for 24 h at room temperature. The extract was then filtered at low pressure under vacuum, a methodology similar to the one performed by Rutherford and Powrie (1993), Hajhashemi et al. (2003) and Boligon et al. (2009). The extraction was repeated three times with the same plant material. The resulting extracts were combined and concentrated in rotary evaporator (model: MA120, Marconi, Brazil) at a mean temperature of 60°C, then the dried residue was used in the biological assays, according to the work of Áquila (2000) and Sadraeietai (2003).

Germination bioassay (pre-emergent)

The bioassay was carried out in Petri dishes (8x8 mm) lined with germination paper, which was dampened with 1 ml of the extract diluted with distilled water for the concentrations of 5, 10 and 20 mg/ml. Fifty achenes of lettuce cv. Grand rapids were sowed by dish, separated in experimental groups and control (distilled water), incubated for 48 h in growth chamber (model: 411/FPD, Nova Ética, Brazil) in relative humidity 80 to 90% and temperature 23±2°C (Alves et al., 2004). The experimental design was fully randomized, with six repetitions of each treatment concentration or control. The germination was monitored every 6 h, with the projection and geotropic curve of the root being the germination evaluation criteria, as described by Ferreira and Áquila (2000), Ferreira et al. (2008) and Maraschin-Silva and Áquila (2006a). With the results obtained, the following indexes were calculated.

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Germination: G% = [∑ni/A].100

Mean time of germination: $\bar{t} = \frac{\sum_{i=1}^{k} niti}{\sum_{i=1}^{k} ni}$

Mean speed of germination: $\bar{v} = \frac{CV}{100} = \frac{1}{\bar{t}}$

Where, A: Total number of achenes sowed; ni: Number of achenes germinated in each instant (ti); CV: coefficient of variation; ti: the time gap between the beginning of the experiment and the observation time and k: last day of observation (Labouriau, 1983; Santana and Ranal, 2004; Pereira et al., 2009).

Physical-chemical characteristics (pH, osmotic potential and electric conductivity)

The pH of the *S. aculeatissimum* diluted extract was determined directly in the treatment solutions, using a pH meter (Tecnopon ®, model MPA210, Brazil). The osmotic potential measurement of the extracts was carried out using dilutions of polyethylene glycol (PEG-6000) to produce the osmotic potentials of -1.0 to -0.02 MPa, such as described my Villela et al. (1991) and Mazzafera (2003). The Brix refraction measurement, for each concentration of PEG-6000 and the extract was determined by a refractometer ABBÉ and the values were used to calculate the water potential, as described by Bakke et al. (2006). The electric conductivity was determined in the treatment solutions, using a conductivity meter (Instrutherm® CD-860).

Length of the radicle and hypocotyl (post-emergent)

In the growth bioassay, thirty seedlings of lettuce (with approximately 2 mm of main root length) were put in petri dishes (8x8 mm) with 1 ml of extract (5, 10 and 20 mg mL^{-1}), and distilled water, as described above. The dishes were put in growth chamber (model: 411/FPD, Nova Ética, Brazil), under the same conditions of the germination assay, as described by Ferreira et al. (2008) and Maraschin-Silva and Áquila (2006b). The experimental design was fully randomized, with six repetitions of each treatment concentration or control. The experiment was monitored at 24 and 48 h, the length of the primary root and hypocotyl were measured with digital caliper rule (modelo: IP65, DIGIMESS ®, Brasil).

Mitotic index

For the mitotic index analysis, the primary roots of *L. sativa* seedlings (approximately 2 mm length of the primary root) were collected and prepared by the squash technique (Guerra and Souza, 2002; Mahajan and Sharma, 2008). First, the roots were fixated in Carnoy solution (ethanol : glacial acetic acid 3:1) for 2 h, hydrolyzed in HCl 5N for 15 min at room temperature, washed with distilled water and colored with 5% of carmin acetic acid. The cells were observed under optic microscope, with magnification of 100x and 5000 cells were analyzed for each treatment. The mitotic index (MI) was obtained from the equation, MI=(m/T)x100, where m= the number of cells in mitosis and T= total number of cells (Pires et al., 2001; Tabur and Oney, 2009).

Quantification of total phenols and flavonoids

The quantification of phenols and total flavonoids was performed in the ethanol diluted extract with the concentrations of 25, 50, 75, 100, 250, 500 and 1000 µg/ml. For the determination of phenols content, the Folin and Ciocalteu (1927) method was carried out. For each 0.5 ml of extract in the different concentrations were added 5 ml of distilled water and 0.25 ml of Folin-Ciocalteu reagent. After 3 min, 1 ml of saturated Na_2CO_3 at 10% was added to the mixture and stored for 1 h. The absorbance was read at 725 nm using UV-Vis spectrophotometer (Model: SP220, BIOSPECTRO, Brazil). All tests were performed in triplicate and the results were expressed in mg of galic acid by gram of extract.

For the quantification of the extracts total flavonoids was carried out determination by mean of UV-Vis spectrophotometer (model: SP220, BIOSPECTRO, Brazil) reading and the samples were prepared as described by Zhishen et al. (1999), based in the complexation of flavonoids and $AlCl_3$. An aliquot of 250 µl of different concentration extracts were mixed with 1.25 ml of distilled water and 75 ml of $NaNO_2$ 5%. After 6 min, 150 ml of a 10% $AlCl_3/H_2O$ solution was added. Passed 5 more min, 0.5 ml of a 1 M NaOH solution was added and following the total volume was completed by the addition of 2.5 ml of distilled water. The samples were agitated in a vortex agitator and the absorbance measure at 510 nm. All the tests were performed in triplicate and the results expressed in mg of rutin by gram of extract.

Determination of DPPH radical scavenging activity

The DPPH radical (1,1-diphenyl-2-picrylhydrazyl, Sigma, EUA) scavenging activity was determined according to the methodology proposed by Bilos (Manian et al., 2008). The dried ethanolic extract of each sample was dissolved in ethanol (75%) in different concentrations (25, 50, 75, 100, 250, 500, 1000 µg/ml), following they were mixed with 5 ml of DPPH solution ($1.5x10^{-4}$M). The extracts reacted with the radical for 30 min at low luminosity, then the readings were performed in an UV-Vis spectrophotometer at 517 nm wavelength. The antioxidant activity calculation was carried out according to the formula: I%=[(control - sample)/control]x100. The galic acid (Vetec-QuímicaFina, Brazil) was used as reference. Triplicates were performed for the analyses. EC50 was calculated using linear regression.

Ferric-ion reducing antioxidant power (FRAP) assay

The FRAP assay was performed as previously described by Pulido et al. (2000) with some modifications. 2.7 ml of FRAP reagent, freshly prepared was mixed with 270 µl distilled water and 90 µl of each sample. Then this mixture was maintained in water bath at 37°C for 30 min. The FRAP reagent contained 2.5 ml of 10 mM TPZ solution in 40 mM HCl, plus 2.5 of 20 mM $FeCl_26H_2O$, plus 25 ml of 0.3 M acetate buffer (pH 3.6). Readings was done at the absortion maximum (595 nm). Solutions of known Trolox concentration was used for calibration. The final results were expressed as micromole Troloxequivalents (TE) per grams of extract (µmol TE/g of E.).

Evaluation pro-oxidant activity by methods relative electrophoresis mobility (REM)

REM was adopted from Hsieh et al. (2005) and Toda (2005). Bovine Serum Albumin - BSA (2 mg/ml) was diluted in PBS (10 mM, pH 7.4) and incubated with Cu^{2+} (2 mM) and H_2O_2 (0.25 mM) at 37°C for 24 h in the presence or absence of the herbal ethanolicextract (1000 and 500 µg/ml). Electrophoresis of BSA was performed using polyacrylamide gels (SDS-PAGE), it was prepared according to the standard technique (Encor biotechnology inc.). Running gel solution was utilized in 12% of Acrylamide, and the

Table 1. Effect of ethanolic extract of *S. aculeatissimum*on the germination and growth of *L. sativa*.

Extract (mg/ml)	Germination (%)	Mean time (h)	Mean speed (h)	Radicle length (mm)	Hypocotyl length (mm)	Mitotic index
0	98.66±1.00[a]	17.08±5.34[a]	0.07±0.04[a]	13.77±4.74[a]	3.49±0.66[a]	11.23±1.23[a]
5	92.00±3.10[a]	21.38±0.86[a]	0.05±0.00[ab]	04.81±0.62[b]	2.38±0.39[b]	10.97±1.83[a]
10	92.00±5.21[a]	26.58±1.19[b]	0.04±0.00[ab]	05.06±0.98[b]	2.52±0.56[b]	09.98±2.09[a]
20	41.33±19.0[b]	38.88±2.82[c]	0.02±0.00[b]	02.86±0.75[c]	2.01±0.55[c]	03.45±0.29[b]

The data was presented in averages±standard deviation. [a]Averages with at least one equal letter, in the column, indicate absence of significant difference (p>0.05), by the Tukey test. [b]Mitotic index = (total number of cells in division / total number of cells analyzed x 100), with at least one equal letter, in the column, indicate absence of significant difference, by the Qui-squared test (χ^2< 0.05).

Table 2. pH, osmotic potential and electric conductivity of the ethanolic extract of *S. aculeatissimum*nas in different concentrations (5, 10 and 20 mg/ml).

Extract (mg/ml)	pH	Osmotic potential (MPa)	Electric conductivity (mS/cm)
0	4.87	0.00	0.50
5	4.78	0.10	0.86
10	4.74	0.46	1.60
20	4.81	1.26	2.91

stacking gel at 5%. Proteins were stained with 0.25% Coomassie Blue R-250. Results were expressed in the REM in mm using that of native BSA as the base.

Extracts phytochemical profile determination

The phytochemical tests were carried out according to the procedures described by Sivasankari et al. (2010) to identify components, such as flavonoids, alkaloids, terpenes, triterpenoids, condensed tannins, hydrolysable tannins, coumarins, saponins, glycosides and phenols.

Statistical analysis

The data was analyzed by variance analysis and Tukey test (α =0.5). These tests were performed using the BioEstat software (version: 5.0), according to Santana and Ranal (2004) and Pereira et al. (2009). For the mitotic index analysis, the qui-squared test was performed to identify a positive answer between the experimental groups and control, according to the analyses proposed by Ribeiro et al. (2003).

RESULTS

In Table 1, the germination indexes were presented. The germinability of seeds treated with the concentrations 5 and 10 mg/ml did not present significant difference (the percentage of germination to the concentrations of 5 and 10 mg/ml was 92%), but presented values with significant difference to the 20 mg/ml treatment (percentage of germination of 41.33%) being that only in the last concentration there was significant difference to the control. Considering the mean germination time, the 5 mg/ml concentration did not present significant difference compared to the control, however it differed to the 10 and 20 mg/ml treatments, while these two treatments differed among their selves, where the 20 mg/ml reached the highest value (38.88 h). For the germination speed all concentrations (5, 10 and 20 mg/ml) did not present significant difference among each other, and the only treatment that differed from the control was the 20 mg/ml.

In the analyses of the radicle and hypocotyl it was observed that all treatments (5, 10 and 20 mg/ml) differed statistically from the control, where the concentrations of 5 and 10 mg/ml did not present significant difference. The concentration of 20 mg/ml expressed the lowest radicle length (2.86 mm) and hypocotyl (2.01 mm), being significantly different from the other concentrations. For the mitotic index, it was observed that the concentrations of 5 and 10 mg/ml did not present significant difference in comparison with the control group, only the 20 mg/ml presented significant difference from the other treatments, obtaining the lowest mitotic index of all (3.45).

Table 2 presents the physical-chemical characteristics of the ethanolic extract of *S. aculeatissimum* leaves. pH, osmotic potential and electric conductivity were measured for each concentration (5, 10 and 20 mg/ml) and the control group (distilled water). In the pH analysis, control presented the highest value (pH=4.87) compared to the treatment, while the 10 mg/ml concentration presented the lowest value (pH=4.74) among the concentrations. For the osmotic potential and electric conductivity, the 20 mg/ml concentration presented the highest values (1.26 MPa for osmotic potential and 2.91

Table 3. Antioxidant activity, total phenols and total flavonoids of the *Solanum aculeatissimum* ethanolic extract in different concentrations (mg/ml).

Extract (mg/ml)	Total phenols[a]	Total flavonoids[a]	% Antioxidant activity[b]	FRAP[c]
25	-	1.64	04.49	-
50	-	1.70	03.78	-
75	-	1.67	05.75	-
100	-	1.70	08.09	-
250	0.81	1.96	23.30	-
500	3.12	2.46	51.25	40.67
1000	3.95	3.42	86.50	55.11

[a]Average values ± standard deviation of triplicates for total phenols (equivalent mg/g of galic acid extract), total flavonoids amount (equivalent quercetin mg/g of extract); [b]Average values ± standard deviation of triplicates for test cleaning of DPPH radical scavenging activity; [c]Micromole Trolox equivalents (TE) per mg of dried extract (µmol TE/g of E.).

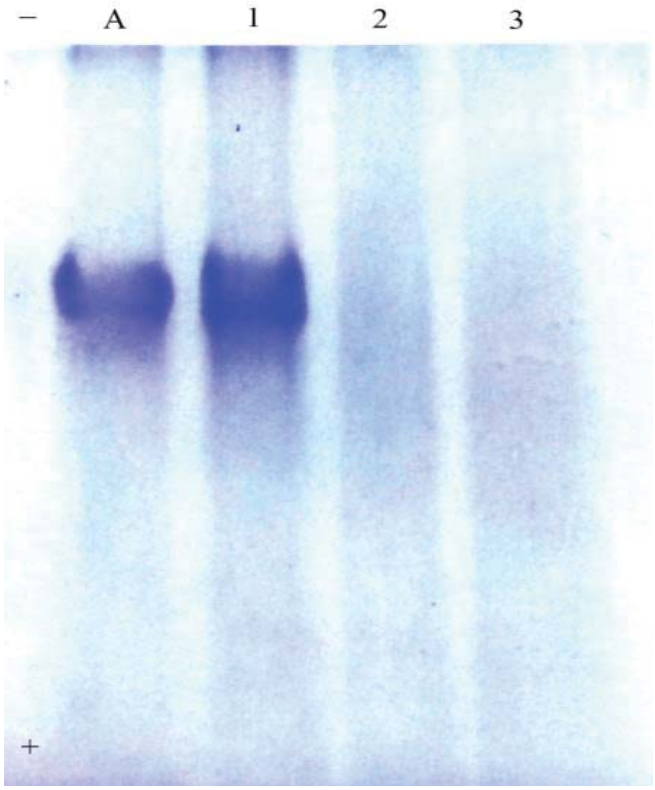

Figure 1. Effect of ethanolic extract from *S. aculeatissimum* and Cu_2+/H_2O_2 on migration of BSA with PAGE (Incubation period was 10 days). A: Native BSA, 1: BSA with Cu_2+/H_2O_2, 2: BSA with Cu2+/H_2O_2 and ethanolic extract (500 µg/ml), 3: BSA with Cu_2+/H_2O_2 and ethanolic extract (1000 µg/ml).

mS/cm for electric conductivity) compared to the other concentrations and control group.

In Table 3 are found the amounts of antioxidant, phenols and total flavonoids of the *S. aculeatissimum* ethanolicextractin different concentration (25, 50, 75, 100, 250, 500 and 1000 µg/ml), for these it was observed a dose dependence for the antioxidant activity and, phenols and total flavonoids determination, the EC50 was calculated at the concentration 556.38 µg for the antioxidant activity in DPPH assay. In the FRAP assay was found 55.11 (µmol TE/g of E.) at the concentration of 1000 µg/mL of extract.

It is possible to view electrophoresis (Figure 1) gel which was extensive fragmentation of the protein resulting from the action of BSA in conjunction extract used in two concentrations of 500 and 1000 µg/ml (lane 2 and 3), fragmentation was much higher compared to positive control who owned copper and hydrogen peroxide only in acting protein (lane 1).

The phytochemical triage of the *S. aculeatissimum* ethanolic extract is presented in Table 4. It is possible to observe the presence of coumarins, hydrolysable tannins and triterpenes, but in low visualization. For alkaloids the visualization was moderate and for flavonoids it was observed the highest visualization and test sensibility.

DISCUSSION

According to Lorenzi (2006), *S. aculeatissimum* presents high competitive capacity due to its invasive characteristics, being characterized as a weed. This peculiar characteristic of the species shows susceptibility in presenting substances of ecological importance, such as allelochemicals, as confirmed in the bioassays of this study. With the exposed, the obtained results of this study indicate that the *S. aculeatissimum* ethanolic extract possesses compounds capable of interfering in the germination index, radicle growth of lettuce and mitotic index of meristematic cells at the same laboratory conditions. Such results corrobarate to studies done by Silva et al. (2012) which showed that the concentrations of 10 and 20 $mg.mL^{-1}$ of the ethanol extract of leaves of *Zanthoxylum rhoifolium* Lam interfered significantly in the germination index and radicle growth of lettuce. According to Ferreira and Aquila (2000) and Inderjit

Table 4. Phytochemical triage of the *S. aculeatissimum* ethanolic extract.

Phytochemical components	Intensity
Alkaloids	++
Coumarins	+
Steroids	-
Flavonoids	+++
Condensed tannins	-
Hydrolysable tannins	+
Triterpens	+

+ Low visualization; ++ moderate visualization; +++ high visualization; - not visualized.

(2001), such changes may be directly linked with cellular and metabolic alterations, including modification in the membranes functioning, absorption of nutrients and water, photosynthetic and respiratory activities, cellular growth, expression and synthesis of nucleic acids and several other alterations.

pH and osmotic potential are factors that can interfere in the germination process, however the results obtained in the present study demonstrate that they stayed inside the acceptable standards of what is considered appropriate for germination and initial growth (Aquila et al., 2012) (Table 2). These evaluations are necessary, because plant extracts can present specific solutes that can change water properties, thereby being able to proportionate a false positive result for the experiment. Studies carried out by Ferreira and Áquila (2000) and Borella (2009) demonstrated that sugars, amino acids and organic acids are solutes that can mask the allelopathic effect of the extracts by pH interference or for being osmotic active.

Whereas the extract activity on the radicle length, Aquila et al. (1999) and Omezzine and Haouala (2013) showed that allelochemicals can act in different manners, depending in the environment that the target plant inhabits, once both reflect different physiological states. The results of the present study show the effects on seeds germination and lettuce seedlings development (Table 1). The results presented in the seedling development (post-emergent test), at the end of the 48 h of experimentation, present a significant reduction in the seedlings radicle and hypocotyl growth treated with the three extracts concentration, when compared to the control group (Table 1). This data showed that the extract, beyond presenting cytotoxic characteristics in the germination process as evaluated by the mitotic index determination, also presented phytotoxic action for the development of seedlings, corroborating study performed by Candido (2013) in different target plants.

Study performed by Inderjit (2011) demonstrated that plant extracts tested in bioassays for the investigation of possible allelopathic potential, are constituted by a blend of substances with primary and secondary origins of plant metabolism. According to Ahmad et al. (2011) the allelochemicals that act in pre-emergent, post-emergent and phytotoxicityare the benzoquinones, coumarins, flavonoids, terpenoids, lactones, mucilage and alkaloids that can be associated with effects on germination, plant development and possibly cell division, as demonstrated in this study.

Considering that allelochemicals are related to oxidative stress, became opportune the antioxidant evaluation of the *S. aculeatissimum* ethanolic extract, present in this study. Mori and Schroeder (2004) demonstrate that oxidative stress is a process that occurs in the plant tissues, where by action of some enzymes the superoxide radical is transformed in water. One of the many effects of allelochemical in plants is the production control and accumulation of oxygen reactive specimens (ORSs), which accumulate in cells as answer to the allelochemicals and are responsible by the cellular death (Gallice, 2011). Another mechanism related to the ORSs formation is the action of allelochemicals over the NADPH oxidase, enzyme responsible for the donation of NADPH electrons to an acceptor (O_2) forming superoxide (Foreman et al., 2003).

Thus, it was possible to analyze the antioxidant activity of the ethanolic extract, presenting activity of 86.50% in the concentration of 1000 µg/ml (Table 3). This activity is related to the presence of phenolic compounds electron donators, characterizing a reduction action, descendent of the vast presence of flavonoids in the *S. aculeatissimum* extract. It is known that flavonoids possess ideal structures for the scavenging of free radicals, being antioxidants with great reduction capacity (Ozçelik et al., 2011). With the exposed, this study also evaluated the presence of phenols and total flavonoids, for the concentration 1000 µg/ml was observed 3.95 mg equivalent grams of galic acid extract of total phenols. According to these results and according to studies mentioned above, is possible sugest that the free radicals elimination activity, just like the elevated level of poliphenols present in the extract, can be correlated with the action mechanism, characterized by the allelopathic effect of *S. aculeatissimum*.

Extracts in the presence of Cu^{2+} / H_2O_2 fragmentation of the protein was more severe, there was an enhancement by the extracts, this is a suggestion the one way how the phytotoxicityis expressed in the extract studied in this work. Considering the invasive characteristic of *S. aculeatissimum* and the results obtained in this study, it is possible to conclude that this species presents allelopathic substances with phytotoxic activity capable of interfering directly in stabilization and development of other species in their natural environment.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Integrated evaluation of soil fertility based on grey correlation analysis at regional scales

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This paper reports on studies of soil samples from the region of Xiaoliang water conservation station, in China, and its naturally restored forest. 32 indices based on the physical, chemical, microbiological and enzyme properties of the soil are studied, from 9 different vegetation patterns at 45 observation points. Grey correlation analysis and integrated evaluation is used for the analysis of soil fertility. From experimental measurements, it is shown that the integrated evaluation of the sample soil fertility by grey correlation analysis provides excellent agreement with the actual soil characteristics. Eucalyptus may be considered as a primary forest choice to recover bare land, however, it is not suitable for long-term planting and cropping since it will eventually result in degradation of soil fertility, and is therefore not favourable for long-term ecological restoration. Nevertheless, if chosen, additional actions can be taken to benefit soil fertility, such as regular changes of breed variety, crop rotation, mixing with broad-leaf forest, protecting undergrowth vegetation and reducing forest litter coverage.

Key words: Grey correlation, integrated evaluation, regional scale, soil fertility, water conservation station.

INTRODUCTION

Soil clearly represents one of the most important constituents of a forest ecological system, and its quality directly affects the health of the whole forest ecosystem, and which influences the sustainable development of the economy of human society (Pamela and John, 2003; Wienhold et al., 2004; Zhong et al., 2005; Dadhwal et al., 2011; Thierfelder and Wall, 2012). Soil affects land productivity and its sustainable use through processes of degradation and protection. It is governed by the interactions of the physical, chemical and biological characteristics of soil, and is also influenced by soil management (Eugène et al., 2010; Pinho et al., 2012). Soil quality assessment is based on the soil function to determine the criteria for quantitative assessment. Quality assessment is the most effective method to determine the dynamic change of soil conditions, reflecting changes of soil management and also the capability of soil to recover from degradation (Huang and Yang, 2009; Bautista-Cruz et al., 2011). The essence of soil quality is soil productivity, and its basis is soil fertility. Soil degradation is a process of decreasing soil productivity, which is affected mainly by mechanical, chemical and biological factors, where the soil characteristics are further affected by artificial interference that is, soil

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management (Huang, 2004; Eni et al. 2010). It is important to give consideration to the selection of soil quality assessment indices and in choosing their relative weightings. According to previously reported research, the indices for soil quality assessment is primarily related to the physical properties, such as unit mass, density and water content, the chemical properties, including pH, cation exchange capacity, and the nutrition content of N, P, K, etc. (Zhang et al., 2002); the biological properties (Hamer et al., 2009), including soil respiration and microbial biomass, and the enzyme activity properties (Kiss et al., 1998).

In this paper, the mechanical, chemical, microbiological and enzyme activity properties are used as a primary methodology for soil quality assessment, and 32 sub-system indices including the water content, unit weight, porosity, etc. are used as a secondary indexing system. The integrated evaluation of soil quality is performed by grey correlation analysis (Liu and Dang, 2008). The aim of the paper is to gain knowledge of the soil quality and its degradation degree in order to provide scientific evidence for ecological restoration, increasing land productivity and promoting the sustainable development of the forest economy.

OVERVIEW OF THE RESEARCH REGION

Xiaoliang water conservation station is on the costal terraced land in the southwest of Xiaoliang town, Dianbai county, Maoming City, Guangdong Province, China, with geographic location of 110°54′18″E, 21°27′49″N. The area of the station is 288.87 m^2. The greatest highest height above sea level is 36 m. It belongs to the northern tropical maritime monsoon climate, and the annual average temperature is 23°C, with the highest temperature 36.5°C, and the lowest 4.7°C. The yearly average precipitation is 1442 mm, and the hyetograph is extremely non-uniform. Patterns of rainfall are normally convectional rain and typhoon rain. Most of the rainfall occurs between June and August. There are obvious divisions between the dry and rainy seasons. The annual average evaporation is 2100 mm. Zonal soils are mostly pebbly clay lateritic, derived from granite parent materials. The top vegetation plant cover is tropical monsoon forest (Liu and Li, 2009).

METHODOLOGIES

Collection of soil samples

The collection of forest soil samples are at the same time period and under similar site conditions. The soil samples are collected from the different vegetation patterns: mixed forest, pure eucalyptus forest and bare land, separately. According to the vegetation patterns and the planting time, 9 sample areas are chosen in, and around, Xiaoliang water conservation station for investigation. 5 sample plots are chosen in each sample area, in an ‘X’ formation. A profile is dug in each sample plot. The soil profile is at the concentrated distribution area of the forest root growth, ~ 1 m from the tree trunk, avoiding the fertilization spot and the manual farmland soil. The soil samples are gathered evenly from bottom to top on the 0 ~ 60 cm soil profile in 3 layers. The mass of the soil sample from every profile layer is 0.2 kg, which is further mixed to form one soil sample with a mass of 1 kg. The soil samples are subsequently taken to the laboratory for physical, chemical, microbiological and enzyme activity testing (Carter, 1993; Sparks, 1996; Sarkar, 2005).

Pre-processing of soil samples

Soil samples gathered from the field are pre-processed in the laboratory prior to subsequent testing and analysis. The purpose of sample pre-processing is, (1) to prolong the service of the samples and reduce deterioration due to microbial activity, (2) to assure the results of analysis represent the original composition of the soil by removing non-soil particles from the samples, (3) to make the analysis samples more representative and to reduce the errors on sample mass by adequate grinding and full blending of the field samples, (4) to maintain consistency of the reactions of the testing soil solutions by increasing the surface area of the soil particles through grinding of the field samples.

Conventional pre-processing of soil samples

Conventional processing of soil samples use air-drying, grinding, screening, blending, separating, weighing and storage. The field forest soil samples are air-dried immediately to avoid the samples becoming moldy and affecting their quality. The processing method is to break the soil sample into small pieces and spread them flat onto a thin layer on clean paper in a cool, well-ventilated environment, turning them regularly to accelerate the drying. Direct sunlight is avoided. The soil samples are then grinded, screened, blended and separated into suitable analysis samples.

Processing for special soil samples

To analyse the physical properties of soil, 100 ~ 200 g of air-dried samples are used, and any large organic materials and stone blocks are picked out, grinded, screened by sieve No. 6 ~ 7 (3 mm) and sieve No. 8 (1 mm). The fine gravels of the scale of 1 ~ 3 mm, are weighed. Meanwhile the soil samples after sieve size of 1 mm are weighed, and the percentage of 1 ~ 3 mm fine gravels, is calculated. Finally, after blending, the soil samples are placed into a wide-mouthed container for an analysis of particles and testing of other physical properties. If iron manganese concretions or calcareous concretions exist, they are carefully removed and weighed for further analysis and testing.

Testing methods for the soil samples

The soil samples, after pre-processing, are used for tests of their physical, chemical, biological and enzyme activity properties. The measurement results are given in Appendix Table 1. The following list provides the testing method of each property:

(i) Water content in soil – oven-drying method;
(ii) Specific weight – pycnometer;
(iii) Physical composition of soil – densimeter;
(iv) pH of soil – potentiometric method;
(v) Organic content in soil – potassium dichromate oxidation-reduction titration method;
(vi) Nitrogen content in soil – Kjeldahl nitrogen determination method;

Table 1. Average testing values of the physical properties of soil in sample areas.

Sample areas	Naturally restored forest	Broad-leaved forest	Eucalyptus pine mixed forest	Bare land	W5.41	W5.44	W5.34	W5.31	U6.31
Unit weight (g cm^{-3})	1.383	1.660	1.673	1.677	1.683	1.717	1.720	1.720	1.770
Specific weight (g cm^{-3})	2.543	2.593	2.540	2.577	2.637	2.660	2.653	2.607	2.607
Rock content (%)	21.233	25.640	21.727	21.213	20.580	20.967	20.573	20.563	20.570
Clay content(%)	35.160	30.340	34.480	38.250	32.397	32.253	40.430	32.153	32.387
Gross porosity (%)	37.743	35.090	37.403	31.953	36.283	36.213	34.293	36.957	37.597
Capillary porosity (%)	28.383	27.410	31.797	26.447	30.070	27.830	28.077	32.807	32.880
Noncapillary porosity (%)	9.360	7.683	5.607	5.507	6.213	8.383	6.250	4.150	4.720
Natural water content (%)	16.910	16.500	12.523	9.090	7.113	7.150	11.373	6.197	6.167
Soil hygroscopic water (%)	1.207	1.003	0.633	0.590	0.853	0.863	1.063	0.867	0.853
Field water capacity (%)	17.927	16.283	19.997	15.083	17.900	16.403	16.107	19.963	20.213

(vii) Variation of alkaline hydrolysis nitrogen – alkali - hydrolyzed diffusing method;
(viii) Total phosphorus in soil – NaOH fusion–Mo-Sb anti spectrophotometric method;
(ix) Available phosphorus in soil – 0.05 mol L^{-1} HCl - 0.025 mol L^{-1} (1/2) H_2SO_4 bleaching solution – anti-spectrophotometric method;
(x) Total potassium in soil – NaOH fusion flame photometry;
(xi) Available potassium in soil – 2 mol L^{-1} HNO_3 bleaching solution-flame photometry;
(xii) Microbial biomass in soil – beef extract plate method to determine the biomass of bacteria, fungus and actinomyces;
(xiii) Soil respiration and induced respiration – isolation tank-alkaline solution absorption method;
(xiv) Urease activity in soil – indophenol blue colorimetric method;
(xv) Soil phosphatase activity – disodium phenyl phosphate colorimetry;
(xvi) Saccharase activity – 3, 5-dinitrosalicylic acid colorimetry.

RESULTS AND ANALYSIS

The physical properties of soil provide a comprehensive reflection of its basic features, and are an amalgamation of many factors. They also affect the soil productivity. The analysis is typically complex with many inter-related factors influencing quality (Wang et al., 2007; Yusuf and Yusuf, 2008; Adesanwo et al., 2009; Belachew and Abera, 2010). The grey system theory is based on an analysis and determination of the interactions between many inter-related factors and their contributions to the principal characteristics, according to relative similarities and differences. It is a quantitative assessment method, providing a magnitude, direction and speed of the factors in the system process. If the relative change between two factors is consistent, they are considered to have high correlation. The grey correlation degree is the index for the uncertainties between the factors, or those between a factor and the principal characteristic. From the sample areas chosen here, the physical properties of soil can be analyzed with respect to correlations between factors such as soil unit mass, specific weight, content of rock fragments, content of clay, porosity, water content, among others.

From a random factor sequence, the correlation can be calculated to determine their contribution to the soil fertility through a quantitative analysis of the physical properties. The method is proved to be effective, efficient and intuitive. The procedure for grey correlation analysis for the 9 sample plots is as follows:

(1) Determine the reference sequence of the factors:

$X_0=\{X_{01}, X_{02}, X_{03}, \ldots, X_{0n}\}$ (K=1, 2,… , n);
X_0={1.383, 2.540, 20.563, 40.430, 37.743, 32.880, 9.360, 16.910, 1.207, 20.213};

(2) Determine the comparative sequence of the factors:

$X_i=\{X_{i1}, X_{i2}, X_{i3}, X_{in}\}$ (i=1,2,3,… , m; K=1,2,… , n;)

Where X_1={naturally restored forest}; X_2={broad-leaved forest}; X_3={eucalyptus pine mixed forest }; X_4={bareland}; X_5={W5.41}; X_6={W5.44}; X_7={W5.34};

Table 2. The physical properties of soil after nondimensionalization.

Sample areas	Naturally restored forest	Broad-leaved forest	Eucalyptus pine mixed forest	Bare land	W5.41	W5.44	W5.34	W5.31	U6.31
Unit weight	1.000	1.200	1.210	1.213	1.217	1.242	1.244	1.244	1.280
Specific weight	1.001	1.021	1.000	1.015	1.038	1.047	1.044	1.026	1.026
Rock content	1.033	1.247	1.057	1.032	1.001	1.020	1.000	1.000	1.000
Clay content	0.870	0.750	0.853	0.946	0.801	0.798	1.000	0.795	0.801
Gross porosity	1.000	0.930	0.991	0.847	0.961	0.959	0.909	0.979	0.996
Capillary porosity	0.863	0.834	0.967	0.804	0.915	0.846	0.854	0.998	1.000
Noncapillary porosity	1.000	0.821	0.599	0.588	0.664	0.896	0.668	0.443	0.504
Natural water content	1.000	0.976	0.741	0.538	0.421	0.423	0.673	0.366	0.365
Soil hygroscopic water	1.000	0.831	0.524	0.489	0.707	0.715	0.881	0.718	0.707
Field water capacity	0.887	0.806	0.989	0.746	0.886	0.812	0.797	0.988	1.000

X_8={W5.31}; X_9={U6.31}*. The average testing values of the mechanical properties of soil are listed in Table 1.

(3) Nondimensionalization of the data: Because the units of the collected data and the physical significance of the factors are different, to make sure the equivalence of each factor, the original collected data is nondimensionalized, by $X_i'= X_i/ X_0$. The data, after nondimensionalization is listed in Table 2.

(4) Calculate the absolute difference sequence – Define $\Delta 1=|A_0(K)-A_1(K)|$; $\Delta 2=|A_0(K)-A_2(K)|$; and so on. The absolute differences are listed in Table 3.

(5) Find the maximum and minimum differences: The maximum difference is: $M=\max_i.\max_k \Delta_i(K)$, M=0.635; and the minimum difference is: $m=\min_i.\min_k \Delta_i(K)$, m=0.000.

*U6.31: Eucalyptus urophylla asexual plantation, the third crop annual eucalyptus; W5.31: Eucalyptus ABL12 asexual plantation, the third crop annual eucalyptus; W5.34: Eucalyptus ABL12 asexual plantation, the third crop four yearly eucalyptus; W5.41: Eucalyptus ABL12 asexual plantation, the fourth crop annual eucalyptus; W5.44: Eucalyptus ABL12 asexual plantation, the fourth crop four yearly eucalyptus.

(6) Calculate the correlation coefficients, as listed in Table 4: p=0.5

$$\eta_{0i}(\kappa)=\frac{P\min.\min\left|\chi_0(\kappa)-\chi_i(\kappa)\right|+P\max.\max\left|\chi_0(\kappa)-\chi_i(\kappa)\right|}{\left|\chi_0(\kappa)-\chi_i(\kappa)\right|+P\max.\max\left|\chi_0(\kappa)-\chi_i(\kappa)\right|}$$

(7) The correlation degrees (Table 5) are calculated from

$$\tau_i=\frac{1}{n}\sum_{k=1}^{n}\eta_i(k)$$

(8) Analysis of the results: The results show that, r1 > r3 > r9 > r8 = r7 > r2 > r5 > r6 > r4, where ri > rj, showing that the grey correlation degree of X_i with respect to reference X_0 is higher than X_j. The higher the correlation degree, the stronger the relationship exists between it and the reference factor. Here, it is shown that the naturally restored forest provides the best physical properties, and bare land yields the poorest. The relative order of the 9 sample areas, with respect of the physical properties of soil, in descending order is: naturally restored forest > eucalyptus pine mixed forest > U6.31 > W5.31 = W5.34 > broad-leaved forest > W5.41 > W5.44 > bare land.

Using the same procedure as above, the grey correlation degrees for the chemical properties of soil are listed in Table 6, for microbiological properties in Table 7, and for enzyme activities in Table 8. The results of the grey correlation degrees for the chemical properties of soil in Table 6 show that, r1 > r2 > r9 > r6 > r3 > r8 > r5 > r7 > r4. The results of the grey correlation degrees for the microbiological properties of soil in Table 7 show that, r1 > r2 > r3 > r7 > r9 > r8 > r5 > r6 > r4. It is shown from the results of the grey correlation degrees for the enzyme activity in soil in Table 8 that, r1 > r2 > r3 > r9 > r5 > r8 > r7 > r6 > r4.

