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Full Length Research Paper

Comparative fungal profile of tea leaves from highland and lowland in Nigeria

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A study to determine the fungal profile in leaves of both highland and lowland tea in Nigeria was performed. Healthy leaves were harvested from nineteen tea clones: T-14, T-19, T-33, T-35, T-61, T-68, T-74, T-108, T-143, T-228, T-229, T-236, T-238, T-318, T-354, T-355, T-367, T-368 and T-369 on the highland and lowland areas. Potato dextrose agar (PDA) was used as the growth medium to culture associated mycoflora from the tea leaves in five replications. The healthy tea leaves of the clones from the highland and the lowland areas show the presence of ten and six fungi respectively which include *Pleurothecium recurvatum, Candida albicans, Aspergillus niger, Aspergillus fumigatus, Itersonilia perplexans, Penicillium italicum, Lasiodiplodia theobromae*, *Trichordema viride, Rhizopus stolonifer* and *Botrytis cinerea*. Clones T-369, T-14, T-108 and T-368 have the highest (4) mycoflora load found on the tea leaves but three were found associated with each of clones T-228, T-68 and T-236 on the highland. Only one fungus was isolated from each of the clones T-108, T-61, T-229 and T-318, two fungi from each of T-355, T-19 and T-236 while the highest mycoflora load on the lowland tea were found in clones T-368 and T-228.

Key words: Tea, two and a bud, clones, highland, lowland, mycoflora.

INTRODUCTION

Tea (*Camellia sinensis* (L) Kuntze) belongs to the family Theaceae; this plant is an evergreen shrub or small tree. Tea is a popular beverage produced from the young leaves of commercially cultivated tea plant and has become one of an important revenue source for tea producing countries in the world (Bandyopadhyay, 2011). Originally cultivated in East Asia, India and China or perhaps both of these countries are native home of tea plant. Today, tea is grown over a wide range of tropical and subtropical region in more than 50 countries throughout Asia and Africa (Balasubramanian et al., 2011).

Tea was introduced into Nigeria by de Bouley from West Cameroon in 1952 (Kassboll -Smith, 1965).

However, the first commercial clones were imported into the Mambilla Plateau in 1975 (Hainsworth, 1981). Since then, more clones have been imported to establish large commercial plantations. Due to limited land area for tea on Mambilla Plateau, tea adaptation trial plots were established in seven lowland locations in Nigeria (Omolaja et al., 2000).

Tea production is greatly hindered due to a number of pests and diseases. Perennial habit of tea plant, peculiar culture condition and warm humid climate of the tea growing areas are highly conducive for disease development (Ponmurugan and Baby, 2007) and majority

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of the diseases are of fungal origin (Muraleedharan and Chen, 1997).

Microorganisms live in association with plants (endophytic microbes) and are commonly observed in nature (Bacon and White, 2000). They are commonly found in Coniferaceae, Gramineae, dan Ericaceae (Okane et al., 2001a), bakau *Bruguiera gymnorrhiza* (Okane et al., 2001b, c), *Theobroma cacao* (Rubini et al., 2005), and *Camelia sinensis* (Agusta et al., 2006). Array of fungi have being isolated from tea plants parts; *Helminthosporium* sp. and *Pestalotiopsis theae* (synonym: *Pestalotia theae*) from the leaves, *Fusarium, Helminthosporium, Cercospora, Botryodiplodia* and *Rhizoctonia* spp. and *Pestalotiopsis theae* from the shoot, *Rigidoporus microporus* (synonym: *Fomes lignosus*), *Colletotrichum coccodes* (synonym: *Colletotrichum camelliae*, *Nemania serpens* (synonym: *Hypoxylon serpens, Macrophoma theicola* and *Lasiodiplodia theobromae* (synonym: *Botryodiplodia theobromae*), *Fusarium* sp. and *Amyloflagellula pulchra* (synonym: *Marasmius pulcher*) Olunloyo, 1985; Filani et al., 1989; Anonymous, 1991; Otieno, 1996; Adedeji, 2006) among others as causing diseases of different tea parts. Fungal infestations and association with tea are of utmost importance as the leaves can be infused and consumed directly.

MATERIALS AND METHODS

Collection of tea leaves

The Mambilla Plateau on coordinate 6.7138N, 11.2500E and Mayo Selbe on 7.283N, 11.133E were the study locations where nineteen clones of tea representing the Indian/China types were collected. Average altitude in Mambilla is 1600 m (5000 ft), mean rainfall 1800 mm and average annual temperature of 16°C. Mayo Selbe has an average altitude 455 m (1496 ft), average temperature of 25.8°C and rainfall of 1518 mm.

The clones of the tea types were T-14, T-19, T-33, T-35, T-61, T-68, T-74, T-108, T-143, T-228, T-229, T-236, T-238, T-318, T-354, T-355, T-367, T-368 and T-369 on the Mambilla Plateau on the highland while T-19, T-61, T-108, T-228, T-229, T-236, T-318, T-355 and T-368 are from the lowland, Mayo Selbe. The healthy two tea leaves immediately below the bud, known in the tea trade as “two and a bud” (TAB), and use for the production of tea were harvested from each of the clones in the selected locations. The tea leaves were kept in a transparent polyethylene bag and transferred to the laboratory for further studies.

Isolation from tea leaves

The tea leaves samples were treated separately on clone basis. The medium, potato dextrose agar (PDA) acidified with 10% lactic acid use for culturing fungi from the leaves samples. The tea leaf samples were cut into small pieces of about 3 x 4 mm using a sterile scalpel; surface sterilized in 1% sodium hypochlorite, washed in three changes of sterile distilled water and aseptically sandwiched to dry in between sterile Whatman No 1 filter paper (150 mm). The tissue pieces were aseptically inoculated into the solidified PDA medium in 90 mm Petri dishes and replicated five times. The inoculated plates were incubated at ambient temperature (28±2°C) and the plates observed daily for colony growth. The fungal colonies were hyphal-tip transferred into new plates to obtain pure cultures and the colonies were thereafter identified base on their morphological features (Pitt et al., 1992).

RESULTS AND DISCUSSION

The selected 17 healthy tea leaf clones from the Mambilla Plateau (Taraba state) and 9 clones from Mayo Selbe in the lowland area of the state show the presence of ten and six fungi respectively. The isolated mycoflora include *Pleurothecium recurvatum, Candida albicans, Aspergillus niger, Aspergillus fumigatus, Itersonilia perplexans, Penicillium italicum, L. theobromae, Trichordema viride, Rhizopus stolonifer* (synonym: *Rhizopus nigrican*) and *Botrytis cinerea* (Table 1). Clones T-369, T-14, T-108 and T-368 have the highest (4) mycoflora load found on the tea leaves but three were found associated with each of clones T-228, T-68 and T-236 on the Mambilla Plateau respectively. Each of clones T-318, T-354 and T-33 have only one fungus isolated while two fungi were culture from each of clones T-35, T-19, T-74, T-61, T-367, T-143 and T-238 on the highland (Table 1).

The six fungi species from the nine tea clones in the lowland include *L. theobromae, I. perplexans, P. recurvatum, A. niger, C. albicans* and *R. stolonifer* (Table 2). Only one fungus was isolated from each of the clones T-108, T-61 and T-229 two fungi from each of T-355, T-19, T-368 and T-228 while the highest mycoflora load on the lowland tea were found in clones T-318 and T-236 (Table 2).

In the highland tea clone samples, the incidences of *L. theobromae* was most prevalent as it was recorded 58.82% of the highland clones and closely followed by *P. recurvatum* having 52.94% incidence which distant from 29.41% occurrence of each of *A. niger* and *R. stolonifer*. The nine lowland clones of tea have both *L. theobromae* as the most common flora with 66.67% occurrence, followed by *R. stolonifer* having incidence of 55.56%. *P. recurvatum* was isolated from 3 clones and each of the rest isolated fungi was found on one clone only and some other flora have zero incidence in the lowland tea leaves but occurred though at low percent incidences in the highland tea clones (Figure 1).

The isolation of *L. theobromae* from ten and six tea clone on the highland and lowland areas respectively corroborates the existence of the fungus as reported by Saha et al. (2008) that *L. theobromae* causes severe damage of tea gardens in the nurseries. It has also been reported as a common pathogen of tea, causing diplodia disease which affects roots, stems and leaves of tea plants of all ages (Sarmah, 1960; Chandramouli, 1999; Singh, 2005). Mbenoun et al. (2007), however, reported *L. theobromae* as most commonly associated with dieback and consistently isolated from various tissues (twigs, bark, vascular tissues and fruits) of symptomatic

Table 1. Frequency of occurrence of fungi associated with leaves of tea clones collected from highland, the Mambilla Plateau.

Clones	*Pleurothecium recurvatum*	*Candida albicans*	*Aspergillu s niger*	*Aspergillus fumigatus*	*Itersonilia perplexans*	*Penicilliu m italicum*	*Lasiodiplodia theobromae*	*Trichoderma viride*	*Rhizopus stolonifer*	*Botrytis cinerea*
T-369		+	+				+			+
T-35						+	+			
T-19							+		+	
T-14	+	+						+		+
T-74			+				+			
T-318	+									
T-61	+						+			
T-354							+			
T- 367	+								+	
T-228		+	+				+			
T-108	+				+		+		+	
T-143	+		+							
T-68			+	+			+			
T-33								+		
T-236	+					+	+			
T-238	+								+	
T-368	+			+	+				+	

Presence (+); Absence (-).

Table 2. Frequency of occurrence of fungi associated with leaves tea clones collected from lowland, Mayo Selbe.

Clones	*Pleurothecium recurvatum*	*Candida albicans*	*Aspergillus niger*	*Itersonilia perplexans*	*Lasiodiplodia theobromae*	*Rhizopus stolonifer*
T-236	+	+	-	+	+	+
T-318	+	-	+	-	+	+
T-108	-	-	-	-	+	-
T-61	-	-	-	-	+	-
T-229	-	-	-	-	-	+
T-355	+	-	-	-	+	-
T-19	-	-	-	-	+	-
T-368	+	-	-	+	-	+
T-228	-	+	+	-	-	+

Presence (+); Absence (-).

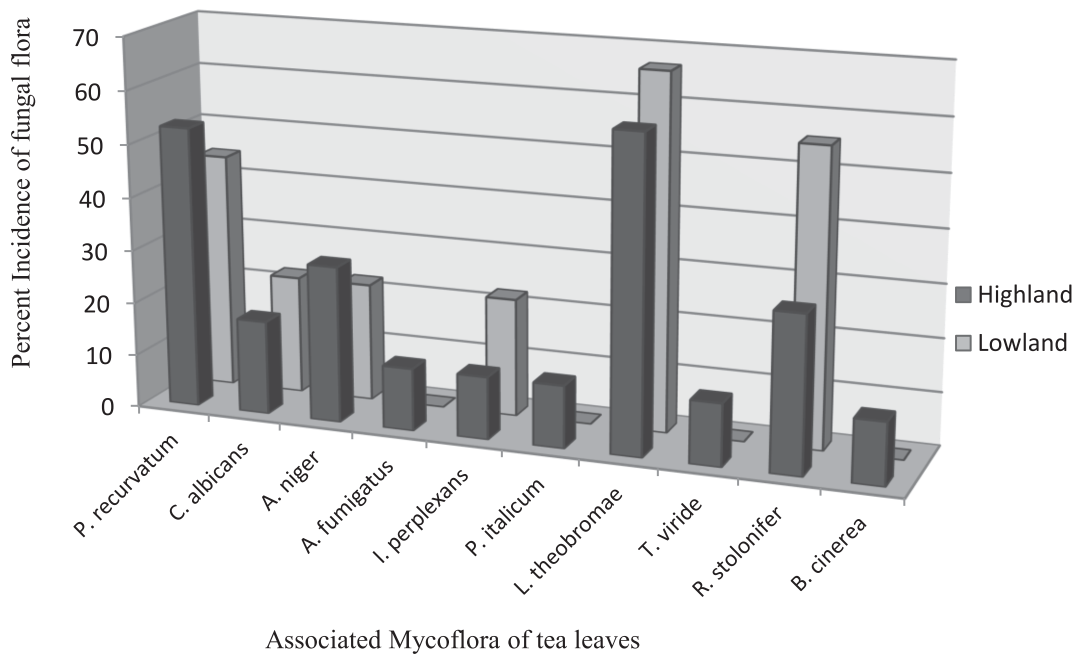

Figure 1. Incidences of Mycoflora in tea clones of the highland and lowland areas.

cacao (*Theobroma cacao*) plants.

This study also agrees with the early findings of Olunloyo et al. (1987) which reported the isolation of *L. theobromae, Penicillium tamari* and *A. niger* among others from the tea cuttings. Adedeji (2000) also reported the incidences of *L. theobromae, Fusarium* spp. and *A. niger* on fresh leaves of tea plants in the lowland. However, the tea leaf samples used in this study did not exhibit any disease symptom when they were collected and processed.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

The pattern of pathogen diversity and abundance in Lentil (*Lens culinaris*) fields in Constantine region, Algeria

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Lenses are a group of pulses having a socio-economic and nutritional significance. The study was conducted on two fields of *Lens culinaris* in Constantine region contaminated with molds, had the objective to put a relationship between pathogenic molds associated with these plants and their environment. This study revealed the presence of 20 genera in soils (*Absidia, Acremonium, Alternaria, Aspergillus, Bysochlamyces, Chaetomium, Cladosporium, Emericella, Eurotium, Fusarium, Mucor, Paecilomyces, Penicillium, Peronospora, Phytophotora, Pseudalesheria, Scopulariopsis, Scytaldium, Trichoderma* and *Ulocladium*) and 20 genera also in plants (*Absidia, Acremonium, Alternaria, Aspergillus, Botrytis, Chaetomium, Cladosporium, Cylindrosporium, Curvularia, Eurotium, Fusarium, Myrothecium, Onychocola, Phytophthora, Pseudalescheria, Penicillium, Peronospora, Rhizoctonia, Trichoderma* and *Ulocladium*). They contribute approximately 54% of the total micropopulation enumerated in studied samples. The development of these pathogenic strains is governed by environmental conditions namely the chemical elements in soil, pH, electrical conductivity, Nitrogen, Carbon and saturation. The results we have obtained shows that the chemical variations ground contribute to the right development fungi and their transfer to plants

Key words: *Lens culinaris*, soil, fungi.

INTRODUCTION

Lentil is a dicotyledonous legume plant and takes the fifth place in production of pulse crop in the world (Hymowitz, 1990). Lentil is still widely used today because of its high fiber, protein, vitamin and mineral content (Hnatowich, 2000) and considered as one of the best vegetable source of Iron especially important for adolescent and pregnant women. Furthermore, lentil is cultivated in sandy loam soil and can be grown in nutrient deficient soil (Summerfield, 1981). Lentils are drought resistant and can be grown in water logged and saline soils (Muehlbaur et al., 2002).

In Constantine, lentil production has tripled in the last six years from 37,120 kg in 2006 to 710,074 kg in 2013. A survey of literature showed that many fungal species

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have been reported for lentil seeds including species of *Pythiumsp, Rhizoctoniasp, Sclerotiumsp, Fusarium*sp, (Muehlbaur et al., 2002), *Alternariasp, Aspergillussp, Mucorsp, Chaetomiumsp, Penicillium sp. Nigrosporasp* (Hussain et al., 2007), *Uromycesfabae* and *Botrytis sp.* (Richardson, 1979). However, the presence of so much pathogenic fungi reduces the quality and quantity of crop which in turn lead to economic losses.

Soil is the most important resource of the nature (Sumithra et al., 2013), it is a thin layer of the earth's crust which serves as a natural medium for the growth of plants because the components of soils are mineral material, organic matter, water and air. Soil has important ecological functions in recycling resources needed for plant growth and microorganisms' development, the soil forms a large reservoir for many potential plant pathogens and especially plants with a decreased vitality (Katarzyna and Christel, 2011). An extremely simplified vegetation, such as a monoculture, selects a specific microbial community, including plant pathogenic microorganisms and sometimes also their parasites or antagonists (Bruggen et al., 2006).

This study aimed to reveal the complex interactions within the infected soil-lentil ecosystem and the aggressive behavior of molds.

MATERIALS AND METHODS

Studied area

The field experiments were conducted in 2011 and 2012 on two agricultural soils: AIN SEMARA and BARAOUIA situated in the North East of Algeria.

Sampling procedures in the study area:

Soil sampling

Soil samples taken at 5, 10 and 15 cm from the soil surface were collected from four locations in the vicinity of the two areas (AIN SEMARA and BARAOUIA), in sterile paper bags. The soils collected are ground and passed through 0.2 mm sieve and were used for the analysis.

Plant sampling

Lentil plants from Metropole variety (20 samples), were collected from different places in fields infected by many types of mold. These plants were collected in sterile paper bags.

Physical and chemical properties analysis

The physiochemical properties are executed in a laboratory of soil chemistry. The important parameters studied are: available Nitrogen, Organic Carbon, Electrical conductivity and pH value.

Available Nitrogen

The dosage of Nitrogen is effected using berthelot reaction (Krom, 1980; Searle, 1984, EN ISO 11732 (2013)) modified by Belahrache (Benlahrache, 2013).

Organic Carbon

The Walkey and Black (1934) method described by Nelson and Sommers in 1982 is used for assaying the organic carbon in simple soils.

Electrical conductivity

The assay method involves wet ground with 100 g of distilled water slurry. The paste obtained was centrifuged at 300 revolutions / min for 30 min. In the end, the temperature of the float is noted in the resistance, is measured after adjusting the temperature to 25°C, using a conductivity meter (Anonymous, 1954).

pH

The method relies on the preparation of a solution (diluted suspension) of ground 10 g and 25 mL of distilled water, after magnetic stirring, the pH is measured at room temperature using a pH meter (Boudoudou et al., 2009).

Mycoflora isolation

From soil (Dilution plating method)

To isolate the fungi from the soil sample, 1 g was diluted in 9 mL of saline solution. 1 mL of the diluted sample was poured and spread on Petri plates containing sterilized PDA medium. The inoculated plates were incubated at a temperature of 27°C for 3 days (Warcup, 1950). To prevent bacterial growth, one milligram of chloramphenicol was added to the medium.

From plants

Infected plants collected from the websites surveyed (AIN SEMARA and ABARAOUIA), was cut into small pieces of about 0.5 cm. The pieces are disinfected with sodium hypochlorite (2%) for 3 min, then rinsed several times with sterile distilled water and ethanol (70%) and dried between sterile filter paper, then placed in Petri dishes (5 piece / Petri plates) containing a culture medium PDA, MEA5 or MS. Finally, the Petri plates were incubated at 27°C for 4 days (Belabid et al., 2000).

Mycoflora identification

Colony colour, odor, morphology and growth rate were noted besides hyphal structure, spore size, shapes and spores bearing structures. They were compared with the standard works of Packer and Thomas (1990), Rinaldi et al. (1998), Gams et al. (1998), Botton et al. (1999), and Snavi (1999).

Presentation of data

The number of genera is referred to as genera diversity. Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factors. In order to assert the dominance of the genus in site, percentage contribution was worked out as follows:

Table 1. Results of physico-chemical analysis of soils in Constantine region.

Parameter	BARAOUIA			AIN SEMARA		
Depth (cm)	5	10	15	5	10	15
Organic matter (%)	1.720	1.978	1.686	1.892	1.858	1.686
Carbon (‰)	10.0	11.5	9.8	11.0	10.8	9.8
Nitrogen (‰)	0.76	0.89	0.70	0.95	0.82	0.80
C/N	13	13	14	12	13	12
pH	7.86	7.76	7.77	7.69	7.79	7.83
EC (milliohm/cm)	0.50	0.93	0.70	0.65	0.52	0.35

$$\% \text{ contribution} = \frac{\text{Number of colonies of the same genus in a sample}}{\text{Total number of all colonies of all the genera in a sample}}$$

RESULTS

Physical and chemical properties analysis

The results of the physical and chemical properties analysis of two soil samples (with different depth), have revealed that soils of AIN SEMARA and BARAQUIA are rich in organic matter (>1) and by consequence Carbon and Nitrogen. In addition, the organic matter is rich in carbon and poor in Nitrogen. Furthermore, results of pH analysis show that values are slightly alkaline (between 7.69 and 7.86). The measure of electrical conductivity (EC) and the salinity factor are indicated to lower than 1; which means that the soils were not salty (Table 1).

