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Full Length Research Paper

An efficient protocol for indirect somatic embryogenesis and shoot organogenesis from leaf segments of date palm (*Phoenix dactylifera* L.) CV. Quntar

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Received 4 November, 2014; Accepted 21 February, 2015

The establishment of an efficient protocol for plantlets regeneration and micropropagation from leaf cultures of date palm cv. Quntar is reported. The two types of leaflet segments used in the experiment (First piece of leaf base which have white color, and second piece of leaf tip which have green color), were taken from micro propagated shoots of date palm cv. These leaf segments were *in vitro* culture on MS media containing various concentrations of 2,4-dichlorophenoxyaceticacid at 1.0 mgl^{-1} and benzyl adenine (BA). Maximum induction of callus was obtained from piece of leaf base (white) in medium supplemented with 5.0 mgl^{-1} 2,4-D and 1.0 mgl^{-1} (BA), as compare with [piece of leaf tip (green)] which did not show response for callus formation. Using culture system with temporary tissue immersions "bioreactor" in Somatic embryo production enriched with 1.0 mgl^{-1} 2,4-D + 500 mgl^{-1} active charcoal (AC) gave the highest number of somatic embryos (172 embryos). These embryos formed from 500 mg initial callus. As well as, the short immersions frequently repeated, 1 min immersions every 4 h, led to the largest quantities of embryos. Indirect shoot organogenesis was achieved from the embryogenic callus using 3.0 mgl^{-1} 2iP + 1.0 mgl^{-1} NAA, where highest number of shoots (8.28 ± 0.71) with maximum frequency (58.34%) was regenerated. The addition of 3 gml^{-1} Gelrite to medium was optimal for the shoots proliferation. The balance of auxin to cytokinin in medium affected on the growth of the date palm tissue. The shoots were successfully rooted (80%) with rapid elongation when cultured on MS medium containing 0.5 mgl^{-1} GA_3 + 1.0 mgl^{-1} NAA. Rooted shoots were successfully acclimatized when planted in plastic pots containing a mixture of peat moss and perlite (2:1) with 80% success.

Key words: Date palm, histology, indirect shoot organogenesis, leaf segment, somatic embryogenesis, temporary immersion 'bioreactor'.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) cultivation is one of the most economically-important activities in the arid zones of the Middle East and North Africa (El Hadrami and Al-Khayri, 2012). One of the major problems in date palm

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cultivation that prevents rapid crop improvement is the lack of an adequate method of asexual propagation.

Traditionally, date palm is propagated vegetatively by offshoots produced by desirable trees. The low rate of propagation (1-20 offshoots/ tree, depending on the variety) has limited the multiplication of healthy plants (Tisserat, 1982). To overcome the propagation problems and to maintain the germplasm, the *in vitro* micropropagation. Large-scale plant production through cell tissue and embryo cultures using bioreactors is promising for industrial plant propagation. Bioreactors are usually described in a biochemical context as self-contained, sterile environments which capitalize on liquid nutrient or liquid/air inflow and outflow systems, designed for intensive culture and according maximal opportunity for monitoring and control over micro environmental conditions such as agitation, aeration, temperature, dissolved oxygen and pH (Paek et al., 2005). The application of liquid cultures for micropropagation in bioreactors using the embryogenic and organogenic regeneration pathways is becoming a more efficient alternative system for scale-up and automation *in vitro* (Ziv, 1995; Tahardi et al., 2003; Ducos et al., 2007). However, somatic embryogenesis appears to be the most appropriate for an automated system in liquid medium (Etienne et al., 2006). The cultivation in liquid media using a temporary immersion system with different frequencies of immersion was reported to improve plant quality and multiplication rates of banana and coffee (Alvard et al., 1993; Teisson and Alvard, 1995). In addition, Othmani et al. (2009) demonstrated the applicability of embryogenic suspension culture for high output of date palm somatic embryos using temporary immersion bioreactor (TIB) system. The objective of the present study is to develop the use of other possible explants (leaf segments) as the sources of *in vitro* culture for date palm cv. Quntar, as well as development of somatic embryos in culture system with temporary tissue immersions "bioreactor", and optimize the protocol for indirect shoot.

MATERIALS AND METHODS

Plant material

The experiment was carried out at the Plant Tissue Culture Laboratory, Basra University, Iraq. Two types of leaf segments (piece of leaf base which has white color, and piece of leaf tip which has green color) (Figure 1A and B). These pieces were taken from plantlets of date palm (*P. dactylifera* L. cv. Quntar) produced in *in vitro* culture. After, the larger cut of leaf was trimming into segments at 1 cm length. To induce callus, these leaf segments were horizontally cultured directly in Murashige and Skoog, 1962).'MS medium', with additional 100 mgl^{-1} glutamine, 5 mgl^{-1}thiamine HCl, 1 mgl^{-1} biotin, 40 mgl^{-1}adenine sulfate, 30 gl^{-1} sucrose, 2.0 gl^{-1} activated charcoal. Also the media were supplemented with different concentrations of 2,4-D (0.0, 1.0, 2.5 and 5 mgl^{-1}) combined with 1 mgl^{-1} BA. The pH of medium was adjusted to 5.8 before adding 7 gl^{-1} agar. The media were sterilised by autoclaving at 1.1 kg cm^{-2} (121°C) for 20 min. All the cultures were incubated at 27±2°C in darkness. The frequency of explant producing callus and fresh weights (gm) of callus were recorded for callusing explants after 6 weeks. The primary calluses were chopped and transferred on the same medium, after two subcultures with six weeks interval, embryogenic calluses were used for the installation of embryogenic suspension cultures and indirect shoot organogenesis .

PLANT DEVELOPMENTAL PATHWAYS FROM EMBRYOGENIG CALLUS

Somatic embryogenesis

Effect of culture media composition in culture system with temporary tissue immersions "bioreactor" on Somatic embryo production

Embryogenic callus pieces (500 mg), [developed from piece of leaf base which has white color of date palm (cv. Quntar)] were culturing in bioreactor with 400 ml liquid medium (Figure 2A). The culture medium contained MS basal salts, 170 mgl^{-1} KH_2PO_4, 10 mgl^{-1} thiamine-HCl, 200 mgl^{-1} glutamine, 30 gl^{-1} sucrose and 0.500 gl^{-1} AC. This medium supplemented with different concentrations of 2,4-D (0.0, 0.5, 1.0 and 2.0 mgl^{-1}). A temporary immersion system (TIS) was conducted using bioreactor. The callus in bioreactors remained in their culture containers at 5 weeks. The fresh mass of callus was evaluated at every subculture. The immersion duration for the latter was 3 min every 8 h. Once the best combination of culture medium was determined, a second experiment was carried out, to evaluate the effect of the immersion period on somatic embryo development as follows: 1 min every 4 h, 5 min every 8 h and 10 min every 12 h (Figure 2B). In both two experiments, three bioreactors containers were used (Figure 2C). The suspension cultures were kept under 16 h light photoperiod (30 µmol m^{-2} s^{-1}) at 27 ± 2°C. For both experiments, the total number of somatic embryos, was determined after 45 days from culture.

Germination of the somatic embryos

Matured somatic embryos of date palm were transferred to MS medium enriched with 0.1 mg l^{-1} NAA. The MS basal medium was added with 30 g/L sucrose, pH 5.7 and solidified with 6 gl^{-1} Agar for shoot and root induction. The cultures were incubated in the culture room at 27 ± 2°C with 16 h light and 8 h dark.

Organogenic pathway

Indirect shoot organogenesis from leaf derived callus

For shoot regeneration, embryonic callus obtained from the above experiments was transferred to MS medium supplemented with various of cytokinins (BA alone, 2iP alone or in combination with auxin NAA, for each experiment a minimum of 12 replicates. The data was recorded after 12 weeks from culturing as following: The percentage of response of callus mass on shoot regeneration; number of regeneration shoots /explant. For improvement of shoot regeneration, the medium was optimized by testing the effect of different concentrations of gelling agents (6, 7, and 8 gl^{-1} agar or 2, 3, and 4 gl^{-1} Gelrite). Cultures were maintained at 27 ± 2°C in a growth chamber with a 16 h photo-period under standard cool white fluorescent tubes (35 µmol s^{-1} m^{-2}) and 60 to 70% relative humidity.

Elongation and rooting

Regeneration shoots were transferred to the MS medium

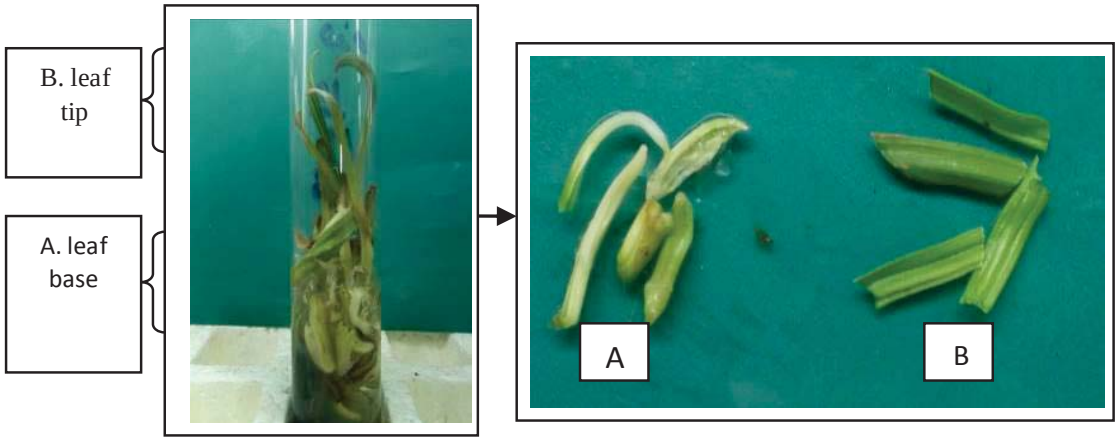

Figure 1. Adventitious shoot induced from apical bud of date palm (*P. dactylifera* L. cv. Quntar)" Two types of leaf segments are used in this study": A) piece of leaf base which has white color. B) Piece of leaf tip which has green color.

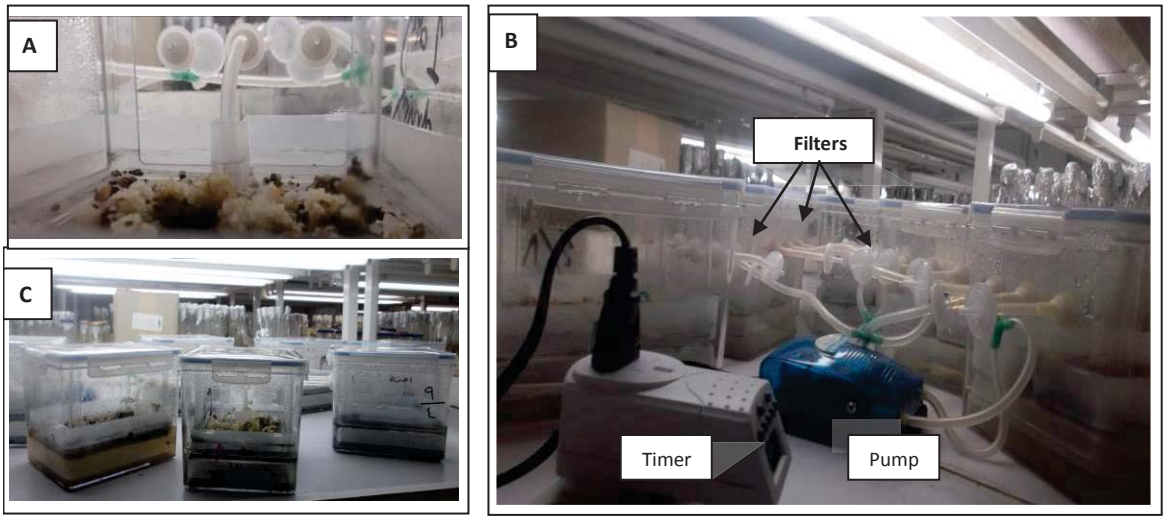

Figure 2. (A) Date palm callus cv. Quntar cultured in bioreactor. (B) The timer organize the immersion periods through the pump in bioreactor. (2-C) Bioreactors that used for induction of somatic embryo from culture of embryonic callus.

supplemented with 0.5 mgl^{-1} GA_3 + 1.0 $mg1^{-1}$ NAA for elongation and rooting.

Acclimatization

All the produced plantlets from indirect regeneration via somatic embryogenesis and shoot organogenesis were acclimatized. The plantlets were gently removed from the vessels, washed initially to remove adhered gelling agent and traces of the medium to avoid contamination. Then, the plantlets were washed with distilled water and treated with fungicide (Benlet 500 mgl^{-1}) for 20 min and transferred to plastic pots containing autoclaved a mixture of peat moss and perlite (2:1) (AL-Mayahi, 2014). The plants were covered with glass bottles to maintain humidity. The plants were initially irrigated with quarter-strength inorganic salts of MS medium for 2 week followed by tap water. Potted plantlets were grown in culture room (25±2°C, 55±5% RH, under 16 h of photoperiod with a light intensity of 40 µmol m^{-2} s^{-1}) for 45 days. The glass bottles were gradually removed upon emergence of new leaves and acclimatized plantlets were transferred to the greenhouse.

Histological studies

Histological examinations during bud formation were carried out using a freezing microtome. Microtome slide preparation and observation were made following the methods as described by Sarker and Awal (1999).

Experimental design and statistical analysis

A complete randomized design was employed in all of the

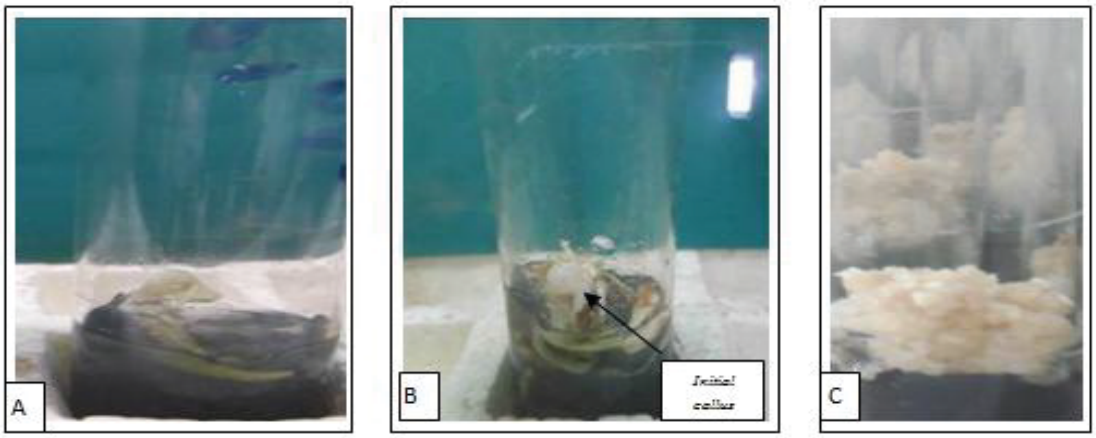

Figure 3. Callus induction from leaf segments of date palm cv. Quntar (A) Swelling of leaf explants. (B) Initial callus tissue in MS 5 mgl-1 2-4,D+1.0 mgl-1 BA (C). granular callus showing the embryogenic structures on same medium after two subcultures with six weeks interval from culture of primary callus.

experiments. Analysis of variance was used to show statistical differences between treatments using the L.S.D. at 5% (Snedecor and Cochran, 1989).

RESULTS

Formation of primary and embryogenic callus

The first evidence of date palm callus induction process was the swollen of leaf segment when it cultured on MS medium supplemented with different concentrations of 2-4,D+ BA after 3 weeks of incubation (Figure 3A). Maximum response callusing response from the [piece of leaf base (white)], reached 53.34% in the medium MS + 5 mgl^{-1} 2-4,D+1.0 mgl^{-1} BA after 5 weeks of incubation (Figure 3B). These same media gave the highest fresh weight of callus reached 1.239 g. (Table 1). The weight in this treatment which was statistically significant compared with the other treatments media.. Also, variations existed between two types of leaves segments in their ability to form callus. The piece of leaf base produced considerably more callus than piece of leaf tip which did not show any response for callus formation (Table 1). When the globular and compact primary callus were cultivated on the same medium. This callus gave rise to friable granular callus composed of embryogenic cells after two subcultures with six weeks interval (Figure 3C). According to the results shown in Table 1, leaves cultured on MS medium supplemented with 5 mgl^{-1} 2-4,D+1.0 mgl^{-1} BA gave better callus formation. The ratio of auxin: cytokinin was critical for inducing callus at a high frequency. Using certain hormonal balance in which the percentage of the auxins is much bigger than the percentage of the cytokenines, results in unbinding and breaking up the cohesive tissue structure of the basic explant, and also in cell division, which led to important swelling in the size of the basic explants, followed by the appearance of the callus tissue. Of all the auxins or auxin-like plant growth regulators, 2,4-D has proven extremely useful, being used in 57.1% of successful embryogenic cultures (Al-Khalifah and Shanavaskhan, 2012). Callus induction has been reported from almost all types of tissues of date palm on various media formulations, which usually contained a combination of an auxin and a cytokinin (Taha et al., 2002; Al-Mayahi 2013). The type of tissue reactions were influenced by many factors but mostly by medium composition and the level of tissue differentiation and lignification (Abahmane, 2013). This good response of explants for callus formation may be due to the efficient selection of cells in an explants (Al-Mallah and Hassan, 1994). Coupled with compatibility between the endogenous hormonal levels and the addition of growth regulator to the induction medium. This may also suggest that levels of endogenous hormones or their sensitivity might vary between organs. These results are similar to those reported by Sane et al. (2012) who confirmed that the aptitude for primary callogenesis appeared to be strongly dependent on the explant nature, the growth regulators used and the genotype.

Effect of culture media composition in culture system with temporary tissue immersions "bioreactor" on Somatic embryo regeneration

The effect of the four liquid media supplemented with different concentrations of 2,4-D in culture system with temporary tissue immersions on somatic embryo formation from embryogenic callus derived from piece of leaf base (white) (Table (2). The highest number of somatic embryos was obtained in media containing 1.0 mgl^{-1} 2,4-D After 45 days of culture reached 172

Table 1. Effect of different combinations of growth regulators on callus proliferation from two types of leaf segments of date palm c.v. Quntar.

Types of leaf segments	Treatments (mgl^{-1})	Induction callus (%)	Fresh weight of callus (g)
Piece of leaf base (white)	0.0	0.0 [c]	0.0 [d]
	(1.0) 2-4,D+ BA(1.0)	6.67 [c]	0.466 [c]
	(2.5) 2-4,D+ BA(1.0)	26.67 [b]	1.017 [b]
	(5.0) 2-4,D+ BA(1.0)	53.34 [a]	1.239 [a]
Piece of leaf tip (green)	0.0	-	-
	(1.0) 2-4,D+ BA(1.0)	-	-
	(2.5) 2-4,D+ BA(1.0)	-	-
	(5.0) 2-4,D+ BA(1.0)	-	-

* ± Standard error (n = 15). *No response. ** Values followed by the same letter are not significantly different at P<0.05.

Table 2. Effect of different 2,4-D concentrations on mean number of somatic embryos per 500 mg callus in a temporary immersion system bioreactor after 45 weeks from culturing of date palm cv. Quntar.

2,4-D conc. ($mg.l^{-1}$)	Number of somatic embryos /500 mg callus
0.0	13.6±0.85 [d]
0.5	26.0 ±0.51[c]
1.0	172.0 ±0.81[a]
2.0	78.0±1.19 [b]

*± Standard error (n = 3) .** Values followed by the same letter are not significantly different at P<0.05.

embryos/ bioreactor (500 mg embryonic callus) (Figure 4A). This treatment was significantly different other treatments after 60 days from culture in system with temporary tissue immersions, (Table 2). Followed liquid media supplemented with 2.0 mgl^{-1} 2,4-D gave rise to only 78 somatic embryos. The germination rates of somatic embryos were 82% after transfer to MS medium enriched with 0.1 mg l^{-1}NAA. (Figure 4C) Also, Figure 4D and E showed the plants proliferation (shoots and roots) in same medium after 60 days from embryos culture.

A histological study showed embryogenic cells are mainly composed of nucleus (N) nucleole (Nu) after 2-3 weeks of culture of the globular callus in the bioreactor. These cells of tissue were in active division (M) showed very small vacuoles and a large quantity of soluble proteins in the cytoplasm stained blue (Figure 4B-1). The somatic embryos started their development of meristematic centers after 6 weeks of culture. Somatic embryos are mainly composed of small compact meristematic cells, characterized by the dense cytoplasm, dense nucleus (N), vacuoles (V), procambium bundles and apical meristem for shoot and root.

Induction of somatic embryos from embryogenic callus cultures of were established in the present study using bioreactor was successfully obtained. Using 2-4,D was important to unbind and break up the components of the tissue structure to separate cell or tissue groups (Margara, 1984). It is well established that 2,4-D plays a role in the induction of somatic embryogenesis which is presumably mediated by a signal cascade triggered by this exogenous auxin (Zuo et al., 2002). Somatic embryogenesis is considered the most efficient regeneration process for date palm micropropagation (Fki et al., 2003). It is reported to be a quick and efficient method for large scale propagation of date palm and could also be highly useful for breeding programs (El Hadrami et al., 1998). The great increment in the number of somatic embryos in culture system with temporary tissue immersions "bioreactor" include better contact between the plant biomass and the medium, no restrictions of gas exchange, the control of the composition of both the medium and the gaseous atmosphere.

The improvement in somatic embryo formation and maturation in culture system with temporary tissue immersions "bioreactor" may be attributed to the increased oxygen diffusion to the liquid medium and eventually to the developing embryogenic cells because of gradually higher agitation as well as air supply in bioreactor. The bioreactor transfers oxygen from gaseous

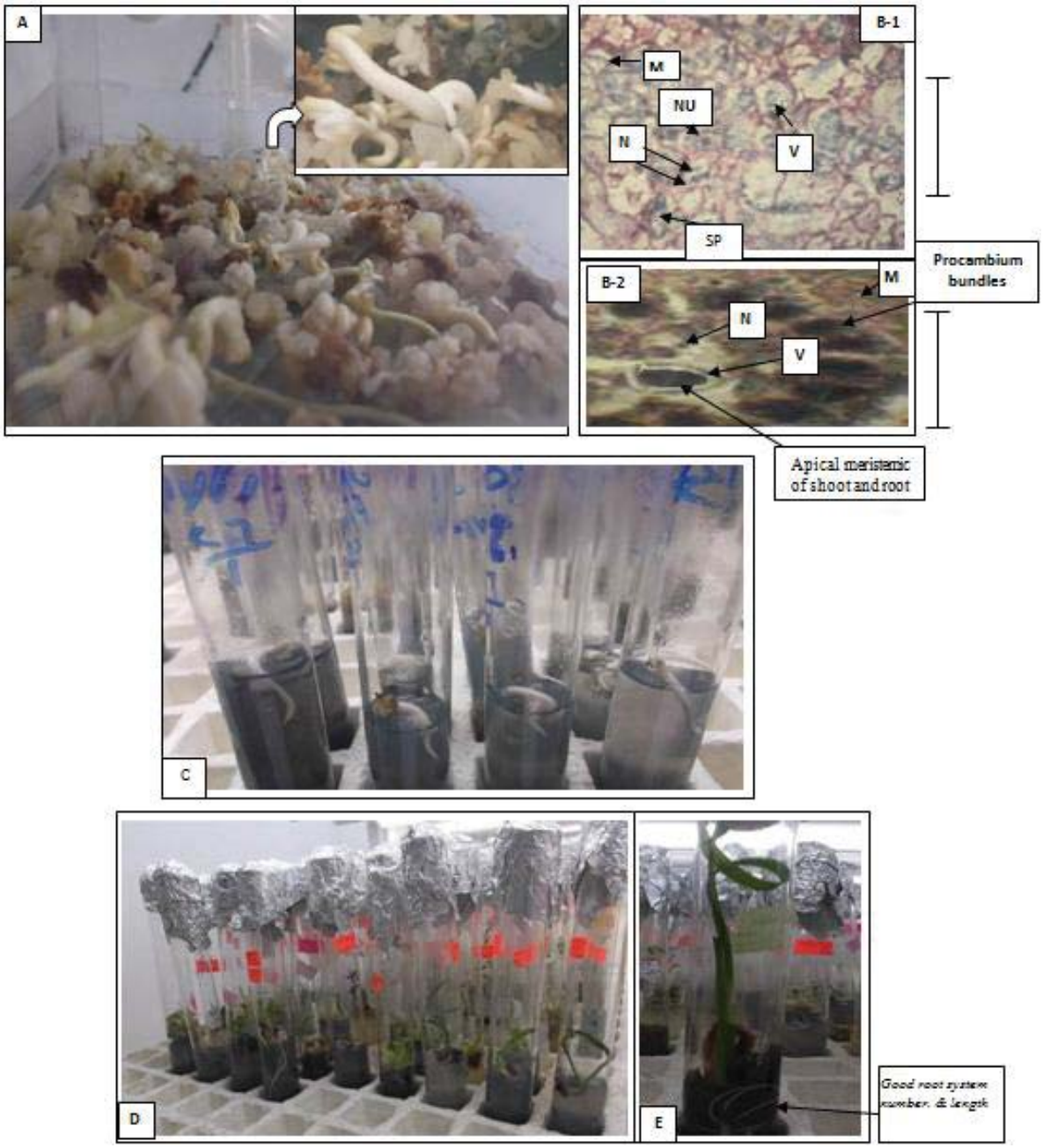

Figure 4. A. Somatic embryos developed from embryogenic callus on MS medium supplemented with 1.0 mgl^{-1} 2,4-D in bioreactor. (B-1) Histological structure of the granular callus showing cells that are very rich in soluble proteins (SP), vacuoles (V), with nucleus (N) and nucleole (Nu). This tissue is in active division (M) (B-2) Somatic embryos are mainly composed of small compact meristematic cells, characterized by the dense cytoplasm, dense nucleus (N), vacuoles (V) procambium bundles and apical meristem of shoot and root. C, D) germination of somatic embryos on MS medium with 0.1 mgl^{-1} NAA. E) Plantlets with good root system after 60 days in same medium.

to the liquid stage, makes oxygen available to cultivating tissues or cells and promotes *in vitro* biomass growth (Sajc et al., 2000; Sane et al., 2006). The optimum presence of several gases like O_2 and CO_2 are very critical for somatic embryo as the requirement varies at different stages of embryo growth (Rosnow et al., 2011). Similar observations have earlier been reported for carrot (Jay et al., 1992). Also, this confirms previous findings reported that temporary immersion culture systems have improved the development of somatic embryos in Musa spp and *C. arabica* F1 hybrid (Caturra x E531), and woody plants (Escalant et al., 1994; Barry-Etienne et al.,

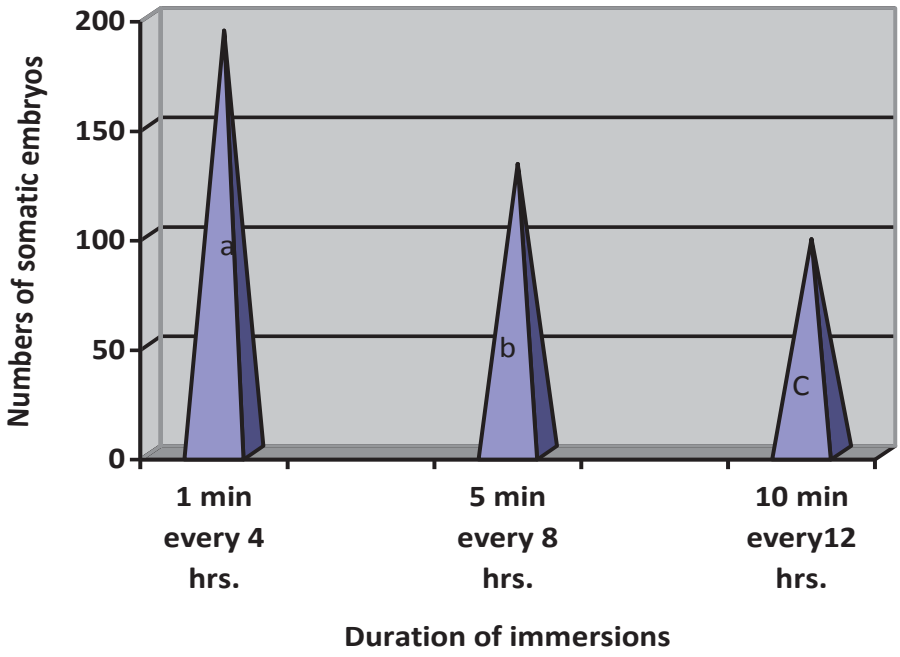

Figure 5. Effect of duration of immersion in bioreactor on regeneration of somatic embryos of date palm cv. Quntar after 45 days of culture in MS medium.

2002; Paek and Chakrabarty, 2003). Ibraheem et al. (2013) reported that the liquid culture system (suspension cultures and RITA® bioreactors), is a promising technique for rapid mass propagation of date palm, somatic embryo production in liquid media is about ten times greater than that on solid media. Ducos et al. (2007), who found that the temporary immersion system has positive effects on micropropagation as somatic embryogenesis for coffee. This system is very efficient, because it reduces the toxicity of phenols emitted by *in vitro* cultivated tissue (Fki et al., 2011). The present study also was demonstrated that the use bioreactor was fostered embryo initiation (Data not shown). This result is in accordance with Ibraheem et al. (2013) who found that the liquid medium accelerated the formation of somatic embryos. These embryos were formed within 3-6 weeks after inoculation in the liquid media. While, they were formed in 12 to 18 weeks on solid medium.

During the transition from embryogenic status to somatic embryogenic status, cells have to dedifferentiate and activate their cell division cycle. Dividing small cells had an embryogenic appearance, with a protein-rich cytoplasm, small vacuoles and a large nucleus. Dividing small cells had an embryogenic appearance, with a protein-rich cytoplasm, small vacuoles and a large nucleus as described in by Sane et al. (2006), the absence of accumulation of reserves during the maturation of the somatic embryos, and finally the development of these embryos into rooted vitro plants (Figure 4B-2).

Effect of the immersion duration on production of somatic embryo

The Figure 5 showed that effect of the immersion period on somatic embryo production of date palm cv. Quntar. The immersion period in the bioreactors significantly affected the formation of somatic embryos. The highest number of somatic embryos, were obtained using an immersion period of 1 min every 4 h. Increasing the immersion duration reduced the number of somatic embryos.

In culture systems with temporary tissue immersions, the immersion time was very important, since it determines nutrient and plant growth regulator uptake and can influence hyperhydricity (Etienne and Berthouly, 2002). Among the different immersion times used for formation of somatic embryos from suspension cultures, it was shown that the largest amounts of somatic embryos. The highest number of embryos were obtained using short immersions (Figure 5). Modifications of immersion frequencies and durations affect the efficiency of the somatic embryo regeneration (Albarran et al., 2005). Short immersions frequently repeated, 1 min immersions every 4 h, led to the largest quantities of torpedo-shaped embryos without hyperhydricity (Ducos et al., 2007). The adjustment of immersion time was critical with this type of bioreactor. According to Teisson and Alvard (1995) with 1 L temporary immersion bioreactors, 15 min' immersion every 6 h led to successive development and germination of coffee

Table 3. Effect of different treatments of plant growth regulators on a response percentage of embryonic callus derived from piece of leaf base for shoot regeneration and number of shoots /100 mg callus after 12 weeks from culturing of date palm cv. Quntar.

Treatments (mgl^{-1})	Response of callus (100 mg weight) for shoot regeneration	Number of shoots/100 mg callus
0.0 (Control)	0.0 [d]	0.0 [d]
3.0 BA	16.67 [b]	1.5 ± 0.21[c]
3.0 2iP	33.34 [b]	2.75 ± 0.39[b]
3.0 2iP+1.0 NAA	58.34 [a]	8.28 ± 0.71[a]

* ± Standard error (n = 12). ** Values followed by the same letter are not significantly different at P<0.05.

embryos, whereas for an identical culture medium, immersions of 1 min every 24 h halted embryo development and stimulated the production of adventive embryos. Besides, in an agitated liquid medium embryogenic callus disaggregated allowing the formation of suspension cultures. This confirms previous findings reported that immersion time, that is, duration or frequency, is the most decisive parameter for system efficiency, the temporary immersion system with other perennial crops which varied from 1 to 15 min immersion every 2 to 12 h (Etienne and Berthouly, 2002). Othmani et al. (2009) reported that the embryogenic callus of date palm cv. Deglet Bey turned brown and died using a RITA® bioreactor with immersion frequency of 5 min every 8 h. The growth is enhanced by forced aeration, since continuous provided sufficient oxygen supply to the tissue, which ultimately led to their faster growth. A similar result was obtained by Albarrán et al. (2005) who showed that short immersion periods (1 min every 12 h) strongly promoted the embryo production in coffee compared to long immersion duration (5 and 10 min every 12 hrs.). Also, this result was in accordance with Gatica-Arias et al. (2008) who showed that the highest somatic embryo of coffee development and fresh weight were obtained with 1 min of immersion every 8 h as compared of immersion with 5 and 10 min every 8 h.

Indirect shoot organogenesis from leaf derived callus

Effect of different treatments of plant growth regulators on a response of embryogenic callus for shoots regeneration

Table 3 showed that from embryogenic callus (100 mg weight) derived from leaf base segments (white) gave the best significant response for shoot regeneration and number of shoot/100 mg weight when was cultured in MS medium supplemented with 3.0 mgl^{-1} 2iP+1.0 mgl^{-1} NAA in comparison with other treatments, reached 58.34% and 8.28 shoots/100 mg weight, after 12 weeks from culture (Figure 6A, B). But, the embryogenic callus cultured in MS medium without hormone (control treatment) did not give any response for shoot regeneration.

The development of adventitious shoots from the undifferentiated callus masses

The process usually occurred after an intervening period of callus growth. Callus is differentiated and unorganized mass of parenchyma cells formed by the proliferation of parent tissue. Callus tissue is a good source of adventitious shoot regeneration (Dodds and Roberts, 1982). The presence of cytokinin along with auxin is necessary for shoot regeneration from callus derived from the leaf explants, a combination of auxin (1.0 mgl^{-1} NAA) and higher cytokinin (3.0 mgl^{-1} 2iP) was the most effective (Table 4). The growth regulators added to medium play an important role in the growth and differentiation of explants (Tisserat, 1984). They might have more metabolically active cells with hormonal and nutritional conditions that are responsible for increased organogenesis (Famiani et al., 1994). The cytokinin-auxin combination has been widely used for organogenic differentiation in various protocols developed for date palm (AL-Mayahi, 2012).

The emergence of the new bud from the primitive meristemic tissue is caused by the superiority of the cytokenines' balance over the auxines', inside the explant's cells, due to the successive cultivation in solution rich with the cytokenines. The importance of the cytokenines in releasing the process of meristems and, consequently, formation, is well-known (Auge, 1984), as well as the importance of the auxin and the cytokenines in enhancing the growth of the date palm buds (Amin, 2001). The formation of the organs in the moncotyledonae is, generally, enhanced by a big and sudden increase in the concentration of the cytokenines (Duhoux, 1988). Organogenesis can be induced by transferring callus or explants to a suitable medium or sequence of media that promote proliferation of shoot, root or both. For investigating the effects of different concentration of gelling agents "agar and Gelrite" on the shoot regeneration of date palm cv. Quntar, in MS medium supplemented with 3.0 mgl^{-1} 2iP+1.0 mgl^{-1} NAA (Figure 7). The best result of shoot proliferation was

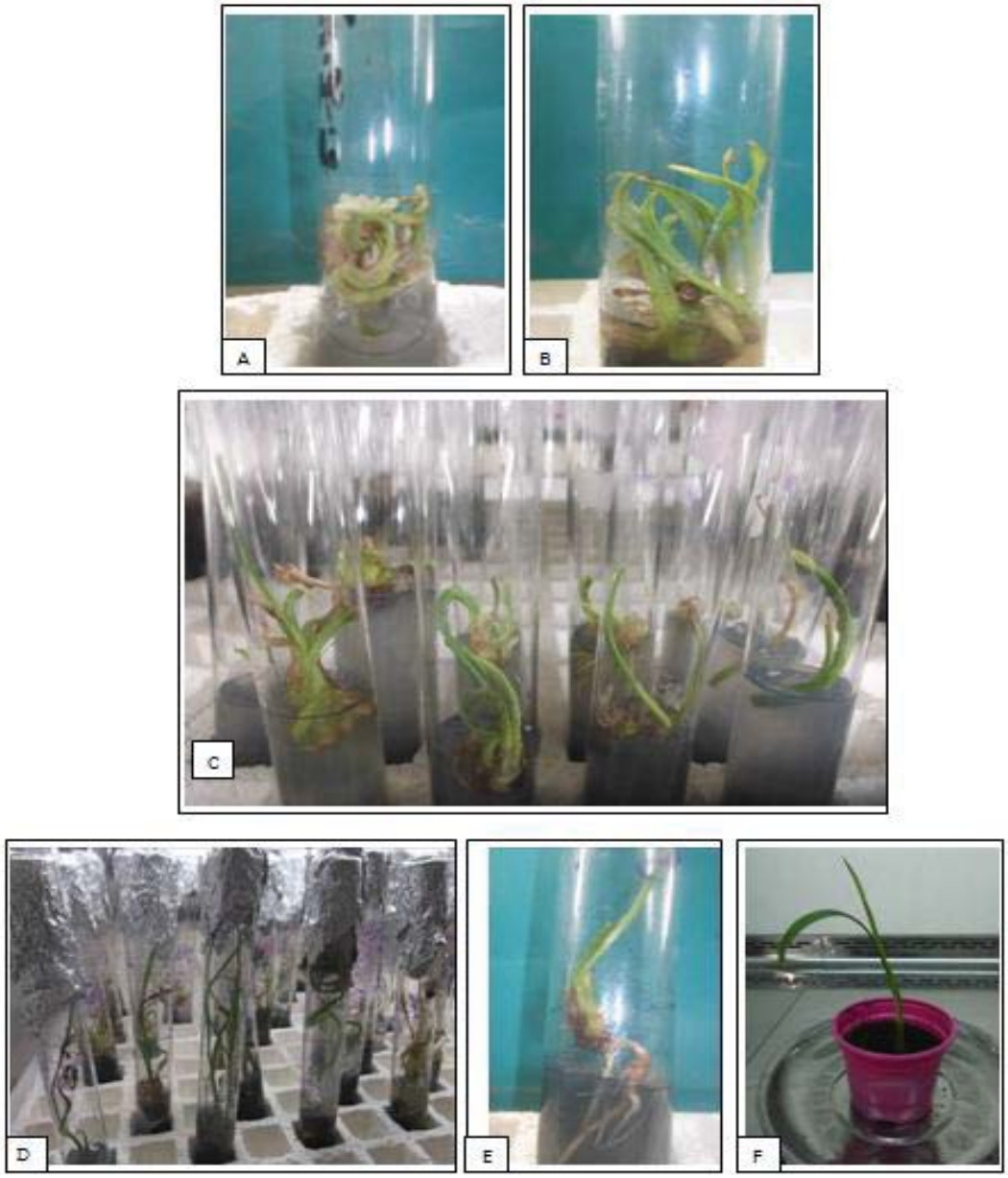

Figure 6. (A, B) Shoot organogenesis from callus derived from leaf segments of date palm cv. Quntar. Shoots regenerated from organogenic callus on MS medium containing 1.0 mgl^{-1} NAA and 3.0 mgl^{-1} 2iP after 10 and 12 weeks from culturing. (C,D,E) separate each shoot from the others and transfer them to the elongation and rooting medium supplemented with 0.5 mgl^{-1} GA3+ 0.1 mgl^{-1}NAA.(F) Hardened plantlet transferred to pot after 45 days.

resulted with 3 gml^{-1} Gelrite. Shoot organogenesis was found to be more efficient when Gelrite was used as the gelling agent as compared with agar. The superiority of Gelrite over agar for the purposes of shoot regeneration has also been reported for apple (Saito and Suzuki, 1999).

Histological investigations revealed the division and growth of callus cells continued for some time resulting in the enlargement of primary callus. Where active cell division occurred, certain cells of the meristems undergo divisions in such a way that, one product of a division becomes a new body cell, called derivative and the other remains in the meristem, called initials (Figure 8-1). Meristemoids formation could be detected on the top of the meristematic area on some peripheral callus sectors. They were constituted of densely cytoplasmic small cells with prominent, enlarged nuclei. Those meristemoids elongated and acquired a well- defined shoot primordia shape (Figure 8-2). The indirect formation of shoot buds from meristematic areas on callus surface. The vascular

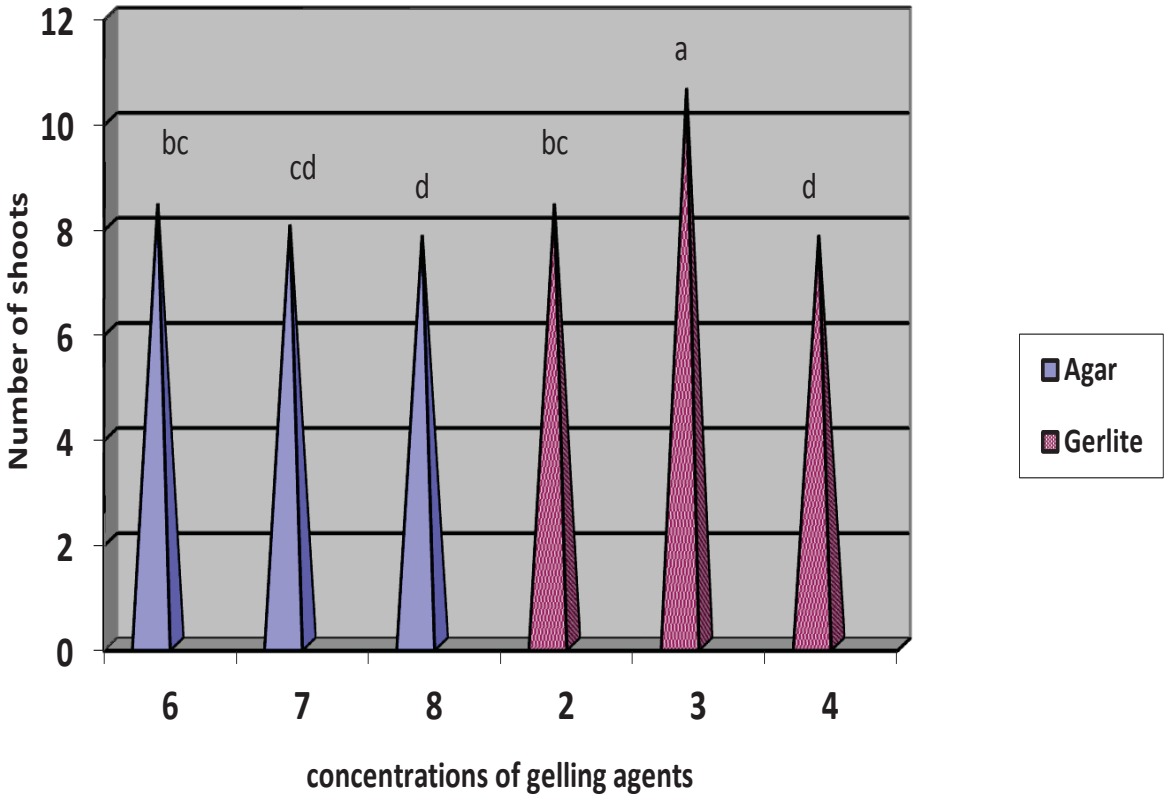

Figure 7. Effect of different concentrations of gelling agents on number of shoots /100 mg callus after 12 weeks from culturing of date palm cv. Quntar, in MS medium supplemented with 3.0 mgl^{-1} 2iP+1.0 mgl^{-1} NAA.

bundles were found initiated in the form of procambium, the cells of which assume a rather narrow elongated form because of the predominance of longitudinal division. The procambium then developed into distinct vascular elements, (Figure 8-3). The adventitious shoot with leaf primordial (L) and shoot meristems (MR) formed (Figure 8-4).

The emergence of the new bud from the primitive meristemic tissue and its transference into bud-generating-tissue, is caused by the superiority of the cytokenines' balance over the auxins. The hormonal balance of the solution made, apparently, the pro meristemic tissue continue their active division which led to an important increase in the mass of this pro meristemic tissue. Consequently, this tissue developed into primitive meristemic tissue, which is characterized by its rapid division and increase in size. The divisions led to the formation of meristematic areas as meristemoids. These consisted of small cells with a dense cytoplasm and prominent nucleus. These structures were more developed. Thus, the adventitious shoot buds were developed from meristemoid areas, as the result of the mitotic activity of cells. Meristemoids differentiated in the callus and are later transformed into cyclic nodules from which shoots or roots develop (Al-Khalifah and Shanavaskhan, 2012).

Elongation and rooting of regenerated shoots

The regenerated shoots, were separated and transferred to the elongation and rooting medium MS + 0.5 mgl^{-1} GA_3 + 1.0 mgl^{-1} NAA (Figure 6C, D, E). The shoots rooted successfully (80%) with rapid elongation, after 6 weeks of culture (Figure 6D, E).

Acclimatization

The plantlets (produced from both two pathways), were acclimatized successfully in a mixture of peat moss and perlite (2:1), reached 80% after 10 weeks of transferred to plastic pots (Figure 6F). All the micropropagated plants were free from external defects.