Integrated evaluation of the soil fertility

The degradation of soil productivity is a process of decreasing soil fertility, which is a comprehensive reflection of the degradation of its physical, chemical, microbiological and enzyme activity properties. Based on the results from the grey correlation analysis, a comprehensive index of soil

Table 3. The physical properties of soil in order of absolute difference.

S/No	Δ1	Δ2	Δ3	Δ4	Δ5	Δ6	Δ7	Δ8	Δ9
1	0.000	0.200	0.210	0.213	0.217	0.242	0.244	0.244	0.280
2	0.001	0.021	0.000	0.015	0.038	0.047	0.044	0.026	0.026
3	0.033	0.247	0.057	0.032	0.001	0.020	0.000	0.000	0.000
4	0.130	0.250	0.147	0.054	0.199	0.202	0.000	0.205	0.199
5	0.000	0.070	0.009	0.153	0.039	0.041	0.091	0.021	0.004
6	0.137	0.166	0.033	0.196	0.085	0.154	0.146	0.002	0.000
7	0.000	0.179	0.401	0.412	0.336	0.104	0.332	0.557	0.496
8	0.000	0.024	0.259	0.462	0.579	0.577	0.327	0.634	0.635
9	0.000	0.169	0.476	0.511	0.293	0.285	0.119	0.282	0.293
10	0.113	0.194	0.011	0.254	0.114	0.188	0.203	0.012	0.000
min	0.000	0.021	0.000	0.015	0.001	0.020	0.000	0.000	0.000
max	0.137	0.25	0.476	0.511	0.579	0.577	0.332	0.634	0.635

Table 4. The correlation coefficients of the physical properties of soil.

S/No	n01	n02	n03	n04	n05	n06	n07	n08	n09
1	1.000	0.613	0.602	0.599	0.594	0.568	0.566	0.566	0.532
2	0.996	0.938	1.000	0.956	0.893	0.870	0.877	0.923	0.923
3	0.907	0.563	0.849	0.909	0.997	0.942	0.998	1.000	0.999
4	0.709	0.560	0.683	0.855	0.615	0.611	1.000	0.608	0.615
5	1.000	0.819	0.972	0.674	0.891	0.887	0.776	0.938	0.988
6	0.699	0.656	0.906	0.619	0.788	0.674	0.685	0.993	1.000
7	1.000	0.639	0.442	0.435	0.486	0.753	0.489	0.363	0.390
8	1.000	0.929	0.550	0.407	0.354	0.355	0.492	0.334	0.333
9	1.000	0.653	0.400	0.383	0.520	0.527	0.727	0.530	0.520
10	0.737	0.620	0.967	0.556	0.735	0.627	0.610	0.963	1.000

Table 5. Grey correlation degrees of the physical properties of soil.

r1	r2	r3	r4	r5	r6	r7	r8	r9
0.905	0.699	0.737	0.639	0.687	0.681	0.722	0.722	0.730

Table 6. Grey correlation degrees of the chemical properties of soil.

r1	r2	r3	r4	r5	r6	r7	r8	r9
0.648	0.605	0.517	0.457	0.488	0.518	0.486	0.495	0.527

Table 7. Grey correlation degrees of the microbiological properties of soil.

r1	r2	r3	r4	r5	r6	r7	r8	r9
0.6792	0.6065	0.5289	0.4696	0.5034	0.4912	0.5256	0.5115	0.5158

fertility assessment is calculated from the contribution scores, in order to evaluate the levels of productivity for the different vegetation patterns. From the comparisons, it is shown that pure eucalyptus forest has negative

Table 8. Grey correlation degrees of the enzyme activities in soil.

r1	r2	r3	r4	r5	r6	r7	r8	r9
0.7085	0.6530	0.6398	0.4098	0.5222	0.5065	0.5085	0.5107	0.5248

Table 9. The integrated evaluation of the soil quality from 9 sample areas.

Sample areas	Physical properties	Chemical properties	Microbiological properties	Enzyme activities	Average	Level
r1	0.9	0.9	0.9	0.9	0.90	Very good
r2	0.4	0.8	0.8	0.8	0.70	Relatively good
r3	0.8	0.5	0.7	0.7	0.68	Relatively good
r4	0.1	0.1	0.1	0.1	0.10	Very poor
r5	0.3	0.3	0.3	0.5	0.35	Relatively poor
r6	0.2	0.6	0.2	0.2	0.30	Relatively poor
r7	0.55	0.2	0.6	0.3	0.41	Relatively poor
r8	0.55	0.4	0.4	0.4	0.44	Relatively poor
r9	0.7	0.7	0.5	0.6	0.63	Relatively good

effect on the soil productivity. According to results from the 9 sample areas, they are categorized into 5 levels: very good (0.8 ~ 0.9); relatively good (0.6 ~ 0.7); moderate (0.5); relatively poor (0.3 ~ 0.4); very poor (0.1 ~ 0.2), and are listed in Table 9.

Conclusion

The proposed analysis techniques have been employed for the first time to provide an integrated evaluation of soil fertility. As shown, the proposed use of grey correlation analysis provides good agreement with the actual properties of the differing vegetation patterns. Key outcomes of the research are:

(1) From the evaluation scores, the soil fertility in descending order is: naturally restored forest (r1 = 0.9) > broad-leaved forest (r2 = 0.7) > eucalyptus pine mixed forest (r3 = 0.68) > U6.31 (r9 = 0.63) > W5.31 (r8 = 0.44) > W5.34 (r7 = 0.41) > W5.41 (r5 = 0.35) > W5.44 (r6 = 0.3) > bare land (r4 = 0.1).
(2) Through use of the proposed approach, the sample areas are categorized into 5 levels: Level 1 (very good) is the naturally restored forest, which shows that the soil productivity of natural forest is better than man-made forest. Increasing artificial interference hastens soil fertility degradation. Level 2 (relatively good) includes the broad-leaved forest, the eucalyptus pine mixed forest and the U6.31 pure eucalyptus forest, which shows that, among the planted forest, broad-leaved forest is better than eucalyptus pine mixed forest, which, in turn, is better than pure eucalyptus forest. Among pure eucalyptus forest, U6 clone forest shows better soil fertility than the W5 clone forest. Level 4 (relatively poor) is the pure eucalyptus forest. For the same species of W5 clone pure eucalyptus forest, the degradation of soil increases with increasing tree age and with cropping and planting. Level 5 (very poor) is the bare land, where due to the lack of vegetation protection, every characteristic that is assessed, is ranked as poor.

In summary, planting pure eucalyptus forest is beneficial for recovering land productivity, but it is not as good for soil fertility when compared with other types of vegetation. It is therefore concluded that eucalyptus can be used as a pioneer forest to recover the bare land, but it is not suitable for long-term planting and cropping, which can have detrimental effects on ecological restoration. When considering the planting of a pure eucalyptus forest, the following actions are advised in order to recover / maintain soil fertility viz. regular changes of tree species, crop rotation, mixing with broad leaf forest, saving forest litter coverage, etc.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Post-harvest effects on beverage quality and physiological performance of coffee beans

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During coffee drying, different temperatures applied to the beans with varied humidity content levels can interfere in the membranes integrity, germination, organic acid and carbohydrate content resulting in coffees with distinct flavors. The quality control of the beans will be much more effective the earlier the alterations provoked in the postharvest are detected. This work has an objective to study alternative methods for the dehydration of the coffee beans using ultra-drying followed by slow drying and its impact on the sensorial quality, chemical composition and physiology. For that purpose, coffee lots were processed by the methods, dry (natural coffee) and wet (fully washed coffee); and sun-dried and machine-dried at a constant 60°C temperature and alternating 60/40°C. The sensory quality of the samples was assessed by the Specialty Coffee Association of America (SCAA) analysis protocol. The sugar, total titratable acidity and the phenolic compound content was also analyzed. The physiological alterations of the coffee beans were analyzed by germination tests, emergence speed index, electrical conductivity and potassium leaching. The temperature of the drying air significantly altered the sensorial quality of the coffee beans. The processing way associated to drying methods causes many physiological alterations with the highest damage observed in the natural coffees. For the first time, we are showing that drying with heated air at 60/40ºC is promising for the fully washed coffee beans, which are more tolerant to dehydration than the natural coffee beans. Conversely, the natural coffee beans were much more sensitive to drying regardless the temperature, with very low performance in the physiological analyses. The drying at the constant 60ºC temperature is inappropriate for the natural coffee as well as for the fully washed coffee beans. In addition, the physiological tests used were shown effective for the early evaluation of coffee beans quality.

Key words: *Coffea arabica* L., processing, drying, sensory analysis, chemical composition, germination.

INTRODUCTION

The quality of coffee is determined mainly by the flavor and aroma formed during the roasting of the beans. Approximately 300 chemical compounds present in green coffee beans originate about 850 compounds after the roasting (Flament, 2001). Many of those compounds act as precursors of coffee flavor and aroma, their presence

being dependent on the combination of genetic, environmental and technological factors (Bertrand et al., 2006; Farah et al., 2006). Other factors, such as post-harvest procedures also interfere in the coffee quality, especially processing and drying (Borém et al., 2008a, 2014; Saath et al., 2010, 2014; Taveira et al., 2012).

There are two processing methods used for the preparation of the coffee: the dry and the wet. In the dry processing, the whole or intact fruits are submitted to drying, without the removal of the exocarp (outer skin), resulting in the natural coffees. In the wet processing, the exocarp of the fruit is removed, creating three types of coffee: the peeled cherry coffee obtained after the peeling and drying of the beans, the remaining mucilage staying adhered to the parchment; the fully washed coffee, produced from the peeling of the fruits and removal of the mucilage by fermentation; and the demucilaged coffee, whose mucilage is removed mechanically.

The chemical composition of the raw coffee beans depends on the processing manner used (Bytof et al., 2004; Knopp et al., 2005; Borém et al., 2008b), effects of which determine distinct quality characteristics. Natural and fully washed coffee beans originate beverage with very distinct profile but with very similar final total score. Usually, the natural coffee beans have denser body and sweet drink compared to the fully washed coffees, which have a more acidy drink. The sugars contribute to the sweetness of the drink, being considered one of the most desirable flavor attributes in the special coffees. The sugars participate in important chemical reactions such as the Maillard reaction giving rise to compounds responsible for the formation of the color, flavor and the peculiar aroma of the drink (Arruda et al., 2012; Liu and Kitts, 2011; Murkovic and Derler, 2006). Several studies indicate that the postharvest operations also exercise influence on the sugar levels (Joët et al., 2010; Knopp et al., 2005).

The main phenolic compounds of coffee are in the form of chlorogenic acids (Farah and Donangelo, 2006; Monteiro and Farah, 2012). Besides contributing to the flavor and aroma of the drink, those acids can present benefits to human health. However, the presence of high amounts of chlorogenic acids increases the astringency of the coffee flavor, contributing to the devaluation of the product (Clifford, 2000).

A soft, fruity or citric acidity, perceived by the tasters in the sensory analysis, has been pointed as a good indicator of the coffee quality. However, acetic or very intense acidity is related to some type of fermentation which occurs in the postharvest and it represents a negative aspect in the sensory evaluation of the coffee. In the literature, it is possible to find works that describe significantly higher total titratable acidity values for coffee processed by the dry method when compared to the values obtained for the fully washed coffees (Leite et al., 1996). However, the total titratable acidity values are not always directly related to the sensorial perceptions, because it involves the analysis of a very large group of organic acids and other chemical compounds.

The Specialty Coffee Association of America (SCAA) sensory evaluation method has stood out for its quality evaluation of the special coffee drinks. That method is based on a quantitative descriptive analysis of the drink, conducted by a team of selected tasters and, making use of a non-structured scale from 6 to 10 points for the evaluation of the fragrance, aroma, flavor, aftertaste, acidity, body, balance, sweetness, absence of defects and drink uniformity, with evaluation of the global quality of the coffee according to the terminology presented by Lingle (2011).

Physiological analyses have been used to evaluate the quality of the coffee beans and can be a valuable tool to evaluate the drink quality indirectly. Important biochemical alterations in the coffee beans during the processing, related to the metabolism of germination, whose extension depends on the preparation means, be it wet or dry (Bytof et al., 2007; Selmar et al., 2006). However, a correlation between the physiological performance and alterations with the drying methods is still missing in the literature.

The drying of coffee, if poorly conducted, can intensify the degradation of cell membranes which can be consistently indicated by potassium leaching and electrical conductivity tests (Prete, 1992). The coffee beans with badly structured, disorganized and damaged membranes leach higher amounts of solutes, presenting higher electrical conductivity and potassium leaching values (Krzyzanowski et al., 1991), indicating loss of quality (Prete, 1992).

In drying studies, the highest damage in coffee bean cell membrane systems occur due to the increase of the drying temperature and the high drying rates provoked by high temperatures can cause damage to the coffee quality due to the damage caused to the cell membranes (Borém et al., 2008b; Marques et al., 2008; Saath et al., 2010). In addition, the cell membranes of the coffee beans are damaged only between the 30 and 20% (w.b.) humidity levels (Borém et al., 2008b), when the natural and fully washed coffees were dried at the 60ºC temperature. Thus, a drying method that uses high temperatures at the beginning of the process, followed by lower temperatures at the end, could become promising, highlighting the lower exposure time of the beans to the drying conditions, contributing to the quality maintenance of the coffee.

The physiological analyses of the coffee during the postharvest can also aid in the elucidation of the

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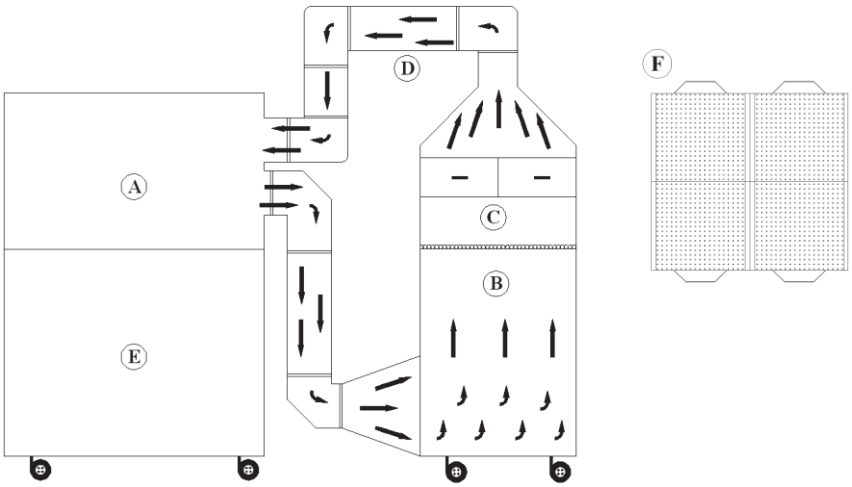

Figure 1. Scheme of the apparatus used in the mechanic drying: (A) compartment of air conditioning (B) plenum; (C) drying compartment; (D) recirculation system of air; (E) electrical system, motor and fan, and (F) removable drawer of the drying compartment.

biochemical events that occur in the beans during the processing and drying, which will result in different chemical compounds and, therefore, in different drink qualities.

The objective of the present research was to assess the effects of different processing and drying methods on the physiological quality and chemical composition of coffee beans, analyzing their interrelation with the quality of the drink.

MATERIALS AND METHODS

Experimental design

The experiment was conducted with handpicked ripe coffee fruits of Catuaí Vermelho IAC 99 (*Arabic Coffea* L. cv.). The fruits were washed and separated by density, eliminating the float portion formed by light, hollow, bored or poorly grained fruit. To guarantee the uniformity of the raw material, new manual selection was carried out in the portion of ripe fruits, eliminating all of the immature and over-ripe fruits still present, remaining only the fruits at the cherry stage. The cherry coffee was processed by dry and wet methods and dried under three different conditions: on an asphalt patio, in a hot air dryer at a constant 60°C temperature and in dryer with air heated to an alternating 60/40°C temperature.

In the dry processing of the coffee samples, the ripe fruits were dried with the exocarp intact, obtaining the natural coffee. In the wet processing, the coffee was mechanically peeled and depulped by natural fermentation in water for approximately 20 h, under ambient conditions at an average temperature of 22°C.

The experiment was conducted in a randomized block design with three repetitions represented by the harvest times and in a 2 × 3 factorial outline (two processing methods and three drying methods):

i. Patio drying: After the processing, the coffee was taken for drying on the patio, where it remained under ambient conditions and was managed according to the procedures proposed by (Borém, 2008). The control treatment portions remained on the patio until they reach a moisture content of 11% (w.b.), for the natural coffee as well as for the fully washed coffee samples.

ii. Drying in mechanical dryer: To minimize the differences in the initial moisture level in the hot air drying, the portions destined for mechanical drying passed through a two day pre-drying period on the patio for the natural coffee, and one day for the fully washed coffee samples. After the pre-drying period, the portions were taken to the 0.15 m fixed bed dryer (Figure 1) coupled to a high-precision air conditioner, according to the model proposed by Fortes et al. (2006), which allows for the precise control of the air flow, temperature (T) and relative humidity (RH) of the drying air.

The air flow was maintained at 20 $m^3.min^{-1}.m^{-2}$, normal values for the commercial coffee dryer models. To maintain the same drying conditions during the whole experiment, independently of the climatic variations, the temperature and relative humidity values of the air were controlled and maintained constant, observing a relative humidity of 7% for the hot air at 60°C and 21% for the hot air at 40°C. Those drying conditions were established based on the thermodynamic studies of the ambient air warming, considering the climatic averages of 20°C and 60% RH during the coffee harvest months.

The portion that received the 60°C hot air treatment remained in the dryer until the coffee reached a moisture content of 11% ± 0.5% (wb). The portion that received the 60/40°C hot air treatment was dried at 60°C until the beans reach 30% (wb) moisture followed by drying with 40°C air until reaching 11% (wb).

The control of the bean moisture level during drying was handled by successive weighing knowing the mass and the initial moisture content of each coffee sample (Equations 1 and 2). The coffee moisture content was determined by the standard (ISO, 6673, 2003) method. To determine the air temperature transition point from 60 to 40°C, each drawer, containing the experimental portion, was weighed every 30 min. When each drawer reached the mass relative to a 30% ± 2% (wb) moisture content, the temperature was changed from 60 to 40°C, remaining at 40°C until the coffee reached 11% (wb).

$$PML = \left[\frac{(MC_i - MC_f)}{100 - MC_f}\right] \times 100 \quad (1)$$

$$M_f = M_i - \left(M_i \times \frac{PML}{100}\right) \quad (2)$$

in which PML: percentage of mass loss (%); MC_i: initial moisture content (% wb); MC_f: final moisture content (% wb); M_f: final mass (kg) and M_i: initial mass (kg).
The moisture reduction rate was obtained by means of Equation 3.

$$MRR = \left(\frac{MC_0 - MC_c}{t_c - t_p}\right) \quad (3)$$

in which MRR: moisture reduction rate (kg H_2O.kg coffee^{-1}.hour^{-1}); MC_0: previous moisture content (kg H_2O.kg coffee^{-1}); MC_c: current moisture content (kg H_2O.kg coffee^{-1}); t_c: total current drying time (hours); t_p: total previous drying time (hours).

Characterization of coffee quality

Sensorial analysis and chemical analyses: The sensorial analysis and the chemical analyses were conducted in the Pólo de Tecnologia em Qualidade do Café da Universidade Federal de Lavras. For the chemical analyses, three subsamples of each experimental portion were used, corresponding to the respective treatments. The chemical and sensorial analyses were conducted in the portions of the bean samples classified above sieve 16, discarding the peaberry and defective beans.

Sensory analysis: The sensorial analysis was carried out according to the protocol established by the Specialty Coffee Association of America (SCAA), (Lingle, 2011). For presenting different sensory characteristics, the sensorial analysis of the natural and fully washed coffees was conducted separately, aiming to minimize possible negatives or positive interferences. The final results of the sensorial evaluation were made up of the sum of the scores of all of the attributes.

Reducing and non-reducing sugars: The total and reducing sugars were extracted by the Lane-Enyon method, cited by (AOAC, 1990) and determined by the Somogy adapted technique (Nelson, 1944). The non-reducing sugars were determined by the difference between the total and reducing sugars.

Total titratable acidity: The total titratable acidity was determined by titration with NaOH 0,1N, adapting the methodology (AOAC, 1990). Two grams of the ground coffee sample were weighed, and added to 50 mL of distilled water, agitating for one hour. Soon afterwards, filtering took place in filter paper and 5 mL of the filtered solution removed, placed in an Erlenmeyer flask with about 50 mL of distilled water. Three drops of phenolphthalein were added and, soon afterwards, it was titrated to turning with NaOH 0,1 N. The result was expressed in mL of NaOH 0,1 N for 100 g of sample.

Polyphenols: The polyphenols were extracted using 80% methanol (U/V) as extractor (Goldstein and Swain, 1963) and identified according to the Folin Denis method (AOAC, 1990). The results were expressed as percentage of dry matter.

Physiological analyses: For the physiological analyses; four seed subsamples were used without visible defects for each repetition of the respective treatments.

Germination test: The germination test was conducted with four subsamples of 50 seeds without parchment, distributed on a paper towel moistened with an amount of water equal to two and a half times the substrate dry mass, and placed to germinate at a temperature of 30ºC. The evaluations were made at thirty days after the sowing (Brasil, 2009) and the results expressed in percentage.

First germination and root protrusion count: The first germination count was carried out together with the germination test; fifteen days after the beginning of the test. The seeds that presented a principle root and at least two lateral roots were considered as normal seedlings (Brasil, 2009) with the results expressed in percentage. Also, at fifteen days from the beginning of the germination test; the counting of root protrusion was done with the results expressed in percentage.

Emergence velocity index test (EVI): Four subsamples of 50 seeds were sowed in plastic trays containing a 2:1 mixture of sand and soil. The trays were maintained in a growth chamber, previously regulated to a temperature of 30°C, under an alternating regime of light and darkness (12 h). The trays were irrigated when necessary and, at the onset of the emergence, the emerged seedlings were computed daily, until stabilization, with the results expressed in percentage of final emergence. ESI was calculated according to the formula proposed by Maguire (1962), using the following equation:

$$EVI = \left(\frac{E1}{T1}\right) + \left(\frac{E2}{T2}\right) + \cdots + \left(\frac{En}{Tn}\right) \quad (4)$$

In which EVI: emergence velocity index; E: number of emerged seedlings per day; T: time from sowing to the respective counting (day).

Open cotyledonary leaves: At the end of the emergence test, the seedlings that presented totally expanded cotyledonary leaves were counted and the results were expressed in percentage.

Electrical conductivity: The electrical conductivity of the raw beans was determined adapting the methodology proposed by Krzyzanowski et al. (1991). Four repetitions of 50 beans of each portion were used, which were weighed to a precision of 0.001 g and immersed in 75 mL of deionized water inside 180 mL plastic cups. Soon afterwards, these containers were taken to a forced ventilation greenhouse at a temperature of 25°C, for five hours, proceeding with the electrical conductivity reading of the soaking water in a Digimed CD-20 apparatus. With the obtained data, the electrical conductivity calculations were made, the result being expressed as $\mu S.cm^{-1}.g^{-1}$ of beans.

Potassium leaching: The leaching of potassium ions was conducted in the raw beans, according to the methodology proposed by Prete (1992). After the electrical conductivity reading, the solutions were submitted to the determination of the amount of leached potassium. The reading was conducted in a Digimed NK-2002 flame photometer. With the obtained data, the calculation of the amount of leached potassium was made and the result was expressed in ppm.

Statistical analysis

The data obtained in the chemical, sensorial and physiological analyses were submitted to the ANOVA using the Sisvar 4.0 computational program (Ferreira, 2011) and the means were compared by the Scott-Knott test (Scott and Knott, 1974) at 5% level of significance.

RESULTS

Drying conditions

During the drying on the patio, the air temperature varied

Table 1. Average final score of the sensorial analyses of coffees submitted to processing and drying.

Processing	Final score	Drying	Final score
Natural	79.04[A]	Patio	80.35[A]
Fully washed	78.98[A]	60/40ºC	79.05[B]
		60ºC	77.64[C]

Averages followed by the same capital letters on the columns do not differ statistically for the Scott-Knott test at 5% probability.

Table 2. Average of total sugars, reducing sugars, non-reducing sugars and total titrable acidity (TTA) of the natural and fully washed coffees.

Processing	Total sugars (%)	Reducing Sugar (%)	Non-reducing Sugars (%)	TTA (NaOH 0.1N/100 g)
Natural	6.47[A]	0.38[A]	6.09[A]	162.16[A]
Fully washed	5.64[B]	0.35[A]	5.29[B]	132.17[B]

Averages followed by the same capital letters on the columns do not differ statistically for the Scott-Knott test at 5% probability.

Table 3. Percentage averages of the phenolic compounds of coffee beans.

Processing	Phenolics (%)	Drying	Phenolics (%)
Natural	6.21[A]	Patio	6.23[A]
Fully washed	6.31[A]	60/40ºC	6.59[A]
		60ºC	5.97[A]

Averages followed by the same capital letters on the columns do not differ statistically for the Scott-Knott test at 5% probability.

between 10 and 28°C and the relative humidity between 34.5 and 61.2%. Those environmental conditions provided an average drying rate of 0.0020 kg $H_2O.kg^{-1}.hora^{-1}$ for the fully washed coffee and 0.0017 kg $H_2O.kg^{-1}.hora^{-1}$ for the natural coffee. It should be emphasized that those values were very inferior to those obtained in the drying with hot air at 60/40°C and 60°C. In this case, the highest drying rates were observed in the hot air treatment at 60°C, being 0.0324 kg $H_2O.kg^{-1}.hora^{-1}$ for the fully washed coffee and 0.0213 kg $H_2O.kg^{-1}.hora^{-1}$ for the natural coffee. In the 60/40°C treatment, in which the hot air used was 40ºC starting from a 30% moisture level (wb), the drying rate was 0.0205 kg $H_2O.kg^{-1}.hora^{-1}$ for the fully washed coffee and 0.0098 kg $H_2O.kg^{-1}.hora^{-1}$ for the natural.

Sensory analyses

Sensory analysis

The highest sensorial analysis scores were observed in t he sun-dried coffees, indicative of the best quality of those coffees (Table 1). Compared to the hot air drying at 60°C, the drying treatment with the 60/40°C temperature resulted in better drinks, for the natural coffee as well as for the fully washed coffee. Although, the natural and fully washed coffees can present different sensorial profiles for some isolated attributes, the final score of the coffee drink was not affected significantly by the processing type.

Chemical analyses

The average total sugars, non-reducing sugars and total titratable acidity (TTA) values were different (α<0.05) for the natural coffee and the fully washed coffee (Table 2). However, significant differences were not observed for the reducing sugar values (Table 2) or for the final the phenolic compound levels (Table 3).

Physiological analysis

The physiological performance of the coffee seeds (Table

Table 4. Physiological quality evaluations of the coffee beans submitted to drying on patio and heated air at 60/40ºC and 60ºC.

Parameter	Drying	Processing (%)	
		Natural	Fully washed
Root protrusion	Patio	76^{bA}	97^{aA}
	60/40ºC	2^{bB}	85^{aA}
	60ºC	9^{bB}	51^{aB}
Germination	Patio	80^{bA}	97^{aA}
	60/40ºC	0^{bB}	85^{aB}
	60ºC	0^{bB}	61^{aC}
Emergence	Patio	67^{bA}	92^{aA}
	60/40ºC	0^{bB}	60^{aB}
	60ºC	0^{bB}	30^{aC}
Emergence velocity index	Patio	1.2^{bA}	1.5^{aA}
	60/40ºC	0^{bB}	1.1^{aA}
	60ºC	0^{bB}	0.5^{aB}
Open cotiledonary leaves	Patio	56^{bA}	80^{aA}
	60/40ºC	0^{bB}	47^{aB}
	60ºC	0^{aB}	16^{aC}

Averages followed by the same capital letters on the columns and the same lower case letter on the rows do not differ statistically for the Scott-Knott test at 5% probability.

4) depends on the interaction between the drying method and the processing type. Significantly lower values for root protrusion, germination, emergence, ESI and open cotiledonary leaves were observed in the natural coffee, indicating that the processing form exposes the beans to more intense physiological damage than the fully washed coffees during the drying. In spite of there being a small percentage of root protrusion in the natural coffee drying treatments at 60/40°C and 60°C, the germination, emergence, ESI and open cotiledonary leaves had drastic reduction indicating that the seeds were already dead or in an advanced deterioration process. The lower root protrusion and germination values in the natural coffee compared to the fully washed coffees, sun-dried, show that more intense physical and physiological damage occurred to the beans in the processing through drying. Conversely, the root protrusion and ESI results for the fully washed coffee dried at 60/40°C did not differ significantly from the sun-dried fully washed coffee. Furthermore, the germination, emergence and open cotiledonary leaf values were significantly superior to the values observed in the coffees dried with hot air at 60°C.

The lowest electrical conductivity values were observed for the sun-drying, while the highest values were observed for the natural coffee hot air drying (Table 5). It is noticed that the electrical conductivity values depend on the interaction between the drying method and processing. In the dry processing method, it is observed that the electrical conductivity values of the coffee dried at 60/40°C did not statistically differ from that dried at 60°C. On the other hand, the drying of the depulped coffee with temperatures of 60/40ºC resulted in an intermediate value, statistically differing from the sun-drying as well as the drying at the temperature of 60°C. As for the processing effect, it was observed that independent of the manner of heating the air, the electrical conductivity values were significantly lower in the coffees processed by the wet method, there was no significant differences for the sun-dried coffees.

The potassium leaching of the coffee beans (Table 6) was significantly lower for the depulped coffee, independent of the drying method. Furthermore, the use of an elevated temperature followed by a low temperature (60/40°C) made possible a significant reduction in the potassium leaching values compared to the continuous drying with warm air at 60°C.

DISCUSSION

The coffee cell membrane integrity can be affected by the seed moisture removal rate. The higher the stress

Table 5. Electrical conductivity averages of the coffee beans submitted to drying on patio and heated air at 60/40ºC and 60ºC.

Drying	Processing	
	Natural (μS/cm/g)	Fully washed (μS/cm/g)
Patio	59.59^{aA}	48.22^{aA}
60/40ºC	114.7^{bB}	72.98^{aB}
60ºC	117.5^{bB}	95.67^{aC}

Averages followed by the same capital letters on the columns and the same lower case letter on the rows do not differ statistically for the Scott-Knott test at 5% probability.

Table 6. Potassium leaching (KL) averages of the coffee beans submitted to drying on patio and heated air at 60/40ºC and 60ºC.

Processing	KL (g/kg)	Drying	KL (g/kg)
Natural	67.52^{A}	Patio	47.63^{A}
Fully washed	57.52^{B}	60/40ºC	62.25^{B}
		60ºC	77.67^{C}

Averages followed by the same capital letters on the columns do not differ statistically for the Scott-Knott test at 5% probability.

provoked by the drying in the seeds, the higher the damage in the membranes (Borém et al., 2008b, 2008a; Saath et al., 2010) and the worse will be its physiological quality. The sun-drying made a slow and continuous drying of the coffee possible, while the drying with hot air at 60°C exposed the seeds to the highest drying rates. On the other hand, the use of high temperatures, when the beans had a humidity level above 30%, followed by the reduction of the air drying temperature to 40°C, resulted in less intense moisture removal rates, favoring the coffee quality compared to the continuous use of high temperatures.

The processing conditions and drying of the beans altered the chemical composition, the physiological and sensorial quality of the coffee. The coffee processed by the wet method presented lower total and non-reducing sugar levels, indicating that the hexose breakdown for the energy production due to the change of the aerobic respiration to alcoholic or lactic fermentation under the lack of oxygen during the depulping (Knopp et al., 2005) might have occurred. The consumption of sugars under anaerobic conditions is very high when compared to the normal aerobic conditions for the production of the same amount of energy. In contrast to the wet processing, the dry processing is maintained under aerated environmental conditions, allowing the normal breathing of the beans during drying, with smaller breakdown and lower sugar consumption.

On the other hand, it is known that the drying speed can exercise an influence on the amount and proportion of the several types of sugars in coffee beans. In general, the slow drying favors the accumulation of sugars of higher molecular weight, such as the oligosaccharides, to the detriment of the reducing sugars (Lima et al., 2004; Rosa et al., 2004). Marques et al. (2008) observed reduction in the levels of non-reducing sugar with the increase of the drying temperature from 40 to 60°C. Similar results showed higher non-reducing sugar levels in the samples that had lower moisture reduction rates (Borém et al., 2006). In that work, although the differences among the levels of sugar were small in the natural and fully washed coffees, it was observ ed that the slowest drying of the beans in the dry processing method favored the accumulation of total and sugars non-reducing sugars.

Higher total titratable acidity values (TTA) have been associated with deteriorative processes and, consequently, with worse drink quality (Franca et al., 2005). It has been verified in coffees processed by the dry manner when compared to the values obtained for the peeled, demucilaged and fully washed coffees. In the present work, although the natural coffee has presented a significantly higher TTA value in relation to the fully washed, this was not reflected in the drink quality of those coffees. Other chemical compounds, besides the organic acids, such as lactic, citric, malic, phosphoric, and acetic or butyric acid can also participate in the determination of TTA. In this case, it is important to distinguish the titratable acidity from that perceived by the cuppers at the occasion of the sensorial evaluation.

The phenolic compounds are responsible for the astringency of the drink, being found mainly when there is a presence of green or immature beans. In this work, due to the selective harvest and rigorous selection of the raw

material for obtaining of cherry fruits, high astringency was not observed in the drink. In spite of the presence of those compounds being undesirable, they can inhibit the oxidation processes in certain systems, but that does not mean that they can protect the cells and the tissues from all types of oxidative damage.

For a precise diagnosis of the immediate and latent effects of the processing and drying on the coffee quality, the use of methods and/or equipment that allow the determination of the chemical composition of the beans is recommended, such as high performance liquid chromatography with (HPLC) and high resolution gas chromatography (HRGC) coupled to a mass spectrometer. However, it stands out that, since most of the time those determinations are accomplished with raw beans, it is not always possible to obtain a good correlation between the chemical composition and the beans quality, especially when dealing with the drink quality. This can occur because the sensorial analysis is conducted with roasted beans, while the determination of the chemical composition is made with raw beans, in other words, completely different beans are analyzed as to their chemical composition.

As an alternative to those more sophisticated and high cost analyses in the present work, unprecedented physiological evaluations in raw coffees beans destined for roasting and consumption took place with the objective of an early diagnosis of possible quality alterations.

The physiological analyses indicated that as the drying conditions became more severe, the damage to the beans also increased, showing that the processing by the wet method and sun-drying were the treatments that favored a better preservation of the beans integrity. The root protrusion and germination results for the fully washed coffee dried under alternate temperature of 60/40°C indicate that the beans were in good physiological conditions in way similar to the fully washed sun-dried coffee. The germination and emergence tests presented solid results and they proved that the combination of treatments that provides the best physiological quality is the wet processing and the sun-drying.

The use of high drying temperatures for the coffee allows a fast removal of moisture from the beans, however it can provoke very large differences between the moisture level of the external part and the centre of the bean, generating a high pressure gradient, which can cause ruptures and cracks within the beans, besides possible thermal damage. Tissues damaged during drying require energy expenses for the reorganization of membranes, with possible increases in the respiration rate and solute leaching, besides the formation of toxins by the non-recuperated parts, among other metabolic events, possibly resulting in the reduction of the seed energy or even total loss of its viability, depending on the extent of the damage and the metabolic activity of the beans.

The increase of the drying temperature causes damage to coffee beans cell membrane system, increasing the electrical conductivity of the bean exudates (Borém et al., 2008a; Coradi et al., 2007). Those authors affirm that with the extravasations of fatty acids present in the cell interior due to the disorganization or breakdown of the cytoplasmic membranes, oxidative or catalytic reactions can occur, forming undesirable by-products and harmful to the sensorial quality of the coffee drink.

In this work, it can be observed that the depulped and machine-dried coffees presented lower electrical conductivity values than the natural coffees, allowing to infer that the damage in the cell membranes was less intense in the depulped coffees.

The potassium leaching test, as well as that of electrical conductivity, evaluates the integrity of the membrane systems. In this work, it is observed that the effects of the treatments are similar to those verified in the electrical conductivity test, and the drying method 60/40°C presented lower intermediate of potassium leaching values when compared to the sun-drying and the continuous drying at 60°C. This can be an indication that there was less membranes damage at the 60/40°C temperature, and that the damage might have happened before the beans reached the 30% ± 2% (wb) moisture level. It stands out that in the drying of the natural coffee at 60°C the exposure time of the beans to the drying conditions was longer than in the depulped coffees, which certainly favored the occurrence of more severe damage to the natural coffee cell membrane systems, similar to the observations found in the literature (Borém et al., 2006, 2008a; Marques et al., 2008; Prete, 1992).