Mycoflora isolation

From soil

Altogether, six soil samples from two different stations representing the entire Constantine district were examined for fungal diversity. The study resulted in the presence of 44 species of fungi belonging to 14 genera from BARAOUIA and 42 species of fungi belonging to 16 genera from AIN SEMARA (Figure 1).

Indeed, the soil sample of BARAOUIA is characterized by the presence of 68.21% Deutromycetes, 20.43% Ascomycetes, 6.82% Oomycetes and 2.27% of Phycomycetes and Zygomycetes. The soil samples of AIN SAMARA contain less Deutromycetes (50%), Ascomycetes (35.72%) and Zygomycetes (9.52%) but more Oomycetes (4.76%) (Figure 2).

The quantitative comparison of genera shows that high percentage belongs to the *Fusarium, Penicilum* and *Aspergilus* in two studied areas. By contrast, the other genera have variable percentages in both sites (Figure 3). The dominant genera from the field soils of BARAOUIA are *Fusarium* and *Penicillium* (9 species each), followed by *Aspergillus* (8 species), *Phytophtora* (3 species), *Acremonium, Alternaria*, *Chaetomium*, *Cladosporium* and *Paecilomyces* (2 species each) and finally *Mucor, Peronospora, Scopulariopsis, Trichoderma* and *Ulocladium* (1 species each); whereas in AIN SEMARA soils, the dominant species were *Aspergillus* (9 species), *Penicillium* (5 species) and *Fusarium* and *Absidia* (4 species each) followed by *Eurotium* and *Scytalidium* (3 species each), *Alternaria, Paecilomyces, Phytophtora* and *Ulocladium* (2 species each), and finally *Acremonium, Bysochlamyces, Cladosporium, Emericella, Pseudallesheria* and *Trichoderma* (1 species each).

From plants

A total number of 110 species belonging to 18 genera of fungi: *Absidia, Acremonium, Alternaria, Aspergillus, Botrytis, Chaetomium, Cladosporium, Cylindrosporium, Curvularia, Eurotium, Fusarium, Myrothecium, Onychocola, Penicillium, Peronospora, Phytophtora, Pseudalescheraia* and *Rhizoctonia* (Figure 4), were isolated from lentil plants (root, stem and leaf) in two areas of BARAOUIA and AIN SEMARA. These genera are classified in six divisions for BARAOUIA (Ascomycetes 66.24%, Basidiomyctes 1.78%, Deutromycetes 12.52%, Oomyctes 1.78%, Phycomycetes 12.5% and Zygomyctes 3.57%) and four divisions for AIN SEMARA (Ascomyctes 79.63%, Basidiomyctes 1.85%, Deutromycetes and Phycomycetes 9.25%).

The major genus in the area of BARAOUIA is *Fusarium* (9 species) and *Alternaria and Apergillus* (8 species each). The other genera have a variable number of species: *Phytophtora* (7 species), *Penicillium* (4 species), *Cladosporium, Curvularia* and *Ulocladium* (3 species each), *Absidia, Acremonium and Chaetomium* (2 species each) and finally *Botrytis, Cylindrosporium, Myrothecium, Peronospora* and *Rhizoctonia* (1 species each). The *Alternaria* was the major genus with 11 species in the area of AIN SEMARA, followed by *Fusarium* (8 species), *Aspergillus* (6 species), *Curvularia, Penicillium* and *Phytophtora* (5 species each), *Ulocladium* (4 species),

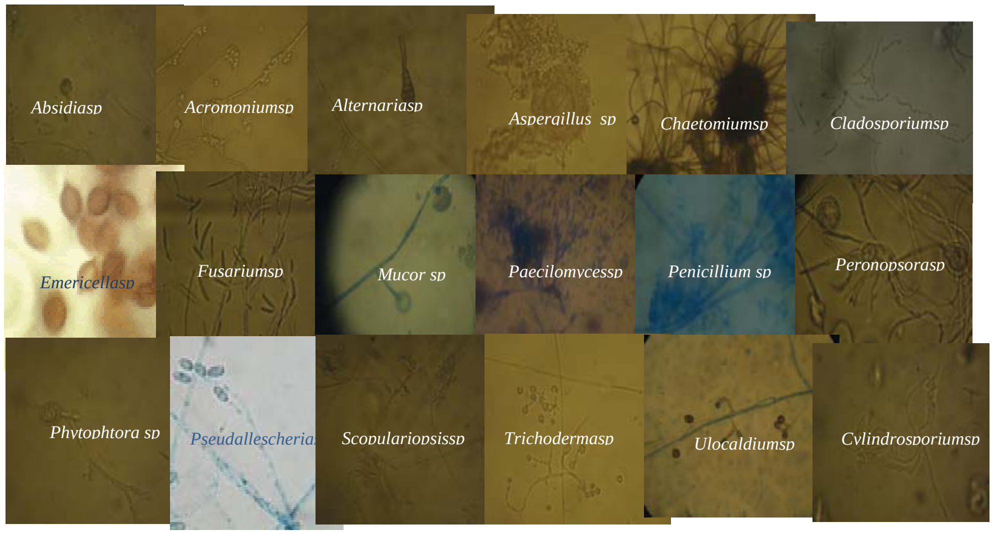

Figure 1. Examples of some species isolation from soils and plants.

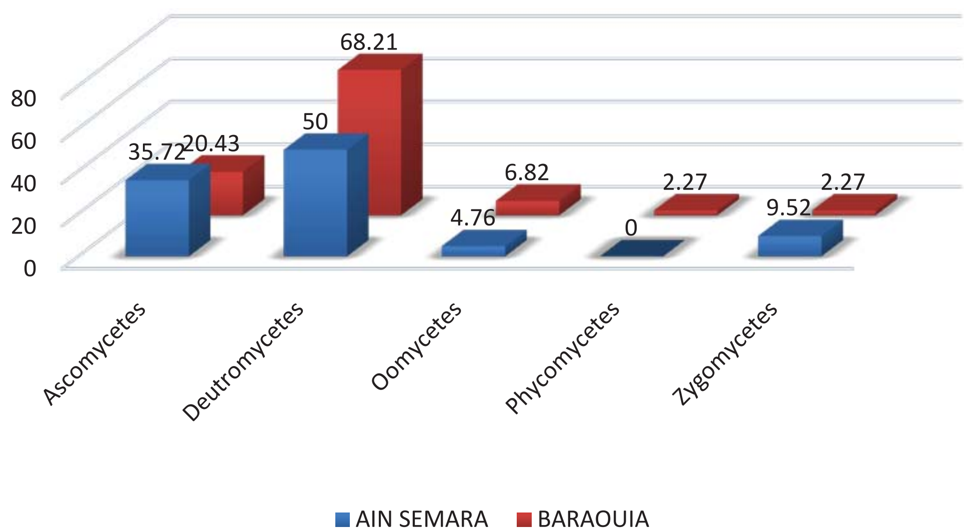

Figure 2. Percentage of different branch of fungi in the soil of BARAOUIA and the soil of AIN SEMARA.

Acremonium, Onychocola and *Trichoderma* (2 species) and finally *Rhizoctonia* (1 species) (Figure 5).

DISCUSSION

The samples of soil analysis were taken from the first horizon (0 to 20 cm) in which the essence of the biological activity is concentrated. This horizon is the most exposed one to the air and it contains mainly aerobic species.

The internal environmental conditions can assign the specific composition of the microbial communities and their chemical potential (Alexander, 1997). Additionally, with an organic-matter equal to 1.79% in (BARAOUIA) and 1.81% in (AIN SEMARA), the studied soils are included in the interval of normal rates (1.5 to 2.5%) which are described in the standards of Duthil (1970).

The comparison of the pH values of the soil studied with the data standards by Madagascar (quoted by the interprocessor of the agronomist (1974)), shows that our soils are slightly alkaline. According to Baise and Jabiol (1995), the alkali soils have a pH between 7.3 and 8.5. These pH values should be perfect for fungi development.

The values of the electrical conductivity (EC) measures

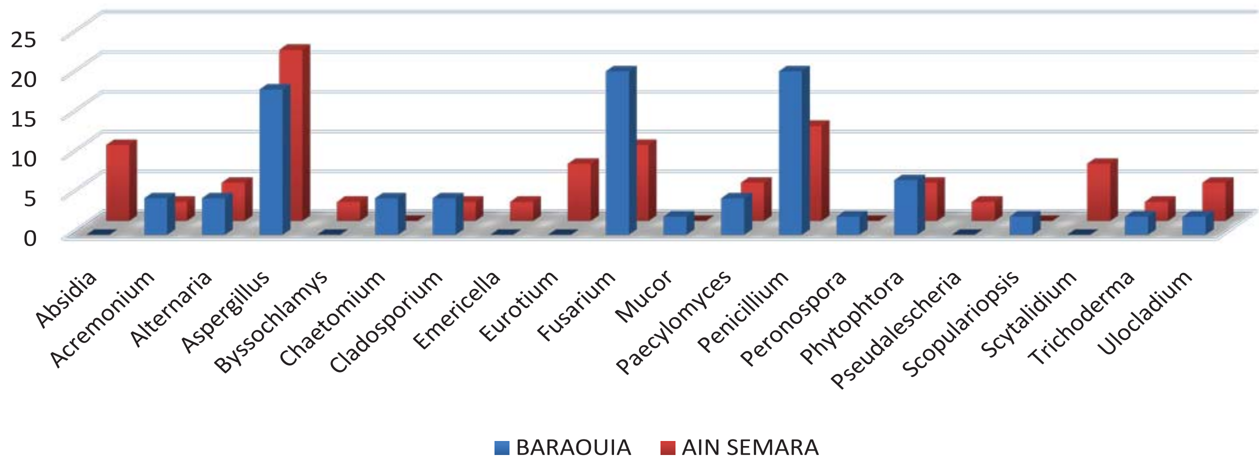

Figure 3. Percentage of contribution of different genera in the soil of BARAOUIA and the soil of AIN SEMARA.

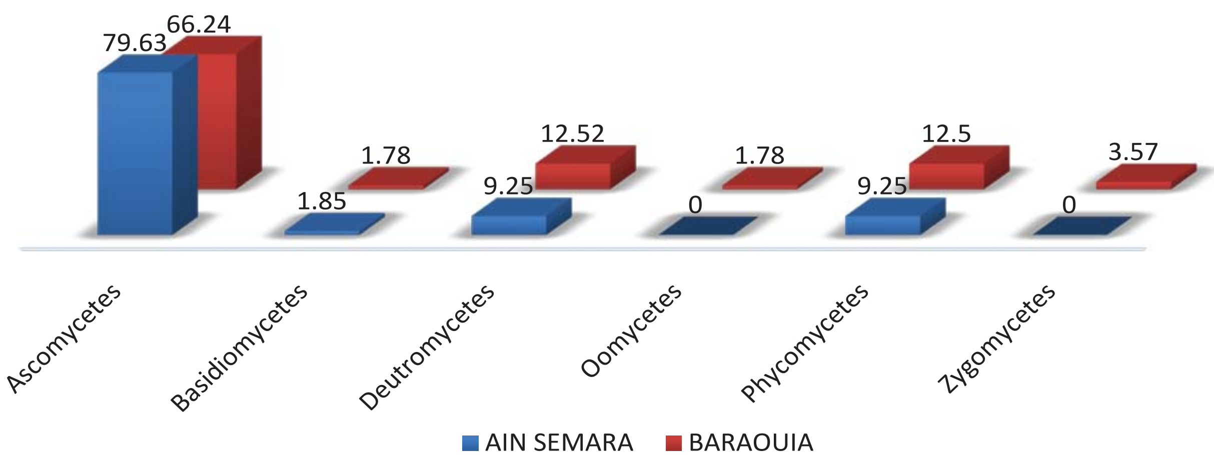

Figure 4. Percentage of branch divisions of fungi in the plants of BARAOUIA and the soil of AIN SEMARA.

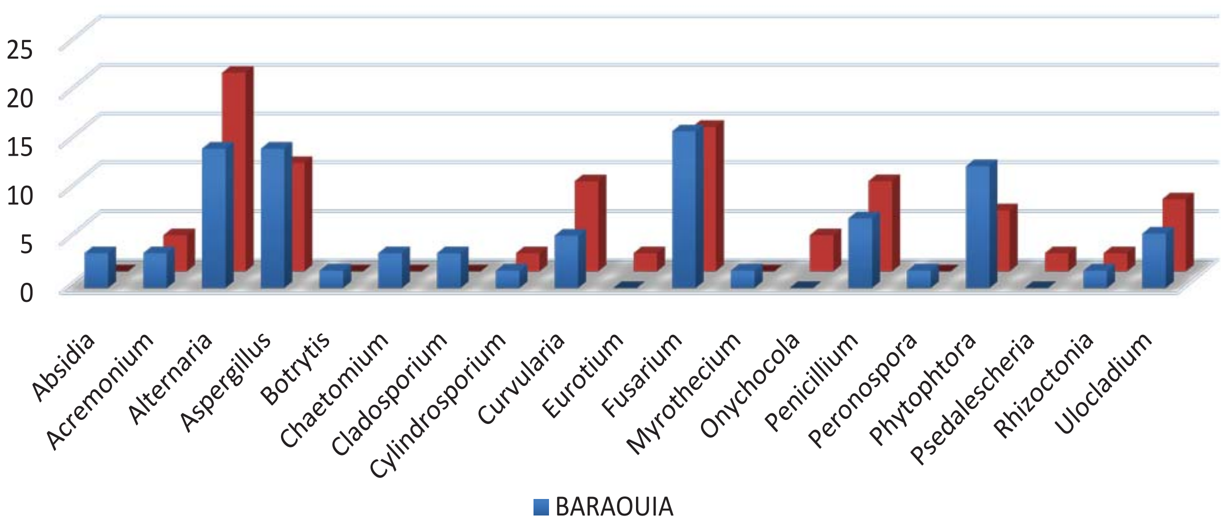

Figure 5. Percentage of contribution of different genera in the plants of BARAOUIA and the plants of AIN SEMARA.

of the soil which are taken from BARAOUIA and AIN SEMARA show that the soils are not saline (EC< 1) and this is according to the norms given by DURAND (quoted by the interprocessor of the agronomist, 1974).

Furthermore, the C/N report that was calculated after the determinations of Carbon and Nitrogen, has given the results <15, which means that the organic material has been normally mineralized. Thus, it provided mineral nitrogen.

The analysis of the results obtained after the isolation of the fungi of the two prospected sites shows that 84.09% of the strains isolated from the BARAOUIA site (genera: *Acremonium, Alternaria, Aspergillus, Chaetomium, Cladosporium, Fusarium, Perenospora, phytophtora, Penicillium,* and *Ulocladium*) and 90.73% of the AIN SEMARA site (genera: *Acremonium, Alternaria, Aspergillus, Eurotium suggests, Penicillium, Phytophthora, Fusarium, Pseudallescheria, Ulocladium* and *Trichoderma*) are isolated from the different parts of infected plants and also from the sampled soils. These results showed that the strains have been transmitted from the soil through the sap which can take for granted the soil as a middle of storage of fungal spores. These results are in relationship with those of Estelle Levetin and also of Abigail Jenkis (2005).

Furthermore, the remains of the soil isolates (15.91% of BARAOUIA and 9.27% of AIN SEMARA) are plants only. It means that these strains are transmitted through the seed or the air (Estelle Levetin).

Actually, the study of the different isolates shows that only 55.34% of the strains of the BARAOUIA site (genera: *Alternaria, Botrytis, Cladosporium, Cylindrosporium, Fusarium, Peronospora, Phytophthora* and *Rhizoctonia*) and 46.3% of the strains of the AIN SEMARA site (genera: *Alternaria, Cylindrosporium, Phytophtora* and *Fusarium*) are of pathogenic strains associated with *Lens culinaris* plants. These results are in coincidence to those of Richardson (1979), Ahmed et al. (1993), Muhlabauer et al. (2002) and Hussain et al. (2007).

The great diversity of isolates obtained after identifications (soil and plants) is mainly due to the environmental conditions favouring the development of molds: rate of rainfall between 19 and 68 mm, 60 to 71 humidity and temperature between 19 and 26^{o}C.

The majority of the identified isolates belong to large families of the Ascomycetes and Deuteromycetes. These strains are indigenous, usually isolated from the ground (Alvarez-Rodriguez et al., 2002).

The obtained varieties after isolation from the ground are: *Absidia, Acremonium, Alternaria, Aspergillus, Bysochlamyces, Cladosporium, Eurotium suggests, Emericella, Fusarium, Mucor species, Peronospora, Paecylomyces,Penicillium, phytophtora, Pseudallesheria, Trichoderma, Ulocladium, Chaetomium* and *Scopulariopsis* species are often dating in the soils, this is related with the results obtained by Prince et al. (2011).

Conflicts of Interest

The authors declare no conflicts of interest.

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Full Length Research Paper

Persistence and retention towards degree completion of BS agriculture students in selected State Universities in Region IV-A, Philippines

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Using descriptive-correlational research design, total enumeration of 56 junior BS Agriculture students uncovered factors that support and challenge students in agriculture degree programs. The study selected the targeted State Universities based on the level of degree accreditation in agriculture. The researcher also incorporated qualitative data by conducting semi-structured interviews. Factors related to personal, academic and career disposition were found to be influential in student persistence. Overall results affirmed that classroom-related factors contribute most to persistence, whereas; the quality of teaching contributes most to the retention of BS Agriculture students. Correlation analysis revealed a relationship between persistence and retention. Analysis of differences across accreditation levels revealed slight to greater variations regarding factors that relate to persistence and retention. The results of this study suggested that the selected State Universities have not gone far enough on a practical level to ensure that BS Agriculture students are supported in an effective manner. Thus, the program should provide an educational environment with adequate, effective and accessible administrative and educational support services specific to the students' academic success towards completing a degree in Agriculture.

Key words: Agriculture, completion, degree, persistence, retention.

INTRODUCTION

Agriculture plays a dominant role in the Philippine economy particularly as 70% of the country's population is rural and two-thirds of these depend on farming for their livelihood (http://www.state.gov/r/pa/ei/bgn/2794.htm). It is the sector that determines economic development and poverty alleviation of the country. It is also a significant sector for employment and labor.

With global competition, a primary consideration is to enhance the overall global competitiveness of Philippine agriculture rather than to attain the traditional and relatively narrow-goal of upgrading the low levels of income as well as the production and productivity of small farmers in the rural areas (http://www.state.gov/). But the

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reality dictates that no matter how the government envisions transforming agriculture into a modern, dynamic and competitive sector; still, the Philippine agriculture suffers from lack of human resources who have the knowledge and skills to be able to adapt to this ever changing world (http://www.philstar.com/Article 20 April 2010). Education is one of the most influential and powerful tools a society has for contributing to advancing knowledge and transforming lives. Thus, change resulting from new agricultural and rural development will continue to depend mainly on agricultural science and agricultural college education.

In a predominantly agricultural country like the Philippines, there is a need for constant supply of well-trained, skilled and knowledgeable agriculture graduates to provide the manpower base for the implementation of the country's agricultural production and development programs. While agricultural education has been recognized as strategic factor in boosting productive and human resource development in the Philippine agricultural sector, it has failed to turn out sufficient number of competent graduates (Directo, 2002 in Aquino, 2005).

Commission on Higher Education (2010) records show that there are slightly over 49,000 students enrolled in agriculture and related courses, only 2% in the overall discipline group in the Philippine higher education (Figure 1). Student persistence and retention are important issues facing Philippine higher education today. Although the number of students attending college continues to grow, improving graduation and completion rates remains a challenge.

To clarify, retention is an organizational phenomenon—colleges and universities *retain* students. Institutional retention rates, the percentage of students in a specific cohort who are retained, are often presented as measures of institutional quality. Persistence, on the other hand, is an individual phenomenon—students *persist* to a goal. That is, to complete the four-year bachelor's degree in agriculture.

Retaining a student is fundamental to the ability of an institution to carry out its mission. A high rate of attrition (the opposite of retention and persistence) is not only a fiscal problem for schools, but a symbolic failure of an institution to achieve its purpose. Thus, it becomes important to understand and act on what research tells about student persistence, retention into the next year level and to graduation.

Although preparation, ability, and motivation are important factors in student persistence and retention, they cannot explain all the reasons that students persist or drop out (Reason, 2009). Braxton (2009) indicates that the lack of student persistence may be labelled the departure puzzle. Given the availability of numerous guides on the selection of colleges and universities by the parents, career counsellors and students and the enormous amount of attention that college officials focus upon the college selection process, it is puzzling that almost one-half of students entering two-year colleges and more than one-fourth of students entering four-year collegiate institutions leave these institutions at the end of their first year (Spedding, 2009).