Conclusion

An efficient protocol for high frequency of plant regeneration of date palm cv. Quntar through indirect

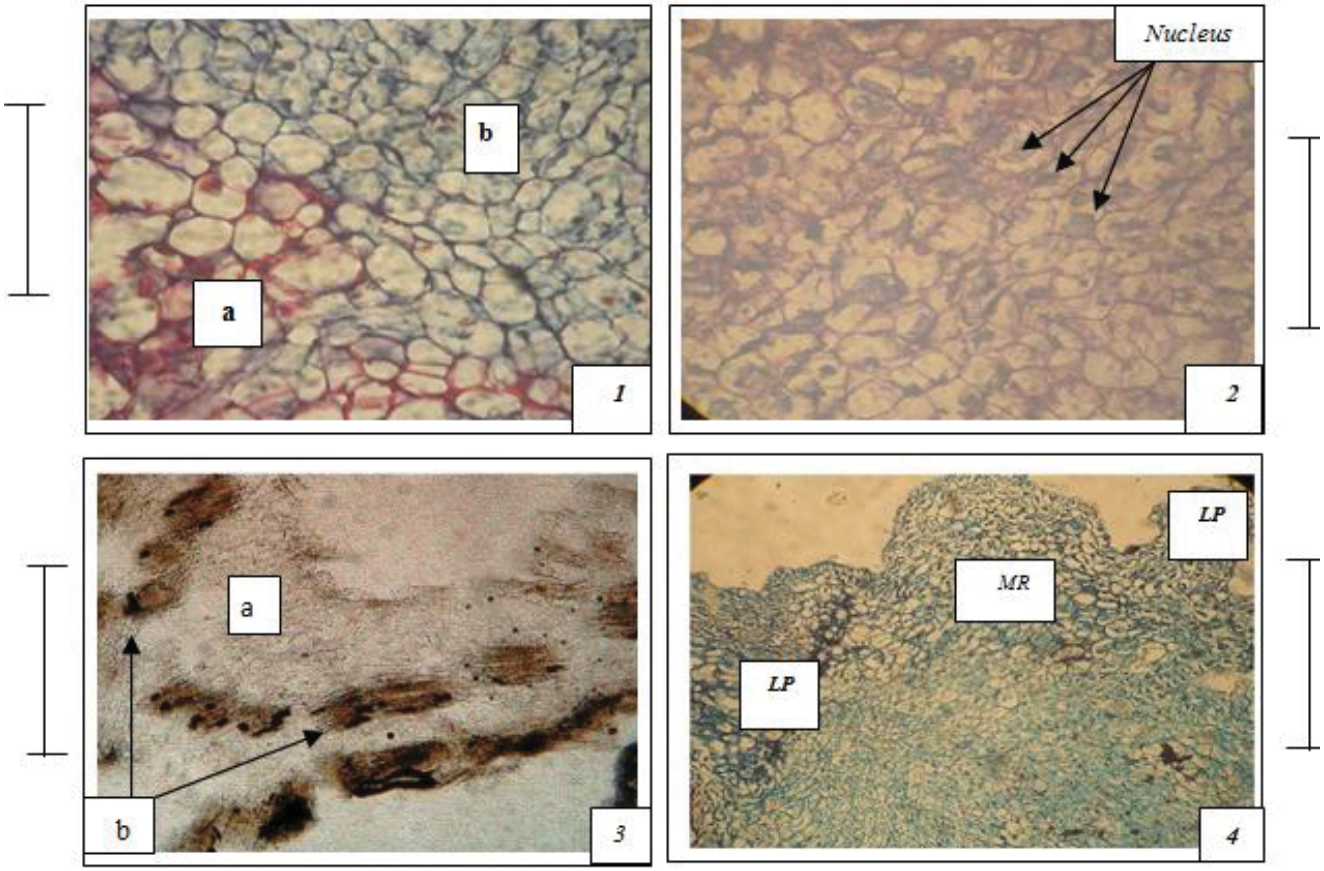

Figure 8. Histological of adventitious shoots developed from leaves derived callus of date palm cv. Quntar 8-1) Meristematic zone showing divided cells (a) and initials (b). 8-2) Cells consist of dense cytoplasm and prominent nucleus 8-3) Callus with procambium cells (a), and vascular nodules (b). (x 100). 8-4) Development of shoot primordia (LP) and shoot meristems (MR) from callus in the medium containing 3.0 mgl^{-1} 2iP+1.0 mgl^{-1} NAA.

somatic embryogenesis and organogenesis from leaf base segments which have white color. Maximum callus induction when cultured on MS Medium supplemented with 5.0 mgl^{-1} 2,4-D + 1.0 mgl^{-1} BA. The exploitation of bioreactor may make the process more efficient in getting large number of somatic embryos. The highest somatic embryo development (193) were obtained on MS medium supplemented with 1 mgl^{-1}2,4-D+0.500 gl^{-1} with 1 min of immersion every 4 h. The balance of auxin: cytokenines in medium, they determined the growth of the date palm tissue.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Physical-chemical characterization of two peanut extract based beverages enriched with fruit pulp

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The goal of this paper is to contribute to research on the peanut crop by studying the making of two beverages based on peanut extract in order to be first by rural communities, which work with familiar agriculture and school meals. In the experiment it was studied the enrichment of the peanut extract with umbu pulp and peanut extract with guava pulp in three formulations as well as the storage of the beverages in polyethylene terephthalate (PET) containers at the temperature of -18°C for 150 days. The beverages were evaluated as to their humidity, ashes, pH, acidity and protein. As a result, there is the possibility of making a product bases on peanut and enriched with fruit pulp representing good market potential.

Key words: *Arachis hypogaea*, umbu and guava pulp, peanut milk.

INTRODUCTION

One of the biggest problems found in developing countries is the deficiency in the intake of protein by part of the poor populations, which demands incentive policies to consume vegetable protein with low cost and good quality.

As an alternative to the problem, soy was used due to its adequate source of protein, accessibility and low cost□ however, variety in feeding habits from the inclusion of different legumes such as peanut may help minimizing that necessity as well as enriching one's diet (Sim et al., 2012□Pretti, 2010□Bede, 2007).

Peanut is a legume of global fame and it is cultivated in all of the national territory. It is widely used in industries and the making of many products due to its nutritional values. It is considered a highly energetic food, as it contains 48.7□ of oil and 80□ of unsaturated fatty acid, amongst which oleic and linoleic. Oil and its proteins have high nutritional quality which determines the expressive economic value in developing countries. Peanut still presents important amounts of vitamin E, vitamin B1 and folic acid (□ranato et al., 2009).

Some types of aliment have been thoroughly studied as alternative source in the making of products for human feeding i n the form of aqueous extract. Therefore, the

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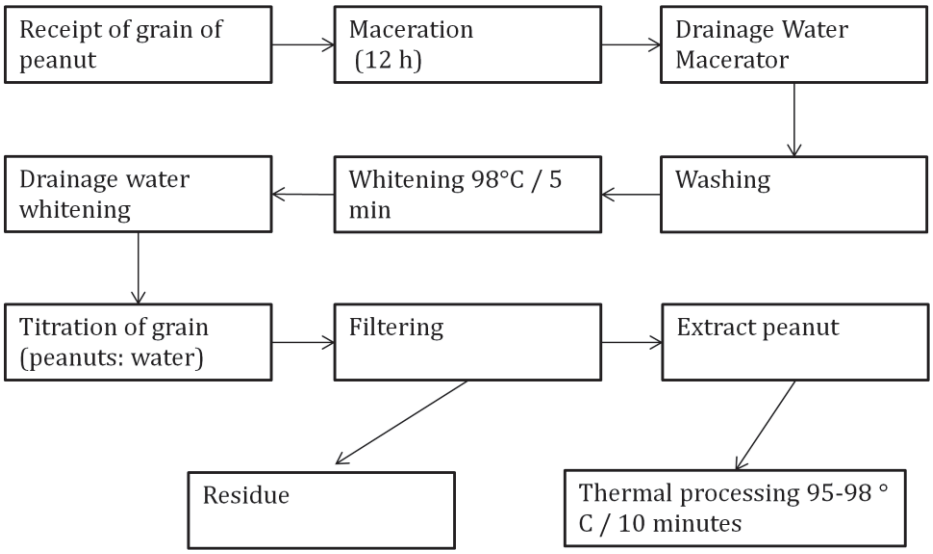

Figure 1. Flowchart of the aqueous extract of peanut grains.

formulation of mixed ready to drink beverages with vegetable basis may be used to improve the nutritional characteristics of certain products (Jain and Khurdiya, 2004) by the complementation of nutrients provided by different vegetables such as peanut and guava, peanut and umbu.

According to Pro-peanut (2010), pro-amendoim and other products derived from peanut such as peanut butter have been prioritized by ONU to be distributed in Haiti, especially amongst children who are more affected by the country shortage, due to its value as energetic food and its rapid action in the human organism as it concentrates three sources of calories (peanut, sugar and oil).

In this context, the present paper aimed at producing two beverages based on peanut extract enriched with guava and umbu fruit pulp as well as studying the effect of storing the beverages at the temperature of -18□C (-0.4F) for 150 days thus characterizing them by raw protein, humidity, pH, total titratable acidity and ashes.

MATERIALS AND METHODS

Location of the experiment

The experiments took place in the Laboratory of Storage and Processing of Agricultural Products of the Academic Unit of Agricultural Engineering at Federal University of Campina □rande and the Department of Research and Extension in Aliment of the Center of Sciences and Technologies at State University of Para□ba, both in Campina □rande, Para□ba, Brazil.

Obtention of raw material

Peanut grains manufactured by loky used in the experiments were obtained from a supermaket store in Campina □rande, Para□ba, Brazil. Umbu (*Spondias* spp.) as well as guava (*Psidium guajava* L.) fruit pulp were obtained in frozen state at a factory of fruit pulp. Both fruit pulp were homogenized and repacked in low density polyethylene bags. Part of the samples were characterized as to humidity, dry extract, pH, acidity, ashes and soluble solids as the other part was stored in a freezer at the temperature of -18□C (-0.4F) until they were used in the experiments.

Preparing of the extract peanut extract

Peanut grain samples were manually peeled, soaked into water at ambient temperature for 8 to 12 h (maceration time) and sub□ected to water heating until ebullition, point in which remained for 5 min□ afterwards the grains were dried and washed in running water. The cooked grains were disintegrated with water at 60□C (140F) in a machine developed for this specific purpose named *vaca mecânica* □mechanic cow□. Regarding the disintegration of the grains, the grain:water ratio was evaluated (1:8 weight□volume) according to physical-chemical and sensorial analysis. The extract produced was sub□ected to whitening (at □ 98□C □208.4F□for 5 min) after which it was stored in polyethylene terephthalate (PET) bags at -18□C (-0.4F). In Figure 1, the flowchart of the preparation of the peanut extract is presented.

Beverage formulation

In order to make the peanut based beverages in the enrichment process, guava and umbu fruit pulps without any treatment were used. Three formulations (Table 1) were elaborated aiming at a selection via physical-chemical analysis. The quantity of pulp and the peanut extract were obtained considering the soy based beverage and the fruit pulps. In each analysis, 480 g of each pulp and 200 g of grains was used.

Analytical determinations

Determinations for humidity and dry extract (Brasil, 1985), protein (IAC, 2003), ashes (AOAC, 2005), acidity (AOCS, 1997) and pH

Table 1. Proportions of peanut extract (□) and guava and umbu pulps used in the selection of the formulation.

Formulation	Quantity of the peanut extracts (PE) (%)	Quantity of pulp (QP) (%)
1	40	60
2	50	50
3	60	40

Table 2. Physical-chemical characterization of guava and umbu fruit pulp and peanut extract.

Determination	Umbu pulp	Guava pulp	Peanut extract
Humidity (□)	92.10	86.54	90.30
pH	2.74	4.17	6.70
Acidity (□)	1.73	0.59	0.30
Ashes (□)	0.30	0.41	0.12
Proteins (□)	-	-	2.46

were achieved in triplicates in the (beverage) formulations obtained. After formulated and characterized, they were stored for 150 at -18□C.

Statistical design

The results were analyzed with Programa Computacional Assistat (Silva and Azevedo, 2006) □Assistat Computational Program□, version 7.4 beta, using the completely randomized design. The results were tested in beverages stored at the temperature of 18□C (-0.4F) and 3x2x4 (3 formulations, 2 pulps, 4 periods of storage). The means were compared with Tukey's test at 1 and 5% probability.

RESULTS AND DISCUSSION

Centesimal composition of the umbu and guava pulps and the peanut extract

The approximate centesimal composition of the pulps (umbu and guava) and the peanut extract is described in Table 1. The values for ashes (0.41□) and humidity (86.54□) found for the guava pulp were very close to the values on the Brazilian Table of Aliment Composition (Taco, 2011) which determines for guava pulp 0.5□ ashes and 85□ humidity. The acidity values (0.59□) were superior to those found by Brunini et al. (2003) who have obtained at the beginning of the storing of guava pulp 0.40□ acidity. Such differences may be related to a variety of causes, such as the location of the orchard, rainfall, light, which influence either directly or indirectly the development of each variety and consequently its components.

Data analysis enabled the statement that pH value (4.17) for guava pulp (Table 2) was superior than the ones found by Machado et al. (2007), who evaluated the quality of four brands of frozen guava pulp and obtained mean pH of 3.90, and by Brunini et al. (2003), who found in the characterization of the guava pH of 3.86. However, the values are acceptable according to the interval from 3.5 to 4.20 established by the Ministry of Agriculture and Provision for beverages with guava.

In regard to the data exposed in Table 2 for the umbu pulp, the values found in this paper for pH analysis (2.74) are superior to the ones found by Carmo et al. (2012), who obtained 2.75 pH for umbu □uice, and Souza et al. (2010) who achieved pH of 2.3. Comparing the quantity of ashes of the umbu pulp in this paper (0.30□), the former is inferior to the value found (0.5□) by Ferreira et al. (2000). Mean values for humidity (92.1□) were superior to the found by Souza et al. (2012) who verified in their study values of 89.70□. The mean value for total titratable acidity of umbu (1.73□) is closer to the one found by Ferreira et al (2000) in their work with frozen umbu pulp (1.30□) during 180 days of storage.

As for the data achieved for the peanut extract, the quantity of ashes and protein is similar to the one found by Kouane et al. (2005) who obtained values of 0.2 and 2.80□ for both aspects, respectively. Also, with respect to protein content (2.46□) was superior to that obtained by Rubico et al. (1987) that was 2.14□.

Centesimal composition of the formulated beverages

Ashes

In Table 3, the mean values for ashes (□) for the beverages formulated by the peanut extract and umbu and guava pulps are presented. It is noted in each formulation the statistical superiority of the peanut extract and guava beverage compared to peanut extract and umbu beverage: 0.16 (57.14□) in formulation 40P:60PE, 0.17 (73.91□) in formulation 50P:50PE, and 0.18 (78.16□) in formulation 60P:40PE. Such result is probably due to a higher concentration of minerals in

Table 3. Ashes mean values (□) of peanut extract enriched with umbu and guava for the interaction beverage x formulation stored in freezer after 150 days.

Beverage (peanut extract - PE)	Formulation % (pulp:peanut extract)		
	P40:PE60	P50:PE50	P60:PE40
PE□Umbu	0.16^{bC}	0.17^{bB}	0.18^{bA}
PE□□uava	0.28^{aA}	0.23^{aB}	0.23^{aB}
LSD Column	0.007		
LSD Line	0.009		

Means followed by the same capital letter in the line and lower cases in the column do not differ statistically by Tukey's test at 5% probability; PE: Peanut Extract; P:Pulp. Least Significant Difference (LSD).

Table 4. Protein mean values (%) for the peanut extract enriched with umbu and guava pulps for the interaction beverage x formulation stored in freezer after 150 days.

Bebida	Formulation % (pulp:peanut extract)		
	40P: PE60	P50: PE50	P60: PE40
PE/Umbu	1.86^{aA}	1.96^{aA}	1.64^{bB}
PE/Guava	1.66^{bC}	2.08^{aA}	1.85^{aB}
LSD Column		0.13	
LSD Line		0.16	

Means followed by the same capital letter in the line and case letter in the column do not differ statistically by Tukey's test at 5% probability; PE: Peanut Extract; P: Pulp. Least Significant Difference (LSD).

guava pulp (0.5%) over umbu pulp (0.4%). Souza et al. (2011) characterized nutritionally the residues of tropical fruit pulps and observed that the residue of guava pulp presented higher percentage of ashes (0.72%).

In respect to the percentage of ashes in the beverage enriched with umbu and guava pulp, in each formulation, the higher percentage is verified to the peanut extract enriched with umbu at P60;40PE (0.18%), followed by the formulations P50:PE50 (0.17%) and P40:PE60 (0.16%), respectively. As to the beverage enriched with guava pulp the higher concentration of ashes was found in formulation P40:PE60 (0.28%) followed by P50:PE50 (0.23%) and P60:40PE (0.23%) which were statistically identical.

Jaekel et al. (2010) verified that beverages composed of 70% soy extract and 30% rice extract (70S:30R) are better nutritional options with higher quantities of protein, lipids, minerals (estimated by the ash percentage) and fibers, over beverages composed of 50% soy extract and 50% rice extract (50S:50R) and 30% soy extract and 70% rice extract (30S:70R).

Proteins

In Table 4, the higher percentage of proteins for the beverage enriched with umbu was noted in formulations P40:PE60 and P50:PE50 (pulp:peanut extract) and the lowest in formulation P60:40PE, which means to say, the larger the quantity of umbu pulp added to the mix, the lower is the percentage of proteins. Such was explained by Souza et al. (2010) who classified umbu as aqueous or juicy, a fruit that presents great humidity, therefore by adding more of the fruit, the quantity of water also increases and, consequently, proteins are dissolved. According to Souza et al. (2011) fruits in general are not potential sources of proteins, however this macronutrient is particularly found in the peel and seed of fruits. The experiment behavior was different for beverages composed of peanut extract and guava pulp as the higher percentage was found in formulation 50P:50PE, followed by formulation P60:40PE and 40P:60PE, respectively.

The percentage values of proteins (Table 4) in each formulation are statistically the same in formulation P50:PE50 for both beverages and its behavior is the inverse to the other formulations, which means for P40:PE60, the higher percentage is related to umbu enrichment, as for P60: PE40, the higher percentage was verified for the beverage enriched with peanut extract and guava pulp.

pH

Based on data displayed in Table 5, it is noticed that the pH of beverages elaborated with peanut extract and guava pulp presented higher values of pH when compared to the beverage elaborated with peanut extract

Table 4. Mean values of pH for the peanut extract enriched with umbu and guava pulp for the interaction beverage x formulation stored in freezer after 150 days.

Beverage	Formulation % (pulp:peanut extract)		
	40P: PE60	P50:50PE	P60: PE40
PE/Umbu	3.60^{bA}	3.35^{bB}	3.25^{bC}
PE/Guava	4.12^{aA}	4.03^{aB}	4.02^{aB}
LSD Column		0.03	
LSD Line		0.04	

Means followed by the same capital letter in line and case letter in column do not differ statistically by Tukey's at 5% probability; PE: Peanut Extract; P:Pulp. Least Significant Difference (LSD).

Table 5. Mean values of acidity (%) for the peanut extract enriched with umbu and guava pulp for the interaction beverage x formulation stored in freezer after 150 days.

Beverage	Formulation % (pulp:peanut extract)		
	P40: PE60	P50: PE50	P60: PE40
PE/Umbu	4.12^{aC}	5.47^{aB}	6.58^{aA}
PE/Guava	0.43^{bB}	0.46^{bA}	0.44^{bB}
LSD Column	0.05		
LSD Line	0.07		

Means followed by the same capital letter in the line and case letter in the column do not differ statistically by Tukey's test at 5% probability; PE: Peanut Extract; P: Pulp. Least Significant Difference (LSD).

and umbu pulp for the three formulations. These results are due to the fact that pH value of the chosen species to enrich the beverages are different, that is, guava pulp presents higher pH values than the umbu pulp. There is support to such situation on Brazil et al. (1995), who found pH of 3.98 for guava pulp and Gouveia et al. (2004) who found it of 3.91; as to the umbu pulp the pH values decrease from 2.21 found by □erreira et al. (2000) to 1.38 by Paula et al. (2012); these lower values indicate the umbu pulp is more acid which justifies the lower pH of the beverage enriched with it.

Abreu et al. (2007) in his evaluation of physical□ chemical characteristics of the soy based beverage from different brands with pineapple, guava, mango, passion fruit and coconut juice mix, commercialized in Brazilian markets, obtained for the guava enriched beverage pH values of 3.98 (GJ1 – soy based beverage and guava (brand A)) and 3.88 (GJ2 □ soy based beverage and guava (brand B)).

Analyzing the pH information for each beverage elaborated in each formulation, the pH decreases as the quantity of umbu pulp increases. Umbu is considered an acid fruit (low pH) thus when its quantity increases, the pH tends to decrease. □or the beverage based on peanut extract and guava pulp the higher pH is verified for the formulation P40: EP60 over the others which were the same statistically (P50:PE50 e P60:40PE).

Mercaldi (2006) studied soy based beverages enriched with soursop and developed in the beginning of the work the preparation of a standard for beverages in which it was detected that as the quantity of soursop juice increased from 2.5 to 4.90 ml, the pH values decreased from 6.05 to 4.09.

Acidity

The values regarding the total titratable acidity of the beverages elaborated with peanut extract and umbu and guava pulps are presented in Table 6. The results in respect to the acidity of both beverages in each formulation showed that the acidity of the beverage elaborated with umbu pulp increased as the quantity of fruit also increased and the quantity of peanut extract decreased. The opposite behavior was found to the pH (Table 5) in the same beverage (enriched with umbu). The beverage enriched with guava pulp presented higher acidity in the formulation 50% pulp and 50% peanut extract, followed by formulations P40:PE60 and P60:40PE, which were the same statistically.

The higher acidity of the fruits used in the mixed beverages in comparison to the pure peanut extract (acidity □ 0.30, Table 2) resulted in higher acidity and lower pH in the products containing guava and umbu pulps.

Abreu et al. (2007) evaluated chemically and physically soy juice with tropical fruit and observed that acidity varied between 0.14 and 0.34 while the soy extract

Table 6. Means (%) of factors beverage, formulation and time of dried extract of guava and umbu present in the "peanut milk" (PE) during the 5 month storage.

Beverages	Mean
PE/Umbu	92.34[a]
PE/Guava	91.23[b]
LSD	0.21
Formulations (%)	**Mean**
P40: PE60	91.80[a]
P50: PE50	91.82[a]
P60: PE40	91.74[a]
LSD	0.31

P: pulp; PE: Peanut Extract.

acidity value was of 0.06 which confirmed the pH value found in the experiments.

Analyzing the acidity for each formulation, the P40:PE60 presents low acidity for the beverage formulated with guava pulp (0.43%) over the one enriched with umbu pulp (4.12%). The remaining formulations presented guava enrichment as the least acid statistically.

Humidity

In Table 6 the mean values for humidity are presented (% $_w$) of two beverages elaborated with peanut extract and enriched with guava and umbu pulp. The humidity for the beverage elaborated with umbu pulp was higher in 1.11% compared to the guava pulp. According to Taco (2011), the humidity of the fruit umbu is of 89.3% and of its frozen pulp 90.20%. For the red guava such value is of 85% and the pulp is of 88.33%, according to Freire et al. (2008).

Analyzing the influence of formulations in the amount of humidity, it noticed the statistical equality between formulations during the experiment which indicates that humidity was not influenced by these factors.

Caus et al. (2008) elaborated beverages based on soy hydrosoluble extract with fruit pulp and noted that the humidity of the soy beverage, variant CD 206, with strawberry was of 83.16%; on the other hand, the passion fruit enriched beverage reached 82.79%. Pettri (2010), on the same topic, found studies with peanut extract for formulations of powder milk (0.2 and 4% powder milk) with humidity of 77.12, 76.15 and 75.66%, respectively.

Conclusions

Both the beverages here analyzed in their three formulations presented quantities of protein expressively inferior to the pure peanut extract (2.46%). The quantity of both ashes and acidity was larger in the beverage enriched with guava pulp over the umbu pulp. The pH for the beverage elaborated with peanut extract and guava pulp presented higher values than the combination of peanut extract and umbu pulp for the three formulations after 150 days of storage.

Conflict of Interest

The authors have not declared any conflict of interest.

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Bio-efficacy of insecticides and biorationals against the incidence of whitefly, *Bemisia tabaci* (Genn.) and yellow mosaic virus in mungbean

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Whitefly, *Bemisia tabaci* (Genn.) and yellow mosaic virus disease incidence in mungbean, *Vigna radiata* (L.) Wilczek is a most serious problem in northern states of India. Bioefficacy of some insecticides and biorational were tested during *kharif* 2010 to 2011 and 2011 to 2012 at Pulses Research Farm, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The results revealed that 30 days after sowing minimum whitefly population was recorded in plots, when seeds were treated with dimethoate 5 ml/kg seeds. At three days after spray application lowest whitefly population (1.6 and 6.4 adults/ cage/ plant) was noted in NSKE 5 per cent sprayed plots. At seven days after spray, NSKE 5 per cent and triazophos 0.04% was the most effective in keeping the whitefly incidence and yellow mosaic virus infection low. Triazophos and NSKE did not help in managing the whitefly population at low level up to 10 days after spray application. Spraying of triazophos 0.04% resulted in higher grain yield as well as net profit. Lower dosage of triazophos 40EC at 0.02% enhanced the whitefly population in 2011 to 2012. However, the maximum incremental cost benefit ratio (1:13.41) was obtained in dimethoate 5 ml/kg seed treated plots followed by seed treatment with dimethoate plus spraying of triazophos 0.04% (1:11.93).

Key words: Bioefficacy, biorational, insecticides, whitefly, yellow mosaic virus.

INTRODUCTION

Mungbean, *Vigna radiata* (L.) Wilczek commonly known as green gram is an important legume crop widely grown in many Asian countries including India, Bangladesh, Bhutan, China, Myanmar, Nepal, Sri Lanka, Thailand and Pakistan. In India, it occupies third place after chickpea and pigeonpea (Ahlawat et al., 2008). Mungbean crop grow once in a year, except some regions of the country where it is grown in summer season for fodder as well as grain purpose. Due to its more luxurious growth and more vegetative canopy, numbers of insect pests attack from seedling to maturity stage which is detrimental factor for their production and cause severe losses in their productivity. Since it is grown in tropical climates, bunches of insect pests play an important role in marketable production. The magnitude of insect pest losses to mungbean has been estimated by Panchabhavi and Chhadam (1990); Rao et al. (1990) and Sharma et al. (1991). Most of these insects are polyphagous and

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feed on a wide range of variety of legumes and non□ legumes. Mungbean is attacked by different species of insect pests but sucking insect pests (aphid, jassids, leaf hopper and whitefly) are of the major importance (Biswass et al., 2008). Among the various constraints, yellow mosaic virus disease caused by yellow mosaic Gemini virus is one of the major factor in the cultivation of mungbean, particularly, in northern states. The vector of this disease is whitefly (*Bemisia tabaci* Genn.). Sastry and Singh (1973) and □hattak et al. (2004) have observed it as major importance and that even low population of whitefly is capable for wide transmission of yellow mosaic virus. *B*. *tabaci* is common in India and cause direct damage by sucking the cell sap (Sehgal and Ujagir, 1987). □ hitefly sucks the cell sap from the leaves and in case of severe damage, it secretes honeydew which causes sooty mould and hampers the photosynthetic activity. □or control of vector transmitted viral diseases, controlling the insect vectors using chemical insecticides has been the common practice among the farmers. Previously many research workers have evaluated synthetic chemicals against sucking pests of mungbean (Gopal and Srivastava 1997). A drastic reduction in the incidence of M□M□ was recorded where whitefly population was reasonably controlled by using chemical insecticides. But indiscriminate use of pesticides resulted in the development of resistance in the target pest species, resurgence of whitefly and environmental pollution. Contrastingly, neem products like neem seed kernel extract (□S□E), 5% was found effective in managing the population of whitefly in mungbean (Hussain et al., 2001). In order to arrest the mungbean damage due to these insects, the insecticides are mainly relied upon to keep the pest population below the economic threshold level (Chhabra and □ooner, 1985). □eeping these facts in view present study was conducted on mungbean to find out the bioefficacy of some insecticides and biorationals against whitefly in order to find an effective and economic control of this pest under agro□ecological conditions of Haryana state.

MATERIALS AND METHODS

Experimental trials were conducted during *kharif* season 2010 to 2011 and 2011 to 2012 at Pulses Research □arm, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Mungbean cultivar 'Asha' (20 kg ha^{-1}) was sown as a test variety, at 30 □ 10 cm spacing in a randomized block design with three replications. □or seed treatment, 60 and 100 ml quantity of imidacloprid 200SL and dimethoate 30EC was mixed separately in water and made a total volume of emulsion 300 ml each. Seed treatment was done, one day before sowing in the evening. The treated seeds were spread on the polythene sheet and left overnight for drying. Sowing was done in the morning hours next day. The crop was raised under recommended agronomical package of practices, except the plant protection measures (Anonymous, 2004). Monitoring was done regularly for recording the incidence of whitefly. □ hen the adult whitefly population reached three adults per plant, treatments were imposed to manage whitefly population. The adult whitefly population per cage per plant was recorded with the help of split cage (□ath, 1994). □bservations on adult whitefly population were recorded 30 days after sowing and one, three, seven, ten and fourteen days after spray. The observation on the total number of plants and the yellow mosaic virus infested plants per plot were also recorded at the same period as for whitefly population and percent plant infection was worked out for each plot. The percent avoidable yield loss was also calculated with the help of following formula given by Sharma et al. (1991):

$$Avoidable\ yield\ loss\ (\%) = \frac{Yield\ of\ protected\ crop - Yield\ of\ unprotected\ crop}{Yield\ of\ unprotected\ crop}\ x\ 100$$

The grain yield obtained and cost of plant protection measures, including labour charges and spraying charges and net profit per hectare was also calculated. Cost Benefit Ratio (ICBR) was worked out as the net profit to the cost of plant protection.

Seeds were treated with Imidacloprid 200 SL at 3 ml/kg seed and dimethoate 30 EC at 5 ml/kg seed. These treatments were compared with seed treatments of these insecticides followed by spraying of triazophos 40 EC at 0.04 and 0.02% and □S□E 5% at 30 days after sowing. □ine treatments *viz*., (T1) seed treatment with Imidacloprid 3 ml/kg, (T2) seed treatment with dimethoate 5 ml/kg, (T3) T1 □ triazophos 0.04%, (T4) T1 □ triazophos 0.02%, (T5) T2 □ triazophos 0.04%, (T6) T2 □ triazophos 0.02%, (T7) T1 □ □S□E 5%, (T8) T2 □ □S□E 5% and (T9) untreated control were evaluated against whitefly population. Data was subjected to analysis of variance given by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Bioefficacy of insecticides and biorationals for management of whiteflies

Data recorded in *kharif* 2010 to 2011 presented in Table 1 revealed that at 30 days after sowing whitefly has reached the ETL level and minimum population of whitefly was in plots where seeds treated with dimethoate 30 EC at 5 ml/kg seed and it ranged from 2.4 to 3.5 adults/ plant. The initial 30 days of the crop is crucial for the well establishment of the seedlings. So, the lower whitefly populations were harboured in those plots, where seeds were treated with dimethoate. Three days after spraying, the lower population of whitefly (1.6 adults/ cage/ plant) was recorded in treatment T8 (seed treated with dimethoate followed by □S□E 5% spray) and it was statistically at par with treatment T7, seed treatment with imidacloprid followed by □S□E 5% spray (2.0 adults/ cage/ plant), T2, seed treatment with dimethoate (2.4 adults/ cage/ plant), T3, seed treatment with imidacloprid followed by triazophos 0.04% (2.3 adults/ cage/ plant) and T5 seed treatment with dimethoate followed by triazophos 0.04% (2.7 adults/ cage/ plant). The higher population of whitefly (4.2 adults/ cage/ plant) was recorded in treatment T1 (seed treatment with imidacloprid 3 ml/kg) which was statistically at par with untreated control.

□bservations recorded 7 days after spray, minimum population of whitefly was recorded in treatments T3 and T5 (2.8 adults/ cage/ plant) where triazophos 40EC at 0.04% was sprayed after seed treatment and these were

Table 1. Bioefficacy of insecticides and biorationals against whitefly in mungbean harif, 2010 to 2011.

S/ No.	Treatments	Population of whitefly/ cage/ plant				Yellow mosaic virus (%)	Yield (q/ha)	Avoidable yield loss (%)
		30 Days after sowing	3 Days after spray	7 Days after spray	10 Days after spray			
T_1	ST Imidacloprid 3 ml/kg	4.2 (2.28)	4.2 (2.27)	5.9 (2.63)	2.8 (1.91)	3.4 (10.65)	13.43	20.34
T_2	ST Dimethoate 5 ml/kg	2.4 (1.84)	2.4 (1.85)	4.2 (2.27)	3.0 (1.99)	2.5 (9.07)	13.85	24.10
T_3	T_1 Triazophos 0.04%	4.3 (2.31)	2.3 (1.82)	2.8 (1.93)	4.3 (2.29)	2.2 (8.57)	18.03	61.55
T_4	T_1 Triazophos 0.02%	5.3 (2.51)	3.3 (2.07)	3.6 (2.14)	4.0 (2.22)	3.2 (10.17)	15.11	35.39
T_5	T_2 Triazophos 0.04%	3.4 (2.10)	2.7 (1.91)	2.8 (1.94)	4.0 (2.21)	1.4 (6.81)	18.75	68.01
T_6	T_2 Triazophos 0.02%	3.5 (2.12)	2.9 (1.97)	3.1 (2.01)	2.3 (1.82)	3.4 (10.57)	16.78	50.35
T_7	T_1 SE 5%	4.3 (2.31)	2.0 (1.72)	2.8 (1.94)	3.1 (2.02)	2.3 (8.72)	16.14	44.62
T_8	T_2 SE 5%	3.3 (2.08)	1.6 (1.58)	3.1 (2.02)	3.1 (2.02)	3.4 (10.61)	17.56	57.34
T_9	Untreated control	4.2 (2.27)	3.6 (2.13)	4.2 (2.28)	3.2 (2.04)	5.5 (13.54)	11.16	
SEm		(0.11)	(0.17)	(0.14)	S	(0.83)	0.67	
CD at 5%		(0.23)	(0.36)	(0.28)		(1.76)	1.43	

ST: Seed treatment, Figures in parenthesis for whitefly population are √n+1 of the population, Figures in parentheses for yellow mosaic virus infestation are angular transformed values.

statistically at par with treatment T4 (T1 triazophos 0.02% spray) 3.6 adults/ cage/ plant, T6 (T2 triazophos 0.02% spray) 3.1 adults/ cage/ plant, T7 (T1 SE 5% spray) 2.9 adults/ cage/ plant and T8 (T2 SE 5% spray) 3.1 adults/ cage/ plant. The similar findings were reported by han et al. (2012), who reported that minimum whitefly population was observed when mungbean seeds were treated with imidacloprid. The results differed from those of Singh and Chourasiya (2013), who reported that whitefly population/ cage was maximum in imidacloprid treated blackgram plots as compared to other treatments at three and seven days after spraying.

Data presented in Table 2, regarding mean adult whitefly incidence during *kharif* 2011 to 2012 revealed that all the treatments were superior over untreated control at 30 days after sowing. The minimum population of adult whitefly (6.0 adults/ cage/ plant) was recorded in treatment T6, seed treatment with dimethoate 5 ml/kg seed. However, maximum population of adult whitefly was found in untreated control plots (12.6 adults/ cage/ plant).

bservations recorded 1 days after spray application revealed that treatment T7, seed treatment with imidacloprid followed by spray of SE 5% and treatment T8, seed treatment with dimethoate followed by spray of SE 5% are equally effective in reducing the adult whitefly population, registering 5.7 and 5.9 adults/ cage/ plant. The highest population of adult whitefly (10.1 and 10.8 adults/ cage/ plant) was observed in treatments T6 and T4, seed treatment with dimethoate followed by spray of triazophos 0.02% and seed treatment with imidacloprid followed by spray of triazophos 0.02%. Similar trends of adult whitefly population. were observed in treatments T7 and T8 after 3rd and 7th days of application of insecticide and recorded 6.4, 6.1 and 6.6 adults/ cage/ plant respectively. The higher population of adult whitefly (14.2, 11.9 and 15.9, 12.3 adults/ cage/ plant) was recorded in treatments T6 and T4, respectively, where lower dosage of triazophos was sprayed. rom the above findings it is clear that seed treatment with imidacloprid 3 ml/kg seed, seed treatment with dimethoate 5 ml/kg seed and spray of SE 5% was effective up to 7 days after spray application. Spraying of triazophos at 0.02% increased the whitefly population.

Ten days after spray, the lower adult whitefly incidence (8.4 and 8.7 adults/ cage/ plant) was recorded in those plots whose seeds were treated with dimethoate 5 ml/kg and imidacloprid 3 ml/kg seed, respectively. Higher adult whitefly population (16.4 and 13.2 adults/ cage/ plant) was recorded treatments T6, seed treatments with dimethoate followed by spray of triazophos 0.02% and T4, in seed treatment with imidacloprid followed by spay of triazophos 0.02%. hitefly adult population increased after 10 days of spray of triazophos at 0.02 and 0.04% and spray of SE 5% indicating that after 10 days, triazophos

Table 2. Bioefficacy of insecticides and biorationals against whitefly in mungbean harif, 2011to 2012.

S/ No.	Treatments	Population of whitefly/ cage/ plant						Yellow mosaic virus (%)	Yield (q/ha)	Avoidable yield loss (%)
		30 Days after sowing	1 Days after spray	3 Days after spray	7 Days after spray	10 Days after spray	14 Days after spray			
T_1	ST with Imidacloprid	7.1 (2.85)	7.8 (2.99)	8.8 (3.13)	8.1 (3.02)	8.7 (3.11)	2.5 (1.87)	29.1 (32.64)	6.65	9.91
T_2	ST with Dimethoate	7.7 (2.94)	8.4 (3.07)	8.0 (3.00)	8.4 (3.07)	8.4 (3.07)	5.5 (2.53)	28.5 (32.26)	6.53	7.93
T_3	T_1 Triazophos 0.04%	7.6 (2.90)	6.0 (2.64)	7.6 (2.93)	11.8 (3.57)	10.5 (3.40)	3.8 (2.19)	29.1 (32.29)	7.75	28.09
T_4	T_1 Triazophos 0.02%	6.3 (2.71)	10.8 (3.45)	15.9 (4.12)	12.3 (3.05)	13.2 (3.77)	3.6 (2.11)	35.4 (36.51)	6.65	9.91
T_5	T_2 Triazophos 0.04%	7.2 (2.86)	5.9 (2.61)	8.1 (3.18)	10.7 (3.40)	9.5 (3.21)	3.2 (2.05)	29.2 (32.69)	7.57	25.12
T_6	T_2 Triazophos 0.02%	6.0 (2.64)	10.1 (3.33)	14.2 (3.90)	11.9 (3.59)	16.4 (4.17)	5.2 (2.44)	34.0 (35.64)	6.84	13.05
T_7	T_1 SE 5%	6.9 (2.81)	5.7 (2.57)	6.4 (2.73)	6.1 (2.66)	8.9 (3.14)	2.9 (1.93)	25.4 (30.28)	7.23	19.50
T_8	T_2 SE 5%	6.7 (2.76)	5.9 (2.63)	6.6 (2.76)	6.6 (2.75)	9.3 (3.21)	5.2 (2.44)	28.3 (32.16)	7.19	18.84
T_9	Untreated Control	12.6 (3.68)	9.4 (3.23)	13.0 (3.74)	8.5 (3.08)	10.6 (3.46)	6.0 (3.46)	39.0 (38.43)	6.05	
SEm		(0.22)	(0.19)	(0.13)	(0.18)	(0.25)	S	(0.48)	0.39	
CD at 5%		(0.47)	(0.39)	(0.27)	(0.38)	(0.52)		(1.02)	0.82	

DAS: Days after sowing, igures in parenthesis for whitefly population are angular transformed values, igures in parentheses for yellow mosaic virus infestation are angular transformed values.

and SE could not protect the crop and resurgence in whitefly population was recorded. The results are in conformity by Panghal et al. (2008), who reported that seed treatment with imidacloprid, dimethoate and spray of SE 5% were the most effective against whitefly in keeping the population low up to 7 days. Masood et al. (2004) observed that seed treatment with imidacloprid 75.03 and Mustafa (2000) 72.76% reduction in whitefly population. Iqbal et al. (2013) also found that imidacloprid was most effective and resulted in a minimum population of whitefly.

The present findings can be compared with those of Afzal et al. (2002) who reported that imidacloprid 25 P at 200 g/acre was found to be most effective for whitefly. Mohan and atiyar (2000) stated that imidacloprid was the most effective in suppressing the whitefly population and its continuous use resulted in increased whitefly population.

Effect of insecticides and biorationals on yellow mosaic virus infection (%)

The data presented in Table 1, revealed that the minimum yellow mosaic virus infection (1.4%) was recorded in treatment T5 (seed treated with dimethoate followed by spray of triazophos 0.04%) and it was statistically at par with T3, seed treatment with imidacloprid followed by triazophos 0.04%. The highest yellow mosaic virus infection (3.4%) was recorded in treatment T1 (seed treatment with imidacloprid 3 ml/kg), T6 (T2 spray of triazophos 0.02%) and T8 (T2 spray of SE 5%). The results are differed from han et al. (2012) who reported that imidacloprid was most effective to control mungbean yellow mosaic virus. These findings were in agreement with those of etting and Anderson (1991) who reported that application of imidacloprid reduces the yellow mosaic virus in greenhouse grown poinsettias as compared to other treatments.

bservations recorded on yellow mosaic virus presented in Table 2 revealed that minimum yellow mosaic virus infection (25.4%) registered in treatment T7, seed treatment with imidacloprid followed by spray of SE 5% it was followed by T8, seed treatment with dimethoate followed by spray of SE 5% (28.3%). Seed treatment with imidacloprid/ dimethoate and spraying of triazophos at 0.02% could not help in reducing yellow mosaic virus infection and 35.4 and 34.0% MM in treatment T4 and T6. These results indicating that the spraying of SE 5% showed long time effect against whitefly as compared to untreated control. The present findings on percent yellow mosaic virus infection are in agreement with those of Panghal et al. (2008), who reported that minimum plant infection by yellow mosaic virus was observed in seed treatment with imidacloprid/ dimethoate and SE 5% sprayed

Table 3. Effect of different insecticidal treatments on yield of mungbean, net profit and their cost benefit ratio.

S/ No.	Treatments	Av. yield (q/ha)	Increase yield over control (q/ha)	Value of the additional grain yield (`/ha)	Cost of treatment (`/ha)	Net profit (`/ha)	Cost benefit ratio
T_1	ST with Imidacloprid	10.04	1.44	2304	239.5	2064.5	1:8.62
T_2	ST with Dimethoate	10.19	1.59	2544	176.5	2367.5	1:13.41
T_3	T_1 □ Triazophos 0.04%	12.89	4.29	6864	627	6237	1:9.94
T_4	T_1 □ Triazophos 0.02%	10.88	2.28	3648	433	3215	1:7.42
T_5	T_2 □ Triazophos 0.04%	13.16	4.56	7296	564	6732	1:11.93
T_6	T_2 □Triazophos 0.02%	11.81	3.21	5136	408.5	4727.5	1:11.57
T_7	T_1 □□S□E 5%	11.68	3.08	4928	630	4298	1:6.82
T_8	T_2 □ □S□E 5%	12.37	3.77	6032	567	5465	1:9.63
T_9	Untreated Control	8.60	□	□	□	□	□
SEm□		0.67	□	□	□	□	□
CD at 5%		1.43	□	□	□	□	□
Input						**Cost (`)**	
Imidacloprid 200SL						1700/l	
Dimethoate 30EC						300/l	
□S□E						25/kg	
Triazophos 40EC						310/l	
Mungbean grain						1400/q	
Labour charges						100/labour/ha	

plots. The similar results are reported by □han et al. (2012), who reported that chemical imidacloprid was most effective to control mungbean yellow mosaic virus. Earlier, reduction in the infection of yellow mosaic virus by spraying of □S□E (Sethuraman et al., 2001) has also been recorded.

Effect of different treatments on the yield of mungbean and cost benefit ratio

The data on seed yield, net monetary returns and cost benefit ratio are presented in Table 3. The data on seed yield showed that the seed yield in all the insecticides and biorational treatments was significantly higher than the untreated control. However, the highest grain yield of mungbean (13.16 q/ha) was realized in treatment T5, seed treatment with dimethoate followed by spray of triazophos 0.04%. It was followed by treatment T3, seed treatment with imidacloprid □ spray of triazophos 0.04% (12.89 q/ha) and treatment T8, seed treatment with dimethoate □ spray of □S□E 5% (12.37 q/ha). The lowest yield 8.60 q/ha was registered in untreated control plots, indicating immense damage potential of whitefly on mungbean.

The contrasting results are observed by Shah et al. (2007), who reported that the highest seed yield of 1563 kg ha^{-1} was recorded from the plots where imidacloprid was applied to control sucking insect pests of mungbean. These results are in conformity with those of Ujagir and Chaudhry (1997), Ahmad et al. (1998) and Deka et al. (1998). They found in their experiments that plots those treated with imidacloprid provide maximum yield. However, □aresh and Thakur (1972), Saxena et al. (1984), Chhabra and □ooner (1986) and □adodaria and □yas (1987) obtained higher grain yield in oxydemeton methyl treatment. Sethuraman et al. (2001) obtained higher seed yield of greengram with the application of □S□E spray. It may be inferred from the present investigation that yield advantages from the mungbean crop under the existing conditions could be achieved by using the imidacloprid against the sucking insect pest complexes of mungbean.