The high moisture removal rate, provoked by the high temperature at the beginning of the drying in the 60/40°C treatment, might have been harmful to the physiological integrity of the beans. Besides, a seed defense mechanism against cell membrane degeneration is the accumulation of sugars, acting in the seed membrane and protein stabilization (Corbineau et al., 2007). However, when the drying is excessively fast, there is no enough time for that phenomenon to happen and, consequently, the maintenance of the physiological integrity of the bean is compromised. These results are coherent with those obtained in the chemical analyses and in the sensorial analyses, indicating that the physiological analyses constitute promising tools for evaluation of coffee quality.

According to the scale of scores for sensorial evaluation of special coffees proposed by the Specialty Coffee Association of America (SCAA), coffees with scores above 80 points are considered very good coffees and are placed in the category of special coffees. In the present work, only the sun-drying made possible the obtaining of coffees with average scores superior to 80 points, independent of the processing type. The machine-drying was unfavorable for the sensorial quality of the

coffees, which were placed in the non-special coffee category, with scores between 75 and 79 points. Damage to the beans provoked by the drying conditions can result in alterations to the flavors and perceptible aromas in the sensorial analysis. Besides the objective evaluation, the SCAA protocol allows a descriptive analysis of the coffee flavor and aroma. Strange flavors of wood and oil were more frequently found in the coffees dried at 60°C. The result of this experiment corroborates the results obtained by several authors who associate the elevation of the temperature with the reduction of the quality of the drink (Borém et al., 2008a; Coradi et al., 2007; Marques et al., 2008).

In the present work, the sensorial and physiological analyses provided an early diagnosis of some of the transformations which occurred in the coffee beans during the processing and drying. Based on the obtained results, it can be affirmed that the coffee drying procedures that provided the best physiological quality of the beans also provided the best drink quality, indicating a close relationship between these two variables. This takes on great importance in the coffee postharvest technology context, with relevant contributions for the definition of coffee postharvest management strategies, be them sun or machine dried, seeking the obtaining of superior quality coffees.

Conclusions

The sun-drying provided coffee beans with the best physiological performance and the best drink quality. However, the fully washed coffees presented better physiological performance than the natural coffees, regardless the drying method. In addition, the innovative drying method with alternate temperature of 60/40°C was only adequate for the fully washed coffees, while the constant temperature of 60°C was inappropriate for the natural coffees as well as the fully washed coffees.

Conflict of Interest

The authors have not declared any conflict of interest.

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Prediction of somatic growth of tropical fish using a simple mathematical formula

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Age determination or growth rate of fishes is a critical component of a proper fishery management. Current methods of fish age determination or growth rate have several drawbacks, including, large numbers of fish needed over time for analyses, subjective interpretations during age determination or the use of expensive instrument for analysis. In this report, a simple theoretically derived mathematical formula is assessed for prediction of fish growth in length. Secondary data of length measures of 16 different species of tropical marine fish was used to assess the accuracy of the formula for predicting fish growth. The data comprised of relative length-at-age of 14 different fishes and absolute length (in centimeters) of 2 other different fishes. For each species, two of the length measures together with the corresponding ages were used to estimate two constants in the formula and the formula used to predict the remaining lengths. The accuracy of the formula for prediction was assessed by evaluating the discrepancies between observed data and corresponding predicted data. The biasness as well as the accuracy of the formula was also assessed. In all the species studied, discrepancies between observed data and the corresponding formula predicted values were minimal and fluctuated between negative and positive values. The mean signed value of the discrepancies (a measure of biasness) for all the 16 species was -0.2 ± 1.7, while the mean of the absolute discrepancies (a measure of accuracy) was 1.2 ± 1.4. The fluctuations of the discrepancies between negative and positive values demonstrate that the discrepancies are not systematic errors of prediction. The signed mean discrepancy of -0.2 is close to 0, thus indicating minimal biasness of prediction. Also the absolute mean discrepancy of 1.2 suggests prediction accuracy within 1 unit of actual measurement, indicating high accuracy.

Key words: Tropical fish, demersal marine fish, fish length prediction, growth rate, age determination, discrepancies.

INTRODUCTION

Age determination of fishes is a critical component of a proper fishery management; and some conventional techniques have been used for direct age determination of fishes. These include the growth zones analysis of skeletal-structures (Bagenal, 1974; Brennan and Cailliet, 1989; McForlane and Beamish, 1995), the tag-recapture technique (Cailliet, 2001) and the length-frequency analysis technique (Ricker, 1975). All these methods

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require collecting large numbers of fish over time (in some cases) for analyses. In addition to this time consuming process of studying large numbers of fish over a period of time in order to determine the age structure, the tag-recapture and the length-frequency analyses are prone to subjective interpretations and/or tagging artifacts, thus leaving the skeletal-structure analysis technique the preferred method (Fowler, 1990), though that is not to say that the skeletal-structure analysis technique is without drawbacks. As noted in a previous report, several factors, such as maturation and spawning, crowding conditions, population density, photoperiod, productivity of the ecosystem, chemical components in the water, temperature fluctuations, amount and quality of food, as well as almost any environmental biotic or abiotic factors, all affect the way fish hard structures can be interpreted during age determination studies (Buesa, 1987). Another method, which utilizes radioactive decay activities of some elements (notably lead-210 (^{210}Pb) and radium-226 (^{226}Ra) in the otoliths of bony fish to determine the age has also been used (Bennett et al., 1982; Campana et al., 1990; Calliet et al., 2001). Though valuable for determining the ages of long-lived fishes and deep water fishes (which are difficult to sample and keep alive), the technique is expensive to use, as it requires the use of spectrometers as well as scintillation counters to measure the radioactivity of ^{210}Pb and ^{226}Ra. In addition to this drawback, three assumptions must be met in order to successfully apply the technique in fish age determination: These assumptions are that: (1) The hard structure of the fish (e.g., otoliths) is a closed system for radium and its daughter products; (2) The initial activity ratio of ^{210}Pb:^{226}Ra in the structure should be close to zero, and (3) uptake rate of ^{226}Ra is proportional to mass growth of the structure during the lifetime of the fish (Calliet et al., 2001). Violations of some of these assumptions resulted in failures of the technique in determining the ages of sharks (Welden et al., 1987) and the sturgeon fish when using it pectoral fin rays (Burton et al., 1999).

In addition to the above mentioned techniques, several mathematical models have been advanced for modeling fish growth (von Bertalanffy, 1938; Kozlowski, 1996; Jørgensen and Fiksen, 2006; Lester et al., 2004; Day and Taylor, 1997; Economo et al., 2005; Dumas et al., 2010). However, all these models contain several parameters, requiring statistical parameter optimization for good fit to growth data to be achieved. Also, in most of these models, prior knowledge of some of the parameters is required in order to do curve fitting.

Therefore, any method of determining fish age and growth rate that could overcome some of the drawbacks of the techniques mentioned above will be very important in fishery management and in research. Buesa proposed the use of regression analyses based on exponential functions for the estimation of growth rate and age of fishes (Buesa, 1987). However, with this method, knowledge of the feeding habit of the fish is needed so that a feeding correction factor can be imposed on the regression estimates to achieve good prediction of age and/or growth rate of the fish. Recently, a simple mathematical formula was derived for prediction of human brain tissue volume re-growth/recovery in sustained abstinent alcohol dependent individuals (Mon et al., 2011) and for prediction of individual child growth (human growth) in both boys and girls across ethnically diverse children (Mon et al., 2013). For individual growth data, the formula relies on an intrinsic factor, known as growth rate factor or growth coefficient (k) to predict growth of the individual. The value of k of an individual is governed by the combined effect of genetics, environmental and any other factors that modulate growth. However, the formula can be used for group data analysis, in which case, k is the growth rate factor assumed for all the members of the group. In this study, the applicability of the formula for prediction of fish growth in length is assessed, using group data of 16 tropical marine fish species.

METHODS

In terms of fish growth in length, the formula proposes a square root dependence of the length of the fish on time (that is, age); as follows:

$$\frac{1}{2}L^2 = kt + C$$

where L is the length of the fish at time t, k is the growth rate factor/growth coefficient, and C is the intercept on the L axis. If L is measured at two well separated time points t_1 and t_2, the values of k and C can be estimated from:

$$k = \frac{1}{2(t_2 - t_1)}\left(L_{t_2}{}^2 - L_{t_1}{}^2\right)$$

and

$$C = \frac{1}{2}L_{t_1}{}^2 - kt_1$$

or

$$C = \frac{1}{2}L_{t_2}{}^2 - kt_2$$

When the estimated values of k and C are substituted back into the formula, all other loci of points on the growth curve of the fish can be predicted. The units of k and C are cm^2 per unit time and cm^2 respectively if L is measured in centimeters (cm).

The formula was assessed for fish growth (in length) using secondary data of linear growth of 16 different species of tropical marine fishes from one published work (Buesa, 1987) and one unpublished work (http://fishing.about.com/od/bassfishing/a/How-Fast-Do-Alabama-Bass-Growth.htm)). The growth measurements for the 16 tropical marine fishes in the two reports were established values obtained from repositories, therefore no sample sizes are provided in the reports. The relative length-at-age data of 14

Table 1. Measured length ($L_{r(meas)}$), formula predicted length ($L_{r(pred)}$) and discrepancy (ΔL_r) between $L_{r(meas)}$ and $L_{r(pred)}$ for 14 tropical demersal fishes.

Fish type	Age in years											
	1			2			3			4		
	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r
Calamus nodosus	24.0	24.0	0.0*	34.0	36.3	+ 2.3	45.0	45.4	+ 0.4	53.0	53.0	0.0*
Lutjanus synagris, Brazil	27.0	27.0	0.0*	44.0	45.2	+ 1.2	58.0	58.0	0.0*	69.0	68.4	-0.6
Cynoscion nebulosus	24.0	22.0	- 2.0	39.0	39.0	0.0*	49.0	50.6	+ 1.6	60.0	60.0	0.0*
Cynoscion nebulosus, Florida	27.0	27.0	0.0*	47.0	46.5	- 0.5	59.0	60.0	+ 1.0	70.0	70.0	0.0*
Cynoscion nebulosus, Ceda, Florida	18.0	15.0	+ 3.0	29.0	29.0	0.0*	37.0	38.2	+ 1.2	45.0	45.6	+ 0.6
Centropornus undecimalis	11.0	11.0	0.0*	29.0	24.6	- 4.4	33.0	33.09	0.0*	39.0	39.7	+ 0.7
Epinephelus morio	22.0	15.0	-7.0	26.0	26.0	0.0*	32.0	33.5	+ 0.5	39.0	39.7	+ 0.7
Tarpon atlanticus	20.0	20.0	0.0*	25.0	25.3	+ 0.3	29.0	29.7	+ 0.7	33.0	33.6	+ 0.6
Lutjanus aya	20.0	20.0	0.0*	25.0	24.9	- 0.1	29.0	29.0	0.0*	31.0	32.6	+ 1.6
Haemulon aurohneatum	23.0	19.6	- 3.4	33.0	33.0	0.0*	42.0	42.3	+ 0.3	50.0	50.0	0.0*
calamus nodosus	23.0	22.1	- 0.9	36.0	36.0	0.0*	45.0	45.9	+ 0.9	53.0	54.0	+ 1.0
Mycteroperca rnicrolepis	29.0	29.0	0.0*	34.0	37.3	+ 3.3	44.0	44.0	0.0*	52.0	52.0	0.0*
Lutjanus purpureus, Brazil	20.0	20.0	0.0*	27.0	27.0	0.0*	38.0	38.9	+ 0.9	47.0	47.0	0.0*

	Age in years											
	5			6			7			8		
	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r	$L_{r(meas)}$	$L_{r(pred)}$	ΔL_r
Calamus nodosus	61.0	59.6	+ 1.3	66.0	65.6	-0.4	71.0	71.0	0.0	76.0	76.1	+ 0.1
Lutjanus synagris, Brazil	78.0	77.5	- 0.5	85.0	85.5	+ 0.5	-	-	-	-	-	-
Cynoscion nebulosus	71.0	68.1	- 2.9	80.0	75.4	- 4.6	-	-	-	-	-	-
Cynoscion nebulosus, Florida	81.0	80.5	- 0.5	88.0	89.0	+ 1.0	97.0	96.7	-0.3	-	-	-
Cynoscion nebulosus, Ceda, Florida	52.0	52.0	0.0*	59.0	57.7	- 1.3	63.0	62.8	- 0.2	68.0	67.6	- 0.4
Centropornus undecimalis	45.0	45.3	+ 0.3	50.0	50.4	+ 0.4	55.0	55.0	0.0*	-	-	-
Epinephelus morio	45.0	45.0	0.0*	50.0	49.7	- 0.3	54.0	54.1	+ 0.1	57.0	58.1	+ 1.1
Tarpon atlanticus	37.0	37.0	0.0*	40.0	40.1	+ 0.1	43.0	43.0	0.0*	46.0	45.8	- 0.2
Lutjanus aya	33.0	32.9	- 0.1	38.0	38.0	0.0	46.0	46.0	0.0	55.0	52.8	- 2.2
Haemulon aurohneatum	57.0	56.6	- 0.4	63.0	62.5	- 0.5	68.0	67.9	- 0.1	73.0	73.0	0.0
calamus nodosus	61.0	61.0	0.0*	68.0	67.3	- 0.7	-	-	-	-	-	-
Mycteroperca rnicrolepis	60.0	60.5	+ 0.5	68.0	68.0	0.0*	71.0	74.7	+ 3.7	86.0	81.0	- 5.0
Lutjanus purpureus, Brazil	54.0	53.9	- 0.1	60.0	60.0	0.0*	65.0	65.5	+ 0.5	69.0	70.4	+ 1.4

different species of tropical demersal fishes (Table 1) was extracted from Buesa's article (Buesa, 1987). Relative length-at-age (L_r: a dimensionless quantity) is defined as $L_r = \left(L_t / L_M \right) \times 100$, where L_t is the length at age t and L_M is the maximum total length of the species for the area where the fish came from (Buesa, 1987). The fishes were from several geographical locations including the United States

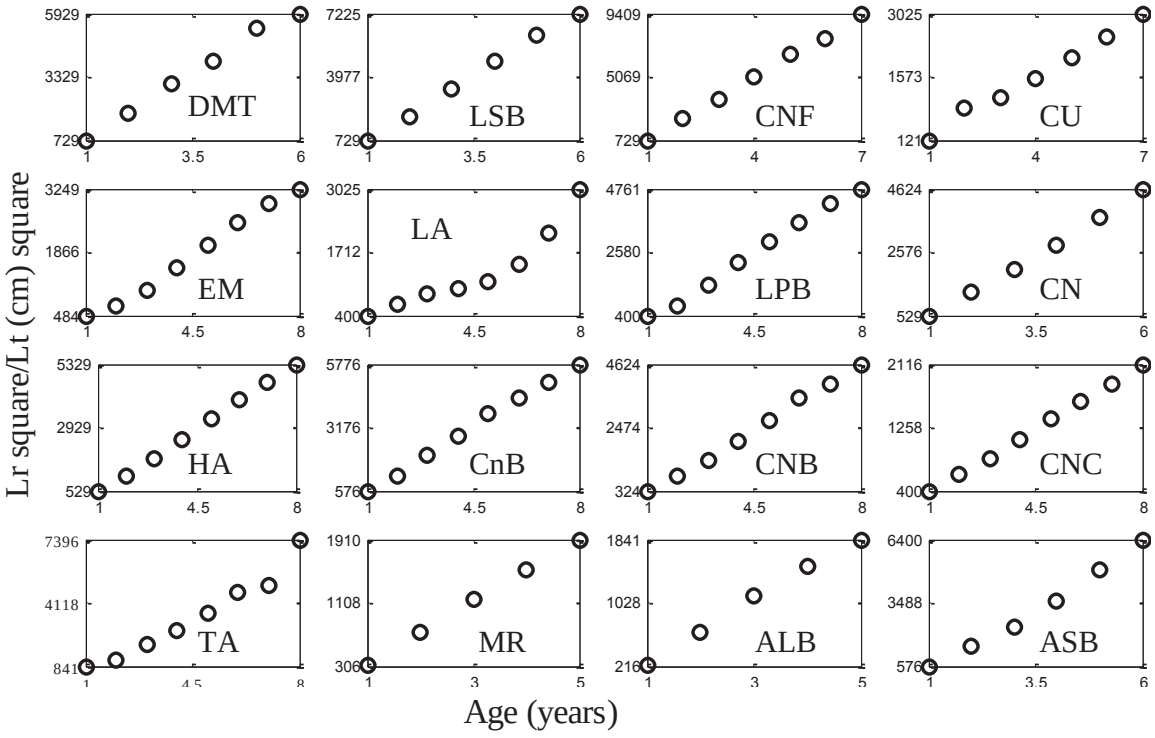

Figure 1. Plot of relative length-at-age square against age (for DMT, LSB, CNF, CU, EM, LA, LPB, HA, CnB, CNB, CNC, TA and MR) and length-at-age (cm) square against age (for CN, ALB and ASB). DMT, *Dentex macrophthalmus*; LSB, *Lutjanus synagris*, Brazil; CNF, *Cynoscion nebulosus*, Florida; CU, *Centropornus undecimalis*; EM, *Epinephelus morio*; LA, *Lutjanus aya*; LPB, *Lutjanus purpureu*s, Brazil; CN, *Calamus nodosus*; HA, *Haemulon aurohneatum*; CnB, *Calamus nodosus*, Brazil; CNB, *Cynoscion nebulosus*, Ceda; TA, *Tarpon atlantiscus*; MR, *Mycteroperca rnicrolepsis*; ALB, Alabama largemouth bass; ASB, Alabama spotted bass.

of America, Japan, Brazil, Cuba, the Atlantic Ocean, the Caribbean, Panama and Venezuela. The growth measures used in this report covered a minimum of 5 years and a maximum of 8 years from hatch. The absolute lengths of 2 species were measured using the marginal increment method, 1 using the length-frequency method and the rest using the growth-zone method (otoliths, scales and urohyal bone).

Absolute length data (in centimeters) for the first 5 years from hatch of 2 other species were extracted from the unpublished report by the Alabama Division of Wildlife and Freshwater Fisheries (http://fishing.about.com/od/bassfishing/a/How-Fast-Do-Alabama-Bass-Growth.htm). These species were the Alabama Largemouth Bass and the Alabama Spotted Bass. The method used to measure the lengths of these two species is not stated in the report.

The proposed formula suggests that $L^2 \propto t$; that is, the square of the length is proportional to age. Thus in the analyses, the linear dependence of the square of length on age was first verified, by plotting the square of the observed length measures against age for each species. Then for each species, two of the length measures together with the corresponding ages at which the measurements were taken, were used to estimate *k* and *C*. The formula was then used with the estimated values of *k* and *C* to predict the lengths for the remaining ages of each species. The discrepancies (ΔL_r) between the measured data and their corresponding predicted data were then estimated from $\Delta L_r = L_{r(pred)} - L_{r(meas)}$, where $L_{r(pred)}$ is the predicted length and $_{L(meas)}$ is the corresponding measured value. The biasness of the formula for prediction of length for all the different fishes across all ages was estimated by summing all ΔL_r (signed values). The accuracy of the formula for predicting growth of all the species was also estimated by taking the sum of the absolute values of all the ΔL_r. The biasness and accuracy of prediction for each species are not included in the results as these can easily be appreciated by inspection of Table 1.

RESULTS

Figure 1 shows plots of the square of length measures against age for all the 16 fish species analyzed. As can be seen, all the plots are fairly linear, indicating that indeed the square of fish length is linearly dependent on age ($L^2 \propto age$). Table 1 shows the observed relative length data and the formula predicted values, as well as the discrepancies between corresponding observed and predicted values for the 14 fish species taken from Buesa (1987). The discrepancies marked "*" (0) correspond to the measures that were used to calculate *k* and *C*. These discrepancies are zero since the fit of the formula passes through the centers of the two measurements used to calculate *k* and *C*. As can be seen in the table, most of the ΔL_r corresponding to all other measurements are minimal, except in a few cases where discrepancies are several centimeters large. For each species, ΔL_r varied between negative and positive numbers, indicating that

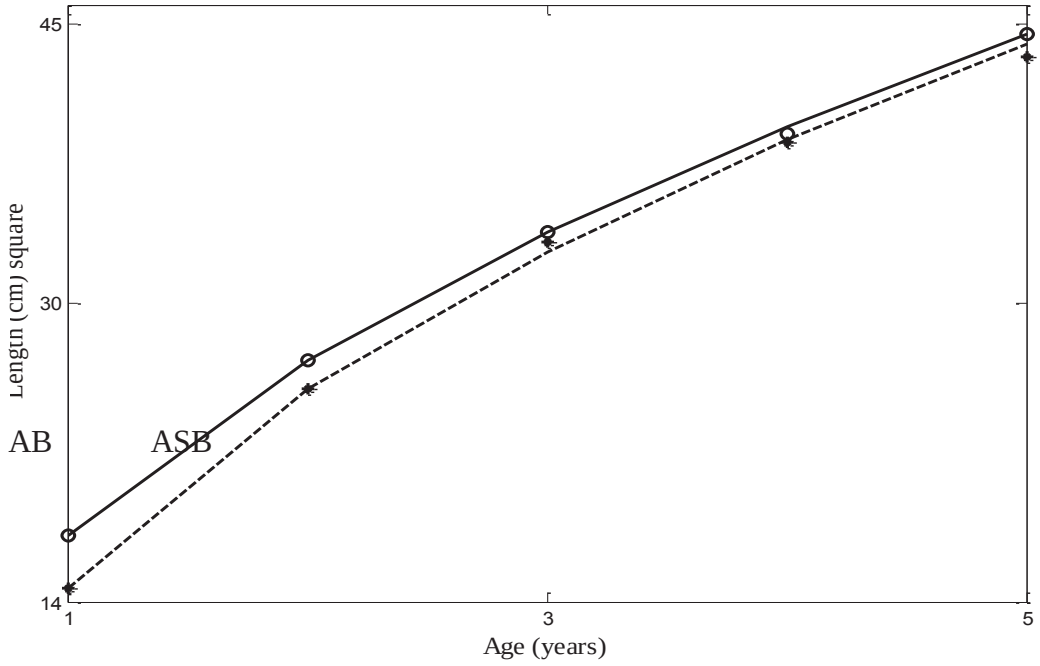

Figure 2. Plot of relative length (in cm) square against age (in years) for the Alabama bass (AB) and Alabama spotted bass (ASB). Solid line represents formula's fit for AB, while the circular marks represent the measured data for AB. Similarly, the broken curve represents the formula's fit for ASB, while the star marks represent the measured data for ASB.

the discrepancies are not systematic prediction errors. The mean signed value of ΔL_r (excluding all ΔL_r marked "*") for all the 14 species was -0.2 ± 1.7, indicating minimal bias. The mean of the absolute ΔL_r was 1.2 ± 1.4, suggesting prediction accuracy within 1 unit of actual measurement. Figure 2 shows a plot of both the observed data and the formula prediction against age for the Alabama largemouth bass (ALB) and Alabama spotted bass (ASB) fishes. The curves represent the formula's description of the growth trajectories of the fishes (solid curve for ALB and dotted curve for ASB), while the marks represent the observed data (circular marks for ALB and star marks for ASB). Similar to the results in Table 1, the figure demonstrates the closeness of the formula's fits to the observed data of the Alabama bass fishes too. The discrepancies between the measured data and corresponding predicted data for these two fishes range from -0.1 –to- +0.4 cm, with a signed mean value of 0.1 ± 0.2 cm and a mean absolute value of 0.2 ± 0.2. These means also demonstrate minimal bias and high accuracy of the formula's prediction of growth for the two Alabama bass fishes.

DISCUSSION

In this report the applicability of a simple mathematical formula for determining fish growth rate was assessed using longitudinal length measures of 16 different marine fish species as proof of concept. As can be seen in Table 1 and Figure 2, the formula predicts growth of all the 16 fish species with high accuracy; and Figure 1, which demonstrates a linear dependence of the square of the observed length data on age, is consistent with the theory that growth rate of a fish is inversely proportional to current length of the fish. It is important to note that prediction accuracy was similar and minimal in most cases across all the species (except in a few cases where discrepancies were several units large) and fluctuated between negative and positive values, demonstrating that the discrepancies were not systematic errors of prediction. The signed mean of the discrepancies was 0.2 ± 1.7 cm, while the mean absolute value was 1.2 ± 1.4 cm. The signed mean, which is close to 0, suggests very little bias, while the absolute mean suggests accuracy within 1.0 cm.

Many conventional techniques of age and/or growth rate determination of fish exist, but they are marred by several drawbacks, such as the need to collect large numbers of fishes over a long period of time for analysis (the case of tag-recapture technique (Cailliet, 2001) or the length-frequency technique (Ricker, 1975)), the influence of environmental and other factors on the accuracy of age determination (in the case of growth-zone analysis of skeletal structures (Cailliet, 1997; Brennan and Cailliet, 1989; McForlane and Beamish, 1995)) or the use of expensive instrument for analysis of activities of radio-isotopes ((the case of the radiometric

technique (Bennett et al., 1982; Campana et al., 1990)).

The mathematical formula presented in this report will help to overcome most, if not all the difficulties encountered in the conventional methods. This is because, for a given geographical location and fish type, the formula requires only two length measurements at two different known ages to determine the growth rate and to predict future growth of the fish. Thus, only two data sets using a conventional method are needed for the calculation of *k* and *C*, after which the formula can be applied to predict future lengths of the fish.

The formula also improves upon the von Bertalanffy's (1938) and its hybrid models (Kozlowski, 1996; Jørgensen and Fiksen, 2006; Lester et al., 2004; Day and Taylor, 1997; Economo et al., 2005; Dumas et al., 2010) used to describe fish growth. This is because, unlike the current formula where only two constants are required and can easily be calculated, all the existing models contain several parameters, requiring time-involving parameter optimization to achieve good fits to growth data. Most importantly, these models are exponential functions based on the assumption that growth rate is proportional to size. This assumption is inconsistent with practical observations of animal growth (including fish), where growth rate generally decreases with age or size. Schmalhausen studied growth of animals based on the assumption that growth rate is inversely proportional to time (Birnholz, 1980). However, since the size of a growing animal is directly proportional to age (that is, time), it is more appropriate to relate growth rate with the size (length) of the animal rather than time, because size of an animal is uniquely influenced by genetics and environmental factors, which all influence growth rate. Indeed, in fish studies, the well known Gulland and Holt plot shows an inverse dependence of growth rate on length. This together with the demonstration of the linear dependence of the square of length on age and the accuracy of the fits of the current formula to growth trajectories of the 16 fish species studied in this research attest that growth of fish obeys the theory of the formula; that is, growth rate is inversely proportional to current length.

Though the formula is assessed here for prediction of fish growth using data of short-lived tropical fishes, it should be able to also predict growth of long-lived or temperate zone fishes, since somatic growth patterns of all fishes are qualitatively similar. Also, weight of some fishes has a dependence on length (Lester et al., 2004; Jørgensen and Fiksen, 2006); thus the formula may be useful in weight trajectory studies of such species of fish. Furthermore, somatic growth patterns of some aquatic animals, such as crocodiles, sharks, whales and seals are qualitatively similar to somatic growth patterns of fishes; and the formula could also be useful in studies involving somatic growth of these animals. However, direct studies using growth data of these animals are needed to support the above supposition.

It must be noted that for accurate prediction of growth using the formula, the interval between the two measurements used to calculate *k* and *C* in the formula must be well separated in time, such that growth between the two time points is greater than measurement error. In this report, the one year time interval between measurements was long enough for two consecutive data points to be used to calculate the constants; however measurements separated by two or more years' interval were also used. Another way to improve upon prediction accuracy is to use the average value of *k* from two or three estimates of the constant from three measurements. This is because, the possibility of imperfect uniform growth coupled with measurement errors may result in slightly different values of *k*; thus an average value of *k* from two or three estimates will describe the growth trajectory better than any of the individual values.

In conclusion, a simple mathematical formula has been successfully assessed for prediction of fish growth. The simplicity and accuracy of the formula makes it an attractive method of predicting fish age and growth in length. The accuracy of the formula for predicting brain tissue volume growth, human linear growth and fish linear growth suggests that these three processes share the same trajectory. Given that generally growth trajectories of most animals are qualitatively similar, growth of other animals could also possibly be studied using the formula.

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Conflict of Interest

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Evaluation of soybean [*Glycine max* (L.)Merr.] genotypes for agronomic and quality traits in Kenya

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The interaction between genotypes and environments results in significant differences in the performance of genotypes when tested in various environments. Fifteen soybean [*Glycine max* (L.) Merr.] genotypes were evaluated for yield and protein content in Kenya aiming to quantifying and identifying high yielding genotypes for human food and livestock feed. The study was conducted in Eldoret (0° 35′N, 35°18′E), Lanet (0°18′S, 36° 09′E), Nakuru West (0° 33'S, 36° 0'E) and for two seasons at Njoro (0° 20′S, 35° 56′E). The results indicated significant ($p \leq 0.01$) effects due to genotype, environment and genotype × environment interaction for days to flowering harvest maturity, plant height, number of pods per plant, number of nodes per plant, seed yield, oil and protein content. The mean seed yield was 1267.8 kg ha^{-1}. Genotype Nyala produced the highest yield across environments. The mean protein content ranged from 40.3% for genotype TGX 1740-2F (DPSB 19) to 35.2% for genotype 931/5/34 across the five test environments with the highest mean protein being recorded at Njoro and the lowest at Nakuru West. Genotype Nyala and SBH 7/1/1 may be recommended for production for their relatively high grain yield across the sites.

Key words: Soybean, yield, genotype, environment, protein.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is an important legume grown as source of vegetable oil and proteins as well as for feed and industrial uses (Wan et al., 2005). Soybean cultivation in Kenya is expected to gain popularity in the near future because of the increasing need for food and fodder (Mugendi et al., 2010). Livestock feed consumption accounts for over 90% of soybean utilization in Kenya and 60% of all livestock feed is compounded with soybean (Chianu et al., 2008.). Soybean improves soil fertility by fixing atmospheric nitrogen and some varieties fix 44 to 103 kg N ha^{-1} annually (Sanginga et al., 2003). This can reduce the need for application of nitrogenous fertilizers that are detrimental to the environment (Zhang et al., 2003). Biological nitrogen fixation is environmental friendly and ideal for sustainable agriculture (Cheng, 2008). Soybean biomass is a source of feed, green manure and mulch (Chianu et al., 2008). Soybean farming is one of most cost effective ways smallholder farmers can maintain soil fertility and reap other benefits from subsequent crops (Osunde et al., 2003). Multi-environment trials are carried out to identify superior and stable soybean genotypes and to

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understand the effects of genotype and environments on soybean performance. The interaction between genotypes and environments results in significant differences in the performance of genotypes when tested in various environments (Gauch and Zobel, 1997). The genotype × environment (G × E) interaction plays a major role in the performance of any genotype and in the success of any breeding program for the development of genetic material, adapted to varying environments.

Quality aspects are affected by interactions between genotypes and the environment. Three aspects that affect soybean quality are seed appearance, protein and oil content and the chemical components of protein and oil (Liu et al., 1995). Primomo et al. (2002) investigated G × E interaction for soybean fatty acids and found that genotype × year interaction was significant for all fatty acids. Due to varying regional ecological conditions in Kenya, it is extremely important to select suitable cultivars for adaptability to specific as well as across environments.

Objectives of this study were to identify genotypes with high grain yield and related agronomic traits, protein and oil content for human food and feed consumption.

MATERIALS AND METHODS

Experimental sites

The study was conducted in Njoro (0° 20′S, 35° 56′E) for two seasons (Njoro I and Njoro II), Eldoret (0° 35′N, 35°18′E), Nakuru West (0° 33'S, 36° 0'E) and Lanet (0°18′S, 36° 09′E). Njoro (2185 m above sea level) is situated about 200 km West of Nairobi in Nakuru County and experiences an average daily minimum temperature of 9.5°C and maximum temperature of 24.2°C with an average precipitation of 1032 mm (average of 21 years 1992-2012 weather station number 9035021; Jaetzold et al., 2010). The soils are mollic andosols in eco-zone III (Jaetzold et al., 2010). Eldoret [2154 m above sea level (m.a.s.l.)] is located in Uasin Gishu County (FAO/UNESCO, 1994) and the soils are predominantly acidic (pH 4.7) rhodic ferrasols that are low in organic matter and deficient in nitrogen (N) and phosphorus (P). Lanet is 16 km South East of Nakuru town at an altitude of 1920 m.a.s.l. and experiences a bimodal rainfall pattern with an annual mean of 800 mm. The minimum and maximum temperatures that prevail in this site are 10 and 26°C, respectively (http://www.kalro.org). Nakuru West is elevated to an altitude of 2003 m.a.s.l. and is on the wind ward side overseeing Lake Nakuru.

Genotypes

Fifteen soybean genotypes obtained from Kenya Kenya Agricultural and Livestock Organization, Food Crops Research Centre (KARLO-Njoro) were used in this study. Eight of these were coded SBH (soybean hybridization) lines (SBH 10/5/6, SBH 1/12/9, SBH 7/1/1, SBH 4/4/4, SBH 10/2/3, SBH 4/6/6, SBH 6/6/6/2, SBH 3/8/4/1). Genotype EAI 3600, a stable genotype, was used as a check. Genotypes Nyala and Gazelle are medium maturing while genotype 931/5/34 gives high oil content. Genotype TGX1835-10E (DPSB 3) is resistant to Asian soybean rust disease [*Phakopsora pachyrhizi* (H. Sydow and Sydow)]. Genotypes TGX 1895-33F (DPSB-8) and TGX 1740-2F (DPSB-19) are promiscuous and have high biomass, trait that aids in improvement of poor soils and provision of feed to livestock.

Experimental procedure

The seedbed was prepared in order to become suitable for planting soybean. Planting was done on 13th June 2011 at Njoro in first season (Njoro I), 22nd June 2011 at Nakuru West, 8th July 2011 at Lanet, 14th June 2011 at Eldoret and on 15th July 2011 at Njoro in second season (Njoro II). The experiment was laid out in a randomised complete block design (RCBD) with three replicates. In all locations, the genotypes were planted in six row experimental units of 3 m × 2.7 m. Seeds were planted at a spacing of 45 cm × 10 cm and at an average depth of 5 cm. At planting time diammonium phosphate fertilizer (DAP) was applied to supply 22 kg of N ha^{-1} and 57.5 kg of P ha^{-1}. A pre-emergent herbicide (Metribuzin) was applied at the rate of 360 g ha^{-1} immediately after planting in order to control weeds. Weeding was done manually when weeds appeared after the waned effect of the herbicide. Foliar fungal diseases were controlled by applying Tebuconazole at the rate of 250 g ha^{-1} weekly from flowering until the beginning of maturity (stage R7) (Fehr and Caviness, 1977). The crop was grown fully under rain fed conditions.

Data collection

Evaluation was done at vegetative and reproductive growth stages as described by Fehr and Caviness (1977). Days to 50% flowering was taken when there was at least one flower in 50% of all plants in the plot. Days to harvest maturity were observed when 95% of the pods had reached their mature pod colour (stage R8). At maturity, plant height, number of nodes $plant^{-1}$ and number of pods $plant^{-1}$ were determined from five plants of each genotype in the four middle rows. Mean seed count from twenty pods was observed as the number of seeds pod^{-1}. Seed yield was determined by weighing as the seed weight from four centre rows. The weight of 100 seeds was recorded by weighing a random sample of 100 seeds. Seed protein content was estimated using Near Infra red Refractometer (NIR) machine (Infratec ™ 1241 Grain Analyzer ISW 3.20: Foss Analytical AB, Sweden).

Data analysis

Analysis of variance to estimate the effect of genotype, environment and G × E interaction on yield and protein content was done using the statistical analysis systems (SAS) general linear mode (GLM) procedure (SAS Institute, 2000 release 8.1). Means of genotypes were separated using least significant difference (LSD) test.

RESULTS

Agronomic performance of the soybean genotypes across the environments

The analysis of variance indicated that there was significant ($p \leq 0.01$) effects due to genotype (G), environment (E) and genotype × environment interaction (Table 1) for all the traits studied. Variation among genotypes was observed in mean values of traits (Table 2). Days to 50% flowering ranged from 74 (DPSB 19) to 101 (DPSB 3). The contribution to the sum of squares

Table 1. Mean squares for the examined traits of soybean genotypes evaluated at Njoro I, Njoro II, Eldoret, Nakuru West and Lanet, Kenya in 2011.

Source	df	50% flowering (days)	Harvest maturity (days)	Plant height (cm)	Pods plant^{-1} (No.)	Nodes plant^{-1} (No.)	Yield (Kg ha^{-1})	100-seed weight(g)	Oil content (%)	Protein content (%)
Environment (E)	4	1120.60 **	10411.87**	6458.81**	6203.01**	94.15**	4983331.16**	97.68**	134.68**	649.34**
Replicates R(E)	10	8.71	67.96	70.25	120.93	1.64	77842.17	1.62	0.81	6.88
Genotype(G)	14	714.72**	3774.52**	2996.69**	339.60**	59.85**	933486.56**	71.41**	26.98**	26.31**
G × E	56	30.24**	142.55**	439.69**	251.51**	8.64**	227898.57**	5.53**	0.97**	4.65**
Error	140	4.91	36.75	37.41	48.97	1.24	50436.56	1.81	0.39	3.02
C.V. %		2.64	3.72	8.72	17.83	8.29	17.71	9.85	3.23	4.61
R^2		0.95	0.95	0.94	0.86	0.90	0.86	0.87	0.94	0.88

** Significant at $p \leq 0.0$l, C.V.% - coefficient of variation, R^2- coefficient of determination, df- degrees of freedom.