Objectives of the study

The purpose of the study reported in this paper was to bring to light factors that relate to persistence and retention of college students in pursuing a Bachelor of Science in Agriculture course towards degree completion. This study also serves as a step toward understanding the mechanisms through which university influences, and other factors that affect the persistence and retention of students.

The study specifically determined the relationship between persistence and retention; examined the difference in persistence and retention of third-year BS Agriculture students across accreditation levels; and recommended policies to improve persistence and retention among BS Agriculture students in selected State Universities.

Theoretical models of the study

Models of persistence

The reasons that students persist became a major area of inquiry for education scholars beginning in the 1960s (Braxton, 2009). To give further elaboration on the issue, the following section presents the theoretical models related to student persistence and retention.

Alexander Astin's input-environment-outcomes model explains the impact of various environmental experiences by determining whether students grow or change differently under varying environmental conditions. The consideration of input characteristics when assessing student retention helps to understand the influence of students' backgrounds and characteristics on their ability to persist. Environmental variables that might influence student success include: institutional characteristics, students' peer group, faculty characteristics, curriculum, financial aid, major field of choice, place of residence, and student involvement; whereas, outcomes are the student's characteristics after exposure to the environment.

Vincent Tinto's theory of student departure states that, to persist, students need integration into formal (academic performance) and informal (faculty/staff interactions) academic systems and into formal (extracurricular activities) and informal (peer-group interactions) social systems. Tinto argues that the institution shares this responsibility for helping students achieve academic and social integration.

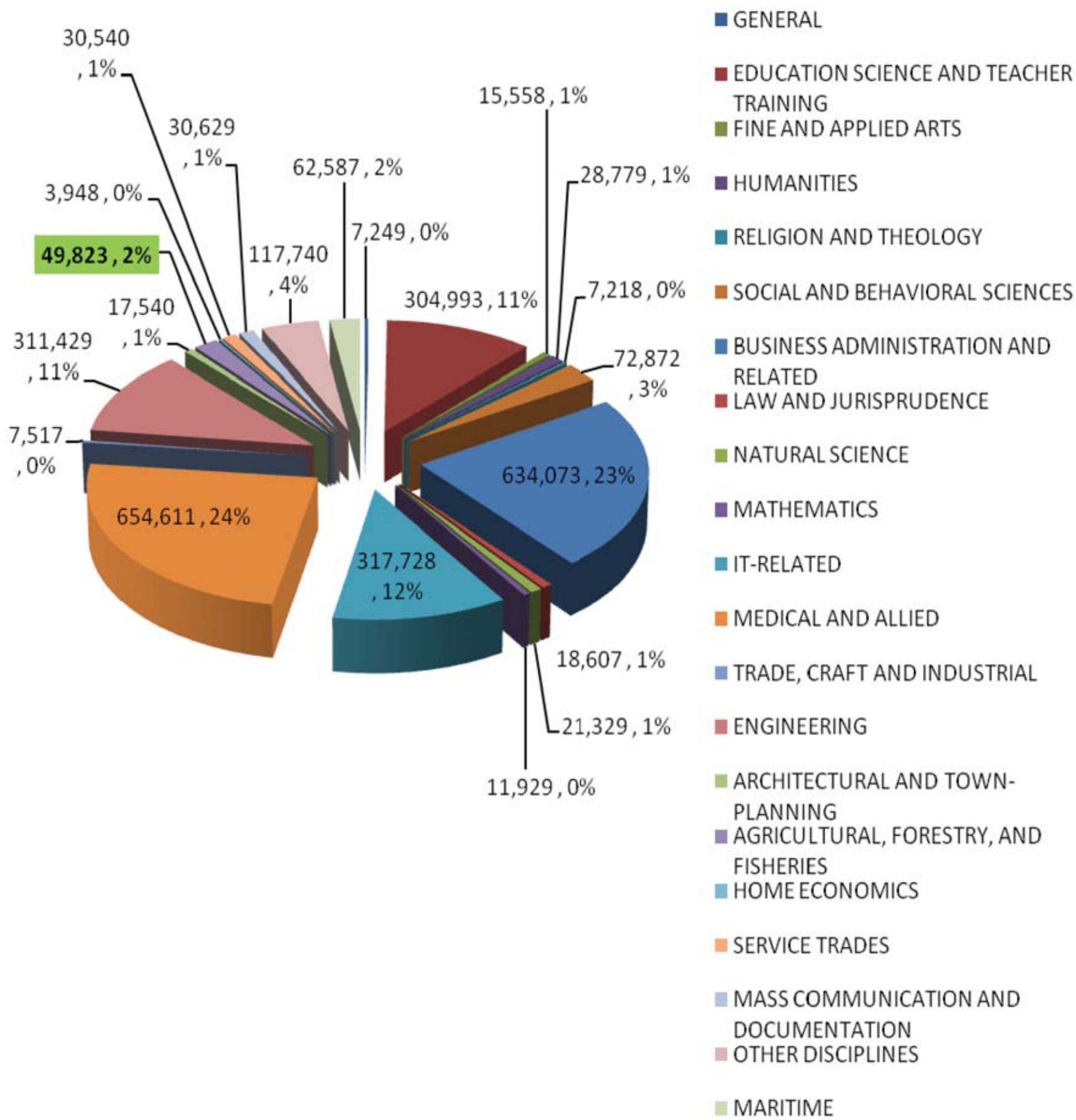

Figure 1. Higher education enrolment by discipline group, Academic Year 2010-2011.

Models of retention

Astin's student involvement theory deals with the factors that are important to the integration of students into the institution such as peer and faculty interactions and involvement in campus activities. In contrast to Tinto's theory concerning integration, this theory of Astin posits that the student plays an integral role in determining his or her own degree of involvement in college classes, extracurricular activities and social activities.

Tinto's student integration model applies the concept of integration to college students. Essentially, students drop out when they have not achieved a sufficient level of integration into the fabric of college life. In other words, the "fit" between person and institution is not conducive to persistence.

These theoretical models have identified factors that contribute to persistence and retention of students in higher education. These factors include individual, institutional, as well as social and academic and their integration into an institution including: interactions with peers and faculty members, and levels of involvement of the student in the institution.

Conceptual framework

To answer the call for a more comprehensive and

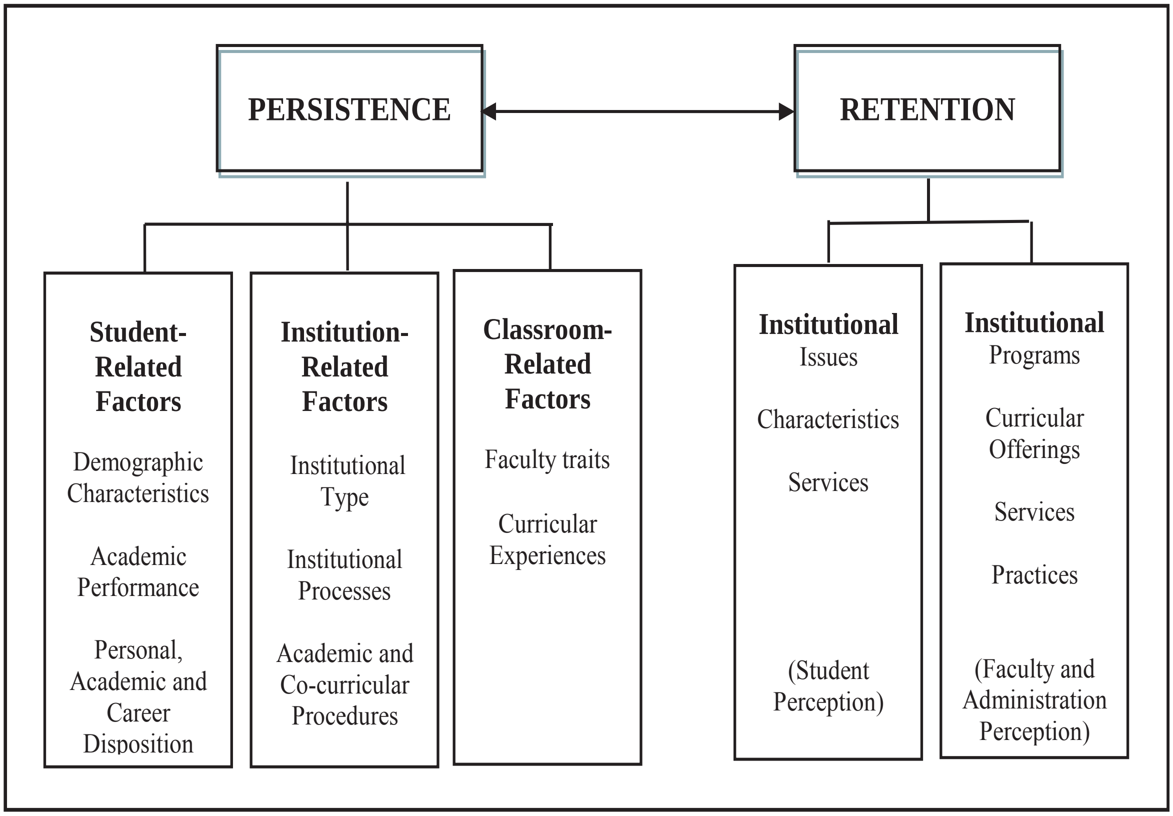

Figure 2. Conceptual framework of factors which explain persistence and retention of BS Agriculture students.

integrated model for studying student persistence and retention, the following conceptual framework was formulated by the researcher. This was based on the models of Astin (1985, 1993) and Tinto (1975, 1993).

The framework incorporates, in five sets of constructs, the wide array of persistence and retention factors (Figure 2). The persistence factors comprise three categories: student-related factors, institution-related factors, and classroom-related factors. These student-related factors including demographic characteristics, academic performance, as well as personal, academic and career disposition shape subsequent experiences upon entering the college through their interactions with the institution-related factors including the type (that is, the level of degree accreditation of the State University such as Level I, II, and III) and processes being implemented by the institution as well as academic and co-curricular procedures; and the classroom-related factors constituting the individual student experience towards faculty traits and the entire curriculum. The framework shows the relationship of these persistence factors to retention factors. Retention factors include the institutional programs and retention practices in addressing the institutional issues to support/enhance persistence and retention of agriculture students towards degree completion.

METHODOLOGY

This section presents the study's research design, locale, respondents, sampling technique, data-gathering instruments and procedures, and the tools used for data analysis.

Research design

A descriptive-correlational research design was utilized to identify persistence and retention factors of agriculture students towards degree completion. This study employed a survey questionnaire to gather data regarding students' demographic characteristics and their perceptions on the persistence and retention factors.

In addition to describing *what is* with respect to the student-, classroom-, and institution-related factors, this study also utilized a correlational research method to identify the strength and direction of relationships among selected variables.

Locale

This study was conducted in three State Universities in Region IV-A. Their respective type in terms of their current level of degree accreditation and location, as shown in Figure 3, are the following:

(1) **The Cavite State University** formerly known as Don Severino (delas Alas) Agricultural College (DSAC) is a state university in the province of Cavite, Philippines. In February 2012, it has a total of 7, 817 students in its nine colleges. College of Agriculture, Forestry, Environment and Natural Resources has 361 students in all its

Figure 3. Map showing the selected State Universities in Region IV-A, Philippines.

degree programs.

During the second semester of the Academic Year 2011-2012, there were 236 students taking Bachelor of Science in Agriculture (that is, 161 in first year, 44 in second year, 21 in third year, and 10 in fourth year). The student-to-faculty ratio is 20 to 1 (CvSU-Indang, Office of the University Registrar AY 2011-2012).

(2) **The Laguna State Polytechnic University (LSPU)** is a state university in the Province of Laguna. It has four regular campuses in Santa Cruz (the main campus), Siniloan, San Pablo City, and Los Baños, two satellite campuses — LSPU-Nagcarlan and LSPU-RECS Complex in Santa Cruz — and two International Language Studies Centres in Thai Nguyen University, Vietnam, and Changwon College, South Korea. The University has a total of 2,596 students in its ten colleges. College of Agricultural Science and Technology has a total of 340 students in its degree programs.

There were 77 students taking Bachelor of Science in Agriculture (that is, 36 in first year, 16 in second year, seven in third year, and 18 in fourth year). The student-to-faculty ratio is 20 to 1 (LSPU-Siniloan, Office of the University Registrar AY 2011-2012).

(3) **The Southern Luzon State University** (SLSU), formerly known as Southern Luzon Polytechnic College (SLPC) is a state university with eight campuses in Lucban (the main campus), Tagkawayan, Alabat, Polillo, Sampaloc, Lucena, Tiaong, and Infanta. The main campus has a total of 9,696 students in its seven colleges. College of Agriculture has a total of 357 students in all its degree programs. There were 122 students taking Bachelor of Science in Agriculture (that is, 54 in first year, 31 in second year, 28 in third year, and nine in fourth year). The student-to-faculty ratio is 20 to 1 (SLSU-Lucban, Office of the University Registrar AY 2011-2012).

Respondents of the study

The sample consisted of the total enumeration of all third-year BS Agriculture students for the second semester of the academic year 2011-2012. These students were purposively selected as the ones who persisted through their second year of college and remained in the same institution to continue their third year. They were identified as persisters as they manifested the desire to pursue bachelor's degree by having reached their current year level, an indication of being adjusted to the system of their chosen field from the beginning year through degree completion.

Sampling technique

The study selected targeted schools based on the level of degree accreditation in agriculture including the SLSU representing the Level I Status, LSPU representing the Level II Status and CvSU representing the Level III Status. *Level I accredited status* is granted for programs after a formal survey, effective for a period of three years; *Level II accredited status* is granted for accredited programs effective for a period of three or five years; while, *Level III accredited status* is granted for programs which have met a reasonably high standard of instruction, highly visible research tradition, strong faculty development tradition, and extensive and functional library and other learning resource facilities (http://www.ched. gov.ph/ched www/ index. php/eng/Information /CHED Memorandum- Orders/2005 Retrieved: 2012 October 08). A total of 56 students enrolled in the BS Agriculture program of the selected State Universities. Specifically, 28 students from SLSU, 21 from CvSU, and 7 from LSPU) completed the questionnaires at the end of their class period.

Data-gathering instrument and procedure

The study was conducted using researcher-made questionnaires which determine the student's demographic characteristics, their persistence and retention. Persistence and retention factors were measured using perception-survey statements. Persistence factors were identified using four-point Likert scale ranging from "strongly disagree" to "strongly agree". Retention factors were determined using four-point degree scale ranging from "no contribution" to "major contribution to my retention".

In addition to the student's self-administered questionnaire, 86 faculty and administrators completed a separate questionnaire during their vacancy period.

The researcher also conducted Key Informant interviews with selected members of the academy and administration including the Course Adviser, College Dean, Office of the Student Affairs (OSA) Director, University Registrar, Campus Director, and the State University's President and Vice Presidents to ask for their personal viewpoints regarding the factors that support/enhance and hinder student persistence and retention towards degree completion. Confidentiality was addressed by assigning a code number to each student as they completed the survey and using only that code to indicate survey responses.

Tools for data analysis

The study made use of the Statistical Package for the Social Sciences (SPSS) software version 16 in analyzing the data. It employed descriptive analysis (that is, weighted means) to analyze items related to personal, academic and career disposition and perceptions regarding institutional processes, academic and co-curricular procedures, faculty traits and curricular experiences; also, faculty and administration evaluations of the retention factors. The study also used Pearson's Product Moment Correlation Coefficient to determine the relationship between persistence and retention. In particular, the nature and degree of relationship were described. No test of significance was conducted.

RESULTS AND DISCUSSION

Here, presents the data gathered, and organized according to the objectives of the study. In order to facilitate analysis and interpretation, quantified data were tabulated and are presented based on the order of overall weighted mean from the highest to lowest values.

Factors that explain persistence

Persistence factors embrace all 15 statements on student-related, 17 on institution-related, and 18 on classroom-related factors. These persistence factors are represented by 50 perception statements in all are represented by S_1 – S_{15} in Table 1. BS Agriculture students from Southern Luzon State University (SLSU) representing Level I Accredited Status, Laguna State Polytechnic University (LSPU) Level II, and Cavite State University (CvSU) Level III identified persistence factors using a four-point Likert scale ranging from "strongly disagree" (1) to "strongly agree" (4).

Student-related factors

Academic disposition

Third-year BS agriculture students strongly believed that they were trying their best to complete their degree, and by taking the right courses in their chosen school. This students' academic disposition was supported by the result of the KI interviews. When asked about the essential characteristics that make BS Agriculture students successful in pursuing their degree, interviewees often described students as very hardworking, highly trainable, motivated, responsible and adaptable, meaning that students would do everything without complaint, and were very persistent to the academic goal. Majority of these students are from low-income group; hence, their aspiration to improve their way of living is always present.

As explained by Lango (1995 in Quinn and Hemmings, 2000), persistence is influenced by a person's sense of self, specific expectations, and a sense of responsibility for one's successes and failures. Likewise, family has an impact on student commitment to complete college. Parents play a key role in instilling in their children a sense of self-efficacy or a relentless drive to persist despite adversity. According to Nora et al. (1999 in Crissman and Uperaft, 2000), fostering a 'culture of possibility' encourages student achievement and influences educational aspirations and expectations.

In line with students' perceptions of being enrolled in the school of their choice, Tinto (1993 in Swail (2004) hypothesized that student commitment to educational goals and to the institution in which one enrols

Table 1. Factors that explain persistence.

Item	Statement	SLSU Level I	LSPU Level II	CvSU Level III	Overall
No.	Persistence Factors		Weighted Means		
S11	I am trying my best to be able to complete this degree.	3.64	3.57	3.71	3.66
S14	I think I would be successful.	3.64	3.57	3.71	3.66
I16	This campus has a commitment to academic excellence.	3.64	3.00	3.86	3.64
C41	Course advisers are knowledgeable about the program…	3.46	3.43	3.76	3.57
C42	The contents of the course are valuable…	3.57	3.29	3.67	3.57
S7	I am taking the right courses.	3.39	3.71	3.71	3.55
C50	I am able to experience intellectual growth here.	3.54	3.29	3.67	3.55
C44	The contents of the course are applicable to the work.	3.36	3.14	3.81	3.50
C48	The course demonstrates the ability to adapt to changes...	3.36	3.43	3.71	3.50
C49	The course helps me to perform the skills…	3.36	3.43	3.71	3.50
S3	I have a family who are supportive of my goals.	3.39	3.57	3.52	3.46
C45	The objectives of the course comply with the purpose…	3.36	3.43	3.62	3.46
C33	Faculty have mastery of the course content.	3.32	3.29	3.67	3.45
C47	The courses help me understand my everyday life.	3.32	3.43	3.62	3.45
S12	I am aware that applicable jobs are in my community.	3.29	3.57	3.57	3.43

Range: 3.26-4.00 - Strongly Agree; 2.51-3.25- Agree; 1.76-2.50- Disagree; 1.00-1.75- Strongly Disagree.

significantly influences student performance and persistence. Evidence from the work of Swail, Pascarella and Terenzini supports this notion that the institution which a college student chooses becomes an important factor in his or her persistence, because some institutions are more conducive to persistence than others. According to Swail (2004), institutional variables include selectivity, course length (two or four year), size, control (public or private), gender composition, and racial composition. However, (Oseguera and Rhee, 2009) suggest that in general, these institution-related variables are less influential than students' experiences once they enrol.

Career disposition

Agriculture students are quite optimistic in believing that they would be successful. The students involved in this study are in their junior years of their degree program, and they have crossed the threshold where they were at risk of switching majors or dropping out. Aside from the findings already cited about academic disposition, more intangible characteristics of these students are instrumental in keeping them on their academic paths. Characteristics such as being hardworking, trainable, motivated, responsible and adaptable are of a more intrinsic nature, yet seem to be held by the majority of successful students.

Personal disposition

Agriculture students credit their parents with instilling in them the importance of a college education. While members of the family can provide emotional support, it can also place demands and responsibilities that extend beyond life as a college student. For students who have to work for a part-time job in order to support the family needs, balancing their roles as a worker with the demands of their degree program can become a source of stress while attending school. Family demands were cited by several faculty members as reasons why some agriculture students have difficulty in keeping up with course work. This verifies the reason why student respondents slightly agreed with a specific statement related to personal disposition as having enough money for education. KI interviews confirmed the importance of scholarships as a source of student support. In a review of research on the relationship between financial aid and persistence (Pino, 2005) concluded that finance-related factors (student aid, tuition, and other costs, including living) explained about half of the variance in the persistence process.