Data on incremental cost benefit ratio presented in Table 3 revealed that cost effectiveness of treatment T2, seed treatment with dimethoate 5 ml/kg with highest ICBR of 1: 13.41. It was followed by treatment T5, seed treatment with dimethoate □ triazophos 0.04% spray (1: 11.93) and treatment T6, seed treatment with dimethoate □ triazophos 0.02% spray (1:11.57). Lowest ICBR was estimated in treatment T7, seed treatment with imidacloprid □ □S□E 5% spray (1: 6.82). □ther promising treatments can be arranged in descending order of cost effectiveness as treatment T4, seed treatment with imidacloprid □ triazophos 0.02% spray (1: 7.42), treatment T1, seed treatment with imidacloprid 3 ml/kg (1: 8.62), treatment T8, seed treatment with dimethoate □ □S□E 5% spray (1: 9.63) and treatment T3, seed

treatment with imidacloprid spray of triazophos 0.02% (1: 9.94). Similar findings on maximum cost benefit ratio (1: 29.5) was reported by Panghal et al. (2008) in greengram when seed treatment was done by dimethoate 5 ml/kg seed. Contrasting results from the present study are reported by Gupta and Pathak (2009) who reported higher ICBR of 1: 16.9 in S E 3% and 1: 11.2 in S E 3% followed by spray of dimethoate 0.03%.

Avoidable yield loss (%)

Data on per cent avoidable yield loss depicted in table 1 and revealed that in treatment T5, seed treatment with dimethoate followed by spray of triazophos 0.04%, per cent avoidable yield loss was maximum (68.01%). It was followed by treatment T3, seed treatment with imidacloprid followed by spray of triazophos 0.04% (61.55%) and treatment T8, seed treatment with dimethoate followed by spray of S E 5% (57.34%).

Data from the Table 2 shows that application of imidacloprid as seed treatment and spray of triazophos 0.04% registered maximum percent avoidable yield loss (28.09%) and it was followed by 25.12% in seed treatment with dimethoate followed by spray of triazophos 0.04%. hile the application of imidacloprid as seed treatment and spray of triazophos 0.02% was unable to reduce the percent avoidable yield loss (9.91%). So, from the findings on per cent avoidable yield loss, it can be concluded that application of triazophos 0.04% could be the first choice against whitefly and reduce the per cent avoidable yield loss in mungbean crop.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Dispersion of proteins from *Moringa oleifera* seeds using pouche immersed in aqueous medium

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Due to the need for proper water quality for human consumption, some studies have developed new treatment techniques. Some have used a natural coagulant based on *Moringa oleifera*, which has the capacity to clarify turbidity water and has anti-microbial properties. This paper aimed at developing a new technique to apply the coagulant. This technique consists of putting the *M. oleifera* powder into pouches. Five different materials were analyzed for the concentration of protein released and the increased turbidity in the water: four different types of nonwoven fabric and one commercial coffee filter paper. It was made some tests of dissolution: during 24 h, the pouches were put in distilled and deionized water and then it was mixed in low velocity in the Jar Test equipment. Through these tests, it was found that the five pouch materials released protein into the water even in the first 60 min of contact. It gave 7.7 mg L^{-1} min^{-1} of dispersion rate and maintained turbidity values lower than the ones observed using the liquid coagulant. From the result, it's possible to conclude that those five types of pouches can be used in water treatment, because all them release similar concentration of protein that was observed when it was used the liquid coagulant.

Key words: *Moringa oleifera*, pouch, protein concentration.

INTRODUCTION

Access to water with good quality for human consumption is the subject of many research studies, because of its implications for the health of a population. That is one of the reasons of the fundamental importance of the consumption of contaminants free water. Thus, subjects such as water quality and its reuse are increasingly being discussed and studied.

An option to improve the efficiency of physical treatment systems is the use of coagulants to aid the shaping of clusters which are easily removed in the process of sedimentation or filtration. However, the use of synthetic coagulants can represent a high financial value

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and can leave unwanted residue in the treated water depending on the conditions of the essay. Because of this reason, the use of plant-based coagulants has become a better option for the clarification of turbid waters.

One of the natural coagulants that are already studied is the one that is produced from the powder of *Moringa oleifera* seeds. Composed of approximately 40% protein, (Gallão, 2006), *M. oleifera* seeds are quite used in the clarification of turbid waters in some regions of the planet, such as Sudan and other African countries, Northeast Brazilian, among others (Borba, 2001). This protein is responsible by the processes of coagulation and flocculation of colloids in natural water that present color and turbidity. According to Ndabigengesere et al. (1995), it is a hydro soluble cationic protein, which has molecular mass between 12 and 14 kDa. However, Broin et al. (2002) and Ghebremichael et al. (2005) saw that when this protein is in aqueous solution and it is responsible by the coagulation process, it has molecular mass between 6.0 and 6.5 kDa. Ndabigengesere et al. (1995), Ndabigengesere and Narasiah (1998) and Amagloh and Benang (2009) saw that the use of *M. oleifera* in water treatment could result in a similar efficiency when it is compared with aluminum sulfate.

The use of *M. oleifera* as a coagulant in rural communities can show advantages like the generation of biodegradable sludge (Bergamasco et al., 2009). Depends of the characteristic of the water that will be treated, this sludge can be applied as fertilizer (Ndabigengesere and Narasiah, 1998). Furthermore, the volume of sludge that the use of the *M. oleifera* had created is lower than the one created by the aluminum sulfate (Bergamasco et al., 2009). Ndabigengesere and Narasiah (1998) verified that there were not significant changes in pH parameter because of the use of *M. oleifera*. For many tested dosages, this parameter remained around 7.6. Valverde et al. (2013) also verified that *M. oleifera* can operate over a wide pH range and it does not have a significant change in the treated water pH. Babu and Chaudhuri (2005), Beltrán -Heredia and Sánchez-Martín (2009) obtained a big reduction in turbidity when they used a *M. oleifera* seeds aqueous suspension as auxiliary on the direct filtration. However, Babu and Chaudhuri (2005) verified the fast colmatation of the filter. Using the coagulant based on the *M. oleifera* seeds as an auxiliary in the slow sand filtration process, Franco et al. (2010) found that its use can result in a smaller duration in the career of filtration. Pritchard et al. (2010), in addition to the conventional method of dosing the coagulant, tested an alternative way of performing this dosage: they confined the *M. oleifera* seed powder into pouches made of muslin.

However, based on the results obtained, the authors found that an optimization of the method is required.Based on the recommendations of Pritchard et al. (2010) and also considering that not all materials of the *M. oleifera* seed operates as a coagulant, the objective of this study was develop a new method for the application of the coagulant solution, reducing the addition of possible residues that are not interesting to the water to be treated.

MATERIALS AND METHODS

The research was developed in the Hydraulics and Irrigation Laboratory and in the Sanitation Laboratory of the Faculty of Agricultural Engineering at the University of Campinas (FEAGRI/UNICAMP). The *M. oleifera* seeds were collected in the Experimental Field of FEAGRI/UNICAMP, dried in a heater at 65°C for 24 h and stored in an incubator with temperature and humidity control.

At the time of preparation of the pouches, the seeds were peeled and processed in manual grinder. All the five types of pouches received 2 g of powder obtained after grinding. The characterization of this powder was made according to Henderson and Perry (1976), and it was established that such powder was not considered a uniform product, being composed of medium and fine grain with an average diameter of 0.388 mm. All pouches had approximately 31 cm^2 of contact area. After the powder was added into the pouches, they were sealed and attached to one side of the jar.

Five types of materials were evaluated for the manufacture of the pouches: four non-woven fabrics weighting 272 g m^{-2} (NW 272), 352 g m^{-2} (NW 352), 117 g m^{-2} (NW 117),42 g m^{-2} (NW 42) and one *Melitta*® filter paper, produced in Guaíba/RS/BR, weighting 54 g $m^{-}2$ (FP). The non-woven fabrics were made with polymers. The characteristics of each of these materials are described in Table 1. The thickness was determined using a pachymeter and the relation between the materials weight and the samples area determined the grammage. The permeation and the resistance to the airflow were determined by the Gurley method (NBR NM:ISSO 5636) developed by Packaging Technology Center/ Institute of Food Technology in Campinas/SP. The porosity (or pore percentage) and the pore size was determined using image analysis: the images were obtained by sweep electrical microscopy and processed using the computational program (Rasband and ImageJ, 2014).

Figure 1 represents each of the five types of pouches used. Besides the pouches, we also evaluated the application of liquid coagulant (LC) based on *M. oleifera* seeds. For the production of the aqueous solution, *M. oleifera* seeds were manual peeled and then they were processed in a manual grinder and were sieved in a sieve with 0.84 mm of opening. It was added that powder to distilled and deionized water aiming to obtain 20 g L^{-1} of concentration. After the homogenization of the powder in water using a magnetic agitator for 3 min, a sieve with 0.125 mm of opening passed through the resultant suspension. At least, it was added 100 mL of this solution to one of the jars.

The assays were conducted in Jar-Test equipment. It were added 2 L of distilled and deionized water in each jar in the equipment. In the first jar, it was added an aqueous solution based on 2% *M. oleifera* seeds, under the hypothesis of representing the most critical situation of turbidity and protein. In each of the other five jars, it was positioned one of five types of pouches, as shown in Figure 2. The assays were carried out for 24 h. It were collected samples at the instants: 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720, 780, 840, 960, 1080, 1200, 1320 and 1440 min. Throughout the period, the Jar-Test was kept under slow agitation, with 20 rotations per min (RPM) as described by Pritchard et al. (2010) in order to maintain the homogeneity of the medium. For the proposed evaluation, turbidity analyses were made by nephelometry and concentration of protein by the method of Lowry (1951), adapted by Madrona (2010). The method of Lowry consists in the reduction of folin reagent when it is in the presence of a protein that was previously treated with copper.

Table 1. Thickness, grammage, porosity, pore size, permeance and air resistance (PRA) of the materials used in the manufacture of pouches.

Material	Thickness (cm)	Grammage (g m^{-2})	Porosity (%)	Pore size (µm)	PRA (sec/100 ml)
NW 272	0.18	272	4	15	0.4
NW 352	0.22	352	3	7	0.4
NW 117	0.08	117	6	13	0.2
NW 42	□0.01	41	10	24	0.1
FP	□0.01	54	1	3	0.7

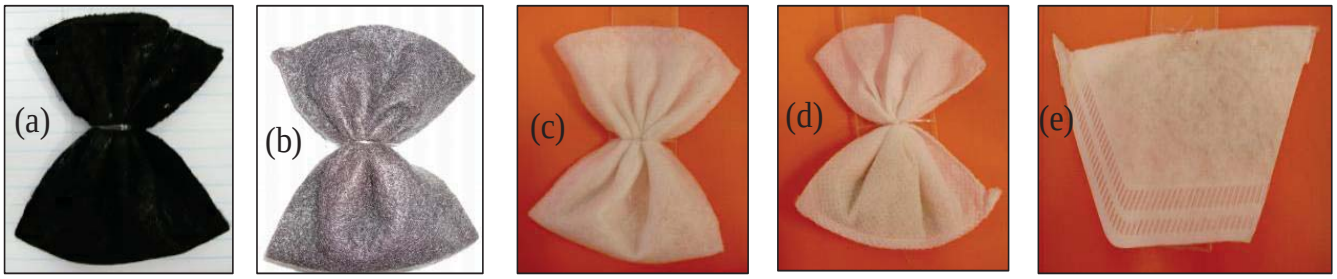

Figure 1. Pouches used: (a) NW 272, (b) NW 352, (c) NW 117, (d) NW 42, (e) FP.

Figure 2. How the pouches in the jars of the Jar-Test.

This reaction can result in a "new" component that gives color to the solution with 550 nm of maximum absorption. The standard curve was obtained using Bovine serum albumin (BSA).

RESULTS AND DISCUSSION

The average turbidity of the distilled and deionized water after the addition of the pouches and liquid coagulant (LC) are represented in Figure 3. With the use of the five pouches, it was found that after 600 min of dispersion, increased turbidity values were observed. There were increased turbidity values with the use of the liquid coagulant since the first min of the dispersion. In the treatment with LC, flakes were probably formed and deposited, fact that can justify the gradual reduction of turbidity over time.

The pouch made with filter paper (FP) was the one that caused the lowest turbidity increase. At the end of the 24 h, the increased turbidity arising from the use of the pouches NW 272, NW 352, NW 117 and NW 41 was respectively, 534, 590, 441 and 296% higher than that observed with the use of filter paper. These percentages are relating to turbidity increase by the pouches NW 272, NW 352, NW 117 and NW 41, when they were compared to the filter paper (the lowest value of turbidity that was observed) after 24 h of contact. However, for all treatments carried out with the use of pouch, it was observed maximum turbidity values below that were verified with the use of liquid coagulant (LC). Comparing the greater turbidity value observed with the use of liquid coagulant (0 min) with such value for the other pouches (1440 min), it was found that the increase in turbidity from the liquid coagulant was 1553, 161, 140, 205 and

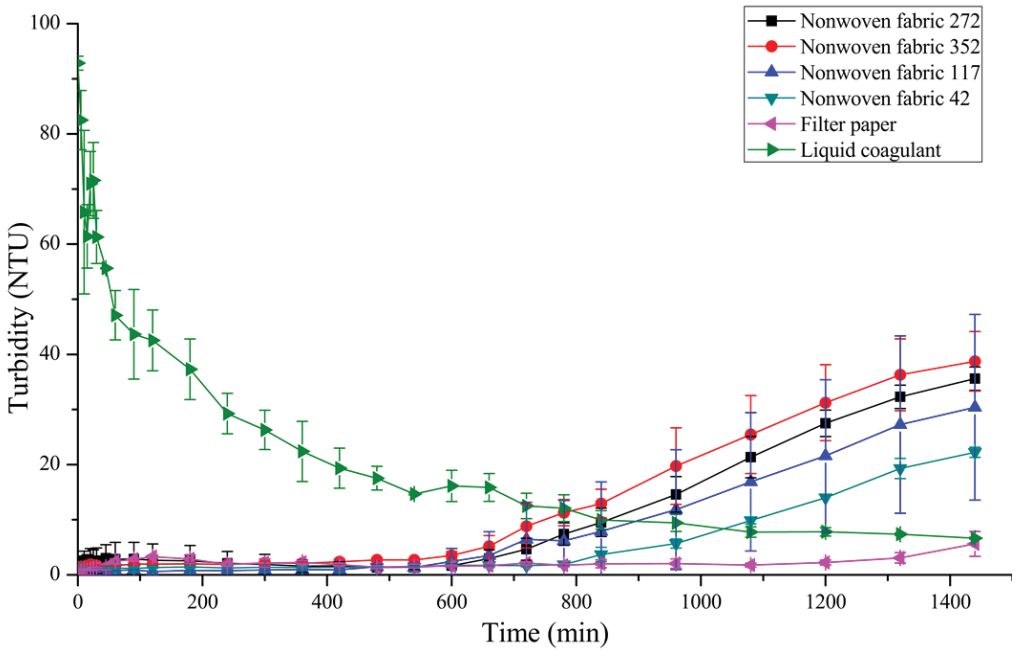

Figure 3. Turbidity over time for the treatments.

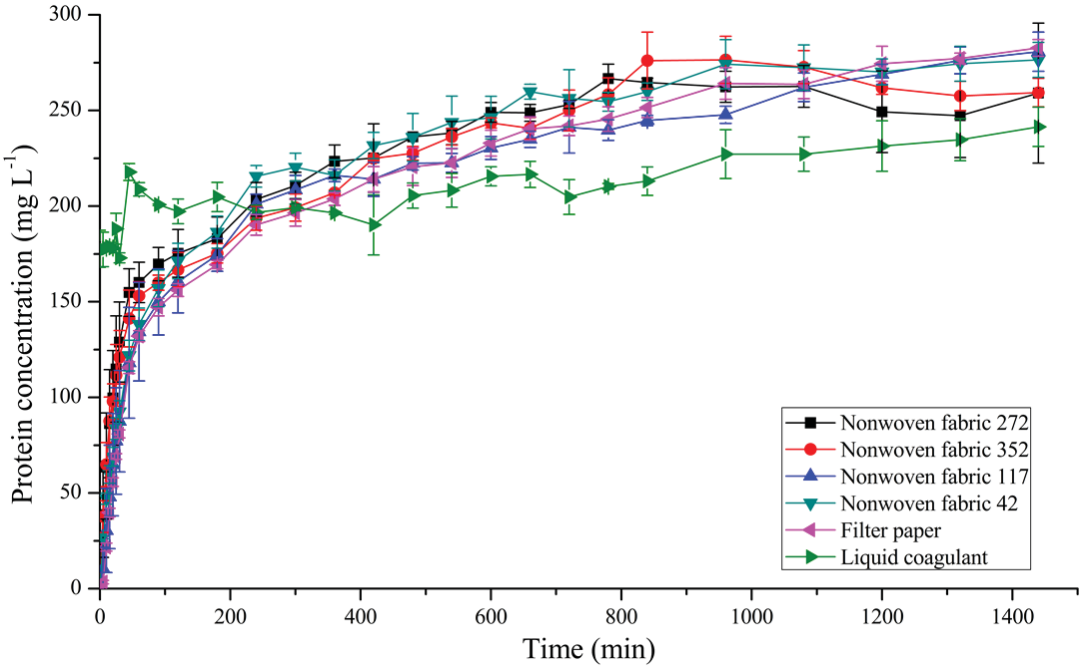

Figure 4. Protein dispersal rate and turbidity: (a) NW 272 (b) NW 352 (c) NW 117 (d) NW 42 (e) FP (f) LC.

317% higher than that observed with the use of the pouches Filter Paper, NW272, NW352, NW117 and NW 41, respectively. In this case, the higher value of turbidity was verified in each treatment were compared, based on the turbidity of original water as reference.

There is the possibility that the reason of the raise in the turbidity during the essay was the solubilization of some substances that were contained in *M. oleifera* seeds. Figure 4 demonstrates that, for the five treatments with pouch, the concentration of protein over time

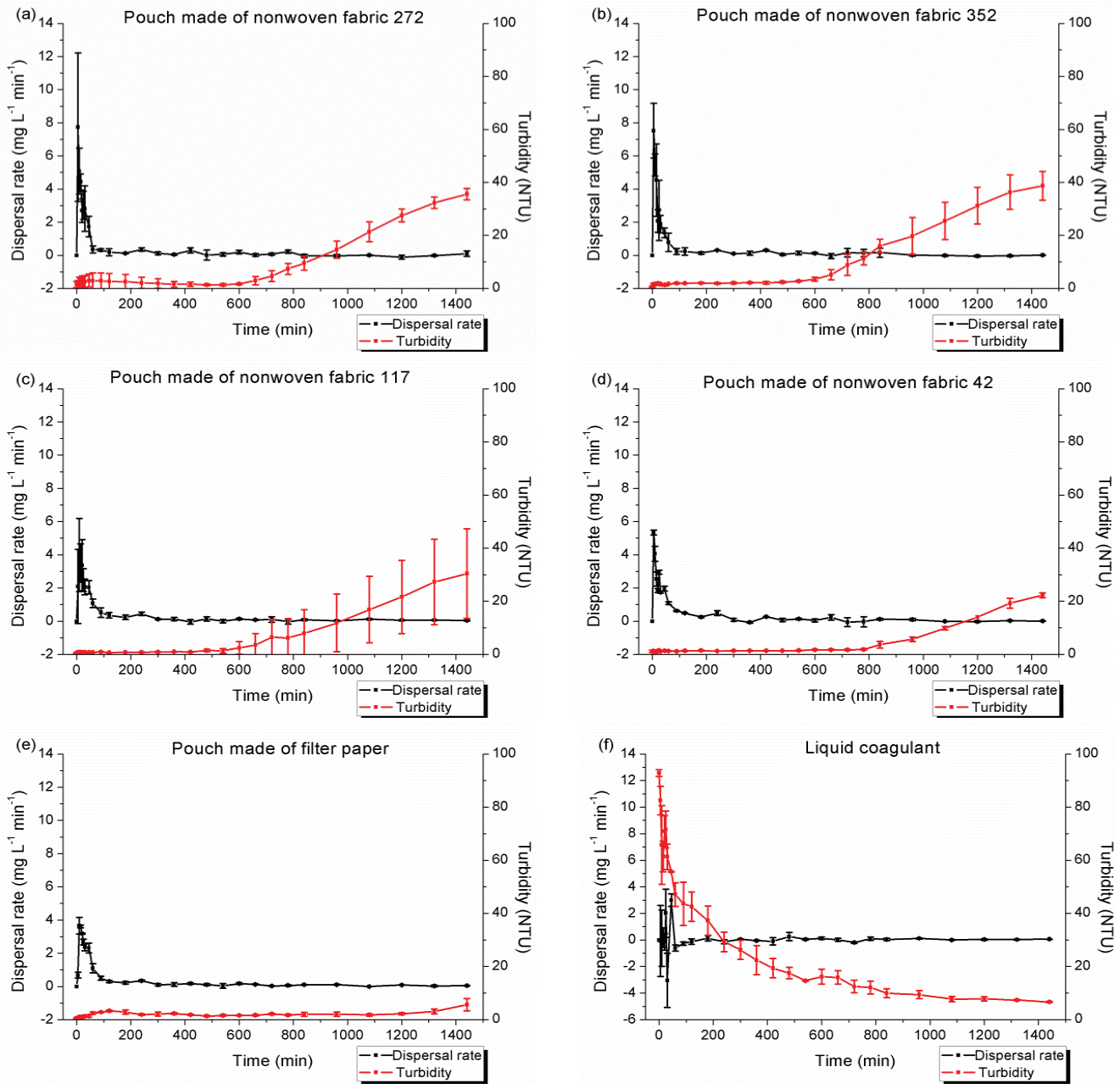

Figure 5. Protein dispersal rate and turbidity: (a) NW 272 (b) NW 352 (c) NW 117 (d) NW 42 (e) FP (f) LC.

presented very similar values, with the greatest increase in concentration at the beginning of the assays and a tendency for stabilization after 480 min. During the preparation of the LC, the *M. oleifera* seed powder was added to the distilled and deionized water, with the agitation of such a mixture. This way, with the use of the LC, it was observed high concentrations of protein at the beginning of the assay.

The average dispersal rate (*DP)* is the rate of change of protein concentration (ΔC_i) for a given period of time (Δt) and is given by the equation:

$$DP = \frac{\Delta C_i}{\Delta t}\left(\frac{\text{mg L}^{-1}}{\text{min}}\right)$$

As shown in Figure 5, the pouches that provided protein for the medium at a higher speed were those made with NW 272 and NW 352. At 5 min, for those pouches, V_{disp} was 7.7 mg L^{-1} min^{-1} and 7.5 mg L^{-1} min^{-1}, respectively. For the other treatments, the maximum dispersion rate did not exceed 5.5 mg L^{-1} min^{-1}. However, in such treatments, the dispersion rate exceeded 1.0 mg L^{-1} min^{-1}

up to 60 min, while, when using the pouches NW 272 and NW 352 at 60 min, the dispersal rate was less than 1.0 mg L^{-1} min^{-1}. For the five treatments, after 60 min, it were observed values lower than 1.0 mg L^{-1} min^{-1} for the dispersal rate, with a predominance of values below 0.1 mg L^{-1} min^{-1}. In the direct application of LC, it was verified that at 30 and 45 min of dispersion there are spikes in the concentration of protein, with V_{disp} equal to 3.0 mg L^{-1} min^{-1} and 2.0 mg L^{-1} min^{-1}, respectively. In the other times, V_{disp} is close to or less than 0.0 mg L^{-1} min^{-1}.

The five types of pouches used provide considerable concentrations of protein in the first minutes of dispersion, which enables their use for the treatment of water, since the protein is the agent responsible for the process of coagulation and flocculation (Madrona et al., 2009). Although the use of the pouch made of filter paper has resulted in a smaller increment of turbidity in the distilled and deionized water, with the use of the other pouches, increased turbidity values were only observed from the times in which the dispersal rate is considerably low, as shown in Figure 5.

Considering the criteria evaluated in this study, we conclude that the five types of pouches can be used. As in all treatments the protein concentration reached values similar to those observed with the use of liquid coagulant in a dispersal period in which the turbidity still had low values. Pritchard et al. (2010) used muslin pouches containing powder of *M. oleifera* seeds in water treatment and they obtained 85% of reduction in turbidity. It shows that this technology has potential to reduce the turbidity in water treatment.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Assessment of variation in pear genotypes through genetic divergence and cluster analysis using D2 statistics

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The genetic divergence among twenty-three pear genotypes under the present study was of medium quantum. The studied genotypes, on the basis of total variability were grouped into three distinct clusters. Maximum number of cultivars were accommodated in Cluster II (Ishiwase, Seckel, Anjou, Beurre Hardy, Lisnova Karavista, Jargonelle, Kala Hathi, Autumn of Yaklove, Kashmir Pear, Hengal, Flemish Beauty, Awal Number and Basuidse Favourite) followed by Cluster III (William Bon Chrétien, Luhimtsa Kalapa, Manning Elizabeth, Red Bartlett, Max Red Bartlett and Starkrimson) and Cluster I (Kiskusui, Yakumo, Bodde Cappiament and Nouveau Poiteau). Cluster II had highest intra cluster value and was therefore the most divergent and Cluster III had least intra cluster value so was least divergent. Highest value for inter cluster distance was recorded between Cluster I and III while it was lowest between Cluster I and II. Cluster means were maximum in Cluster II. Kala Hathi was found to be best cultivar for fruit yield/tree and tree height while Red Bartlett was for fruit length and breadth. Cultivars Beuree Hardy and Ishiwase proved best for different vegetative characters.

Key words: Pear, *Pyrus commmunis* L., cluster analysis, D^2 statistics, genetic divergence.

INTRODUCTION

The cultivated pear (*Pyrus commmunis* L.) is a member of family Rosaceae and sub family Amygdaloideae. The pear is native to coastal and mildly temperate regions of the old world, from Western Europe and North Africa east right Asia. For the success of any breeding programme the basic requirement is the variability found within the members of the population (Bell et al., 1999). It is this variation which if heritable could be used for cultivar improvement, as improved cultivars are the backbone of any orchard system. Therefore, prior to initiation of any breeding programme they should be tested and extent of variability present must be adequately assessed so that the breeding programme could yield the desired results (Sharma and Sharma, 2006). To use or exploit the available variability present in the genetic material in the form of some specific groups or classes, the divergence studies based upon some desirable/suitable parameters is of very essential and of utmost significance (Bellini and Nin, 2002). eeping in view the above, genetic divergence and cluster analysis using D^2 statistics was

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undertaken with the objective to assess the variability present among the twenty-three pear genotypes and potential use of this variability for hybridization programmes. Use of Mahalanobis D^2 statistics to estimate or evaluate the net/total divergence has been indicated by number of workers in different fruit crops (Saran et al., 2007). The use of genetically divergent parents in hybridization under transgressive breeding programme is dependent upon categorization of breeding material on the basis of appropriate criteria (Santos et al., 2011). Apart from providing requisite assistance or help in selection of divergent parents in hybridization, D^2 statistics also adequately assists in the measurement of diversification and the contribution of the relative proportion of each component trait towards the total genetic divergence or variation.

MATERIALS AND METHODS

Studies were carried out in the Department of Fruit Science, Dr □S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India on twenty-three pear genotypes viz□ Lisnova □aravista, Jargonella, □ala Hathi, □iskusui, □akumo, Bodde Cappiament, Nouveau Poiteau, Ishiwase, Seckel, Anjou, Beurre Hardy, Autumn of □aklove, □ashmir Pear, Hengal, Flemish Beauty, Awal Number, Basuidse Favourite, William Bon Chr□tien, Luhimtsa □alapa, Manning Elizabeth, Red Bartlett, Max Red Bartlett and Stakrimson. Tree height and spread (North-South and East) were measured with the help of measuring pole and were expressed in meters. The trunk girth was measured at a height of 9 cm above graft union with the help of measuring tape and was expressed in centimetres. The length and diameters of ten fruits was measured with the help of digital vernier calliper and mean was worked by weighing ten fruits selected randomly from each tree and weighed on a single pan kitchen balance and mean was expressed in grams (g). Flesh firmness of fruit was measured after removing the skin (0.8 cm) and using effigy penetrometer (model FT 327) with plunger of 11 mm diameter. The results were expressed in lb per sq inch. Total soluble sold contents of five uniformly ripened fruits of each tree were determined with an Erma hand refrectometer (0 to 32° Brix) by placing few drops of juice on the prism and reading was taken. The weight of entire fruits harvested from per tree was recorded for each cultivar and the results were expressed as yield per tree. Mahalanobis D^2 statistic was used for assessing the genotypic divergence between populations (Mahalanobis, 1936). The generalized distance between any two populations is given by the formula:

$$D^2 = \sum\sum \lambda ij\ \sigma_{ai}\ \sigma_{aj}$$

Where D^2 □ square of generalized distance□λij □ reciprocal of the common dispersal matrix, σ_{aj} □ (□$_{i1}$-□$_{i2}$); σ_{aj} □ (□$_{j1}$-□$_{j2}$)□□ □ general mean. Since, the formula for computation requires inversion requires inversion of higher order determinant, transformation of the original correlated unstandardized character mean (□$_s$) to standardized uncorrelated variable (□s) was done to simplify the computational procedure. The D^2 values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (gs) values of any two uncorrelated genotype of D^2 value. All n(n-1)/2 D^2 value were clustered using Toucher's method described by Rao (1952). The intra cluster distances were calculated by the formula given by Singh and Chaudhary (1997):

Square of the intra cluster distance = $\sum D^2_i/n$

Where $\sum D^2 i$ is the sum of distance between all possible combinations of the entries included in a cluster and n is number of all possible combinations.

The inter cluster distances were calculated by the formula described by Singh and Choudhary (1997):

Square of the inter cluster distance□ $\sum D^2_{i\,/}\, n_i\, n_j$

Where, $\sum D^2_i$ is the sum of distances between all possible combinations (n_i n_j) of the entries included in the cluster under study. n_i is number of entries in Cluster I and n_j is number of entries in Cluster j.

The criterion used in clustering by this method was that any two genotypes belonging to the same cluster, at least on an average, show a small D^2 value than those belonging to two different clusters.

RESULTS AND DISCUSSION

The clustering pattern of twenty-three cultivars of pear for tree, shoot, fruit and yield characters are presented in Table 1. The genetic divergence in the present study observed among the twenty three cultivars is of medium quantum. The twenty-three cultivars on the basis of net variability were grouped into three distinct clusters. Maximum number of cultivars (13) were accommodated into Cluster II (Ishiwase, Seckel, Anjou, Beurre Hardy, Lisnova □aravista, Jargonelle, □ala Hathi, Autumn of □aklove, □ashmir Pear, Hengal, Flemish Beauty, Awal Number and Basuidse Favourite) while the minimum number (4) were in Cluster I which included □iskusui, □akumo, Bodde Cappiament and Nouveau Poiteau. Inter and intra cluster divergence values (D^2) between and within four clusters are presented in the Table 2. The intra cluster distance was found to be maximum (2.330) for Cluster II and minimum (1.950) for Cluster III. Highest value (4.192) for inter cluster distance was recorded between Clusters I and III while it was lowest (2.965) between Clusters I and II. On the basis of results, it is inferred that subsequent hybridization between the genotypes having broad genetic base should result in maximum heterotic performance and eventually the desirable transgressive recombinants, as broad genetic base is a fundamental requirement for any crop improvement programme (Sharma et al., 2013□ Srivastava et al., 2012). The Cluster II accommodating cultivars Ishiwase, Seckel, Anjou, Beurre Hardy, Lisnova □aravista, Jargonella, □ala Hathi, Autumn of □aklove, □ashmir Pear, Hengal, Flemish Beauty, Awal Number and Basuidse Favourite was more divergent, followed by Cluster III having six cultivars namely William Bon Chr□tien, Luhimtsa □alapa, Manning Elizsbeth, Red Bartlett, Max Red Bartlett and Starkrimson. Wide diversity in the progeny is expected when hybridization is attempted within cultivars which are more divergent. Since inter cluster distance is maximum (4.192) between Clusters I and III so maximum variability will be achieved when hybridization between the cultivars accommodating these clusters is attempted.

The cluster means of the various trees, shoot, fruit and

Table 1. Clustering pattern of 23 genotypes of pear on the basis of genetic divergence.

Cluster	Number of genotypes	Genotypes
I	4	□iskusui, □akumo, Bodde, Cappiament and Nouveau Poiteau
II	13	Ishiwase, Seckel, Anjou, Beurre Hardy, Lisnova □aravista, Jargonelle, □alaHathi, Autumn of □aklove, □ashmir Pear, Hengal, Flemish Beauty, Awal Number and Basuidse Favourite
III	6	William Bon Chr□tien, Luhimtsa □alapa, Manning Elizabeth, Red Bartlett, Max Red Bartlett and Starkrimson

Table 2. Intra and inter cluster distance (D^2).

Cluster	I	II	III
I	1.970		
II	2.965	2.330	
III	4.192	3.623	1.950

Table 3. Cluster means for different characters among 23 genotypes of pear.

Character	Clusters		
	I	II	III
Tree height (m)	5.12	7.21	4.74
Tree girth (cm)	66.83	86.15	52.61
Tree spread (m)	4.27	5.68	4.51
Fruit length (mm)	46.77	52.47	69.66
Fruit breadth (mm)	47.95	53.81	63.75
Fruit weight (g)	74.13	102.90	151.24
TSS (°Brix)	11.17	13.59	14.13
Fruit firmness (lb/sq inch)	18.71	18.88	18.52
□ield (kg/tree)	16.29	36.88	36.84

yield characters are presented in Table 3. The average cluster means revealed highest values for characters like tree spread (7.21 m), tree girth (86.15 cm), tree spread (5.68 m), fruit firmness (18.88 lb/inch^2) and yield (36.88 kg/tree) in Cluster II. The Cluster III had better mean performance for the traits like fruit length (69.66 mm), fruit breadth (63.75 mm), fruit weight (151.24 g) and TSS (14.13°Brix). Cluster I did not show mean value superior than Cluster II and for any character studied. While studying the clonal variability in mango Manchekar et al. (2011) reported substantial variation after applying D statistics. Hence, it is concluded that genotypes with wide variation accompanied with useful characteristics could be effectively employed in intra specific crossed with the hope that this would lead to the transmission of higher genetic gain for different putative traits major being yield from practical utility perspective. On the basis of the performance of different cultivars and the cluster analysis, the twenty-three pear genotypes have been identified for different characters (Table 4) which are potential parents for hybridization programmes. 'Kala Hathi' is best cultivar for fruit yield/tree and tree height. However, cultivar Red Bartlett outperformed all other cultivars for the characters fruit length and breadth. Cultivars Beurre Hardy and Ishiwase prove best for vegetative characters, that is, tree girth and spread.

Table 4. Promising cultivars of pear for different character.

Character	Highest cultivars	Lowest cultivars	Promising cultivars at par with highest
Tree height (m)	□ala Hathi	Luhimtsa □alapa	-
Tree girth (cm)	Beurre Hardy	Manning Elizabeth	Ishiwase
Tree spread (m)	Ishiwase	□iskusui	□ala Hathi and Basuidse Favourite
Fruit length (mm)	Red Bartlett	□iskusui	Max Red Bartlett
Fruit breadth (mm)	Red Bartlett	□iskusui	-
Fruit weight (g)	Manning Elizabeth	□iskusui	Red Bartlett
TSS (°Brix)	Basuidse Favourite	Nouveau Poiteau	-
Fruit firmness (lb/sq inch)	Anjou	□ala Hathi	Ishiwase, □iskusui and □ashmie Pear
□ield (kg/tree)	□ala Hathi	Seckel	

Sweetness was highest in cultivar Basuidse Favourite.

Conflict of Interest

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

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Full Length Research Paper

Indigenous knowledge system weather forecasts as a climate change adaptation strategy in smallholder farming systems of Zimbabwe: Case study of Murehwa, Tsholotsho and Chiredzi districts

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The smallholder sector is vulnerable to climate change due to its reliance on rainfed agriculture and has the least ability to adapt. Based on appropriate weather forecasts, farmers can mitigate and adapt to climate change through sound crop management decisions. A study was conducted to explore indigenous knowledge system (IKS) weather forecasts as a climate change adaptation strategy in smallholder farming systems of Zimbabwe. Eighty six farmers from three agro-ecological regions with different agricultural potential and cultural backgrounds were involved in the study. Questionnaires and focus group discussions were used to collect data on climate change perceptions, access and interpretation of meteorological forecasts and IKS weather indicators and their use in crop production. Most farmers (93%) believed that there is climate change, citing low rainfall, late rains and rising temperatures as some of the indicators. Sixty five percent of farmers had access to and can interpret the meteorological forecasts disseminated through print and electronic media, though arguing that the forecasts are not timely disseminated. Sixty seven percent of the respondents were using IKS weather indicators such as wild fruits, trees, worms and wind for predicting seasonal quality in addition to meteorological forecasts. Basing on IKS forecasts, farmers are changing varieties, staggering planting dates, varying fertilizer rates and cropping land area. The study showed that IKS forecasts indicators are different in the three agro-ecological regions, are being used by farmers in making farming decisions and if properly documented, disseminated and integrated with scientific seasonal climate forecasts can be used as a climate change adaptation strategy.

Key words: Climate change adaptation, indigenous knowledge systems, meteorological predictions, seasonal climate forecasts, smallholder farming.

INTRODUCTION

Most of Zimbabwe's population (70%) live in communal areas and derive their livelihoods from rainfed agriculture and natural resources (FAO, 2010). Regrettably, the smallholder communal farmers grow crops against a background of limited, erratic and poorly distributed rainfall in space and time resulting in frequent crop

failures. This makes smallholder farmers food insecure especially now when the varying and changing climate makes droughts more common. The smallholder sector is particularly vulnerable to climate change and variability due to its reliance on rainfed agriculture and has the least ability to adapt. It is therefore important for farmers to integrate the issues of climate variability into resource use and development decisions (Siyakumar, 2006).

Seasonal climate forecasts (SCFs) had been used by farmers to make appropriate crop management decisions related to crop choice, planting time, fertiliser use, area planted to a given crop, timing and tillage type (Patt and Owata, 2002; Ziervogel, 2004; Patt et al., 2007). Siyakumar (2006) also argued that the introduction of seasonal climate forecast into management decisions can reduce the vulnerability of rainfed agriculture to droughts and reduce its negative impacts. Seasonal climate forecasts can be conducted based on indigenous knowledge systems (IKS) indicators or scientific meteorological predictions.

The term 'indigenous knowledge' is used to describe the knowledge systems developed by a community as opposed to the scientific knowledge that is generally referred to as 'modern' knowledge (Ajibade, 2003). Indigenous knowledge is the basis for local-level decision-making in many rural communities. It has value not only for the culture in which it evolves, but also for scientists and planners striving to improve conditions in rural localities. Incorporating indigenous knowledge into climate-change policies can lead to the development of effective adaptation strategies that are cost-effective, participatory and sustainable (Robinson and Herbert, 2001). Therefore, indigenous knowledge system forecasts are conducted using the local biophysical and mystical knowledge that has been gained through many decades of experience and has been passed down through generations about cropping decisions based on weather and climate patterns of a specific area (Ziervogel and Opere, 2010).

A study in Nigeria, shows that farmers are able to use knowledge of weather systems such as rainfall, thunderstorms, windstorms, harmattan (a dry dusty wind that blows along the north-west coast of Africa) and sunshine to prepare for future weather (Aibade and Shokemi, 2003). A similar study in Zimbabwe and Zambia shows that smallholder farmers depend mostly on their indigenous knowledge systems for forecasting seasons which they make use of to develop crop management adaptive strategies (Ugabe et al., 2010). The study shows that farmers have several indicators for weather forecasting and some of these are similar in both Zambia and Zimbabwe. Some of these indicators include; floods or excessive rains in the preceding season, strong winds around October, an extended cold season that goes up to August and sometimes September and abundance or scarcity of certain fruits (Ugabe et al., 2010).

The meteorological office offers SCF that is scientific and based on models and empirical data which require skill in interpretation for seasonal quality assessment. The scientific seasonal forecast is based on the fact that lower-boundary forcing, measured by sea surface temperatures, drives future atmospheric perturbations (Murphy et al., 2001). These boundary conditions evolve slowly and so enable predictions of rainfall and temperature to be produced (Parmer and Anderson, 1994). The forecast is usually issued for a period of one, three or six months and suggests the total amount of rainfall expected over that period, but not the distribution of rainfall within that period. If the amount of rainfall forecast were to fall over a few days, the seasonal forecast would still be correct but the impact could be catastrophic (Agrawala et al., 2001). Most smallholder farmers, however, have no access, cannot interpret and use meteorological predications as they live in marginal areas where electronic and print media are inaccessible (Nicholls, 2000).

Despite seasonal forecasts having been issued by national meteorological offices in Southern Africa (O'Brien et al., 2000; Basher et al., 2001), the extent of uptake is limited (Walker et al, 2001; Ziervogel, 2001; Archer, 2003). With an increase in climate stress in most communal farming areas, it is important to establish how best forecasts of variable skill might be integrated into the decision making of vulnerable groups to facilitate improved adaptation to climate variability and change (Washington and Downing, 1999; Sivakumar et al., 2000). However, most studies reported on how farmers are trained to understand, interpret, use the meteorological forecasts and to integrate the IKS and meteorological forecasts in their farming operations (Patt et al., 2005; Ugabe et al., 2010). Only a few studies had looked at the use of IKS forecasts as an adaptation strategy in the varying and changing climates by smallholder farmers. The study sought to increase awareness of the IKS weather forecasts that are being used by communal farmers to adapt to the varying and changing climate in crop production. The objective of this study was to explore indigenous knowledge system weather forecasts as climate change adaptation strategy in smallholder farming systems of Zimbabwe.

METHODOLOGY

Study sites

The study was conducted in Gurehwa, Tsholotsho and Chiredzi

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Figure 1. Location of the study sites in Zimbabwe.

districts of Zimbabwe (Figure 1). Murehwa district which is 130 km north-east of Harare (17°45'S and 31°31'E) lies in agro-ecological region (AgR) II, where average rainfall is 1000 mm per annum. The soils in the area are classified as Typic Sandiustaff derived from granitic parent material with relatively low water holding capacity and consists of sandy to loamy sands (Vogel et al., 1994). Tsholotsho district is 110 km north-west of Bulawayo (19°45′59.77″S 27°45′00.00″E) and falls in AgR III, where average rainfall is 650 mm per annum, mostly restricted to the summer season from November to April (Surveyor General, 2002). Tsholotsho is composed of mainly three types of soils, the Kalahari Sands, fertile black clay soils and the red soils. Respondents were located on purely Kalahari sands. Chiredzi district is 384 km south-east of Harare (18°55′S 29°49′E) in AgR IV, where average rainfall received is less than 450 mm per annum through erratic showers (Vincent and Thomas, 1962) and has predominantly vertisols. The three districts were chosen because of their different agro-ecological locations and cultural traditional practices.

Data collection and analysis

A total of 86 farmers were selected through stratified random sampling technique from Chiredzi (25), Murehwa (30) and Tsholotsho (31) districts respectively. The selection of respondents was limited to wards in which our research partner Community Technology Development Trust (CTDT) conducts farmer field schools (FFS) to promote crop diversity. Thus respondents were composed of FFS participants and non-participants. Looking to the nature of study, the qualitative approach, which is more appropriate in case of studying indigenous knowledge systems and natural resources, has been chosen for the explanation of qualitative data. Personal interviews and participatory methods were used complimentarily to carry out the study. Both approaches have their place and their results can be complementary and possibly cross-validate each other. Using a structured questionnaire and in-depth interviews; quantitative and qualitative data were collected on farmers' perceptions on climate change, their responses to climate change and variability, access to and interpretation of meteorological predictions, IKS seasonal weather indicators and their use in farming operations. Farmers responded either at their homesteads or during farmer field school sessions. Responses from the questionnaires were gathered from August to November 2010. During the data collection process Agricultural Technical and Extension (AGRITEX) officials were engaged in all the three districts. Data collected in the study were analysed and inferences have been drawn using statistics like percentage, mean and rank using descriptive frequencies in the Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Perception of farmers on causes and effects of climate change

Most farmers (93%) in all the three districts believed that the climate is changing. Their indicators are low rainfall being received, change in planting season from October to late November or early December, late rains, rising temperatures, disappearance of very cold winters, erratic rainfall distribution and drying of wetlands. The responses are in agreement with the Intergovernmental Panel for Climate Change (IPCC) Report (2007) which dispelled any uncertainty about climate change and gave detailed projections for the 21st century, which showed that climate change and variability exists, will continue and accelerate.

Farmers stated different causes of climate change which can be grouped into scientific, cultural, and spiritual categories (Figure 2). Scientific causes of climate change given by farmers (25%) were deforestation, ozone layer depletion, technological advancement and release of industrial gases into the atmosphere. These findings are supported by IPCC Report (2007) which highlighted that humans play a major role in causing global warming, hence, climate variability and change.