Table 2. Mean values of soybean agronomic traits of 15 genotypes evaluated at Njoro I, Njoro II, Eldoret, Nakuru West, and Lanet, Kenya in 2011.

Genotype	50% Flowering	Harvest maturity (days)	Plant height (cm)	Pods plant^{-1}	Seeds Pod^{-1} (No.)	Nodes plant^{-1} (No.)	Grain yield (kg ha^{-1})	100 Seed weight (g)	Oil content (%)	Protein content (%)
SBH 10/5/6	79.0^{f}	158.2^{fe}	87.4^{b}	35.3^{d-f}	2.4^{a-c}	15.7^{bc}	1303.5^{c-e}	13.3^{cd}	20.5^{b}	36.6^{gh}
SBH 1/12/9	79.8^{fe}	155.3^{f-h}	78.1^{d}	40.6^{bc}	2.4^{a-c}	14.4^{de}	1434.7^{bc}	12.6^{de}	19.6^{e-g}	37.0^{e-h}
SBH7/1/1	79.6^{fe}	162.5^{e}	87.9^{b}	39.4^{b-d}	2.4^{a-c}	16.8^{a}	1543.9^{ab}	12.9^{de}	20.1^{b-d}	36.8^{f-h}
Gazelle	89.3^{c}	168.5^{d}	81.6^{cd}	32.9^{ef}	1.9^{f}	14.8^{d}	1327.5^{c-e}	17.5^{a}	19.9^{c-f}	36.1^{hi}
SBH 4/4/4	79.2^{fe}	156.7^{fg}	85.7^{bc}	33.9^{ef}	2.4^{a-c}	15.1^{cd}	1367.4^{cd}	12.8^{de}	20.3^{bc}	36.9^{e-h}
SBH 10/2/3	83.1^{d}	154.2^{f-h}	60.5^{fg}	39.8^{b-d}	2.3^{b-d}	11.4^{h}	1124.9^{f}	14.5^{b}	19.7^{ef}	38.8^{bc}
SBH 4/6/6	82.6^{d}	153.8^{gh}	62.1^{ef}	37.3^{c-f}	2.3^{a-d}	11.1^{h}	1289.5^{c-e}	14.4^{b}	19.7^{d-f}	38.4^{bcd}
Nyala	84.3^{d}	174.5^{c}	60.7^{fg}	46.1^{a}	2.2^{de}	13.4^{f}	1600.9^{a}	17.6^{a}	19.5^{fg}	37.2^{d-h}
EAI 3600	83.2^{d}	155.8^{fg}	66.3^{e}	32.6^{f}	2.4^{a}	11.4^{h}	1375.2^{cd}	14.1^{bc}	20.0^{bc-e}	37.8^{c-g}
SBH 6/6/6/2	80.6^{e}	151.1^{ih}	57.8^{f-h}	43.2^{ab}	2.3^{a-d}	11.3^{h}	1197.7^{ef}	12.2^{e}	19.2^{g}	37.9^{c-f}
DPSB 8	91.7^{b}	185.1^{b}	95.8^{a}	37.5^{c-f}	2.4^{ab}	16.3^{ab}	1414.8^{bc}	12.3^{e}	17.9^{h}	38.0^{b-e}
DPSB 19	74.0^{g}	140.5j	59.6^{f-h}	37.9^{c-e}	2.3^{c-e}	12.5^{g}	807.9^{g}	10.7^{f}	17.0^{i}	40.3^{a}
931/5/34	93.1^{b}	188.9^{b}	55.3^{h}	47.1^{a}	2.4^{ab}	12.3^{g}	1323.3^{c-e}	16.7^{a}	21.9^{a}	35.2^{i}
DPSB 3	100.9^{a}	194.2^{a}	57.2^{gh}	39.1^{b-d}	2.1^{e}	13.8^{ef}	661.7^{g}	10.5^{f}	16.6^{i}	39.2^{ab}
SBH 3/8/4/1	80.6^{e}	147.3^{i}	56.8^{gh}	46.4^{a}	2.3^{a-d}	11.1^{h}	1243.5^{d-f}	12.9^{de}	19.2^{g}	38.9^{bc}
Mean	84.1	163.1	70.2	39.3	2.3	13.4	1267.8	13.7	19.4	37.7
C.V.%	2.6	3.7	8.7	17.8	8.8	8.3	17.7	9.8	3.2	1.3
LSD(0.05)	1.6	4.4	4.4	5.1	0.1	0.8	162.1	1.0	0.5	1.3

Means designated by the same letter within columns are not significantly different at p=0.05, CV%- coefficient of variation, df-degrees of freedom, LSD-least significance difference.

was 59% by genotypes, 26% by the environment while genotype by environment interaction (GEI) contributed 9% for the days to 50% flowering. The SBH lines were medium maturing with a range of 147-163 days while the TGX genotypes had the earliest maturing genotype ranging from 141 (DPSB19) to 194 days (DPSB 3). The check variety (EAI 3600) was medium in maturity. Genotype 931/5/34 had the highest number of pods plant^{-1} and the shortest in height. Genotypes with pods plant^{-1} above the mean of 39.3 were SBH 1/12/19, SBH 7/1/1, SBH 10/2/3, Nyala, SBH 6/6/6/2, SBH 3/8/4/1 and 931/5/34. There was minimum variation on seeds pod^{-1} since 30% of the genotypes (Gazelle, SBH 10/2/3, Nyala, DPSB 19, DPSB 3) had significantly different number of seeds pod $^{-1}$. Genotypes SBH 7/1/1 and DPSB 8 had the highest plant height and number of nodes plant^{-1} while genotypes SBH 3/8/4/1, SBH 4/6/6, SBH 6/6/6/2, EAI 3600 and SBH 10/2/3 had significantly lower number of nodes plant^{-1}. Genotype Nyala had the highest mean grain yield across sites with the highest yield observed at Njoro in the second season (2397 kg ha^{-1}) while genotype DPSB 3 yielded the least (Table 2).

There were significant differences in seed weight among the genotypes. Genotypes Nyala had the highest seed, weight; however, this was not significantly different from Gazelle and 931/5/34. Only 40% of the genotypes (Gazelle, SBH 10/2/3, SBH 4/6/6, Nyala, EAI 3600, 931/5/34) observed seed weight above the mean (Table 2). Protein content was highest on genotype DPSB 19 while genotype 931/5/34 had the least. The contribution of environment sum of squares to yield, oil content, protein content, seed weight, maturity and pods plant^{-1} was 37, 52, 70, 20, 38 and 48%, respectively while the genotype contributed 24, 37, 10, 51, 49, and 9%, respectively. The G × E interaction contributed 24, 5, 7, 16, 7 and 27% respectively. Based on their contribution, it is evident that protein and oil content are influenced by environment compared to other traits.

Performance of the genotypes in individual test environments

There was considerable variation in the performance of genotypes in individual test environments (Table 3). At Eldoret, genotype SBH 7/1/1 yielded highest while DPSB 19 yielded least (Table 3). Highest seed yield was observed at Lanet. Protein content was marginally higher at Njoro in the first season (Njoro I) compared to second season (Njoro II) (Table 3). The highest protein content (45%) was observed on genotype 931/5/ DPSB 19 at Njoro in the first season. Variability in the protein content among the genotypes was least in Nakuru west.

DISCUSSION

Significant genotype and G × E interactions for all the traits studied suggested that genotypes responded differently to environments and there was G × E interaction, allowing for selection of genotypes for specific eco-zones. However, contribution to the total sum of squares differed depending on the trait. In a combined analysis of variance, Pfeiffer et al., (1995) observed that soybean genotypes differed significantly for seed yield and seed composition, however the genotype by environment interaction was not significant for seed yield but was significant for seed composition. The mean plant height range observed in the current study agrees satisfactorily with that observed by Aditya et al. (2011) while testing 31 soybean genotypes. A study by Karasu et al. (2009) indicated a similar range of mean plant height among the eight soybean genotypes tested in two locations for two years. The genotypes height differences in their study and the current study could be attributed to genetic and environmental influences.

Pods plant^{-1}, seeds pod^{-1}, number of nodes plant^{-1} and seed weight are important components of seed yield. The results of the study on these traits are consistent with those of Aditya et al. (2011) and Karasu et al. (2009); however, Ojo (2003) recorded a higher range for pods plant^{-1}. The variations could be attributed to differences in genotypes and test environments. The oil content was significantly different among the genotypes. The percentage oil content was affected more by the environment and the genotype and less by G × E interaction effects. Protein in the current study varied among the genotypes and the variation was attributed to environment, genotypes and influence of environment on genotype. Protein and oil content are influenced by both genotype and environment cues (Clemente and Cahoon, 2009). The inverse relationship between oil and protein content exists, typically a 15% reduction in total oil content leads to a 2% increase in protein content (Clemente and Cahoon, 2009).

Environments and G × E interaction play a significant role in the performance of genotypes in a particular environment. There was significant G × E interaction for all traits studied which underscores the importance of selection for specific adoption to the given regions.

CONCLUSION AND RECOMMENDATIONS

The study has revealed that there are high yielding genotypes for different environments with high oil and protein content. Genotype SBH 7/1/1 and Gazelle, may be recommended for Eldoret and Lanet, respectively. DPSB 8 a late maturing genotype maybe recommended for Njoro in the first season. Genotype DPSB 19 and DPSB 3 had high mean protein content However, they had low grain yield and maybe useful in a breeding program to improve protein content. Among the sites tested seems to favor soybean production. Production targeting high protein content needs to be done in higher

Table 3. Variation in yield and protein content of soybean genotypes grown at Njoro I, Njoro II, Eldoret, Nakuru West, and Lanet experimental sites, Kenya in 2011.

Genotypes	Seed yield (kg ha^{-1})						Protein content (%)					
	Eldoret	Lanet	Nakuru West	Njorol	Njoroll	Mean	Eldoret	Lanet	Nakuruwest	Njoro I	Njoroll	Mean
SBH 10/5/6	1222.8^{a-c}	1748.8^{a-c}	912.3^{b-e}	1370.0^{b-d}	1263.0^{cd}	1303.5^{c-e}	35.5^{bc}	38.6^{a-d}	31.4^{a-c}	38.7^{d-f}	38.7^{e-f}	36.6^{gh}
SBH 1/12/9	1204.3^{a-c}	1932.1^{a-c}	920.4^{b-e}	1495.1^{bc}	1621.6^{b-d}	1434.7^{bc}	37.5^{a-c}	36.9^{de}	31.3^{a-d}	38.6^{d-f}	40.8^{bc}	37.0^{e-h}
SBH7/1/1	1492.0^{a}	1975.3^{a-c}	942.0^{b-d}	1542^{b}	1768.5^{b}	1543.9^{ab}	39.1^{ab}	37.7^{a-e}	31.2^{a-e}	37.8^{ef}	38.1^{f}	36.8^{f-h}
Gazelle	1197.5^{a-c}	2179.0^{a}	959.2^{b-d}	827.8^{fg}	1474.1^{b-d}	1327.5^{c-e}	36.3^{bc}	36.1^{e}	30.8^{d-f}	37.9^{ef}	39.3^{c-f}	36.1^{hi}
SBH 4/4/4	1217.3^{a-c}	1668.5^{bc}	918.5^{b-e}	1459.9^{b-d}	1572.8^{b-d}	1367.4^{cd}	39.1^{ab}	37.2^{b-e}	31.1^{a-e}	38.6^{d-f}	38.2^{f}	36.9^{e-h}
SBH 10/2/3	812.3^{de}	1804.9^{a-c}	682.7^{ef}	997.5^{e-g}	1327.1^{b-d}	1124.9^{f}	38.0^{a-c}	40.0^{ab}	31.5^{ab}	43.3^{ab}	41.1^{cb}	38.8^{bc}
SBH 4/6/6	943.8^{c-e}	1933.9^{a-c}	1070.4^{a-c}	1156.8^{d-f}	1342.6^{b-d}	1289.5^{c-e}	37.3^{a-c}	39.8^{a-c}	31.1^{a-e}	41.8^{bc}	41.9^{ab}	38.4^{bcd}
Nyala	1165.4^{a-c}	1992.6^{ab}	1229.0^{a}	1237.7^{b-e}	2379.6^{a}	1600.9^{a}	39.5^{ab}	37.1^{c-e}	31.0^{b-f}	39.9^{c-e}	38.4^{ef}	37.2^{d-h}
EAI 3600	1125.9^{b-d}	2036.4^{ab}	1129.6^{ab}	1205.6^{c-e}	1378.4^{cd}	1375.2^{cd}	37.7^{a-c}	37.9^{a-e}	31.1^{a-e}	41.6^{b-d}	40.4^{b-f}	37.8^{c-g}
SBH 6/6/6/2	1085.8^{b-d}	1538.9^{cd}	871.6^{c-e}	800^{g}	1692.0^{bc}	1197.7^{ef}	36.7^{bc}	38.6^{a-e}	31.4^{ef}	41.7^{b-d}	41.7^{ab}	37.9^{c-f}
DPSB 8	927.2^{c-e}	1816.1^{a-c}	873.5^{c-e}	1919.1^{a}	1538.3^{c-d}	1414.8^{bc}	39.2^{ab}	39.1^{a-d}	31.4^{a-c}	40.6^{b-e}	39.9^{b-f}	38.0^{b-e}
DPSB 19	675.3^{e}	1175.9^{ed}	768.5^{d-f}	246.9^{h}	1172.9^{d}	807.9^{g}	41.4^{a}	39.9^{a-c}	31.6^{a}	45.1^{a}	43.4^{a}	40.3^{a}
931/5/34	1293.2^{ab}	1930.8^{a-c}	587.73^{f}	1569.1^{b}	1235.8^{cd}	1323.3^{c-e}	34.2^{c}	35.9^{e}	30.6^{f}	36.9^{f}	38.5^{ef}	35.2^{i}
DPSB 3	1055.0^{b-d}	792.6^{e}	887.0^{bc-e}	827.8^{fg}	124.7^{e}	661.7^{g}	41.6^{a}	40.5^{a}	30.9^{c-f}	42.1^{bc}	40.8^{b-d}	39.2^{ab}
SBH 3/8/4/1	1184.0^{a-c}	1778.4^{a-c}	680.9^{ef}	937.7^{e-g}	1636.4^{b-d}	1243.5^{d-f}	39.2 ^{ab}	40.2^{a}	31.2^{a-e}	43.6^{ab}	40.7^{b-d}	38.9^{bc}
Mean	1106.8	1153.62	895.6	1147.7	1435.2	1267.8	38.2	38.4	31.1	40.5	40.1	37.7
C.V.%	18.0	15.30	16.7	17.3	19.5	17.7	7.1	4.3	1.0	4.5	3.1	1.3
Lsd	333.9	448.8	248.9	331.8	469.2	162.1	4.5	2.8	0.5	3.0	2.1	1.3

Means designated by the same letter within columns are not significantly different at p=0.05, CV %- coefficient of variation, df-degrees of freedom, LSD-least significance difference.

altitudes such as Njoro and Eldoret while production targeting high oil content should focus on lower altitude areas such as Nakuru west. The low G×E interaction of the genotypes for oil and protein content implies that there is still need to develop genotypes with high oil and protein content. Genotype Nyala and SBH 7/1/1 maybe recommended for production across studied environments due to their relatively high grain yield. Further evaluation in low and medium altitudes with more diverse soybean genotypes, especially the promiscuous ones is recommended.

Conflict of Interest

The authors have not declared any conflict of interest.

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Associations of traits with yield in Dekoko *(Pisum sativum var. abyssinicum)* accessions in the highlands of southern Tigray, Ethiopia

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Dekoko (*Pisum sativum var. abyssinicum*) is a unique crop developed and cultivated in Ethiopia. The objectives of the study were; understanding the genetic variability present in Dekoko population, determining the correlation between grain yield, protein content and other traits and understanding traits that can be used for indirect selection for high grain yield and high protein content. Local collections of Dekoko were planted in 3 replications of the RCBD design at Mekhan farmers' Training Centre in Endamekhoni during 2010.Traits such as days to flowering and maturity and leaf width had low phenotypic coefficient of variation and genotypic coefficient of variation and low genetic advance (<20%). The genotypic correlation between and the direct effect of days to flowering, plant height and biomass on seed yield was positive suggesting that the traits can be used for indirect selection of high yielding accessions. Seed yield and protein content had perfect negative genotypic correlation (-1.00). The direct effect of protein content on seed yield was also negative implying that simultaneous selection towards increased seed yield and increased protein content at the same time may be difficult.

Key words: Associations, Dekoko, *Pisum sativum* var. *abyssinicum*, accession, crude protein.

INTRODUCTION

Field pea (*Pisum sativum* L.) is the fourth most important legume crop in Ethiopia after faba bean, haricot bean and chick pea in terms of both area and total amount of production. Field pea covers over 203,990.64 ha with a total production of 257,031.41 tons which accounts for 13% of the total grain legume production (CSA, 2011).

Both field pea and Dekoko are consumed as a protein supplement in the cereal-based diets of Ethiopia Sentayehu (2009). However , the average yield is very low, that is, 1.26 t/ha (CSA, 2011) for field pea and there is no record for Dekoko. The low yield in field pea is because of limited number of high yielding and disease resistant varieties, growing of pulses on marginal soils, poor management practices, absence or low fertilizer rate of application, and high insect and disease problems.

Even though the origin of field pea is controversial, Ethiopia is undoubtedly the centre of diversity for this crop since wild and primitive forms are known to exist

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in the high elevations of the country. Ethiopia is one of the major Vavilovian centers of diversity for several grain legume crops including lupine, field pea and wild ancestors of cow pea (Ali et al., 2003).

Cultivated *Pisum* is dominated by *P. sativum* species but *P. sativum* species *abyssinicum* (or simply *P. abyssinicum*) is a unique sub-species independently developed and cultivated in Ethiopia. The existing germplasm in the country shows tolerance to disease (IBC, 2007); (Sentayehu, 2009); (Jing et al., 2010). *Pisum sativum* is widespread across the Middle East and has affinity with the wild *P. elatius* while *P. abyssinicum* is restricted to highland regions of Ethiopia (South Tigray and North Wello) and Southern Yemen and shows a greater affinity to *P. fulvum* Yemane and Skjelvåg (2002); Jing et al. *(*2010). However*, P. fulvum* is found around the eastern edge (Syria, Lebanon, Israel, Palestine and Jordan) and not common in Ethiopia (Maxted and Ambrose, 2001).

P.s. abyssinicum is locally known as Dekoko (minute seeded) in Tigrigna and Yagere Ater (pea of my country) or Tinishu Ater (the smallest pea) in Amharic. According to Yemane and Skjelvåg (2002) Dekoko is capable of producing seed yield of up to 1.95 t/ha under phosphorus fertilization and is known for its high market price and for its food preference. Farmers and consumers in the study area call it as the “Dero-Wot of the poor” (chicken stew of the poor) probably to express its high nutritional value. Most often, the dry seeds of Dekoko are decorticated and split (‘split peas’) before boiling. Similarly, the seeds are boiled without decortications and consumed as soup and is preferred by most users (personal observation). In Ethiopia the annual consumption per person of field pea including Dekoko seeds is estimated at 6 to 7 kg (Messiaen et al., 2006; Sentayehu, 2009).

The genetic diversity of a species is the outcome of cumulative mutation, recombination and selection on individuals by the environment and selection by man for traits desirable for cultivation or consumption (Ali et al., 2007). The largest collection of *P. sativum* germplasm in Africa is located at the Institute of Biodiversity Conservation, Addis Ababa, Ethiopia, with over 1600 accessions (Messiaen et al., 2006).

A large genetic diversity has been found in *P. sativum* collections from both Africa (e.g. Ethiopia) and Asia. High to medium field pea genetic diversity in Ethiopia was observed in collections from Shoa, Gojam, Gondar, Wello, and Tigray while low to trace genetic diversity was observed in collections from Arsi, Gamo-gofa, Wellega, Illubabur and Kafa (Ali et al., 2003).

An understanding of morphological characters facilitates the identification and selection of desirable traits, designing new populations, transferring the desirable genes into widely grown food legumes through biotechnological means, resistance to biotic and abiotic stresses that are known to individual accessions increase the importance of the germplasm (Santall et al., 2001; Tar'an et al., 2005; Jorge, 2006).

Land races are the genetic wealth that a crop acquires over many years of its existence and have considerable breeding values as they contain valuable adaptive genes to different circumstances (Messiaen et al., 2006; Ali et al., 2003). In Ethiopia, more than 15 cultivars of field pea, with better yield potential, seed size, seed color and disease resistance than the farmers’ varieties, have been released for different agro-ecological conditions (MoARD, 2008). Some of these varieties were obtained from local collections while others were obtained through hybridization of landraces with introduced germplasm.

Even though Dekoko (*P. sativum var. abyssinicum*) is important both for the local farmers and consumers, the existing germplasm was not studied for its diversity; neither was there any improvement work on this crop so far. The study was, therefore, conducted with the objectives of determining the correlation between the various quantitative traits and identifying those traits having high correlation with grain yield so that they can be used in indirect selection.

MATERIALS AND METHODS

Description of the study area

The research was conducted in Southern zone of Tigray regional state, Wereda Endamekhoni at tabia Mekhan farmers’ training center which is one of the mandate areas of Alamata Agricultural Research Center (AlARC).

Mekhan is only five kilo-meters South of Maichew and located about 660 km North of Addis Ababa and about 120 km south of Mekelle. Tabia Mekhan has a total population of 1249 households of which 741 are male headed and 508 are female headed. Major crops grown in the area include wheat, barley, maize, lentil, field pea and faba bean. The soil of the experimental site is black clay loam. The Wereda has a temperature range of 9 to 18°C with a mean annual rain fall of 600 to 700 mm (Endamekhoni BoANR, 2010).

Accessions evaluated

Twenty four local collections of Dekoko collected from two regions; South-Tigray and North-Wello were tested at Mekhan farmers training center (FTC) at an elevation of 2100 m above sea level. The accessions were collected in 2008 by Alamata Agricultural Research Center from weredas: Alamata, Ofla, Endamekhoni, Alaje, and Hintalo-Wejerat in South Tigray, and Kobo, Guba-lafto, Srinka and Habru in North-Wello (Table 1).

Experimental design and trial management

The trial was conducted using Randomized Complete Block Design with three replications and the plot size for each accession was 1.5 m^2 with inter- and intra-row spacing of 25 and 5 cm, respectively. Accessions were sown in six rows each 1m long. Phosphorus and nitrogen fertilizers with normal recommendation rates to other pulse crops 46 kg P_2O_5 and 18 kg N ha^{-1}, that is, 100 Kg DAP (Di-Ammonium Phosphate ha^{-1}) and seed rate of 150 kg ha^{-1} were applied.

Table 1. Accessions of Dekoko Included in the Study and Their Sources with their Altitude

S/No.	Accession name	Source of accessions			
		Region	Wereda	Major agro-ecology	Altitude of wereda (masl)
1	T-001/08 Of	Tigray	Ofla	High land	2457
2	T-002/08 Of	Tigray	Ofla	High land	2457
3	T-003/08 Of	Tigray	Ofla	High land	2457
4	TK-004/08 Al	Tigray	Alamata	Low land	1178-3148
5	TK-005/08 Al	Tigray	Alamata	Low land	1178-3148
6	TK-006/08 Al	Tigray	Alamata	Low land	1178-3148
7	TK-008/08 Al	Tigray	Alamata	Low land	1178-3148
8	T-023/08 Mw	Tigray	Endamekhoni	High land	2100
9	T-022/08 E/A	Tigray	Emba-Alaje	High land	2116
10	T-024/08 E/A	Tigray	Emba-Alaje	High land	2116
11	T-021/08 H/W	Tigray	Hintalo-Wejerat	Mid –altitude	1400-3050
12	T-007/08 Ko	Amhara	Kobo	Low land	1100-3000
13	T-009/08 Ko	Amhara	Kobo	Low land	1100-3000
14	T-010/08 Ko	Amhara	Kobo	Low land	1100-3000
15	T-017/08 Ko	Amhara	Kobo	Low land	1100-3000
16	T-018/08 Ko	Amhara	Kobo	Low land	1100-3000
17	T-019/08 Ko	Amhara	Kobo	Low land	1100-3000
18	T-020/08 Ko	Amhara	Kobo	Low land	1100-3000
19	T-012/08 G/L	Amhara	Guba-lafto	High land	2061
20	TA-013/08 Sr	Amhara	Srinka	Mid –altitude	1868
21	TA-014/08 Sr	Amhara	Srinka	Mid –altitude	1868
22	TA-015/08 Sr	Amhara	Srinka	Mid –altitude	1868
23	T-011/08 Hb	Amhara	Habru	Low land	700-1900
24	T-016/08 Hb	Amhara	Habru	Low land	700-1900

Table 2. Form of analysis of covariance between quantitative characters.

Source of variation	Df	MSCP	EMS‡
Replication	r-1	$MSCP_{rxy}$	
Accession	a-1	$MSCP_{gxy}$	$\sigma^2_{exy} + r\sigma^2_{gxy}$
Error	(r-1) (a-1)	$MSCP_{exy}$	σ^2_{exy}

Df= degree of freedom, r= number of replications, a= number of genotypes; $MSCP_{rxy}$ = Mean sum of cross product of replication for variable x and y; $MSCP_{gxy}$ = Mean sum of cross product of accessions for variable x and y; $MSCP_{exy}$ = Mean sum of cross product of error for variable x and y; $\sigma^2_{exy} = MSCP_{exy}$ = environmental covariance between trait x and y; $\sigma^2_{g\,xy} = (MSCP_{gxy} - MSCP_{exy})/r$ = Genotypic covariance between trait x and y; σ^2_{pxy} = phenotypic covariance between traits x and y = $\sigma^2_{axy} + \sigma^2_{exy}/r$; σ^2_{gxy} = genotypic covariance between character x and y.

Statistical analysis

Analysis of covariance (ANCOVA), correlations and path coefficient analysis

Analysis of covariance (ANCOVA) was conducted for the quantitative data (Table 2).

Phenotypic and genotypic correlation coefficients

Phenotypic correlation, the observable correlation between two variables, which includes both genotypic and environmental components between two variables, was estimated using the formula suggested by Miller et al. (1958):

$$r_{pxy} = \frac{\sigma^2 pxy}{\sqrt{(\sigma^2 px)(\sigma^2 py)}}$$

Genotypic correlation was computed as:

Table 3. Genotypic correlation coefficients of 12 characters of Dekoko accessions.

	YLD	DE	DF	DM	LW	PPP	BIO	TSW	PH	HI	PEST	PROT
YLD	1.00											
DE	1.00**	1.00										
DF	1.00**	1.00**	1.00									
DM	1.00**	1.00**	1.00**	1.00								
LW	-1.00**	-1.00**	-1.00**	-1.00**	1.00							
PPP	1.00**	-0.828*	-0.994**	-0.971**	0.901*	1.00						
BIO	0.985**	0.798*	0.943**	0.886*	-0.760*	-1.00**	1.00					
TSW	-0.853*	-0.387	-0.517*	-0.573*	0.288	1.00**	-1.00*	1.00				
PH	0.833*	1.00**	1.00**	0.956**	-1.00**	-0.476	0.423	-0.010	1.00			
HI	-0.225	0.845*	0.695*	0.326	-1.00**	0.361	-0.454	-0.454	1.00**	1.00		
PEST	-0.481	-0.948*	-0.846*	-0.822*	1.00**	-0.140	0.327	0.327	-0.920*	-1.00**	1.00	
PROT	-1.00**	-0.757*	-0.889*	-0.903*	0.888*	1.00*	-1.00**	1.00**	-0.421	0.666*	-0.139	1.00

- * = significant at 0.05 probability level; ** = highly significant at 0.01 probability level. DE=days to emergence, DF=days to flowering, DM=Days to maturity, LW=leaf width, PPP=pods per plant, BIO=above ground biomass, TSW= thousand seed weight, PH= plant height, HI=harvest index, PEST= Insect pest, PROT= percent of crude protein

$$r_{gxy} = \frac{\sigma^2 gxy}{\sqrt{(\sigma^2 gx)(\sigma^2 gy)}}$$

Where r_{pxy} is phenotypic correlation coefficient and r_{gxy} is genotypic correlation coefficient between characters x and y; σ^2_{pxy} and σ^2_{gxy} are phenotypic covariance and genotypic covariance between characters x and y, respectively. σ^2_{px} and σ^2_{gx} are phenotypic and genotypic variances for character x and σ^2_{py} and σ^2_{gy} are phenotypic and genotypic variances for character y.

The coefficient of correlation at phenotypic level was tested for its significance with table for simple correlation coefficient using n-2 df as suggested by Gomez and Gomez (1984) or using‘t’ table, with observed t expressed as:

$$t = \frac{r_{pxy}\sqrt{n-2}}{\sqrt{1-r^2_{pxy}}}$$

The calculated ‘t’ value was compared with the tabulated ‘t’ value at n-2 degree of freedom, at 5% and 1% level of significance (where n is the number of genotypes).

The coefficient of correlation at genotypic level was tested according to Robertson (1959):

$$t = \frac{r_{gxy}}{SEr_{gxy}}$$

Where, r_{gxy} = genotypic correlation coefficient, SEr_{gxy}= standard error of genotypic correlation coefficient:

$$SEr_{gxy} = \sqrt{(1-r^2 gxy)^2 / 2h_1^2 h^2_2}$$

Where; h^2_1 and h^2_2 are broad sense heritability for character 1 and 2.

The calculated ‘t’ value was compare with the tabulated ‘t’ value at the 5 and 1% level of significance using n-2 DF (where n is the number of accessions).

Path coefficient analysis

Partitioning of the cause and effect relationship of different traits will help to see what is contributing to the observed correlation. In some conditions, correlation alone does not give the exact picture of direct and indirect effect of characters up on each other, thus path coefficient analysis is preferable, since it can identify the direct and indirect causes of associations and can measure the relative importance of each (Singh and Chaudhary, 1977; Sharma, 1998).

Association of yield with its components was determined by the application of correlation and path analysis. The use of path analysis requires a cause and effect situation among the variables. Path coefficient analysis is usually calculated using the formula suggested by Dewey and LU (1959) to assess direct and indirect effects of different traits on grain yield (dependent trait j) as:

$$r_{ij} = p_{ij} + \sum r_{ik}\ p_{kj}$$

Where r_{ij} is mutual association between the independent trait (i) and the dependent trait (j) as measured by the correlation coefficient r_{ij}, p_{ij} is component of direct effect of the independent trait (i) on the dependent variable (j); and r_{ik} p_{kj} is the components of indirect effect of a given independent trait (i) on the dependent traits (j) via all other independent traits (k).

The residual effect (U) which is the unexplained variation of the trait that is not accounted for by path coefficient is calculated using the formula of Dewey and LU (1959) as:

$$U = \sqrt{1-R^2}$$

Where $R^2 = \sum r_{ik}\ p_{kj}$

RESULTS AND DISCUSSION

Associations between characters in Dekoko

Genotypic correlation coefficients

Genotypic correlations between traits indicate the direction and magnitude of correlated responses to selection, the relative efficiency of indirect selection, and permit calculation of optimal multiple trait selection indices (Falconer and Mackay, 1996). Plant breeders traditionally have estimated genotypic and phenotypic correlations between traits using the method of moments on the basis of a multivariate extension of ordinary least squares referred to as multivariate analysis of variance (MANOVA) (Anderson, 1958; Mode and Robinson, 1959).

Phenotypic values of different traits in the same plant are often correlated, such as height and yield. Environmental factors and genetic effects are two reasons for correlations.

A set of closely linked genes present on one chromosome tend to be inherited together (not easily separable by recombination). If two genes are in linkage disequilibrium, a genetic covariance may arise between traits X and Y. In this study, 24 Dekoko accessions were analyzed for genotypic coefficients of 13 characters. Grain yield exhibited significant and positive associations with days to emergence (1.00***), days to flowering (1.00***) and maturity (1.00***), and biomass (0.985***) (Table 3). These positive and strong associations with grain yield revealed the importance of these characters in determining grain yield and indicated that selection for one or all of these traits would result in superior yield (Pandey and Gritton, 1975). It showed significant and negative associations with leaf width (-1.00***), pods per plant (-1.00***) and crude protein content (-1.00***), indicating the difficulty of improving both yield and protein content simultaneously. Days to emergence exhibited significant and positive association with grain yield, days to flowering and maturity, and plant height while it expressed significant and negative associations with pest and pod per plant (Table 3). Similarly crude protein content showed significant and positive associations with number of pods per plant (1.00***), and 1000 seed weight (1.00***) while it exhibited significant and negative association with grain yield (-1.00***) and biomass (-1.00***).

Even though, there is no report on breeding of Dekoko to cross check, this result is in agreement with the reports of various researchers on field pea that indicated the existence of strong associations between agronomic characters and grain yield (Muhammad et al., 2009; Ali et al., 2007).

Phenotypic correlations coefficients

Phenotypic correlation is a function of genetic and environmental correlation. The expression can be simplified by substituting the square root of variances as suggested by Walsh (1981). In this study, 24 Dekoko accessions were analyzed for phenotypic correlation coefficients of 12 quantitative characters (Table 4). Accordingly, grain yield exhibited highly significant and positive associations with days to emergence (0.944***), flowering (0.967***) and maturity (0.977***), biomass (0.894**) and plant height (0.771**). However, it expressed highly significant and negative associations with pods per plant, leaf width, 1000 seed weight and crude protein content. Generally, the phenotypic correlation coefficients were lower than their corresponding genotypic values indicating that the influence of environmental factor up on the accessions is lower than the inherent genetic effects. However, grain yield showed no significant relationships with harvest index and pest score. Results of similar trend in field pea have been reported by many researchers (Muhammad et al., 2009; Singh et al., 2007; Sharma et al., 2007).

Path coefficient analysis

Information on correlation and path coefficient analysis is of much use to plant breeders for selection and breeding genotypes with increased yield potential. Correlation analysis for seed yield provides opportunity for selection and leads to a directional model based on yield and its components in field experiments. Path coefficients have been used for complex characters in several crop species to provide information on interrelations of complex characters and to develop selection criteria (Kang et al., 1993; Gravois et al., 1991; Diz et al., 1994). In this investigation, 11 characters of Dekoko were analyzed for their direct and indirect effects up on grain yield (Table 5).

Direct effects of various characters on grain yield

Five out of 11 characters had positive direct effect on grain yield. They were number of pods per plant, biomass, plant height, days to flowering and pest score (11.358, 7.182, 7.148, 5.182, and 1.292) respectively (Table 5). Characters with negative direct effects were days to emergence, crude protein content, days to maturity, harvest index, leaf width, and thousand seed weight (-8.801, -8.057, -1.292, -0.762, -0.394, -0.232), respectively. Number of pods per plant had the highest (11.358) direct effect while days to emergence had the highest negative (-8.801) direct effect up on grain yield. The direction of the correlation coefficient between grain yield with the trait and the direct effect of the trait on seed yield coincide and are positive for biomass yield, plant height, and days to flowering. Selecting taller plants that flower late and produce high biomass can lead to increase in seed yield. However the indirect negative effects of these traits through other traits must be taken

Table 4. Phenotypic correlation coefficients of 12 characters of Dekoko accessions.

	YLD	DE	DF	DM	LW	PPP	BIO	TSW	PH	HI	PEST	PROT
YLD	1.00											
DE	0.944**	1.00										
DF	0.967**	0.997**	1.00									
DM	0.977**	0.992**	0.999**	1.00								
LW	-0.853*	-0.977**	-0.957**	-0.944*	1.00							
PPP	-0.908**	-0.719*	-0.772*	-0.799*	0.556*	1.00						
BIO	0.894**	0.695*	0.750	0.778**	-0.527*	-0.999**	1.00					
TSW	-0.640*	-0.350**	-0.423	-0.462	0.144	0.903**	-0.917*	1.00				
PH	0.771**	0.938**	0.908**	0.889**	-0.990**	-0.434	0.403	-0.004	1.00			
HI	0.153	0.471	0.399	0.359	-0.647*	0.274**	-0.307	0.662	0.747	1.00		
PEST	-0.318	-0.614*	-0.549*	-0.512*	0.767**	-0.108	0.141	-0.525*	-0.849*	-0.986**	1.00	
PROT	-0.879**	-0.671*	-0.728*	-0.758*	0.450	0.998**	-0.999**	0.929**	-0.374*	0.337	-0.173	1.00

- * = significant at 0.05 probability level; ** = highly significant at 0. 01probability level.

into account. Although the genetic correlation between seed yield and pods per plant is negative, number of pods per plant has big positive direct effect on seed yield. The negative correlation is due to its big negative indirect effects through other traits which will be discussed later. Although days to emergence and days to maturity had positive genotypic correlation with seed yield their direct effect is negative. One should be cautious in using these traits for indirect selection for seed yield. The positive genotypic correlation of these traits with seed yield is through their positive indirect effect through other traits. Crude protein had negative genotypic correlation with seed yield and also a big negative direct effect on seed yield. This indicates that it is impossible to increase crude protein content and seed yield of Dekoko simultaneously.