Institution-related factors

Institutional processes

Here, it is shown that institution's commitment to academic excellence constitutes most to the experiences of agriculture students. This is followed by the personal safety and security that they experience on their campus; likewise, for making every student feel welcome at all times. This is supported by a study of the Azusa Pacific University, by Noel-Levitz, a higher education consulting firm (2009) (www.nasfaa.org), who found that students who feel welcome, know what is happening on campus, and feel that they belong are more likely to return the following year. Schreiner (2009 in NASFAA, 2010) found that increased student satisfaction, particularly with the campus experience, can increase student persistence and retention. According to the report of the Azusa Pacific University, satisfaction indicators almost doubled the ability to predict retention beyond demographic characteristics and institutional features (NASFAA, 2010).

On the other hand, student respondents slightly agreed with the statements related to campus procedures for student registration, and for regularly communicating student satisfaction and important data.

It became very clear in the KI interviews that BS Agriculture students not only faced transition to college life, but also to becoming part of the sub-culture of agricultural education. Successful students come to understand and navigate the "campus college system." Those who do not, they weed themselves out. Several faculty members speculated that inability to figure out such "campus college system" was most likely a large factor in losses of agriculture students at the end of the freshmen year. One interviewee spoke of the need for students to learn to organize, plan, and target the work for their classes, and that it takes time and peer mentoring to do so. As he put it: "by the time they hit the junior year the ones who have figured it out are in agriculture classes. The ones who haven't are gone." Aside from learning content, learning the process of surviving in the content classes and degree program is an expectation for a student to continue with the course. (Leach and Zepke, 2009) found that, to enhance retention, institutions must change their processes so that financial services, procedures for adding and changing courses and academic advice are easily available and offered in non-bureaucratic ways. Academic advising is one of the arenas through which a student has a chance to have quality interaction with a concerned person on campus, a primary factor affecting college retention (Lotkowski et al., 2004).

Academic and co-curricular procedures

Based on the perception of third-year BS Agriculture students, they were able to avail academic programs from quality teaching and manageable workloads. These two institution-related factors highlight academic and co-curricular procedures and explain why agriculture students remain enrolled and persist to complete their degree program.

Academic programs such as academic coaching, in which faculty members move beyond voicing support of students in the classroom to putting into practice teaching practices and interactions that foster student success. Academic coaches help students to enhance their learning, and learning outcomes, recognizing that not all students are equipped with academic, study, and social skills at the college. They can also motivate students with changes in rules, regulations, and course requirements to better meet student expectations and needs.

Classroom-related factors

Faculty traits

The majority of classroom-related factors particularly instructional skills of the teachers were experienced by agriculture students. Primarily, they acknowledged their course adviser, as being knowledgeable about the program requirements, having mastery of the course content, and treating them with respect.

However, not all of the student respondents agreed with classroom-related factors. This was indicated in their response to the statement related to the kind of treatment they received from their teachers. Basically, it shows that not all of them experienced fair and unbiased treatment from their teachers.

Faculty treatment manifests the kind of campus experience, one that mediates students' academic and social experiences in college (Vallerand and Menard, 2000). By establishing good relationships with the students, the institution shares this responsibility for helping students achieve academic and social integration (Tinto, 2002).

On the other hand, faculty and administrators view this relationship similar with that of Astin in his *Student Involvement Theory*. For them, it is the student's role to build good relationships with faculty members. They believed this is something that distinguished successful students from those who were less successful: (What makes them successful is) the degree to which they work collaboratively and consort with their peer students and take advantage of the relationships they can build with faculty. Those that tend to try to do it alone – that's a real trial for them. They learn to become more responsible. So, the degree to which they integrate with their peer students and take advantage of building faculty relationships outside of the structured class environment is a factor (in persistence). This idea is attested by the findings of Pascarella and Terenzini (Quinn and Hemmings, 2000) that both the frequency and quality of students' interactions with faculty and peers were positively associated with persistence.

Curricular experiences

Students also noted their favourable experiences towards the curriculum. Agriculture students perceived that their curriculum is valuable and sufficient, applicable to their

Table 2. Students' perceptions on factors that explain retention.

Item No.	Institutional issues, characteristics, and services (Students' Perceptions)	SLSU Level I	LSPU Level II	CvSU Level III	Overall
		Weighted Means			
R12	Quality of teaching	3.57	3.14	3.86	3.63
R16	Student engagement in classroom (active learning)	3.39	3.14	3.52	3.41
R15	Student employment opportunities	3.43	3.14	3.29	3.34
R17	Student-institution "fit"	3.25	3.00	3.43	3.29
R14	Student assessment strategies	3.11	3.14	3.48	3.25
R3	Attitude of faculty toward students	3.00	3.29	3.43	3.20
R5	Career exploration services	3.04	2.86	3.52	3.20
R20	Rules and regulations governing student behaviour	3.18	3.14	3.19	3.18
R13	Social environment	3.00	2.86	3.38	3.13
R1	Academic support services (learning centres, similar resources)	2.96	2.57	3.48	3.11
R18	Student involvement in campus life	3.14	3.00	3.10	3.11
R11	Personal contact between students and faculty	3.07	3.00	3.10	3.07
R4	Attitude of staff toward students	2.93	2.86	3.14	3.00
R6	Cultural environment	2.82	3.14	3.19	3.00
R7	Curriculum issues	2.86	3.00	3.05	2.95

Range: 3.26-4.00 - Major Contribution; 2.51-3.25- Moderate Contribution; 1.76-2.50- Little Contribution; 1.00-1.75- No Contribution.

future work, helpful in understanding everyday life, adaptable to changes in society and technology, and helpful to students in performing the skills needed. Also they experienced intellectual growth in their campuses. For agriculture students, these are the influential factors inside the classroom setting. Three interviewees who talked about the need for social relevance of the Agriculture curriculum as a factor related to persistence for students; connections to the "real-world" and to relevance to society may be factors in helping to retain BS Agriculture students towards degree completion.

Social relevance of the curriculum corresponds with the views of FAO (Maredia, 2007) that for the higher educational system to be effective in fulfilling its role in supplying well-trained and productive work force for the agricultural economy, the curriculum must adapt to changes in society and technology to prepare students for taking up or creating new employment opportunities. It must be flexible in creating unique interdisciplinary majors to allow students to meet individualized curriculum program needs. A curriculum should focus on imparting skills and abilities that are transferable to a wide range of occupations; and puts emphasis on processes and abilities of students to critically think and solve problems which are relevant to societal needs.

Social relevance of the curriculum as advocated by the faculty and administration, guides them in dealing with curricular issues. Basically, the results point to the positive actions of the State Universities to better serve the community and/or society by way of promoting the intellectual, personal, social-, and physical development of the students.

Table 2 presents the factors that explain retention of third-year BS Agriculture students. These 20 items on retention factors deal with the student perceptions of the institutional issues, characteristics, services, also their evaluation of their institution's ability to maintain the enrolment by implementing some student retention "best" practices. Student respondents identified the retention factors using four-point degree scale ranging from "no contribution" (1) to "major contribution to my retention" (4) Retention factors are represented by $R_1 – R_{20}$ in Tables 2 and 3.

Factors that relate to retention

Students' perceptions

(i) Quality of teaching: Consistent with the results gathered on the institution-related factors, it is the quality of teaching that had the highest perception rating from the student respondents. This indicates that the selected State Universities were supportive of quality teaching as reflected in the students' high perception rating of classroom-related factors in their curricular experiences. Accordingly, students most appreciated classes where "real-world" connections were made. One of the teachers interviewed in the study talked about being very explicit in making those connections. That was, his way of connecting the learning within the course to other classes they were taking. Four faculty members discussed the need for more visual presentation of material as a pedagogical technique benefiting students. When prompted

Table 3. Faculty and administration perceptions on factors that explain retention.

Item No.	Programs, curricular offerings, services, practices (Faculty and Administration)	SLSU Level I	LSPU Level II	CvSU Level III	Overall
		Weighted Means			
R6	Curriculum review and revision practices	3.64	3.45	3.76	3.62
R9	Faculty development program	3.68	3.45	3.52	3.58
R1	Academic support services (learning centres, similar resources)	3.86	3.29	3.64	3.57
R2	Admissions practices/requirements	3.68	3.26	3.58	3.49
R13	Honour students programs	3.27	3.35	3.42	3.40
R3	Assessment programs	3.32	3.29	3.48	3.37
R14	Interactive, relevant, hands on, exploratory instructional practices	3.14	3.23	3.52	3.35
R10	Financial aid services	3.36	3.23	3.45	3.35
R4	Career planning and placement programs	3.23	3.58	3.12	3.31
R8	Extracurricular programs	3.27	3.29	3.33	3.30
R18	Student services (housing, personal counselling, academic advising)	2.82	3.03	3.55	3.21
R15	Rules and regulations governing student behaviour	3.00	3.06	3.36	3.20
R11	First-year programs	2.55	3.03	3.67	3.19
R16	Social activities programs	2.95	3.13	3.36	3.17
R17	Social skills course/program	3.00	3.13	3.18	3.12

Range: 3.26-4.00 - Major Contribution; 2.51-3.25- Moderate Contribution; 1.76-2.50- Little Contribution; 1.00-1.75- No Contribution.

for what they meant about "visual" material, faculty referred to material that allows the students to manipulate things, or draw what they are learning. Two faculty members expressed awareness of the need for instruction that incorporated multiple modes of learning. An instruction that accords with versatility (that is, meeting needs of diverse groups), which is a desired characteristic of the curriculum (FAO, 2000 in Maredia, 2007).

(ii) Student engagement: Student engagement in classroom or their exposure to active learning was found to be the second in the list of the retention factors among the third-year BS Agriculture students. This agrees with the finding of Tinto (1993 in Tinto, 2002) that academic engagement activities have a positive influence on retention.

(iii) Institutional fit. Institutional fit means that institution's curricular and co-curricular programs fit with the student's personal, academic and career interests. Institutional fit was another factor to BS Agriculture students. Many interviewees found that taking on leadership roles in organizations, being active in various campus activities, mentoring younger students and working closely with their teachers contributed to student retention towards degree completion.

This finding agrees with those of several researchers including McClanahan (2004) and Habley (2010), who affirmed that institutional fit and campus integration are important to retaining college students towards degree completion (Vallerand and Menard, 2000). Similarly, Lotkowski, 2004 noted that most definitions of fit exhibit characteristics of students' interactions with the academic and the social, or non-academic, systems of the college. They further noted that these academic and social interactions affect both student retention and educational attainment. Several authors indicated that the roots of student attrition lay both with students and with the institution; in other words, the success of an institution and its students are inseparable (Levitz et al., 1999; Tinto, 1999 in Tinto, 2006).

Table 3 presents the factors that explain retention of third-year BS Agriculture students. These 20 items on retention factors deal with the faculty and administrators perceptions of the institutional programs, curricular offerings, services and their evaluation of their institution's ability to maintain the enrolment by implementing some student retention "best" practices. faculty and administrators identified the retention factors using a nominal scale (no/yes), and a four-point degree scale ranging from "no contribution" (1) to "major contribution to retention" (4).

Faculty and administration perceptions

With reference to the perceptions of faculty and administration, the high-rated retention factors have to do with curriculum review and revision practices, faculty development programs, and the availability of academic support services as contributions to the retention of BS Agriculture students towards degree completion. It should be noted here that there is conformity between the way that students and members of the faculty and administration view these factors as contributing to institutional retention. The lowest perception ratings for students were the availability of student services in a form of personal/academic counselling/advising, and financial aid services have something to do with the way that faculty and administration perceived the availability of early-alert and intervention programs and the tutoring/mentoring program as the lowest among the retention factors provided in the questionnaire.

Issues related to course availability, content, and instruction affect a student's ability to persist; hence, institutional programs and services should have support mechanisms such as tutoring, mentoring, and career counselling to have positive effect on the student retention (Swail, 2004). The significance of taking into account institutional factors equally with student-related factors and social/external factors is to underscore the importance of campus participation and knowledge in students' social and academic development. It is in fact the college that forms the foundation for college success. It is the institution that can identify and match the needs of individual students, a student cohort group, or the student body as a whole (Swail, 2004).

Faculty and administration made reference to students needing to "learn the system" with respect to being successful college students. Learning the system occurs on several levels. A successful student learns how to navigate university and campus life including locating and utilizing campus support programs. A successful student must learn productive study and work habits. Likewise, a successful student must come to comprehend the way learning takes place within agriculture. This includes adapting to or being naturally inclined toward traditional pedagogical methods, and being comfortable with the expectation that a large part of their learning occurs outside the classroom where they experience actual wading through mud and exposure to sunlight.

Faculty members, and especially academic advisers were clear that students "must undergo an orientation" during the registration process. Faculty members in the study approved the idea of Pino, 2005 and Leach and Zepke, 2009 seeing it as the responsibility of the student to figure things out on their own or with the help of their peers. If students have figured out the system of the degree program, have formed productive peer relationships in the form of study groups and/or mentors, and have strong intrinsic motivation and goal-commitment, the challenges of the curriculum can be overcome and they are successfully retained. The good students figure it out, but some change majors or leave the university. Furthermore, where the students' cultural practices are deemed inappropriate, incongruent with that of the institution (Pino, 2005), deficient, or invalidated (Leach and Zepke, 2009), students are more likely to experience acculturative stress (Leach and Zepke, 2009) and to leave. Acculturative stress happens when students experience psychological stress resulting from imposing other culture (that is, institutional practices) to them. Hence, as the integration model suggests, in order to succeed, college students should abandon their cultural background (i.e., student personal and academic interest) and adapt to the institutional culture (Tinto, 1975, 1993; Vallerand and Menard, 2000).

Relationship between persistence and retention

The analysis indicates a linear relationship among the weighted means of the given variables. The degree of correlation of retention with overall and individual persistence factors including student, institution, and classroom-related ones, ranges from substantial to very high, as reflected by r values indicated in Table 4.

Overall, there is a very high positive relationship between institution-related factors and retention of BS Agriculture students ($r = 0.786$), a substantial relationship between classroom-related factors and retention ($r = 0.684$), and a moderate relationship between student-related factors and retention of BS Agriculture students ($r = 0.480$).

This indicates that students' persistence in completing their degree changes (either increases or decreases) with the level of institution's retention practices. BS Agriculture students who find their personal attributes, institution and classroom experiences relevant and meaningful to their chosen degree, and realize that these persistence factors are being supported by the institution's way of implementing the student retention practices in providing better education, much more likely to develop definite aspirations towards completing the four-year bachelor's degree in the same institution.

This adds to the substantial evidence of Kulik and Schwab (1983 in Lau, 2003) that, when students participate in the services and programs designed to enhance their success, they are more likely to persist and remain in the same institution. Similarly, the stronger the goal and institutional commitment, the more likely that the student will graduate (Tinto, 1993 in Tinto, 2002).

However, it has to be noted that the relationship is low between retention and academic performance. This affirms how students' retention is related to or influenced by factors other than the hope of getting a better grade from the institution. This further supports the perception of BS Agriculture students regarding issues on faculty traits concerning fair and unbiased treatment to students inside and outside the classroom.

The results of analysis of the differences in persistence and retention across accreditation levels revealed slight variations in the perception rating of students in some statements related to physical fitness, academic performance, and knowledge about career opportunities. Nonetheless, there seem similarities in most of the statements concerning personal and academic disposition. Financial support plays an important role in bringing students to college and in retaining them; and

Table 4. Correlation coefficients between retention and persistence factors.

Variables	SLSU	LSPU	CvSU	OVERALL
Student-Related Factors	0.633	0.577	0.168	0.480
Institution-Related Factors	0.742	0.666	0.883	0.786
Classroom-Related Factors	0.675	0.448	0.676	0.684
Overall Persistence	0.749	0.607	0.829	0.765
Academic Performance	-0.228	0.268	0.155	-0.094

r value: 1.0- Perfect; 0.70 to 0.99- Very High; 0.50 to 0.69- Substantial; 0.30 to 0.49- Moderate; 0.10 to 0.29 Low;0.01 to 0.09- Negligible.

faculty and administration perceived the effectiveness of their institution's scholarship program in helping students to find financial assistance to offset the costs of their education. But still, BS Agriculture students –particularly low-income students- find it increasingly difficult to afford their college education and they see it as one of the major barriers towards degree completion.

Additional similarities have been depicted in most of the perception rating towards classroom-related factors, although the effect of institution-related factors appear different across the accreditation levels of the selected State Universities in Region IV-A, Philippines. This signifies how accreditation level plays a crucial role in the student's perception of the institution-related factors that contribute to their persistence towards degree completion. Degree accreditation level indicates how institutional processes, academic and co-curricular procedures are structured and implemented enough so as to achieve educational objectives. This was attested by the results on the factors that support retention of BS Agriculture students across accreditation levels based on the perceptions of students along with the faculty and administration. The higher the level of degree accreditation of an institution (that is, CvSU), the higher is its evaluation rating in terms of the way students perceive and experience upon staying in their institution. While the opposite is true for LSPU and SLSU, in that the lower the level of degree accreditation status, the more its need to focus on addressing institutional issues, programs, curricular offering, services and practices to be able to support/enhance persistence and retention of BS Agriculture students towards degree completion.

Conclusions

The results of this study suggest that, as indicated by the responses of the students as well as the faculty and administrators interviewed, the selected State Universities in the Philippines have not gone far enough to ensure that BS Agriculture students are supported in an effective manner. The junior and senior university officials need to more directly support this curricular program so that Agriculture serves as these agricultural state universities' major thrust.

However, the conclusions should not suggest that reenrolment or retention alone should be the goal of an institution for its students. For if retention alone becomes the goal, institutions will find themselves engaged in trying to hold students at all costs. Pressuring students to stay when it is not in their best interests to do so is not only wrong morally but also counterproductive: it often results in an accelerated attrition rate (Noel in Braxton, 2009).

Instead, as Noel argued, "The more students learn, the more they sense they are finding and developing a talent, the more likely they are to persist; and when we get student success, satisfaction, and learning together, persistence is the outcome' (P. 1).

RECOMMENDATIONS

Students who persist beyond their sophomore years are often highly motivated individuals with the ability to adapt to the challenging system of the degree program. For agriculture students, all possible effort should be made to support those who have found the right choice in an agriculture field. State Universities have the opportunity to make positive changes in persistence and retention of these special populations of agriculture students. Several areas point toward the potential to make an impact. The following section presents some of the suggested policies for improving the institutional programs, curricular offerings, services and practices of the selected State Universities in Region IV-A, Philippines:

Combining academic and non-academic factors

Integrating academic and non-academic information enables colleges to design and implement courses and programs that address both types of needs. Such programs may include first-year orientation programs, academic advising and tutorials, workshops in study skills, time management skills, critical thinking, planning, assertiveness training, library use, and cultural awareness. These programs should aim to increase levels of academic self-confidence, achievement motivation, goal and institutional commitment, and social involvement and support. These programs should strengthen ties between faculty and students and between students and their peers, through the creation of a socially inclusive and supportive academic environment; a campus environment characterized by fairness toward students.

Intentional institutional interventions

State Universities can use various types of academic and non-academic information to develop and design their retention programs. Non-academic information may be derived from formal college surveys such as Your First College Year Survey questionnaire, first-year college experience orientation programs, and college student inventories and profiles. Academic and non-academic information enables State Universities to develop and maintain a comprehensive student profile that can serve as both a performance indicator and a way to identify potential dropouts. This information alerts institutions to students who may have potential difficulties and enables them to direct these students into retention programs before their risk of dropping out increases.

Evaluation of early-alert and intervention programs

The economic impact of college retention programs should be determined through a cost-benefit analysis of student dropout, persistence, assessment procedures, and intervention strategies to enable informed decision-making with respect to types of interventions required academic and non-academic, including remediation and financial support. To make informed decisions, State Universities need to assess the costs of student dropout and time to degree completion with the benefits of improved student retention and graduation rates to determine the cost effectiveness of retention strategies, assessment procedures, and interventions-including remediation and financial support. Additionally, resource availability and allocation must be assessed with respect to the costs of program provision and the benefits accrued from improved college graduation rates.