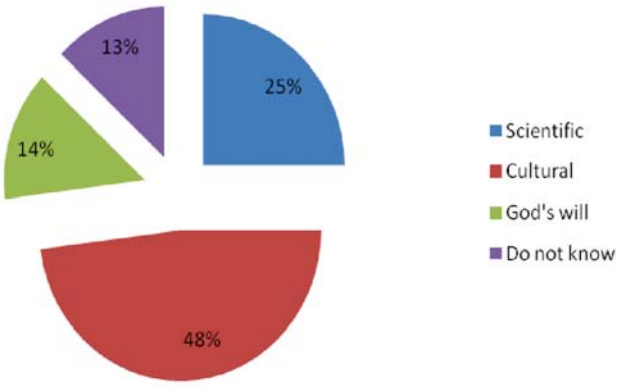

Figure 2. Smallholder farmers' perceptions on the causes of climate change.

However, □u□ari et al. (2014) noted that greater proportion of smallholder farmers in Zimbabwe perceives climate change as a purely natural phenomenon, without any human intervention being responsible for climate change. Forty eight percent of farmers believed that change and disrespect of cultural and traditional values like bathing at sacred places, incestuous behaviour, committing suicide, abortion, not brewing ceremonial traditional beer and inappropriate burials could be the causing factors of climate change. However, 14□ of the farmers are of the mindset that it was God's will that the climate is changing and nothing can be done about that. They believe that the Almighty might be angry at the way people are living as they no longer follow his ways□commandments. These assertions are supported by □ubaya et al. (2010) who noted that farmers in □ower Gweru in Zimbabwe "state that causes of climate change have also been due factors such as the wrath of cultural spirits and □od who have meted out punishment to Zimbabwe. The punishment has been for the failure of people to continue to appease their spirits and conduct traditional rites such as the rainmaking ceremony (*mukwerera*) for asking for rain from □od and for showing gratitude for the rains in the previous season".

Communal farmers in the three districts studied noted different effects of climate change being experienced in their areas (Table 1). Some of the effects mentioned were decreased yields, changes in planting time and less water for livestock. Other studies done in smallholder areas of Zimbabwe have observed the following changes in climate in recent decades. First, rainfall patterns have become highly unpredictable. Secondly, the prevalence of mid-season dry spells has increased (□utekwa, 200□). Thirdly, years of below-normal rainfall are becoming more frequent, worsening the food insecurity situation among smallholders. Fourth, it is not uncommon to experience droughts and floods back-to back during the same season (□ubaya, 2010). Fifth, semiarid areas are getting drier and rainy seasons have become shorter (□utekwa, 200□). Sixth, temperatures have increased (□ubaya et al., 200□), and farmers have started experiencing warmer winters than before (□u□ari et al., 2014). This means climate change is affecting food and water resources that are critical for livelihoods and survival in these smallholder sectors of Zimbabwe. This observation is also supported by Nhemachena and Hassan (2008) who noted that changing climates impact negatively in areas where populations rely on local supply systems that are sensitive to climate variability.

Use of seasonal climate forecasts by communal farmers

Sixty-two percent of respondents in all the three districts studied were using SCFs to predict the quality of a season so as to make informed farming decisions and manage risk in the changing climate. From those farmers who do SCFs, 67□ use □□S whilst 56□ utili□e meteorological forecasts on assessing the potential of a season to rain. The use of these SCFs may be necessary in helping farmers to minimi□e risks during dry seasons and maximi□e opportunities during wet conditions. This is supported by Tarhule (2005) and Washington et al. (2006) who noted that the use of SCFs may reduce the impact of climate variability and change on food production and livelihoods through anticipating and managing annual climate related risks.

Indigenous knowledge systems indicators on the quality of a season

48, 72 and 83□ respondents in Tsholotsho, Chired□i and □urehwa districts respectively use □□S indicators to predict the quality of the season before the onset of the rains. The climate indicators that are used to predict if there is rain or drought in the three districts include birds, wild fruits, trees and worms (Table 1). Chired□i district had many indigenous knowledge weather indicators being used by farmers compared to □urehwa and Tsholotsho (Table 1). □opani worms (*Imbrassia belina)* (which are found in the drier areas of Zimbabwe), winter, dew and wild animals' indicators are specifically in use in Chired□i and are not used in the other two districts. The district lies in A□R □, where rainfall is through erratic showers and therefore sub□ect to periodic droughts (□incent and Thomas, 1□62). So farmers could be using many □□S indicators as a responsive measure to minimi□e risks in drier years and maximi□e opportunities in a wet season. Tsholotsho farmers based their weather predictions on birds and wild fruits. However, wild fruits that are naturally found in the district which is mostly covered by the □alahari sands, such as untundulula *(Facourtia indica)*, umviyo *(Vangueria infausta)*, inhlokotshiyane *(Ximenia arnericana)* are mostly used.

Table 1. □□S indicators farmers in different districts use to predict the quality of a season.

District	Indicator	Wet year	Drought year
□urehwa	Births	Birth of too many girl child.	Too many boy child.
	Wild Fruits	Plenty of wild fruits such as hacha *(Perinari capensis*) or ma□han□e (*Vapaca kirkiana*) in October and November.	□ess wild fruits such as hacha *(Perinari capensis*) and ma□han□e (*Vapaca kirkiana*) in October and November.
	Trees	□arly flowering of trees such as msasa *(Brachystegia spiciformis)*, munhondo (*Julbenardia globiflora).*	□ate flowering of trees such as musasa *(Brachystegia spiciformis)*, munhondo (*Julbenardia globiflora).*
Tsholotsho	Birds	Prevalence, singing and movement of certain type of birds like stocks.	No singing and movement of birds like stocks
	Wild fruits	Plenty of wild fruits □ust before the rain season like untundulula *(Facourtia indica)*, umviyo *(Vangueria infausta)*, inhlokotshiyane *(Ximenia arnericana)* umhakawuwe (*Securinga virosa),* isigogwane *(Pappea capensis)*	□ery few wild fruits □ust before the rains like untundulula *(Facourtia indica)*, umviyo *(Vangueria infausta)*, inhlokotshiyane *(Ximenia arnericana)* umhakawuwe (*Securinga virosa),* isigogwane *(Pappea capensis)*
Chired□i	Wild animals	Heavy sounds of wild animals such as lions around October	□ess or no sounds of certain wild animals like lions around October.
	Birds	Prevalence, singing and playing of many different types of birds at the beginning of the season.	□ess singing and playing of different types of birds at the beginning of the season.
	Dew	Falling of dew early in the morning after the first rains.	No dew in the morning after the first rains.
	Trees	□arly flowering of certain trees such as mupfura *(Sclerocarya cattra).*	□ate flowering of certain trees such as mupfura *(Sclerocarya cattra)*.
	Worms	□any mopani worms *(Imbrassia belina)* □ust after the first rains.	Few mopani worms (*Imbrassia belina)* after the first rains.

The fruits are common in the district and are rare in most parts of Zimbabwe. □n □urehwa; births, wild fruits such as hacha *(Perinari capensis)* and ma□han□e *(Vapaca kirikiana)* and trees are being used as weather assessment indicators. The trees and wild fruits in use are specific to the district and are different to those being used in the other two districts.

The results show that indicators being used by farmers are different for the different districts as natural resources available, cultural□traditional and social backgrounds vary with location of an area. Ziervogel and Opere (2010) also noted that □□S forecasts are conducted based on local biophysical and mystical knowledge that has been gained through many decades of experience and are specific to an area. However, there are some indicators such as wind and clouds that are common to the districts. The interpretation of these indicators in use is almost the same amongst the different farmers in the different districts (Table 1). Strong easterly winds around August and September indicates a good season whilst weak winds are a reflection of a dry year. □n all the districts, heavy dark clouds before a rain season are interpreted to mean a wet season whereas light white clouds mean a dry year.

Basing on the □□S predictions, most farmers in □urehwa and Chired□i districts anticipated a good 2010□2011 rainfall season. □ndicators like many mopani worms, early flowering of certain trees, strong easterly winds and many wild fruits indicated that the season was going to be good. This was in agreement with the rains received and scientific forecasts of □eteorological Services Department of Zimbabwe which predicted normal to above normal rains for the two districts for 2010□2011 season. Farmers in Tsholotsho district predicted that the 2010□2011 season would have below normal rains. This was supported by most local indicators such as very few wild fruits, light winds and late flowering of some trees which indicated that the season was not going to be good. However, this was in disagreement with the rains received and scientific forecasts of □eteorological Services Department of Zimbabwe which predicted normal to above normal rains for the district for 2010□2011 season.

Use of IKS weather predictions in smallholder farming operations

All respondents in Tsholotsho and Chired□i districts, who do □□S weather forecasts, grow crops and adopt specific farming operations basing on the quality of the season. Surprisingly, □urehwa district which had 83□ of

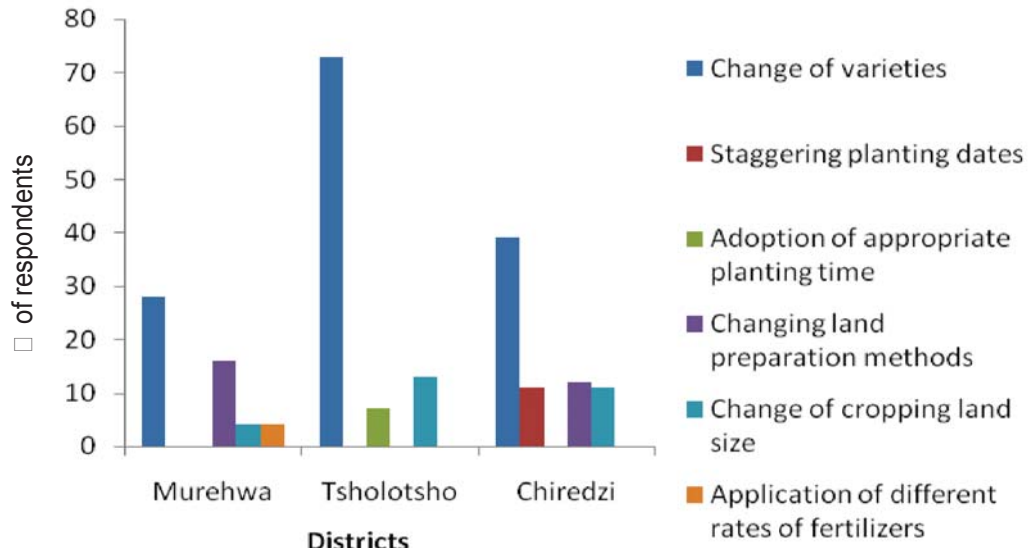

Figure 3. Different farming operations being adopted by farmers basing on □□S forecasts.

respondents conducting □□S weather predictions had the least farmers (24□) growing crops basing on the forecasts. □aybe it is because most □urehwa farmers who are only 130 km from Harare (87□) have access to meteorological forecasts compared to 52□ and 61□ for Chired□i and Tsholotsho respondents respectively through print and electronic media hence base their farming decisions on that.

The different farming operations that are being adopted by farmers basing on □□S forecasts include changing varieties, staggering planting dates, dry planting and use of hoes for land preparation (Figure 3). This is correct as seasonal climate forecasts have been used before for assisting farmers make appropriate crop management decisions like correct crop choice, fertili□er rates, planting dates and tillage type (Patt and □wata, 2002; Ziervogel, 2004; Patt et al., 2007). Ziervogel and Calder (2003) also demonstrated that farmers who used SCF forecasts for planning purposes significantly improved their yields.

73, 3□ and 28□ of respondents in Tsholotsho, Chired□i and □urehwa districts respectively are changing varieties to suit the quality of the season as per □□S predictions they could have done (Figure 3). Short season varieties are being adopted for drier years whilst medium to late maturity varieties can be chosen for a good year depending on the agro-ecological region of the district. This is so because different agro-ecological regions have different potential for different varieties (Surveyor □eneral, 2002). For example, □urehwa farmers used to grow medium to late maturity varieties but are now changing to short season varieties if they forecast a dry year. Tsholotsho and Chired□i farmers who used to prefer medium maturity varieties are now shifting to the short season varieties in drier years so as to minimi□e production risks. Bourdillan et al. (2003) also noted that short season varieties are attractive to farmers who are risk-averse as they have superior tolerance to heat and drought, as they silk faster, making them less susceptible to mid-season drought and mature quickly. Some farmers also noted that open pollinated varieties (OP□s) perform better compared to hybrids in drier years. This could be because OP□s are more drought resistant compared to hybrids which are sensitive to moisture stress (Chidu□a et al., 1□□4).

□n Chired□i district 11□ of respondents are staggering planting dates as a response to the □□S season quality assessment done. □f the forecast indicates good rains, farmers usually plant all their fields with the first effective rains. However, if indicators forecast a dry year farmers may start planting the first fields as early as end of October with the first rains, then wait and assess how the season proceeds. Depending on the rainfall being received, they then stagger planting of the remaining fields accordingly. □ast season (200□2010) most farmers planted the first fields in mid November and only planted all the other fields in □anuary (Personal communication, 2010). This was because the district witnessed a dry spell from November after the first effective rains to □anuary.

13, 11 and 4□ of respondents in Tsholotsho, Chired□i and □urehwa districts are changing the cropping land si□e depending on the □□S predictions on the quality of the season. □f indicators predict a wet year, maximum possible land area is planted so as to maximi□e opportunities of a good harvest. □n drier years farmers tend to reduce the cropping land thereby concentrating their labour and inputs on small pieces of land. This is done to reduce drought costs and to implement soil water conservation strategies to small pieces of land where it is manageable. This implies that there will be less wastage on resources and land maybe rested.

Few respondents (4□) in □urehwa district are applying different fertili□er rates depending on the quality of the season. □f predications are of a wetter season, recommended fertili□er rates are applied. However, this will depend on the ability to buy and availability on the market. □ubaya et al. (2010) observed that apart from SCF and how to respond, it is equally important to understand the socio-economic status of the recipient because wealth endowment decides on which adaptation strategy to adopt to cope with the predicted climate risk. This means farmers may be willing to apply recommended fertili□er rates in a good year but cannot because they have no ability to buy. □f the season is dry, micro dosing is done. This is because the use of low levels of fertili□ers especially N has consistently given better grain yields in dry years (Hove et al., 2010).

16 and 12□ of respondents in □urehwa and Chired□i districts, respectively are changing land preparation methods basing on IKS forecasts. In drier years' soil water conservation strategies that include basins, tied ridges and infiltration pits are being implemented. Use of these techniques in combating the drought by crops has been reported to be effective (Unganai and □urwira, 2010; □erie and □ugiya, 2010). There is also an increase in use of hoes for land preparation in the study sites that can be attributed to the promotional work by Community Technical Development Trust a Non-□overnmental Organisation (N□O) which is advocating for conservation agriculture. □ncreased uptake of hoes can also be due to the decline in draught power due to droughts and socio-economic hardships of 2008 which resulted in most farmers selling livestock so as to survive.

Knowledge of the meteorological season climate forecasts by communal farmers

□ost respondents in □urehwa (87□) and 61□ in Tsholotsho districts knew about the SCFs that are done by the □eteorological Department office through print and electronic media, A□R□T□□ officials and friends. Therefore it can be noted that meteorological SCFs have become increasingly available to most farmers over recent times (O'Brien and Vogel, 2003; Roncoli et al., 200□). However, less than of 50□ of the farmers in Chired□i district have heard about meteorological forecasts. □ost farmers in the district do not have access to the SCF since radio and television transmissions are poor. Nicholls (2000) also observed that use of SCFs is impaired by several constraints that include inappropriate content from a user perspective and communication problems.

□n all the three districts most farmers (□2□) who know meteorological forecasts understand the meaning of the predictions from the meteorological office. This is because of late the □eteorological Department is simplifying the scientific language of models and empirical data it uses in forecasting to the language that is understood by most communal farmers. The meteorological department after coming up with the percent probabilities and translating them into Above Normal, Normal and Below Normal do not release the percent probabilities to the public. Farmers then understand the predictions as the terms below normal, normal and above normal are easier to understand than the probabilities used in SCF. This however, can mislead farmers in that if a forecast is below normal to normal the take home message is normally that the coming year is a drought for it neglects the probabilistic nature that is associated with the seasonal climate forecast (□ugabe et al., 2010).

Seventy percent of farmers in all the three districts were in agreement that meteorological predictions are similar and useful as the □□S assessments they do. However, they argued that although the meteorological forecasts are scientific there are not timely disseminated, which, makes them not that useful compared to the □□S predictions. For seasonal climate forecasts to have an impact to farmers, they have to be reliable, timely, well presented and readily accessible (O'Brien and Vogel, 2003), which, is not the case with the meteorological forecasts. The □□S indicators are observed early before the season commences whilst the meteorological department can sometimes disseminate the forecasts way after the season had started. This makes the farmers have much more faith in the □□S forecasts compared to the meteorological predictions. □crea et al. (2005) showed that adoption and use of SCF by farmers depends on the level of understanding of the forecast, the format of presentation of the forecast, time the forecast is disseminated and the attitude of farmers towards the usefulness of the forecast as an indicator of future rainfall.

Farmers' suggestions to meteorological department for better SCFs dissemination

Farmers in all the three districts were in agreement that the meteorological forecasts can only be useful after being simplified and reaching the farmers before the onset of the rains. They suggested that the forecasts from the meteorological department should be passed through A□R□T□□ offices and N□Os. They argued that these organi□ations are always in contact with them before, during and after the rains, so it will be easy and effective to receive the forecasts from them. □oreover these organisations offer them inputs, extension and teach them new farming practices to adopt in the changing climate. Farmers also advised the meteorological department to distribute weather forecasts pamphlets, release the forecasts every month and to do community communication through councillors and chiefs. Chired□i farmers suggested that radio communication should be

improved through disseminating forecasts in different vernacular languages that are used in different areas of the country. As a long term strategy farmers proposed that the meteorological department should build substations closer to farmers so that they give specific predictions for specific areas not to generalise.

Conclusions

The study showed that most IKS forecasts indicators in use in the three agro-ecological regions are different, are being used by farmers in making farming decisions and if properly documented, disseminated and integrated with scientific seasonal climate forecasts can be used as a climate change adaptation strategy. The indicators differ with districts as natural resources, traditional and social backgrounds vary with location. The climate indicators in use include wild fruits, trees, worms and birds. Depending on the interpretation of the indicators farmers are making different farming decisions such as changing varieties, staggering planting dates, changing cropping land size and applying different fertilizer rates to suit quality of a specific season. Most farmers also understood the forecasts from the meteorological office because the department is now simplifying the scientific language it uses in forecasting to the language that is understood by most communal farmers. They agreed that meteorological predictions are similar and useful as the IKS assessments they do. However, farmers argued that although the meteorological forecasts are scientific and more reliable, there are not timely disseminated, which, makes them not that useful compared to the IKS predictions they do. Farmers advised the meteorological department to disseminate the forecasts early before the rains through ARTs and NGOs, distribute weather forecasts pamphlets and to release the forecasts every month.

Conflict of Interest

The authors have not declared any conflict of interest.

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Genetic analysis in a six parent diallel cross of cassava (*Manihot esculenta* Crantz)

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There are concerted efforts in promoting cassava production in sub Saharan Africa as a staple food crop as well as income earner at both household and commercial level. These efforts can bear meaningful results if breeding strategies are properly coordinated. Efficient breeding methods require a thorough understanding of the genetic background of planting materials. Therefore, a genetic study was conducted to establish the genetic basis of cassava genotypes for root weight, root number/plant, root length (cm), above ground weight, harvest index, cassava mealy bug and cassava green mite by mating six cassava genotypes using a diallel method. The generated F1s were evaluated using a randomized complete block design (RCBD) replicated thrice. The analysis of variance revealed highly significant (P< 0.001) genetic variation among the genotypes for all the traits except for cassava mealy bug, indicating the presence of high variability among genotypes. Thus, selection among these genotypes could lead to good progress for the improvement of the target traits. Mean squares for both general combining ability (GCA) and specific combining ability (SCA) were highly significant for all traits except for harvest index and cassava green mite for GCA and SCA respectively which suggested involvement of additive and non-additive component of heritable variance in the inheritance of all these traits. However, the proportions of additive variance to the total genetic variances (that is, $2\delta^2gca/(2\delta^2gca + \delta^2sca)$ showed that crossing for these traits and making selection from such crosses, about 41 to 100% of the improvement would be expected to come from SCA and less than 59% from GCA which indicated a greater role played by non-additive genes. Overall, results indicated that it would be readily possible to breed for high yield from this set of genotypes.

Key words: Breeding, combining ability, gene action.

INTRODUCTION

The importance as well as challenges of cassava production are well known worldwide. The acclaimed advantages accrued to cassava production include staple food crop and source of income for the rural communities, industrial raw material, and earner of foreign exchange (Cock, 1□85). Furthermore, cassava is regarded as a "low risk crop" as it adapts readily to a wide range of agro ecological conditions, utili□es efficiently mineral reserves of marginal soils, withstands climatic variations and is highly efficient in the conversion of solar energy to starch. □n addition, cassava has low requirements of inputs like fertili□ers, its flexibility in planting and harvesting, convenient in ground storability, diversified modes of utili□ation and higher dry matter yield

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per hectare (Westby, 2002; Ministry of Agriculture, Irrigation and Food Security [MoAFS], 2007). Thus, cassava has tremendous potential for future exploitation to contribute to food security, rural income, and the economy in the African continent.

However, major threats of cassava in terms of yield and quality are pests and diseases infestation (Nahungu et al., 1994). The major insect pests are cassava mealy bug (CMB) and cassava green mite (CGM) while cassava mosaic disease (CMD), cassava bacterial blight (CBB) and cassava brown streak virus disease (CBSD) are the important diseases (Dixon et al., 2003). These diseases can affect plant establishment and vigour, inhibit photosynthetic efficiency and cause pre-harvest or postharvest deterioration. Severe occurrences of both insect pests and diseases often lead to a considerable loss in yield potential of cassava. Insect pests can cause damage to cassava by reducing photosynthetic area, damaging stems, which inhibit nutrient transport causing low production (IITA, 1990).

Combining ability is the relative ability of an inbred line or a clone, when crossed to another inbred line or clone, to transmit desirable traits or specific trait to the next generation (Chaudhari, 1971). It helps to predict the performance of a particular line when used as a parent in a hybrid and facilitates the selection of superior parents for hybrid combination and for studying the nature of genetic variation (Russel, 1985). It is reported that Griffing (1956) introduced a method of analysing combining ability using the genetic estimates of the parent and hybrid components of diallel analysis through general and specific combining ability. Falconer and Mackay (1996) defined general combining ability (GCA) as the mean performance of the line in all its crosses and it is expressed as a deviation from the mean of all crosses. This average performance of parents in crosses GCA, estimates the breeding value of a given genotype due to additive gene effects (Ceballos et al., 2004). Specific combining ability (SCA) is defined as the deviation of individual crosses from the average performance of parents, and this is due to dominance effects.

Understanding of gene action would be useful for the formulation of breeding strategies to improve desired traits. Therefore, information on combining ability is needed in order to identify suitable parents (among the following; Kulola, 01/1313, Depwete, 01/1316, Silira and Kaunili) and superior genotypes which can be hybridized for the development of elite cultivars and hybrid varieties that would ultimately ensure sustained production and productivity by smallholder farmers in Malawi.

MATERIALS AND METHODS

The present study was conducted during 2011 to 2012 season at Chitala Agricultural Research Station. Chitala Agricultural Research Station lies on latitude 13°40' South and on longitude 34°15' East. It is at an altitude of 606 m above sea level. The station is on the lakeshore areas of Malawi and therefore has hot weather with mean annual temperatures of 28°C maximum and 16°C minimum. The soils are sandy clay to clay with the pH range of 5.8 to 6.4 (Figure 1).

Plant materials

Six cassava genotypes namely, Kulola, 01/1316, 01/1313, Depwete, Silira and Kaunili and their F1 derived through half diallel during 2009 to 2010.

Seedling evaluation trial

Seeds were germinated and grown on 9th November, 2010 in a glasshouse in floating trays at Chitedze Agricultural Research Station. The water was given twice a day to ensure good germination and development. Two months after sowing, the seedlings were transplanted on 13th January, 2011 for field evaluation in a seedling trial. The seedlings were planted at spacing of 100 × 50 cm. Parents (mature stems 25 cm long) were also planted at the same spacing but 20 plants per genotype/variety of each parent. No irrigation and fertilizers were applied at this stage.

Clonal evaluation trial

Fifteen crosses and six parents were used in the clonal trial. Twenty plants were selected from each cross. The selection of plants per cross from seedling field trial was based on the ability of the plant to produce six good vegetative cuttings to proceed with clonal evaluation. A randomized complete block design with three replicates was used to plant a total of twenty one entries consisting of fifteen F_1s and six parents. The planting date was 14/01/2012. Each replication contained twenty one entries (parents and crosses), planted together in the respective plots of each replication. The plant spacing was 1 m between rows and 0.10 m within rows. The field was weeded manually and not irrigated or fertilized.

Agronomic characters measured

During the growing period, data was collected on CGM and CMB which were scored quarterly at 3, 6, and 12 months after planting (MAP) using a scale of 1 to 5, where, 1 means no visible symptoms while 5, severely attacked (IITA, 1990). At harvest, 12 MAP, the individual plants were assessed for their number of storage roots per plant, root weight (kg per plant), above ground weight (kg/plant), root length (cm) and harvest index. Plants were hand-harvested individually and the results were averaged across plants. The roots were counted and weighed separately.

Data analysis

Data was analysed using SAS version 9.3 (SAS Institute Inc, 2011). Different analytical tools were employed and these included general analysis of variance, analysis of variance for combining ability and estimates of genetic parameters.

General analysis of variance

The general analysis of variance was used to test if the sources of variation had any significant influence on the characters under study using the following model:

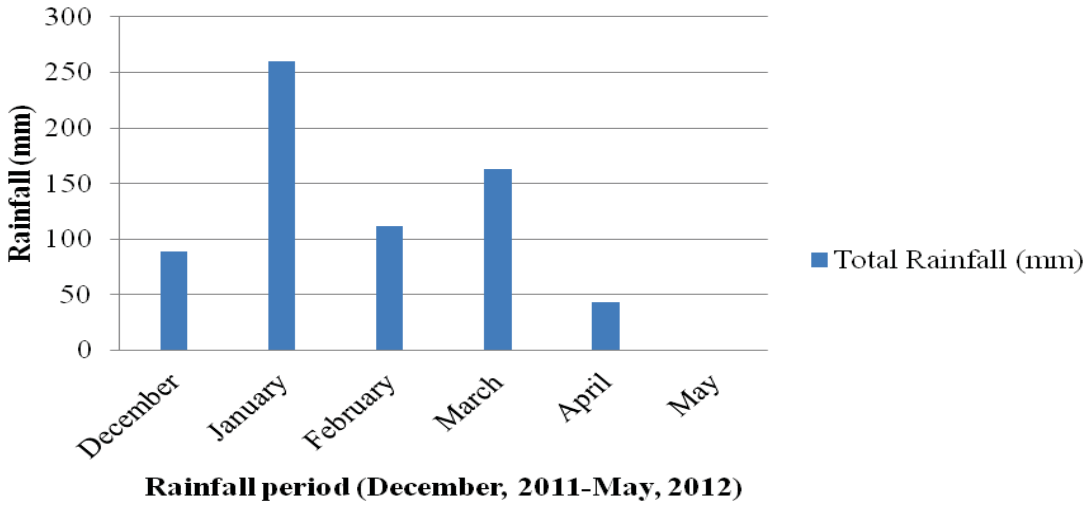

Figure 1. □onthly rainfall (mm) during trial experimentation period.

□$_{ik}$ = μ + T_i□ B_k □□$_{ik}$

Where□□$_{ik}$□observation of the i□cross in block k, □ □overall mean associated with the data, T_i□□effect of the cross i□, B_k□effect of block and □$_{ik}$□effect of the random error associated with the i□cross in block k.

Combining ability effects

□CA effects and SCA effects were estimated according to □riffing (1□56) model 1 method 2 using D□A□□□-SAS05 program developed by Zhang et al. (2005). Griffing's fixed model for the second method is presented below as outlined by □ayo (1□87).

□$_{ij}$□ □ □ gca_i □ gca_j□ sca_{ij}+ E_{ij}□

Where□□$_{ij}$□ is the observed response, □ □ is population mean, gca_i □ the general combining ability (□CA) of the i^{th} parent, gca_j□ the general combining ability (□CA) of the j^{th} parent, sca_{ij}□ the specific combining ability (SCA) associated with the i^{th} and j^{th} cross and E_{ij}□ the error associated with each observation.

Test of significance for combining ability effects

Significance of combining ability effects were determined by using *t*-test at 0.05, 0.01 and 0.001 levels of probability. *t* (calculated) for □CA effect □ $\frac{GCA}{S.E}$, and *t* (calculated) for SCA effects □ $\frac{SCA}{S.E}$. Standard error (S.□) was adapted from the combining ability analysis of variance output. The distribution of crosses in relation to □CA and SCA effects was determined by denoting significant positive combining ability effects as high, non-significant as average and significant negative as low for average root number per plant, fresh root weight (kg□plant), above ground weight (kg□plant), root length (cm) and harvest index. For C□□, significant positive combining ability effects were considered as low, non-significant as average and significant negative as high (Saleem, 2008). □CA and SCA values with negative effects show contribution towards resistance, and positive values show contribution towards susceptibility for C□□ (Owolade et al., 2008). Baker's prediction ratio (PR) (Baker, 1□78) was used in determining progeny performance, that is, $PR=\frac{2\delta^2_{gca}}{(2\delta^2_{gca}+\delta^2_{sca})}$. The closer this ratio is to one the greater the chances of predicting progeny performance based on □CA, that is, value less than 1, was taken as predominance of non-additive type of gene action, and greater than 1 as additive gene action.

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant (P□0.001) genetic variation among the genotypes for all the traits except for cassava mealy bug, indicating the presence of variability among genotypes (Table 1). High variability for different characters had also been reported in cassava (□unga, 2008; Da Silva, 2008; □tunda, 200□; Akinwale et al., 2010; □amau et al., 2010; Parkes, 2011). This implies that selection among these genotypes could lead a good progress for the improvement of the target traits.

The significant values of mean squares for □CA and SCA for all traits except harvest index and cassava green mite were observed (Table 1). The significant differences among the □CA and SCA effects suggested the involvement of additive and non-additive component of heritable variance in the inheritance. □CA and SCA variance components were used to determine their relative importance in predicting progeny performance for the traits under study. The general combining ability variances (δ^2gca) for all the traits were lower than specific combing ability variances (δ^2sca) (Table 1) which indicated that SCA was more important in predicting progeny performance for expression of the traits. □t corroborates with the findings of Da Silva (2008) and Parkes (2011) wherein higher SCA variances than □CA variances in cassava for various traits was reported and concluded that SCA was more important in predicting progeny performance. To determine the relative

Table 1. Mean squares from analysis of variance of root weight, root number, above ground weight, root length, harvest index, CGM and CMB.

Source	DF	RTN	RWT	AWT	RTL	HI	CGM
Genotype	20	7.7***	1.54***	0.6***	104.14***	0.02***	0.26
GCA	5	11.81**	2.00***	0.73**	8.03	0.01	0.53**
SCA	15	6.83	1.18***	0.60**	124.11***	0.02***	0.21
Error	40	3.16	0.26	0.22	22.31	0.01	0.14
$\delta^2 g$		1.54	0.43	0.16	27.28	0.004	0.04
$\delta^2 e$		1.05	0.0	0.07	7.44	0.002	0.05
$\delta^2 p$		2.5	0.51	0.23	34.71	0.01	0.0
H^2 (%)		5.44	83.50	68.46	78.58	71.72	46.74
$\delta^2 gca$		1.08	0.22	0.06	8.34	0.00	0.05
$\delta^2 sca$		3.67	0.2	0.38	101.80	0.01	0.07
PR		0.37	0.32	0.24	0.14	0.00	0.5

*, **, ***Significant at 5, 1, and 0.1% respectively. RTN=Root number per plant, RWT=Root weight (kg/plant), AWT = Above ground weight (kg/plant), RTL = Root length (cm), CGM = Cassava green mite, CMB = Cassava mealy bug, HI= Harvest index, GCA = General combining ability, SCA = Specific combining ability, PR=Prediction ratio.

importance of GCA and SCA in the genetic control of the different traits, as well as the expected amount of improvement from GCA and SCA, the proportions of additive variance to the total genetic variances were calculated (that is, PR= $2\delta^2 gca/(2\delta^2 gca + \delta^2 sca)$ (Table 1). The ratios ranged from 0 to 0.5. It suggests that crossing for these traits and making selection from such crosses, about 41 to 100% of the improvement would be expected to come from SCA and less than 50% from GCA. Thus by far the largest part of the improvement for these traits comes from SCA and ultimately from non-additive genes. In other words, since the prediction ratio (PR= $2\delta^2 gca/(2\delta^2 gca + \delta^2 sca)$ for all the traits was less than 1, it means that non additive gene effects were more predominant in the expression of the studied traits. Therefore, the study suggests that an efficient cassava breeding program should be based on breeding strategies that exploit dominance effects.

Similarly, the preponderance of non-additive gene effects for fresh root weight have been reported earlier (Da Silva, 2008; Kamau et al., 2010; Parkes, 2011). However, the predominance of additive gene effects for average number of roots was reported by Da Silva (2008), Ntunda (200) and Kamau et al. (2010) which contradicted our findings.

General combining ability effects quantitatively measure the comparative performance of parents in relation to one another. Therefore, GCA effects were computed to show both the magnitude and direction of the genetic effects (Table 3). GCA effects for average root number showed significant positive values for Kulola (0.87) and 01/1316 (0.75), which means that these genotypes contributed most for the high root number so these were the superior genotypes for this particular trait, while 01/1313 had significant negative value (-1.00), which means it performed below average of this set of parents. Kulola and Kaunyili were the good general combiners for fresh root weight with significant positive values. For above ground weight, Kulola was the best general combiner as it exhibited a significant positive value (0.20).

Silira proved to be the best combiner for root length as shown by a significant positive value (2.78) while 01/1313 performed below average of this set of parents as shown by a significant negative value (-3.57). Among the six parents, Kaunyili performed above average harvest index with a significant positive GCA effect of 0.04 while 01/1313 and Silira exhibited significant negative GCA effect of -0.02. The estimates of GCA effects for CGM showed that Kaunyiri had a significant negative value while Silira had a significant positive value. Therefore, Kaunyili can be a desirable parent for hybrids as well as for inclusion in breeding program aimed at developing varieties resistant to CGM due to its significant negative value (Table 3). The foregoing suggestion is in tandem with the report by Owolade et al. (2008) and Da Silva (2008). The study has found that the best rated general combiners based on GCA effects were Kulola for average root number, fresh root weight and above ground weight and Kaunyili for fresh root weight, harvest index and cassava green mite which means that these could be good parents to use when breeding for genotypes for the said traits. 01/1313 was the worst combiner for all the traits studied except cassava green mite.

The highest magnitudes of significantly positive and desirable SCA effects for average root number were recorded on four crosses (Table 4). The results also indicated that two hybrids had significant negative SCA effects. On average, 50% of the hybrids were desirable for the development of cassava genotypes with large number of roots. The distribution of hybrids based on their parental GCA effects revealed that best hybrids emerged from parents with varying levels of GCA effects

Table 2. □ean values of root number, root weight, above ground weight, root length, harvest index, cassava green mite and cassava mealy bug.

ENTRY	Root number/plant	Root weight (kg/plant)	Above ground weight (kg/plant)	Root length (cm)	Harvest index (0-1)	CGM mean severity score	CMB mean severity score
1x1	7.80	2.53	2.61	2□.51	0.50	3.27	1.47
1x2	7.73	2.86	1.61	34.2□	0.65	2.47	1.00
1x3	8.60	1.51	1.32	23.6□	0.52	3.00	1.73
1x4	□.□3	1.51	1.11	26.84	0.56	3.13	1.60
1x5	8.27	1.47	1.36	30.64	0.51	2.87	1.27
1x6	6.17	1.64	1.04	1□.67	0.63	2.38	1.00
2x2	6.□3	1.27	1.47	25.62	0.46	3.00	1.00
2x3	6.□3	0.87	0.88	24.50	0.51	2.87	1.00
2x4	8.00	1.71	1.57	26.67	0.52	3.13	1.47
2x5	10.03	1.48	1.03	25.22	0.60	3.50	2.00
2x6	8.13	1.4□	1.66	28.04	0.48	2.47	1.27
3x3	5.20	1.0□	1.03	32.56	0.52	3.27	1.33
3x4	4.88	1.46	1.05	21.67	0.57	3.08	1.00
3x5	5.33	1.01	0.75	1□.67	0.57	2.□3	1.67
3x6	5.73	0.7□	0.8□	1□.66	0.47	2.□3	1.00
4x4	5.00	1.03	0.83	27.81	0.56	3.13	1.00
4x5	8.00	2.04	2.34	31.58	0.46	3.33	1.53
4x6	6.07	1.27	1.01	45.25	0.53	3.03	1.33
5x5	4.33	1.51	1.76	28.53	0.46	2.□3	1.40
5x6	7.83	2.□7	1.33	28.67	0.6□	2.63	1.00
6x6	7.07	3.45	1.44	22.63	0.71	2.73	1.00
□ean	7.05	1.66	1.34	27.27	0.55	2.□6	1.2□
S□	0.27	0.10	0.08	0.□5	0.01	0.35	0.06
□SD	2.□3	0.83	0.77	7.7□	0.11	0.61	0.74
F-Prob	0.0075	□.0001	0.000□	□.0001	0.0003	0.0445	0.1□76

1□ □ulola, 2□ 01□1316, 3□ 01□1313, 4□ Silira, 5□ Depwete, 6□ □aun□li, C□ □ □ Cassava green mite, C□B□ Cassava mealy bug, □SD□□east significant difference (5□).

such as Depwete □ □aun□li (average □ low parental combinations), □ulola □ Silira (high □ low), and 01□1316 □ Depwete (high □ average). These combinations suggest that best progenies might not only be derived from crosses with genotypes having the greatest positive □CA effects. This suggests that these hybrids could be exploited for heterosis in a breeding program in order to develop genotypes with high number of roots. Several crosses exhibiting significant SCA effects for average root number per plant in cassava have been reported in previous studies (DaSilva, 2008; □tunda, 200□; □amau et al., 2010).

Five of 21 crosses exhibited significant SCA effects for fresh root weight (Table 4). Of these, SCA effects were in desirable positive direction for two crosses, and in undesirable negative direction for three crosses. The distribution of crosses in relation to □CA effects of parental combinations revealed that almost all types of SCA effects were derived from any type of □CA values. For example, □ulola □ 01□1316 obtained from (high □ low), Depwete □ □aun□li (average □ high), parental combinations. Therefore, the performance of hybrids was independent of parents'. A similar trend was observed in several previous studies (Da Silva, 2008; □tunda, 200□; □amau et al., 2010). Based on mean performance (Table 2) and SCA effects; two hybrids namely, □ulola □ 01□1316 and Depwete □ □aun□li were designated as best hybrids and are suggested for hybrid vigour breeding.

For above ground weight, thirteen crosses had positive □CA effects but only one had a significant positive value (Silira □ Depwete). On the other hand, two crosses exhibited significant negative values for above ground weight which means that these two (Table 4) were the most undesirable crosses for this trait. The most desirable cross for this trait was derived from average □ average parental combination.

□agnitude and direction of SCA effects of crosses for root length were variable ranging from significant (five crosses) to non significant and negative to positive values (Table 4). The highest desirable SCA effects were

Table 3. □eneral combining ability effects for root number, root weight, above ground weight, root length, harvest index and cassava green mite.

Parent	Root number/plant	Root weight (kg/plant)	Above ground weight (kg/plant)	Root length (cm)	Harvest index	Cassava green mite
Mulola	0.87□	0.28□□	0.20□	0.25	0.01	-0.0□
01/1316	0.75□	-0.03	0.07	0.20	-0.01	-0.04
01/1313	-1.00□□	-0.52□□□	-0.32□□	-3.57□□	-0.02	0.07
Silira	-0.23	-0.14	0.01	2.78□□	-0.02	0.20□□
Depwete	0.0□	0.11	0.12	0.1□	0.002	0.0□
Maunjili	-0.38	0.2□□□	-0.08	0.13	0.04□□	-0.24□□
SE (GCA)	0.35	0.0□	0.0□	1.02	0.01	0.07

□, □□, □□□ Significant at 5□, 1□, and 0.1□ respectively.

Table 4. Specific combining ability effects for root number, root weight, above ground weight, root length, harvest index and cassava green mite.

Cross	Root number/plant	Root weight (kg/plant)	Above ground weight/plant	Root length (cm)	Harvest index	Cassava green mite
1x1	-1.16	0.33	0.□0	1.82	-0.07	0.50□
1x2	-1.0□	0.□7□□	0.04	6.64□□	0.10□□	-0.35
1x3	1.62	0.11	0.13	-0.1□	-0.02	0.08
1x4	2.08□	-0.27	-0.41	-3.38	0.02	0.08
1x5	0.0□	-0.56□	-0.27	3.01	-0.05	-0.08
1x6	-0.38	-0.□1□	-1.30□□	-□.73□□	0.10□□	-0.73□
2x2	-1.78	-0.32	0.04	-1.□7	-0.07	0.13
2x3	0.07	-0.22	-0.17	0.68	-0.004	-0.11
2x4	0.27	0.23	0.1□	-3.51	-0.001	0.03
2x5	1.□8□	-0.24	-0.47□	-2.36	0.06	0.50□□
2x6	2.33□	-0.0□	0.33	2.4□	-0.03	-0.33
3x3	0.1□	0.4□	0.36	12.50□□□	0.01	0.18
3x4	-1.00	0.47	0.05	-4.738	0.06	-0.14
3x5	-0.87	-0.22	-0.36	-4.153	0.05	-0.17
3x6	-0.1□	-1.12□□	-0.38	-16.5□□□	-0.10□	-0.02
4x4	-1.75	-0.34	-0.50	-4.□4	0.04	-0.21
4x5	0.□3	0.43	0.□0□□	1.41	-0.07	0.10
4x6	1.21	-0.1□	0.27	20.0□□□	-0.08	0.34
5x5	-3.06□□	-0.35	0.21	0.□5	-0.0□□	-0.1□
5x6	3.□7□□	1.27□□	-0.23	0.1□	0.1□□□	0.04
6x6	-6.□3□□	1.04	1.30	3.55	-0.0□	0.6□
S□ (SCA)	0.84	0.40	0.22	2.47	0.03	0.18

1□ □ulola, 2□ 01□1316, 3□ 01□1313, 4□ Silira, 5□ Depwete, 6□ □aun□ri, SCA□ Specific combining ability. □, □□, □□□ Significant at 5□, 1□, and 0.1□ respectively.

observed on Silira □ □aun□li (20.0□), followed by 01□1313 □ 01□1313 (12.50). Of the five significant crosses, two were in the undesirable direction, the worst being 01□1313 □ □aun□li (-16.5□). The distribution of the crosses in relation to the □CA effects of the parents showed that the best cross (Silira □ □aun□li) emerged from high □ average □CA parental combination and the second best cross (01□1313 □ 01□1313) emerged from low □ low parental combination. This means that the best crosses are not necessarily derived from the best general combiners.

The best SCA effects were recorded on three crosses for harvest index (Table 4), namely, Depwete □ □aun□li (0.1□), □ulola □ □aun□li (0.10) and □ulola □ 01□1316 (0.10). These best crosses were derived from any parental combination such as average □ low (□ulola □ 01□1316), and average □ high (Depwete □ □aun□li).

The crosses manifested varying degrees of resistance

to C□□ (Table 4). One cross (□ulola □ □aunili) exhibited desirable significantly negative SCA effects for this trait while two crosses recorded significantly positive SCA effects. One of the parents in the desirable cross (□aunili) was the best general combiner for this trait.

The performance of the crosses with respect to the parental genotypes suggests that progeny performance cannot largely be dependent on performance of parents *per se.* Therefore, these results are consistent with previous studies by Ceballos et al. (2004) that not only additive effects are important in determining the performance of derived progenies but also that there is a large component of dominance effects that translate into significant heterosis for traits such as fresh root yield of cassava, and C□AT (1□87) that any breeding method in cassava should maintain hetero□ygosity and take into account both additive and non-additive genetic variance. Ceballos et al. (2004) and □ebot (200□) argued that since cassava is a highly hetero□ygous crop, dominance effects are likely to play an important role in the performance of materials being selected and any breeding program should exploit dominance effects because, once an elite clone is identified, it can be propagated vegetatively thereby carrying along the dominance effects. This however, does not preclude the actuality that the predominance of the additive gene effects imply that the best progeny might be derived from crosses with genotypes having the greatest □CA effects (Arunga et al., 2010).

Conclusion

Significant □CA and SCA effects indicated importance of both additive and non-additive gene action in controlling the traits studied. Predominance of SCA effects, however, highlighted the greater role of non-additive relative to additive gene action. Overall, results indicated that it would be readily possible to breed for high yield from this set of genotypes.

Conflict of Interest

The authors have not declared any conflict of interest.

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An agronomic and economic evaluation of lettuce cultivars intercropped with rocket over two cultivation seasons

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The objective of this study was to investigate the agronomic and economic viability of lettuce intercropped with rocket over two cultivation seasons. The experimental designs were in randomized blocks in 3 × 3 + 2 factorial arrangement with four repetitions. The treatments were made up of a combination of three lettuce cultivars (Elisa, Veronica and Lucy Brown) and three cultivation systems (monocrop, intercrop with transplanted rocket and intercrop with seeded rocket) plus two other treatments (monocrop of seeded rocket and monocrop of transplanted rocket). Measurements of plant height were taken for the lettuce, number of leaves per plant were taken for both and stand count for the rocket. Total fresh matter per hectare was evaluated for both cultures. To evaluate the efficiency of the intercrop systems, the land equivalent ratio, total operating cost, gross revenue and economic results were determined. The intercropped rocket produced higher values for leaf area, and productivity and revenues were higher for the transplanted rocket mono cropping in both cultures. The characteristics of the lettuce were affected by the different cultivation systems and the treatments with loose-leaf lettuce, mainly in the first cultivation cycle, demonstrated higher productivity, land equivalent ratio and economic results compared to the lettuce mono cropping.