In a similar study on field pea, grain yield was found to have a highly significant positive correlation with number of pods per plant and above ground biological yield (Togay et al., 2008; Ali et al., 2007). Number of pods per plant showed highest degree of diversity and can be used directly for the improvement of the crop. The same results were reported by the work of Turi (2004) quoted in Ali et al. (2007) and Mehrani (2002). Malik et al. (1987) and Ghafoor et al. (1998) also reported positive correlation of grain yield with the above ground biological yield, which proved the complete association of the two traits. Many researchers like Donald (1962), Lal (1967), Sing et al. (1980), and Khan and Malik (1989) have already suggested that selection on the basis of best performance is vital in improving field pea. Leleji et al. (1972) examined significant negative correlations between grain yield and protein percent in dry beans. Kelly and Bliss (1975) found negative correlation between grain yield and percent protein in beans. Similarly, Camacho (1978) explained that protein concentration in legumes had negative correlation with grain yield. In this study, it was seen that there exists an association between the different quantitative traits of Dekoko.

Indirect effects of various characters on grain yield

Days to emergence

Days to emergence had negative indirect effect up on grain yield at genotypic levels through number of pods per plant, pest and days to maturity (Table 5).

Days to flowering

Days to flowering had positive direct effect up on grain yield at both genotypic and phenotypic levels exhibited via biomass, plant height and crude protein content.

Days to maturity

Days to maturity had positive direct effect up on grain yield at both genotypic and phenotypic levels which was expressed via days to flowering, biomass, plant height and crude protein content.

Leaf width

Leaf width had a positive indirect effect up on grain yield at both genotypic and phenotypic levels that was exhibited via days to emergence and maturity, number of pods per plant and pest.

Number of pods per plant

Number of pods per plant had a positive direct effect up on grain yield at both genotypic and phenotypic levels that is exhibited through days to flowering, biomass, plant height, and crude protein content.

Table 5. Direct (bold and underlined diagonal) and indirect (out of diagonal) correlation coefficients of 11 Characters of Dekoko Accessions grown at Mekhan (Endamekhoni).

	DE	DF	DM	LW	PPP	BIO	TSW	PH	HI	PEST	PROT
DE	**-8.801**	5.182	-1.292	0.394	-9.408	5.733	0.09	7.141	-0.644	-1.225	6.098
DF	-8.801	**5.182**	-1.292	0.394	-11.294	6.775	0.120	7.141	-0.530	-1.093	7.160
DM	-8.801	5.182	**-1.292**	0.394	-11.033	6.362	0.133	6.829	-0.249	-1.062	7.278
LW	8.801	-5.182	1.292	**-0.394**	10.229	-5.457	-0.067	-7.141	0.762	1.292	-7.155
PPP	7.290	-5.153	1.255	-0.355	**11.358**	-7.182	-0.232	-3.401	-0.275	-0.181	-8.057
BIO	-7.026	4.888	-1.145	0.299	-11.358	**7.182**	0.232	3.019	0.346	0.422	8.057
TSW	3.403	-2.677	0.7405	-0.114	11.358	-7.182	**-0.232**	-0.075	-0.762	-1.189	-8.057
PH	-8.801	5.182	-1.236	0.394	-5.409	3.036	0.002	**7.141**	-0.762	-1.292	3.391
HI	-7.436	3.601	-0.421	0.394	4.100	-3.263	-0.232	7.141	**-0.762**	-1.292	-5.370
PEST	8.348	-4.384	1.06	-0.394	-1.595	2.345	0.213	-7.141	0.762	**1.292**	1.116
PROT	6.660	-4.605	1.167	-0.35	11.358	-7.182	-0.232	-3.006	-0.508	1.116	**-8.057**

DE=days to emergence, DF=days to flowering, DM=Days to maturity, LW=leaf width, PPP=pods per plant, BIO=above ground biomass, TSW= thousand seed weight, PH= plant height, HI=harvest index, PEST= Insect pest, PROT= percent of Crude protein. Number of pods per plant, biomass, plant height and days to flowering have significant and positive direct effects up on yield while days to emergence, protein content and days to maturity had significant but negative effect up

Biomass

Biomass had positive direct effect up on grain yield at both genotypic and phenotypic levels that is expressed via days to flowering, plant height, and crude protein content.

Thousand seed weight

Thousand seed weight had negative direct effect up on grain yield at both genotypic and phenotypic levels. It was revealed via days to flowering, biomass, pest and crude protein content.

Plant height

Plant height had positive direct effect up on grain yield at both genotypic and phenotypic levels expressed via days to flowering, biomass, and crude protein content. The indirect effect of plant height up on grain yield was exhibited through days to emergence and maturity, number of pods per plant, and pest.

Harvest index

Harvest index had negative direct effect at genotypic level and positive direct effect at phenotypic level. The indirect effect of harvest index was revealed via days to emergence and maturity, biomass, 1000 seed weight, pest and crude protein content.

Pest

Pest score had negative direct effect up on grain yield at both genotypic and phenotypic levels expressed via days to flowering, number of pods per plant, and plant height. The positive and indirect effect of pest up on grain yield was expressed via days to emergence, days to maturity, and crude protein content.

Crude protein content

Crude protein content had negative direct effect up on grain yield at both genotypic and phenotypic levels exhibited via days to emergence, biomass, and plant height.

Conflict of Interest

The authors have not declared any conflict of interest.

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Review

Review of the pest status, economic impact and management of fruit-infesting flies (Diptera: Tephritidae) in Africa

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Fruit flies are a major threat to the horticulture industry in Africa owing to their damage incidence and economic losses to fruit and vegetable crops, and their quarantine implications. Numerous studies with different research interests have been conducted on fruit flies throughout the African continent. Despite these studies, there is little knowledge among stakeholders about fruit fly pests in terms of the economically important species, their pest status, economic impact and control strategies. These parameters are prerequisites in designing management tools for addressing the fruit fly problem in the continent. This paper reviews the status of the fruit fly menace in Africa by reporting some of the findings of previous researchers while laying emphasis on what needs to be done.

Key words: Tephritid fruit flies, pest status, economic losses, management, Africa.

INTRODUCTION

Fruit and vegetable production is one of the fastest growing sectors of the horticulture industry in Africa (Weinberger and Lumpkin, 2007). The sector contributes to poverty alleviation by promoting food security while helping to increase total export earnings for African countries. It has been integral to any thinking of economic growth and development. A strengthened horticultural sector can have a positive impact on the Millennium Development Goals (MDGs) of Sub-Saharan Africa (SSA) (World Bank, 2008). Throughout Africa, several fruit and vegetable crops are grown for both domestic and export markets. The major ones include mango, citrus, pineapple, papaya, avocado, banana, tomatoes, peppers, okra, garden eggs and the cucurbits. More than 900,000 t of fruit and vegetables are exported annually while an unestimated volume is comsumed domestically (Weinberger and Lumpkin, 2007). For example, in 2007, the horticulture industry generated over US$ 16 billion in foreign exchange from exported commodities and over US$ 6,500 million domestically to the continent; directly and indirectly employing over 40 million people (World Bank, 2008). More than 50% of the production volume is affected by fruit fly infestation (USDA-APHIS, 2008). Several constraints however, hinder the sector from realizing its full potentials. Among them include: insufficient investments, inadequate basic and applied

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research, poor extension of existing innovations and methods of dissemination, porous quarantine borders and economic treaties, limited knowledge of the incidence and management of key pests and diseases (Norman, 2003). Currently, the key insect pest constraint for the increased and sustainable production of fruit and vegetable crops in Africa is infestation by fruit flies (Jaeger, 2008). Tephritid fruit flies have been recognized as one of the most economically important group of insects which pose serious threat to the horticultural industry in Sub-Saharan Africa (SSA) (White and Elson-Harris, 1992; Ekesi and Billah, 2006; De Meyer et al., 2012).

Fruit fly infestation has led to heavy losses in yield and quality of fresh fruits, and restrictions to quarantine-sensitive markets throughout Africa. Fruit fly research and management is yet to be fully optimized in most parts of Africa. There is limited knowledge and awareness among stakeholders along the fruit value chain in terms of the species concerned, their economic impact and management strategies (STDF, 2009). It is necessary to collate existing research information which is prerequisite for developing action plans and formulating management decisions for addressing the menace posed by these problem pests in the region. This paper presents a review of the economically important fruit fly species in Africa, the damage and losses they cause, their economic impact on the horticultural sector of SSA and available management options.

PEST SPECIES OF FRUIT FLIES

According to White and Elson-Harris (1992), Sub-Saharan Africa (SSA) is a reservoir of 915 fruit fly species from 148 genera, with over 299 species developing in both wild and cultivated fruits. Most species of fruit fly which attack commercially grown fruit and vegetable crops belong to two genera; *Ceratitis* and *Dacus* (White and Goodger, 2009). A few species belong to other genera such as the coffee fruit flies (*Trirhithrum* species) which are close relatives of *Ceratitis,* or the genus *Bactrocera,* which are close relatives of *Dacus* (White and Elson-Harris, 1992). De Meyer et al. (2012) classified pest species of fruit flies in Africa into indigenous and invasive species, which belong mainly to four genera: *Bactrocera, Ceratitis*, *Dacus,* and *Trirhithrum* (Table 1).

Indigenous fruit fly pest species in SSA

Sub-Saharan Africa is the home of several species of highly damaging fruit flies. For example, on mango, the results of several surveys across SSA show the crop is attacked by native fruit fly species such as *Ceratitis cosyra* (Walker), *C. quinaria* (Bezzi), *C. fasciventris* (Bezzi), *C. rosa* (Karsch), *C. anonae* (Graham) and *C. capitata* (Wiedemann). Traditionally, yield loss on mango due to native fruit flies can range between 30% and 70% depending on the locality, season and variety (Lux et al., 2003). Other important native *Ceratitis* species in the region include *C. rubivora* (Coquillet), *C. puntata* (Wiedemann), *C. discussa* (Munro), *C. ditissima* (Munro) and *C. pedestris* (Bezzi) that attack a variety of important fruits and vegetables. Several native *Dacus* species (e.g. *D. bivittatus* (Bogot) *D. lounsburyii* (Coquillet) *D. ciliatus* (Loew), *D. puntatifrons* (Wiedemman), *D. frontalis* (Becker), *D. vertebratus* (Bezzi) etc) also inflict considerable losses on vegetable crops especially the cucurbits (White and Elson-Harris, 1992; De Meyer et al., 2012; Ekesi and Billah, 2006).

Exotic fruit fly pest species in SSA

Although Africa is known to be the place of several fruit fly introductions and establishments worldwide (the most notorious species being the Mediterranean fruit fly, *C. capitata*), with the intensification of fruit trade, the continent has also become highly vulnerable to introduction of alien fruit fly species. In 1997, *B. zonata* was introduced into Egypt (De Meyer et al., 2012). In 2003, *B. invadens* was detected and described for the first time in Africa as a junior synonym of *B. dorsalis* (Drew et al., 2005). In 2006, the Solanum fruit fly *B. latifrons,* a primary pest of solanaceous crops, was detected in Tanzania (Mwatawala et al., 2009). Although damages caused by *B. latifrons* are currently centered on local solanum species such as *Solanum aethiopicum* and *S. macrocarpon* (Mwatawala et al., 2009); damage to tomato (*Lycopersicon esculentum*) seems to be the largest (De Meyer et al., 2012). The melon fly *B. cucurbitae* has also been in Africa for years without a clear date of introduction (White and Elson-Harris, 1992).

Among all the native and exotic fruit fly species, one specie, *B. invadens,* that is commonly referred to as the African invader fly, is thought to be responsible for causing extensive economic losses to horticultural crops throughout Africa since its first detection in 2003 (Lux et al., 2003). The rapid spread and devastating impact of *B. invadens* in SSA has been a matter of serious concern to the horticulture industry (De Meyer et al., 2012).

DAMAGES CAUSED BY FRUIT FLIES

Because of their polyphagous habit, fruit flies inflict serious damages on a wide range of fruit and vegetable crops. Direct damage begins with female fly puncturing the fruit skin and ovipositing underneath it. Fruit injury results from the ovipositional punctures on the skin which reduces the quality and market value of the fruit. Damage symptoms vary from fruit to fruit. During oviposition, fruit-rottening bacteria from the intestinal flora of the fly

Table 1. Economically important fruit fly species in Sub-Saharan Africa.

Genera	Species	Notes on pest status
Ceratitis	*Ceratitis cosyra* (Walker)	Commonly called the mango/marula fruit fly. Major mango pest across Africa, causing 20-90 crop loss (av. 30%). Present in central, Eastern and West Africa. Primary host plants include mango, marula, guava and custard apple, but attacks variety of other plants. A major quarantine pest.
	Ceratitis capitata (Wiedemann)	Commonly called the Mediterranean fruit fly. Most widespread of all fruit fly species in Africa. Attacks over 300 host plants. Very important quarantine pest, capable of withstanding low temperatures.
	Ceratitis rosa (Karsch)	Commonly called the natal fruit fly. Occurs in Eastern, Central and Southern Africa. Very competitive African species. Known distribution is mainly southern and eastern Africa. Very important pest of mango and papaya species. It should be considered as a potential invasive species in other parts of Africa, outside its current range, and in other parts of the world. A pest of quarantine significance.
	Ceratitis fasciventris (Bezzi)	Formerly regarded as a variety of C. rose. Occurs in Central, East and West Africa. Major pest of mango and guava but also attacks a variety of other host plants. Capable of withstanding low temperatures.
	Ceratitis anonae (Graham)	Distributed across East, Central and West Africa. Attacks over 50 fruit species but principal pest of mango in West Africa. Females are extremely difficult to differentiate from those of C. rosa and C. fasciventris
	Ceratitis rubivora (Coquillet)	Commonly called the blackberry fruit fly. A rare species occurring in Eastern and southern Africa. Principal pest of berries such as rasp berry and black berry.
Dacus	*Dacus bivitattus* (Bigot)	Commonly called the pumpkin fruit fly. Occurs in eastern and West Africa. Mainly pest of cucurbits.
	Dacus ciliatus (Loew)	Commonly called the lesser pumpkin fly. Reported from East, West and Southern Africa. Primary pest of cucurbits recorded from nearly 20 commercial host plants.
	Dacus frontalis (Becker)	Occurs mainly in East and Southern Africa. Pest of cucurbits principally on cucumber, pumpkin and watermelon.
	Dacus vertebratus (Bezzi)	Commonly called the jointed pumpkin fly. Occurs in East, West and Southern Africa. Pest of cucurbits with special preference for watermelon.
	Dacus lounsburyii (Coquillet)	Occurs in East and Southern Africa. Recorded mainly on sweet melons, watermelons and pumpkins.
Trirhithrum	*Trirhithrum coffeae (Bezzi)*	Small black dark species. Occurs mostly in Central and West Africa. Mainly found in coffee growing areas and in members of the Rubiaceae family. Attacks variety of species including arabica coffee

Table 1. Contd.

	Trirhithrum nigerrimum (Bezzi)	Small black dark species. Occurs mostly in Central and West Africa. Mainly found in coffee growing areas and in members of the Rubiaceae family. Attacks variety of species including arabica coffee.
Bactrocera	*Bactrocera cucurbitae* (Coquillet)	Commonly called the melon fly. Reported from East and West Africa since1930s. Primary pest of both cultivated and wild cucurbits.
	Bactrocera latifrons (Hendel)	First detected in Tanzania (2006) and in Kenya (2007). Restricted to Solanaceous plants. Does not respond to methyl eugenol, Only responds to Alpha-oinol+cade oil (Lati-Lure)
	Bactrocera zonata (Saunders)	Commonly called the peach fruit fly. Pest in Egypt, Libya and Indian Ocean Island of Mauritius. It has wide host range and an important pest of mango and citrus. Responds well to methyl eugenol. Constantly monitored across the frontiers of Sudan and other bordering countries.
	Bactrocera invadens (Drew, Tsuruta and White)	Commonly called the African invader fly. Originally detected in Kenya in 2003 and in Ghana in 2006. Now reported from 23 African countries. Over 39 host records in 21 plant families (and rising) but mango is most preferred. Major devastating quarantine pest.

Adapted from Ekesi and Billah (2006); De Meyer et al. (2012).

are introduced into the fruit. These bacteria reproduce and cause the tissues surrounding the egg to rot (Vayssières et al., 2009). When the eggs hatch, the rotten fruit tissues make it easier for the larvae to feed inside the fruit, resulting in a soft, mushy mess. The puncture and feeding galleries made by developing larvae also provide entry points for pathogens to infect, develop and increase the fruit decay. From a quantitative point of view, the damage is caused by larvae at the second and especially third instar stages, by the removal of the significant proportion of the pulp which consequently results in reduction in the yield and quality of the harvestable fruits. Generally, the fruit falls to the ground as, or just before the maggots pupate and emerge as adult to continue the cycle (Ekesi and Billah, 2006).

The invasion of alien species can cause extensive economic and ecological damage, with unpredictable negative effects on native populations. Alien species' impact on environment is believed to be second only to habitat destruction. Invasive species can alter succession patterns, mutualistic relationships, community dynamics, ecosystem functions and resource distributions. Invasive species that cause extinction of native species will ultimately reduce local and global species diversity (Lyon and Miller, 2000).

ECONOMIC LOSSES AND IMPACT OF FRUIT FLIES

Fruit producing communities in Africa have experienced heavy losses due to fruit fly

infestation. This occupies a large proportion of the profitable fruit crop production in the region (Lux et al., 2003). Annual damage to fruit and vegetable crops caused by fruit flies is worth millions of dollars (NRC, 1992). Prior to the invasion of Africa of *B. invadens*, the major fruit fly pests in Africa were the *Ceratitis* species, whose average damage was estimated at about 20-30% on mango and citrus (Lux et al., 2003). In South Africa, *C. rosa* ranked second in importance to *C. capitata* (CDFA, 2007). In many West African countries, *C. cosyra* featured prominently on mango (Lux et al., 2003). In recent years however, *C. cosyra* and other related species has continuously suffered competitive displacement by the invasive *Bactrocera* species (Ekesi and Billah, 2006). For example, the African invader fly, *B. invadens* is believed to be native to Sri Lanka and currently deported from 28 African countries including the Comoros Island and Cape Verde (Drew et al., 2005). It has rapidly displaced several of the indigenous fruit fly species and currently ranked as the most important fruit fly pest in the African continent (Ekesi et al., 2009). Currently, *B. invadens* is found in almost all countries in sub-Saharan Africa. It is thought to be responsible for causing production losses to horticultural crops throughout Africa since its first report in 2003.

The export of potential host species of *B. invadens* such as mango, citrus, avocado and cucurbits from Kenya, Tanzania and Uganda are already banned in Seychelles, Mauritius and South Africa. Trade of several horticultural produce between Africa and the US has been severely hampered by recently issued Federal Order by the US banning importation of several cultivated fruits and vegetables from African countries where *B. invadens* has been reported. In the case of avocado, Kenya lost US$ 1.9 million in 2008 due to *B. invadens* quarantine restriction imposed by South Africa (USDA-APHIS, 2008). The current export volume for Mozambique is estimated at 35,000 t per year with a foreign exchange value of US$ 17.5 million, but South Africa, its major trading partner has closed its markets to fresh fruits from the northern part of the country due to the presence of *B. invadens*. At the Vanduzi Company in the Central province of Manica, about US$ 1.5 million has been lost due to the presence of *B. invadens* and quarantine restrictions on the export of various fresh fruits and vegetables (Cugala et al., 2009). The direct damage caused by *B. invadens* and other tephritid pests seriously threatens the income, food security and livelihood of millions of families that produce and sell fresh fruit and vegetables across Africa.

Bactrocera invadens is an overabundant, highly polyphagous species attacking 40 host fruit and vegetable crops in 22 families. Yield loss due to fruit fly damage may exceed 70% on mango and 40% on citrus (COLEACP-CIRAD, 2009). An assessment of damage of *B. invadens* on mango in Benin showed yield loss averages varying from 10 to 57% between the months of April and June (Vayssières et al., 2009).

In Senegal, fruit growers reported an average yield loss of about 40% all year round (Video Senegal, 2007). Production losses due to *B. invadens* in Ghana have been estimated to over 40% (UDSA-APHIS, 2008). The Phytosanitary Council of the African Union has described *B. invadens* as a devastating quarantine pest (French, 2005). It has a broad temperature range, has been trapped at high altitudes (>1600 m above sea level) and has the capability for invading other regions of the continent (Ekesi et al., 2009). Several countries in Africa continue to suffer significant loss in revenue due to lost export markets associated with the presence of *B. invadens*. A concerted effort is required by the fruit fly research communities to provide better understanding and technologies, build capacity and create awareness on the significance of this economically important pest to improve horticultural production in Africa and beyond.

Indirect losses caused by fruit flies results from quarantine restrictions that are imposed by importing countries to prevent entry and establishment of unwanted fruit fly species (STDF, 2009). The effect of these pests has led to barriers to trade in fresh fruit commodities, costly surveys, control and eradication programmes throughout the world and thus, imposing limits on the export market (Ekesi and Billah, 2006). The introduction of uniform and strict maximum residue levels across Europe exacerbates the problem and further jeopardizes export of fruits and vegetables. Of greater concern, is the fact that even in countries where fruit fly management methods are undertaken, rejection by European markets is increasingly largely because with global trade and passenger travelling, they are easily translocated and the risk of majority of African fruit flies as key and potential quarantine pests is becoming increasingly realized (Ole-Moi Yoi and Lux, 2004). Quarantine regulations imposed by an importing country can either deny a producing country a potential export market, or force the producer to carry out expensive disinfestation treatments against fruit flies (White and Elson-Harris, 1992).

Since 2007, the African continent has experienced several interceptions of fresh mangoes imported to the European Union (EU) (Table 2). Though the updated data is currently unavailable, results should be regarded as a fluctuation rather than a reduction of the fruit fly problem. The rapid spread and devastating impact of *B. invadens* in SSA has been a matter of serious concern to the horticulture industry. Trade of several fruit and vegetable crops between Africa and the US has been severely hampered due to the Federal Order by US banning importation of several fruits from African countries where *B. invadens* has been reported (USDA-APHIS, 2008). These restrictions seriously threaten the income, food security and livelihood of millions of families that produce and sell fresh fruits and vegetables across Africa. With increasing emphasis on quality of fruit and vegetable produce, and the possibility of expansion of

Table 2. EU interceptions of infested mangoes from Africa.

Importing Country	2007		2008		2009		2010		2011		2012	
	No. Interceptions	Entry point	No. Interceptions	Entry point	No. interceptions	Entry point	No. interceptions	Entry point	No. interceptions	Entry point	No. interceptions	Entry point
Burkina Faso	3	FRA	4	FRA	5	FRA	9		5	FRA	8	FRA
Cote d'Ivoire					2	FRA	1	FRA				
Gambia			1	GBR							1	GBR
Ghana	1	GER	2	NLD	2	NDL(1) GBR(1)	1	NDL	1	NDL		
Guinea			1	FRA								
Mali	14	FRA	5	FRA(3) NDL(1)	13	FRA	4	FRA	3	NDL	7	FRA(2) NDL(2)
Senegal	15	FRA	2	FRA(1) NDL(1)	4	FRA(2) GBR(2)	1	NDL			1	FRA
Cameroon	17	FRA	5	FRA	9	FRA	2	FRA	2	FRA	4	FRA
Cent. Afric. Rep.	1	FRA							2	FRA		
Kenya	2	FRA	3	FRA(1) GBR(2)	1	FRA	1	GBR	4	FRA	1	FRA
Egypt	1	FRA			1	FRA			2	FRA		
TOTAL	54		26		37		22		19		22	

Adapted from COLEACP-CIRAD (2009); EUNSPH (2012).

trade in horticultural commodities, importing and exporting countries are giving increasing attention to fruit fly management at pre-harvest and post-harvest levels (Drew et al., 2005).

MANAGEMENT STRATEGIES FOR FRUIT FLIES

Fruit fly management generally involves two basic approaches: the eradication approach and the IPM approach (Lux et al., 2003; Ekesi and Billah, 2006).

The eradication approach

This approach usually involves an area-wide action to eliminate the target fruit fly population in order to create a fruit fly-free area/zone. Such a pest-free zone may however, be liable to future re-infestation (Myers et al., 1998). Eradication is costly and is justified only when a highly productive industry is threatened, or when the pest has just arrived in the area (Wilson, 2006). It has been observed that eradication of a pest from an agricultural region is theoretically challenging and depending on the method used, can be socially and environmentally unacceptable. For instance, eradication in an urban area using aerial and ground application of pesticides can evoke public opposition.

The major means by which eradication can be achieved is through a "birth control" method based on genetic manipulation, known as the Sterile Insect Technique (SIT) (IAEA, 2003). This control method makes use of artificially sterilized populations of the male fruit fly pest to mate with fertile female in the wild, and thereby interfere with the normal reproductive efforts of the target species (Van der Vloedt and Klassen, 2006). Irradiation is presently the most practical way to sterilize insects. Reproductive sterility is induced by exposure of the flies to X-rays, electron beams, and most commonly gamma rays from a Cobalt-60 or Caesium-137 source (Robinson, 2005). It is among the most nondestructive pest control methods and unlike biologically-based methods, it is species-specific and does not release toxic agents into the environment (Hendrich et al., 2002). The method is effective especially if the sexually mature males are aggressive and

effectively compete with wild males in searching for and mating with wild females. It also has the advantage of being compatible with other control methods and has increased efficiency with decreased target population density (IAEA, 2003).

Although SIT for the African continent are currently unavailable, many attempts have been made worldwide in controlling these pests using SIT. For instance, SIT was successfully applied against the Mediteranean fruit fly, *C*. *capitata* from areas it had already infested in Southern Mexico (Hendrich and Hendrichs, 1998). This fused initially on the concept of eradication, following the successful example of the screwworm which was eradicated from the United States, Mexico and Panama (Wyss, 2000). Since then, a sterile fly barrier had been maintained in that region. The successful application of SIT in Chile to eradicate *C*. *capitata* in 1995 opened trade opportunities estimated over five years at a benefit to Chilean fruit industry of $500 million (SAG, 1996). Also, SIT was successfully used to eradicate *B*. *dorsalis* (Hendel) from Okinawa and neighbouring islands in the Ryukyu Archipelago, Japan (FFEPO, 1987). A more recent study conducted by Ogaugwu (2007) indicated the possibility to produce sterile, viable and competitive males of *B*. *invadens* in Ghana and Africa as a whole.

The IPM approach

Previous experience with exotic and native fruit fly species in Africa has shown that management of fruit fly pests in general is unlikely to be successful if based on a single management technique (Allwood and Drew, 1997; Lux et al., 2003). An IPM strategy offers the best method to improve the economics of the production system by reducing yield losses and enabling growers to comply with stringent quality standard of the export market (Allwood and Drew, 1997). The approach that is being promoted across Africa by the ICIPE-led African fruit fly program (AFFP) is to use a combination of management techniques that is based on at least two or more of the available tactics as discussed below:

Monitoring with attractants

Information on the seasonal population fluctuation and peak period of pest activity is an important component of any pest management strategy because a warning of the timing and extent of pest outbreak can improve efficiency of control measures (Ekesi and Billah, 2006). The estimate of pest abundance or a change in numbers provides an essential measure by which control decisions can be made. According to Manrakhan (2006), fruit fly monitoring helps to:

(i) Determine fruit fly pests in an area,
(ii) Determine distribution of pest species,
(iii) Determine local hot spots with high populations of the pest,
(iv) Track changes in population levels,
(v) Determine the efficacy of control measures, and
(vi) Facilitate early detection of new fruit fly pests in a particular area.

Tools used in monitoring fruit flies consist of attractants, traps and insecticides (used in traps as killing agents). The two main types of attractants used in fruit fly monitoring include parapheromones (male lures) and food baits (Lux et al., 2003).

Use of parapheromones

Parapheromones are lures that attract only male fruit flies. They are highly species-specific and are known to have a high efficacy in attracting fruit flies from long distances. The use of parapheromones in fruit fly control is a technique commonly referred to as the male anihilation technique (MAT). MAT aims at reducing male fruit fly populations to low levels such that mating does not occur or are reduced to low levels. Parapheromones are available in both liquid form and polymeric plugs (in the form of a controlled-release formulation). The major types of attractants include; Methyl eugenol (ME) (benzene, 1,2-dmethoxy-4-2-propenyl); Cuelure (CUE) (4-(p-hydroxyphenyl-2-butanone acetate); Trimedlure (TML) (tert-butyl-4-5-chloro-2-methylcyclohexane-1-carboxylate); Terpinyl acetate (TA) (alpha, alpha,4-trimethyl-3-cyclohexene-1-methanol); and Vertlure (VL) (methyl-4-hydroxybenzoate). ME and CUE attract several species of *Bactrocera*, TML and TA attract several species of *Ceratitis*, while VL attract some species of *Dacus* (IAEA, 2003; Manrakhan, 2006). These attractants are currently being used in fruit fly management in many countries in Africa (Ekesi and Billah, 2006; COLEACP-CIRAD, 2009). The traps and trapping procedures for monitoring fruit flies are dependent on the attractant and the nature of the area (IAEA, 2003).

Use of Foodbaits

Fruit fly suppression is mainly based on the use of food baits (hydrolyzed proteins or their ammonium mimics) mixed with a killing agent. These are lures that attract both male and female fruit flies. They are not species-specific and are known to have a low efficiency compared to male lures (White and Elson-Harris, 1992). The use of food baits for fruit fly control is a technique commonly referred to as the Bait Application Technique (BAT). They are available in both liquid and dry synthetic forms. Available food baits include liquid proten hydrolysates, yeast products, ammonium salts, and the three-

component lure (consisting of putrescine, ammonium acetate and trimethylamine) (Lux et al., 2003; IAEA, 2003; Ekesi and Billah, 2006). A number of commercial baits are now available in the market, such as GF-120 (Success® Apart), Nulure, Buminal and SolBait that are premixed with insecticides like spinosad for direct application (Ekesi et al., 2009; Vayssières et al., 2009). The bait system is an integral component of IPM in horticultural crops because it reduces pesticide usage with minimum effect on predators, parasitiods and pollinators. Protein bait application is less time consuming and less demanding of labour. However, a major problem in the use of baits in Africa is that they are quite expensive and inaccessible to a large number of fruit and vegetable growers.

Soil inoculation

An important component of fruit fly control is soil treatment with fungal pathogens to kill the mature maggots and puparia. This is a new method of fruit fly control, targeting the immature stages of the fruit flies (maggots and puparia). The active ingredient is the fungus *Metarhizium anisoplie*, a naturally occurring fungus isolated from the soil that is being used worldwide as a biological pesticide for controlling different kinds of insect pests. The fungus is formulated as granules and can be dispersed by hand and then raked into the soil where it can persist for over a year (Ouna, 2010). The soil can also be inoculated with neem cake and other botanical formulations to kill pupating larvae (Ekesi and Billah, 2006).

In recent studies, several potent isolates have been identified against *B. invadens* both for soil inoculation targeting pupariating larvae and adult using auto dissemination devices (Ekesi et al., 2009; Ouna, 2010). The ultimate goal is to reduce oviposition by gravid female fruit flies, and the overall effect of fungal infection on adult fecundity and fertility has been shown to be very high. While soil inoculation with *M. anisopliae* cannot be considered as a stand-alone strategy, the fungus has a significant role to play when combined with other IPM component for *B. invadens* suppression. This technique of fruit fly control is expected to be environmentally friendly and easily adoptable by farmers, and can be used as a supportive measure to the bait sprays. Further research on formulation of the fungus is underway and the product would be available for use in the near future.

Post-harvest fruit treatment

Without post-harvest treatment to provide quarantine security, exports of fruit and vegetable crops to lucrative markets abroad is limited due to quarantine restrictions. Therefore, effective post-harvest quarantine treatments that are not harmful to either the product or people coming in contact with or consuming the fruits, must be applied to the export commodities. The available quarantine treatment technologies (as alternatives to toxic fumigation) include: i) heat treatment to increase temperature of host fruits above thermal limits of the fruit fly, ii) cold treatment to decrease temperature of host fruits below the thermal limits of the fruit fly, and iii) irradiation with gamma rays from a Cobalt-60 or Caesium-137 source to kill the developing flies (Robinson, 2005). Currently, there is no known published research on the use of post-harvest fruit treatment methods against tephritid pests in Africa, but once developed, these treatments should increase the export potential of tropical fruit and vegetable crops in the region (Ekesi and Billah, 2006).

CULTURAL CONTROL

Poorly managed or abandoned fruit crop farms and a variety of wild hosts can result in high population build up of fruit flies. Cultural control method relies on farm sanitation and crop hygiene targeted at breaking the reproductive cycle of the pests. It is based on an understanding of the developmental biology of the flies, which ensures that larvae in the dropped fruits do not mature in the soil. It entails the collection and destruction of all infested fruits found on the trees and all falling fruits containing fruit fly maggots and puparia. Fruit destruction is achieved by crushing the infested fruit in a grinding machine or burying them deep (at least >50 cm) under the soil surface with addition of sufficient time to kill the developing larvae. This can contribute significantly to reduction in fruit fly populations on the farm. Rwomushana (2008) was able to demonstrate that the density of *B. invadens* was significantly higher in fallen mango on the ground compared with that sampled from the tree signifying the important role of orchard sanitation in the management of the insects. The collection and deposition of fallen, damaged and unwanted fruits in an augmentorium is being strongly advocated among fruit and vegetable growers across Africa.

Cultural control for fruit flies is a laborious exercise but can be quite effective if the fruits are regularly collected and destroyed throughout the season. Collection and destruction of infested fruits is strongly recommended to reduce resident populations of fruit flies in orchards. One effective means of achieving cultural control against fruit flies is collection of infested fruits, tying them in black plastic bags and exposing them to the heat of the sun for a few days untill the fruits are rotten and all the maggots in the bags are dead. The control of *B. zonata* using killing bags has been successfully reported in Egypt (Mohamed and El-Wakkad, 2003). To eliminate or reduce resident population reservoirs, crop sanitation has been an essential component of fruit fly management

programmes in the Integrated Tamale Fruitts Company in Ghana. The adoption of sound crop sanitation practices has helped to release pressure on other components of control systems, particularly protein bait sprays whose effectiveness are threatened under high fruit fly population pressure. Under quality assurance schemes being adopted for production of export commodities, sound crop sanitation has become a prerequisite for any farm that is global-gap certified for export production.

Mechanical fruit protection

Notwithstanding the presence of fruit flies in the farm, wrapping or bagging of individual fruits with newspaper or paper bags to prevent adult female flies from laying eggs on the fruits is also a practice of producing fruits that are free from fruit fly infestation. To be effective, the fruits must be wrapped or bagged well before fruit fly attack that is, at least, one month before harvest. Although laborious, it is an effective method for high value fruit produced for export or fruits produced in backyard gardens for family use. At present, there is no known published research on the use of mechanical fruit protection methods against tephritid pests in the continent.

Prompt harvesting of fruits

Avoidance of fruit fly infestation is possible by harvesting fruits at the stage of maturity when fruits or vegetables are not very vulnerable to fruit flies. Fruit flies do not attack certain fruits such as papaya, banana and sapodilla when they are 100% green. Only the ripe fruits are susceptible. Bananas, for example, have been exported around the world because they are not susceptible to fruit flies at the mature green stage. An unsuccessful example of using early harvesting to control fruit fly is on mango where species like *B. invadens* and *C. cosyra* are still capable of infesting even immature or mature green mangoes (Ekesi and Billah, 2006). However, early harvesting to evade fruit fly infestation is an important technique in the production of these fruits.

Biological control

Biological control is the use of parasitoids, predators or pathogens to control pest populations. Fruit fly parasitoids are insects that develop by laying their eggs in fruit fly eggs or larvae. The host is killed when the parasitoid larval development is completed. Parasitoid wasps are introduced into fruit farms for fruit fly control. They are fairly specific to certain fruit fly species or genera. Natural enemies must be conserved so that they can contribute to the control of all stage of the fruit flies (Stibick, 2004). A major hazard to natural enemies is the blanket spray of pesticides. Limited application of blanket sprays and use of localized spot treatment will assist in the conservation of important natural enemies for use in biological control programs. Biological control practices are advantageous in that the natural enemies are programmed to search for the target pests. It is also relatively safe, permanent and economical (Ekesi and Billah, 2006).