Evaluation of student programs and services

As a means of empowering agriculture students, develop a way of gathering student feedback on programs on a yearly basis. This could be accomplished through exit interviews with seniors and open-ended anonymous surveys that offer a way to gather in-depth information from students for program improvement. Until faculty and administration listen to and recognize student concerns, change will not occur.

However, to bring about change, requires leadership from industry, from educational providers and from government, and the need to improve agricultural productivity and competitiveness must be the driver for this change. There is a need to see the efforts of the following stakeholders:

Agricultural industry leaders

The industry marketing itself positively, promoting not only its future importance to the country's food supply and environmental stewardship, but also its requirement for highly motivated people with high levels of skill at all levels.

Education sector leaders

State Universities and Colleges working in close coordination with the industry to develop and promote courses that will be relevant in this new and challenging era of the global marketplace and emphasizing the importance of land-use to food and energy supplies. This should be aimed not only at young people, but also at providing training opportunities for those in work.

Policy makers

Government providing the necessary impetus and incentives for educational institutions to follow this path. Most importantly, the government's programs for providing more jobs/items that are related to the studies of those students who successfully graduated the degree in agriculture. Such jobs are not necessarily being a farm worker to promote agricultural sector as a competitive career destination of young people. It is by this way that the government will be responsive enough to the importance of attracting and training the next generation of farmers and employees in the agricultural industry. Workers who are progressive, entrepreneurial and have outstanding business management skills crucial to the economic growth of the country. Student in the program and workforce in agriculture is an issue which has global implications in a world that is becoming smaller due to advances in technology and communications. Insights into reasons for the continued lack of representation are paramount to changing the landscape of the agriculture workforce.

This study has helped to illuminate ways in which students are supported and challenged in their academic pursuits in BS Agriculture program. Many of the issues that surfaced in the study support much of what has been written in the literature related to students in the selected State Universities' BS Agriculture programs. Where the study offers its most significant contributions is in contextualizing the findings to the institutions under study and offering data on which to base programmatic improvements. This study has implications for not only educational processes during the course or in particular to the university but also the marketing of agriculture all over the country, and the targeting of that marketing effort.

The opportunity to enrol in a college level and the ability to complete educational objectives (e.g., occupational training, certificate or degree attainment) should define college access and success. Only a concerted effort by policy-makers, educational providers and other interested stakeholders can lead to equity and excellence in college education. By improving students' awareness and academic preparation, changing college finance structures and enhancing institutional responsibility, the nation can extend this vital opportunity to a larger, more diverse population of agricultural practitioners.

Conflict of Interest

The authors have not declared any conflict of interest.

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Cellulases produced by the endophytic fungus *Pycnoporus sanguineus* (L.) Murrill

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Cellulases are enzymes that act on the hydrolysis of cellulosic substrates and comprise a complex capable of hydrolyzing cellulose and producing glucose. This conversion can be performed by an enzymatic complex found in secretions of such microorganisms as fungi and bacteria. The challenge is to transform this conversion into a quicker and more cost-effective process. The objective of this study was to evaluate the activity of the cellulase produced by the fungus *Pycnoporus sanguineus* (L.) Murrill, strains D1-FB, D3-FB, D5-FB and D10-FB, isolated from *Baccharis dracunculifolia* D. C. (*Asteraceae*), in the production of cellulolytic enzymes. This study was conducted using sugarcane bagasse enriched with carboxymethylcellulose at 1% as a substrate. The material was kept in an incubator at a temperature ranging from 25 to 45°C for 43 days, with the cellulase activity being quantified every seven days. The indirect quantification method was employed to quantify reducing sugars that were determined by reaction with Domain Name System (DNS). After evaluation, it was observed that the fungus strain *P. sanguineus* (L.) (D10-FB) presented the highest cellulase activity, with values of 16.32±2.65 IU/g of fermented substrate after 29 days of fermentation, using sugarcane bagasse as the substrate, at a temperature of 30°C and pH 5.5.

Key words: Cellulase, sugarcane bagasse, cellulose, *Pycnoporus* sp.

INTRODUCTION

Among naturally occurring materials, cellulose is the most abundant biopolymer in the world (Bayer and Lamed, 1992) and can be hydrolyzed by acids into glucose. Microbial degradation of cellulose is total and specific and has encouraged the use of cellulolytic fermentation processes by man. In nature, these

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processes represent the greatest source of carbon for soil (Sakumaran et al., 2009; Sanchez, 2009).

The hydrolysis of cellulose by cellulases results in the final product of glucose. Because these are proteins, however, they cannot easily penetrate the lignin barrier of plant cells and, as such, the difficult access of these enzymes to the cellulose fibers is the main problem in triggering of the degradation process (Thiemann et al., 1980). The hydrolysis of cellulose can occur through physical, chemical, and enzymatic processes. Enzymes produced by fungi stand out among the enzymatic processes (Castro and Junior, 2010; Onofre et al., 2014).

In recent years, there has been growing interest in the use of agricultural waste to produce renewable fuels such as cellulosic ethanol. In addition to the large-scale production of ethanol from sugarcane in Brazil, the production of alcohol derived from lignocellulose is also a viable and sustainable alternative, especially in the context of an impending world energy crisis (Sakumaran et al., 2009; Sun, 2009; Mussatto et al., 2010; Shrestha et al., 2010; Bansal et al., 2012).

Currently, the surplus of sugarcane bagasse available for the production of second-generation ethanol lies between 7 and 10% of the total bagasse generated in Brazil. In the harvest of 2010/2011, the total production of sugarcane bagasse was estimated at 163 million tons. The surplus bagasse could reach as high as 50% after the proper optimization of the combustion system of independent distilleries (Balat and Balat, 2008; Bressan Filho, 2011; Onofre et al., 2014).

In addition to sugarcane bagasse, other substrates have been used to obtain large quantities of cellulolytic enzymes from microorganisms (Vitti, 1988). Agricultural waste can be properly milled or crushed to serve as a source of nutrients, mostly carbon in submerged fermentation. Among this waste, however, sugarcane bagasse has been the most widely employed because of the quantity of enzymes and protein enrichment produced by it. The bioconversion of bagasse has been proven to be potentially economically advantageous in such cases as the production of enzymes (Pandey et al., 2000; Pandey et al., 2005; Goncalves et al., 2008; Sakumaran et al., 2009; Arantes and Milagres, 2009; Limayem and Riker, 2012; Onofre et al., 2014).

The fungi that decompose cellulosic substances occur in the soil, colonizing plants, their roots and residues, and playing an important part in the recycling of nutrients. Microorganisms have a high capacity for multiplication; they can adapt to various nutritional media, and synthesize a variety of chemical substances such as enzymes (Andreaus, 2002; Goncalves et al., 2008).

The enzymes produced by fungi are biological catalysts consisting of protein molecules produced by living cells. These biocatalysts have high catalytic activity and specific selectivity on the substrate (Said and Pietro, 2004; Onofre et al., 2014).

Among microorganisms, fungi stand out in the production of enzymes of industrial interest. They are eukaryotic, uni- or multicellular, heterotrophic, chemoorganotrophic, and aerobic or microaerophilic organisms. Some have cell walls composed of chitin and cellulose. With respect to nutrition, fungi absorb food, accumulate glycogen as reserve substance, and occur in diverse life forms ranging from saprobionts, commensal organisms, and symbiotes to parasites (Teixeira et al., 1999; Limayem and Riker, 2012).

The fungus *Pycnoporus sanguineus* (L.) Murrill stands out for having applications ranging from popular medicine to alternative plant disease control methods, with a potential for inducing resistance when the extract of its basidiocarps is used (Viecelli et al., 2009). This fungus is a basidiomycete and an efficient producer of a polyphenol oxidade that acts on a variety of aromatic hydrogen donors called laccases. It has been successfully used in the fermentation of agro-industrial waste, the decolorization of Kraft effluent, and in various dyes (Valeriano et al., 2007).

Despite the limited number of publications on this fungus, there is a growing trend to employ it in biotechnological processes. The most recent report on the use of this fungus was in the fermentation of agro-industrial waste, with good results being obtained regarding the growth of the fungus, highlighting the cellulolytic, proteolytic, lipolytic, and amylolitic compounds (Bononi and Grandi, 1999; Ferraz, 2004; Garcia, 2006).

It is therefore imperative that more research be done on this fungus to obtain new products that can be applied in productive activity, especially the enzymes, which can be applied to various fields of agro-industry.

It is in this context that this study evaluated the capacity of the endophytic fungus *P. sanguineus* (L.) Murrill, isolated from *Baccharis dracunculifolia* D.C. (*Asteraceae*), to produce cellulolytic enzymes.

MATERIALS AND METHODS

Study location

The experiments were carried out in the Chemistry and Microbiology Laboratories of the institutions União de Ensino do Sudoeste do Paraná - UNISEP - Francisco Beltrão, Paraná, Brazil and Universidade Comunitária da Região de Chapeco - UNOCHAPECÓ – Chapecó, Santa Catarina, Brazil.

Microorganisms

The strains of the endophytic fungus *P. sanguineus* (L.) Murrill D1-FB, D3-FB, D5-FB, and D10-FB, isolated from *B. dracunculifolia* D.C. (Asteraceae), kept in the mycology collection of the Microbiology Laboratory of the Paranaense University - UNIPAR - Campus Francisco Beltran - PR. All the collections were authorized by IBAMA (Brazilian Institute for the Enviroment) under protocol number 13.234-2, August 1st 2006.

Determination of cellulolytic activity

Sugarcane bagasse, washed successively in running water to completely remove the sugars, was used as the basic support to determine cellulolytic activity. The bagasse was dried in a drying oven until a constant weight was obtained. It was subsequently ground in a mill, packed, and stored in a dry place for further testing (Onofre et al., 2014).

Fermentation in the supplemented medium

A semi-solid culture medium consisting of 10 g of dehydrated sugarcane bagasse supplemented with 0.5 g/L carboxymethylcellulose, 1 g/L glucose, 3 g/L $NaNO_3$, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, 0.5 g/L KCl, 10 mg/L $FeSO_4.7H_2O$, 5.0 mg/L $ZnSO_4$, and 3 g/L urea was used to assess the cellulolytic activity. All medium components were added to 120 mL of distilled water and then autoclaved for 15 min. A total of 30 mL of a spore suspension (±10^8 mL) was used for inoculation. The material was incubated at 28°C for 21 days, after which the first analysis of enzyme activity was carried out. Further analyses were then carried out every three days until 43 days of incubation were completed (Onofre et al., 2014).

Fermentation in the non-supplemented medium

A semi-solid culture medium containing 10 g of dried and ground sugarcane bagasse was used. 0.5% carboxymethylcellulose and 0.1% glucose in 120 mL of distilled water was added to it. The medium was then autoclaved for 15 min. A spore suspension (±10^8 mL) was used for inoculation. A total of 30 mL of this inoculum suspension was added to each vial containing the culture medium. The material remained in an incubator at 30°C for 22 days, when the first enzyme quantification occurred. The cellulolytic activity was measured every seven days until day 43 of fermentation. As the first step to 22 days, after that every seven days.

Substrate fermentation analysis

Aliquots of 5 g of the medium of each strain were weighed and placed in Erlenmeyer flasks. A total of 50 mL of deionized sterile water was added to each flask, which were stirred for 30 min at 30°C. The solution was filtered to remove the solids and the pH was determined after adding 2 mL of a 7.0 phosphate buffer. The extract was centrifuged at 3,000 rpm for 15 min and the supernatant was the enzyme source to determine the reducing sugars by the indirect spectrophotometric method. This method was used to determine enzyme activity based on the release of glucose molecules by the action of the cellulolytic enzyme complex (Moura et al., 2012; Santos et al., 2012; Onofre et al., 2014).

Determination of reducing sugars

Reducing sugars were determined by reaction with 3,5-dinitrosalicylic acid (DNS) (Onofre et al., 2012, 2014). In a basic medium and at high temperatures, this acid becomes 3-amino-5-nitrosalicylic acid, developing a red color and absorbing light at 540 nm. One unit of cellulase was defined as the amount of enzyme released on the substrate that releases 1 μmol of reducing sugar, expressed as glucose, *per* minute, under test conditions (Alazard and Raimbault, 1981; Onofre et al., 2011; Onofre et al., 2014).

pH optimization

The enzymatic activity of the strains was evaluated under different pH values (4.0, 5.0, 5.5, 6.0, and 6.5). These tests were conducted to determine the optimum pH for subsequent assays.

Effect of temperature

Tests were carried out at temperatures of 25, 30, 35, 40, and 45°C, under optimal pH conditions, to verify the optimum temperature for the production of the cellulolytic complex.

Statistical analysis

Results were submitted to variance analysis using the statistical analysis system assistant (Silva and Azevedo 2002). The comparison of means was done by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

After obtaining the data for the production of cellulases by the four endophytic strains of *P. sanguineus*, this information was grouped together and entered into Tables 1, 2, 3, and 4, and in Figures 1 and 2. The values contained in Table 1 are linked to the enzymatic volumes produced by strains at different pH values.

Among the physical parameters, the pH of the growth medium played an important role by inducing morphological changes in the organism and also in the enzyme secretion. The change in pH observed during the growth of an organism also affected the stability of the product in the medium (Pandey, 2003; Sales et al., 2010).

The pH of the cultures varied because of reactions that occur during the metabolic activities of the microorganism. When organic acids such as acetic or lactic acid are secreted, they cause a decrease in pH. However, the consumption of these acids, when they are present in the medium, causes an increase in pH. The nitrogen source used may also cause variations in pH. Ammonia salts, for example, usually decrease pH during cell growth because of the formation of hydrogen ions during the consumption of ammonia (Doelle et al., 2002).

The performed tests revealed that the greatest production of the cellulolytic complex occurred in the four studied strains when the pH was 5.5. Decreases occurred at pH levels below 4.0, 6.0, and 6.5.

The solid state fermentation (SSF) processes are exothermic, as a lot of heat is released in direct proportion to the metabolic activity of the microorganism. In filamentous fungi, the temperature has a direct influence on the germination of spores, growth, and product formation. Temperature is a critical factor in practically all SSF processes because of the accumulation of generated metabolic heat (Pinto, 2003).

Sato and Sudo (1999) reported that the temperature affects both the growth and the production of enzymes, making an efficient removal of heat necessary. In this sense, Bianchi et al. (2001) add that, because of the metabolic activities of microorganisms and depending on the substrate layer, a large amount of heat can be

Table 1. Behavior of the four endophytic strains of *Pycnoporus sanguineus* in the production of cellulases under the influence of various pH values.

Isolates	pH assessed				
	4.0	5.0	5.5	6.0	6.5
D1-FB	2.20±0.22^{bB}*	3.21±0.40^{bB}	11.44±1.02^{aA}	4.49±0.15^{aB}	3.45±1.04^{aB}
D3-FB	4.12±0.13^{aB}	3.42±0.72^{bB}	7.33±1.33^{bA}	3.47±0.24^{aB}	2.14±0.13^{aB}
D5-FB	5.53±1.09^{aB}	6.38±1.04^{aB}	9.52±1.65^{aA}	3.74±0.28^{aC}	3.63±0.21^{aC}
D10-FB	5.59±0.05^{aB}	4.42±0.54^{bB}	10.40±1.78^{aA}	3.85±0.33^{aC}	2.22±0.32^{aC}

*Means followed by lowercase letters vertically and capital letters horizontally do not differ from one another for the level of 5% by the Tukey Test.

Table 2. Behavior of the four endophytic strains of *Pycnoporus sanguineus* in the production of cellulases under the influence of various temperatures at pH 5.5.

Isolates	Temperature evaluated (pH 5.5)				
	25	30	35	40	45
D1-FB	0.90±0.23^{bC}*	10.03±0.20^{bA}	9.31±0.69^{aA}	6.33±1.01^{aB}	1.55±0.33^{aC}
D3-FB	1.03±0.20^{bB}	12.25±1.78^{bA}	8.25±0.35^{aA}	5.43±0.78^{aB}	2.22±0.31^{aB}
D5-FB	1.22±0.45^{bC}	13.42±2.87^{aA}	9.88±0.93^{aA}	5.23±0.86^{aB}	3.67±0.23^{aC}
D10-FB	3.21±0.37^{aB}	9.21±2.09^{aA}	7.44±0.08^{aA}	6.21±0.68^{aA}	3.34±0.19^{aB}

*Means followed by lowercase letters vertically and capital letters horizontally do not differ from one another for the level of 5% by the Tukey Test.

produced during the SSF process. Because temperature directly affects the germination of spores, the growth and sporulation of microorganisms, and product formation, the produced heat should be immediately dissipated if increases in temperature are not to affect the process.

The data in Table 2 reveal that 30°C is the optimum temperature for the growth and production of celulolytic enzymes for the four endophytic strains of *P. sanguineus* studied. For temperatures below and above 30°C, one can observe that the enzymatic volumes decrease for all the strains under study. With the data obtained for optimum pH and temperature, it was possible to continue the study using a temperature of 30°C and a pH of 5.5.

It should be stressed that temperatures close to 30°C comprise a range of temperatures in which mesophilic microorganisms develop. These data are in line with the concepts of Buswell et al. (1998), who showed that 30°C is an ideal temperature for the production of the celulolytic enzymatic complex by fungi of the genus *Pycnoporus.* This behavior is consistent with those found by Jan and Chen (2003), Dalsenter et al. (2005), Mitchell et al. (2006), Gomes et al. (2007), Carvalho et al. (2008), and Taneda et al. (2012).

Regarding the optimal pH for the production of cellulolytic enzymes, Camassola et al. (2004) stated that the largest production of these enzymes by *Penicillium echinulatum* occurs at pH 5.5. Alam et al. (2008) optimized the cellulase production process by *P. sanguineus* through the bioconversion of the liquid state of domestic sewage sludge and also found maximum activity at pH 5.5. This same behavior was observed by Qin et al. (2008), Zhang et al. (2012), Bendig and Weuster-Botz (2012), and Puglisi et al. (2012).

An analysis of the data in Table 3 and Figure 1 revealed that the four evaluated strains presented the same behavior in the production of the cellulolytic complex, with the production of enzymes starting at the 22nd day of fermentation and reaching maximum production values at the 29th day of the process. From then on, a decline occurred in the volumes of produced enzymes.

The highest production occurred at the 29th day, with values of 14.33±2.56; 12.73±2.34; 13.73±1.86; and 16.32±2.65 IU/g of substrate for D1-FB; D3-FB; D5-FB; and D10-FB, respectively, with no significant differences in the Tukey test at the level of 5%.

When the behavior of the four strains of *P. sanguineus* shown in Tables 3 and 4, subjected to growth in a non-supplemented culture medium is considered, one can observe that the volumes of enzymes produced over the 43 days of fermentation were lower than the volumes produced by the four strains grown in a medium supplemented with salts.

The enzymatic volumes produced at 22 days were 1.58±0.76, 6.42±1.23, 9.50±2.20, and 7.65±1.95 for the strains D1-FB, D3-FB, D5-FB, and D10-FB, respectively. These enzymatic indices differ at a significance level of 5%, as the strain D1-FB produced volumes that were lower than the other strains under study.

It should be pointed out that these volumes are lower than the volumes produced by the same strains at the same time but in the presence of supplements based on

Table 3. Cellulase activity (IU/g of substrate) produced by endophytic strains of *Pycnoporus sanguineus* in a supplemented medium.

Isolates	22	29	36	43
D1-FB	1.58±0.76^{bC}*	14.33±2.56^{aA}	7.99±0.47^{bB}	3.14±1.45^{aB}
D3-FB	6.42±1.23^{aB}	12.73±2.34^{aA}	5.93±0.78^{cC}	2.21±0.36^{aC}
D5-FB	9.50±2.20^{aB}	13.73±1.86^{aA}	6.56±1.58^{aC}	3.06±0.82^{aD}
D10-FB	7.65±1.95^{aB}	16.32±2.65^{aA}	4.82±0.74^{cC}	2.12±0.89^{aC}

*Means followed by lowercase letters vertically and capital letters horizontally do not differ from one another for the level of 5% by the Tukey Test.