Key words: *Lactuca sativa*, *Eruca sativa*, viability.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the leafy vegetable with the highest consumption in Brazil and is cultivated across practically the whole country. According to the Brazilian Seed and Seedling Association (ABCSEM, 2012), its production during the 2010/2011 season was 1.3 million tons, making it the fifth largest vegetable crop in terms of economic importance.

Rocket (*Eruca sativa* M.) belongs to the Brassicaceae family and is an annual herbaceous plant with a height of 15 to 25 cm and a growth cycle and management similar

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to those for lettuce and coriander crops. Its leaves are thick and divided with green leaf blades and light-green ridges, it has rapid vegetative growth and a short life cycle and its tender leaves are consumed as a salad (Arrangeiro et al., 2011).

Leafy vegetables constitute a diverse group of plants made up of over one hundred species cultivated on a temporary basis, and in Brazil are predominantly produced using conventional cultivation systems and monocropping techniques. In recent years, however, the growth of different cultivation systems has been observed, in particular those using greenhouses and intercropping techniques, both being options used to increase productive yield (Purquerio et al., 2007).

Intercropping systems constitute optimizing labor and exploiting the plant architecture, considering variations in cultural cycles, size and nutritional requirements, and facilitating the use of raw materials and irrigation, reducing the occurrence of weeds and increasing production through the use of easy-to-use technology (Rezende et al., 2005). According to Montezano and Peil (200), the main advantage of intercropping in olericulture is that it leads to higher productive efficiency while reducing the impact on the environment.

However, it is recommended that the efficiency of intercropping be evaluated through economic analysis, as this helps in the interpretation of the results obtained from cultivation systems. Rezende et al. (2005) have demonstrated that the joint cultivation of carefully selected and managed vegetable crop species provides higher net revenues than those associated with monocrops.

Batalha (2010) claims that globalization has impacted the whole of Brazil's production chain, providing technological and structural improvements and exposing the obstacles that need to be overcome in order to increase competitiveness. In order to do this, the search for alternative production technologies, such as intercropping, is of great value to Brazil's horticultural production system.

Therefore, the objective of this study was to evaluate the use of lettuce intercropped with rocket as an alternative and sustainable cultivation system for use in vegetable crop production in the Northwest region of Paraná - Brazil.

MATERIALS AND METHODS

The experiments were carried out on the Umuarama-PR Regional Campus of the State University of Maringá (UEM), located at latitude 23 47'28.4"S and longitude 53 15'24.0"W and at an altitude of 379 m. The soil used was a typical sandy Dystrophic RED LATOSOL (EMBRAPA, 2013). During the experimental period, the minimum and maximum temperatures were 15.7 and 2 .1 C and rainfall was 5 mm for the first cultivation cycle (April to June 2012) and 18.1 and 30.9 C with a rainfall of 3 mm for the second cultivation cycle (August to September 2012) (SEAB, 2013). The physical and chemical characteristic of the soil are shown in Table 1.

Table 1. Main soil characteristics of Dystrophic RED Latosol (Umuarama, PR. 2012).

Soil characteristics	Soil layer (cm)
	0-20
Clay ( )	10.75
Silt ( )	2.95
Sand ( )	8 .3
p (CaCl$_2$)	. 9
rganic matter (g dm^{-3})	2 .17
P (mg dm^{-3})	1. 1
(cmol$_c$ dm^{-3})	0. 1
Ca^{2} (cmol$_c$ dm^{-3})	5.2
Mg^{2} (cmol$_c$ dm^{-3})	1.75
Al^{3} (cmol$_c$ dm^{-3})	0.10
Al (cmol$_c$ dm^{-3})	5. 2
CEC- cation exchange capacity (cmol$_c$ dm^{-3})	13.23
Base saturation inde ( )	57.52

The experimental designs were in randomized blocks in 3 3 2 factorial arrangement with eleven treatments, combining three lettuce cultivars (Veronica - loose-leaf, Elisa - crisp-leaf, and Lucy Brown - American) and three cultivation systems (monocrop, intercrop with transplanted rocket and intercrop with seeded rocket), as well as transplanted and seeded rocket monocrops, with four replications.

The experimental plots covered 1.5 1.5 m, totaling 2.25 m , with a useful area of 0.5 0.5 m or 0.25 m being used for the evaluations. The lettuce seedlings were formed in styrofoam trays with 128 cells and transplanted with a spacing of 0.35 0.35 m.

For the plots containing transplanted rocket, the seedlings were formed by depositing eight seeds into each cell, and each cell was transplanted at 0.05 m intervals in rows according to the treatment. The plots containing seeded rocket were thinned to maintain a uniform population across all of the treatments, the arrangement of which is shown in Figure 1.

The plots were maintained using crop treatments and the required agrotechnological management during the experimental period. Thiamethoam (1 g 100 L^{-1}) and Azoxystrobin (20 g 100 L^{-1}) were applied to control insects and fungus. Irrigation was carried out via sprinkling. Fertilization and correction of soil acidity were carried out via soil analysis and using recommendations for the crops used as described by CFS (200). Fertilization was carried out in the two cultivation systems using 100 kg ha^{-1} of P, 87 kg ha^{-1} of Ca and 53 kg ha^{-1} of S as simple superphosphate (20. P, 18 Ca and 11 S) and a top dressing of 150 kg ha^{-1} nitrogen ( ) from urea and 200 kg ha^{-1} potassium ($_2$) in the form of potassium chloride, divided into three applications. These fertilizers were mixed, homogenized and applied 1 0, 390 and 550 growing degree days (DD).

The variables used to evaluate the effect of the treatments on the development of the crops were: fresh mass production (rocket lettuce), number of leaves of the plant (rocket), final stand count (rocket), as well as plant growth characteristics (number of leaves and height of the plant) for lettuce.

Fresh mass production was determined for the lettuce and rocket by collecting and weighing the plants from the useful plot area (0.25 m). The stand count of rocket was determined directly by counting (0.25 m) of plants in the plots.

Direct measurement was used to determine the growth characteristics of the lettuce, using a 30 cm graduated rule to measure heart height and directly counting the number of leaves, discarding leaves with characteristics undesirable to the consumer.

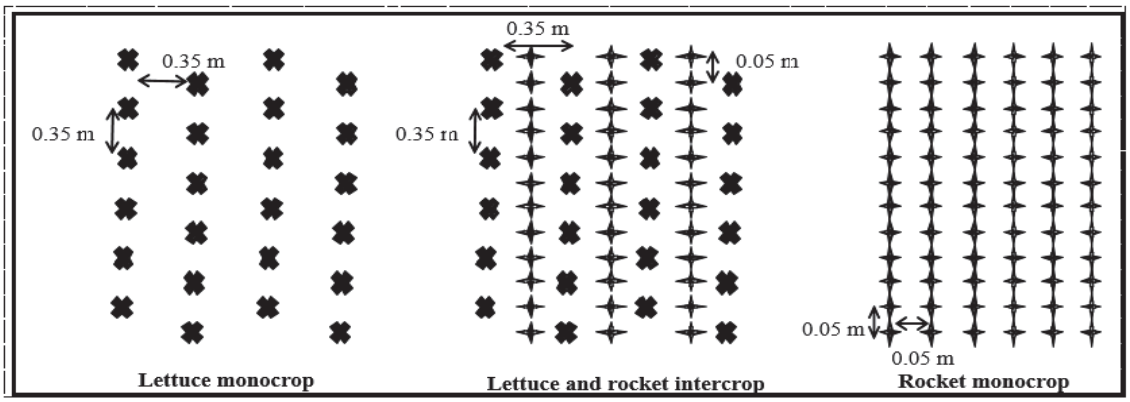

Figure 1. Arrangement and configuration of the monocrop and intercrop fields (Umuarama, PR. 2012).

Table 2. □uantity and cost of inputs used, according to crop arrangement (Umuarama, PR. 2012).

Quantity of inputs	L+TR	L+SR	L	TR	SR
Dolomitic limestone (kg ha^{-1})	3.500	3.500	3.500	3.500	3.500
Simple Superphosphate (kg ha^{-1})	555	555	555	555	555
Urea (kg ha^{-1})	307	307	307	307	307
Potassium chloride (kg ha^{-1})	□55	□55	□55	□55	□55
Pelleted seeds (g ha^{-1})	330 □ 1.3□0	330 □ 2.0□0	330	1.3□0	2.0□0
□rays (units ha^{-1})	□12	20□	20□	□08	0
Substrate (kg ha^{-1})	□□3	221	221	□□2	0
□nsecticide A□□ARA 250 □ □□ (g ha^{-1})	200	200	200	200	200
Fungicide AM□S□AR □ □□ (g ha^{-1})	100	100	100	100	100
Leaf fertilizer (L ha^{-1})	0.3	0.3	0.3	0.3	0.3

L□□R, Lettuce □ transplanted rocket; L□ SR, lettuce □ seeded rocket; L, lettuce; □R, transplanted rocket; SR, seeded rocket.

Economic evaluation of the crops was carried out by calculating production costs for lettuce and rocket in both a monocrop and an intercrop. □he production cost structure used by the Agricultural Economy □nstitute (□EA) (Matsunaga et al., 197□) was used to determine the □otal □perating Cost (□□C), taking into account the all of the producer's outlays during the production cycle, such as e□penses associated with workforce, repair and maintenance of machinery, implements and specific improvements, machine and implement operation, inputs and the depreciation in value of the machinery, implements and specific improvements used in the production process.

□echnical coefficients related to operations associated with soil preparation (plowing and harrowing) and the application of herbicides and other technical coefficients were obtained during the e□periment. □he inputs used during the study were □uantified for each treatment as shown in □able 2.

From this data, the following information on production technology was determined: type and time in use for the machinery and e□uipment used in each operation, the types and □uantities of inputs and workforce re□uirements from the preparation of the soil up to harvest, with the per unit values of each item being calculated according to the description provided below.

□ orkforce salaries were obtained from the Umuarama Rural Worker's Union, considering a monthly salary based on 25 working days and a working day of eight hours. □he information re□uired for the implantation and management of the crops was determined using technical coefficients and monitoring during the e□periment. □he average prices □uoted by the Central Supply Center for Paraná (CEASA-PR) and the São Paulo □eneral □ arehousing and Centers Company (CEA□ESP) for □une and September 2012 were used to calculate gross revenues. □et revenues were obtained from the difference between gross revenues and □□C for each crop.

Activities to develop the seedlings included the washing of trays, preparation of the substrate (humidifying followed by homogenization), filling of trays and manual sowing. □he terrain was cleared using herbicide to remove weeds.

□he marking of transplant locations involved determining the planting arrangement of the lettuce and rocket seedlings using the spacing determined for each treatment. Manual weeding was carried out within the plots and in the spaces between them. Spray irrigation was used for both of the crops. □he estimated production cost did not take into account any e□penses associated with the commercialization of the products.

Land E□uivalent Ratios (LERs) were calculated according to the method used by □ illey (1979), using the formula: UE□ □ (□ab/□aa) □ (□ba/□bb), where □ab is the production of crop "a" intercropped with crop "b", □ba is the production of crop "b" intercropped with crop "a", □aa is the production of crop "a" in a monocrop and □bb is the production of crop "b" in a monocrop.

□he data obtained was submitted to analysis of variance and averages were compared using the Scott-□nott test with a 5□ level of probability.

Table 3. Analysis of variance of variables analyzed, total fresh mass production for lettuce and rocket in kg/m^2 (□FM), number of leaves per plant for rocket (□LPP) and final stand count in plants m^{-1} for rocket (SC) and number of leaves per plant (□LPP) and heart height in cm for lettuce (□□) (Umuarama/PR, 2012).

SV	Variables analyzed									
	TFM		NLPP (rocket)		SC (rocket)		NLPP (lettuce)		HH (lettuce)	
	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle
□L	10	10	7	7	7	7	8	8	8	8
S□	2.78	2.□8	□.□□	0.2□	735.22	7□2.□7	721.38	2023.25	5.35	118.50
MS	278391235	2□8371788	0.□7	0.0□	105.03	108.92	90.17	252.91	0.□7	1□.81
Fc	13□.□0□□	5□.□2□□	0.88 ns	0.0□ ns	3.78□□	2.51□	15.□1□□	□8.5□□□	2.□□□	□.□1□□
CV (%)	□.50	13.5□	20.□2	19.□7	15.8□	20.35	10.1□	11.33	10.90	15.1□

S□, Source of variation (treatments); □L, grau de liberdade; S□, Soma de □uadrados; MS, Mean s□uares; Fc, F calculado; C□, coefficient of variation. □Significant (5□); □□Significant (1□); ns□on - significant.

Table 4. □otal fresh mass production for lettuce and rocket in kg/m^2, number of leaves per plant for rocket and final stand count (plants/m) for rocket and number of leaves per plant and heart height (cm) for lettuce (Umuarama/PR, 2012).

Treatment	Fresh mass (kg/m^2)		Number of leaves per plant (rocket)		Stand count (plants m^{-1})		Number of leaves per plant (lettuce)		Heart height (cm)	
	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle
ELL□□R	2.□□0^c	1.793^b	3.□7^a	□.9□^{a}	□7.50^b	57.50^b	29.13^a	3□.50^a	□.15^b	□.17^b
ELL□SR	2.□00^c	1.781^b	□.99^a	□.91^a	59.50^b	□9.50^a	30.5□^{a}	21.9□^{b}	□.17^b	3.18^d
ELL	1.133f	1.755^b	-	-	-	-	30.25^a	23.□□^{b}	□.22^b	7.27^a
□CL□□R	2.191^c	1.□52^c	3.8□^{a}	□.81^a	59.□□^{b}	53.50^b	20.□9^b	2□.□□^{b}	□.82^a	□.92^c
□CL□SR	1.870^d	1.□28^c	□.32^a	□.□□^{a}	71.5□^{a}	73.00^a	20.5□^{b}	13.0□^{c}	□.37^b	5.22^c
□CL	1.17□f	1.5□1^c	-	-	-	-	23.13^b	13.25^c	□.72^a	2.87^d
LBAL□□R	2.2□9^c	1.□85^c	□.33^a	□.87^a	□□.50^b	□2.50^b	19.38^b	22.38^b	□.75^a	3.80^d
LBAL□SR	2.358^c	1.580^c	□.29^a	□.80^a	72.50^a	77.00^a	20.0□^{b}	1□.81^c	5.0□^{a}	2.8□^{d}
LBAL	1.□17^e	1.□88^c	-	-	-	-	20.5□^{b}	11.50^c	5.29^a	3.15^d
□R	3.7□2^a	1.903^a	□.3□^{a}	□.87^a	52.00^b	□9.50^b	-	-	-	-
SR	3.1□0^b	1.781^b	3.93^a	□.87^a	85.50^a	75.00^a	-	-	-	-
CV %	□.5	13.5□	20.□2	19.□7	15.8□	20.35	10.1□	11.33	10.9	15.1□

Averages followed by the same letters down the column did not differ according to the Scott-□nott test (P□0.05). C□ □ Coefficient of variation. □reatments: Elisa loose-leaf lettuce □ transplanted rocket (ELL□□R), Elisa loose-leaf lettuce □ seeded rocket (ELL□SR); Elisa loose-leaf lettuce (ELL); □eronica crisp-leaf lettuce □ transplanted rocket (□CL□□R); □eronica crisp-leaf lettuce □ seeded rocket (□CL□SR); □eronica crisp-leaf lettuce (□CL); Lucy Brown American lettuce □ transplanted rocket (LBAL□□R); Lucy Brown American lettuce □ seeded rocket (LBAL□SR); Lucy Brown American lettuce (LBAL); transplanted rocket (□R); seeded rocket (SR)

RESULTS AND DISCUSSION

According to □able 3, it can be inferred that there was a higher production of fresh mass in the first crop cycle (April - □une) for the rocket monocrops due to a significant influence on the total production of fresh mass, since the plots containing only rocket presented the highest averages compared to the lettuce monocrop and intercrop, independent of the type of implantation used (□able □).

□hese results agrees with the results obtained by □liveira et al. (2010), who verified a higher production of fresh mass in rocket monocrops when studying rocket and lettuce intercrops. □n this study, the higher production associated with the rocket monocrop was probably due to the arrangement of the rocket plants allowing for more efficient use of space and to the absence of lettuce, permitting higher vegetative growth. □hese factors led to two harvests being reaped. □he highest production among the monocrops was observed for transplanted rocket.

□ hen analyzing the production of the intercrops in relation to the lettuce monocrops, it was observed that there was a significantly higher production for the intercrops (□able □), as has already been observed by Costa et al. (2007), who obtained a higher production of fresh mass when studying intercrops of lettuce and rocket.

Table 5. Production of fresh mass of lettuce and rocket crops as a function of intercrop system and land e□uivalent ratio (LER) in the two cultivation cycles. (Umuarama/PR, 2012).

Treatments	Cycle	Lettuce (kg/m^2)	Rocket (kg/m^2)	LER
ELL□□R	1st	1.0□8	1.398	1.30
	2nd	0.23□	1.5□0	0.95
ELL□SR	1st	1.153	1.2□8	1.□1
	2nd	0.517	1.2□□	1.00
□CL□ □R	1st	1.025	1.1□□	1.18
	2nd	0.2□9	1.183	0.81
□CL□□R	1st	0.817	1.053	1.03
	2nd	0.21□	1.21□	0.87
LBAL□□R	1st	1.152	1.117	1.11
	2nd	0.39□	1.088	0.72
LBAL□SR	1st	1.□7□	0.88□	1.32
	2nd	0.32□	1.25□	0.92

□reatments: Elisa loose-leaf lettuce □ transplanted rocket (ELL□□R), Elisa loose-leaf lettuce □ seeded rocket (ELL□SR); Elisa loose-leaf lettuce (ELL); □eronica crisp-leaf lettuce □ transplanted rocket (□CL□□R); □eronica crisp-leaf lettuce □ seeded rocket (□CL□SR); □eronica crisp-leaf lettuce (□CL); Lucy Brown American lettuce □ transplanted rocket (LBAL□□R); Lucy Brown American lettuce □ seeded rocket (LBAL□SR); Lucy Brown American lettuce (LBAL); transplanted rocket (□R); seeded rocket (SR)

For the second cultivation season (□uly - September) higher production was observed for the transplanted rocket monocrop again, however high temperatures (28 - 32□C) during cultivation negatively influenced lettuce and rocket production, and higher competition within the intercrops reduced production. □his lower production for the rocket monocrops in the second cycle was due to there being only one harvest, as higher temperatures reduce the lettuce growth cycle by up to 20 days.

□igher values for the rocket number of leaves per plant were obtained in general for the intercrop in both cultivation cycles, independent of the method of implantation (□able 3), when compared to the monocrops, possibly due to the larger amount of space available for the plants to develop and the lower population density, different than demonstrated by □ascimento et al. (2013). □owever, this differs to the results obtained by □liveira et al. (2010), who found no difference in rocket number of leaves per plant, independent of the spatial arrangements used.

□he intercrop of lettuce with seeded rocket and the mono cropping of seeded rocket demonstrated significantly higher stand counts, the plots containing seeded rocket presenting higher averages than the transplanted rocket monocrop and the intercrops containing transplanted rocket. □his is due to the increased difficulty in controlling the number of seeds used during sowing compared to that possible through the development of the seedlings in trays (□able 5).

Looking at the lettuce growth characteristics, it can be verified that the number of leaves in the first cultivation cycle was higher for loose-leaf lettuce (□able □), which may be attributed to the morphological characteristics of the material, in agreement with the results obtained by Costa et al. (2007). □n the second cycle, the plots containing the intercrop with transplanted rocket had the highest number of leaves, and again the loose-leaf lettuce demonstrated the highest numbers due to the morphological characteristics of the material and reduced competition with rocket at the end of the cycle, providing better conditions for vegetative growth and a higher number of leaves.

For heart height (□able □), loose-leaf lettuce presented the lowest values in the first cycle due to the precocity of the material. □n the second cycle, this material presented the largest values for height due to the climatic characteristics of the period and the competition with the rocket at the end of the cycle, which caused the crop to surpass the vegetative stage more rapidly, as demonstrated in a study by Silva et al. (2000), in which plant height was influenced by increased plant density.

□herefore, it was verified that LER (□able □) was higher for the intercrops in the first cycle, which was also observed by Rezende et al. (2005) and Costa et al. (2007), especially for the intercrop of Elisa lettuce with seeded rocket, which presented a LER □1□ higher than that for the monocrop due to the fact that two harvests were reaped, agreeing with the results obtained by □liveira et al. (2010), who observed higher efficiency in the intercrop due to the regrowth of the rocket.

During the second cycle, in which temperatures in the region are higher, it was observed that values for LER

Table 6. □otal hours, costs of operations and inputs, total operating cost, revenues and economic result. CAU-UEM, (Umuarama-PR, 2012).

Treatment	Cycle	Total hours (h/ha)	Cost of operations (R$/ha)	Total cost of inputs (R$/ha)	Total operating cost (R$/ha)	Revenues (R$/ha)	Result (R$/ha)
ELL□□R	1st	817.93	□.282.03	□.277.79	8.559.82	31.□83.13	22.923.31
	2nd	788.□0	□.1□2.□1	□.1□3.□9	8.28□.10	28.1□□.7□	19.880.□□
ELL□SR	1st	□3□.75	3.55□.95	3.□81.□2	7.03□.37	29.□88.95	22.□52.58
	2nd	□05.32	3.□15.53	3.3□7.12	□.7□2.□5	25.190.5□	18.□27.89
ELL	1st	□98.09	2.771.09	3.318.12	□.089.21	8.15□.95	2.0□7.7□
	2nd	□□8.□□	2.□31.□7	3.183.82	5.815.□9	12.□□0.□7	□.82□.98
□CL□□R	1st	817.93	□.282.03	□.277.79	8.559.82	29.1□7.20	20.587.38
	2nd	788.□0	□.1□2.□1	□.1□3.□9	8.28□.10	22.35□.92	1□.2□8.82
□CL□SR	1st	□3□.75	3.55□.95	3.□81.□2	7.03□.37	25.3□0.3□	18.303.99
	2nd	□05.32	3.□15.53	3.3□7.12	□.7□2.□5	22.558.07	15.795.□2
□CL	1st	□98.09	2.771.09	3.318.12	□.089.21	10.711.80	□.□□2.59
	2nd	□□8.□□	2.□31.□7	3.183.82	5.815.□9	1□.235.23	8.□19.7□
LBAL□□R	1st	817.93	□.282.03	□.277.79	8.559.82	2□.915.57	18.355.75
	2nd	788.□0	□.1□2.□1	□.1□3.□9	8.28□.10	21.21□.23	12.928.13
LBAL□SR	1st	□3□.75	3.55□.95	3.□81.□2	7.03□.37	25.180.73	18.1□□.3□
	2nd	□05.32	3.□15.53	3.3□7.12	□.7□2.□5	23.5□2.0□	1□.779.□1
LBAL	1st	□98.09	2.771.09	3.318.12	□.089.21	9.777.□8	3.□88.27
	2nd	□□8.□□	2.□31.□7	3.183.82	5.815.□9	10.2□7.23	□.□51.7□
□R	1st	5□3.53	3.253.05	3.091.58	□.3□□.□3	□3.5□0.52	57.195.89
	2nd	51□.10	3.113.□3	2.957.28	□.070.91	32.315.91	2□.2□5.00
SR	1st	3□0.□5	2.250.2□	2.37□.8□	□.□27.10	53.□□0.23	□9.033.13
	2nd	331.02	2.110.82	2.2□2.5□	□.353.38	30.239.92	25.88□.5□

Source: Data for the region of Umuarama – PR, A□R□A□UAL (2012), CEASA – PR and CEA□ESP (2012). □reatments: Elisa loose-leaf lettuce □ transplanted rocket (ELL□□R), Elisa loose-leaf lettuce □ seeded rocket (ELL□SR); Elisa loose-leaf lettuce (ELL); □eronica crisp-leaf lettuce □ transplanted rocket (□CL□□R); □eronica crisp-leaf lettuce □ seeded rocket (□CL□SR); □eronica crisp-leaf lettuce (□CL); Lucy Brown American lettuce □ transplanted rocket (LBAL□□R); Lucy Brown American lettuce □ seeded rocket (LBAL□SR); Lucy Brown American lettuce (LBAL); transplanted rocket (□R); seeded rocket (SR).

were generally lower for the intercrops when compared to the lettuce monocrops due to higher competition between the crops and the fact that the second cycle was around 20 days shorter and did not allow for a second rocket harvest. □his affected LER for the intercropped treatments.

Analyzing □able □, □□C was higher in both cycles for the intercrops with seeded and transplanted rocket when compared to the monocrops of lettuce. □ hen comparing the two cycles, the first has higher values of □□C. Previously, Cec□lio Filho et al. (2008), studying chicory and rocket, observed □□C values 33 and □2□ higher for the intercrop compared to monocrops of chicory and rocket respectively. □owever, Rezende et al. (2009) obtained higher values for lettuce and rocket compared to intercrops of these with pepper.

□ncreased labor re□uirements were observed for the development, thinning and transplant of the seedlings, which supports data presented by Rezende et al. (2005) studying a range of intercropped vegetable crops.

For the treatments containing transplanted rocket, □□C was higher compared to the values obtained for seeded rocket, lettuce monocrops and transplanted and seeded rocket monocrops for both cultivation cycles (□able □), and this is due to the need to develop rocket seedlings, re□uiring more hours of work per hectare compared to these three other systems. □hese results are supported by the findings of Barros □unior et al. (2008), who observed a large influence of □□C for lettuce seedlings. □his study differs from the present study, as they ac□uired seedlings and did not develop them themselves. □owever, author did demonstrate that the seedlings had a significant influence on □□C, e□plaining the results obtained in this study for transplanted rocket.

Use of transplanted rocket also affects input costs and the total cost for the intercrop, considering that this cost represented 50 to 55□ of □□C for the treatments. □his was also observed by Rezende et al. (2005), who observed that 55 to □2□ of total cost was due to inputs. □herefore, the higher □□C observed for the treatments with transplanted rocket was due to the development of the seedlings, the marking of the field and the transplant of the rocket, supporting the results obtained by Rezende et al. (2011) on the impact of labor on □□C when studying a lettuce and cucumber intercrop.

□n addition, the intercrop with transplanted rocket involved higher input costs for seedling development (trays and substrate), representing 9 and 10□ of □□C for the first and second cultivations cycle respectively, assuming that the cost of the trays is spread over five yearly crop cycles.

Analyzing □able □, higher gross revenues were observed for the rocket monocrops in both cycles. □n the first cycle, this measurement was 59, □0 and 8□□ higher for American lettuce compared to the intercrop with transplanted rocket, seeded rocket and the monocrop respectively. For the second cycle these values were 3□, 25 and □9□ higher.

□he higher production observed for the rocket monocrops (□able 3) affected the gross revenues obtained for the crops, which were higher for the lettuce intercrops and monocrops. Similar results were obtained by Rezende et al. (2009), who observed gross revenues for a rocket monocrop that were 8 and 5□ higher compared to a lettuce monocrop and an intercrop with pepper respectively. Similar results were obtained by Silva et al. (2008) when studying lettuce and cucumber, where the lettuce monocrop provided higher gross revenues.

Analyzing the lettuce monocrops and intercrops, the loose-leaf lettuce intercropped with transplanted rocket obtained □ and 11□ higher gross revenues than the intercrop with seeded rocket in the first and second cycles respectively.

□t is worth highlighting that among the different types of lettuce, loose-leaf lettuce provided the highest gross revenues, which was e□pected due to its higher fresh mass production. Similar data was obtained by Mota et al. (2012), who obtained higher gross revenues for the ma□ority of their loose-leaf lettuce and carrot intercrops compared with the carrot monocrops, confirming the potential of intercropped loose-leaf lettuce in high-temperature regions such as those observed for the second cultivation cycle in this study.

Analyzing □able □, it can be observed that in the first cycle loose-leaf lettuce intercropped with transplanted rocket obtained an economic result around 91□ higher than the monocrop and 2□ higher than the treatment intercropped with seeded rocket, and in the second cycle the treatment with transplanted rocket obtained a result 7□ higher than the intercrop with seeded rocket and □□□ higher than the lettuce monocrop.

Crisp-leaf lettuce intercropped with transplanted rocket had a higher economic result in the first cycle compared to the monocrop and the intercrop with seeded rocket. American lettuce intercropped with transplanted rocket also obtained a higher result than the monocrop. Comparing the rocket monocrops, transplanted rocket had a result 1□□ higher in the first cycle and 1□ higher in the second cycle.

□he transplanted rocket monocrop obtained the highest economic result, which were 1□ and 1□ higher than seeded rocket for the first and second cycles respectively. Compared to the treatments with loose-leaf lettuce (loose-leaf □ transplanted rocket, loose-leaf □ seeded rocket and loose-leaf), the results for this treatment were □0, □1 and 9□□ higher for the first cycle and 2□, 30 and 7□□ higher for the second cycle respectively. For the treatments with crisp-leaf lettuce (crisp-leaf □ transplanted rocket, crisp-leaf □ seeded rocket and crisp-leaf) results were □□, □8 and 92□ higher for the first cycle and □□, □0 and □8□ for the second cycle respectively.

□ hen analyzing the lettuce monocrops and intercrops, economic results for the loose-leaf intercrops were higher than those obtained for the monocrops, supporting data obtained by Rezende et al. (2005) and Catelan et al. (2002), who observed higher net revenues for intercrops compared to monocrops. Analyzing □ust the intercrops, results for loose-leaf lettuce were higher than those obtained for crisp-leaf and American lettuce by 1□ and 20□ in the first cycle and 22 and 23□ in the second cycle. Considering the above, the best intercrop in terms of economic results was loose-leaf lettuce with transplanted rocket.

Barros □unior et al. (2008) obtained higher net revenues for the same cultivars of crisp-leaf and American lettuce intercropped with rocket, especially for the American lettuce intercrop. □herefore, different types of lettuce present different economic results when cultivated in an intercrop system, as was observed in the present study where crisp-leaf outperformed America lettuce and where both were outperformed by loose-leaf lettuce when intercropped with rocket.

□he rocket monocrop obtained a higher production than the other treatments, with results for transplanted rocket higher than those for seeded rocket. □his was reflected in the economic results for this crop, which were consistently higher for the transplanted rocket, generating higher net revenues. Considering lettuce to be the main crop, the higher results obtained for production and revenues through the use of intercrops demonstrate the importance of the use of intercropping as an alternative cultivation system. □his is supported by various studies, such as those by Rezende et al. (2005), Barros □unior et al. (2008) and Catelan et al. (2002), who also observed higher results for intercrops, confirming the viability of their use.

The viability of a cultivation system involving lettuce intercropped with rocket has been confirmed, with the best results being obtained for treatments involving transplanted rocket, even if it may be inferred that the intercrop in this study was representative only during the first crop cycle and demonstrated restrictions during the second cycle due to the region's climatic conditions during this period, this information is supported by Porto et al. (2011), were both lettuce and rocket crops had better productive performance in the second cycle.

Conclusion

Intercropping has been proven to be a viable, productive and economic alternative for use in the Northwest Region of Paraná. The higher results for intercrops, confirming the viability of their use for lettuce and rocket. There was interference on meteorological conditions within the crop cycles.

Conflict of Interest

The authors have not declared any conflict of interest.

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Growth of *eucalyptus* plants irrigated with saline water

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The study was aimed at evaluating the growth of eucalyptus seedlings irrigated with saline water. It was conducted on full-sun bench in the Goiás State University. The experiment was set up following the completely randomized design, with five treatments (plants irrigated daily with water with an electrical conductivity equal to 0; 2; 4; 6 and 8 dS m^{-1}) and six replications. The eucalyptus plants showed small alterations of the growth variables (height, diameter and biomass). Vigorous vegetative growth and retention of tissue moisture indicate that eucalyptus is tolerant to salinity.

Key words: Tolerance, electrical conductivity, biomass.

INTRODUCTION

The increase in greenhouse gas concentrations in the Earth's atmosphere, resulting from anthropic actions, has been jeopardizing natural resources and the survival of mankind. The constant pressure for sustainable development and preservation of natural resources entails the necessity of exploiting planted forests.

The constant increase in the economic value and shortage of high-quality timber intensified the multiple use of the genus *Eucalyptus* (Souza et al., 2012). In Brazil, the genus *Eucalyptus* found ideal growth conditions. Plantation productivity in Brazilian lands is higher than that of traditional countries like Australia (origin of the species).

Brazil has great potential for exploitation of planted forests in most of its territorial extent, as a result of its climate and soil conditions, biodiversity, land and manpower availability, as well as documented technical competence in the field of forestry. The competitiveness of the Brazilian forest sector, which stems from the climatic conditions and from the technology developed by companies and research institutions, places the country at a unique position in the international scene (Ferreira et al., 2012). The Brazilian forestry sector accounts for 3.5% of the gross domestic product (Abraf, 2013). Currently, planted forests in Brazil comprise about 7.9 million hectares, 5.9 million of which with eucalyptus (about 25% of the world plantation) and 1.8 million with pine (Gonçalves et al., 2013).

Despite its high potential in the forestry sector, Brazil can produce even more and transfer wealth to other economy segments. The increased interest in the use of eucalyptus for diverse purposes, as timber or otherwise

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and for the recovery of degraded areas has expanded the species exploitation. Population growth increases the demand for timber and cultivation areas, requiring the exploitation of marginal areas (Lopes and Vilar, 2009). Eucalyptus is quite adaptable to different conditions of heat, light and dryness. However, production can be better ensured by the use of irrigation, and even the possibility of cultivating it in salinity conditions should be researched (Queiroz et al., 2011). The quality of the water from many sources is low, especially water from wells and surface reservoirs. Since the water used for periodic irrigation contains soluble salts, it causes incorporation of salts into the soil profile. Without leaching, salt settles in the root zone and on the soil surface as a result of water evaporation (Queiroz et al., 2011).

Salinity in soils of arid and semi-arid regions is an issue of social concern, since salts affect millions of hectares in the whole world, which reduces the capacity for production in these areas (Lima et al., 200). Both in soils and waters salinity is one of the main causes of crop yield reductions (Flowers, 200; Matos et al., 2013) due to the osmotic, toxic or nutritional effect (Viana et al., 200). Salinity affects about 10% of the Earth's surface. According to Shannon et al. (199), water electrical conductivity equal to or higher than dS m^{-1} drastically reduces agricultural productivity. Globally, the production in about 00 million hectares of land used for agriculture is severely limited by salinity (Bot et al., 2000).

According to Lopes et al. (2012), the *Eucalyptus platyphylla* plants are tolerant to irrigation water salinity, as they present small variation in diameter and plant height when irrigated with low quality water. However, little is known about the tolerance of Eucalyptus plants to salinity. In view of the need for such information for the production of seedlings for field planting, and for better understanding the attributes for salinity tolerance, which would allow for commercial exploitation with saline water irrigation, the present study is aimed at evaluating the cultivation of eucalyptus seedlings irrigated with saline water.

MATERIALS AND METHODS

Experimental design

The study was conducted on a transparent cover bench, located in the State University of Goiás, Ipameri Unit (Lat. 17 3 19 S, Long. 8 09 35 , Alt. 773 m), Ipameri, Goiás. According to the ppen classification, this region has Aw-type climate.

The experiment was conducted following the completely randomized design, with five treatments (plants irrigated daily with water with electrical conductivity (ECw) equal to 0, 2, , and 8 dS m^{-1}) and six replications. Seedlings of *Eucalyptus urophylla* aged 100 days were planted in pots containing 8 L of a substrate composed of red-yellow oxisol, sand and manure at ratios of 3: 1: 0.5, respectively. The chemical analysis of the mixture showed the following values: p ; 19 g dm^{-3} of M; 2. mg dm^{-3} of P, 109 $cmol_c$ dm^{-3} of , 1.5 $cmol_c$ dm^{-3} of Al, 3.2 $cmol_c$ dm^{-3} of Ca, 1. $cmol_c$ dm^{-3} of Mg, 27.7 mg dm^{-3} of n, 77.20 of BS, and 58 of CEC. After analysis of the mixture composition, fertilization and p correction of the substrate were performed according to technical recommendations for eucalyptus cultivation. The seedlings were irrigated for 30 days with a solution volume corresponding to the daily evapotranspiration. The choice for *Eucalyptus urophylla* was due to its high commercial exploitation in Brazil and use for various purposes, resistance to eucalyptus cancer (*Cryphonectria cubensis*), high plasticity and adaptation to diverse ecosystems. The solution volume supplied to the plant was calculated according to recommendations of Allen et al. (200). aCl was added to the local water supply, in order to obtain solutions with different electrical conductivity, the uantity ( ) being determined by the equation (mg L-1) 0 ECw (dS m^{-1}), according to Rhoades et al. (2000), where ECw represents the desired electrical conductivity value of the water. Subsequently, the conductivity values were checked and verified with a conductivity meter.

At 130 days and 30 days after treatment, the following variables were analyzed: umber of leaves, plant height, stem diameter, relative water content (R C), total chlorophyll and carotenoid contents, root mass ratio (RMR), stem mass ratio (SMR), leaf mass ratio (LMR), transpiration and total biomass.

Growth variables

The plant height and stem diameter were measured using a graduated ruler and a digital pachymeter. The number of leaves was obtained by counting all the plant leaves. The destructive analyses were carried out next, after leaves, roots and stems were separated, put to dry in an oven at 72 C until they turned into a steady dry mass, and finally weighed. With the dry mass data, the leaf mass ratio (LMR), root mass ratio (RMR), stem mass ratio (SMR), leaf foliar area ratio (LAR) and total biomass were calculated.

Relative water content in leaf (RWC)

In order to determine the relative water content, ten 12 mm leaf disks were extracted, then weighed and placed for four hours in Petri dishes with distilled water to saturate. After that, the disks were weighed again and put to dry at 70 C for 72 h, the dry weight in grams being obtained afterwards.

Transpiration

The daily transpiration was estimated through gravimetry, by measuring the difference in the pots weight at one-hour intervals between 07:00 and 18:00 hours, as described by Cavatte et al. (2012).

Photosynthetic pigments

Leaf disks of known area were collected and placed in ars containing dimethyl sulfoxide (DMS), to determine the total carotenoid and chlorophyll concentrations. Later, the extraction was carried out in water bath at 5 C for one hour. Aliuots were removed for spectrophotometric reading at 80, 9 and 5 nm.

The chlorophyll *a* (Cl *a*), chlorophyll *b* (Cl *b*) and carotenoid (Car) contents were determined following the equation proposed by ellburn (199).

Data analysis

The experiment followed the completely randomized design with

Table 1. Analysis of variance, averages and percentages of height, diameter, number of leaves and branches, transpiration and relative water content (R□ C) of eucalyptus seedlings irrigated with saline water in comparison with control plants.

Variation source	DF	Mean squares					
		Height	Diameter	No. of leaves	No. of branches	Transpiration	RCW
Doses	□	7.5^{ns}	1.07^{ns}	7815.8□	□2.9□	□,1□8.□□	1□8.1^{ns}
Residue	25	37.□	0.7	1,185.9	□.3	258.3	□□.□
C□ (□)		9.8	9.□	13.□	9.3	22.1	8.1
Electrical conductivity (dSm^{-1})		**Averages and percentages**					
0		□3.3 (100)	8.8 (100)	31□.3 (100)	2□.2 (100)	125 (100)	90.8 (100)
2		□3.9 (101)	9.2 (105)	2□7.□ (85)	25.0 (103)	80 (□□)	7□.7 (8□)
□		□1.5 (97)	8.7 (99)	2□0.0 (83)	21.3 (88)	□5 (52)	87.3 (9□)
□		□2.3 (98)	9.1 (103)	23□.3 (75)	21.8 (100)	□0 (□8)	81.9 (90)
8		□1.□ (97)	8.7 (99)	183.3 (58)	17.0 (70)	□0 (32)	7□.□ (8□

□□Significant at 5□ probability; ns □ not significant according to the F test.

Table 2. Analysis of variance, averages and percentages of the biomass, root mass ratio (RMR), stem mass ratio (SMR), leaf mass ratio (LMR), leaf concentrations of carotenoids (Car) and total chlorophylls (Cl *a*□*b*) of eucalyptus seedlings irrigated with saline water, in comparison with control.

Variation source	DF	Mean squares					
		Biomass	RMR	SMR	LMR	Car	Cl (*a*□*b*)
Doses	□	2□.7^{ns}	0.0005^{ns}	0.0005^{ns}	0.003^{ns}	0.08□	0.5□^{ns}
Residue	25	22.3	0.00□	0.00□	0.003	0.03	0.35
C□ (□)		12.5	22.5	18.1	13.9	2□.3	18.□
Electrical conductivity (dSm^{-1})		**Averages and percentages**					
0		□1.1 (100)	0.2□ (100)	0.32 (100)	0.□2 (100)	0.□□ (100)	3.5 (100)
2		39.□ (9□)	0.28 (108)	0.32 (100)	0.□0 (95)	0.□8 (15□)	2.9 (83)
□		37.9 (92)	0.27 (10□)	0.30 (9□)	0.□3 (102)	0.□9 (157)	2.9 (83)
□		35.9 (87)	0.33 (127)	0.30 (100)	0.37 (88)	0.73 (1□□)	3.7 (10□)
8		3□.□ (8□)	0.29 (112)	0.30 (100)	0.□1 (98)	0.77 (175)	3.1 (89)

□Significant at 5□ probability; ns □ not significant according to the F test.

five treatments and si□ replications. □he data were submitted to variance analysis and then to regression analysis using S□S□AR 5.3 software (Ferreira, 2011).

RESULTS

During the e□periment period, the temperature varied between 12 and 30□C and the relative air humidity between 50 and 80□ . □n the evaluation day, the average air temperature was 30□C and the relative humidity □5□ . □he variance analysis and the averages of all analyzed variables can be found in □ables 1 and 2. Regardless of the electrical conductivity of the irrigation water (ECw), the plant height, stem diameter and leaf water content showed non-significant variation. □owever, the R□ C showed a 1□□ decrease when the treatment with deionized water irrigation was compared to the treatment using water with ECw of 8 dS m^{-1}. □he number of leaves, branches and level of transpiration decreased as the ECw increased, showing a significant statistical difference. □he highest variation in the number of leaves was □2□ when comparing the treatment with deionized water irrigation to the treatment using water with ECw of 8 dS m^{-1}. □he number of branches was 30□ lower in plants irrigated with ECw of 8 dS m^{-1} in comparison with the control irrigated with deionized water. □he total plant transpiration was □8□ lower in plants irrigated with ECw of 8 dS m^{-1} in comparison with the control.

Regardless of the electrical conductivity of the irrigation water (ECw), the biomass, root mass ratio, stem mass ratio, leaf mass ratio and total chlorophyll contain little variation (□able 2). □owever, even without statistical difference, it can be observed that the biomass showed a 1□□ decrease when the deionized water irrigation treatment is compared to the irrigation treatment using water with ECw of 8 dS m^{-1}. □he foliar concentration of total carotenoids was 75□ lower in the control plants

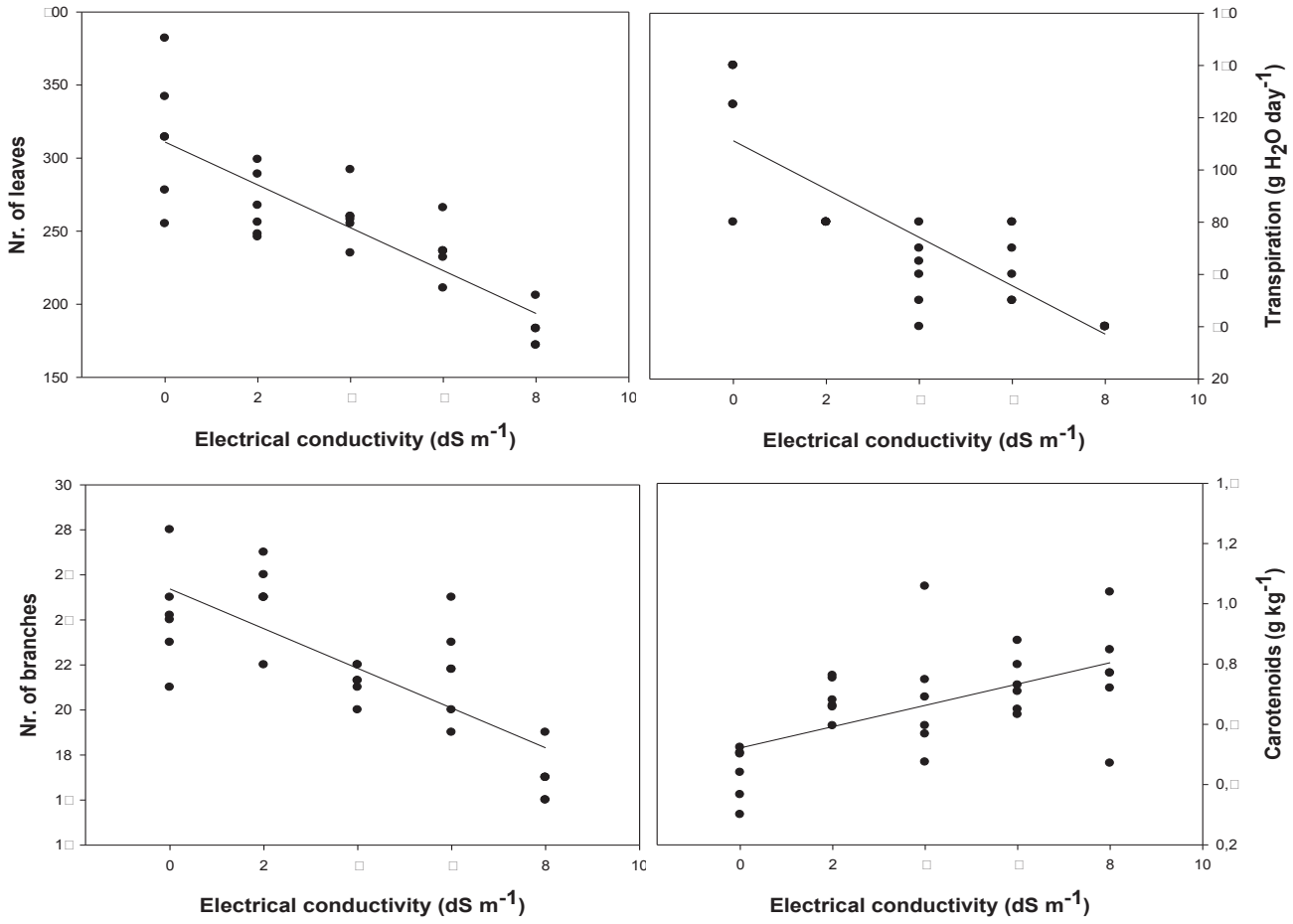

Figure 1. Linear regression e□uations for number of leaves (□ □ 310.9□ - 1□.□□□, R^2 □ 0.72□), transpiration (□ □ 111.17 - 9.25□, R^2 □ 0.95□), number of branches (□ □ 25.37 - 0.88□, R^2 □ 0.99□), and carotenoids (□ □ 0.52 - 0.035□, R^2 □ 0.9□□) of eucalyptus seedlings irrigated with saline water with different electrical conductivity (0; 2; □; □ and 8 dS m^{-1}).

irrigated with deionized water than in the plants irrigated with ECw of 8 dS m^{-1}.