Among the parasitoid species the Opines, which are koinobionts, are the most abundant parasitoid group most frequently used in IPM and biocontrol programmes. They parasitize the young larvae that are developing under the skin of fruits. *Terastichus* spp (Eulophids) are larvae-pupal parasitoids and they can complement the activity of the Opines (Stibick, 2004). *Fopius* (*Opius*) *longicaudatus* var. *maliaensis* Fullaway, *Fopius* (*Opius*) *vandendoschi* Fullaway, and *Fopius* (*Opius*) *ariasanus* Fullaway, have become established in Hawaii and are primarily effective against the oriental and the Mediterranean fruit flies in cultivated crops. Releases that have taken place in Hawaii and noted that all the benefits are almost entirely due to *F. arisanus* (*Opius oophilus*) have been given extensive review. He observed a decreased infestation of about 80% in guava as a result of reduction in *B. dorsalis* populations through the effects of parasitism. Use of parasitic hymenoptera to control tephritid fly populations can be dated back to the 20th Century when natural enemies were sought in Africa to control *C. capitata* in Hawaii (Wharton 1989). Following successful establishment of some species in the Island, *Diachasmimorpha longicaudata* has been introduced and established in many parts of the world. Extensive work has been conducted to the development of mass rearing systems for some species (Stibick, 2004), and incorporation of augmentative releases in conjunction with the sterile insect technique to eradicate Medfly, *Ceratitis capitata*; Melon fly, *Bactrocera cucurbitae*; Mexican fruit fly, *Anastrepha ludens*; West Indian fruit fly, *A, oblique* and Caribbean fruit fly, *A . suspensa.* A recent study conducted in Benin on the use of predator ants to manage fruit flies revealed that *Oecophylla longinoda* significantly reduced the number of fruits damaged by deterring fruit flies. Although predation on adults of fruit flies took place, deterrence and disturbance by ants during fruit fly oviposition seemed to be the most important causes of reducing fruit fly damage. Also, birds and rodents have been reported to cause a high level of larval mortality by consuming infested fruit (Drew et al., 2005). Similar groups of predators are likely to play a role in restricting fruit fly populations throughout Africa, and their conservation may be of practical importance. However, a thorough assessment of their impact on fruit fly population in various regions in Africa, and in various production systems needs to be validated.

Other biological control agents that have been used against Tephritids include Nematodes, protozoan

bacteria and fungi. *Anastrepha* larvae are susceptible to the entomopathogenic nematode, *Neoaplectana* spp (Rhabditida, Steinematidea) and *Heterorhabditis* spp. Pathogens like protozoa, *Bacillus thuringensis* (Bt) and fungi have also been used. Fuji and Tamashiro (1972) made the first observations of pathogens attacking fruit flies in Hawaii. They reported infection of *B. dorsalis* and *C. capitata* by the protozoa *Nosema tephritidae*. Although successes have been generally limited, the use of natural enemies (pathogens, parasitoids and predators) for the suppression of fruit flies has always had a wide appeal because it is relatively safe, permanent and economical. Several species of parasitoids and predators abound in fruit and vegetable agro ecosystems, which can contribute to the suppression of fruit flies. Efforts to conserve these natural enemies through efficient management based on the fruit fly IPM components described above may contribute to the overall suppression of fruit flies. The search for and research on biological control of fruit flies, especially the invasive species, in Africa should also remain an integral part of the fruit fly suppression effort.

Chemical control

Tephritid fruit flies have been controlled since the beginning of the 20th Century by combining baits with different insecticides. Hydrolyzed proteins and partially hydrolyzed yeast at a 4:1 ratio with organophosphates (malathion) have been applied aerially at ultra-low volume in a number of eradication efforts around the world. Bait spray is generally applied on bands or spots reducing thus the area of coverage by exploiting the attractive properties of the bait and adult fly mobility. Current research focuses on replacing Malathion with more specific and environmentally friendly products such as Spinosad, or Phototoxic dyes. Detailed review on this subject has been given by Moreno and Mangan (2000). For flies in the genus *Bactrocera,* male annihilation programs have been successfully implemented in several parts of the world (Ekesi and Billah, 2006; Mwatawala et al., 2009; COLEACP-CIRAD, 2008). This technique consists of spreading wooden blocks impregnated with Methyl Eugenol and Malathion that massively attract male *Bactrocera* when feeding on the bait. The attractive effect of Methyl Eugenol is so potent that males can be annihilated, severely affecting the reproductive capacity of the pest population. Releases of sterile insects can follow male suppression to guarantee eradication.

CONCLUSION

Despite extensive research, fruit flies still remain a major threat to fruit and vegetable production in Africa. The damage and economic impact of fruit flies should be of great concern to all stakeholders along the fruit value chain. Smallholder farmers in the African continent could be suffering from higher losses due to fruit fly infestation. The export potential of fresh fruits and vegetables from Africa could also be more threatened by these quarantine pests. A concerted effort is required by the fruit fly research communities to provide technologies, build capacity and create awareness on the importance of these pests for improving the horticulture industry in Africa. More research information is needed to document the species inventory, host plant diversity economic status and population dynamics of fruit flies in all ecological zones of Africa. There is the need to establish national committees in all member countries to help increase stakeholder awareness, and to develop and disseminate more adaptive and sustainable management strategies for addressing the fruit fly menace in Africa.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Chemical properties of a soil cultivated with color cotton irrigated with wastewater in the brazilian semi-arid

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Research found the effect of treated wastewater on chemical properties of the soil cultivated with cotton. The soil fertility was studied by using a randomized blocks experimental design in factorial scheme (4 x 2) with three replications, whose factors were four treated wastewater depths (278, 416, 554 and 692 mm) and two soil sampling times (before and after cultivation of colored cotton, with a rainfall of 89 mm during the crop cycle); while for the soil salinity, it was also used the experimental design in randomized blocks, but in factorial scheme (4 x 3) with three repetitions, being the factors the same four treated wastewater depths used in the fertility study, and three times of soil sampling (before and after the irrigation and after the rainfall period). After irrigation with effluent of stabilization pond and a rainfall of 89 mm, the contents of P, K^+, Ca^{2+}, H^+ and the capacity of cationic change of the soil increased, while the contents of Na^+, Mg^{2+} and the sodium exchangeable percentage decreased. However, the electrical conductivity, the sodium adsorption ratio and the contents of calcium, magnesium, sodium, chlorides of the soil saturation extract decreased after five months of rains, which totalized 646 mm.

Key words: Reuse, irrigation, soil.

INTRODUCTION

The urban domestic wastewater reuse aiming fertirrigation industrial crops as cotton is considered an alternative use and final disposition of this appeal and, at the same time, a way to increase the productivity of crops in fertirrigation areas, given the presence of nutrients as Ca^{2+}, Mg^{2+}, N, P, K^+, and S, and micronutrients in their

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chemical composition. However, it is unknown that the antagonistic effects of its implementation on the chemical characteristics of the soil, as well as the chemical characteristics that the soil will pass with the effects of seasonality.

Mapanda et al. (2005), study the effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables, found that the magnitude of contamination, regulatory compliance and annual loadings of soils with copper (Cu), zinc (Zn), cadmium (Cd), nickel (Ni), chromium (Cr) and lead (Pb) where wastewater was used to irrigate vegetable gardens for at least 10 years. According to these authors, the annual heavy metal loading rates showed that within 5 to 60 years, all studied heavy metals would have exceeded their permitted limits in soils, depending on site. It was concluded that the use of wastewater in urban horticulture enriched soils with heavy metals to concentrations that may pose potential environmental and health risks in the long-term. Soils, as filters of toxic chemicals, may adsorb and retain heavy metals from wastewater. But when the capacity of soils to retain toxic metals is reduced due to continuous loading of pollutants or changes in pH, soils can release heavy metals into groundwater or soil solution available for plant uptake (Mapanda et al., 2005). Kimberly and William (1999), report that the amount of heavy metals mobilized in a soil environment is a function of pH, clay content, organic matter content, cation exchange capacity and other soil properties making each soil unique in terms of pollution management.

While Smith (1996) reports that with the exception of Mo, Se and As, heavy metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes. The idea of reuse has been developing to turn an integral factor of preservation and rational use of water resources, besides being an important practice for semi-arid areas as the one of the Brazilian northeast, representing a form of water supply for irrigation and sources of minerals nutrients and organic composed (Arthur, 1983).

According to Jnad et al. (2001), the significant increase of the amount of Na^+ and P in the soil was the main alterations in the chemical characteristics of the soil, resulting from application of wastewater of domestic origin, through underground drip irrigation system, in areas cultivated with gram. However, significant increases were not observed in the amounts of total-N, Mg^{2+}, K^+, and electrical conductivity of the extract of soil saturated paste. In the work of Speir et al. (1999), although the content of Na^+ has increased by application of secondary effluents of treated sewer, the inverse happened when the irrigation ceased, due to the effect of rainfalls on lixiviation of that cation. In the work of Day et al. (1979), the irrigation with effluent of treated sewer did not alter the pH of the soil. However, in this situation, the soil was from a semi-arid area naturally alkaline. However, in work of Sou/Dakouré et al. (2013), found that plots irrigated with wastewater showed important structural damages, especially in the subsurface horizon where the soil pore network collapsed dramatically, resulting in a compact impermeable layer. Fluorescence spectra revealed that the organic matter contained in the wastewater was largely dissolved due to a sharp soil pH increase, resulting in black alkali formation at the surface; the soil became sodic, with an exchange complex dominated by sodium, whereas plots irrigated with fresh water kept properties comparable to that of non irrigated plots. Such a rapid soil sodication was seldom reported so far.

The authors emphasizes the need to carefully examine irrigation water quality and particularly calcite residual alkalinity and suggests that shrinkage analysis could be used to monitor the physical changes of soil properties upon sodication. Inadequate wastewater quality is likely to cause deep and irreversible damages to irrigated soils.

Christou et al. (2014), study the assessment of long-term wastewater irrigation impacts on the soil geochemical properties and the bioaccumulation of heavy metals to the agricultural products, found that the heavy metal content quantified in the forage plants' above-ground parts was below the critical levels of phytotoxicity and the maximum acceptable concentration in dairy feed, whereas heavy metals quantified in orange fruit pulp were below the maximum permissible levels (MPLs), and that heavy metal phyto availability was confined due to soil properties (high pH and clay content), as evidenced by the calculated low transfer factor (TF).

On the other hand, Vazquez et al. (1996) verified decrease of the pH in soil cultivated with corn and irrigated with effluent of treated sewer. The authors suggested that the fall in the pH of the soil was due to nitrification, once that effect was increased by addition of nitrogen mineral fertilizer. According to Pizarro (1990), the soluble salts contained in the irrigation waters can, in certain climatic conditions, generate salt problems in the soil and to modify the ionic composition in the sorption compound, altering the physical and chemical characteristics of the soil, as the humidity regime, aeration, nutritious, vegetative development and productivity.

Normally the rate of potential nitrification presents temporal variation, or better, a close relationship between the concentration of NO_3^- and rainfall. Thus, during the dry period, there is low rate of mineralization, however, with the onset of rains, the increased rate of mineralization of organic matter provides greater release of NH_4^+, substrate of nitrification and, consequently, increased in concentration of NO_3^- in soil (Wheatley et al., 2001). The objective of this study was to verify the effect of irrigation with different treated wastewater depths on some chemical attributes of the soil at the end of irrigation and rainfall seasons.

Table 1. Variance analyses of chemical attributes of the soil for different wastewater depths (W) and soil sampling time (T).

Sources of variation	DF	Mean squares							
		P	K^+	Na^+	Ca^{2+}	Mg^{2+}	H^+	CCC	PES
W	3	10.61**	2007.3**	1.873*	43.62^{ns}	13.70^{ns}	0.527^{ns}	10.62^{ns}	2.544^{ns}
T	1	422.5**	21716.5**	176.3**	50.86^{ns}	90.28*	264.2**	21.31^{ns}	290.9**
W x T	3	1.610^{ns}	426.4^{ns}	4.122**	3.26^{ns}	3.438^{ns}	3.098^{ns}	13.43^{ns}	5.598*
Blocks	2	45.76**	6944.1**	5.114**	11.78^{ns}	5.687^{ns}	97.53**	130.60^{ns}	4.144*
Residue	14	1.748	310.2	0.375	35.23	10.55	1.529	52.78	1.053
Total	23								
C.V (%)		14.70	14.66	11.58	13.86	23.81	8.25	9.11	15.35

*, **, ns - Significant, respectively, for 5% and 1% and no significant by the Test F. DF – degrees of freedom.

MATERIALS AND METHODS

This research was developed under field conditions, in the Station of Sewer Treatment of the Company of Water and Sewer of Paraíba state, Campina Grande city, Brazil, in an area irrigated with wastewater cultivated with colored cotton. The soil of the area was a sandy-clay-loam with contents of sand, silty and clay of 62.9, 16.11 and 20.98%, respectively, and content of organic matter of 7.7 g kg^{-1} and percentage of exchangeable sodium of 4.28%. The humidity at field capacity and at wilting point was, respectively, 124.7 and 45.3 g kg^{-1}. For the fertility analysis it was used the experimental design randomized blocks in factorial scheme (4 x 2), with three replications, whose factors were four irrigation water depths (692, 554, 416 and 278 mm) and two times of soil collection (before and after cultivation of cotton, with a rainfall of 89 mm during the crop cycle), while for salinity, it was used also an experimental design in randomized blocks, but in factorial scheme (4 x 3), with three replications, whose factors were four irrigation water depths (692, 554, 416 and 278 mm) and three times of soil collection (before irrigation; after irrigation with a rainfall of 89 mm during the crop cycle; and after the rainy season of 646 mm). The experimental plot consisted of an area of 20 m^2, and the arrangement of plants was in simple rows with spacing of 0.20 m between plants and 1 m between rows.

The water from the stabilization pond was stored in 2 PVC reservoirs of 5000 L, and a pumping was used for delivering the water up to the drip irrigation system with lateral lines of polyethylene and self compensating emitters spaced 50 cm with discharge of 4 L h^{-1}. The water from these two reservoirs passed with a discharge of 10 m^3 h^{-1} by a sand filter, a disk filter of 130 micron, and a screen filter of 130 micron.

The effluent of treated domestic sewer had the following characteristics: electrical conductivity of 1.40 dS m^{-1}, sodium 109.79 mg L^{-1}, maniacal nitrogen 60.5 mg L^{-1}, nitrate 3.3 mg L^{-1}, potassium 23.01 mg L^{-1}, calcium 25 mg L^{-1}, magnesium 23.4 mg L^{-1}, bicarbonate 195.81 mg L^{-1}, chloride 199 mg L^{-1}, phosphorus 4.6 mg L^{-1}, and soluble orthophosphate 3.2 mg L^{-1}. The soil collections were accomplished before and after the cultivation of cotton. After the soil has been dried and drizzled, samples were taken for determining the values of P, K^+, Na^+, Ca^{2+}, Mg^{2+}, H^+, pH, capacity of cationic change (CCC) and percentage of exchangeable sodium (PES), according to methodology recommended by Embrapa (1997). The soil analyses were accomplished in the Laboratory of Chemistry and Fertility of the Soil, belonging to Paraíba Federal University.

RESULTS AND DISCUSSION

The variance analysis for chemical attributes of the soil revealed significant effect of the tested wastewater depths on values of P, K^+ and Na^+; thus, not exercising effect on values of Ca^{2+}, Mg^{2+}, H^+, CCC and PES (Table 1). For the times of soil collection the effect was significant only for values of P, K^+, Na^+ and PES. There was only interaction among the wastewater depths and the soil collection times for Na^+ and PES. Great variation coefficients were obtained for all the appraised variables.

According to Table 2 the mean values of phosphorus, potassium, sodium, calcium, magnesium, hydrogen, CCC and PES of the soil for different wastewater depths (W) and soil sampling time (before and after cultivation – T). It is verified that for the factor soil sampling time the values of P, K^+, Ca^{2+}, H^+ and CCC increased after the irrigation with wastewater. There was decrease of the values of Na^+, Mg^{2+} and of PES after the irrigation and 89 mm of rainfall. The pH before the cultivation varied from 7.03 to 7.19 and after from 6.23 to 6.43. There was elevation of phosphorus contents after the cultivation, certainly due to the contribution through irrigation water; there were not great differences among the water depths, being the content of exchangeable phosphorus in the soil of 11.42 mg dm^{-3}. The increase of the contents of calcium caused larger fixation of phosphorus to clay particles.

Usually, the pH of the irrigation water has not been affecting the pH of the soil significantly, because of its power lid; thus, it is not expecting direct effect of the effluent in the pH of the soil, even with the widespread occurrence of HCO_3^- (one of the present forms of alkalinity) in the wastewaters. However, there is the possibility of that alkalinity associated to high concentrations of Na^+ and CO_3^{2-}, in alkaline waters, to cause increase of the value of pH of the soil (Bouwer and Idelovitch, 1987). In soils treated with biodegradable residues (as the sewer effluent), through the degradation of these materials by the microorganisms, it can have decrease in the value of pH of the soil due to the production of CO_2 and organic acids (Bouwer and Chaney, 1974).

Duarte et al. (2008) evaluating the effects of disposal of treated domestic sewage on some chemical characteristics of the soil, found that the used effluent showed physical and chemical quality suitable for irrigation and the use

Table 2. Mean values of phosphorus, potassium, sodium, calcium, magnesium, hydrogen, capacity of cationic change (CCC) and percentage of exchangeable sodium (PES) of the soil for different wastewater depths (W) and soil sampling time (before and after cultivation – T).

Factors	Mean values							
	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	H^+	CCC	PES
	W (mm)	----- mg dm^{-3}-----		----------$mmol_c$ dm^{-3}----------			%	
781	8.60	112.06	4.98	44.86	12.52	15.11	80.36	6.28
643	7.37	105.89	5.44	38.95	15.83	15.23	78.17	6.94
505	10.50	146.87	6.01	44.45	12.76	14.55	81.22	7.47
367	9.50	115.60	4.73	43.05	13.46	15.04	79.20	6.04
LSD	2.21	29.56	1.02	9.96	5.45	2.07	12.19	1.72
				T				
Before	4.79 [b]	90.02 [b]	8.00 [a]	41.37 [a]	15.58 [a]	11.67 [b]	78.79 [a]	10.16 [a]
After	13.18 [a]	150.1 [a]	2.58 [b]	44.28 [a]	11.70 [b]	18.30 [a]	80.68 [a]	3.20 [b]
LSD	1.15	15.42	0.53	5.19	2.84	1.08	6.36	0.89

Mean values followed by the same letter in the column don't differ amongst themselves, by the test of Tukey to 5% of probability. LSD – least significant difference.

of wastewater favored the rapid mineralization of organic matter as a result of the concentration of carbon and nitrogen existing in these waters. The use of wastewater not caused significant changes at pH, nor in the levels of phosphorus and potassium of the soil.

According to Figure 1, the contents of phosphorus and exchangeable potassium in the soil increased after the cultivation, being attributed that increase to the contribution of these elements through irrigation water (Figure 1a and b). The largest and smallest phosphorus and potassium contents were found, respectively, for treatments with water depths of 505 and 781 mm. The calcium had a light increment after the cultivation, staying in the soil in large contents, in spite of being an element of easy lixiviation (Figure 1c); among the water depths the calcium did not vary so much, which mean value was 44.33 mmolc dm^{-3}. However, the magnesium decreased after the cultivation certainly due to rains that easily drag certain amounts of nutrients (Figure 1d). Among the water depths there were not great variations, being its mean value of 11.04 mmolc dm^{-3}.

Fonseca (2001) found that the disposal of treated effluent in fertilized soil not exercised any influence on the content of phosphorus, but there was decrease of magnesium, independent of the irrigation water used (potable or treated effluent). Kouraa et al. (2002) watered potato and lettuce with raw sewage, treated wastewater and drinking water and found that in a year of cultivation there's been no change in phosphorus levels in the cultivated soil. The authors reported that to occur changes in soil chemical characteristics are required several years of irrigation, whereas the dynamics of this occurs very slowly; on the other hand, Al-Nakshabandi et al. (1997) contradict the authors mentioned above, because in just five months cultivating Eggplant irrigated with treated effluent containing 28 mg L^{-1} of PO_4^-, there have been significant increase in soil phosphorus levels.

Increases in levels of exchangeable potassium in the soil had been evidenced by Adekalu and Okunade (2002); however, agreeing with the existing conditions in this study, Kouraa et al. (2002), using raw sewage, treated effluent and drinking water for irrigation of potato and lettuce, not found significant differences in the levels of potassium in soil receiving these three types of water. The sodium decreased after the cultivation, certainly once again due to the precipitation of 89 mm happened during the experiment, and also by using a drip irrigation system, which forms a humid bulb in the soil profile, and so the salts contained in the soil or in the irrigation water tend to address for the periphery of the bulb, reducing a lot the contents of salts inside of the humid bulb; there were not great variations among the applied water depths, being the mean value of 2.45 mmolc dm^{-3} (Figure 2a).

The contents of hydrogen increased after the cultivation, due to microbial activity, which liberates a lot of hydrogen, and also to precipitation that elevates the contents of hydrogen in the soil; however, it is verified that among potassium, calcium and magnesium, the element that was more expressively reduced with the entrance of hydrogen was magnesium (Figure 2b). The capacity of cationic change (CCC) of the soil was already very high before the beginning of irrigation, with a minimum value of 76.49 mmolc dm^{-3}; generally, the irrigation with wastewater, which gave a great contribution of nutrients, increased CCC; in relation to water depths, the increase of CCC was larger for 367 mm (Figure 2c). The calculations of the percentage of exchangeable sodium based on CCC decreased after the cultivation, being the reductions larger in water depths of 505 and 643 mm (Figure 2d).

In Table 3 the variance analysis for soil chemical

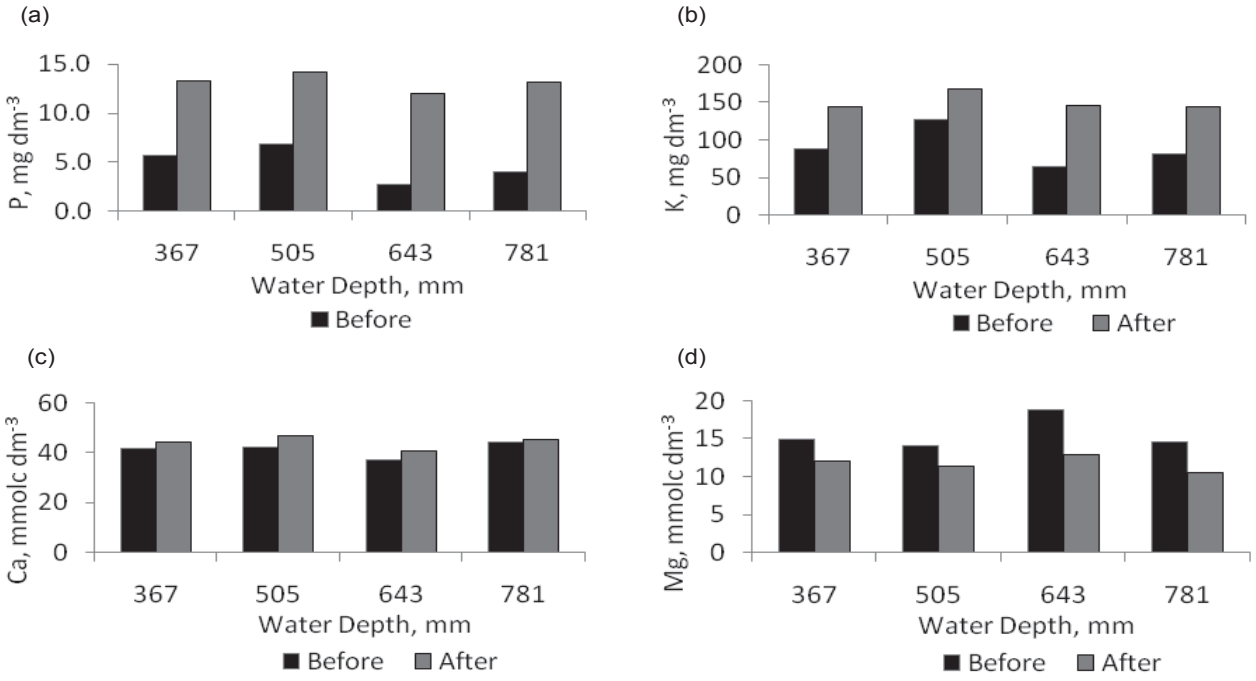

Figure 1. Contents of phosphorus, potassium, calcium and magnesium of the soil, before and after cultivation of cotton irrigated with wastewater.

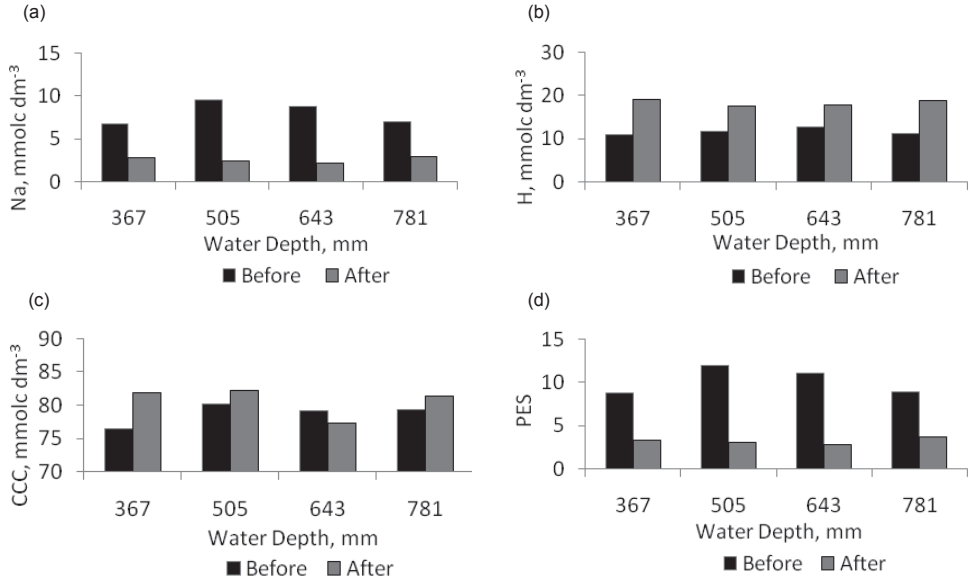

Figure 2. Contents of sodium, hydrogen, capacity of cationic change (CCC) and percentage of exchangeable sodium (PES) of the soil, before and after cultivation of cotton irrigated with wastewater.

attributes reveals significant effect of tested wastewater depths on electrical conductivity of extract of saturation of the soil (EC), Na^+, K^+, HCO^-_3, Cl^-, and sodium adsorption ratio (SAR). The effect was also significant among the times of collection of the soil for all appraised chemical attributes. There was not interaction of water depths and of soil collection times for EC and for contents of bicarbonate (HCO^-_3).

Cavallet et al. (2006) studying the fertilizer value of wastewater of an industry of enzymes in a Yellow Red

Table 3. Variance analyses of chemical attributes and of sodium adsorption ratio (SAR) of the soil for different wastewater depths and times of soil sampling (E), that is, before and after irrigation and after the rainy season.

Sources of Variation	DF	Mean Square							
		EC	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SAR
Water Depths (W)	3	0.144*	0.456*	0.724*	19.13**	0.167**	6.203**	8.611**	2.329**
Times (E)	2	1.075**	1.662**	3.893**	180.0**	1.658**	39.23**	84.72**	21.79**
W x E	6	0.083^{ns}	1.177**	0.900**	9.924**	0.091**	0.904^{ns}	6.811**	1.443**
Blocks	2	0.161	0.317	0.669	6.245	0.011	1.847	5.288	0.275
Residue	22	0.041	0.104	0.229	1.854	0.008	0.559	1.469	0.081
Total	35								
C.V (%)		16.22	9.62	12.82	20.13	15.40	8.94	25.14	11.40

*, **, ns - Significant for 5%, 1% and no significant, respectively, by Test F. DF – degrees of freedom.

Table 4. Mean values of chemical attributes of salinity and of sodium adsorption ration (SAR) of the soil irrigated with different wastewater depths, before and after irrigation and after the rainy season.

Factors	EC	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SAR
Water Depths (mm)	dS m^{-1}	----------	mmol$_c$ L^{-1}	----------				
692	1.36	3.32	4.14	8.04	0.80	9.18	6.03	2.78
554	1.32	3.15	3.51	7.24	0.51	7.21	4.95	2.77
416	1.08	3.48	3.57	4.66	0.58	8.60	3.65	1.74
278	1.23	3.20	3.71	7.10	0.52	8.46	4.65	2.72
LSD	0.26	0.42	0.62	1.78	0.12	0.97	1.58	0.37
Time	dS m^{-1}	----------	mmol$_c$ L^{-1}	----------				
E1	1.58^{a}	3.25^{b}	4.31^{a}	10.92^{a}	0.22^{c}	6.41^{c}	7.87^{a}	3.97^{a}
E2	1.16^{b}	3.78^{a}	3.72^{b}	6.09^{b}	0.61^{b}	9.97a	3.52^{b}	2.22^{b}
E3	1.00^{b}	c3.06^{b}	3.17^{c}	3.26^{c}	0.97^{a}	8.71^{b}	3.06^{b}	1.32^{c}
LSD	0.20	0.33	0.49	1.39	0.09	0.76	1.24	0.29

E1 - before irrigation; E2 - after irrigation with 89 mm of rain during the crop cycle; E3 - after the rainy station with 646 mm. Means followed by the same letter in the column don't differ amongst themselves, by the test of Tukey for 5% of probability. DF – degrees of freedom. LSD – least significant difference.

Argisols with 8, 16 and 32 mm of water, evaluating the levels of extractable K and P, organic carbon, pH ($CaCl_2$), Ca^{+2}, Mg^{+2}, (H^{+} $^{+}$, Al^{+3}) and saturation by bases, found that due to the presence of nutrients in the water; the soil pH increased and the levels of Al^{+3} decreased with the application of the treatments by virtue of the properties of soil acidity neutralization. Soil fertility increase occurred at the same levels as the mineral fertilization, when applying the dosages of 16 and 32 mm in wastewater. There were correction of acidity, insolubilisation of the levels of exchangeable aluminum in the soil and availability of the phosphorus element.

It is observed in Table 4 the mean values of chemical attributes of salinity and of SAR of the soil irrigated with different wastewater depths, before and after the irrigation and after the rainy season. In the factor time, the electrical conductivity of the saturation extract decreased with time, certainly due to precipitations happened in the period. Also, the concentration of salts in the humid bulb formed by the emitter and the contents of magnesium, sodium, chloride and the sodium adsorption ratio decreased along the time, while the content of potassium increased. The pH before irrigation varied from 8.18 to 8.35; after irrigation and the rainfall of 89 mm the pH increased from 8.2 to 8.4 and after the rainy season of 646 mm the pH was among 8.1 and 8.3. The EC decreased with the precipitation of 89 mm and later with 646 mm.

According to Figure 3, the tendency of the contents of calcium after irrigation with wastewater and the rainfall of 89 mm was linear, increasing with increments of 0.0033 mmolc L^{-1} mm^{-1} (Figure 3b). With the rains of 646 mm happened the inverse, that is, the contents of calcium decreased in larger water depths. With magnesium

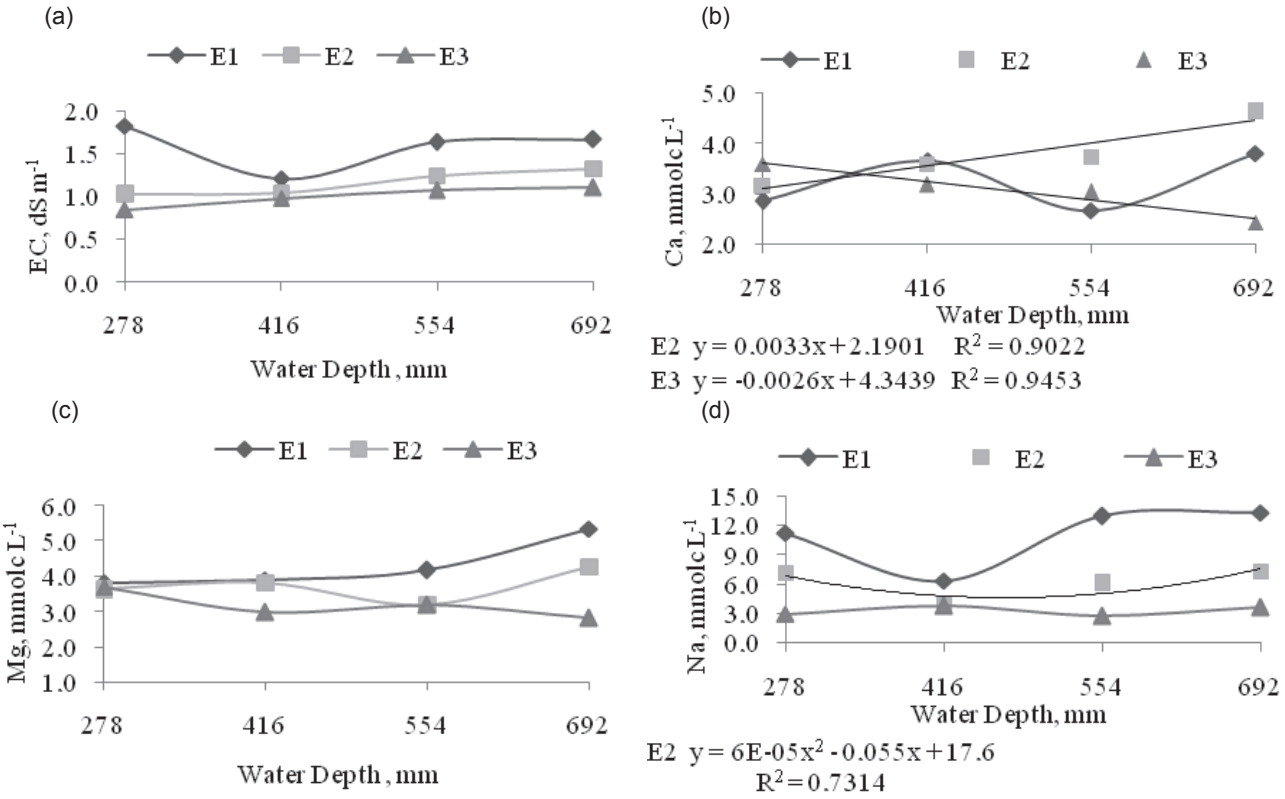

Figure 3. Electrical conductivity of the extract of saturation of the soil (EC), and contents of calcium, magnesium and sodium, before irrigation (E1), after irrigation (E2) and a rainfall of 89 mm, and after the rainy season of 646 mm (E3), in area irrigated with effluent.

something similar happened (Figure 3c); until the water depth of 554 mm there was not a tendency, from which up to the wastewater depth of 692 mm and with the precipitations of the five months, the contents of magnesium decreased.

The sodium decreased soon after the precipitation of 89 mm, decreasing much more with the precipitation of 646 mm (Figure 3d); in agreement with the regression analysis the smallest content of sodium of 4.95 mmolc L^{-1} would happen in the wastewater depth of 461 mm. Thus, it is observed that there was a wash of the soil profile in the layer of 20 cm, where the soil samples were collected; possibly the great amounts of sulfates in the soil profile, originating from wastewater, might have contributed to a larger lixiviation of sodium. The potassium content after irrigation increased; in agreement with the regression analysis the smallest content of 0.37 mmolc L^{-1} would be found in the wastewater depth of 439 mm (Figure 4a). There was an increase of the contents of potassium even with the precipitations happened in the five months; this increase certainly is linked to the application of the effluent rich in potassium.

There was an increase of bicarbonate soon after the irrigation with effluent, decreasing afterwards with the pluvial precipitation of 646 mm (Figure 4b). The contents of chloride decreased after the irrigation in the area, not existing a satisfactory tendency in relation to the amount of applied water (Figure 4c), decreasing with the precipitation in five months, with a larger reduction in the water depth of 692 mm, where the contents were higher. In general there was, however, a tendency of uniformity of the contents of the studied elements with the happened precipitations. The SAR decreased after irrigation and the precipitation of 89 mm, decreasing much more in five months of rainfall with precipitation in that period of 646 mm (Figure 4d).

Due to the fact that the effluent is usually saline, the irrigation with wastewater has been taking to increase of soil salinity (Cromer et al., 1984; Smith et al., 1996), which can affect the absorption of water by plants due to presence of a larger concentration of the ions Na^+, Cl^- and HCO_3^- in soil solution (Bielorai et al., 1984). However, some authors have been shown decrease in soil salinity with irrigation by effluent (Day et al., 1979; Stewart et al., 1990). In the first case, soil naturally saline was undertaken, and in the second one the authors verified that in a forest soil irrigated with treated sewer effluents for more than four years, the salinity was reduced due to lixiviation and to salts absorption by trees.