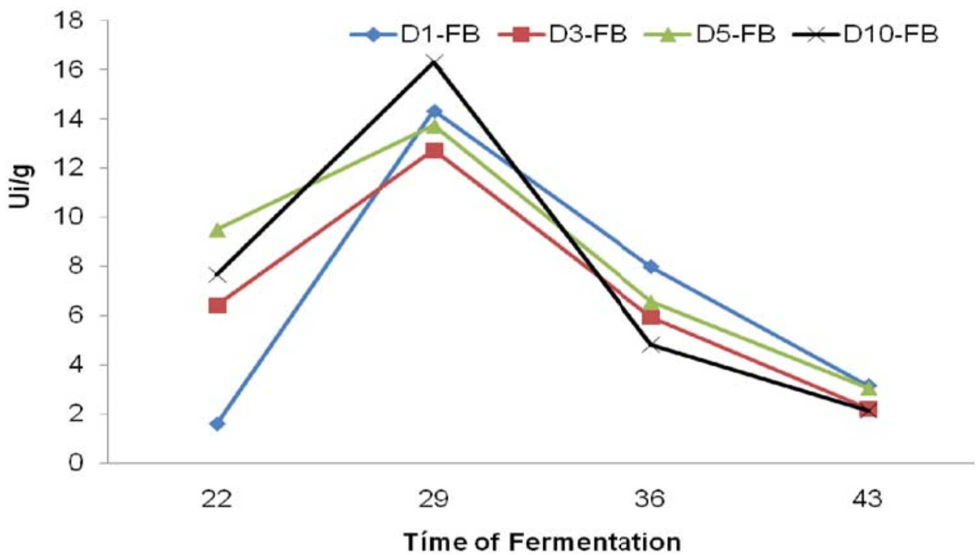

Figure 1. Behavior of the four endophytic strains of *Pycnoporus sanguineus* in the production of cellulases in a supplemented medium.

salts. The data in this study can be compared with data obtained by Aguiar (2008), who used the same culture conditions in 30 days of fermentation and observed that the fungus *P. sanguineus* produced 16.21 IU/g of the cellulolytic complex in a supplemented medium and 5.76 IU/g in a non-supplemented medium.

One can see similar data at 29 days when the endophytic strain FB4 produced 16.32 IU/g in a medium supplemented with salts and 7.93 IU/g in a medium that was not supplemented with salts. Basso et al. (2010), on the other hand, evaluated the cellulolytic activity of strains of *P. sanguineus* isolated from decomposing sugarcane bagasse and wood and observed values of 12.58 IU/g of non-supplemented substrate at a temperature of 28°C at 35 days. Once again, the data are similar to those found in this study, but when the behavior in the non-supplemented medium is considered, the values obtained in this study are lower, having reached 7.93 IU/g at 29 days (Table 4 and Figure 2).

The data obtained in this study are also similar to those obtained by Rifat et al. (2003) and Falkoski et al. (2012), who evaluated the crude enzymatic extract produced by *P. sanguineus* and observed that this fungus produces xylanases, cellulases, mananases, α-galactosidase, α-arabinofuranosidase, and pectinase in a culture medium, using a substrate based on sugarcane bagasse, at the same temperature and pH.

With respect to the substrate based on sugarcane bagasse supplemented with glucose, one can see by its having been present in both systems that it does not interfere with the production of cellulase. These results are in line with those of Rifat et al. (2003) and Castro (2006). They compared the production of endoglucanases and β-glucosidase by *P. sanguineus* and *Humicola grisea* when grown on insoluble (cellulignin from sugarcane bagasse and avicel) and soluble (carboxymethylcellulose and cellobiose) substrates.

They observed slower kinetics in the production of enzymes when insoluble sources were used, especially those of lignocellulosic origin, as a result of an initial period of acclimation of cells to this raw material. Regarding carboxymethylcellulose and cellobiose,

Table 4. Cellulase activity (IU/g of substrate) produced by endophytic strains of *Pycnoporus sanguineus* in a non-supplemented medium.

Isolates	22	29	36	43
D1-FB	1.79±0.23^{aC}*	10.85±0.34^{aA}	9.18±1.25^{aA}	3.24±0.65^{aB}
D3-FB	2.27±0.46^{aC}	8.02±1.45^{aA}	5.02±0.89^{bB}	3.95±0.79^{aB}
D5-FB	1.69±0.32^{aB}	9.88±0.79^{aA}	7.04±1.02^{bA}	3.67±0.54^{aB}
D10-FB	1.43±0.78^{bC}	7.93±0.68^{aA}	5.56±0.45^{bB}	2.83±0.34^{aC}

*Means followed by lowercase letters vertically and capital letters horizontally do not differ from one another for the level of 5% by the Tukey Test.

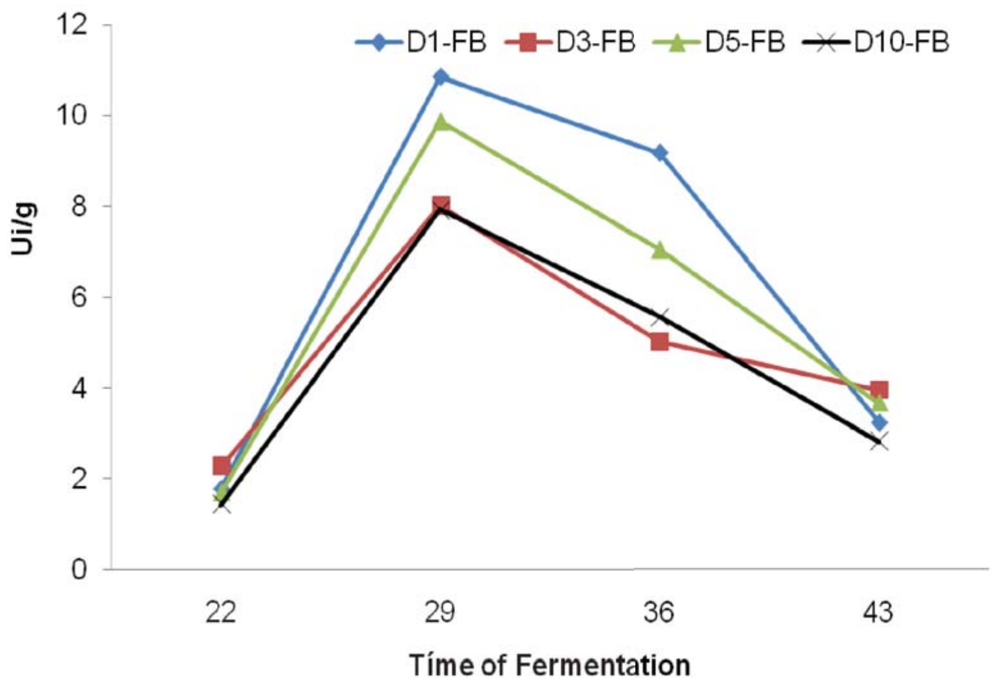

Figure 2. Behavior of the four endophytic strains of *Pycnoporus sanguineus* in the production of cellulases in a non-supplemented medium.

however, the times corresponding to the enzyme production were anticipated by approximately 50 h. It could therefore be suggested that the synthesis of the enzymes of the cellulolytic complex is not directly related to the carbon source used for induction, as cellulases were produced even when the strains were grown in the presence of cellobiose as a substrate, a synthesis that does not fit in the constitutive character.

The results obtained here were also in line with those reported by Rodríguez-Zú-iga et al. (2011). Working with *Aspergillus niger,* they found that the largest volumes of cellulases were obtained in a medium supplemented by salts, denominated as the modified Mandels and Weber medium (Mandels and Reese, 1960) with the addition of the inducer carboxymethylcellulose. Under these conditions, the activities of total cellulase and endoglucanase were 0.4 and 21.0 IU/g of substrate, respectively. These values represent increases of 2.6 and 4 times their respective enzymatic activities when compared to the medium without salt supplementation. This same behavior was observed by Buswell et al. (1998), Camassola et al. (2004), Spier (2005), Zhang et al. (2006), Chandra et al. (2007), Sanchez (2009), Ahamed and Vermette (2010), and Gao et al. (2011).

The results presented here suggest that the fungus *P. sanguineus* produces a cellulolytic complex with suitable characteristics for application in the saccharification of biomass and additional studies should be conducted to maximize the production of cellulases and hemicellulases.

Conclusions

The obtained results permit us to conclude that the endophytic fungus *P. sanguineus* isolated from *B.*

dracunculifolia, strains D1-FB; D3-FB; D5-FB and D10-FB are producers of cellulases in mediums based on sugarcane bagasse, whether supplemented or not, at pH 5.5 and a temperature of 30°C.

We can also conclude that the medium supplemented with salts proved to be a more appropriate medium to induce the production of cellulases at 29 days of fermentation and that the endophytic strain D10-FB of *P. sanguineus* stood out, producing 16.32±2.65 IU/gram of fermented substrate.

Conflicts of Interest

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

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Full Length Research Paper

Respiratory activity, intensity of processes of ammonification and nitrification in soil subjected to the effect of chemical preparates Reglone 200 SL and Elastiq 550 EC

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Plant protection agents applied in agriculture deserve special attention, given their toxicity, accumulability, and persistence in the soil environment, where they frequently induce disturbances in biochemical processes. Assessment of the risk associated with introduction of chemical preparations to agricultural cultivation is an essential element of protection of the natural environment against the harmful effects of these substances. Therefore, a three-year field study (2010 to 2012) was carried out to assess the long-term effect of soil contamination with the Reglone 200 SL herbicide and Elastiq 550 EC preparation (limiting rapeseed loss) on the course of the ammonification and nitrification processes and soil respiratory activity. The experiment was established in the split-block design on soil classified as black earth proper (WRB-Mollic Gleysols) with pH= 6.1. Soil for the analyses was sampled in 8 periods, that is, after 2, 10, 12, 14, 22, 24, and 26 months of the experiment. The experiments demonstrated that the amount of emitted CO_2 and the content of ammonium and nitrate ions depended largely on the period of the analysis and the type of the chemical agent. The optimal dose of 200 SL and Elastiq 550 EC applied caused periodic statistically significant changes in the respiratory activity and the intensity of the ammonification and nitrification processes in the tested soil.

Key words: Soil, respiratory activity, ammonification, nitrification, Reglone 200 SL, Elastiq 550 EC.

INTRODUCTION

Agriculture contributes considerably to the increase of the pool of soil contaminants. This is related, among other things, with the introduction of chemical agents of plant protection, alternatively referred to as pesticides, in field cultivations. A problem encountered in the cultivation of rapeseed (*Brassica napus* L.) is its non-uniform ripening and yield losses resulting from excessive seed shedding in the course of harvest of that crop plant. The treatment of desiccation, performed with the use of various chemical agents commonly known as desiccants, is aimed at the preparation of a plantation for harvest through acceleration and uniformity of the process of ripening of seed. It reduces excessive cracking of pods during ripening, thanks to which it is possible to achieve

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the maximum yield (Markowski et al., 2003; Choszcz et al., 2005; Konopka et al., 2005).

Pesticides applied in field cultivations are not neutral for the soil environment (Chowdhury et al., 2008). Improper application of chemical preparations which often have the ability of long-term persistence is frequently the cause of their excessive accumulation in the soil. Agricultural chemicals undergo various transformations in the soil. The intensity and directions of those transformations depend largely on the kind of chemical applied, its properties, rate of decomposition, and also on the properties of the soil environment (Onet, 2009). This constitutes a serious threat to the natural environment. Such chemical may interfere with the run of microbiological and biochemical processes which play a key role in the correct functioning of ecosystems. In the study of the soil environment the utilisation of enzymatic activity permits comprehensive identification of changes taking place in that environment (Margesin et al., 2000; Zahir et al., 2001; Janvier et al., 2007; Kucharski et al., 2011) under the effect of contaminants, e.g. chemical substances (Radivojević et al., 2012). It permits the estimation of the ecological status of the soil, its biological activity, as well as its fertility and productivity (Kieliszewska-Rokicka, 2001; Quemada and Menach, 2001; Russel, 2005).

Microorganisms inhabiting the soil have the capability of producing enzymes, thanks to which they are involved in most of the processes taking place in the soil. They participate in the degradation and mineralisation of organic matter, in binding nitrogen, and also in the cycle of elements in nature (Calderon et al., 2000; Chowdhury et al., 2008; Jezierska-Tys and Frąc, 2008). Moreover, the activity of soil microorganisms has also an effect on the degradation and detoxification of various substances contaminating the soil, including e.g. the chemical agents of plant protection, commonly known as pesticides (van Eerd et al., 2003; Beck et al., 2005).

Various chemical contaminants migrating into soil are of particular importance in the aspect of environmental studies. Taking into account the problem of soil contamination with chemical agents, a study based on a field experiment was performed, the objective of which was to determine the long-term effect of the chemical preparations Reglone 200 SL and Elastiq 550 EC on the respiratory activity and on the intensity of the processes of ammonification and nitrification in soil.

MATERIALS AND METHODS

The chemical preparations used in the field experiment were the plant protection agents Reglone 200 SL and Elastiq 550 EC. In crop plant plantations, the preparation Reglone 200 SL is used for weed control, and also for the desiccation of aboveground parts of plants containing chlorophyll. The biologically active substance in the composition of the preparation is diquat ion, a compound from the group of pyridyls. Elastiq 550EC glues the pods together, preventing excessive seed shedding and losses prior to and during the harvest of rapeseed. Elastiq 550 EC contains two biologically active substances in its composition: synthetic latex and alkoxylated alcohol.

Table 1. Basic characteristics of the soil used in the experiment.

Parameter	Value
pH (HCl)	6.1
Corg. (mg kg^{-1})	12.3
Norg. (mg kg^{-1})	0.11
Cu (mg kg^{-1})	133
P (mg kg^{-1})	235.6
K (mg kg^{-1})	0.025

The study was conducted in the years 2009-2011, at the plant variety evaluation experimental station in Głębokie, Kujawsko-Pomorskie Province (52° 38'41''N, 18° 26'18''E). The three-year field experiment was set up in the split-block design on a soil classified as black earth proper (WRB-Mollic Gleysols), with pH 6.1.

In the first year of the experiment, all the treatments were sown with winter rapeseed cv. “Californium”, in the second year – with sugar beet, and in the third – barley. The basic characteristics of the soil are given in Table 1.

In the first year of the experiment spraying with the preparations Reglone 200 SL and Elastiq 550 EC was made. The preparations were applied at technological doses recommended by the manufacturer. The model of the experiment comprised the following treatments: K - control soil, with no chemical preparations; R - soil with an addition of the preparation Reglone 200 SL at the optimum dose (2 dm^3 ha^{-1}); E - soil with the preparation Elastiq 550 EC applied at the optimum dose (1 dm^3 ha^{-1}). The application of the preparations was made by means of a knapsack spraying system. In the case of Elastiq 550 EC the spraying was made 4 weeks, and of Reglone 200 SL - 10 days before the harvest of winter rapeseed. In all the treatments, the basic tillage operations were performed, and the same level of fertilisation was applied, as recommended for the crop plant cultivated.

The effect of the chemical preparations Reglone 200 SL and Elastiq on the soil environment was studied for three years. In 2009 soil samples for microbiological and biochemical assays were taken twice, that is, in August and October. In 2010 and 2011 soil samples were taken three times, in May, August and October. The analyses correspond to the following terms of analyses: term 0- immediately after the harvest of winter rapeseed (in the first decade of August), and then after 2, 10, 12, 14, 22, 24 and 26 months of the experiment. Soil samples for the analyses were taken from the arable horizon of each plot.

Respiratory activity of the soil was assayed with the method of respiration induction through the addition of substrate (glucose) to the soil, according to Rühling and Tyler (1973). Carbon dioxide evolved from the soil during incubation was bound by 0.2 M solution of NaOH used in the experiment, forming Na_2CO_3. Next, after the addition of $BaCl_2$, insoluble sediment of BaCO3 was precipitated, and excess of non-bound soda lye was titrated with 0.1 M HCl. The respiratory activity expressed by the amount of evolved CO_2 was assayed with the titration method.

The rate of the process of ammonification was determined on the basis of the content of NH_4^+ ions, with the Nessler method. For the determination of the content of N-NH_4, portions of soil were weighed from the average soil samples. After 7 days of incubation at room temperature, 0.03 M acetic acid was added and, to extract ammonium ions the samples were shaken and then filtered. For the assay of N-NH_4 the filtrate was collected, sodium-potassium tartrate and Nessler reagent were added, and the whole was topped up

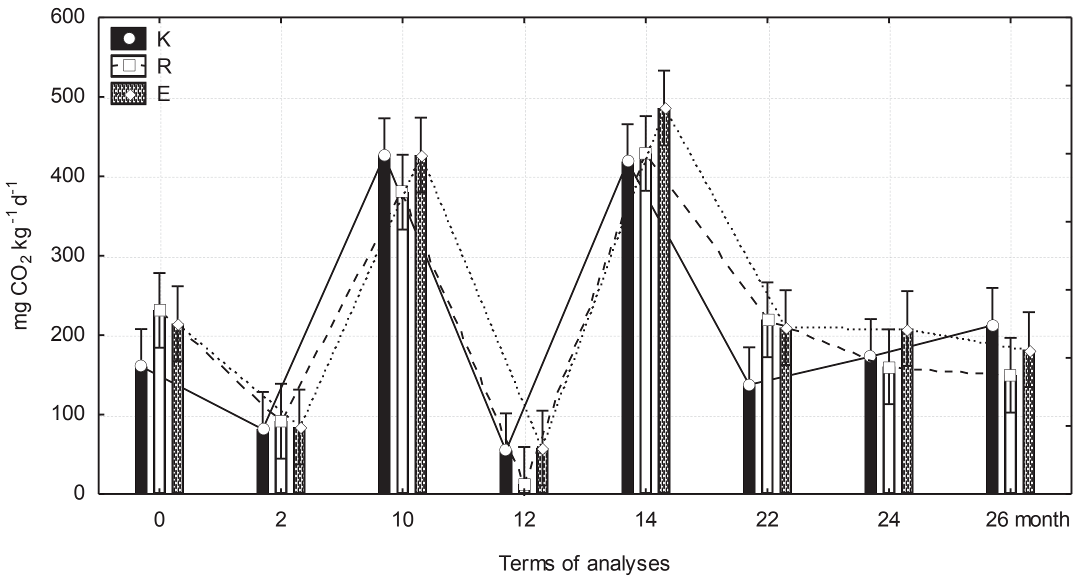

Figure 1. Respiratory activity of soil subjected to the effect of the chemical preparations Reglone 200 SL and Elastiq 500 EC. Explanations: K- control soil, with no chemical agent applied, R- soil with an addition of Reglone 200 SL at the dose of 2 dm^3 ha^{-1}, E- soil with an addition of Elastiq 550 EC at the dose of 1 dm^3 ha^{-1}. Vertical bars denote 0.95 confidence intervals.

with distilled water. The solution obtained in this manner was mixed thoroughly and subjected to colorimetry at wavelength of 410 nm. The zero sample was a mixture of 0.03 M acetic acid, sodium-potassium tartrate, Nessler reagent and distilled water. The results were converted to mg N-NH_4 kg^{-1} d.m of soil 7 day^{-1}.

The intensity of the process of nitrification was determined on the basis of the content of NO_3^-, with the brucine method. For that purpose filtrate was taken, prepared in the same manner as in the assays of the process of ammonification. The filtrate was placed in test tubes to which brucine dissolved in concentrated sulphuric acid was added. After setting aside for 24 h (for the stabilisation of colour), colorimetry was performed at wavelength of 470 nm. The zero sample was a solution containing distilled water and brucine. The results were converted to mg N-NO_3 kg^{-1} d.m. of soil 7 day^{-1}.

The results obtained were processed statistically using the analysis of variance (ANOVA). The least significant differences were calculated with the Tukey test at significance level of $\alpha = 0.05$. The statistical analyses of the results were performed with the use of the program STATISTICA 7.1.

RESULTS AND DISCUSSION

One of the primary indices used in the estimation of the status of the soil environment is the respiratory activity (Yao et al., 2006) which indicates the level of general activity of soil microorganisms (Dutta et al., 2010). It is related with the processes of degradation and oxidation of organic compounds, in the course of which carbon dioxide is evolved. Measurement of the respiratory activity permits the estimation of potential disturbances in the processes of carbon transformations taking place in soils subjected to the effect of pesticides or other xenobiotics (Gil-Sotres et al., 2005).