□he variables number of leaves, number of branches, transpiration and foliar concentration of carotenoids showed significance at the 5□ probability level in F test and, therefore, regression was carried out for each one as shown in Figure 1.

DISCUSSION

Under natural conditions, abiotic stress can cause irreversible damage to plants. And tolerance to salinity can represent an e□pansion of the agricultural frontier to obtain economic payback in areas that used to be inade□uate. □igh adaptability to climate and soil variation, combined with steady growth under stress conditions (Pinto et al., 2008), may have helped the eucalyptus to tolerate the salinity of the irrigation water with little alteration in growth variables, as discussed subse□uently

Regardless of the ECw, eucalyptus plants showed an intense vegetative growth that resulted in similar biomass accumulation among treated plants. □he increase in electrical conductivity of the irrigation water and conse□uent reduced potential of the solution were not sufficient to generate significant alterations to growth variables (height, diameter and biomass). □he results confirm those of Lopes et al. (2012) when evaluating the growth of eucalyptus irrigated with saline water. □he sustained growth under the conditions imposed represents an important sign of tolerance to salinity (Matos et al., 2013). □he eucalyptus plant may have an efficient mechanism for soil water e□traction, since even under conditions of low water supply; it manages to e□tract enough water from the soil to sustain growth.

□he electrical conductivity variation in the irrigation water did not cause any significant alteration in the distribution of biomass to the root system, stem and leaf (RMR, SMR and LMR). □he physiological drought resulting from the reduction in the osmotic potential is a direct effect of saline stress. Under such conditions, plants sub□ected to high electrical conductivity allocate

the little existing biomass to the development of the root system, since vigorous and deep roots have higher potential of extracting water and nutrients from the soil. Greater biomass distribution to the root system under reduced osmotic and water supply conditions are a common response of various species (Góes et al., 2009; Matos et al., 2013).

The common response of greater growth of the root system under water restriction conditions was not found in this study, which shows that saline stress did not limit water availability to the eucalyptus plants to the point that it would induce more root growth. The reduced variation in growth of the root system of eucalyptus plants irrigated with saline water has been documented and seems to be common in irrigations with water electrical conductivity lower than 8 dS m^{-1} (Lopes et al., 2012; Feikema and Baker., 2011).

Reduction in the number of leaves, small variation of the relative water content and reduction in transpiration show that eucalyptus slows down dehydration as a strategy to endure drought. For this purpose, the plant reduces water loss by possessing small transpiring leaf area, thus keeping itself hydrated for a longer period. Water shortage induced by the osmotic effect, which is characteristic of the seasonal drought, causes morphological and anatomical alterations in the plants that unbalance water absorption and transpiration. Among the morphological changes, the most significant one is the reduction in the number and size of the leaves (Santana *et al.*, 2011). Sprouting and early development of the leaves depend on the water supply to the plant. Shorter water availability to irrigated plants with high ECw resulted in lesser water availability for the plant metabolism, such as formation and development of new leaves. Reducing the number of leaves is an important strategy to endure the soil low water supply, as it will reduce the transpiration area. Slowing down dehydration allows for immediate survival. Adjusting the total leaf area and the number of leaves is necessary to bear the imposed stress and to adapt to the new climatic conditions (Matos et al., 2013). Retaining leaf hydration may also be associated to an efficient mechanism for the species stomatal control, since the transpiration rate is reduced with the increase in ECw.

The lower number of branches in plants treated with saline water without significant alteration in the stem mass shows that eucalyptus in salinity condition gives priority to the main stem, possibly to facilitate the hydraulic conductivity of the soil solution along the Xylem.

The reduction in transpiration because of lesser stomatic conductivity may give rise to a photosynthesis disorder as a result of lesser CO_2 influx and increase in leaf temperature. In such circumstances, photosynthesis inhibition and oxidative stress with damage to proteins and membranes are common. Visual symptoms of oxidative stress were sporadically seen in some leaves of plants irrigated with ECw higher than 2 dS m^{-1}. We suggest that the photoprotection of the eucalyptus plants occurred as a result of the higher leaf concentration of total carotenoids in plants irrigated with high ECw. The small variation in chlorophyll leaf concentration is a sign of lack of toxicity caused by salt.

The minor changes in growth variables combined with a strategy of slow dehydration and a photoprotection mechanism indicates that eucalyptus plants are tolerant to salinity. However, further studies with different salinity levels of irrigation water for longer periods of time are required to evaluate the accumulation of salts in the soil and the toxicity of salts in long-term field irrigation before the use of saline water in commercial eucalyptus plantations is recommended.

Conclusions

1. Eucalyptus plants show vigorous vegetative growth when irrigated with saline water with electrical conductivity lower than 8 dS m^{-1}.
2. Eucalyptus plants slow down dehydration reducing the number of leaves and the transpiration level.
3. Eucalyptus plants are tolerant to salinity.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Factors influencing maize crop production at household levels: A case of Rukwa Region in the southern highlands of Tanzania

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Production of staple food occupies an important part in Sub-Saharan Africa's agricultural production. Maize crop in particular is the most important staple food in this area. The article mainly examines households' socio-economic characteristics affecting maize production in Rukwa in the context of the market reforms carried out in Tanzania in the mid 1980's. Rukwa region is one of Tanzania's most reliant maize producers. The article explores the importance of maize to household's crop production, its production levels and the determinants of its productivity. A number of specific issues are explored including the importance of factors such as farm size, education, and access to key inputs such as seeds, fertilizers and agricultural extension services. The study on which the article is based uses data collected from three districts of Rukwa. The findings showed that maize crop continues to play an important role in most households' livelihood. However, the crop production levels were low. Education was observed to be an important factor in raising yields, suggesting that non-agriculture policies may also be important for improving productivity and welfare of farmers. Despite the importance of maize crop to household livelihoods, several constraints were reported to hinder its productivity including access to fertilizers, improved seeds and other chemical inputs necessary for higher production, and extension services. Therefore, efforts need to be taken by both the local and central government to raise households maize productivity and hence increase the possibility of improving their well-being.

Key words: *Zea mays*, productivity and farm households.

INTRODUCTION

Agriculture is the economic backbone, engaging about 90% of Rukwa region's economically active human population. According to URT/NBS/RRCO (2004), agricultural production accounts for about 65% of the region's GDP. The major crops grown by households in the region include food crops such as maize, finger millet, beans, rice, and cassava; and cash crops such as tobacco, sunflower, groundnuts, coffee and wheat. Despite the diversity of crops cultivated in Rukwa, most of the region's cash income comes from maize, which accounts for 35% of the region's total annual food output (URT/NBS/RRCO, 2004). Rukwa's maize production and

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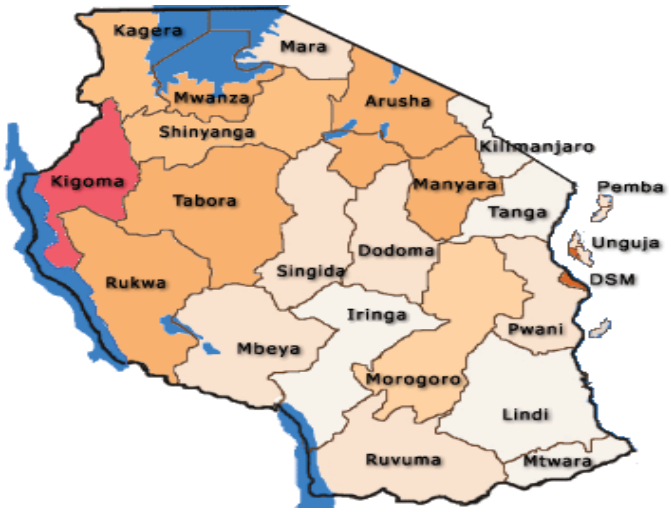

Figure 1. Map of Tanzania showing administrative regions (Source; http□//www.tanzania.go.tz/census/regions.htm□4/5/200□).

Figure 2. Rukwa region's administrative districts (Source: http□//www.tanzania.go.tz/census/rukwa.htm□4/5/200□).

its overall contribution to Tanzania's total maize output over the past years led the region along with three other regions, Iringa, Mbeya and Ruvuma to be nicknamed "the big four" (URT/NBS/RRCO, 2004).

In addition to the above, Rukwa is however different from the others, being much more remote (Figure 1) and its farmers have less access to commercial markets for outputs and inputs. Compared to Rukwa, the other three regions have some better road networks with some or many of the roads having tarmac (asphalt) (Figure 2).

Iringa and Mbeya are also well served by the Tanzania □ambia Railway line (TA□ARA) which connects Dar es Salaam Tanzania's commercial capital to Kapiri Mposhi in □ambia, providing a good way of transporting food, cash crops and other commodities from the regions. Ruvuma can also easily access TA□ARA through Makambako in Iringa region which is not very far from Songea the regions capital. Furthermore, Rukwa is more reliant on maize as a source of income than other regions which have other crops such as tea, coffee, oil palm, and bananas (Mbeya), coffee and tobacco (Ruvuma) and Irish potatoes, tea, and coffee in Iringa (NBS/MAFS/M□ □D/MCM/□ORA□□/MF□A□□, 2006).

In the mid 1980's Tanzania undertook some policy reforms aimed at stimulating crop market incentives and opportunities. Based on the reforms it was e□pected that regions such as Rukwa would be able to seize the opportunity and raise their agricultural productivity based on their inherent agricultural potential. Moreover, it was assumed that under perfect market conditions households would easily access input and output markets. Though some studies (Ashimogo, 1□□5, 1□□7; Bisanda et al., 1□□□) have been done, concentration has been on grain storage, marketing and adoption of maize production technologies. Furthermore, based on the reviewed literature no study was found to have dealt with production constraints following the liberalization of the agricultural input and output markets. Therefore, the study aimed at e□ploring maize crop production constraints in Rukwa region where its production predominates. It mainly dwelt on analysis of farm households' data collected during the fieldwork and compared with previous yield estimates of Rukwa region from the official statistics.

METHODOLOGY

Study area

Rukwa is found in the south□western part of Tanzania on the shores of □ake Tanganyika. The region lies between latitudes 3□ and □□ south of the e□uator and between longitudes 30□ and 33□ east of □reenwich (URT/NBS/RRCO, 2004). Rukwa is bordered to the North by □igoma and Tabora regions, to the □ast by Mbeya region, to the South by □ambia and to the □ est by □ake Tanganyika, which lies between the region and the Democratic Republic of Congo (DRC). Rukwa is situated on the Central African □lateau and is bordered by the □ estern arm of the □reat Rift □alley. Access to Rukwa region is a bit restricted when compared to the other three regions bordering it; as it lacks good all□weather roads.

Research design

The study employed a mi□ed research design during data collection. □owever, a cross□sectional research design (Creswell, 2003) was mostly adopted, whereby data were collected at one point in time. The choice of this approach was partly warranted by its ability to meet the ob□ectives of the study, and also due to time limitation and financial constraint.

Table 1. Socio□economic characteristics of households.

Characteristic		Rukwa region (n = 200)	Districts		
			Sumbawanga (n = 72)	Mpanda (n = 67)	Nkansi (n = 61)
Household head's average age		44.4	43.6	45.1	44.4
Respondents' sex	Male	153 (76.5)	53 (73.6)	57 (85.1)	43 (70.5)
	Female	47 (23.5)	19 (26.4)	10 (14.9)	18 (29.5)
Household head's gender	Male	169 (84.5)	59 (81.9)	61 (91)	49 (80.3)
	Female	31 (15.5)	13 (18.1)	6 (9)	12 (19.7)
Household head's educational level	Primary school leaver	121 (60.5)	47 (65.3)	46 (68.7)	28 (45.9)
	Secondary school leaver	6 (3.0)	3 (4.2)	1 (1.5)	2 (3.3)
	Adult education	9 (4.5)	6 (8.3)	0 (0.0)	3 (4.9)
	No formal education at all	24 (13.0)	10 (13.9)	4 (6.0)	12 (19.7)
	Lower levels of Primary School (< std 7)	38 (19.0)	6 (8.3)	16 (23.9)	16 (26.2)
Household head's main occupation	Crop farming	193 (96.5)	67 (93.1)	65 (97.0)	61 (100)
	Salaried employment	6 (3.0)	4 (5.6)	2 (3.0)	0 (0.0)
	Other (e.g. fishing)	1 (0.5)	1 (1.4)	0 (0.0)	0 (0.0)
Household size	Average household size	5.88	5.29	6.22	6.21
Household's land ownership (ha)	Average land owned	3.19	2.45	4.24	2.93
	Average farmed land	2.29	2.10	2.35	2.45
Household income (Tsh)	Average Household income (2005)	521,947.00	475,069.00	704,411.00	376,867.00
	Income range (2005)	9,000 – 5,284,000.00	9,000-3,000,000.00	10,000- 5,284,000.00	12,000 – 2,670,000.00

Source□Survey Data (2006). Number in brackets indicate percentage.

Data collection and analysis

Data were collected from the three rural administrative districts of Rukwa region. Collection of data from the three districts aimed at ensuring a broader understanding of the phenomena under study in a range of localities. This is because most households in Sumbawanga and Nkansi districts depend mainly on the maize crop as a ma□or means of livelihood, whereas households in Mpanda are also involved in tobacco farming. Data were analysed using S□SS (version 17.0) to generate different descriptive statistics.

Sampling procedures and sample size

The current study involved an interview of 200 randomly selected farm households that are involved in maize crop production from the three districts (72 from Sumbawanga, 67 from Mpanda and 61 from Nkansi). Random sampling was based on official village registers made available to the researchers by leaders of the selected villages. A total of 16 focus group discussions (F□Ds) were further conducted, four each in Sumbawanga and Nkansi districts and eight in Mpanda district. In each case, only one village was selected to ensure a comparison of opinions between the different age and se□ groups. A total of 107 individuals participated in the F□Ds. Apart from individual □uestionnaires and the F□Ds, in□ depth interviews were also conducted with the district agricultural officers of the three districts.

Primary data collection

The study used both primary and secondary data whereby primary data were collected using pre□structured □uestionnaires. These were designed in a way to enable collection of information capable of answering the primary □uestions aimed at determining Rukwa region maize crop production levels after a period of about twenty years of agricultural trade reforms including liberalization of the maize market. The □uestions mainly aimed at determining the importance of maize crop in the livelihoods of surveyed households and their maize production characteristics, for e□ample, the uses of modern technologies in production process. The □uestionnaire also sought information on the farm households' farming practices as well as other types of crops, amount of land dedicated to each and whether households were actively seeking e□tension services in relation to maize crop farming. These lines of en□uiry were based on the prediction that open markets make access to technologies and innovations easier. Secondary data collected came mainly from the Ministry of Agriculture and Food Security and FAOSTAT (online). Data from the 2002/03 National Agricultural Census was also used in the current study.

RESULTS AND DISCUSSION

Socio-economic characteristics of surveyed households

The respondents' major socio□economic characteristics are shown in Table 1. Three □uarters of the respondents were male; however the actual number of male headed households (M□□s) was greater than this. The lower number of female headed households (F□□s) was probably caused by the fact that some of the female respondents were only representing their spouses who could not be available for the interviews, and by the current study's approach of using random sampling

method, whereby names were drawn randomly from the village registers. The low percentage of FHHs observed in the current study is contrary to other observations of rural Tanzania, where higher percentages of them have been reported. For example, FAO (1997) reported 30% of FHHs for Ileje district in Mbeya, 32% for Mvumi division in Dodoma, 25% in Zanzibar north and 17.5% in Tanzania mainland.

A very small proportion of the household heads had attained secondary education (Table 1). Many household heads were primary school leavers, one in five had attended primary schools without completing and 10% of household heads had no formal education. However, education levels can influence households/individuals' choice of a livelihood strategy (Marzano, 2002; Malab et al., 2006). In addition, Minot et al. (2006) pointed out that an increase in an individual's years of education is expected to increase one's range of work-related skills and hence the ability to acquire new skills. According to Minot et al. (2006). a high level of education is expected to be associated with the production of higher value crops, more commercially oriented agriculture, and greater participation in non-farm activities. Crop production was the households' main occupation and this is in line with what has been reported for Rukwa region (URT/NBS/RRCO, 2004). In Nkansi district, it was the main occupation of all the household heads visited. The results conform to official estimates for the Rukwa region, according to NBS/MAFS/MLD/MCM/LORAS/MFA (2006). About 99.8% of the region's households generally grow crops, with about 34% of them growing crops and keeping livestock. Average household size was relatively higher than 5.1 and 4.6 reported for Rukwa region and Tanzania, respectively (NBS, 2002). However, some relative differences between districts, with Sumbawanga having the smallest and Mpanda the highest household sizes, respectively were observed.

Average land owned by households was 3.1 ha and was in conformity with the official estimate of about 3.0 ha observed for Rukwa region during 2002/03 National Sample Census of Agriculture (United Republic of Tanzania URT, 2006). However, when compared at the district levels, Mpanda districts had relatively more land than the other two districts, Sumbawanga having the least one. On ownership of cropping land by gender of household heads, there was significant (0.05) difference between the two genders. On average, FHHs owned less land than MHHs.

This observation is in line with the actual situation in most parts of Tanzania. Land ownership is mainly a male right as a result of the highly entrenched patriarchal system.

Maize crop production in Rukwa region

Here, statuses of maize production at the study households were examined in the context of 20 years of agricultural reforms based on farmer views about the importance of maize crop and the levels of maize production recorded in 2005. The study also examined constraints facing maize producers in the region. Regression analysis was also done in order to assess how important individual constraints were and to shed light on some of the debates referred to in the introductory part.

Levels and trends of maize production

Maize crop is important to most households in Rukwa region and this was clearly illustrated during the Focus Group Discussions (FGDs). During these discussions, the crop was consistently ranked as one of the most important crops cultivated by the study households. Both the FGDs and interviewed farmers referred to its dual use as a food crop for family consumption and a source of households' income. Only where cash crops such as tobacco were well established, maize crop had less importance as cash crop. Generally, apart from differences in maize crop's importance in Mpanda relative to Sumbawanga and Nkansi districts, all the FGDs in Sumbawanga and Nkansi ranked maize as the number one crop in their areas followed by beans and then sunflower. The FGDs that ranked maize as the most important crop based their arguments on its importance both as their main staple and for its income earning commodity when surplus produce is sold. According to the FGDs and study participants, all age groups and genders were equally involved in its production.

The greater importance of maize crop to the farmers in Nkansi and Sumbawanga districts was based on the quantitative estimates of maize output and area cultivated with maize. Table 2 shows that the averages of 1237. kg/ha and 2020.34 kg for maize yield and output, respectively observed for households in Nkansi district in 2005 were relatively higher than the averages observed for both Sumbawanga and Mpanda districts. However, observed levels were relatively low when compared to levels observed elsewhere in the world (Table 2a). The levels were even lower than those reported for some other areas in Tanzania, for example Kilimanjaro, Mbeya, Ruvuma and Iringa regions when maize yield levels ranging between 1264.52 and 143.40 for the 2004/05 cropping season (United Republic of Tanzania URT, 2006). The findings further show that about half of the study households producing maize in 2005 had farm sizes larger than 2 ha and less than fifty percent of households had their farms as a single unit. The observation that yields are highest in Nkansi, where average farm size is slightly larger than elsewhere, suggests that small farms are not more productive. However, other factors might be involved on maize yield differences noted.

Table 2. Households' maize production in Rukwa for the 2005 cropping season.

Characteristics		Rukwa Region (n = 191)	Districts		
			Sumbawanga (n = 66)	Mpanda (n = 66)	Nkansi (n = 59)
Average households total maize production (kg) in 2005		1460.10	1420.00	.3	2020.34
Average households' maize yield (kg/ha) 2005		1057.4	0.12	63.47	1237.
Average farm size (ha) under maize production		1.31	1.35	1.12	1.47
Is crop farm a single plot of land	es	1 (47.6)	22 (33.3)	33 (50)	36 (61)
	No	100 (52.4)	44 (66.7)	33 (50)	23 (3)

Source Own Survey (2006). Numbers in brackets indicate percentage.

Table 2a. Average maize yields (kg/ha) across the world.

Season	Eastern Africa	Northern Africa	Southern Africa	Asia	Eastern Europe	South America	Mexico	World
1 6 –1 0	13 5.54	3627.36	1 3 .76	2 4 .74	3603. 2	20 3.66	1772.4	3502. 4
1 1 1 5	12 1.42	3 4 .56	1 54.16	34 3.46	3236.1	2533.62	2270.02	3 24.4
1 6 2000	1441.7	5260. 6	23 0.16	3722. 0	342 .3	306 . 6	237 . 0	430 .30
2001 2005	1360.2	577 .10	276 .60	3 23.52	4050.66	3673.20	2757.40	4623.54

Source of data FAOSTAT (http //faostat.fao.org/site).

Table 3 shows summary statistics on the use of modern farm inputs and technologies. ith regards to the use of improved technologies, the use of them in maize production in 2005 was low. Most households relied on traditional seeds and recycled hybrid seeds. Only about ten percent of the households bought improved seeds from stockists every year. This observation is partly in line with available statistics by NBS/MAFS/M D/MCM/ ORA /MF A (2006) which on the 2002/03 National Sample Census of Agriculture reported that only 1 % of crop growing households in Tanzania used improved seeds and only 5% of households in Rukwa region used improved seeds. The study's estimate of 9.9% for use of improved seeds by the surveyed households is of a similar magnitude to the official estimate for Rukwa region. Most of the households relied on the hand hoe in their land preparation.

The use of fertilizers was limited amongst the surveyed households (Table 3). enerally, about 42.1, 52.6 and 5.3% of the study households which reportedly use fertilizers applied farmyard manure, chemical fertilizers and both, respectively. The low use or total non use of chemical fertilizers by the surveyed households was reportedly caused by many factors. ariation of reasons between age groups with regards to their non use of fertilizers was observed. Many elderly and middle aged respondents based their arguments on their e periences and traditions while the younger was blamed on unavailability and lack of money. Some younger respondents were also caught up with the problems of traditions and customs of their communities. owever, all the respondent categories were e ually affected by the price of fertilizers and economic hardships as factors hindering their use of fertilizers in maize production. The information provided during the interviews gave a mi ed picture; whereas some respondents seemed very genuine in their concern given the economic and infrastructural conditions of many rural areas of Tanzania which make it impossible or very e pensive to access agricultural inputs, observations from the study suggest that the respondents' crop husbandry may be limited and more education is re uired if the situation is to improve. hile some respondents said they were not using fertilizers as their lands were uite fertile, nonetheless some were ready to use them if they were given for free.

Over one third (3 .2%) of the households which produced maize crop in 2005 sought agricultural e tension services from ualified staff (Table 3). owever, in each district, levels of access to e tension services by households were 2 . , 53 and 32.2% for Sumbawanga, Mpanda and Nkansi, respectively. Mpanda district had relatively high access to e tension services due to the fact that farmers who grew had direct access to tobacco e tension staff who were employed by the tobacco companies. They were also useful for maize production whenever consulted. The low access to e tension services observed could in part be due to inade uacy of e tension staff or the distance between the households concerned and the location of the e tension

Table 3. Rukwa households' use of modern technologies in maize production in 2005 by numbers and percentage.

Characteristics		Rukwa Region (n = 191)	Districts		
			Sumbawanga (n = 66)	Mpanda (n = 66)	Nkansi (n = 59)
Tillage method used	Hand hoe	109 (57.1)	35 (53.0)	61 (92.4)	13 (22.0)
	Ox-plough	82 (42.9)	31 (47.0)	5 (7.6)	46 (78.0)
Use of fertilizers in maize production	Yes	57 (29.8)	9 (13.6)	23 (34.8)	25 (42.4)
	No	134 (70.2)	57 (86.4)	43 (65.2)	34 (57.6)
Maize seeds used	Traditional/Local seeds	134 (70.2)	50 (75.8)	46 (69.7)	38 (64.4)
	Hybrid seeds bought from input shops every year	19 (9.9)	3 (4.5)	10 (15.2)	6 (10.2)
	Hybrid seeds recycled from previous season	38 (19.9)	13 (19.7)	10 (15.2)	15 (25.4)
Household's use of extension services	Yes	73 (38.2)	19 (28.8)	35 (53.03)	19 (32.2)
	No	118(61.8)	47 (71.2)	31(46.97)	40 (67.8)

Source□Own Survey (2006). Numbers in brackets indicate percentage.

staff office and/or residence. According to NBS/MAFS/M□ □D/MCM/□ORA□□/MF□A□□ (2006), Rukwa and □indi rank last in terms of access to crop e□tension services, with only 17% of households having access to e□tension services. The highest access reported was about 64% in Dar es Salaam and Iringa regions. In some areas, the e□tension staff served more than one village. This was □uite challenging to manage due to the geographic orientation of some areas in Rukwa region, with the western rift valley passing through the region. Other factors are the underdevelopment of infrastructure and lack of cheap means of transport like motorcycles. Despite some officers having bicycles, the terrain made them inefficient mode of transport.

Despite the fact that access to e□tension services was a problem to some of the households, others were less keen to access the services. The use or non□use of agricultural e□tension services among the surveyed households varied between the age groups. Many elderly and middle□aged respondents believed that they had appropriate e□pertise and e□perience to produce maize, mainly based on their years of farming or the e□perience of others in their villages. On the other hand, many young respondents mentioned ignorance to be a ma□or factor in not seeking e□tension advice. This observation is supported by Dos et al. (2003) who argued that young farmers are more open to new technologies compared to old ones who had accumulated enough e□perience. The belief acted as an obstacle to adoption of new technologies compared to the younger who had less e□perience. Apart from these two fairly clear distinctions, the findings show that other factors, including lack of money for adoption of technologies advised and unavailability of e□tension staff, affected all age categories in similar ways. □owever, some of the elderly and middle□aged respondents, due to their traditions and/or e□periences, lacked motivation for seeking e□tension services for their maize production due to the crop being perceived as a food crop, and/or other reasons that are beyond the scope of this study.

Factors influencing maize production at household levels in Rukwa region

Table 4 details 2005 surveyed households' maize yields, output, farm characteristics, household characteristics and location on maize yields and output. Two models were estimated using ordinary least s□uares. The first model aimed at investigating the importance of farm, household and location characteristics on yields and the chosen measure of productivity in this area. The second model used total output as its dependent variable. A common set of independent variables was used. In e□ecuting the analysis, the natural logarithm of yields and outputs was chosen to be used in the above models. This was mainly prompted by the fact that the relationship between the above dependent variables is not linear. In addition, there is a likelihood that natural limits could be in operation for both maize yields and outputs.

First, a set of farm characteristics and farming method variables were specified. They include□

i) Total farm size (in ha), which was intended to shed light on the small farm efficiency hypothesis, a dummy variable indicating if the farm land is in a single plot, which may reveal whether economies of scale are available from having consolidated plots.
ii) Three further variables, the proportions of land dedicated to maize production and to cash crops, and whether the household sells maize, capture the e□tent to which farms rely on maize or have access to other forms of incomes. It might be e□pected that households with a large part of land dedicated to cash crops put less effort into maize production, and so have lower maize yields.
iii) The number of crops and livelihood strategies adopted

Table 4. Simple linear regression of the natural logarithms of the surveyed households' 2005 maize yields (kg/ha) and output (kg) in 2005.

Farm characteristics and farming methods	Natural logarithm of households' maize yield (kg/ha) in 2005	Natural logarithm of households' maize output (kg) in 2005
Households farm size (ha)	0.006 (0.040)	**0.355*** (0.042)**
Is crop farm a single plot of land (1=yes; 0=no)	-0.121 (0.125)	-0.095 (0.131)
% of a household's farm land allocated to maize production	-0.006 (0.004)	**0.010** (0.004)**
% of a household's farm land allocated to cash crops	-0.001 (0.004)	0.000 (0.004)
Household's sale of maize (1=yes; 0 = no)	**0.376** (0.164)**	**0.399** (0.171)**
Number of crops grown by household in 2005	-0.041 (0.078)	0.007 (0.081)
Number of livelihood strategies adopted by household	**-0.148** (0.060)**	**-0.137** (0.062)**
Household's use of fertilizer in maize production (1=yes; 0=no)	0.202 (0.133)	**0.256* (0.139)**
Household's use of improved maize seeds (1=yes; 0: no)	0.010 (0.130)	-0.081 (0.136)
Household use of extension services (1=yes; 0 = no)	-0.156 (0.130)	-0.208 (0.136)
Household's tillage method (1=oxen; 0= hand hoe)	**0.423** (0.156)**	**0.499** (0.163)**
Household head's age	**-0.010** (0.005)**	-0.008 (0.005)
Sex of household head (1=male; 0=female)	0.095 (0.176)	0.129 (0.184)
Actual school years of household head	**0.046* (0.026)**	**0.062** (0.027)**
Household size	0.003 (0.023	0.024 (0.024)
Sumbawanga district (1= Sumbawanga; 0= otherwise)	-0.116 (0.178)	-0.186 (0.186)
Nkansi district (1: Nkansi; 0= Otherwise)	-0.141 (0.186)	-0.168 (0.194)
	Constant: 7.435* (0.562)**	**Constant: 5.223*** (0.587)**
	N = 191;	N = 191;
	R^2 = 0.243 and	R^2 = 0.557 and
	F Stat 2.814* **	**F Stat 11.016*** **

Standard errors in brackets and ***Significant at 1% (0.001) level, **Significant at 5% (0.05) level and *Significant at 10% (0.1) level.

by the household were also included as this may also indicate whether farmers put less effort into maize production.

iv) A set of variables that capture input use were also included: use of fertilizers, type of maize seeds, use of extension staff, and use of oxen for tilling land, aiming to capture the benefits of improved farming practices that are associated with higher yields elsewhere.

A second set of variables captures key household characteristics of age, gender and education levels of the household head and household size. There is evidence elsewhere that education and experience may lead to higher yields, although it is not clear if these are important in the cultivation of a staple crop grown by the vast majority of the population. Gender may be important if for example, FHH are constrained to production for home consumption, or have difficulty in acquiring hired labour. Household size aimed to capture the notion that large sized rural households would be able to easily supply the labour required for their crop production due to abundance of own labour. A final set of variables are the dummies for the districts, which capture other characteristics that are not captured by the above, for example variations in soil quality between the districts, or proximity to markets. Results of the simple linear regressions are presented in Table 4.

The first point to note is that the fit of the yield model was quite low especially compared to the output model. This observation suggests that there were other factors determining variations in yields that were not included in this model. One important omission is soil quality but unfortunately, that variable was not possible to collect in this survey. Nevertheless, the F statistics showed that the models are useful for shedding light on maize yields and output levels.

Regarding farm characteristics and farming methods, there were lots of consistency and many appeared to be significantly associated with outputs, but a few were not. For example, farm size, the proportion of farmland allocated to maize production and use of fertilizers. These factors were statistically significant. On the other hand, the age of a household's head was significantly associated with yield but not output. There was no evidence on the inverse farm size-productivity relationship: smaller farms had marginally and statistically insignificant higher yields. The lower yields, due to an increase in amount of land allocated may be due to factors such as households failing to do their weeding in time. According to Bisanda et al. (1998), late weeding can also lead to seriously low maize yields. However, the study generally shows that households with more land and those allocating a higher proportion of their land to maize production despite the lower yields observed they

got significantly higher maize outputs in 2005 compared to those with less land and those allocating a smaller proportion of the land to maize production. The number of crops grown and number of livelihood strategies in place had the expected outcome and were associated with lower maize yields and maize outputs, although the results showed that only the latter influence were statistically significant. One very strong result is that households that sold maize had much higher yields and output than those that did not sell – suggesting that commercialisation of staples may be associated with higher productivity. This observation is further supported by Davis et al. (2006) who argued that market access is very important if households are to get greater returns from their crop and livestock production activities. In a similar way, households that devote more land to cash crops also have lower maize yields, supporting the hypothesis that effort on food crops may be lower.

Surprisingly, despite the literature available (Kaliba et al., 2000; Gemeda et al., 2001; Sasakawa African Association, 2006; World Bank, 2006a) showing that access to extension services may boost maize and other crops yields, there was no significant influence of access to extension services on either higher maize yields or output in 2005 (Table 4). However, according to the information indicated in Table 3, only about one-third of the surveyed households had access to extension services. This may explain the insignificant levels observed. In addition, access to agricultural extension services without access to fertilizers and other inputs may have no effect on crop yields. Some of the households also reported to not doing so because of failure to follow the advices.

Regarding household characteristics, there were no statistically significant effects of age, gender or household size on yields or output. Education level however, had statistically significant impact on maize yields. This may reflect greater awareness of good farming practices, e.g. how and when to apply fertilizers or pesticides, or store seeds from one harvest to the next. Certainly, there seem to be some support for the idea that educating farmers who grow food crops would have positive impacts.

The district dummies had no significant influence in maize yields and outputs. This suggests that unobserved differences between the districts (such as soil quality) are not significant, although of course the latter may be already picked up in the variables that capture use of fertiliser etc (because there may be a relationship – positive or negative - between soil quality and whether a farmer uses fertilisers).

Generally, observations from the regression analysis were consistent with the information provided by the surveyed farmers. For example, households had raised in the interviews the issue of lack of access to modern inputs (fertilizers and improved seeds) due to either unavailability or price restrictions being constraints to their maize production. This finding is supported generally by the regression analysis results on maize output – lots of the corresponding variables have statistically significant coefficients, suggesting that farmers who had access to these inputs had higher outputs. However, the yield regression analysis is less conclusive: using modern inputs and methods do raise yields but not to a statistically significant extent. It is possible that the lack of access to extension services, and the re-use of seeds rather than purchasing new seeds each season, reduces the effect that inputs have on yields.

The regression analysis further suggests that households that sell maize, rather than keep the whole harvest for home consumption, have higher yields than those that do not. This does not necessarily imply causation but is suggestive of the importance of boosting productivity in order for farmers to generate surplus output. Yields also tend to be higher among farmers that are less diversified while the more livelihood strategies are adopted, the lower the maize yield. Again this does not necessarily imply causality but is suggestive that while diversification may be an appropriate response to risk and vulnerability, it will not necessarily enable farmers to escape from poverty traps. Education is also a very important factor associated with higher yields. Some of these results confirm expectations, while others are more surprising. For example, the lack of a significant positive influence of access to extension services on increased yields and output, or access to extension services is normally expected to help raise productivity levels. This was the case with the SG 2000 project implemented in the northern part of Tanzania, where access to reliable extension services and the promotion of input use, for example chemical fertilizers, led to a rise in smallholders' maize yields to between 4.5 and 5.1 tonnes/ha, compared to the national average yield of around 1.5 tonnes/ha (Sasakawa African Association, 2006).

Conclusions

Generally, the study has revealed that productivity of maize and the other crops produced in Rukwa region were relatively lower than levels reported in other parts of the world in general, but broadly in line with official estimates for the region. This may have been due to a multitude of factors. For example, both survey observations and observations from the focus group discussions (FGDs) have shown maize to be one of the most important crops for farmers. Nevertheless, there was a consensus that farmers faced significant barriers in improving yields and output levels. Farmers reported, for example, that accessing important inputs such as new seeds, fertilizers and extension services was difficult, either because these inputs were expensive, not available

locally or not available altogether or because they felt their soil was fertile enough already and they distrusted modern inputs (that is, sought no advice on fertilizer). However, the lack of statistical significance in the yields regression results of impacts of fertilizer, extension services advice and seeds suggests that a combination of factors is at work: simply using the inputs does not raise yields, as they need to be used in the right quantities, at the right time and in combination with other factors. □n addition, the data on fertilizer, for example, was not detailed enough to reveal whether farmers did use fertilizers in the right quantities, or the right types of fertilizer. The lack of statistical significance might be because so few used fertilizer that the regression cannot identify an effect.

The study also concludes that households' heads' education level was relevant to the commercialization of maize production and that education level was positively related to higher maize yields. For example, results from the regression suggest that households that sold maize rather than keep the whole harvest for home consumption had higher yields than those that did not. This, in itself is an interesting result, suggesting that raising productivity is key to establishing well-functioning markets in key food staples. Furthermore, the finding that education is positively related to both yields and commercialisation, suggest that improvements in education may also be a fruitful way of improving performance of the agricultural sector. Although this does not necessarily imply causation, it does suggest the importance of education in boosting productivity in order for farmers to generate surplus output. Higher levels of education were also observed to be positively related to higher maize output. This finding suggests that with more education farmers can easily overcome constraints of poor agricultural extension services and access to market information□ they can therefore, easily get information from printed media and other sources such as agricultural pamphlets, brochures and posters which are locally available or could be obtained during agricultural shows.

Conflict of Interest

The authors have not declared any conflict of interest.

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Natural vulnerability of water resources in the Formoso River basin, Northern Brazil

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The natural vulnerability characterization provides fundamental support for identification of areas more susceptible from the hydrology point of view, assuring the maintenance of water resource features. In this context, this study aimed to characterize the natural vulnerability of water resources based on long-term streamflows, base flow and aquifers' susceptibility to contamination, for Formoso River basin, located in southwestern Tocantins. For that, subdivision by level 5 Ottobasins and discharge and precipitation data sets, both available from the "Brazilian National Water Agency", and the geological map developed by "Tocantins State Bureau of Planning" (SEPLAN) were used. The final natural vulnerability of water resources presented degrees varying from low to very high. Areas with lower vulnerability were observed in the basin headwaters, mainly due to the greatest both long-term (SY_{qlt}) and 90% of the exceedance ($SY_{90\%}$) specific discharges. "High" vulnerability were identified in most of the middle basin course while "Very High" vulnerability in the lower basin course given by lower SY_{qlt} and $SY_{90\%}$ and by the occurrence of the alluvial aquifers which present high natural susceptibility to contamination.

Key words: Natural resources, hydrology, watershed management.

INTRODUCTION

One of the greatest contemporary environmental concerns is the preservation of water resources given the numerous human activities potentially harmful to this feature, since the demand for them has increased exponentially in the last decade. Among several aspects related to water resources, there is the concern to ensure the maintenance of its availability and quality.

Agricultural and livestock are the human economic activities that spatially have greater demand for landing, □hanging significantly the landscape. Normally, these activities are based on the alteration of the natural ecosystems, and they have redefined the land use in large scales throughout the last three □enturies (Ramankutty and Foley, 1999). According to Bartholom□ and Belward (2005) almost 16% of the planet's surface is occupied by tillage. However, these activities have been important sources of water pollution mainly if they are developed based on inadequate soil conservation practices (□hang et al., □009□Orzepowski et al., □014), affecting the water quality by soil erosion that carries

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sediments, nutrients, pathogens, pesticides, heavy metals and others (USEPA, 2005). Thus, the groundwater contamination studies have been recognized as essential for mitigation strategies adoption for adequate land use, reducing the impacts on water resources.

The world population reached up 7.2 billion in 2014, with an expansion estimated around 9.6 billion for 2050 (United Nations, 2014). Irrigated agricultural lands cover approximately 15% of cultivated areas, being fundamental for food production aiming to supply this accelerated growth population, as this system has greater productivity (Simonovic, 2002). Worldly, according to Doll and Siebert (2002), irrigated fields are responsible for 90% of all water consumption and more than 40% of food production as well. This way, it is essential to create tools that allow the estimation both surface and groundwater availability as it is associated to the geomorphology of the basins, which is characterized by the climate, geology, soils and topography attributes. In this context, the identification of basins (and sub-basins) with reduced water availability permits to find places in which are priority for encouragement of great management practices adoption, aiming to increase groundwater recharge rate as well as identification of the most strategic sections for dams building for water storage.

Based on these features, the agricultural and livestock planning, especially the integration between water resources management and appropriate land uses, requires detailed studies about natural vulnerability, searching not only for maintenance of water quality and quantity but also for optimization its use. General way, the natural vulnerability can be defined as a set of soil, climate, hydrology and geology attributes of a given hydrograph basin, which control its capacity for self-restoration (Gomes et al., 2002). In the approach of natural vulnerability of water resources, it is considered the environmental fragility of these attributes, however, without taking into account the anthropic activities (Carvalho et al., 2008; Ribeiro et al., 2011).

Vulnerability can be represented in cartography form, which enables better preparation of proposals for sustainable watershed development by management agencies. This type of study is fundamental for decision maker and directs the choices of areas for the conducting of potentially degrading activities (Szlafsztein and Sterr, 2007; Rahman, 2008; Ribeiro et al., 2011). The determination of the vulnerability indicators provides assistance for the prevention and recognition of the more sensitive areas from a hydrological point of view, ensuring the maintenance of the qualitative and quantitative aspects.

A hydrograph basin is defined as a group of lands drained by a main river and its tributaries. The Formoso River is a major tributary of the right arm of the Araguaia River (Javaés River), in the southwest of the Bananal Island, Tocantins state, northern Brazil. It belongs to the Tocantins-Araguaia hydrographic region according to the hydrographic division of the Brazilian National Water Agency (ANA). The Formoso River basin has a drainage area of 11,018.5 km², which corresponds to approximately 7.2% of the total area of the State of Tocantins and 5.2% of the Araguaia River basin, according to the State of Tocantins Basin Plan.

In Brazil, the states of Minas Gerais and Espírito Santo, both in Southeastern region, have worked with Ecological-Economic Zoning (EEZ), which have as primary purpose to guide the sustainable economic development of these states, allowing the characterization of regions more vulnerable from both socio-economic and ecological point of views (www.zee.mg.gov.br; www.ti.lemaf.ufla.br/zeees.html). The vulnerability of water resources has been fundamental in these zoning studies, as they have allowed the characterization of regions in terms of their natural water supplies and, in same time, areas with greater potential for irrigation, become the use of water resources more suitable for agricultural purposes (Coello et al., 2008). In this context, this study aims to determine the natural vulnerability of water resources taking the surface and subsurface runoff, and aquifer susceptibility to contamination as a reference, for the Tocantins portion of the Formoso River basin. To determine the regions that are more susceptible to contamination, the objective is to provide directional technical support for the integrated management of water resources in this important basin for the state of Tocantins, which already has its own Watershed Committee and which has been suffering the increasing effects of water shortages during drier periods of the year.

MATERIALS AND METHODS

Characterization of the studied site and database

The studied area comprises the Formoso River basin, which is located in the southwest region of the State of Tocantins, Northern Brazil, between 10°28'S and 13°16'S latitude and 48°50'W and 49°57'W longitude. The Formoso River basin is formed by 8 major sub-basins: Escuro, Cau Seco, Taboca, Javante, Fuer, Fago Verde, Urubu e Áreas Marginais ao rio Formoso (SEPLAN, 2002).

The Formoso River basin covers parts of the territory of 11 municipalities, respectively, 18 in the state of Tocantins and 1 in the state of Goias, however, the involvement of the latter are limited to less than 3% of the basin's area (Figure 1). Livestock and agricultural are the most important land uses, covering 59% of its territory. It is worth mentioning the presence of the "Formoso River Irrigation Project" in the mid-western region of the basin, which has a useful area of 18,000 ha in which rice is grown in the rainy season (October to April), and soybean, corn, beans and watermelon in the dry season (May to September) (http://seagro.to.gov.br). The remaining natural vegetation mainly consist of Brazilian Savanna (34.2%), Savanna Forest (4.2%), Gallery and Riparian Forest (3.4%), Semidecidual Seasonal Alluvial Forest (4.9%) and Savanna Parkland (4.5%) (SEPLAN, 2012).