Santos et al. (2006) studying the increasing salinity of

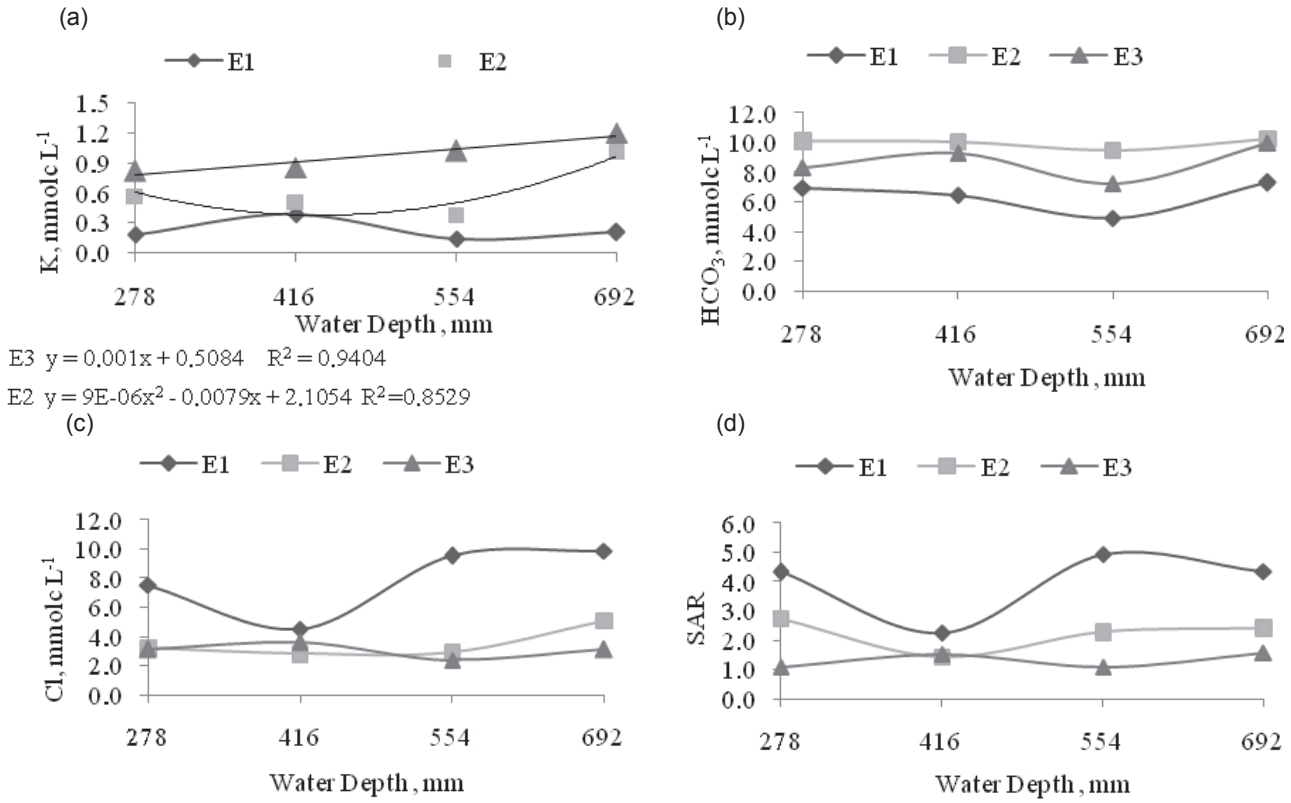

Figure 4. Contents of potassium, bicarbonate and chloride and the sodium adsorption ratio (SAR), before irrigation (E1), after irrigation (E2) and a rainfall of 89 mm, and after the rainy season of 646 mm (E3), in area irrigated with effluent.

the soil irrigated with wastewater and water supply and fertilized with biosolids and mineral manuring found that the wastewater increased the electrical conductivity of the soil by more than 130% in relation to the water supply; the doses of biosolids did not influence the concentrations of cations and anions. The highest values for the SAR and for the exchangeable sodium percentage (ESP) in the soil were observed in treatments with wastewater and biosolids doses of 75 kg ha^{-1} of N. Irrigation with wastewater contributes to make the soil from not saline to saline-sodic.

Conclusions

The contents of P, K^+, Ca^{2+}, H^+ and the capacity of cationic change of the soil increased after irrigation with wastewater, while the pH, the contents of Na^+ and Mg^{2+} and the percentage of exchangeable sodium of the soil were decreased. There was a tendency of the contents of the studied elements, and the electrical conductivity and the sodium adsorption ratio of the extract of saturation of the soil to increase after irrigation with wastewater. The electrical conductivity of the extract of saturation of the soil, the contents of calcium, magnesium, sodium and chlorides, and the sodium adsorption ratio decreased after five months of rainfall of 646 mm. A rainfall of 89 mm in six days was not enough to reduce the contents of calcium and bicarbonate of the extract of saturation of the soil.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Studies on some non-genetic factors affecting reproductive performance of Holstein Friesian × Deoni crossbred cows

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The present study was undertaken to evaluate the effect of non-genetic factors on reproductive performance of Holstein Friesian × Deoni crossbred cows. Data representing 256 HF × Deoni crossbred cows from cattle crossbreeding project with 1485 total records of lactation over a 30 years period were analyzed to determine the effects of period of calving, season of calving, age at first calving and parity on reproductive performance. The parameters used as indicators of reproductive performance in this study were age at first calving (AFC), days open (DO), dry period (DP) and inter calving period (ICP). The overall least squares mean of age at first calving (AFC), days open (DO), dry period (DP) and inter calving period (ICP) were 1198.54±8.18, 149.15±3.87, 126.90±1.76 and 422.95±2.53 days, respectively. All sources of variations except season of calving had significant effect on AFC and ICP. The AFC as seen in this study was somewhat longer. Cows calving in summer had reduced reproductive performance, as measured by DO and DP. First lactation cows had longer DO, DP and ICP, which were poorest values as reproductive traits. It is therefore concluded that, the reproductive performance of these crossbred cows is affected by some non-genetic factors and hence, additional reproductive strategies are needed to improve their performance.

Key words: Reproductive traits, period, season, parity, crossbred cows.

INTRODUCTION

Reproductive traits are crucial factors in determining the profitability of dairy production (Lobago et al., 2007). The reproductive performance of the breeding female is probably the single most important factor that is a prerequisite for sustainable dairy production system and influencing the productivity. The size of the calf crop is all-important for herd replacement and the production of milk depends heavily on the cow's reproductive activity (Kiwuwa et al., 1983). Reproductive performance of cattle is influenced by feed, genetics, disease and management practices (ILCA, 1990).

In India there are about 37 breeds of cattle. In spite of the presence of large and diverse cattle genetic resources, the productivity of cows remains low in the country, for various reasons, such as inadequate nutrition, poor genetic potential, inadequate animal

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health services, the harsh climatic conditions and other management related problems. The milk productivity in India remains one of the lowest as compared to the many leading countries of the world. In the country, the average milk productivity of crossbred cows, indigenous cows and buffaloes is about 6.44, 1.97 and 4.3 kg/day, respectively (GOI, 2007). The indigenous cattle breeds are low producers; they mature late and have a delayed conception coupled with long calving intervals. The productivity of dairy animals could be increased by crossbreeding the low yielding non-descript cows with high yielding suitable exotic breeds. This, systematic crossbreeding of indigenous and temperate dairy breeds is principally undertaken to combine the high milk yield and early sexual maturity of European dairy breeds with hardiness, disease resistance and adaptability of zebu cattle. Thus, crossbreeding of local non-descript cattle with exotic breeds of high genetic potential is considered to be a rapid and effective method of improvement.

Deoni is an important indigenous cattle breed of India. These animals are mainly found in the Latur district and the adjoining area of Parbhani, Nanded and Osmanabad districts of Maharashtra. Deoni is a medium heavy animal. It is found in three-colour variations viz. Wannera (clear white with black colour at the sides of the face), Balankya (clear white with black spots on the lower side of the body) and Shevera (white body with irregular black spots). The body is moderately developed and symmetrical with distinct muscles. Deoni cattle are hardy and well adapted to their breeding tract and constitute an important cattle genetic resource of India. The breed was found to be hardy and well adapted to tropical draught prone areas. The animals are docile and calm. Traditionally, Deoni cattle are maintained under a semi-intensive system of management.

Marathwada Agricultural University has taken a project for improvement of Deoni cattle breed by cross breeding local Deoni cows with Holstein Friesian. The success of dairy production in general and crossbreeding programmes in particular needs to be monitored regularly by assessing the productive and reproductive performance under the existing management system. The aim of the present study is, therefore, to investigate the effect of non-genetic factors on the reproductive performance of HF × Deoni crossbred cows kept at cattle crossbreeding project (CCBP).

MATERIALS AND METHODS

Study area

This study was conducted at CCBP of Marathwada Agricultural University, Parbhani India. It is located at an altitude of 407 m above the mean sea level and is situated between 17° 35' N and 20° 40' N latitude and between 70° 40' E and 78° 15' E longitude. The mean daily maximum temperature varies from 29.1°C in December to 42.5°C in May. The mean daily minimum temperature varies from 6.9°C in December to 25.4° C in May. The relative humidity ranges from 11 to 90%. Normally the summer becomes hot and general dryness persists throughout the year except during south-west monsoon. The region is essentially a subtropical one and it comes under assured rainfall zone with an average rainfall of 900 mm spread in about 70 rainy days mostly received from June to September.

Study animals

The Deoni cattle are an important dual-purpose breed of cattle in India. These animals are quite popular in the tracts of former Hyderabad State which now forms the north-western part of Andhra Pradesh and adjoining districts of Karnataka and Maharashtra. Their crosses with Holstein and Jersey are very good milk yielders. Deoni cattle are hardy and well adapted to their breeding tract and constitute an important cattle genetic resource of India. They are medium-sized animal. Cows of this breed are moderately good milkers, yielding about 1135 kg in a lactation period of 300 days. Deoni bulls are good for heavy work and are particularly suitable for intensive cultivation. Traditionally, Deoni cattle are maintained under a semi-intensive system of management. This study is thus focusing on the reproductive performance of HF × Deoni crossbred cows kept at CCBP (Figures 1 and 2).

Management of animals

The management of animals at CCBP becomes identical with variation due to reason beyond control. The daily routine management activity for lactating animals starts at 8 a.m. After calving, the calves remain with their dams for about 5 to 7 h. The calves then weighed, tagged and bucket milk fed twice a day until weaning. All the calves are separated from their dam at birth and weaned at around 3 months of age. The milk recording starts after 4th day from calving. The dams remain in barn for the first five days during which they provided with green fodder, concentrate meal, and transferred to the milking herd afterwards. Cows are hand-milked twice a day, early in the morning (6:00-7:00 am) and late in the afternoon (5:00-6:00 pm) after feeding concentrate mixture regularly. The cows are allowed for grazing in fallow land from 9.00 a.m. to 5.00 p.m. on a regular basis. However, in summer season (March-June) the cows are allowed for grazing from 9.00 a.m to 12.00 a.m. after that the animals are tied and stall-fed with required quantities of dry and green fodder under the shed.

All animals are routinely checked for any incident of health problem and treatments given if any abnormality exists. Additionally, animals regularly vaccinated against major diseases such as FMD, Black Leg and Haemorrhagic Septicaemia. The milking cows are washed and groomed regularly and fed individually. The project used teaser bull for regular heat detection. Upon heat detection, cows mated naturally to a bull. From conception up to 7 months of pregnancy, cows are grazed on natural pasture after which they are kept indoor and offered roughage and concentrate feed.

Sources and nature of data

Data representing 256 HF × Deoni crossbred cows from CCBP with 1485 total records of lactation over a 30 years period (1981-2010) were collected and organized to study the effects of period of calving, season of calving, age at first calving and parity on reproductive performance. The parameters used as indicators of reproductive performance in this study were ge at first calving (AFC), days open (DO), dry period (DP) and inter calving period (ICP). The complete year was divided into 4 seasons, according to season prevailing climatic conditions and that of the durations of study into 6 time-periods having 5 years each. The seasons

Figure 1. Atypical representative of a Deoni cow at CCBP, MAU.

Figure 2. Atypical representative of HF × Deoni crossbred cow at CCBP, MAU.

considered were winter (December to February), summer (March to May), monsoon (June to September) and post-monsoon (October to November). Six levels of age at first calving (AFC) were coded as A_1 for ≤ 1000 days to A_6 for ≥ 1601 days with a class interval of 150 days. The parties included L_1 to L_{10}. The four seasons namely winter (Dec-Feb), summer (Mar-May), monsoon (Jun-Sept) and post monsoon (Oct-Nov) were coded as S_1, S_2, S_3 and S_4. Cows having at least three offspring's were considered in this study (Tables 1 to 4).

Statistical analysis

System (SAS, 2002 version 9.1.3). When the analysis of variance indicated the existence of significant variation among groups, Duncan's Multiple Range Test (DMRT) was employed to test and locate means significantly differed from the rest. The following statistical model was employed to analyse the data.

$$Y_{ijklm} = \mu + T_i + S_j + A_k + P_l + e_{ijklm}$$

Where, Y_{ijklm} – is the Days Open (DO), Dry Period (DP) and Inter Calving Period (ICP) record of a cow calved during i^{th} period, in j^{th} season, at k^{th} age of first calving, on l^{th} parity, μ - is the population mean common to all the observations, T_i – is the effect of i^{th} time-period of calving (where i = 1, 2, 3, 4, 5and 6), S_j – is the effect of j^{th} season of calving (where j = 1, 2, 3 and 4), A_k – is the effect of k^{th} Data were analyzed by linear models using Statistical Analysis

Table 1. Period of calving and number of crossbred cows calved.

S/No.	Time-period of calving	Years	No. of calving	No. Animals	Percentage
1	P_1	1981-1985	405	103	27.27
2	P_2	1986-1990	408	138	27.47
3	P_3	1991-1995	364	130	24.51
4	P_4	1996-2000	124	62	8.35
5	P_5	2001-2005	90	29	6.06
6	P_6	2006-2010	94	27	6.33
Grand total			**1485**		**100**

Table 2. Frequency of seasonal calving and number of crossbred cows calved.

S/No.	Season of Calving	No. of calving	No. of animals	Frequency of calving	
				From season	From the year
S1	**Winter (Dec-Feb)**				
1	December	155		37.53	10.44
2	January	152		36.80	10.24
3	February	106		25.67	7.14
	Total	413	187		27.81
S2	**Summer (Mar-May)**				
1	March	103		29.26	6.94
2	April	144		40.91	9.70
3	May	107		30.40	7.21
	Total	352	157		23.84
S3	**Monsoon (Jun-Sep)**				
1	June	69		16.55	4.65
2	July	79		18.94	5.32
3	August	105		25.18	7.07
4	September	164		39.33	11.04
	Total	417	199		28.08
S4	**Post monsoon (Oct-Nov)**				
	October	161		53.14	10.84
	November	143		47.19	9.63
	Total	303	165		20.47
Grand total		**1485**		**100**	**100**

AFC (where k = 1, 2, 3, 4, 5 and 6) groups, P_l – is the effect of l^{th} parity (where l = 1, 2……10), e_{ijklm} – Random error associated with the measurement, which is assumed to be normally, identically and independently distributed with a zero mean and common error variance i.e., IND ($0.\sigma^2 e$).

RESULTS AND DISCUSSION

Age at first calving

Age at first calving is one of the important factors contributing to economic return. A reduction in AFC will minimize the raising costs, shorten the generation interval, and subsequently maximize the number of lactations per head. Earlier first calving increases lifetime productivity of cows. It is an important factor in determining the overall productivity of dairy cows (Singh et al., 1986). The least square means and ANOVA of AFC as affected by season and period of calving are presented in Tables 1 and 2, respectively. The overall mean of AFC in HF × Deoni crossbred cows was observed as 1198. 54±8.18 days. Considerably, lower

Table 3. Classification of crossbred cows based on AFC during the study period.

S/No.	Age of first calving (AFC)	Codes	No. animals
1	≤ 1000 days	A_1	48
2	1001-1150 days	A_2	64
3	1151-1300 days	A_3	53
4	1301-1450 days	A_4	41
5	1451-1600 days	A_5	32
6	≥ 1601 days	A_6	18
		Total	256

Table 4. Parity and number of lactation records of crossbred cows.

Parity	No. of records	Percentage
L_1	256	17.24
L_2	255	17.17
L_3	224	15.08
L_4	190	12.79
L_5	168	11.31
L_6	147	9.90
L_7	109	7.34
L8	74	4.98
L_9	41	2.76
L_{10}	21	1.41
Total	1485	**100**

estimate of 974.64±17.99 days was reported by Patil (1983) in same crossbred cows. It is recommended that heifers calve between 23 and 25 month of age, however, average AFC observed in this study (about 40 months) is higher than the optimum AFC. Mureda and Mekuriaw (2007) reported environmental factors, especially nutrition, determine pre-pubertal growth rates, reproductive organ development, and onset of puberty and subsequent fertility. Substantial evidence exists that dietary supplementation of heifers during their growth will reduce the interval from birth to first calving, probably because heifers that grow faster cycle earlier and express apparent oestrus.

The effect of period of birth and season on age at first calving

The age at first calving was affected significantly by period of birth ($P<0.01$), but not by season of birth. Similar result was reported by Komatwar et al. (2010). The significant effect of period of calving on AFC could be attributed to the changes in feeding and managerial systems and environmental conditions which occurred from year to year as well as differences between years in the quantity and quality of forage availability. The non-significant effect of season indicates that, HF × Deoni crossbred heifers are at same level to adopt seasonal variation in the study area (Table 5).

Days open

Days open is the interval between date of calving and date of conception. It is one of the best indicator variables, which is most commonly used to measure fertility performance in dairy cattle (Arbel et al., 2001). Days open directly affect CI, which plays an important role in the profitability of dairy farms. Days open is the part of the calving interval that can be shortened by improved herd management. Long days open and consequently, prolonged CI may affect the overall economic revenues of the dairy herd.

The least square means and ANOVA of days open as affected by season, period of calving, AFC and parity are presented in Tables 6 and 7 respectively. The overall mean of days open in HF × Deoni crossbred cows was recorded as 149.15±3.87 days. This result is in close agreement with the finding reported by Thombre (1996) as 151±4.9 days. Considerably, shorter estimate of

Table 5. Least square means and standard errors for age at first calving on season and period of birth.

Source	Code	LSM ± SE
Overall Mean	μ	1198.54±8.18
Period	P_1	977.27±12.61^{a}
	P_2	1103.71±12.48^{b}
	P_3	1297.06±13.20^{c}
	P_4	1336.97±22.71^{cd}
	P_5	1288.69±26.60^{c}
	P_6	1387.69±26.06^{d}
Season	S_1	1199.99±13.55
	S_2	1178.19±14.44
	S_3	1192.35±13.06
	S_4	1223.64±15.22

Means connected with different superscripts in a column differ significantly (P<0.01).

Table 6. Least square means and standard errors for days open, dry period and calving interval.

Source	Code	DO (Days)	DP (Days)	ICP (Days)
Overall Mean	μ	149.15±3.87	126.90±1.76	422.95±2.53
Period	P_1	163.62±4.91^{c}	121.05±3.57^{a}	440.67±5.15^{c}
	P_2	162.51±4.29^{c}	135.52±2.43^{bc}	440.45±3.50^{c}
	P_3	147.42±4.31^{b}	136.80±2.62^{c}	425.43±3.77^{b}
	P_4	123.19±4.84^{a}	124.16±4.15^{a}	401.15±5.98^{a}
	P_5	152.27±5.51^{bc}	125.75±4.91^{ab}	430.24±7.08^{bc}
	P_6	124.11±5.12^{a}	118.09±4.74^{a}	400.76±6.83^{a}
Season	S_1	144.28±4.26^{b}	122.31±2.57^{a}	422.23±3.70^{a}
	S_2	152.31±4.30^{a}	131.53±2.72^{b}	416.29±3.92^{a}
	S_3	144.42±4.21^{b}	131.02±2.46^{b}	422.39±3.54^{a}
	S_4	139.10±4.39^{b}	122.72±2.84^{a}	418.89±4.09^{a}
AFC	A_1	129.54±4.76^{a}	114.98±3.84^{a}	406.51±5.53^{a}
	A_2	127.12±4.62^{a}	117.85±3.58^{a}	403.92±5.16^{a}
	A_3	149.50±4.59^{bc}	123.01±3.17^{a}	427.48±4.56^{bc}
	A_4	147.52±4.57^{b}	132.05±3.13^{b}	424.91±4.51^{b}
	A_5	159.12±4.63^{c}	106.94±3.10^{b}	437.24±4.47^{c}
	A_6	160.25±4.71^{c}	141.55±3.25^{c}	437.66±4.68^{c}
Parity	L_1	161.18±4.64^{d}	135.52±3.10^{c}	438.86±4.46^{d}
	L_2	160.16±4.60^{d}	133.4±3.04^{bc}	437.98±4.38^{d}
	L_3	146.25±4.59^{abc}	126.34±3.20^{ab}	424.10±4.60^{abc}
	L_4	154.61±4.73^{cd}	131.27±3.36^{b}	431.70±4.84^{cd}
	L_5	155.49±4.81^{cd}	130.17±3.50^{bc}	432.66±5.04^{cd}
	L_6	151.71±4.89^{bcd}	124.72±3.72^{ab}	429.18±5.35^{bcd}
	L_7	140.61±5.09^{abc}	122.65±4.31^{ab}	417.60±6.21^{abc}
	L_8	133.95±5.43^{ab}	113.23±5.19^{a}	411.03±7.47^{ab}
	L_9	132.29±6.20^{abc}	119.78±6.89^{abc}	410.42±9.93^{abc}
	L_{10}	118.23±7.14^{a}	131.9±9.61^{abc}	395.99±13.85^{a}

Means connected with different superscripts in a column differ significantly.

Table 7. Analysis of variance of means for days open, dry period and inter calving period on period, season, AFC and parity.

Sources	DF	DO		DP		ICP	
		MSS	F value Cal.	MSS	F value Cal.	MSS	F value Cal.
Period	5	12759.85	9.53**	13350.00	7.11**	37210.00	9.55**
Season	3	3708.50	2.77*	9359.00	4.99**	4945.00	1.27^{NS}
AFC	5	8804.40	6.58**	12620.00	6.72**	25690.00	6.60**
Parity	9	3982.35	2.98**	4368.00	2.33*	11620.00	2.98**
Error	1462	1338.33		1877.00		3895.00	
Total	1484						

*P<0.05, ** P<0.01 & NS = Non significant effect.

113.33±7.4 days was reported by Mudgal et al. (1986). However, Chauhary et al. (1989) and Thombre (1991) had reported longer days open than the present estimation.

The effect of period of calving on days open

Days open was affected by period of calving (P<0.01). The days open was longer during 2nd and 3rd period while shortest days open was observed in the 4th and 6th periods (Table 7). Similar results were reported by Chauhary et al. (1989) and Thombre (1996). However, Basu and Ghai (1980) reported non-significant effect of period of calving on days open in different crossbred cows.

The effect of season of calving on days open

Days open was also affected significantly (P<0.05) by season of calving. The cows calved in summer season had the longest days open than the other seasons (Table 7). Similar findings were reported by Pyne et al. (1988) and Chauhary et al. (1989). However, Thombre (1996) reported non-significant effect of season of calving on days open. The variation due to season of calving on days open could be attributed to the changes in climatic conditions and feeding regimes during different seasons.

The effect of age at first calving on days open

The days open was significantly (P<0.01) affected by AFC. On the contrary, Patil (1983) has reported non-significant effect of AFC on DO. The maximum days open was observed from the cows which had AFC group of A_6 followed by cows which had AFC group A_5 and the minimum for those cows that had AFC group A_2.

The effect of parity on days open

Days open was influenced significantly by parity (P<0.01). First parity cows had longer DO which was the poorest value as reproductive trait. This result is in close agreement with reports of Mudgal et al. (1986) and Chauhary et al. (1989). The variation in mean values of DO seems to be largely due to environment (management, feeding and breeding).

Dry period

The dry period is necessary for compensation of the depleted nutrients during lactation and gaining stimulation for a new lactation period. Thus, an optimum dry period is essential for maximum production of milk in subsequent lactation. A dry period, typically 40 to 60 days, between lactations believed to be required to maximize milk yield in the subsequent lactation. The least square means and ANOVA of DP as affected by season, period of calving, AFC and parity are presented in Tables 6 and 7, respectively. The overall mean of dry period in HF × Deoni crossbred cows was recorded as 126.90±1.76 days. This observation was above optimum level. Thus, a considerable reduction should be achieved through improved management practices. This result is in close agreement with the finding of 125.4±4.21 days reported by Jadhva et al. (1991). Considerably, shorter and longer estimates of dry period in different crossbred cows were reported by Komatwar et al. (2010) as 82.54±3.62 days, and by Deshmukh (1996) as 167.63 days, respectively.

The effect of period of calving on dry period

The dry period of HF × Deoni crossbred cows was significantly affected by period of calving (P<0.01). The dry period was longer during the 2nd and 3rd period of study as compared to other periods, the difference between the other periods was non-significant (Table 7). Similar results were reported by Mudgal et al. (1986) and Komatwar et al. (2010). On the contrary, Auradkar (1999) reported non-significant effect of period of calving on dry period.

The effect of season of calving on dry period

The dry period of HF × Deoni crossbred cows was significantly (P<0.01) affected by season of calving. The cows calved in summer and monsoon seasons had the longest dry period, while the cows calved in winter and post monsoon season had the shortest dry period (Table 7). Auradkar (1999) and Chenyambuga and Mseleko (2008) reported similar findings in different crossbred cows. In contrast, Mudgal et al. (1986) and Komatwar et al. (2010) reported non-significant effect of season on dry period.

The effect of parity on dry period

The dry period of HF × Deoni crossbred cows was significantly (P<0.05) affected by parity. The longest mean dry period was observed in the first lactation while; the shortest was noted in the 8th lactation. The present finding was in agreement with reports of Mudgal et al. (1986) and Komatwar et al. (2010). In contrast, Patil (1997) reported non-significant effect of parity on dry period in different crossbred cows.

Inter calving period

The calving interval is the period between two consecutive parturitions, and ideally should be in the range of 12 to 13 months. Calving interval has a great economic importance on the lifetime milk production and productive life of dairy animals, which ultimately affects the economics of the owners. It is known that the extended calving intervals negatively affect the longevity as a productive life, because the cow with longer calving interval has fewer lactation numbers during the same period of herd life compared with cows with shorter calving intervals.

The least square means and ANOVA of ICP as affected by season, period of calving, AFC and parity are presented in Tables 6 and 7, respectively. The overall mean of calving interval in HF × Deoni crossbred cows was recorded as 422.95±2.53 days. This is in close agreement with the findings reported by Auradkar (1999) and Yifat et al. (2009) as 412.07±1.46 and 418 days, respectively. Considerably, shorter estimates of calving interval were reported by Rao et al. (1984) and Chavan (2001) as 384.50±42.70 and 381.40±1.77 days, respectively. In contrast, longer estimates of calving interval were reported by Deshmukh (1996) and Dahiya et al. (2003) as 511.11 and 432.42±8.19 days, respectively.

The effect of period of calving on inter calving period

The calving interval of HF×Deoni crossbred cows was significantly (P<0.01) affected by period of calving. The longest calving interval was observed in the cows calved during period 1, while the shortest calving interval was found in period 6. Similar results were reported by Dahiya et al. (2003). But, Auradkar (1999) and Chavan (2001) reported non-significant effect of period of calving on calving interval in different crossbred cows.

The effect of season of calving on inter calving period

The effect of seasons of calving on the calving interval of HF×Deoni crossbred cows was not significant (P>0.05). Similar finding was reported by Deshmukh (1996). However, Auradkar (1999) and Dahiya et al. (2003) reported significant effect of season of calving on calving interval in different crossbred cows.

The effect of parity of calving on inter calving period

The calving interval of HF × Deoni crossbred cows was significantly (P<0.01) affected by parity. The longest calving interval was observed in the first lactation, while the shortest was noted in the 10th lactation. Similar finding was reported by Nehra et al. (1987). However, Latpate (1995) and Deshmukh (1996) reported non-significant effect of parity on calving interval in different crossbred cows. A calving interval of 365 days is usually considered ideal (Khan et al., 1992). Therefore, the calving intervals, as seen in this study were somewhat longer which needs an improvement in overall management of the dairy cows. Sewalem et al. (2008) indicated that poor reproductive performance, manifested as prolonged calving intervals, which can result in reduced milk yield and increased culling rates and replacement cost.

Conclusions

The present study shows that AFC in HF × Deoni crossbred heifer is comparably longer, so it should be further reduced by proper management and selection. Moreover, days open was somewhat longer than optimal level of 80-105 days. Dry periods were also above optimum level and the calving intervals were fairly longer. Thus, a considerable reduction should be achieved in these reproductive traits through an improvement in overall management of the dairy cows. Besides, these crossbred cows were susceptible for periodical and seasonal changes on their fertility performance particularly on days open, dry period and calving interval where it will be difficult for them to thrive and maintain their reproduction potential. Therefore, additional reproductive strategies like improving environmental factors and reproductive management of cows are needed to reduce the adverse effect of periodical and

seasonal changes.

Conflict of Interest

The author have not declared any conflict of interest.

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Performance of rice varieties in relation to nitrogen levels under irrigated condition

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The field experiment was conducted at the research farm College of Agriculture, JNKVV, Rewa M.P. during *kharif* season 2011 to study the performance of rice varieties and relation to nitrogen level under irrigated conditions. Twenty one treatment combinations, comprising three nitrogen levels (40, 80 and 120 kg ha^{-1}) allotted to the main plots and seven varieties (IET 21288, Jaldidhan, Varalu, IET 21296, IET 21278, Aditaya and Dantensavari) to the subplots, were tested in split-plot design with three replications. Application of 120 N kg ha^{-1} proved significantly superior to 40 kg N and produced maximum grain yield (49.88 q ha^{-1}), straw yield (93.10 q ha^{-1}) and net income (Rs. 30836 ha^{-1}) over 80 N kg ha^{-1}. Among different rice varieties, Dantensavari produced significantly higher grain yield (45.56 q ha^{-1}), straw yield, fetched highest net return (Rs. 32037.46 ha^{-1}) and B: C ratio during the study; While, the rest of the varieties remained differed for different traits. The other promising varieties for the region were IET 21278, Varalu, and IET 21278. The treatment combination of Dantensavari with 120 N kg ha^{-1} was found to be best in producing grain yield (58.39q ha^{-1}), followed by IET 21278 (56.78 q ha^{-1}) and obtained net income Rs. 40871 ha^{-1} and Rs. 38983 ha^{-1}, respectively.

Key words: Nitrogen, varieties, rice, grain yield, straw yield.

INTRODUCTION

Nitrogen is the key element in the production of rice and gives by far the largest response. It is the most essential element in determining the yield potential of rice and nitrogenous fertilizer is one of the major inputs to rice production (Mae, 1997). Almost every farmer has the tendency to apply costly N fertilizer excess to get a desirable yield of Aman rice (Saleque et al., 2004), but imbalance use of N fertilizer causes harm to the crop and decreases grain yield. It is also a fact that improper use of nitrogenous fertilizer, instead of giving yield advantage, may reduce the same.

Nitrogen fertilization and proper time of its application is the major agronomic practice that affects the yield and quality of rice crop which requires as much as possible at

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an early and mid tillering stages to maximize panicle numbers and during reproductive stages to produce more number of spikelets per panicle and percentage filled spikelets (Lampayan et al., 2010). Different varieties may have varying responses to N-fertilizer depending on their agronomic traits.

The introduction of high yielding varieties of crops and utilization of various chemical fertilizers has brought a revolution in the crop production. In M.P. rice is grown in the area of about 15.59 lakh ha with production of 14.62 lak ha tons and productivity 989 kg ha^{-1} which is far below than the average national productivity (2,010 kg ha^{-1}).

In Madhya Pradesh around 5,000 ha is under hybrid rice particular in under irrigated production system (Anonymous, 2012). Now a days the identification and release of high yielding rice varieties, it becomes imperative to make a comparative assessment of the growth studies and their influence on grain yield under different nutrient combination. Of the mineral nutrients, nitrogen plays a major role in utilization of absorbed light energy and photosynthetic carbon metabolism in many biochemical and physiological activities of plant (Kato et al., 2003; Huang et al., 2004). Its deficiency or excess application may adversely affect these processes and ultimately reduces crop yield. Keeping these points in view the present research was taken up.

MATERIALS AND METHODS

The field experiment was conducted at the research farm JNKVV College of Agriculture, Rewa M.P. during *kharif* season 2011. The soil of the experimental site was clay-loam in texture with pH 7.7, low nitrogen, medium phosphorous and high in potash. Twenty one treatment combinations, comprising three nitrogen levels (40, 80 and 120 kg ha^{-1}) allotted to the main plots and seven varieties (IET 21288, Jaldidhan, Varalu, IET 21296, IET 21278, Aditaya and Dantensavari) to the subplots, were tested in split-plot design with three replications. A uniform dose of 60 kg P_2O_5 + 40 kg K_2O ha^{-1} was applied in all plots through SSP and MOP, respectively. Nitrogen was applied through urea in 3 split doses that is 50% at basal, 25% at tillering and 25% at panicle initiation. The 21st days old seedlings were transplanted on July 21, 2011 keeping 20 cm spacing between row and 15 cm between hills. The crop was raised as per recommended package of practices. The crop varieties were harvested from 14th to 29th October 2011. The results of yield trait analysis are narrated below:

(1). The Observations on plant height and total number of effective tillers were taken from hills of each plot randomly selected 5 plant and tagged were recorded at 30 days interval, starting from 30 days after transplanting upto at harvesting stage. The height was measured in centimeter from the ground level to the apex of the shoot. The mean height was computed by dividing the summation with five.
(2). Number of tiller m^{-2} were counted and then divided by plant population m^{-2} for obtaining number of tiller $hill^{-1}$.
(3). The length of panicle was taken from ten panicles selected randomly from harvested produce. It was measured from the neck node to the tip of the apical grain. After this, average length of panicle was determined.
(4). Grain samples were taken from the produce of each net plot. Out of the samples, 1000 grains were counted and the same were dried in an oven at 60°C to constant weight. Thereafter, weight was recorded on an electronic balance.
(5). The grain yield was observed at 14 per cent moisture content and converted to q ha^{-1}.
(6). The straw yield calculated by deducting the grain yield from bundle weight and converted to q ha^{-1}.
(7). The harvest index was calculated following formula given by Synder and Carlson (1984):

$$\text{Harvest index (\%)} = \frac{\text{Economic yield (Grain yield)}}{\text{Biological yield (Grain + straw yield)}} \times 100$$

The following calculation further has been made to analysing data for benefit income of different varieties during investigation:

Net Return (Rs. ha^{-1}) = Gross return (Rs. ha^{-1}) – Cost of cultivation (Rs. ha^{-1})

$$\text{Benefit cost ratio} = \frac{\text{Net return (Rs. ha}^{-1}\text{)}}{\text{Total cost of cultivation (Rs. ha}^{-1}\text{)}}$$

RESULTS AND DISCUSSION

The yield attributes of rice were found to be differed significantly due to applied nitrogen levels. Each increase in the N-level significantly increased plant height and number of effective tillers resulting higher yield attributes (Table 1). Thus, at 120 kg N ha^{-1}, the yield attributes recorded maximum number of effective tillers (238.50 kg m^{-2}), panicle length (25.04 cm), panicle weight (2.83 g), number of fertile grains (106.51seeds $panicle^{-1}$) and 1000-grains weight (25.89 g) than lower fertilities. While, 40 N kg ha^{-1} was remained closed to 80 N kg ha^{-1} in number of effective tiller and returned lower net value during investigation.

The improvement in yield attributing traits may be ascribed to the improved vegetative growth due to N fertilization, facilitating photosynthesis, thereby increasing translocation of organic food materials towards the reproductive organs; which enhanced the formation of panicles with fertile grains. The improvement in yield components due to increased N levels have also been reported by many workers Gunri et al. (2004), Parihar (2004), Singh et al. (2005), Mittoliya (2006), Lar et al. (2007), Pandey et al. (2007) and Singh et al. (2008).

The rice varieties had exhibited significant differences in yield attributes. All yield attributing characters were remained differed with different varieties. The var. IET 21288 recorded maximum length of panicle (cm) and weight of panicle (g) and remained at par in number of effective tillers of rice var. Dhanteshwari during the study. While, the tallest plant height noticed with rice var. IET 21278.

However, variety Dantensavari obtained highest number of effective tillers and filled grains/panicle over the rest of the varieties; exerted second promising yield attributing characters during investigation. The climatic

Table 1. Yield attributes, yield and economics of transplanted rice as influenced by different N levels and Varieties.