The periodic respiratory activity in the soil of the experimental treatments, measured by the amount of evolved CO_2, is presented in Figure 1. Based on the analyses performed, both activation and inhibition of the amount of evolved CO_2 were observed in the treatments studied. This was related with the kind of chemical preparation applied and with the time of analyses. In the opinion of Johnsen et al. (2001), plant protection agents can constitute a source of energy and nutrients for certain microorganisms. With the passage of time, organic compounds contained in the preparations, introduced in the soil, were undergoing degradation and probably gradually used up by soil microorganisms. A stimulating effect of both chemical preparations was noted on the first date of analyses, that is, immediately after the harvest of winter rapeseed, and after 22 months of their effect on the soil. Elastiq 550 EC also caused a stimulation of the process in question after 14 and 24 months of the experiment. At other times of analyses, the respiratory activity in the experimental treatments oscillated at a fairly stable level. Analysis of the mean values (Figure 2) for the whole three-year-long period of the experiment revealed that only Elastiq 550 EC caused an increase in the amount of evolved carbon dioxide compared to the control treatment. The results obtained did not differ statistically significantly.

Vig et al. (2008), studying samples of soil contaminated with insecticides (triazophos and endosulphan), also did not observe their negative effect on soil respiration. There are also reports on research in which negative effects of xenobiotics on the process of evolution of CO_2 was observed. Niewiadomska et al. (2009) studied the effect of plant protection agents – a herbicide with the name

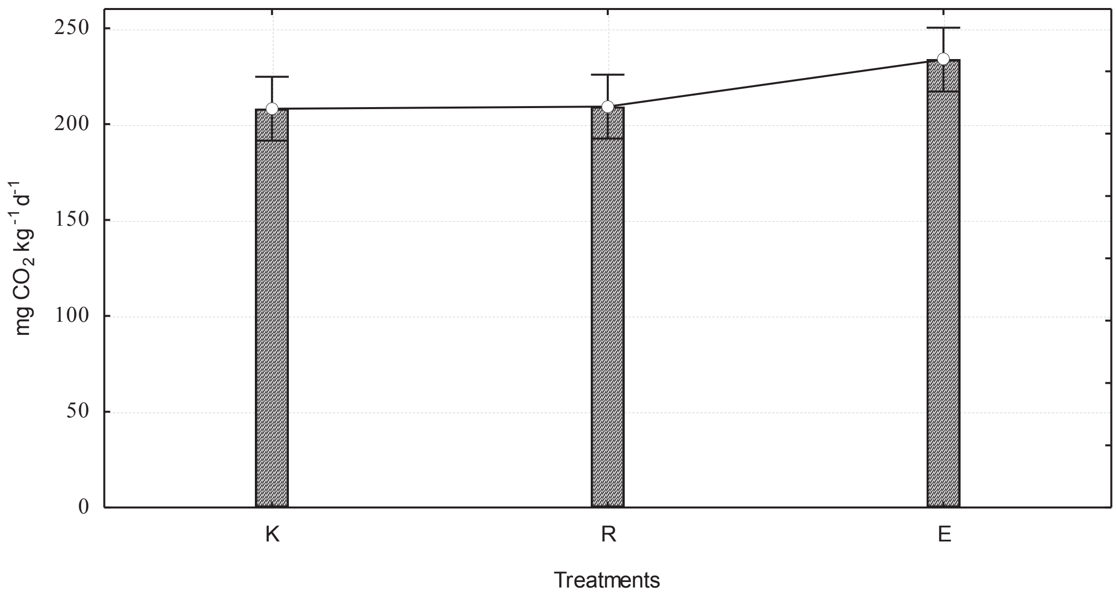

Figure 2. Mean values of respiratory activity in the soil during the whole period of the study. Explanations: K- control soil, with no chemical agent applied, R- soil with an addition of Reglone 200 SL at the dose of 2 dm^3 ha^{-1}, E- soil with an addition of Elastiq 550 EC at the dose of 1 dm^3 ha^{-1}. Vertical bars denote 0.95 confidence intervals.

Fox 480 (active substance - bifenox) and a fungicide – seed primer whose active substance is flutriafol – on the intensity of CO_2 evolution in soil. In that study, the authors observed a negative effect on the herbicide on the process of CO_2 evolution. The fungicide under study had a positive effect on the respiratory activity of the soil, but only on the last date of analyses. In a study by Araujo et al. (2003), glyphosate caused a 10 to 15% increase in the amount of evolved CO_2 in the soils studied.

Nitrogen is one of the most important biogenic elements in nature. Soil microorganisms participate actively in nitrogen transformations. Ammonification and nitrification are among those processes that provide information on nitrogen transformations in soil (Barabasz, 1992; Eemmerling et al., 2002). They also play an important role in the cycle of that element in the soil and are accepted as an important indicator of biological activity of soil. They are commonly used for the determination of the effect of various factors on the biological status of the soil environment (Jezierska-Tys, 2002; Shi et al., 2004).

Available domestic and foreign literature provides a wide range of information on the impact of pesticides on the ammonification and nitrification process in soil.

The study demonstrated that throughout the whole period of the experiment the chemical preparations applied caused periodic changes in the content of ammonium ions (Figure 3). Immediately after the harvest of winter rapeseed (first date of analyses), both Reglone 200 SL and Elastiq 550 EC induced significant inhibition of the intensity of the process of ammonification in the soil studied. A statistically confirmed significant increase in the content of ammonium ions was caused by Reglone 200 SL in the 10th and 26th months of the experiment, and by Elastiq 550 EC in the 22nd and 26th months. In the 0, 14th and 24th months of the experiment, both these preparations had an inhibiting effect on the process studied. Analysing the mean values of intensity of the process of ammonification for the experimental treatments (Figure 4) for the whole three-year-long period of the study, one can conclude that the chemical agents applied did not have any significant effect on the process in question. In their investigations of the effect of contamination of soil with different doses of Harpun 500 SC, Faworyt 300 SL, Akord 180 OF, and Mocarz 75 WG herbicides on the course of the ammonification process in soil, Kucharski et al. (2009) observed that the highest inhibitory effect on the amount of $N\text{-}NH_4$ was exerted by the Mocarz 75 WG herbicide. In turn, in an experiment with Roundup herbicide applied in a field dose, Krzyśko-Łupicka (2008) reported an increase in the concentration of ammonia nitrogen in soil.

Analysis of the data presented in Figure 5 shows that the chemical preparations Reglone and Elastiq used in the experiment caused period variations in the content of nitrate ions in the soil. The preparation Reglone caused a significant increase in the content of nitrate ions in the 2nd, 14th and 22nd months as compared to the control treatment. In the final phase of the experiment a significantly the greatest drop was observed in the intensity of the process of nitrification, both in the soil with Reglone 200 SL and in that with Elastiq 550 EC, a stronger effect being noted in the treatment with Reglone 200 SL. The mean content of $N\text{-}NO_3$ (Figure 6) for the

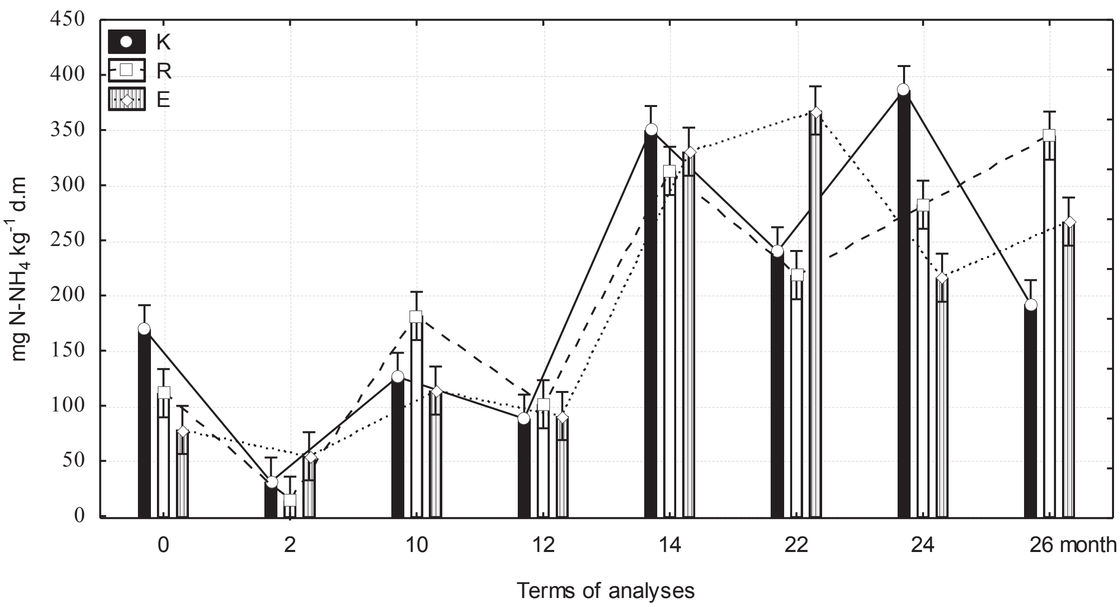

Figure 3. Intensity of the process of ammonification in soil subjected to the effect of the chemical preparations Reglone 200 SL and Elastiq 500 EC. Explanations: K- control soil, with no chemical agent applied, R- soil with an addition of Reglone 200 SL at the dose of 2 dm^3 ha^{-1}, E- soil with an addition of Elastiq 550 EC at the dose of 1 dm^3 ha^{-1}. Vertical bars denote 0.95 confidence intervals.

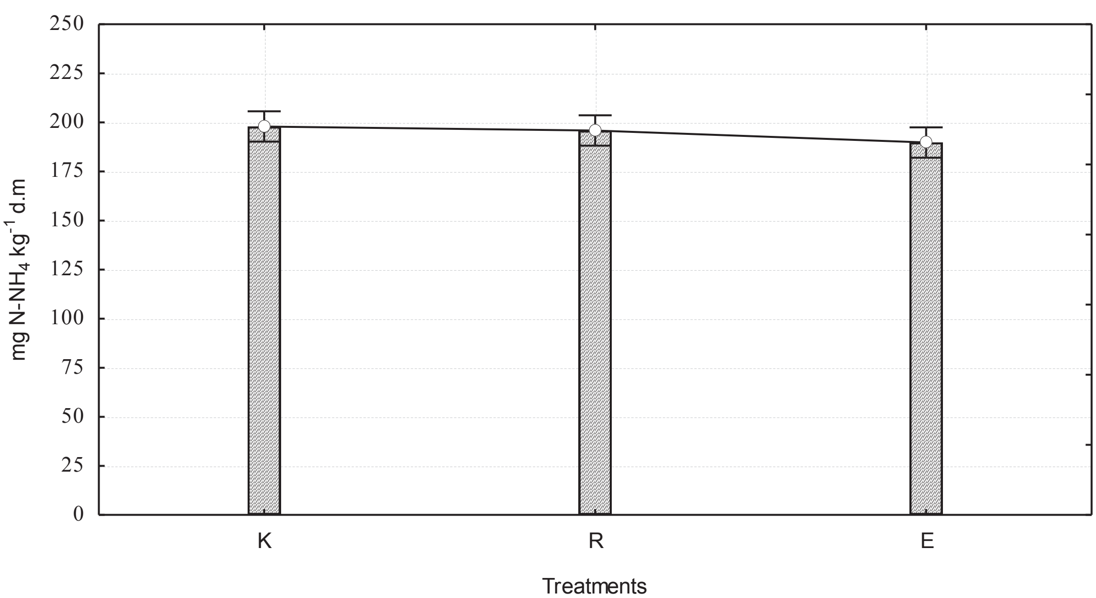

Figure 4. Mean values of intensity of the process of ammonification in the soil during the whole period of the study. Explanations: K- control soil, with no chemical agent applied, R- soil with an addition of Reglone 200 SL at the dose of 2 dm^3 ha^{-1}, E- soil with an addition of Elastiq 550 EC at the dose of 1 dm^3 ha^{-1}. Vertical bars denote 0.95 confidence intervals.

experimental treatments (from the three-year experiment) was at a similar level. In a study by Przybulewska and Nowak (2003), the preparation Reglone 200 SL had an inhibiting effect on that process. Upon application of chlorotalonil herbicide, Lang and Cai (2009) also reported decreased nitrification in soil.

The study demonstrated that the plant protection agents Reglone 200SL and Elastiq 550 EC, applied to the soil at doses recommended by the manufacturer in the process of desiccation, did not cause any significant disturbance of the processes of ammonification and nitrification.

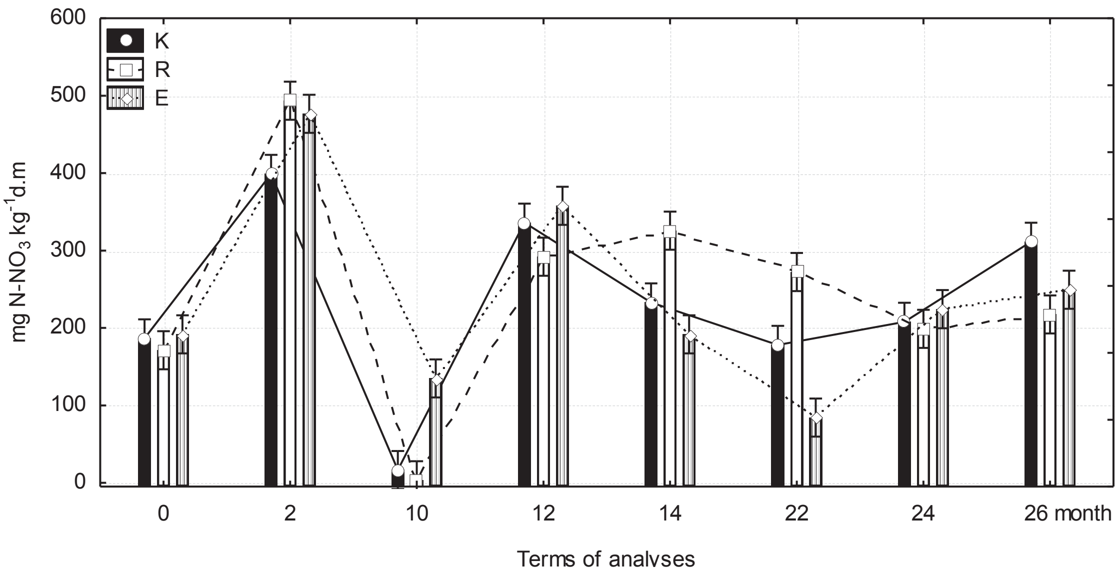

Figure 5. Intensity of the process of nitrification in soil subjected to the effect of the chemical preparations Reglone 200 SL and Elastiq 500 EC. Explanations: K- control soil, with no chemical agent applied, R- soil with an addition of Reglone 200 SL at the dose of 2 dm^3 ha^{-1}, E- soil with an addition of Elastiq 550 EC at the dose of 1 dm^3 ha^{-1}. Vertical bars denote 0.95 confidence intervals.

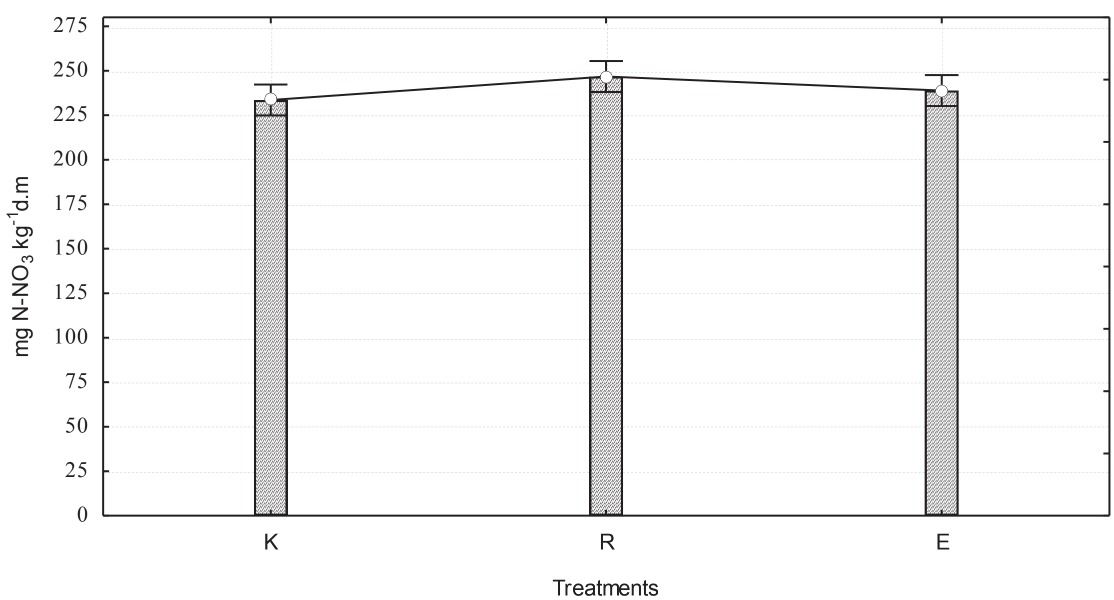

Figure 6. Mean values of intensity of the process of nitrification in the soil during the whole period of the study. Explanations: K- control soil, with no chemical agent applied, R- soil with an addition of Reglone 200 SL at the dose of 2 dm^3 ha^{-1}, E- soil with an addition of Elastiq 550 EC at the dose of 1 dm^3 ha^{-1}. Vertical bars denote 0.95 confidence intervals.

Conclusions

Intensive plant protection treatments in crop fields based on application of chemical plant protection agents is now and will still be a basic method for weed, pest, and disease control in plant cultivations. The benefits of using chemical agents are undeniable, although the risk related to excessive or improper use thereof should not be underestimated. Application of these agents exerts an effect on the soil environment yielding disturbances in biochemical processes. The investigations have shown that the amount of emitted CO_2 and the content of ammonium and nitrate ions depended largely on the period of the analysis and the type of the chemical agent applied.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Glycerin effluent from the biodiesel industry as potassium source to fertilize soybean crop

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This research is aimed at evaluating the effects of the glycerin effluent from the biodiesel industry resulting from chemical processes involving the transesterification of triacylglicerides in bio-oils, a reaction catalyzed with potassium hydroxide. This glycerin material (hereinafter referred to as "K-glycerin") may be assumed to be a potential source of potassium to increase the yields of soybean crops and to promote corresponding alterations in some chemical and microbiological properties of the soil. Two field assays were conducted on two soils classified (i) Typical Hapludox (Perox suborder of the Oxisol order, according to the USDA Soil Taxonomy System. In this work, this site was identified simply as TH) and (ii) Typical Quartzipsamment (Aquents suborder of the Entisols order; TQ), in the state of Minas Gerais, Brazil. The used treatments were: (i) four doses (corresponding to 0, 40, 80 and 160 kg ha^{-1} K_2O) on the TH soil and four doses (corresponding to 0, 60, 120 and 240 kg ha^{-1} K_2O) on the TQ soil form, and (ii) two potassium sources (specifically, KCl and K_2SO_4) at a K_2O equivalent doses of 80 kg ha^{-1} on the TH soil and 120 kg ha^{-1} on the TQ soil. The soybean yields resulting from the application of K-glycerin were found to be related to the availability of K in the soil and this source supplied in part the need of potassium by soybean crop. The K-glycerin does not cause any readily detectable harmful or environmental effect to these cropping sites; however, further detailed studies are needed to better evaluate the long-term use to understand the soil ions dynamics under the soybean crop.

Key words: Organic residue, yield, nutrition, soil, microbial biomass.

INTRODUCTION

The residual crude glycerin from the biodiesel industry corresponds to about 10 to 15% of the total biodiesel mass production. In the lack of any specific legislation indicating how to dispose such residue, much of this by-product is more commonly accumulated in areas of the industrial plants. The expected expansion of the worldwide production of biodiesel will in consequence tend to increase the stock of glycerin (Ooi et al., 2004).

The residual crude glycerin from the biodiesel industry usually contains about 50 mass% of pure glycerin (Zhou et al., 2008; Carvalho et al., 2012) and is rather dark, relatively to the pure glycerin; it needs to be purified for further use in the fine chemistry industry (Yong et al., 2001). However this process is expensive and the effluent is usually discarded as waste glycerin by small industries producing biodiesel (Dasari et al., 2005).

Following the increasing production of biodiesel worldwide, the glycerin waste is thought to be a potential environmental problem although it may also represent an opportunity to get environmental and economical profits depending only on new technological developments favoring its rational use. Many challengeable alternatives may be put under considerations, in attempts to confer better technological uses, reduce the environmental impacts and consolidate biodiesel as an environmentally and economically competitive biofuel (Ito et al., 2005). Some technological uses of crude industrial glycerin as feedstock have been reported (Zhou et al., 2008), although its potential use as soil fertilizer for agricultural lands is still unknown.