On the Araguaia plain, Plintosols and Gleysols are predominating, both with drainage impediment and with frequency

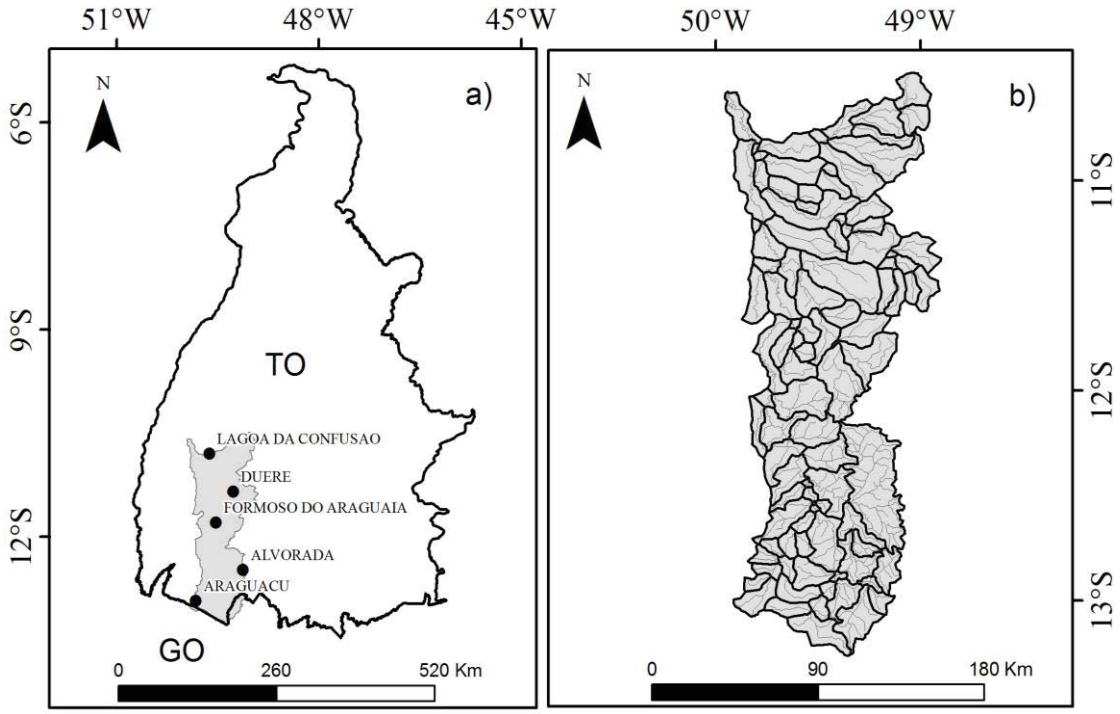

Figure 1. □ocation of the Formoso River basin in the states of Goi□s (GO) and Tocantins (TO) (a) and level 5 Ottobasins of national hydrographic division and hydrography (b).

of floods in rainy season (□BA□ A, 1994).

□etasediment□□etavolcanic, crystalline and cenozoic formations are the predominant geomorphological domains in the basin. □etasediment□□etavolcanic and crystalline domains are related to the so-called fissure aquifer, and presents limited hydrogeological favorability. The cenozoic formations, in turn, have a porous aquifer, characterized by having a primary porosity and better hydrogeological favorability (□□R□, □014). Figure 1a shows the location of the Formoso River basin with emphasis on the municipal centers inserted in the basin.

Aiming to develop the zoning of the natural vulnerability of water resources in the Formoso River basin, subdivision by level 5 Ottobasins was considered, available from Brazilian National □ ater Agency (ANA, □01□). The level 5 Ottobasins of the Formoso river are shown in Figure 1b.

One of the main challenges for the development of hydrological studies for the basin is the low availability of discharge gauging stations. □n order to minimize this limitation, we selected stations from other Araguaia River sub-basins, near the Formoso River, with homogeneous climatic and physiographic characteristics (S□□□AN, □01□). Based on these sub-basins, we proceeded to fit the equations for the hydrological regionalization of the hydrological indicators considered in this study. □ong-term discharges data sets of the following stations were obtained from the Hidroweb□ANA (ANA, □01□): □50□0000, □5090000, □5□50000, □□□□0000, □□□50000, □□□90000 and □□□□0000.

Natural vulnerability of water resources

The water resources has been treated within the natural vulnerability concept established by the □inistry of □nvironment, as advocated in the □ecological-□conomic □oning (□□□) developed for the states of □inas Gerais and □sp□rito Santo (□arvalho et al., □008). This concept relies on the natural capability of the environment to recover itself when affected by anthropic activities. This means that the concept of vulnerability established concerns biotic (native flora and fauna) and abiotic characteristics (soil, climate and hydrology), that is, human activities are not considered. Thus, regarding to water resources the variables that can express its natural vulnerability are water availability and the possibility of geological aquifers contamination, just like applied by □ello et al. (□008) in the □□□ of □inas Gerais state.

Regarding to natural availability, this study considered two components, which are easier to obtain for entire the basin. The first is associated with surface water availability and has been associated to the long-term average specific yield indicator ($S□_{qlt}$), which is widely used in studies on surface water use, especially for flow regularization through dams. The second component aimed to characterize water availability during the dry season, which is markedly severe in the basin, and during which the streamflows are essentially controlled by the groundwater sources whose availability reflects the natural regulatory capacity of the sub-basins. This component was portrayed by two indicators: (a) Specific discharge associated with 90% flow retention ($S□_{90\%}$), which is widespread in water resources management in Brazil and considered the benchmark for the granting of water resources use in the state of Tocantins and also in the context of federal rivers□(b) Regulatory reserve volume of the aquifer (□) (□astany, 19□□), which allows inferences about the natural storage capacity. Thus, it is understood that the greater the water availability, the less natural vulnerability of the sub-basin.

To infer about the aquifer susceptibility to contamination, geological and geomorphological units were interpreted as function of their potential for leaching of contaminants, like the type of rock, and existence of faults. For this indicator, it was considered that the higher the susceptibility to contamination, the higher the natural vulnerability of the basin. Figure □ shows a schematically

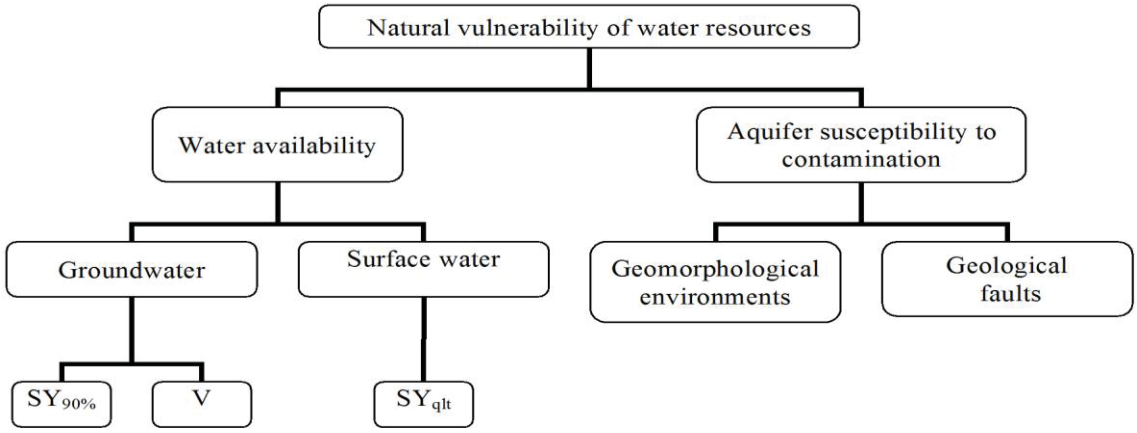

Figure 2. Organogram of the indicators used to assess the natural vulnerability of water resources in the Formoso River basin, Tocantins state, northern Brazil.

organogram, detailing the procedure adopted for characterization of indicators used to express the natural vulnerability of water resources.

Specific yield with 90% retention ($SY_{90\%}$)

The natural water availability can be evaluated by the behavior of the base flow, which is the main component of the streamflow during the dry period. This indicator is linked to the capability of the basin to groundwater recharge (□ello et al., □008).

The minimum flow associated with percentiles of 90% (□$_{90\%}$) identifies the amount of flow that is equaled or exceeded in the watercourse 90% of the time, and is obtained from the flow retention curve. Having obtained □$_{90\%}$, in m□s^{-1}, the regionalization of these flows, considering as an explanatory variable, the drainage area of the sub-basins, was performed as described below:

$$Q_{90\%_{sub-basin_i}} = a \cdot Ad_{sub-basin_i}{}^{b} \quad (1)$$

□ here *a* and *b* are regression adjustment parameters and *Ad* is the drainage area of the sub-basin in km□

The adjusted regionalization equation was used to calculate □$_{90\%}$ for each level 5 Ottobasin of the Formoso River (Figure 1b). Subsequently, the specific discharge, in □s^{-1} km^{-}□, was obtained for each sub-basin, according to □quation □:

$$SY_{90\%_{sub-basin_i}} = \frac{Q_{90\%_{sub-basin_i}}}{Ad_{sub-basin_i}} \cdot 1000 \quad (\square)$$

After obtaining the regionalization equations, hydrological zoning was conducted using ArcG□S 9.1□ software. For each indicator, the value intervals for the interpretation of vulnerability as “Very High”, “High”, “Medium” and “Low”, were defined based on irregular intervals, maintaining the same number of observations (sub-basins) in each class.

Aquifer regulatory reserve at the beginning of the recession period (V)

This indicator allows inferring on the volume available for aquifer discharge at the start of the recession period. □n this sense, the greater the stored volume, the greater it’s natural regulatory capacity and therefore the lower its natural vulnerability. □ts quantification for each fluviometric station was carried out according to the method of Barnes (□astany, 19□□), as presented in □quations □, 4, 5 and □. Subsequently, the □ regionalization was calculated using the drainage area as the explanatory variable. Then, this equation was applied to calculate □ values for the level 5 Ottobasins of the Formoso River. Furthermore, it presents the procedure for obtaining □ in hm□:

$$Q_t = Q_0 \cdot e^{-\alpha \cdot t} \quad (\square)$$

$$\ln(Q_t) = \ln(Q_0) - \alpha \cdot t \cdot \ln(e) \quad (4)$$

$$\alpha = \frac{\ln(Q_0 / Q_t)}{t} \quad (5)$$

$$V = \frac{Q_0 \cdot 0.0864}{\alpha} \quad (\square)$$

in which Q_t is the streamflow rate at time *t* in m□s^{-1}□□$_0$ is the streamflow rate at the start of the recession, m□s^{-1}□ *α* is the coefficient of discharge of the aquifer, day^{-1} and *t* are equivalent to the number of days elapsed between Q_0 and Q_t.

Long-term average specific yield discharge (SY_{qlt})

The long-term average discharge (□$_{lt}$) is the average flow observed at a particular gauging station. Having obtained the □$_{lt,}$ in m□s^{-1}, the regionalization of the streamflow was also conducted considering the drainage area as an explanatory variable as described below:

$$Q_{LT_{sub-basin_i}} = a \cdot Ad_{sub-basin_i}{}^{b} \quad (\square)$$

in which *a* and *b* are the regression adjustment parameters and *Ad* is the drainage area of the sub-basin in km□

The regionalization equation was applied to calculate □$_{lt}$ for each level 5 Ottobasin of the Formoso River. Subsequently, it was

Table 1. Geomorphological environments, corresponding scope and degree of vulnerability considered in the Formoso River basin.

Geomorphological domain	Hydrogeological domain	Hydrogeological subdomain	Occurrence (%)	Vulnerability to contamination
□nconsolidated sedimentary deposits	□enozoic formation	Alluvium	□.4	□ery High
□nconsolidated sedimentary deposits	□enozoic formation	Araguaia	11.□	High
□nconsolidated sedimentary deposits	□etasediments□ metavolcanic	□etasediments□ metavolcanic	0.4	Average
Basements on complex styles	□rystalline	□rystalline	□.□	□ow
Basements on complex styles	□enozoic formation	Alluviums	0.0	□ery High
Basements on complex styles	□etasediments□ metavolcanic	□etasediments□ metavolcanic	0.□	Average
Bands of folds and metasedimentary cover	□rystalline	□rystalline	□5.□	□ow
Bands of folds and metasedimentary cover	□enozoic formation	Alluviums	5.□	□ery High
Bands of folds and metasedimentary cover	□enozoic formation	Araguaia	0.□	High
Bands of folds and metasedimentary cover	□etasediments□ metavolcanic	□etasediments□ metavolcanic	41.9	Average

converted into specific yield discharge, in □ s^{-1} $km^{-□}$, according to □quation 8:

$$SY_{qlt_{sub-basin_i}} = \frac{Q_{LT_{sub-basin_i}}}{Ad_{sub-basin_i}} \cdot 1000 \quad (8)$$

Aquifer contamination susceptibility (ACS)

The potential for aquifer contamination corresponds to susceptibility of groundwater contamination by toxic substances that may reach the aquifer mainly by the leaching process (□ello et al., □008). The geological map of the basin was used to qualitatively characterize the A□S indicator, following the methodology proposed by □ello et al. (□008) for the □□□ of □inas Gerais state. Sites with geological faults had their vulnerability increased one step up, since they represent points that facilitate the leaching process and subsequent contamination (□ello et al., □008). Table 1 presents the existing geomorphological environments and hydrogeological domains in the Formoso River basin, their subdomain and the interpreted vulnerability to the contamination.

Natural vulnerability of water resources in the Formoso River basin

As the vulnerability was treated qualitatively, values were allocated for the final weighting of the vulnerability of the indicators for each vulnerability level as follows: "Low" = 1; "Medium" = 2; "High" = 3 and "Very High" = 4. Based on the EEZ of Minas Gerais (Mello et al., □008), the composition of the final vulnerability weighted by the indicators described above, was carried out considering the following weights: $S□_{qlt}$ □□0% □$S□_{90\%}$ □ □□.5%□□ □ □□.5% and A□S □ □5%. Thus, the final vulnerability (F□) was obtained according to □quation 9:

$$VF = \sum_{i=1}^{4} Vi \cdot Pi \quad (9)$$

in which □i is the value of the vulnerability of indicator i and □i is the weight associated with the indicator i. The final vulnerability classification was considered as: "Low": 1 ≤ FV <1.75; "Medium": 1.75 ≤ FV <2.5; "High": 2.5 ≤ FV <3.25 and "Very High": ≥ 3.25. □ualitatively, in terms of the final results of the vulnerability, □ello et al. (□008) observed that different weights produced similar results, that is, for a given sub-basin, the F□ will change if other weights are considered but its vulnerability classification remains the same. This occurs because the geographic reference is the same, equivalently describing the physical behavior of the indicators in qualitative terms.

RESULTS AND DISCUSSION

Surface water availability

The vulnerability associated with the long-term average specific yield discharge ($S□_{qlt}$) is of great importance due to the widespread use of this flow in studies on flow regulation and quantification of the watershed hydraulic energy potential. The regionalization equation obtained for □ $_{□T}$ is presented in the following sequence:

$$Q_{LT\,sub-basin_i} = 0.0184 \cdot Ad_{sub-basin_i}^{0.9651} \quad r□□\,0.99 \quad (10)$$

The $S□_{qlt}$ value classes in establishing the vulnerability were: "Very High": SY_{qlt} ≤ 14.16; "High": 14.17 ≤ SY_{qlt} ≤ 14.83; "Medium": 14.84 ≤ SY_{qlt} ≤ 15.31; "Low": SY_{qlt} ≥ 15.□□.

Figure □a shows the $S□_{qlt}$ map for level 5 Ottobasins of the Formoso River. To interpret this figure, it is considered that the larger the $S□_{qlt}$ value, the lower the vulnerability, namely, the higher the water availability.

□t can be observed that small headwater basins showed less vulnerability. This can be attributed to the higher specific yield inherent in headwater regions, where the total precipitation is higher and there is preservation of

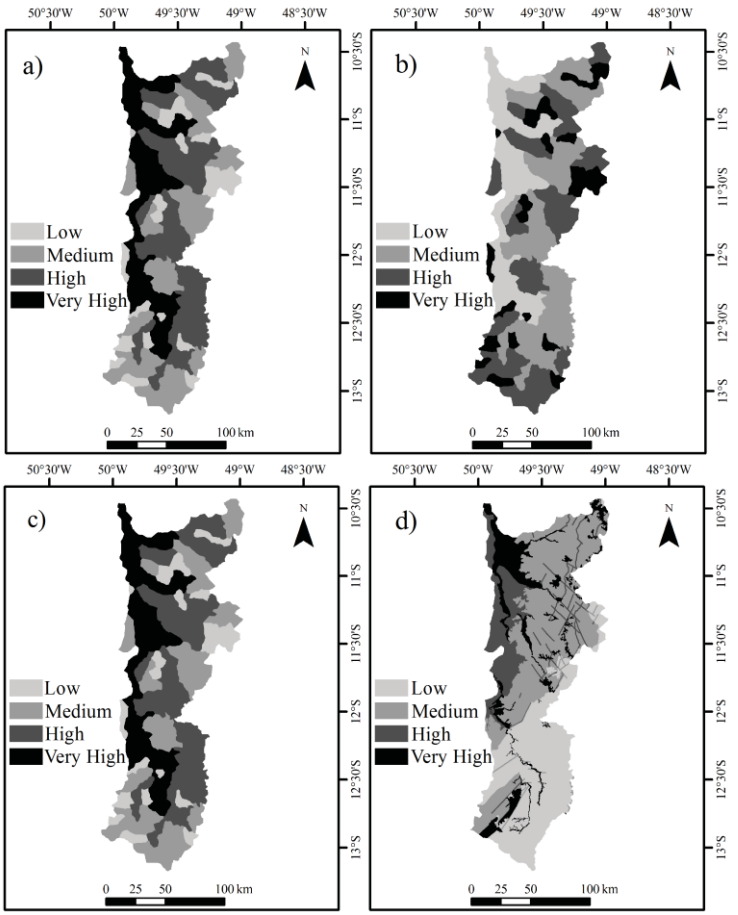

Figure 3. Natural vulnerability associated with the components: long-term average specific yield discharge (a), aquifer regulatory reserve volume (b), specific yield with 90% retention (c) and aquifer natural susceptibility to contamination (d).

native vegetation, favoring the recharge process, and runoff on slopes (Alvarenga et al., □01□□□enezes et al., □009□ □ello and □uri, □01□). Also in this context, for the middle and lower course of the Formoso River basin, have higher drainage area and the vulnerability ranged from “High” to “Very High”. In these areas, the soils are deeper and flat, favoring evapotranspiration and possibly stretches where the watercourses become influent. □n these regions, the construction of dams would be a plausible option, since they would allow the storage of water in the rainy season and its use to become the watercourses perennial during the severe drought period that occurs in the region.

Groundwater vulnerability

The identification of vulnerability associated with the volume of the aquifer regulatory reserve at the beginning of the recession is of great importance in the context of the Formoso River basin, in which the lower order watercourses have an intermittent flow regime. The regionalization equation of this variable shows a good adjustment, as shown in the following sequence:

$$V_{sub-basin_i} = 834765 \cdot Ad_{sub-basin_i}^{0.5491} \quad r^2 = 0.98 \qquad (11)$$

To assess the vulnerability associated with □, it was taken into account that sub-basins that have lower regulatory reserves, are more vulnerable. To classify the vulnerability of this component the following classes have been applied: “Very High”: V ≤ 15.02 hm³; “High”: 15.02 hm³ < V ≤ 24.85 hm³; “Medium”: 24.85 hm³ < V ≤ 51.55 hm³; “Low”: V > 51.55 hm³.

□n Figure □b, it is presented the vulnerability map associated to □. As can be seen, the headwater regions were more vulnerable than the middle and lower watercourse of the Formoso River basin. □n this context, it is clear that this indicator is able to capture the vulnerability

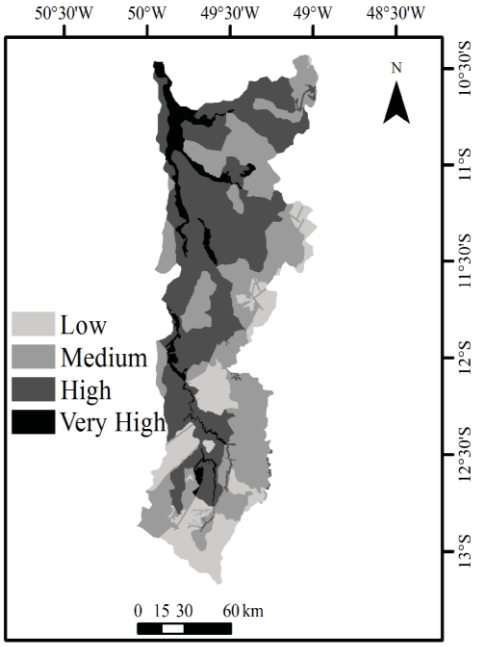

Figure 4. Final natural vulnerability of water resources to Formoso River basin, TO.

of the aquifer discharges, where intermittent watercourses occur in the headwater regions. This is essential to weigh the vulnerability associated with the groundwater availability, jointly with the $S_{90\%}$ indicator.

The second vulnerability attribute associated with the groundwater component was the specific yield discharge with 90% retention ($S_{90\%}$). The $_{90\%}$ calculation for the Ottobasins was performed according to equation 1:

$$Q_{90\%sub-basin_i} = 0.0059 \cdot Ad_{sub-basin_i}^{0.633} \quad r = 0.81 \qquad (1)$$

For this indicator, the lower the flow value, the greater the vulnerability. In this sense, the vulnerability associated with $S_{90\%}$ the following value classes were considered: "Very High": $SY_{90\%} \leq 0.38$; "High": $0.39 \leq SY_{90\%} \leq 0.61$; "Medium" : $0.62 \leq SY_{90\%} \leq 0.85\%$; "Low": $SY_{90\%} \geq 0.86\%$. As can be seen in Figure c, the lower watercourse of the Araguaia River showed greater vulnerability. In other words, headwater basins, despite higher recharge (as detected by the $S_{90\%}$ indicator), have less regulation ability, that is, they drain faster due to the topographical conditions, storing water for less time, which makes them more vulnerable from this point of view.

Aquifer susceptibility to contamination

The degree of vulnerability due to the aquifer contamination susceptibility was established through the interpretation of the source material, as portrayed in Table 1. The higher the aquifer contamination susceptibility, the higher its vulnerability will be, especially in locations with geological faults, because these are points that facilitate the leaching process and subsequent contamination.

Figure d presents the map of aquifer contamination susceptibility. Higher hydrogeological vulnerability can be seen in the alluvium subdomain, which extends along the main river basin channels, since this litological unit is more susceptible to contamination. The lower vulnerability inherent in the crystalline subdomain, can be observed in the southeastern part of the basin, while the "Medium" vulnerability inherent in the metasedimentary subdomain, prevails in almost the entire middle watercourse.

Final vulnerability

The final vulnerability of water resources, which is the product of the weighting of the four components previously treated, showed a classification from "Low" to "Very High" vulnerability degree, as shown in Figure 4.

Some regions with "Low" vulnerability were found in the headwater region of the south of the Formoso River basin. This result is primarily related to the combination of "Low" vulnerabilities for the SY_{qlt} and $S_{90\%}$ indicators and the presence of the crystalline hydrogeological subdomain, which presents "Low" vulnerability to aquifer contamination. "High" vulnerability prevailed in most of the middle watercourse region of the Formoso River, indicating that special cares are needed for the sustainable development of these sites. The "Very High" vulnerability class occurred mainly in the lower watercourse of the Formoso River. Although this region had presented "Low" vulnerability for the indicator associated with the aquifer regulatory reserve volume, it resulted in "Very High" vulnerability for the SY_{qlt} and $S_{90\%}$ indicators and "High" for aquifer contamination susceptibility, as it inserted within of alluvium hydrogeological subdomain, as described by hae et al. (004) and hae et al. (009). In this context, the alluvium subdomain region, especially in the lower watercourse of the Formoso River, can be considered as a priority for water resource conservation in the Formoso River basin. However, this study allows the detection of other points of great importance. The reduced aquifer regulatory reserve volume in the Formoso River headwaters region indicates these areas as priority for the application of basin management techniques. ngineering techniques to stimulate the terracing, construction of infiltration basins, direct planting and adopting techniques that favor water infiltration into the soil can be implemented by water resource managers, aiming to increase the water yield and especially to perennial the headwater watercourses.

Conclusion

1. It was possible to indicate the areas with “Very High” final vulnerability degree as priority for the planning and management of water resources and land use in the Formoso River basin.

□. The interpretation of vulnerability associated with the aquifer regulatory reserve volume allowed the identification that the headwater regions are priorities for the development of watershed management techniques, seeking to become the watercourses perennial.

□. □t was evident that in the middle and lower watercourse, where the specific surface and groundwater discharges are reduced, the construction of dams aimed at water storage is essential to promote water use during severe period of drought, which occurs in the Formoso River basin.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Can an anti-erosive *Andropogon gayanus* live hedge help regulate millet stem borer infestation in neighbouring millet fields?

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From 2008 to 2010, the effect of a wind erosion-alleviating practice, that is, planting of windbreaks of the perennial grass *Andropogon gayanus* Kunth on the populations of millet stem borer (MSB) *Coniesta ignefusalis* (Hampson) (Lepidoptera: Pyralidae) and the damage induced by this pest to pearl millet, was studied at Sadore (Niger). The extent of bored stems in the *A. gayanus* hedge bordering millet plots (23% on average) was significantly lower than in the millet plots (76% on average), with no MSB larva recovered, compared to an average of 2.2 larvae per stem in the millet plots over the three years. *Andropogon* grass was thus ruled out as a trap crop for MSB management. Under the conditions of this study, a pest regulation service by *Andropogon* grass hedges, synergistic with a potential wind erosion alleviating service, cannot be put forward.

Key words: *Coniesta ignefusalis*, *Andropogon gayanus*, trap cropping, wind erosion, Niger.

INTRODUCTION

In the Sahel, there is a major soil erosion problem due to windy conditions that commonly occur at the time of the dry season to rainy season shift (Rajot et al., 2009). The winds can also have a destructive effect on millet crops sown in sandy soils. Several agroecological management practices have been implemented or proposed to alleviate this erosion and thus contribute to soil conservation and pearl millet crop protection. In earlier studies conducted by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Niger, borders of the perennial grass *Andropogon gayanus* Kunth were shown to efficiently protect millet against violent winds (Renard and Vandenbelt, 1990), although there were also contradictory reports (Michels et al., 1998).

A. gayanus was also found to be an alternate host of several cereal stem borers in Africa (Gounou and Schulthess, 2004), including the millet stem borer (MSB),

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Abbreviations: **ICRISAT**, International Crops Research Institute for the Semi-Arid Tropics; **MSB**, millet stem borer.

Table 1. Millet (cultivar ICMV IS 99001) and *Andropogon gayanus* stem damage by stem borers (Sador□, 2008, 2009 and 2010).

Parameter	Bored stems (%)		
Year	2008	2009	2010
Millet	81.7[a]	67.6[a]	78.1[a]
Andropogon	23.8[b]	2□.6[b]	17.7[b]

Means followed by the same letter in the same column are not significantly different at the □□ threshold in a bilateral means comparison test.

Coniesta ignefusalis (Hampson) (□epidoptera□Pyralidae), a major pest throughout Sahelian countries (□oum et al., 1996).

The use of this grass as a 'trap crop' for stem borer management was thus suggested, and promising results were obtained in preliminary studies conducted in southern Mali (INTS□RMI□, 2008). □e therefore conducted trials in Niger from 2008 to 2010 to confirm whether the use of a *A. gayanus* border as a soil conservation measure, that is, an essential ecosystem service, could also have a diversionary effect on the MSB, resulting in "killing two birds with one stone".

MATERIALS AND METHODS

In 2008 and 2009, at the Sadore ICRISAT research station in Niger (13°15'N, 2°17'E), two rows of four experimental millet plots were planted at the onset of the rainy season. Millet plot dimensions were 12.8 □ 12.8 m, with alleys of 1.6 m. They were located north of a □3.6 m long □ 2 m wide perennial *A. gayanus* hedge, which had been planted more than 10 years before. The southern sides of millet plots were parallel to this border, at distances of respectively 28.8 and 43.2 m for the two rows. □ithin the plots, pearl millet grain cultivar ICMV IS 99001 (Ministère de l'Agriculture de la République du Niger, 2012) was planted at a spacing of 0.80 □ 0.40 m, at appro□imately 6-8 seeds per hill (namely appro□imately 3.□ □g/ha). Plots were fertili□ed via broadcast application of calcium-ammonium nitrate at 100 □g/ha at the time of thinning, according to the research station recommendations.

Both years, at millet harvest, two samples of appro□imately 20 millet stems (each corresponding to an area of 1 m□, or si□ millet hills) were randomly ta□en in the central part of each millet plot, and compared to eight samples of 40 stems randomly ta□en along the *A. gayanus* hedge. Stems were observed for the presence of stem borer holes and, after dissection, the number of stem borer larvae and pupae (*C. ignefusalis* or others) were recorded. □arvae were tentatively identified using the □ey reported by Meijerman and Ulenberg (1996).

In 2010, the section immediately north of the *A. gayanus* border (from 2.0 to 27.2 m) was sown with pearl millet fodder cultivar Souna 3 (Ministère de l'Agriculture de la République du Niger, 2012). Four strips were set up orthogonally to the *A. gayanus* border in which five subplots of si□ millet hills, that is, 1 m□ with each located respectively 2, □, 10, 20 and 2□ m from the border, along with a subplot of 30 stems in the *A. gayanus* hedge, in the prolongation of each strip. Each millet si□-hill subplot corresponded to appro□imately 30 stems, due to the higher tillering ability of fodder cultivar Souna 3 as compared to grain cultivar ICMV IS 99001. In every strip, all stems in each subplot were dissected at harvest, and numbers of bored stems and stem borer larvae or pupae were recorded as in 2008 and 2009. Data were analy□ed using the □□STAT t-test module (Addinsoft, 2011).

RESULTS

In all three years from 2008 to 2010, the e□tent of bored stems in the *A. gayanus* hedge bordering millet plots was significantly lower than on the millet plants (Table 1). Not a single *C. ignefusalis* larva was recovered in *A. gayanus*, compared to averages of 1.8, 1.6 and 3.1 larvae per millet stem, in 2008, 2009 and 2010 respectively.

All three years, the lepidopteran (pyralid) caterpillars responsible for stem boring on *A. gayanus* could not be reared to adulthood for identification, but, based on the □ey reported by Meijerman and Ulenberg (1996) they were clearly not *C. ignefusalis*.

Furthermore, no trends were noted in the infestation and damage levels on pearl millet according to the distance to the *A. gayanus* hedge, which could have reflected a positive or negative effect of this hedge on the MSB (Table 2).

DISCUSSION

MSB pressure at Sadore during the study period was high, at the same level as that reported in 1996 both at this site with a MSB susceptible pearl millet cultivar (Drame-□aye et al., 2003; Sastawa et al., 2002) and, for damage rate, as that reported from farmers' fields (on supposedly local pearl millet cultivars) in the Upper East Region of Ghana, and for MSB infestation, from the Upper □ est Region (Tan□ubil and Mensah, 2000), and higher than the damage rate observed in 2010 to 2011 at Maiduguri in Northern Nigeria, with a MSB-susceptible pearl millet cultivar (Degri et al., 2014). Pearl millet cultivar Souna 3 infestation and damage by MSB observed in 2010 at Sadore were higher and more variable than those observed on the same site on cultivar ICMV 99001 (Ratnadass et al., 2014).

A. gayanus was thus ruled out as a trap crop for MSB management under the conditions of Sadore from 2008 to 2010, contrary to preliminary results reported earlier from Mali (INTS□RMI□, 2008). □n the other hand, the

Table 2. Millet (cultivar Souna 3) stem damage and infestation (means ±standard deviations) by MSB according to the distance from the *Andropogon gayanus* hedge (Sadore, 2010).

Distance from *A. gayanus* hedge	2	5	10	20	25
Bored stems (%)	[illegible]2.9 ±33.4	49.3 ±20.9	43.2 ±27.2	[illegible]8.2 ±24.0	49.9 ±28.3
No. MSB larvae/stem	3.9 ±4.1	3.8 ±1.0	1.6 ±1.7	4.1 ±2.3	3.[illegible] ±2.8

only parameter reported from that earlier study was percent dead hearts, and *A. gayanus* was planted as a perimeter trap crop surrounding millet. In addition, *A. gayanus* was specifically planted for the purpose of the study, while the *A. gayanus* hedge at Sadore had been planted more than 10 years before. Actually, one considers that it requires one year before *A. gayanus* can act as a windbreak (Renard and Vandenbelt, 1990). In addition, both in this study and in that of Mali, Andropogon hedges were much narrower than those reported to actually act as wind breaks (Renard and Vandenbelt, 1991; Bielders et al., 2004), except the study by Michels et al. (1998), which revealed that a 2 m tall to 3.[illegible] m wide windbreak of *A. gayanus* reduced annual soil flux by 6 to [illegible]. Also, differences in the results obtained in Niger as compared to those of Mali could be partly due to varietal differences. Actually, the dominant variety used in the relatively humid Sudanian zones of both Mali and Burkina Faso, where wind erosion is less of a problem, is cultivar *bisquamulatus*. Conversely, the only variety adapted to the Sahelian zone, e.g. that of Sadore in Niger, where wind erosion is a crucial problem, is cultivar *tridentatus* (Kaboré-Zoungrana et al., 1994).

In our study, the Andropogon hedge was not ideally planted, neither for wind erosion alleviation purposes, nor for pest regulation purposes via trap cropping effect, since it was displayed parallel to dominating wind direction. There was no "control" millet field (without Andropogon border) either, for comparison purposes. However, the fact that not a single MSB larva was recovered from the Andropogon border, and that there was no effect/trend of the distance from this border on MSB infestation and damage across the millet field (unlike what was found in the case of sugarcane with an Erianthus border Nibouche et al., 2012), are sufficient to reject a trap cropping potential of *A. gayanus* vis-à-vis *C. ignefusalis*.

The previously reported stem borer reduction from Mali could also have been due to a barrier effect, since *A. gayanus* surrounded millet plots, or to top-down control by natural enemies sheltered in the *A. gayanus* hedge. Regarding the bottom-up trap cropping effect, further to the role of attractant volatile organic compounds (Khan et al., 2010), it is interesting to note that the most emblematic cases of successful use of trap plants for stem borer control pertain to Napier grass (*Pennisetum purpureum*) and vetiver grass (*Vetiveria zizianoides*) for *Chilo partellus* management on maize (Khan et al., 2006; Van den Berg et al., 2006), while the results are less clear-cut for *Busseola fusca* (Khan et al., 2007; Van den Berg et al., 2006). The two maize stem borer species could be distinguished by the fact that *C. partellus* females lay their eggs on the leaf surface, while those of *B. fusca* insert them under the leaf sheath, like those of *C. ignefusalis* which seldom deposits them on the leaf surface (Youm and Gilstrap, 1994). This could explain why, based on this behavioral trait, *C. partellus* is a better candidate than either *B. fusca* or *C. ignefusalis* for regulation via trap cropping. Also, while *C. partellus* is indeed a polyphagous species, both *B. fusca* and *C. ignefusalis* are rather oligophagous (Le Ru et al., 2006; Youm et al., 1996).

The results reported here highlight the importance of documenting the impact of agroecological management options on all factors likely to influence crop yields, in both the short and long run, including soil erosion and pest damage. However, they do not question the advantage of *A. gayanus* borders for anti-erosion control, and for protecting pearl millet seedlings from sand and wind damage. Furthermore, *A. gayanus* has some value as a fodder (Kaboré-Zoungrana et al., 1994), and it could be partly used as such without affecting its anti-wind erosion function. Moreover, although erosion alleviation and pest regulation services were not found to be synergistic in this case, at least they were not found to be antagonistic, unlike in the case of anti-erosion techniques based on stem residue management (Ratnadass et al., 2014).

Conflict of Interest

The authors have not declared any conflict of interest.

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Physical and chemical characteristics and instrumental color parameters of passion fruit (*Passiflora edulis* Sims)

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The aim of this study was to evaluate the physical and chemical characteristics and variations of instrumental color parameters of passion fruit (*Passiflora edulis* Sims) at different maturation stages. Fruits were harvested directly from passion fruit plants, selected according to maturation stage and distributed into three distinct groups: Fruits with 1/3 yellow peel (stage 1); fruits with 2/3 yellow peel (stage 2) and yellow fruits (stage 3). Physical characteristics (length, equatorial diameter, length / equatorial diameter ratio, volume, weight and peel thickness), aryl, peel, pulp and seed yield, maturation index and physicochemical characteristics (pH, total soluble solids (TSS), titratable acidity and vitamin C) were evaluated. Instrumental color parameters (L *, a *, b *, chroma and H °) of peel and pulp were evaluated for passion fruits at the three maturation stages. The results were analyzed using a completely randomized design. Passion fruits showed high peel yield. Parameters pH, titratable acidity and TSS of passion fruit pulp were consistent with minimum values established by Brazilian legislation. Peel and pulp lightness were inversely proportional and pulp decreased lightness with increasing maturation stages. Color parameters could be used to assess color changes during maturation of passion fruit with more suitable results for maturation stages 2 and 3.

Key words: Passiflo*raceae*, chroma, maturation stage, yield.

INTRODUCTION

The passion fruit (*Passiflora edulis* Sims) are produced in all Bra□ilian states, the Northeast□s largest producer, followed by the Southeast, North and South of the country, such production e□tends worldwide (Silva et al., 200□). Thus, the passion fruit has significant social and economic importance in Bra□il. It is a versatile fruit that can be mar□eted in different forms, such as raw fruits, fro□en pulps, juices, jams, yogurts, mil□ drin□s and ice cream.

The e□otic and distinct aroma of passion fruit is a

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Figure 1. Passion fruits (*Passiflora edulis* Sims) at different maturation stages (Stages 1, 2 and 3).

determining factor for the mar□eting of it. This fruit present with rounded shape, diameter between 4 and 6 cm and e□ternal appearance of green color. The interior of the fruit is characteri□ed by the presence of seeds surrounded by yellow s□uash with gelatinous consistency bittersweet taste and intense flavor (Jim□ne□ et al., 2011; □□pe□-Vargas et al., 2003).

The visual characteristics of passion fruits are e□tremely important for mar□eting (Abreu et al., 2009). Fruits of commercial □uality are those that meet e□pectations of different customer segments, both in e□ternal (brightness and firmness) and internal characteristics (flavor, soluble solids and acidity), and for industriali□ation, juice yield (Cavichioli et al., 2011).

Silva et al. (200□) reported that there is little information on growth, development, fruit ripening passion causing delay in the shelf life of this product. Criteria to assess the harvest of the fruit were evaluated by Marchi et al. (2000) providing better □uality industrial products to the passion fruit base.

The □nowledge of the physical characteristics of passion fruits at different maturation stages assists in defining the harvest point without the use of destructive methods and contributes for the performance of selection criteria in the field. Thus, management and crop productivity are improved (Alves et al., 2012).

□n the above, the aim of this study was to evaluate the physical and chemical characteristics and variations of instrumental color parameters of passion fruit (*Passiflora edulis* Sims) at different maturation stages.

MATERIALS AND METHODS

Passion fruits were obtained from a farm located in the municipality of Santa Helena de Goi□s, G□, Bra□il, with □2□ m of altitude (17□ 37□□8□S and □0□33□20□□) in months from April to June 2013. Fruits were classified as *P. edulis* Sims because it is a mi□ed culture with predominantly yellow cultivar. The municipality of Santa Helena de Goi□s has climate that fits into Aw of K□ppen. □ ith gently rolling topography and prevailing cerrado vegetation. The soil has eroded and is rated □ □isoil clayey.

The crop had drip irrigation, with seedling renewal of two years, four rows of 200 m in length, 1.80 m in height and spacing of 2.□0 m between rows. Flowering pollination was manually performed every day in the afternoon.

Harvest was done with passion fruits fallen to the ground or still on the branches of plants, pulling away easily from the stal□, without injuries or wrin□led bar□. Then, passion fruits were sent to the □aborat□rio de Frutas e Hortali□as, Setor de Engenharia de Alimentos, Instituto Federal de Educa□□o, Ci□ncia e Tecnologia de Goi□s, Rio Verde Campus, G□, Bra□il, for e□periments.

Fruits were selected according to maturation stage and distributed into three distinct groups□Fruits with 1/3 yellow peel (stage 1, n □ 31); fruits with 2/3 yellow peel (stage 2, n □ 30) and yellow fruits (stage 3, n □30) (Figure 1).

To assess the physical measurements of passion fruits, dirt was removed from fruits with paper towels. □ength (mm) and e□uatorial diameter (mm) was measured using digital caliper. The length /diameter e□uatorial ratio was determined by dividing the length by the diameter. Volume was determined by immersing the fruit in graduated polypropylene jar with distilled water, recording the volume (ml) of li□uid displaced.

Fruit weight was determined by weighing on analytical scale with accuracy of three decimal places, and the results were e□pressed in grams (g). Subse□uently, passion fruits were saniti□ed with chlorine solution at 100 ppm / 10 min and dried at room temperature. Fruits were aseptically cut in half (Figure 2), separating peels, aryls with seeds and pulp. Pulps with seeds were submitted to pulping using industrial fruit depulper. Then, aryl, peel, pulp and seed weight was determined, with results e□pressed in grams (g) to determine the yield of these variables in relation to the weight of passion fruits, whose results were e□pressed in percentage (□). To estimate the aryl, peel, pulp and seed yield, peel thic□ness (mm) was measured using a digital caliper.

Parameters pH, total soluble solids (□Bri□), total titratable acidity (□) and Vitamin C (mg ascorbic acid.100g^{-1}) according to A□AC (200□) were evaluated. Maturation inde□ was obtained by dividing total soluble solids by total titratable acidity.

To evaluate the peel color of passion fruits at maturation Stages 1, 2 and 3, fruits were divided into two hemispheres towards the e□uatorial diameter and each hemisphere was divided into four □uadrants (Figure 3) for the evaluation of three distinct points per □uadrant, totaling 24 measurements per fruit.

Passion fruit pulps corresponding to the different maturation stages, after pulping, were stored in polyethylene bags for color assessment with measurement at 12 different points.

Passion fruit peel and pulp color was determined with a colorimeter (Color□uest II, Hunter □ab Reston , Canada). The

Figure 2. Passion fruits at different maturation (Stages 1, 2 and 3).

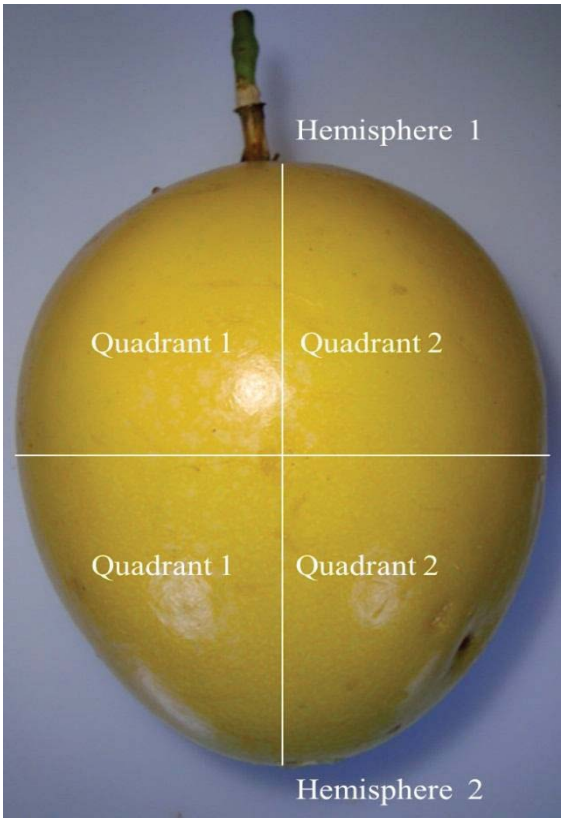

Figure 3. Schematic representation of the division of passion fruit for assessing peel color.

results were e□pressed in □□, a□and b□, with □□values (lightness or brightness) ranging from blac□(0) to white (100), chroma a□values from green (-60) to red (□ 60), and chroma b□from blue (-60) to yellow (60), as reported by Paucar-Menacho et al. (2008). Chroma was calculated using Formula 1, and the Hue Angle (□H) was calculated using Formula 2.

$$C = \sqrt{a^2 + b^2} \quad (1)$$

$$H^\circ = \tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

Statistical evaluation of physical, chemical and color parameters was performed using the SISVAR software (Ferreira, 2011) in a completely randomi□ed design. Means were compared by Tu□ey test at □□ probability.

RESULTS

According to Table 1, it was possible to verify that there is no influence of the maturation stages (p□ 0.0□) on length, e□uatorial diameter, length / e□uatorial diameter ratio, volume, weight, aryl, peel, pulp and seed yield, thic□ness, pH, total soluble solids and vitamin C. The results showed that passion fruits can be harvested at different maturation stages without affecting such physical characteristics.

The lengths of passion fruits with 1/3 yellow peel, 2/3 yellow peel and yellow peel were 82.6□ mm, 88.13 mm and 88.04 mm, respectively. Cavichioli et al. (2011) observed passion fruits averaging 101.0 mm in length, higher than values determined for the different maturation stages of passion fruits in this wor□. However, the length of passion fruits resulted in average value similar to that analy□ed by Alves et al. (2012), with average of 8□3□ mm.

The e□uatorial diameter of passion fruits was 78.37, 79.19 and 79.1□ mm for fruits at maturation stages of 1/3 yellow peel, 2/3 yellow peel, and yellow peel, respectively. Cavichioli et al. (2011) analy□ed passion fruit with diameter of 79.□ mm, similar to value found in this wor□. Dissimilarly, Ara□jo et al. (2008) obtained values lower than those found for fruits analy□ed in this study, 76.64 mm.