Treatment	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
N-levels kg/ha											
40	88.47	160.88	21.93	2.22	80.40	24.55	37.69	71.27	34.48	20256	1.81
80	96.13	182.37	23.34	2.55	92.73	25.19	42.68	80.36	34.61	25501	2.0
120	103.91	238.50	25.04	2.83	106.51	25.89	49.88	93.10	34.84	33356	2.27
C.D. (P= 0.05)	0.85	38.90	0.71	0.08	4.08	0.46	0.60	0.75	0.22	-	-
Varieties											
IET 21288	106.58	195.95	26.82	2.96	95.89	27.67	46.91	86.35	35.22	30448	2.19
Jaldidhan	72.33	157.15	21.32	1.71	55.98	27.91	33.76	67.27	33.36	15031	1.59
Varalu	100.98	252.13	23.63	2.43	121.02	19.93	45.19	84.05	34.94	28438	2.11
IET 21296	107.74	151.09	23.23	2.59	95.61	25.00	39.41	75.56	34.21	21658	1.85
IET 21278	109.24	195.65	23.78	2.66	93.17	25.98	47.17	87.46	34.95	30789	2.20
Aditaya	93.9	191.20	22.33	2.59	87.19	25.62	41.13	78.01	34.51	23675	1.93
Dantensavari	82.41	214.23	22.92	2.82	103.64	24.34	50.37	92.35	35.30	34557	2.35
C.D. (P= 0.05)	1.27	32.59	1.12	0.26	4.97	0.48	2.23	3.61	0.28	-	-
Remarks:	I-Plant height (cm), II- No. of effective tiller, III- Length of panicle (cm), IV- Wt. of panicle (g), V- Filled grains/panicle (No.), VI- 1000 grain Weight (g), VII- Grain yield (q/ha), VIII- Straw yield (q/ha), IX- HI (%), X-Net return (Rs./ha) and XI- B:C ratio.										

condition and genetic makeup of variety had better interaction under which could be enhanced growth and development of panicles. The photoperiodic responses and genetic potentiality on variation of yield attributes of improved varieties have also been reported by Lar et al. (2007) and Singh and Tripathi (2008).

Productivity of rice

The productivity of rice was found to be differed with different level of N and significantly affected the yield of rice. The application of 120 kg N ha^{-1} produced highest grain yield (49.88 q ha^{-1}) and straw yield (93.10 q ha^{-1}) over lower N-levels. This might be due to overall better growth in plant height and appreciable improvement in yield attributing characters. Similar result have also evinced by Luikhan et al. (2004), Sabir et al. (2007), Pandey et al. (2007) and Singh et al. (2008). But the value of harvest index under 80 kg N ha^{-1} remained at par to 40 kg N ha^{-1} and closed to 100 kg N ha^{-1} in present study. The significant rise in HI might be because of the increased grain production as compared to that of straw. Moreover, the improvement in the HI might have also been due to being increased in grain yield through greater partitioning of assimilates from shoot to grain.

The rice variety had also found to be significant differences in yield of rice and var. Dhanteshwari produced significantly higher grain and stove yield that is 50.37 and 92.35 q ha^{-1}, respectively over IET 21278. While, vars. IET 21288 and Varalu were remained at par to each other and found closed to IET 21278 during investigation.

Furthermore, the value of var. Aditaya and IET 21296 found to be non- significant differences among themselves and found to be higher than variety Jaladidhan in study. The increased in grain yield by the varieties due to overall respective performance in growth and appreciable improvement in the yield- attributing characters. Significant variations in the grain yield of rice varieties have also been reported by many workers (Ajeet et al., 2005; Mittoliyam 2006; Lar et al., 2007; Singh and Tripathi, 2008). The increase in the straw yield of var. Dhanteshwari might be due to the superiority of growth parameters. The harvest index also had significantly higher

(35.30%) as compared to rest of the varieties. Jaldidhan recorded the lowest harvest index (33.36%). So much difference in HI among the varieties from different origins reveals that there was greater variations in the partitioning of assimilate from shoot to grain.

Economic gain

Among different levels of N, the application of 120 N kg ha^{-1} resulted in maximum net income (Rs.33356 ha^{-1}) and B: C ratio (2.27) followed 80 N kg ha^{-1} and 40 kg N ha^{-1}. The higher net income is due to higher grain the straw yield at the highest of N level. The different rice varieties also gave promising yield and net returned in var. Dantensavari (Rs. 34557 ha^{-1}) was found maximum with B: C ratio 2.35 than rest of varieties. While, the var. IET 21278 and 21288, the economical gain up to the same extent was obtained Rs. 30789 and 30448 ha^{-1} with 2.20 and 2.19 B: C ratio during the study. The remaining series of varieties for net return and B: C ratio were i.e. Aditaya> Varalu> IET 21296> Jaldidhan, respectively. Such differences in the net income from rice varieties were differences grain and straw yields.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Full Length Research Paper

Management of non-timber forest products harvesting: Rules and regulations governing (*Imbrasia belina*) access in South-Eastern Lowveld of Zimbabwe

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Non timber forest products such as *Imbrasia belina* (mopane worms) provide a wide range of benefits to the rural dwellers in the semi-arid areas of South East Lowveld of Zimbabwe. Rules and regulations governing mopane worm use and access in the study area that comprised of a communal area, resettlement area, small-scale farms and a national game park and the relationship of these rules to harvesting of mopane worms were analysed using questionnaire, observational studies and one way analysis of variance (ANOVA).This paper examines the extent to which access to non-timber forest products especially the harvesting of mopane worms is governed by the existence of institutional arrangements and common property management regimes. The results of the study indicated that possession of a permit is a pre-requisite requirement to harvest timber and non-timber forest products in Gonarezhou National park. Harvesters in Mwenezi resettlement areas and Chikombedzi communal area indicated that rules for mopane worm harvesting were either weak or non-existent. In Gonakudzingwa Small Scale Farms, harvesters had to seek permission from the farm owner before any harvesting of mopane worms could take place. The results from the study showed that there were significant differences in the quantity of mopane worms harvested between Gonarezhou National Park and Chikombedzi Communal Area (p = 0.036), Gonakudzingwa Small Scale Commercial Farms and Chikombedzi Communal Area (p = 0.001), Mwenezi Resettlement Area and Chikombedzi Communal Area (p = 0.001). However, there were no differences between the Gonarezhou National Park, Gonakudzingwa Small Scale Commercial Farms and Mwenezi Resettlement Area. Findings of this study suggest the need for adaptive local management systems that enhance sustainable use of the resource and at the same time regulates the harvesting and the market structure of non-timber forest products.

Key words: *Imbrasia belina,* mopane worm, harvesting, tenure regime, access; non-timber forest products.

INTRODUCTION

There is a growing evidence that non-timber-forest-products contribute significantly to livelihoods in rural Asia, Africa and other developing countries (Campbell and Luckert, 2002; Cavendish, 2000; Cocks et al., 2008; Shackleton and Shackleton, 2004; Viet Quang and Nam Anh, 2006). The three main functions of non timber forest products in the rural economy is that they help to fulfill households' subsistence and consumption needs and

secondly, they serve as a safety-net in times of crises (e.g. crop failure) and thirdly to provide regular cash income (Angelsen and Wunder, 2003; Cavendish, 2002; Chileshe, 2005; Shackleton et al., 2007).

By definition non-timber-forest-products includes fruits and nuts, vegetables, fish and game, medicinal plants, resins, essences and a range of barks and fibers such as bamboo, rattans, and a host of other palms and grasses (CIFOR, 2011). This definition covers (*Imbrasia belina)* mopane worms the subject of this research where it is assumed that their harvesting is threatened by lack of rules of access in the South-East Lowveld of Zimbabwe. The mopane worms are endemic to parts of central and southern Africa and are associated with the distribution of *Colophospermum mopane* (mopane tree). *C. mopane* is confined to countries that include northern part of South Africa, northern Namibia, Botswana, Zimbabwe, Zambia, Mozambique, Malawi, and Angola (Timberlake, 1995).

Mopane worms play an important role in the nutrition of rural communities as they provide them with the vital crude proteins (61%), crude fats (17%) and carbohydrates which are more than equal amounts of beef or fish, and a higher energy value than soybeans, maize, beef, fish, lentils, or other beans that are often lacking in their diets (Hobane, 1994, 1995; Wilson, 1989; Banjo et al., 2006; Defoliart, 1995). In some African countries, children are fed with flour made from dried caterpillars to curb malnutrition, while pregnant and breast feeding women and those who are anemic are encouraged to eat caterpillars to improve their protein, calcium, and iron levels (FAO, 2004; Illgner and Nel, 2000). Toms et al. (2003) recommended that people who are HIV-positive eat the caterpillars to boost their immune system.

Non timber forest products (are the most accessible source of products and incomes for many economically marginalized people, and are consequently under considerable pressure to provide both production and environmental benefits (Darlong and Barik, 2005). Poverty, low income and survival needs often drive local people to over-harvest non timber forest products like mopane worms at the expense of environmental sustainability. Studies done by FAO (1996) indicated that there is a strong link between resource degradation and vulnerability to livelihoods.

The common-pool resources, such as forest resources and mopane worms are considered to have an inelastic supply and their sustainable utilization may be threatened by externalities associated with individual actions in the harvesting of such resources (Mutenje et al., 2010). The sustainable management of non-timber forest products is an important issue facing both development planners and policy makers (FAO, 2003). Hall and Bawa (1993) define sustainable harvesting of natural resources as the level of harvest that does not impair the ability of the harvested population to replace itself. However, Ticktin (2004) pointed out that ignoring the potential variation in harvest strategies and their drivers can lead to spurious conclusions about resource use sustainability.

Agrawal and Gibson (1999) have shown that local people resource management is the most viable option for common pool resources like mopane worms. The premise is based on assumptions that local communities not only understand their problems but also have greater incentive to find workable solutions to problems because their livelihoods depend on these natural resources (Belcher and Schreckenberg, 2007). The incentive to conserve the natural resource base and their sustainable management thereof comes from economic opportunities which the non-timber forest products offer (FAO, 2003).

Despite the recognized importance of mopane worms to the economy and human welfare, the institutional arrangements and rules governing mopane worm use and access were found to be weak and poorly understood (Hobane, 1995). The objective of this study is therefore to investigate the extent to which mopane worm harvesting and management is driven by institutional arrangements and rules of access in the South East Lowveld, Zimbabwe. Secondly, to find out who is harvesting the mopane worms, where, how much, and for what purpose in the South East Lowveld of Zimbabwe.

MATERIALS AND METHODS

Study area

The study was conducted in the South-East Lowveld of Zimbabwe (Figure 1). The area occupies the region 21° 00´-22°15´S and 32° 30´-32°15´E, and covers about 300 000 ha in extent. The study was conducted in the four different property regimes: Gonarezhou National Park (state property), Gonakudzingwa Small Scale Farms (+- 700 hectares in extent), (Private Property), Chikombedzi Communal Area in (Vivinya village) (Communal Property), and Mwenezi Resettlement Farms (Edenvale, Jabula, Nardice and Iroonwood) (State and Private Property).

The study area falls in the Natural Ecological Region 5 classification of Zimbabwe and rainfall ranges between 400 to 500 mm per annum with an annual temperatures of 18 to 24°C (Low and Rebelo, 1996). The study area experiences three climatic seasons: a hot dry period from August to October, a cold dry period from May to July and a hot wet period from November to April. The altitude of the study area varies between 165 and 575 m above sea level (Mlambo, 2006).

The soils are predominantly shallow sands of the siallitic group derived from sandstone (Nyamapfene, 1991). Mopane is a dominant tree found in the study area in association with *Kirkia acuminata, Dalbergia melanoxylon, Adansonia digitata, Combretum apiculatum, Camptostoma imberbe, Acacia nigrescens,* and *Commiphora* species.

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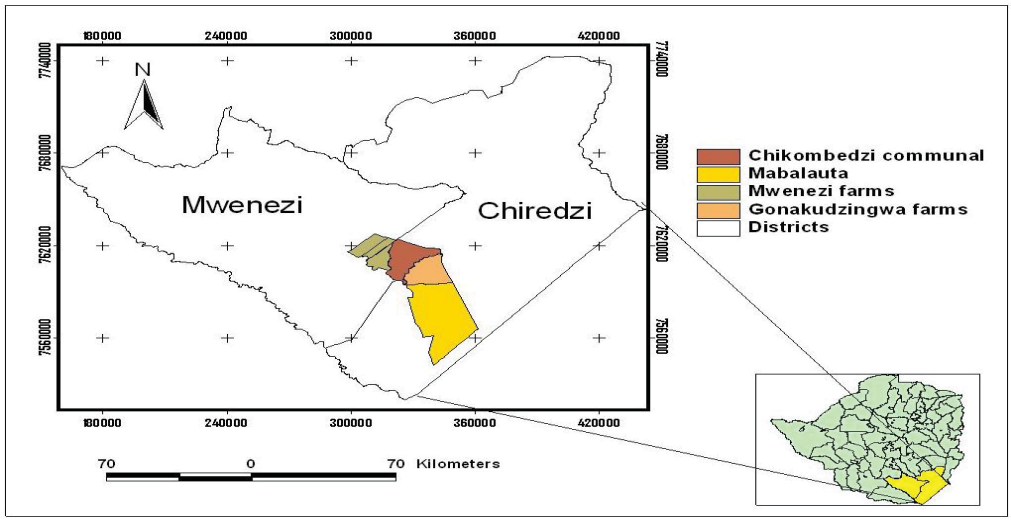

Figure 1. Location of the study area.

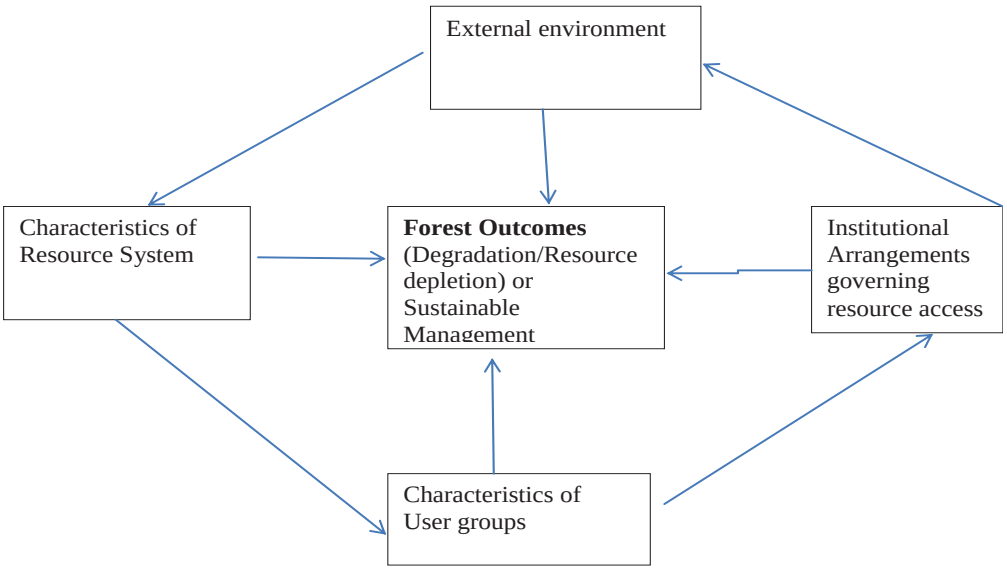

Figure 2. Conceptual framework for analysing the impact of local management institutions on Common Pool Resources management (Heltberg, 2001).

Theoretical framework of analysis

Wade (1988), Ostrom (1990) and Baland and Platteau (1996) suggested favorable conditions for sustainable governance of common pool resources. Agrawal (2001) summarized these factors, and identified four critical factors for sustainable governance of Common Pool Resources which are: characteristic of the resource system, user group, the institutional arrangements, and the external environment (Figure 2).

This paper draws on Agrawal's (2001) synthesis and relates to the study of mopane worms in the south east Lowveld of Zimbabwe. Institutions are important parameters for effective governance of the forest commons. Institutions are defined as a set of accepted social norms and rules for making decisions about resource use. Institutions define who controls the resource, how conflicts are resolved, and how the resources are managed. In addition they shape the resource users' actions and expectations. For sustainable common pool resource governance rules should be easy to understand and enforce, and should hold users accountable for their actions (Agrawal, 2001). The external environment deals with demographic, cultural, technological, market related factors and their influence on resource access and use.

Data collection

The research was carried out from December 2009 to March

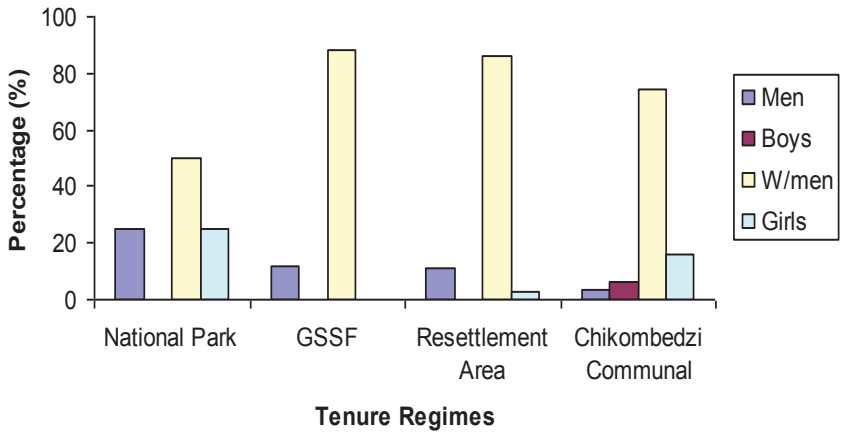

Figure 3. Gender of Harvesters found in the four tenure regimes.

2010, in Gonarezhou National Park (GNP), Gonakudzingwa Small Scale Commercial Farms (GSSCF), Chikombedzi Communal Area (CCA) and Mwenezi Resettlement Area (MRA) in the South East Lowveld, Zimbabwe. The period December 2009 to March 2010 corresponds to mopane worm eruption periods in the study area. Questionnaires were administered on 108 harvesters in all the study areas, 31 harvesters in Chikombedzi Communal Area (CCA), 37 harvesters in Mwenezi Resettlement Area (MRA), 26 harvesters in Gonakudzigwa Small Scale Commercial farms (GSSCF) and 14 harvesters in Gonarezhou National Park (GNP).

The harvesters were followed to harvesting areas and interviewed on the ground by the researcher whilst harvesting. The researcher and mopane worm harvester interaction on the ground helped to valid the correctness of information which was provided by the harvester. Data on quantities of mopane worms harvested, distances covered by harvesters from their homes, the time harvesters spend harvesting, harvesting period, rules governing access, enforcement mechanisms in place and frequency of harvesting were collected from 108 harvesters in the four tenure areas studied. Convenience sampling of harvesters was done and any harvester encountered in the study area harvesting mopane worms was interviewed. Harvesters moved in small groups of about 5 to 15 individuals and were difficult to get due to the size and remoteness of the area.

However, in very few cases harvesters moved alone and were treated as such. In all cases, information regarding where harvesters could be located was obtained through the National Parks Office in the case of Gonarezhou National Park, homestead in the case of Gonakudzingwa Small Scale farms or sabhuku (kraal head) in the case of Chikombedzi Communal and Mwenezi resettlement areas. In the field, getting information on the next group of harvesters was through asking the last group if they had seen other harvesters in the area (snow ball method). The quantity harvested (wet mass) by each harvester was weighed in kilograms (kg) using a 40 kg Dahongying scale. Quantities of mopane worm harvested were analyzed using one way analysis of variance (ANOVA).

RESULTS

Socio-demographic data

Mopane worm harvesting in the study area was mainly a female activity (Figure 3). In Gonarezhou National Park about 55% of harvesters were women, 80% in Gonakudzingwa Small Scale Farms, 80% in Mwenezi Resettlement and 65% in Chikombedzi Communal. The majority of the mopane worm harvesters (27.8%) were aged between 30 to 40 years, whilst (4.6%) of the harvesters were above 70 years of age (Table 1). Results showed that 13% of the mopane worm harvesters harvested mopane worms for own use (consumption) whilst 15.7% harvest for sale and 71.3% for both consumption and for sale (Figure 4)

Quantities of mopane worm harvested in the four tenure regimes

Table 2 shows descriptive statistics, the mean, standard deviation and 95% confidence intervals for the dependent variable (Mopane worms harvested) for each separate group (Gonarezhou National Park (GNP), Gonakudzingwa Small Scale Farms (GSSCF), Mwenezi Resettlement Area (MRA) and Chikombedzi Communal Area (CCA). There were significant differences ($p<0.000$) in the mean quantities of mopane worms harvested per day between the different tenure regimes (Table 3).

Multiple comparisons table

Multiple comparison analysis (Table 4) showed that there were significant differences in the quantity of mopane worms harvested between Gonarezhou National Park and Chikombedzi Communal Area (p = 0.036), Gonakudzingwa Small Scale Commercial Farms and Chikombedzi Communal Area (p = 0.000), Mwenezi Resettlement Area and Chikombedzi Communal Area (p = 0.000). However, there were no differences between the Gonarezhou National Park, Gonakudzingwa Small Scale Commercial Farms and Mwenezi Resettlement Area. The results showed that there were significant

Table 1. The percentage of mopane worms harvested by different age groups.

Age of mopane worm harvester	Percentage
Less than 30 years	13.0
30 - 40 years	27.8
41- 50 years	21.3
51 – 60 years	18.5
61 – 70 years	14.8
More than 70 years	4.6

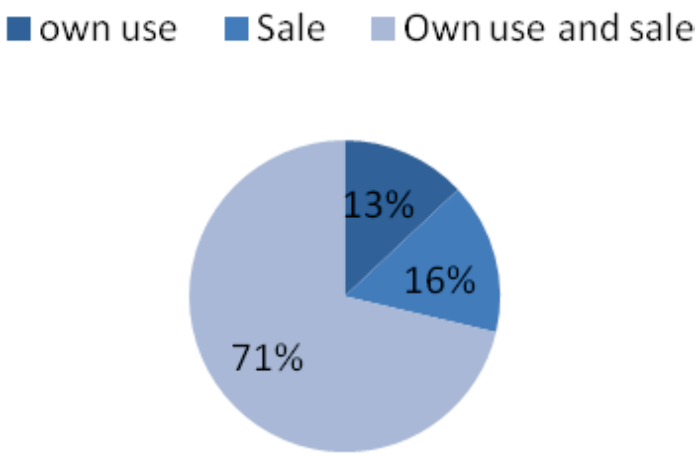

Figure 4. Reasons for harvesting mopane worms in the four tenure regimes

difference between groups as determined by one-way ANOVA (F (3.104) = 13.302, p = 0.000).

A Tukey post-hoc test revealed that the quantity of mopane worms harvested per day was statistically significantly lower after taking the Gonarezhou National Park (20.9 ± 7.7 min, p = 0.036) and Gonakudzingwa Small Scale Farms (32.31 ± 6.33 min, p = 0.000) compared to Mwenezi Resettlement Area (33.43 ±5.80 min). Similar results were found through the Games – Howell analysis.

The rules and regulations governing mopane worm harvesting in the four tenure regimes of South East Lowveld, Zimbabwe

In Gonarezhou National Park (state property) and Gonakudzingwa Small Scale Farms (Private Property) rules governing mopane worm harvesting and access existed. The majority of harvesters indicated that there were no rules governing the harvesting of worms in Chikombedzi Communal Area and Mwenezi Resettlement Farms.

Thirty percent of the respondents in Gonarezhou National Park (GNP) indicated that harvesters of mopane worms should seek for permission from the parks authority prior to harvesting taking place. Whilst (70%) of the respondents said that a permit issued upon payment by the National Parks is required before harvesting of mopane worms or any other products from the park estate (Figure 5). Ten percent of mopane worm harvesters in Gonakudzingwa Small Scale Farms (GSSCF) suggested that permission to harvest the worms should be sought from the farm owner before any harvesting could be done whilst about 5% indicated that any outsider harvester is required to pay to the farm owner first before any harvesting could resume.

The majority (85%) of mopane worm harvesters in (GSSCF) said that tree cutting as a harvesting method is not allowed by the farm owners. In Mwenezi Resettlement Area (MRA), about 95% of the respondents indicated that a permit issued by the occupier of the farm is required before any harvesting whilst 5% indicated that outsiders had to pay first before they could be allowed to harvest the worms. Meanwhile thirty percent of the respondents in Chikombedzi Communal Area (CCA) confirmed that harvesters needed to seek permission from the “owner” where harvesting is taking place whilst 70% indicated that harvesters should report or seek permission from the Sabhuku (Village Headman).

However, a strong relationship existed between tenure regime and the presence of rules governing mopane worm harvesting (χ^2 =54.456; p=0.000; DF=3). There were also significant differences (χ^2 =111.846; p=0.000; DF=12) between rule type and tenure regime where harvesting of mopane worms is taking place. In addition, the research found out that there were strong relationship between tenure regime and the person giving the rules (be it park official, farm owner, or sabhuku in the case of Chikombedzi or Mwenezi (χ^2 =118.000; p=0.000; DF=9). The results showed that a strong association existed between tenure regime and the propensity of rule breaking. The majority of offenders preferred to harvest in Gonarezhou National Park or Mwenezi than in Chikombedzi Communal (χ^2 =82.864; p=0.000; DF=9). The private farms of Gonakudzigwa were the most preferred source of mopane worms compared to Mwenezi and Gonarezhou (χ^2 =38.554; p=0.000;DF=12).

Table 2. Tenure versus the quantities of mopane worms harvested.

Descriptive statistics								
Mopane worm harvested per day (kg)								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
GNP	14	30.0000	18.11077	4.84031	19.5432	40.4568	7.00	65.00
GSSCF	26	41.3462	28.02562	5.49628	30.0264	52.6659	10.00	87.00
MRA	37	42.4649	31.02680	5.10077	32.1200	52.8097	8.00	84.00
CCA	31	9.0323	3.63762	.65334	7.6980	10.3665	3.00	19.00
Total	108	30.9833	27.61098	2.65687	25.7164	36.2503	3.00	87.00

Table 3. Anova output on mean quantities of harvested mopane worms in different tenure regimes

Anova table					
Mopane worms Harvested per day (kg)					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22620.513	3	7540.171	13.302	0.000
Within Groups	58952.697	104	566.853		
Total	81573.210	107			

DISCUSSION

The findings that the majority of harvesters were women is in agreement with findings of Kozanayi and Frost (2002) who reported that more than 70% of mopane harvesting and processing has traditionally been a women and children activity. Our findings indicated that in Gonarezhou National Park about 55% of harvesters were women, 80% in Gonakudzingwa Small Scale Farms, 80% in Mwenezi Resettlement and 65% in Chikombedzi Communal.

Mopane worm play an important role in the nutrition of rural communities as they provide them with the vital crude proteins (63.5%), crude fats (18%), carbohydrates 11.4 (g/100g), minerals 3.5 (g/100g) and 543 of energy (kcal/100g), (Hobane 1994, 1995; Defoliart, 1995). Mopane worms have considerable potential for alleviating nutritional inadequacies in poor rural communities.

An important distinction must be made between the common pool resources and the management regimes under which the resources are held. Resource management regimes are often based on the basis of property rights under which the resources are held. In the study area, there are four types of property rights; which are semi-state property (with some open access rights), common property, private property and state ownership (Heltberg, 2001).

The categorization of property rights regimes into four broad categories may be misleading giving a misconception that there are clear cut divisions between the property regimes, yet there is often overlap across regimes. Different tenure systems can apply in one locale simultaneously or at different times, for example, at one time it's a grazing area where locals harvest mopane worms, and at the other it's someone's crop field. Classification of natural resources under the broad common property regimes is therefore a theoretical ideal as this study in the South East Lowveld of Zimbabwe has shown that there are common-property-like and open-access-like scenarios in mopane worm harvesting and management.

Property rights regimes perform the function of limiting use, coordinating users and responding to changing resource condition. Thus management regimes have two main functions of flow and stock management. They define and enforce rules of resource access (flow management) and limit aggregate output from the resource to ensure continued benefits (stock management) into the future. Common pool resources are natural resources for which it is difficult to exclude potential users and which can be depleted through over–use (McKean, 2000), like in the case of mopane worms. Most of common property resources which are found in southern Africa (including Zimbabwe) are largely held under common property arrangements, (Mutenje, et al 2010), like those found in Chikombedzi. Common property resources belong to the community, and access rules are defined with respect to community membership.

In tenure regimes where there is some form of ownership of the land for example in Gonarezhou National Park (state property) and Gonakudzingwa Small Scale Farms (Private Property) rules governing mopane

Table 4. Multiple Comparisons Analysis

Multiple comparisons							
Dependent variable: Mopane worms harvested per day (kg)							
	(I) Tenure	**(J) Tenure**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Tukey HSD	GNP	GNP					
		GSSCF	-11.34615	7.89250	0.479	-31.9539	9.2616
		MRA	-12.46486	7.47060	0.346	-31.9710	7.0413
		CCA	20.96774*	7.66649	0.036	.9501	40.9854
	GSSCF	GNP	11.34615	7.89250	0.479	-9.2616	31.9539
		GSSCF					
		MRA	-1.11871	6.09281	0.998	-17.0274	14.7900
		CCA	32.31390*	6.33148	0.000	15.7820	48.8458
	MRA	GNP	12.46486	7.47060	0.346	-7.0413	31.9710
		GSSCF	1.11871	6.09281	0.998	-14.7900	17.0274
		MRA					
		CCA	33.43261*	5.79706	0.000	18.2961	48.5691
	CCA	GNP	-20.96774*	7.66649	0.036	-40.9854	-.9501
		GSSCF	-32.31390*	6.33148	0.000	-48.8458	-15.7820
		MRA	-33.43261*	5.79706	0.000	-48.5691	-18.2961
		CCA					
Games-Howell	GNP	GNP					
		GSSCF	-11.34615	7.32377	0.420	-31.0567	8.3644
		MRA	-12.46486	7.03182	0.301	-31.3118	6.3821
		CCA	20.96774*	4.88420	0.004	6.7013	35.2342
	GSSCF	GNP	11.34615	7.32377	0.420	-8.3644	31.0567
		GSSCF					
		MRA	-1.11871	7.49846	0.999	-20.9615	18.7241
		CCA	32.31390*	5.53497	0.000	17.1182	47.5096
	MRA	GNP	12.46486	7.03182	0.301	-6.3821	31.3118
		GSSCF	1.11871	7.49846	0.999	-18.7241	20.9615
		MRA					
		CCA	33.43261*	5.14245	0.000	19.6038	47.2614
	CCA	GNP	-20.96774*	4.88420	0.004	-35.2342	-6.7013
		GSSCF	-32.31390*	5.53497	0.000	-47.5096	-17.1182
		MRA	-33.43261*	5.14245	0.000	-47.2614	-19.6038
		CCA					

*. The mean difference is significant at the 0.05 level.

worm harvesting and access existed. In tenure regimes where there was no real ownership of the land, the majority of harvesters indicated that there were no rules governing the harvesting of worms that is, in Chikombedzi Communal Area and Mwenezi Resettlement Farms.

In Gonarezhou National Park, the Parks and Wildlife Act of 1975 entrusts the management of the park to the National Parks and Wildlife Authority. Access to mopane worm harvesting in the park is made possible through the

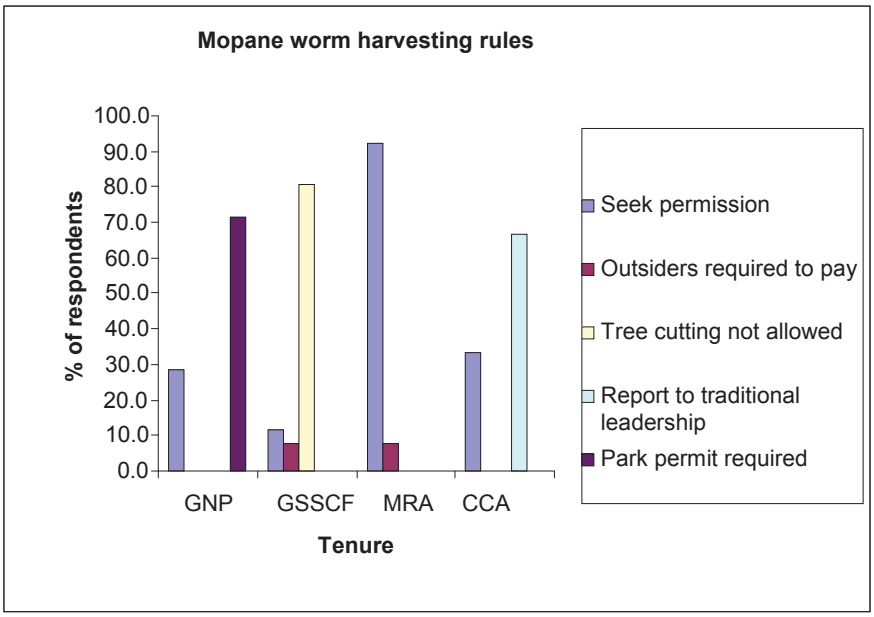

Figure 5. Rules governing mopane worm harvesting in the South East Lowveld.

use of the permit. The park authority has got the power and authority to exclude those people who do not follow their management rules. The major problems faced by harvesters in Gonarezhou are the high fees charged by the park authority to the harvesters about $2 per day. Even people, who live adjacent to the park, have to pay the same fees as people from other areas far from the park. Due to the fee structure, other harvesters sneak into the park to harvest without making a payment. On the other hand the park authority is not able to efficiently patrol the park because of the area it had to cover and secondly because of insufficient financial and material resources. A user friendly and sustainable policy framework should be put in place to enable free access of the local people into the park. Local people feel that they should have a stake in the management of natural resources in the park rather than the current menial benefits, for example, thatch grass and meat from problem animal control operations, which are far from meeting their needs.

In Gonakudzingwa Small Scale Commercial Farms, the farm owner is the sole rule giver with access, withdrawal, management, exclusion and alienation rights. Upon granting of harvesting permission by the farm owner, the mopane harvesters are informed accordingly and tree cutting as a method to harvest worms is not permitted across all the tenure regimes. Any offenders caught would be expelled from the farm or the axe confiscated. In practice it's difficult for farm owners to properly enforce the rules because of many resource constraints which militate against them. In extreme cases farm owners report the violations to the police.

Mwenezi is a resettlement area (semi-state regime) with both elements of state and private property. The majority of mopane worm harvesters in Mwenezi indicated that there were no mopane worm harvesting rules. The new farmers in Mwenezi had the access and withdrawal rights to mopane worms found in their area but do not have full management and exclusion rights to outsiders. The locals and the local leadership in Mwenezi had little or no power to exclude outsiders to harvest mopane worms, the same situation is prevailing in Chikombedzi communal area. The locals could neither sell, transfer or mortgage state property. In addition farmers in Mwenezi got their farms through the land reform program of 2002. The major point therefore, relates to security of tenure. In addition the natural resource governance in Mwenezi is affected by the wrangles between two traditional chiefs in the area, Chiefs Mpapa and Chitanga, over the control of the resettlement area. Mopane worms in Chikombedzi are treated as an open-access resource (no controls over access or use). Open access property regime implies different property-rights governing access to and use of the resources.

In Chikombedzi, villagers had usufruct rights of access and withdrawal to mopane worms found in the village. These villagers had management rights to mopane worms around their immediate homesteads and graveyard areas. However, the rights of Chikombedzi villagers to exclude others are weak and in most cases non-existent. People from other villages had to seek for permission from the local headman (Sabhuku) to harvest mopane worms as a matter of formality. Due to the nature of the statutes that govern access to land and natural resources, the headmen had no full powers to

deny people access to the mopane worms. Our findings are consistent with other studies that some Non Timber Forest Products were being depleted rapidly than others for various reasons (De Beer and McDermott, 1996); Chaudary (1998); Olson (1998) and IFAD (1999). Natural resources with high economic value are depleted more rapidly than resources with low economic value (Schlager and Ostrom, 1992). Hardin's (1968) view that resources being freely accessible to all leads to competition between users in the pursuit of maximizing their personal benefits is related to our findings in the South East Lowveld of Zimbabwe.

Conclusions

The objective of this paper was to examine the rules and regulations governing mopane worm (mw) access in South-Eastern Lowveld of Zimbabwe and their effect on sustainable management. The study results show that there is a correlation between the quantities harvested to protection of the property regimes from wantom harvests especially in Gonarezhou National Park and Mwenezi Resettlement Area.

In general common property resources belong to the community as in the case of Chikombedzi where rules of access are defined with respect to community membership. In Chikombedzi, rules of conservation are difficult to regulate and access is limited to community membership where rules are unenforceable. Such property regimes form good candidates for resource degradation. In this study we managed to show that the rules governing the harvesting and access to mopane worm management are in place in the Gonarezhou National Park and Gonakudzigwa private farms. But such rules do not exist in Chikombedzi communal and Mwenezi resettlement area where mopane worm is treated as open access resource. Further work is needed to establish how the rules could be used to reduce conflict on natural resources in the south east lowveld so that there are "win win" outcomes between state, private, resettlement and the communal people.

Conflict of Interest

The authors declare that they have no conflict of interest.

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