Although no significant differences (p□ 0.0□) were observed for the length / e□uatorial diameter ratio, the results demonstrated that passion fruits with 2/3 yellow peel showed larger si□e, followed by 1/3 yellow peel and

Table 1. Mean length (□), e□uatorial diameter (ED), length / e□uatorial diameter ratio (□. ED^{-1}), volume, weight, aryl, peel, pulp and seed yield, peel thic□ness, pH, total soluble solids (TSS), titratable acidity (TA), maturation inde□(MI) and vitamin C values passion fruits (*Passiflora edulis* Sims) at three maturation stages.

Parameter	Peel color			VC (%)
	1/3 yellow (n = 31)	2/3 yellow (n = 30)	Yellow (n = 30)	
Length (mm)	82.65[a]	88.13[a]	88.04[a]	13.07
ED	78.37[a]	79.19[a]	79.15[a]	11.19
L. ED^{-1}	1.06[a]	1.12[a]	1.11[a]	10.35
Volume (ml)	300.58[a]	330.37[a]	328.37[a]	30.99
Weight (g)	182.38[a]	191.00[a]	195.39[a]	32.71
Aryl yield (%)	5.26[a]	4.73[a]	4.56[a]	34.69
Peel yield (%)	43.25[a]	45.62[a]	42.63[a]	19.75
Pulp yield (%)	33.97[a]	33.86[a]	37.05[a]	18.89
Seed yield (%)	17.51[a]	15.78[a]	15.75[a]	26.61
Peel thickness (mm)	5.88[a]	5.84[a]	5.66[a]	23.13
pH	2.65[a]	2.72[a]	2.67[a]	5.38
TSS (°Brix)	14.77[a]	14.81[a]	15.02[a]	13.23
ATT (%)	0.81[a]	0.67[b]	0.63[b]	27.33
MI	19.72[b]	22.36[ab]	24.69[a]	22.43
Vitamin C (mg ascorbic acid.100 g^{-1})	25.98[a]	24.22[a]	26.55[a]	27.70

Different lowercase letters in line differ significantly by the Tukey test at 5% probability.

yellow fruits. This fact was confirmed with passion fruit volumes, which obtained the same sequence of results, fruits with 2/3 yellow peel with higher volume and fruits with 1/3 yellow peel and yellow fruits with lower volume.

Passion fruits evaluated in this study showed lower weight (182.38, 191.00 and 195.39 g, at maturation stages 1, 2 and 3, respectively). Cavichioli et al. (2011), reported a mean weight of 218.44 g. Values close to those found in this study were observed by Alves et al. (2012), with mean weight of 194.83 g for ripe passion fruits.

The aryl yield of passion fruits was 5.26, 4.73 and 4.56% at maturation stages 1, 2 and 3. A different result was obtained by Oliveira et al. (2011), who observed that the aryl development occurs concomitantly with the fruit development, and ripe fruits had an average of 9.39% aryl yield, whereas for fruits at intermediate maturation stage, aryl yield was 8.30%.

Passion fruit peel showed yield of 43.25, 45.62 and 42.63% for 1/3 yellow peel, 2/3 yellow peel and yellow fruits, respectively. These values were lower than those found by Coelho et al. (2011) (53.00%) and Reolon et al. (2009), with average of 69.40, 73.70 and 63.30% for green, yellow-green and yellow fruits. However, aimed at the highest peel yield (52.07%), results corroborate those by Ferreira et al. (2010), who reported that peel thickness is a relevant factor for fruit classification, being inversely proportional to juice yield.

The pulp yield was lower than that observed by Farias et al. (2007), with average of 44.43%. Values similar to those of this study were reported by Silva et al. (2008), with average values of 31.44 to 41.28% of pulp yield. Santos et al. (2009) reported that the quantification of juice mass per seed had the disadvantage of adhering juice in the sieve used in the extraction, causing measurement errors. The lower pulp yield observed in this study may be related to the maturation stage of fruits, because harvest was performed with fruits at different maturation stages, which can be observed by the high proportion of passion fruit peel.

In this study, seed yield was higher than that observed by Oliveira et al. (2011), who found that the proportion of total residue was higher in ripe fruits with smaller size and seed yield was lower for larger fruits, but did not vary with shape, with average of 4.23% of seeds. Coelho et al. (2011) found that the amount of seeds with aryls was not influenced by fruit size, and fruits with averages of 251.54 and 123.26 g, resulted in 11.5% yield of this residue.

Passion fruit peel thickness was 5.88 mm for fruits with 1/3 yellow peel, 5.84 mm for fruits with 2/3 yellow peel and 5.66 mm for yellow fruits. Values lower than those found in this work were observed by Santos et al. (2009), with average peel thickness between 3.24 and 3.54 mm. However, Freire et al. (2010) and Cavalcante et al. (2007) evaluated average peel thickness values of 7.11 and 6.0 to 7.0 mm, respectively, which were higher than those found in this study.

The pH of the passion fruit was 2.65, 2.72 and 2.67 for fruits with 1/3 yellow peel, 2/3 yellow peel and yellow fruits, respectively. Medeiros et al. (2009) reported values higher than those found in this study, between 3.11 and 3.17, as well as Uchoa et al. (2008) 4.17. However,

Table 2. Mean L, a □, b □, Chroma, and Hue Angle (H °) values of passion fruit peel(*Passifloraedulis* Sims) at three maturation stages.

Parameters	Peel color			VC (%)
	1/3 yellow peel (n = 240)	2/3 yellow peel (n = 240)	Yellow peel (n = 240)	
L	49.64[c]	55.80[b]	65.09[a]	9.69
a□	-5.91[c]	-2.12[b]	0.83[a]	-136.33
b□	30.76[c]	44.67[a]	39.86[b]	18.70
Chroma (C□)	31.40[c]	44.91[a]	40.06[b]	18.17
H°	-1.37[c]	-0.64[b]	0.53[a]	-229.29

Different lowercase letters in the line differ significantly by the Tukey test at 5% probability.

Silva et al. (2005) found values similar to those of this study, ranging from 2.5 to 2.7.

The total soluble solids content was consistent with the results of Silva et al. (2005), who reported that during the maturation of passion fruits, there is an increased amount of TSS, with values ranging from 10.2°Brix to 16.8°Brix. However, in the mature stage, Uchoa et al. (2008) reported values of 20.56 °Brix for passion fruit pulp.

t was observed that titratable acidity was higher in green fruits, 0.81%, Silva et al. (2005) showed the values for titrable acidity of the 4.99 to 5.53 %□Pinheiro et al. (2006) found the range values 0.45-1.26 g.100g^{-1} citric acid□when compared to fruits with advanced maturation stage, 2/3 yellow, 0.67% and yellow,0.63%.

Passion fruit analyzed by Raimundo et al. (2009) and Cavichioli et al. (2011) showed much lower values for the TSS / TTA ratio, varying between 3.07 and 4.40 and 3.4, respectively. The difference in these values can be associated with the maturation stage of fruits and the mode of preparation of passion fruit pulp samples.

Passion fruits showed Vitamin C contents of 25.98 mg of ascorbic acid.100 g $^{-1}$ for fruits with 1/3 yellow peel, 24.22 mg of ascorbic acid.100 g $^{-1}$ for fruits with 2/3 yellow peel and 26.55 mg ascorbic acid.100 g $^{-1}$ for yellow fruits. Farias et al. (2007) found higher vitamin C values, 38 mg ascorbic acid.100g $^{-1}$ for. Uchoa et al. (2008) found values lower than those obtained in this work, 11.76 mg ascorbic acid.100 g $^{-1}$.

In the study by Farias et al. (2007), the average total soluble solids content was 11.02°Brix, titratable acidity of 5.28%,and vitamin C of 35.77 mg 100 g^{-1}, which is higher than the average value observed in this study.

The results of the physicochemical analyses are within Standards of Identity and □uality (SI□) for passion fruit pulp with minimum acidity level of 2.5 g of citric acid /100 g, pH between 2.7 and 3.8 and minimum total soluble solids content of 11° Brix (Brasil, 2000).

Maturation stages of passion fruit peel significantly influenced (p □0.05) the L□, a□, b□, Chroma and H ° results (Table 2). Lightness (L) was higher in fruits with yellow peel, followed by fruits with 2/3 and 1/3 of yellow peel. The average a□values indicated decreased green color with increasing maturation stage of passion fruits, which was expected□however, the highest intensity of yellow color (b□) was verified in fruits with 2/3 yellow peel, followed by yellow fruits and fruits with 1/3 yellow peel.

Coelho et al. (2011) found that lightness and parameter b□of passion fruit peel occur progressively and linearly with fruit maturation. The evolution of lightness was observed in this study□however, the development of parameter b□does not occur homogeneously and had lower values for yellow fruit compared to fruits with 2/3 yellow peel. According to Silva et al. (2008), the yellow color distribution on the entire fruit surface occurs irregularly from the base to the peduncle and defines the upper region near the peduncle to characterize the typical coloring of each maturation stage of the fruit, a fact that explains the difference in the values of variable b□ reported in this work, which were presented as an average of several points of the fruit. Vianna- Silva et al. (2008) reported that greater sun exposure on the fruit top causes differences in parameters b□and a□

The chroma values were higher than 31.40, which indicated that the amount of pigment in passion fruit peel is considerable. However, it was observed that yellow fruits showed lower chroma values than fruits with 2/3 yellow peel. This difference can be explained by the decomposition of pigments during maturation. In addition, color is completely saturated when the Hue Angle indicates no presence of white and black coloring (Barbosa, 2010).

According to CIELAB, the Hue angle (Mcguire, 1992) defines red color as 0°, yellow as 90° green as 180° as blue as 270°, and data confirmed that passion fruits had red-yellowish color according to the maturation stage.

Instrumental parameters pulp color of passion fruits are shown in Table 3. The L□values of passion fruit pulp decreased (p □0.05) with increasing maturation stage of fruits, indicating greater lightness in fruits with 1/3 green peel. For the chromaticity coordinate a□, fruits with 1/3 yellow peel showed mean values similar to yellow fruits, and greater than fruits with 2/3 yellow peel. The yellow color intensity of pulps was higher in the intermediate maturation stage, when compared to other maturation stages.

Fruits with 2/3 yellow peel also showed more intense

Table 3. Mean L, a □, b □, Chroma, and Hue Angle (H °) values of passion fruit pulp (*Passiflora edulis* Sims) at the three maturation stages.

Parameters	Peel color			VC (%)
	1/3 yellow peel (n = 600)	2/3 yellow peel (n = 432)	Yellow peel (n = 584)	
L□	34.78[a]	33.88[b]	33.07[c]	10.43
a□	9.11[a]	8.42[b]	8.71[ab]	45.06
b□	19.93[b]	28.99[a]	19.24[b]	46.30
Chroma (C□)	22.35[b]	30.60[a]	21.52[b]	41.34
H°	1.11[b]	1.25[a]	1.07[c]	17.97

Different lowercase letters in the line differ significantly by the Tukey test at 5% probability.

pulp color, and for yellow fruits and fruits with 1/3 yellow peel, the results were similar (p□ 0.05). Medeiros et al. (2009) also found predominance of yellow color for passion fruit pulp.

Chroma values of passion fruits were 22.35 for fruits with 1/3 yellow peel and 21.52 for yellow fruits, with significant difference (p □0.05) from fruits with 2/3 yellow peel, with an average of 30.60. Flores et al. (2011) observed chroma values between 13.67 and 27.85 for passion fruit pulp. Therefore, fruits with 2/3 yellow peel showed higher amounts of pigment than yellow fruits, which is due to the loss of pigment with fruit maturation.

The H ° results indicate greater average value for fruits with 2/3 yellow peel, followed by fruits with 2/3 yellow peel and yellow fruits and in general, the values obtained indicate pulp color ranging from yellow to red with greater H ° intensity for fruits at intermediate maturation stage.

DISCUSSION

□egreiros et al. (2007) found that the selection of fruits with larger equatorial diameter allowed obtaining heavier fruits with higher pulp yield. For Morgado et al. (2010), the selection of heavy passion fruits can be made directly in the field by measuring fruit length. However, Oliveira et al. (2011) reported that the juice yield increased with the maturation stage, but was not influenced by shape and size.

According to Reolon et al. (2009) the use of passion fruit peel as raw material with functional properties becomes relevant when fruits are harvested with green peel or starting yellowing without major damage to juice yield. The use of passion fruit peel in food products contributes to increase their fiber content since passion fruit peel is rich in soluble fiber, with large amounts of pectin, which is beneficial to the organism (□uertzenstein and Sabaa-Srur, 1999□□apo and □offi, 2006).

An alternative for food industries is the processing of passion fruit peel for use in food formulations, due to the high proportion of peel with high nutritional properties. Byproducts obtained from tropical fruits contain high levels of bioactive compounds (vitamins, minerals, polyphenolic antioxidants and dietary fiber), which may have positive health effects and contribute for the prevention of cardiovascular diseases, cancer and diabetes (Ayala-□avala et al., 2011□Viuda-Martos et al., 2010). Passion fruit seeds are of pharmacological and nutritional interest because they are rich in polyunsaturated fatty acids such as ω-3 and ω-6 (□eraik et al., 2010). Cavichioli et al. (2008) reported that passion fruits with thinner peel have greater amount of pulp, which is interesting for the food industry.

Chitarra and Chitarra (2005) reported that acidity decreases in fruits at final maturation stage and beginning of the senescence stage due to the use of organic acids in biochemical processes of respiration. The maturation index increased with increasing yellow color in the peel of passion fruits and showed significant differences among them (p □0.05). Based on results, passion fruits with 2/3 yellow peel are less acidic and have higher maturation stage.

□im□nez et al. (2011) reported that passion fruit undergo an increase in pH, soluble solids content and a decrease in titratable acidity during ripening. According to those authors, the increase in soluble solids content is a result of starch hydrolysis to sugars that relates to the Carbohydrates in fruit ripening. Since the acidity relates to the use of organic acids during respiration of the fruit (□im□nez et al., 2011).

However, even with fruit ripening passion fruit, Oliveira et al. (2014) state that this is a result which is characterized by high acidity. Also related to the acid content in fruits, Silva et al. (2005) state that the ratio of soluble solids content with the titratable acid content is interesting to analyze the maturation index and during ripening such content tends to increase as a result of the fall of acidity.

According to Azzolini et al. (2004) and □ongatti □eto et al. (1996), the maturation index can be analyzed by the TSS values of fruits, which indicates the presence of substances dissolved in the pulp, with prevalence of sugars. However, for Manfroi et al. (2004), the maturation index should be analyzed with caution, since decrease of acidity and increase of sugars do not always occur in equal proportions.

By combining the pulp color and titratable acidity results, passion fruits would be able to be harvested and consumed after reaching maturation stages 2 and 3, which according to Coelho et al. (2010) would be the optimal point to harvest passion fruits of the winter harvest when fruits present 30.7% of peel surface with yellowish coloration and juice quality parameters suitable for consumption in the fresh form, thus corroborating with the present study.

Conclusion

The results indicated that passion fruits presented high peel yield, regardless of maturation stage, but fruits with lower titratable acidity must be harvested at more advanced maturation stages, which would be more suitable for industrial processing. The pH, titratable acidity and total soluble solids of passion fruit pulp were consistent with the minimum values established by Brazilian legislation. Lightness of peel and pulp were inversely proportional, and with increased maturation stages, pulp decreased lightness. Color parameters can be used to evaluate peel and pulp color of passion fruits with more suitable results for maturation stages 2 and 3.

Conflict of Interest

The authors have not declared any conflict of interest.

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Prevalence of major ectoparasites of calves and associated risk factors in and around Bishoftu town

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A cross sectional study was carried out from October 2013 to March 2014 in and around Bishoftu town with the objectives of determining the prevalence of major external parasite species of calves, preferred predilection sites and determine the associated risk factors. The calves were selected randomly from small holder farms in and around the town. According to this study; out of 384 calves observationally examined, and identified using stereomicroscope/hand lenses, 126(32.8%) were found infested with one or more ectoparasites species. The major ectoparasites identified were lice 26.8% followed by ticks 13.5% and mites 0.5%. Overall, three genera and one subgenus of tick comprising five species and one species of lice and one species of mite were identified in the study. Among the ticks, *Rhipicephalus* (*Boophilus) decoloratus* (7.6%), *Amblyomma variegatum* (5.2%) and *Rhipicephalus evertsi evertsi* (2.9%) were the most prevalent and the least identified tick species was *Rhipicephalus pulchelus* (0.5%). In this study, *Linognathus vituli* was the solitary species of lice identified and the prevalent (26.8%) among all ectoparasite identified. With regard to mites, *Psoroptus ovis* was the only mite species identified with a prevalence of 0.5%. The difference in the prevalence of ectoparasite species in male and female calves was not statistically significant (p>*0.05*). There was significant (p<0.05) association between *L. vituli* infestation and breed, management system, house cleanness and milk feeding practice. The association between tick prevalence and sex, breed, management system, housing condition, house cleanness, and milk feeding practice of calf was no statistically significant (p>0.05). This study demonstrates that ectoparasites are among the most important health constraints of calves in and around Bishoftu leading to important economic losses and attention should be given to control interventions, developing the knowledge of the farmers and further study on the burden of ectoparasites and their effects on calves was recommended.

Key words: Bishoftu, calf, ectoparasite, prevalence, small holder farms.

INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to play great role in the economic development of the country and in the sampling procedure□Formula□1.96^2 (square). There are about 53.4 million cattle, 48.28 million small ruminants, 1.1 million camel, 8.58 million equines and 49.3 million

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poultry in the country. Out of this total cattle population, the calf under six months constitute about 9.27% (CSA, 2011). Ethiopia's economy is dominated by agriculture and cattle play a vital role in agricultural production. In addition to the products of meat and milk, cattle provide draught power for cultivation of the agricultural lands of many peasants. Skins and hides are also important components of the livestock sector in generating foreign currency (Tessema and Zeshaw, 2011). However, the poor health and productivity of animals due to disease remained the major constraint to the significant contribution of livestock industry to the economy (Mokennen et al., 2001). Though, the low productivity of livestock may also be attributed to the low genetic potential of indigenous cattle, poor nutrition and inadequate management, livestock disease have numerous known influences on productivity and fertility of herds including losses due to mortality and morbidity, loss of weight, slow growth rate, poor fertility performance and decrease physical power (Abraha, 2007; Baker, 2007). The productivity of cattle depends largely on their reproductive performance and the survival of calves (Amuamuta et al., 2006). Among other things, the potential of any animal production system depends, on the successful program of raising calves for replacement. Under most conditions, the average length of time a cow stays in a milking herd is about four years and, therefore, 25% of the milking herd must be replaced each year (Bath et al., 1985). But, several infectious and non-infectious disease, environmental and managemental factors affect the health of calves which have a significant effect on replacement of the herd. Some of the main problems affecting cattle production are parasite infestations, particularly ectoparasites, which are responsible for important losses in the productive indices as a result of direct and indirect effect on their hosts (Callegos and Rodríguez, 2011; Onu and Shiferaw, 2013). In tropical regions, where ambient temperature and solar radiation are high, forage production fluctuates seasonally and parasitic diseases are abundant, parasitism poses a serious threat to the development and utilization of animal resource. Ectoparasites commonly ticks, mites and lice affect the host by inflammation and through the damage they inflict on the skin and on the host physiology. They are also very important in different disease transmissions such as parasitic, bacterial, rickettsial, and viral diseases to man and animals. They also serve as vectors or intermediate hosts for some parasite species (Baker, 2007; Taylor et al., 2007). The damages inflicted by ectoparasites are annoyance, stress, or blood loss. Lice and tick worry are recognized conditions that reduce feed efficiency and weight gains in livestock (Baker, 2007). In Ethiopia, ectoparasites in ruminant causes a serious economic loss to small holder farmers, the tanning industry and the country as a whole through mortality of the animals, decreased production, down grading and rejection of skin and hide (Tiki and Addis, 2011). Calves have poorly developed defense mechanism and hence they are highly susceptible to diseases (Demberu, 2004). Ectoparasites affect calves via reducing feed efficiency and weight gain, retarding their growth and causing death. They also predispose the calves to several diseases through skin damaging, immune-suppression and transmission of various diseases. For institution an appropriate intervention, animal production development requires a sound knowledge of the magnitude, the cause and predisposing factors of disease. Any producer should be aware of how parasites grow, reproduce and pass from one animal to other animals. This allows producers to develop management practices that minimize the spread of external parasites and the disease they transmit. Information on the prevalence of ectoparasites of calves facilitates the development of sustainable control strategies that enable farmers to reduce the burden of these parasites on their stock productivity. The existence of various ectoparasites and skin disease affecting cattle are frequently reported from different parts of Ethiopia. Bishoftu town is one of the urban centers where market oriented smallholder farms are flourishing rapidly. A number of dairy farms have been established in the last five years (Tassaye, 2002).

As a result information on the external parasites of calf is required. However, limited efforts have been made so far to investigate the status of ectoparasites of calves in the area. Therefore, the objectives of this study were to determine the prevalence of major ectoparasites of calves, and associated risk factors and identify the composition, and site preference of external parasites in the body of the calves.

MATERIALS AND METHODS

Study area

The study was carried out in and around Bishoftu town. This town is located in Ada'a woreda of East shoa zone of Oromia regional states on southeast of Addis Abeba at distance of 47 km. Bishoftu lies between 9° N latitude and 39° E longitude and an altitude of 1860 m above sea level. It gets an annual rainfall of 871 mm of which 80% is received during long rainy season starting from June to September and the remaining in short rainy season extending from March to May, and the dry season from October to February. The mean annual maximum and minimum temperature are 26 and 14°, respectively with a minimum relative humidity of 63.8% (NMSA, 2013).

Study animals

The study animals were calves below six months of age of both local and cross breed kept under different management systems owned by small holder farms in and around Bishoftu town.

Study design

The study was a cross sectional study and extended over six months from October to March. Within this period, the samples

were collected from sampling units that is, all calves designed as a representative of the study population. The calves belonging to a single farm was visited only once during the study period to collect samples and record animal level data.

Sampling procedure and sample size

The numbers of calves owned by small holders were first registered at milk marketing post where the owners submit their milk to create sampling frame and sampling units were selected by lottery system from sampling frame until required number of calves were incorporated. The required sample size were determined by assuming the expected prevalence of 50% external parasites infestation of calves using 95% confidence interval and at 5% absolute precision according to Thrustfield (2007).

$$n = \frac{1.962 pexp\ (1-pexp)}{d^2}$$

Where, n required sample size, pexp expected prevalence, d desired absolute precision. Because there was no previous study conducted in the area in the same age group in the area, 50% expected prevalence and 95% confidence level and 5% precision was considered. By substituting all the values, a total of 384 calves were considered for this study.

Data collection

Collection of animal level data

Information on host determinants like age, sex, breed and other relevant data such as management system, housing condition and cleanness were collected by interviewing owners and observing the animals and the farms. Breeds of calf were determined by physical characteristics of respective breeds and asking the owners. The management system classified as extensive, semi-intensive or intensive by asking the owners and observing the farms. The housing condition classified as separate or common barns with calves mixed with the dam or other animals. The cleanness of the farm was assessed by interrogating the farmers about the cleaning frequency and personal observation.

Collection and preservation of samples

To collect samples, first the calves were properly restrained and examined by close visual inspection, palpation and parting the hairs against their natural direction for easy observation of ticks and lice and any damage on the skin. Tick and lice were collected from different body parts of calf by hand picking. The whole half of the body was examined with special emphasis given to body parts known to be commonly infested such as head including ear, neck, dewlap, posterior back and rump, ventral body part (scrotum, axillae, belly and groins) and under the upper part of the tail and the tip of the tail. Samples from different body parts were collected in different labeled vials containing 70% alcohol and transported to the laboratory for morphological study. Samples for mites were taken by deep skin scrapping from the edge of the active lesion. The skin were scraped until blood oozes using a clean scalpel blade soaked in water putting on a sheet of paper under a site being scraped to avoid the loss of mites. The collected sample was transferred to a vial containing 10% formalin as a preservative and processed according to the method described by Urquhart et al. (1996). The collected samples were examined by stereomicroscope and identification was performed according to the identification key given by Walker et al. (2013) for tick and Urquhart et al. (1996) and Wall and shearer (2001) for lice and mites.

Data analysis

All data were collected and recorded in a sheet of paper and transferred to Microsoft excel spread sheet and encoded in SPSS version 20 statistical software and analyzed. The Chi-square (x^2) test was used to assess the differences in the prevalence of ectoparasites among sex, breed, management system and housing condition. In all cases, 95% of confidence intervals and p 0.05 were set for significance.

RESULTS

Overall prevalence of ectoparasites

Out of the three hundreds and eighty four calves examined in this study, 126(32.8%) were found infested with one or more ectoparasite species. The major ectoparasites identified were lice, 26.8% followed by ticks 13.5% and mites 0.5% (Figure 1).

Overall three genera and one sub-genus of ticks (*Amblyomma, Hyalomma, Rhipicephalus* and *Boophilus*) comprising five species and one genus of louse (*Linognathus*) and one species of mite were identified in this study. Among the ticks, *Rhipicephalus* (*Boophilus) decoloratus* (7.6%), *Amblyoma variegatum* (5.2%) and *Rhipicephalus evertsi evertsi* (2.9%) were the most prevalent and the least identified tick species was *Rhipicephalus pulchelus* (0.5%). In this study, *Linognathus vituli* was the solitary species of lice identified and its prevalent was (26.8%) among all ectoparasite identified. With regard to mites, *Psoroptes ovis* was the only mite species identified with a prevalence of 0.5% (Figure 2).

Prevalence of ectoparasite by sex and milk feeding practice

In present study, the prevalence of ectoparasite species in male and females calves were found to be (33.33%) and (32.40%), however, it was not statistically significant (p>0.05). In both sexes *L. vituli*, *Rhipicephalus (Boophilus) decoloratus* and *A. variegatum* were the dominant ectoparasites. High prevalence of *L. vituli* was recorded in calves kept in farms with suckling feeding practice (33.47%) than bucket feeding practice (14.28%) and it was statistically significant (p 0.05) while the other ectoparasite species were found statistically insignificant (p 0.05) (Table 1).

Prevalence of ectoparasites in breeds and housing conditions

In this study in most cases, there was no statistically significant (p 0.05) association between breeds and

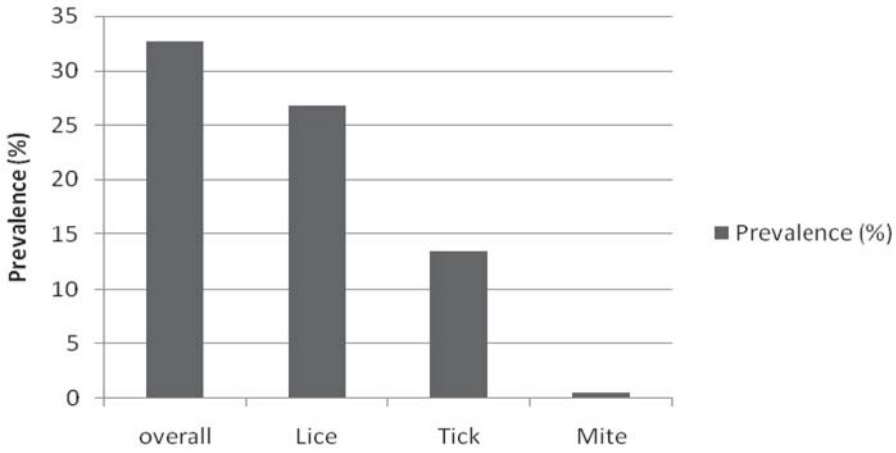

Figure 1. Overall prevalence of ectoparasite.

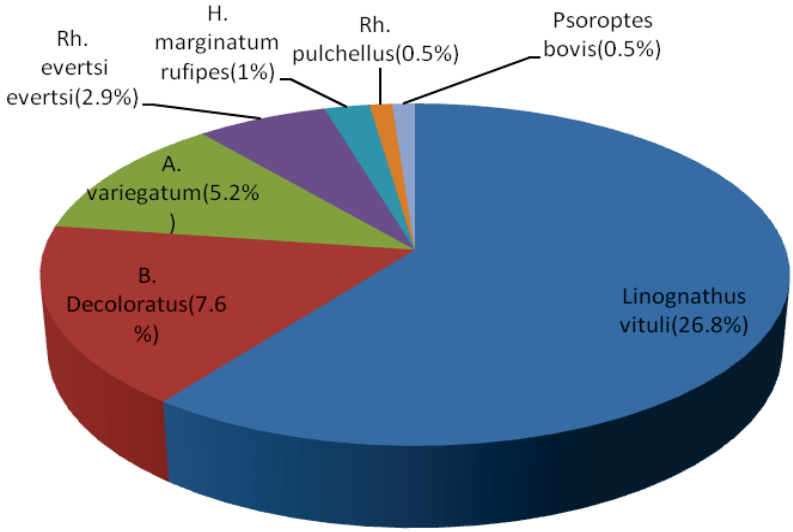

Figure 2. Prevalence of ectoparasite species.

ectoparasite species. However, there was except to *L. vituli*, which was most frequently recorded in local breeds (32.31%) than cross breeds (18.70%) of calves and was statistically significantly (p□0.05). The prevalence of ectoparasites was 33.97 and 31.42% in calves share common housing with dam and in calves kept alone but it was not statistically significant (p□0.05) (Table 2).

Prevalence of ectoparasites in management system and house cleanness

The prevalence of *L. vituli* higher in calves kept under extensive (37.41%) than those kept in semi intensive (29.41%) and intensive (15.43%) management system. This was statistically significant (p□0.05). Similarly, the prevalence was also statistically significant (p□0.05) on calves kept in unclean house (52%) than those kept in clean house (15%) while the infestation of other ectoparasite species were not statistically significant (p□0.05) in both management and house cleanness (Table 3).

Predilection site of ectoparasite

A total of 211 adult ticks and 986 lice were collected from different body parts of the calves. Ectoparasites tend to prefer specific site of attachment on the animal body. The common infestation sites of Rhipicephalus (*Boophilus)*

Table 1. Prevalence of ectoparasites species in relation to sex and milk feeding practice of calves.

Ectoparasite species	Sex of calf				Milk feeding practice			
	Male (n=168)	Female (n=216)	X^2	p-value	Sucking (n=251)	Bucket (n=133)	X^2	p-value
A.variegatum	10(6.00)	10(4.60)	0.34	0.56	15(6.00)	5(3.76)	0.87	0.47
B.decoloratus	12(7.14)	17(7.87)	0.07	0.79	24(9.56)	5(3.76)	4.19	0.04
H.mrufipes	1(0.6)	3(1.38)	-	0.64	2(0.6)	2(1.5)	0.42	0.52
R.e.evertsi	4(2.38)	7(3.24)	0.25	0.62	9(3.58)	2(1.5)	1.35	1.35
R.pulchelus	0	2(0.92)	-	0.51	0	2(1.5)	3.79	0.05
Tick overall	25(14.88)	27(12.5)	0.46	0.499	40(15.94)	12(9.02)	3.549	0.060
L.vituli	46(27)	57(26)	0.05	0.83	84(33.47	19(14.28)	16.25	0.00
P. bovis	1(0.6)	1(0.46)	-	1.00	1(0.4)	1(0.71)	0.21	0.65
Over all	56(33.33)	70(32.40)	0.04	0.85	101(40.23)	25(19.55)	18.13	0.00

Table 2. Prevalence of ectoparasite species in different breeds and housing condition.

Ectoparasite species	Breed				Housing condition			
	Local (n=229)	Cross (n=155)	X^2	p-value	Alone (n=175)	Mixed (n=209)	X^2	p-value
A. variegatum	13(5.67)	7(4.52)	0.25	0.62	14(8)	6(2.87)	5.07	0.24
B. decoloratus	20(8.73)	9(5.80)	1.13	0.29	7(4)	22(10.53)	5.81	.016
H.mrufipes	2(.87)	2(1.29)	-	1.00	1(.57)	3(1.44)	-	0.63
R. e.evertsi	9(3.93)	2(1.29)	2.32	0.13	1(0.6)	10(4.78)	6.08	0.01
R. pulchelus	0	2(1.29)	-	0.16	2(1.14)	0	-	0.21
Tick overall	36(15.72)	16(10.32)	2.300	0.129	20(11.42)	32(15.31)	1.226	0.268
L. vituli	74(32.31)	29(18.70)	9.62	0.00	47(26.86)	56(26.79)	0.00	0.98
P. bovis	1(0.43)	1(6.67)	-	1.00	1(.57)	1(.48)	-	1.00
Over all	90(39.30)	36(23.22)	10.84	0.001	55(31.42)	71(33.97)	0.28	0.59

Table 3. Prevalence of ectoparasite species in management system and house cleanness.

Ectoparasite species	Management system					House cleanness			
	Intensive (n=175)	semi intensive (n=34)	Extensive (n=175)	X^2	p-value	Clean (n=257)	Not clean (n=127)	X^2	p-value
A.variegatum	8(4.6)	2(5.88)	10(9.71)	0.27	0.86	13(5.06)	7(5.51)	0.04	0.85
B.decoloratus	8(4.6)	4(11.76)	17(9.71)	4.26	0.12	15(5.84)	14(11)	3.28	0.07
H.marginatum rufipes	3(1.71)	0	1(.57)	1.50	0.47	2(.8)	2(2)	0.52	0.47
R.e.evertsi	5(2.86)	1(2.94)	5(2.86)	0.001	1.00	9(3.50)	2(1.58)	1.13	0.29
R.pulchelus	1(0.6)	0	1(0.6)	0.19	0.91	1(.4)	1(.8)	0.26	0.61
Tick overall	18(10.29)	5(14.70)	29(16.57)	2.99	0.224	28(10.89)	24(18.89)	4.649	0.031
L.vituli	27(15.43)	10(29.41)	66(37.41)	22.27	0.00	37(14)	66(52)	61.13	0.00
P.bovis	1(0.6)	0	1(0.6)	0.19	0.91	2(0.78)	0	0.99	0.32
Over all	36(20.57)	11(32.35)	79(45.14)	23.97	0.00	57(22.18)	69(54.33)	39.86	0.00

decoloratus were dewlap and neck. *A. variegatum* and *Hyalomma marginatum rufipes* were most frequently observed on ventral body parts of the animals. The favorable predilection sites for *R. evertsi evertsi* were under tail and ventral body parts. Even though it was observed in head, ear, back, rump and base tail in heavy

Table 4. Distribution tick species in different body region of calves.

Attachment site	A. *variegatum* (%)	*B. decoloratus* (%)	*Rh. evertsi evertsi* (%)	*Rh. Pulchellus* (%)	*H. marginatum rufipes* (%)
Head and hear	-	6(6.59)	1(3.13)	-	-
Dewlap	12(24)	57(62.64)	1(3.13)	4(100)	-
□eck	-	26(28.57)	2(6.25)	-	-
Back and rump	-	-	-	-	-
Ventral body parts	36(72)	2(2.2)	18(56.25)	-	24(70.58)
Base and tip of tail	2(4)	-	10(31.25)	-	10(29.41)
Total count	50	91	32	4	34

infestation, the common infestation site for *L. vituli* were dewlap, neck and ventral body parts (Table 4).

DISCUSSION

Ectoparasites are the prevalent diseases of calves in study area and it may causes high economic losses in the area. Several factors such as absences of improved husbandry practices, inadequate utilization of veterinary services and poor awareness of the owners on the effects of ectoparasites on calves have high contribution for wide occurrence of infestation ectoparasites of calves. In the present study, an overall ectoparasites prevalence of 32.8% was found in the study area. This result was higher than □acob et al. *(*2008) who reported to be 21.28% in young cattle (calf less or equal to one year) in Adama. In contrast, it was lower than the report of Bilkis et al. (2011) from Bangladesh who was reported to be 46.15% in calves less or equal to one year. This difference in prevalence of ectoparasites might be due to the presence of different factors like animal husbandry practice like feeding and management, utilization of veterinary service and it might be due to the difference in sample size of different researchers. Furthermore, it may be also associated with the agro-ecological difference where the researches were conducted their research which could favors or limits the distribution of ectoparasites in the area as well as in the animal. In the current study, overall prevalence of lice was found to be 26.8% and it was the most prevalent ectoparasite recorded in calves in this study. The higher prevalence of lice infestation among the ectoparasites of calves might be due to the indoor keeping nature of calves, poor grooming behavior of calves, keeping of calves in dirty and unhygienic conditions, lack of awareness about the effect of lice on calves, other health problems, and nutritional stressors. This might also be aggravated due to underlying problems such as malnutrition and disease stressors (Radostits et al., 2006). This result was higher when compared to the reports of □acob (2008) (9.57%) on calves less than or equal to one year and □harbi et al. (2013) who reported 21.6% prevalence of lice infestation on calves less than eight months in region of □abeul in □ortheast Tunisia. On the other hand, the prevalence reported in this study was lower than that reported by Colwel et al. (2001) who reported 69.2 and 71.1% prevalence of lice on calves in two successive winters, a research conducted in Southern Alberta in Canada. This variation in prevalence lice were might be related to climatic variation, managemental practice and sample size used. The seasonal variation of the study period may also contribute to this difference as the lice populations on animals vary seasonally, depending on the condition of the host. Lice populations on animals are greater during the rainy months (walker, 2007). *L. vituli* was the only lice species identified in the current study. This was in close agreement with a research conducted in Hungary by Hornok et al. (2010) that reported only *L. vituli* on calves and its heavy infestation than other adult animals. This was also supported by the finding of Townsend (2006) who had observed heavy infestation of calves with *L. vituli*. The overall prevalence of tick in current study was 13.5%. This was relatively higher than that was reported by □acob et al. (2008) who reported to be 7.45% in Adama and Fantahun and Mohamed (2012) reported 2.6% on calves less than one year in Assosa. However, this finding was lower than the reports of Wirz (2005) and Singh and Rath (2013) who were reported 24 and 72.59% prevalence of tick on calves between 0 to 6 months of age in Mali and India respectively. These differences may be due to differences in agro-ecology, husbandry practice, the season and length of study period and the sample size might be contributed to this variation in the prevalence of tick. The low prevalence of tick in this study when compared to lice might be due to absence of factors that worsen the occurrence of ticks such as calves maintained apart from adult animals at low population densities and calves graze around home on the pasture with no more animal graze and most of the times they are managed in zero grazing were thus possibly exposed rarely to tick and reduced its transmission between the calf and other animal. The prolonged dry season in the study period might be contributed for this low prevalence of tick. The current study identified five species of ticks belonging to three genera and one sub genus□*Ambylomma, Rhipicephalus, Hyalomma* and *Rhipicephalus (sub genus Boophilus)*. Out of the five species of ticks recorded, the most

prevalent was *Rhipicephalus* (*Boophilus) decoloratus* (7.6%) followed by *Ambylomma variegatum* (5.2%) and *R. evertsi evertsi* (2.9%). This was in line with the reports of Onu and Shiferaw (2013) and Mokennen et al. (2001) who were reported *Rhipicephalus* (*Boophilus) decoloratus* followed by *Ambylomma variegatum* to be the dominant tick species in cattle in Bench Maji zone, southwest Ethiopia and central Ethiopia respectively. There was no statistically significant association were found between tick prevalence and the risk factors assessed. This was in agreement with report of acob et al. (2008) who report statistically insignificant association between tick prevalence and sex and breed of cattle. Moreover, the overall prevalence of mite on calves was 0.5% and genus *Psoroptes* was the only genus of mange mites that was identified in calves in the study area. The current finding on prevalence of *P. bovis* was in agreement with the reports of Tadesse et al. (2011), Onu and Shiferaw (2013) and acob et al. (2008) who were reported 0.4, 0.9 and 0.68% prevalence of *P. bovis* on cattle respectively. The low prevalence of mite infestation in this study could be due to the fact that the length of time mites can survive off its host is strongly affected by ambient temperature and humidity. Time of the year may have an impact on off host survival of mites. This has important implication for the potential for the transmission from the environment to the host, transmission being greater considerably in the winter (Wall and Shearer, 2001). The number of cases was too small for analyzing the effects of sex, breed, management system, housing condition, house cleanness, and milk feeding practice of calf. There was an association of the prevalence of lice with different risk factors in this study except, the prevalence of lice in male and female calves was found to be almost the same 27% in males and 26% in females. This is in agreement with report by Onu and Shiferaw (2013) on cattle. Furthermore, the comparable prevalence of lice in both sexes could be related to the absence of the stresses such as using male for draught purpose and production stresses in female that disrupt the immunity of the animal against ectoparasite and lead to the difference in the infestation of lice on sex group in adult animals. By contrast, Bilkis et al. (2011) had been reported significantly higher prevalence in females (64.63%) than in males (41.86%) and he hypothesized that higher level of prolactin and progesterone hormones makes the female individual more susceptible to any infection and stresses of production such as pregnancy and lactation make the females more susceptible to such infestation. The prevalence of lice on suckling and bucket feeding calves were 33.47 and 14.28% respectively. In this study, it was found a strong association between milk feeding practice and prevalence of *L. vituli*. This might be due to the increased contact rate of calf with dam which favors the transmission of lice. Lice are spread from animal to animal when animals are in close contact with one another such as during feeding, breeding, or shipping (Radostits et al., 2006). This study revealed that the prevalence of *L. vituli* was higher in local breed calves (32.31%) than in cross breed calves (18.70%). This was in line with the findings of acob et al. (2008) and Bilkis et al. (2011) who were reported a positive correlation between prevalence of lice and breed of cattle. This could be associated with taking more care to crossbreeds due to their high productivity nature than local breeds. So, heavy infestation of local breed calves may be associated with the unhygienic handling condition of their housings. On the other hand, the milking procedure in local breeds is done in the presence of calf as a stimulant for milking throughout the lactation period which contributes for the transmission of lice through prolonged contact with infested dam. However, there was no association between the prevalence of other ectoparasite species and breeds of calves. The prevalence of *L. vituli* was almost similar 26.86 and 26.79% in calf's share common housing with dam and in calves kept alone respectively. This comparable prevalence was might be related to the tie of calves in specific place in the house to prevent from suckling the milk. It was also found that the prevalence of *L. vituli* was higher in calves kept in unclean house (52%) than those kept in clean house (15%). This may be due to lice and louse eggs drop off onto bedding or are rubbed off, along with hair, onto fences and feed bunks as a result, other calf may then become infested from contaminated bedding, bunks, sheds, or trucks (Radostits et al., 2006). Even though, the probability of animals to pick up infestations from dirty house from lice drop off their host is limited it could not be ignored. Unhygienic house might predispose calf to other health problems that makes calves more susceptible to lice infestation. There might be underlying factors such as malnutrition and chronic disease in calves kept under unclean house (Wall and Shearer, 2001). *L. vituli* infestation in different management systems was found to be higher in calves kept under extensive (37.71%) than those kept in semi intensive (29.41%) and intensive (15.43%) management system. This agreed with the reports of igatu and Teshome (2012) that were reported lice infestation to be significantly higher in the extensive production system than in the intensive production system. This could be due to the poor management systems applied, prolonged contact with dam during suckling and milking time. The dam might have been in contact with animals from other herds at communal grazing and serve as source of infestation to the calf. More or less, early release of calves to pasture and inadequate utilization of veterinary service in extensive management system might exacerbate the condition. Ectoparasites tend to prefer specific site of attachment on the animal body. The common infestation sites of *Rhipicephalus* (*Boophilus) decoloratus* were dewlap and neck. *A. variegatum* most frequently observed on ventral body parts of the animals. The favorable predilection sites for *R. evertsi evertsi* were under tail and ventral body parts. This corroborated with

those reported by other authors like Tessema and Tashaw (2010) and Tiki and Addis (2011). Furthermore, a verity of factors such as host density, interaction between tick species, time and season, and inaccessibility for grooming, determine the attachment site of ticks (Debre et al., 2001). Lastly, in the present study, *L. vituli* was confirmed commonly to infest dewlap, neck and ventral body parts of the calf. This was similar to the reports by Urquhart et al. (1996) who were indicated that *L. vituli* prefer the head, neck and dewlap but also may be found anywhere in the hair coat.

CONCLUSION AND RECOMMENDATIONS

Ectoparasite infestation in calves has significant economic importance due to direct and indirect loss of production, reduce weight gain, retard growth, skin damage, immunosuppression, nuisance and biological vectors of different bacteria, viruses and protozoa. The present study showed high prevalence of ectoparsites on calves in the study area. The problem of external parasite seems to be crucial as they are widely distributed in relation to breed and management system. The important and prevalent ectoparasite species investigated in this study were *L. vituli, Rhipicephalus (Boophilus) decoloratus, A. variegatum, R. evertsi evertsi, H. marginatum rufipes, Rhipicephalus pulchellus* and *P. bovis.* However, the attentions given to the infestation ectoparasites was not sufficient and the lack of available information on ectoparasite species and the consequence behind its infestation aggravates the infestation of the calves by ectoparasites in the area. Based on this the following recommendations were forwarded There should be a close attention by all stakeholders farmers, veterinarians and government on control and prevention measures to lessen losses caused by ectoparasites infestation and transmission of pathogens to domestic animals. Awareness should be created in small holder farmer concerning the cause, transmission, and effect and control methods of ectoparasites in order to minimize the loss incurred due to ectoparasites in the study area. Further detailed study should be conducted to have appropriate information on the seasonal occurrence, burden and the effect of these ectoparasites on calves and economic losses caused by them in the study area.

Conflict of Interest

The authors have not declared any conflict of interest.

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