Soybean oil is the most widely used for the production of biodiesel (Freedman et al., 1986; Noureddini and Zhu., 1997). Thus the lower the cost of the soybean production the more interesting would be its industrial use. A conceivable alternative for the agricultural use of crude glycerin from the transesterification of triacylglycerides of bio-oils to produce biodiesel, using potassium hydroxide as catalyst, would be to neutralize the effluent with phosphoric or sulfuric acid. The potassium phosphate or sulfate formed during this step would improve the use of the effluent as fertilizer (Zhou et al., 2008).

Potassium is the second most promptly absorbed macronutrient after nitrogen. The plant nutritional requirements may be variable, depending on innumerous conditions of soil and the plant itself. On average, it assumes an uptake of 81 kg N and 54 kg K to produce 1,000 kg of soybean grains (Borket and Yamada, 2000). Some studies have shown that when levels of available potassium in soil are above 60 mg dm^{-3}, the yield responses of the soybean plant to potassium fertilization are usually not significant (Scherer, 1998a, b). Maximum yields of soybeans were reportedly attained with application of 60 kg ha^{-1} (Scherer, 1998b); 80 and 120 kg ha^{-1}, if the level of available K in soil was between 16 and 40 mg dm^{-3} (low content) and between 41 and 70 mg dm^{-3} (mean content), respectively (Novais, 1999) and 85 and 90 kg ha^{-1} K_2O in case of no-tillage land management (Foloni and Rosolem, 2008).

In tropical and subtropical soils, organic matter has a close relationship with other physical, chemical and biological soil properties, chemical among the cation exchange capacity (CEC), is of fundamental importance for maintaining the productive capacity of the soil longer term (Ciotta et al., 2003; Araújo et al., 2007; Moreti et al., 2007; Carvalho et al., 2011). Thus, industrial glycerin, as an organic residue, would tend to increase the usually low CEC of tropical soils, to reduce loss of cations, particularly K^+, and improve their fertility.

In this work, it was used sulfuric acid to neutralize the material of the glycerin effluent. The resulting raw product containing potassium sulfate was assayed as fertilizer for soils supporting the growth of soybean plants. The study thus aimed at evaluating the use of the glycerin effluent (the material will be hereinafter referred to as "K-glycerin"), a waste product of the biodiesel industry, from the transesterification of triacylglycerides in a vegetable oil catalyzed with KOH, as a potassium source on the yield and nutrition of soybean plants and on alterations of the chemical and microbiological properties of the soil.

MATERIALS AND METHODS

Description of the studied area

Two experiments were carried from December 2009 to April 2010 at two different soil sites, both in the state of Minas Gerais, Brazil: (i) of a Typical Hapludox (Perox suborder, Oxisol order, according to the USDA Soil Taxonomy System; in this work, this site was identified simply as TH) in an area (18° 45' S 44° 25' W; 633 m a.s..l.; Köppen climate, Aw, with a mean annual rainfall of 1,200 mm; mean temperature 24°C) located at the Moura Experimental Farm of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), in the municipality of Curvelo; (ii) of a Typical Quartzipsamment (Aquents suborder, Entisols order; TQ), in an area (18° 15' S 43° 36' W; altitude of 1,400 m a.s.l.; Köppen climate, Cwb; mean annual rainfall of 1,082 mm; mean temperature 19,4 °C) located in the Campus JK UFVJM, in the municipality of Diamantina.

The soil samples were air-dried, sieved (2.0 mm) and characterized according to Embrapa (1997) (Table 1). The pH was potentiometrically measured (soil:water 1:2.5, v/v); phosphorous and potassium were extracted with the Mehlich-1 solution and determined by colorimetry (for phosphorous) and flame photometry (potassium); calcium, magnesium and aluminum were extracted with a solution of 1 mol L^{-1} KCl and determined by flame atomic absorption spectrophotometry (calcium and magnesium) and titrated with 0.025 mol L^{-1} NaOH, for aluminum; the acidity (H + Al) was determined by extracting with 0.5 mol L^{-1} calcium acetate buffered at pH 7.0 and quantified by titration with 0.025 mol L^{-1} NaOH. The organic carbon (OC) was determined by Walkley-Black method (Walkley and Black, 1934). The sulfate (SO_4^{2-}) content was determined according Hoeft et al. (1973). Values of cation exchange capacity (CEC), Al saturation (m) and base saturation (V) were then calculated. The pipette method was used to determine the soil texture (Embrapa, 1997).

Planting and agronomic practices

The preparation of the experimental area was made by the

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Table 1. Chemical composition and soil texture as measured before treatments.

Solo	pH_{Water}	P	K	$S\text{-}SO_4^{2-}$	Ca	Mg	Al	CEC	m	V	OC	Areia	Silte	Argila
		mg dm^{-3}			mmol$_c$ dm^{-3}				%		g kg^{-1}			
TH	5.8	1.4	70	4.8	23	11	7	83	16	43	17.4	90	450	460
TQ	5.6	1.8	16	7.1	5	2	11	37	60	20	4.0	890	60	50

pH $_{water}$: Soil-water 1:2.5. P e K: Mehlich-1 extractor; Ca, Mg e Al: KCl 1 mol L^{-1} extractor; $S\text{-}SO_4^{2-}$: Calcium phosphate diacid ($Ca(H_2PO_4)_2$) extractor; CEC: Cation exchange capacity; m: Aluminium saturation; V: Bases saturation; OC: Organic carbon by the Walkey-Black method; Sand, silt and clay: Pipette method; TH: Typic Hapludox e TQ: Typic Quartzipsamment.

conventional system with disc plow to 0.4 m deep and two disking with disc harrow. Liming was carried out three months before planting, by distributing the powder dolomitic limestone (90% total neutralizing power) all over the area with; the material was incorporated at 0.30 m depth at a dose corresponding to 2.4 x 103 kg ha^{-1}, for both soils.

The soybean population of the cultivar MSOY-8001, during the spring of 2008; the seeds were manually spread over to get a stand of 444,444 plants ha^{-1}, with spacing of 0.45 m between rows and 20 seeds per meter along rows. The phosphorus fertilization was done at the planting time at a dose 120 kg ha^{-1} P_2O_5 as superphosphate (18 mass% P_2O_5 and 10 mass% S) in all treatments and cultivation places. The seeds were inoculated with concentrated liquid inoculant (125 mL of liquid inoculant to 50 kg of seeds) o *Bradyrhizobium japonicum*. All cultural practices were followed as recommended by Embrapa (2008).

Treatments and experimental design

The crude glycerin was obtained from the conventional chemical industrial process of transesterification of triacylglycerides, in this case, of the cooking soybean oil after being used in frying foods. The transesterification was catalyzed with potassium hydroxide and the glycerin effluent was finally neutralized with sulfuric acid. The resulting liquid material will hereinafter be referred to as "K-glycerin". The chemical composition for this “K-glycerin” (pH = 8.3 and density = 1.01 kg dm^{-3}), as could be determined according to analytical procedure described by Melo and Silva (2008), in kg m^{-3}, is: P_2O_5 = 0.5; K_2O = 24.9; S = 1.1; B = 1 x 10^{-3}; Cu = 4 x 10^{-3}; Fe = 54 x 10^{-3}; Mn = 2 x 10^{-3}; Zn = 96 x 10^{-3} and organic carbonic = 3 x 10^{-3}.

The field experimental design was a randomized block design with four replications. Each experiment plot consisted of four 3.0 m-long rows, spaced of 0.45 m, comprising a total area of 5.4 m^2. The useful area of each plot was considered by taking two central rows, excluding 1.0 m from each row-end.

The treatments consisted of four K_2O doses on the TH (0; 40; 80 and 160 kg ha^{-1} K_2O) and TQ (0; 60; 120 and 240 kg ha^{-1} K_2O) sites. These K_2O doses correspond to 0; 1.6; 3.2 and 6.4 m^3 ha^{-1} “K-glycerin” on TH, and 0; 2.4; 4.8 “K-glycerin” and 9.6 m^3 ha^{-1} on TQ. Two additional treatments were made to supply inorganic sources of potassium (KCl, corresponding to 58 mass% K_2O, and K_2SO_4, 48 mass% K_2O) at a dose of 80 and 120 kg ha^{-1} K_2O on TH and TQ, respectively. These doses of potassium were based on recommendations for the soybean crop (Novais, 1999) and on the availability of potassium in the soil, evaluated according to standard procedures recommended for the soil chemical analysis (Table 1). The liquid “K-glycerin” was manually distributed all along the sowing rows at a depth of 7 cm.

Inputs and measurements

To evaluate the soybean crop yield, the grain moisture was corrected to 13 mass%. After harvesting the soybean grains, the soil samples were taken at a depth between 0 to 0.20 m from the soil surface, for each plot, in order to determine all other chemical properties. About 20 soil samples were collected from each plot in order to prepare one mixed sample. There were determined the pH in water, P and K (Mehlich-1 extractor), Ca and Mg (KCl 1 mol L^{-1} extractor), H + Al (calcium acetate 0.5 mol L^{-1} extractor) and the organic carbon (OC) according to the Walkley-Black method (Embrapa, 1997); the sulfate (SO_4^{2-}) content was determined according to the procedure described by Hoeft et al. (1973). Values of cation exchange capacity (CEC = Σ(K, Ca, Mg, H + Al) in mmol$_c$ kg^{-1}) and saturation in bases (V% = [Σ(Ca, Mg, K)/(CEC)] × 100) were then calculated.

The nutritional status of the soybean leaf samples was determined at the stage R2 (full flowering) per useful plot, in order to determine the nutrient composition. The amount of nitrogen was determined by the semi-micro Kjeldahl method (Cunniff, 1995). The P, K, Ca, Mg, S, Cu, Fe, Mn and Zn contents were obtained by nitric perchloric acid digestion (Miller, 1998). The contents of Ca, Mg, Cu, Fe, Mn, and Zn were determined by atomic absorption spectrometry; K was determined by flame photometry (Isaac and Kerber, 1971). S was determined by the barium sulfate turbidimetry (Beaton et al., 1968). The B content was determined by colorimetry (azomethine method) after dry digestion (incineration) (Wolf, 1974).

Ten soil sub-samples were randomly collected at a depth between 0 and 0.10 m deep. The first emergence soybean plants were collected, packaged in plastic bags and transported in thermally isolated boxes. The materials were sieved (2 mm) in order to remove organic residues and roots. Then sub-samples were stored at 4ºC until microbial analyses were made. Flurescein diacetate hydrolysis (FDA) by soil microorganisms was evaluated according to Frighetto and Valarini (2000). Soil basal respiration (Rbasal) was estimated based on CO_2 released from four sub-samples (20 g each) taken from each mixed soil sample (water content, 60 mass%). The samples were sealed in a 1.0 L flask with 10 mL of 0.3 mol L^{-1} KOH and titrated, after three days, with 0.1 mol L^{-1} HCl, according to the methodology described by Alef and Nannipieri (1995). Soil microbial biomass C (Cmic) was determined by fumigation and incubation, as described by Jenkinson and Powlson (1976). The metabolic quotient (qCO_2) was determined by the ratio between Rbasal and Cmic (Anderson and Domsch, 1993), expressed in µg CO_2 µg $Cmic^{-1}$ day^{-1}.

Statistical analysis

The numerical data were subjected to analysis of variance for the following factors: blocks, soil type, K_2O doses as “K-glycerin” and inorganic sources of potassium (KCl and K_2SO_4), as an additional nutrient supply. The microbiological analysis of soil, and the factors above, added two evaluation periods (early emergence and harvest) of the soybean. The separation of the mean values was done using the criterion of the least significant difference (LSD) at a 5% probability level. The fitted equations for the variables evaluated in terms of K_2O doses applied as “K-glycerin”.

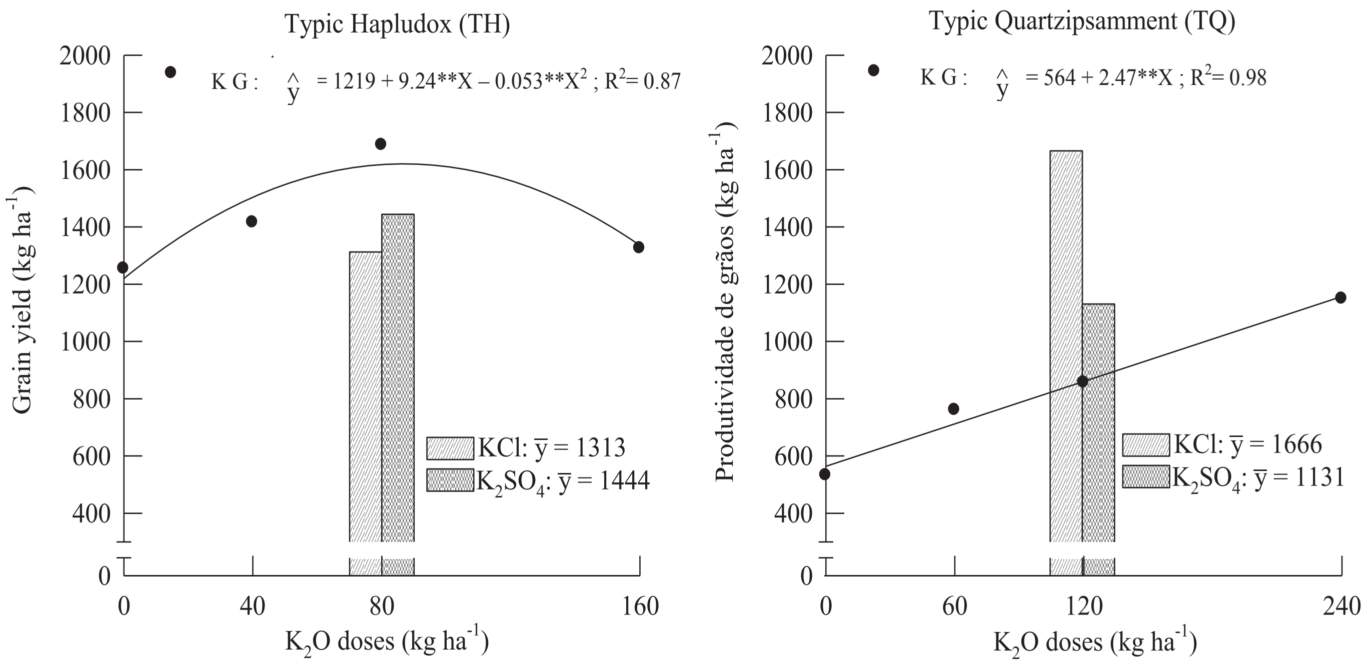

Figure 1. Grain yield of soybeans as a function of K_2O doses in the form of "K glycerin" (KG) and K inorganic sources (KCl and K_2SO_4) into two types of soil (*Significant at 1% by F-test).

RESULTS AND DISCUSSION

Grain yield of soybean influenced by the K_2O doses in the form of "K glycerin" to increase quadratically when cultivated in soil TH and linearly when cultivated in soil TQ (Figure 1). The inorganic sources (KCl and K_2SO_4) applied in TH did not differ and when applied in TQ were superior and differed doses of K_2O in the form of "K glycerin" (Figure 1). The K_2O doses in "K glycerin" in applied TH caused reductions in soybean yield due to nutritional imbalance caused by competition cationic and anionic nutrients (Parker and Norvell, 1999). The lack of increase in grain yield of inorganic sources relative "K glycerin" and the latter by reduction with increasing K_2O doses in TH due to the initial K content in soil (Table 1), with a value above 40 mg kg^{-1} of soil (Rosolem et al., 1993; Scherer, 1998a; Borket and Yamada, 2000). Differently than soybean cultivation in TH, response to K fertilization with application of "K glycerin" and inorganic sources occurred when exchangeable soil K was below 40 mg kg^{-1} in TQ soil. Responses to K fertilization in soybean were found in Typical Hapludox with K content of 27.3 mg kg^{-1} of soil (Mascarenhas et al., 2000).

The maximum yield (1,157 kg ha^{-1}) of soybean attained with the maximum dose applied K_2O (240 kg ha^{-1}) in the form of "K glycerin" on the ground TQ (Figure 1). In soil TH, maximum yield of 1,622 kg ha^{-1} attained with 87 kg ha^{-1} of K_2O with "K glycerin" (Figure 1). The K_2O dose were higher than the determined by Scherer (1998b) and the near recommended for soybean (Novais, 1999) and obtained in succession millet-soybean no-tillage (Foloni and Rosolem, 2008) to achieve maximum productivity in TH soil. In TQ soil, the K_2O dose as "K glycerin" to achieve maximum yield was more elevated than in TH soil.

The yield of soybean crop in Brazil in 2012/2013 was 2,938 kg ha^{-1} and the Minas Gerais State, Brazil was 3,010 kg ha^{-1} (Conab, 2013) with fertilizer application in soybean seeding. The conditions of this study, the yields on both cultivated soils were significantly lower than the national and regional average. The results attributed to climatic conditions during the experimental period with a mean temperature of 24.2 and 20.0°C and rainfall of 735 and 710 mm in Curvelo and Diamantina, respectively. The favorable climatic factors are essential for proper yield of soybean with water requirement of 7 to 8 mm per day, totaling in cycle from 450 to 800 mm, depending on the duration and management of the crop cycle and optimal temperature around 30°C (Embrapa, 2008). The temperature interfered on soybean yield in both cultivation places, with greater effect in Diamantina, besides the difficulty of K fertilizer management due to the low CEC in TQ soil (Table 1), which provides low yield with higher K dose applied to the soil. The need for application of dose above 60 kg ha^{-1} of K_2O in soils with low CEC and in areas subject to intense rainfall, K should be applied broadcast at doses allowing maintenance of adequate levels in soil and that return the quantities exported by crops (Guareschi et al., 2008).

The direct application of "K glycerin" was evaluated as an alternative source of nutrients for the soybean crop, thus the soil chemical attributes and levels in the leaves

Table 2. Effect of K_2O doses in the form of "K Glycerin" (KG) and inorganic sources (KCl and K_2SO_4) on soil chemical attributes applied to two types of soil.

Treatment	pH_{Water}	P	K	$S\text{-}SO_4^{2-}$	Ca	Mg	CEC	V (%)	OC (g kg^{-1})
		mg dm^{-3}			mmol$_c$ dm^{-3}				
Typic Hapludox: KG doses (kg K_2O ha^{-1})									
0	6.0^a	13.7^a	72.5^c	13.2^c	37^a	15^a	101^a	55^a	13.0^a
40	6.0^a	12.1^a	120.7^b	23.7^b	36^a	18^a	98^a	59^a	13.0^a
80	5.9^a	12.6^a	127.3^b	28.9^a	38^a	19^a	102^a	60^a	14.0^a
160	6.0^a	12.3^a	115.0^b	18.5^b	38^a	13^a	97^a	56^a	14.0^a
KCl (80 kg K_2O ha^{-1})	5.7^a	18.7^a	176.0^a	27.6^a	36^a	11^a	100^a	50^a	14.0^a
K_2SO_4 (80 kg K_2O ha^{-1})	5.7^a	19.1^a	131.5^a	26.9^a	38^a	10^a	100^a	50^a	13.0^a
F-test	NS	NS	*	*	NS	NS	NS	NS	NS
Typic Quartzipsamment: KG doses (kg K_2O ha^{-1})									
0	5.5^a	22.0^a	16.8^d	19.5^c	11^a	3^a	43^a	33^a	4.2^a
60	5.4^a	20.0^a	29.1^c	22.9^c	8^a	3^a	40^a	31^a	4.5^a
120	5.4^a	21.0^a	39.2^c	27.3^c	8^a	3^a	43^a	29^a	4.1^a
240	5.5^a	21.0^a	52.8^b	32.3^b	13^a	4^a	45^a	39^a	4.2^a
KCl (120 kg K_2O ha^{-1})	5.3^a	21.3^a	59.6^b	38.4^b	9^a	5^a	49^a	31^a	5.4^a
K_2SO_4 (120 kg K_2O ha^{-1})	5.3^a	20.9^a	71.5^a	49.7^a	10^a	3^a	46^a	32^a	4.8^a
F-test	NS	NS	*	*	NS	NS	NS	NS	NS
CV (%)	7.8	37.8	13.5	7.2	17.7	29.9	9.4	19.0	19.5

NS, Non-significant at $p < 0.05$; * Significant at $p>0.05$. Means with the same treatment and column sharing the same letters are not significantly different at $p<0.05$. CEC: Cation exchange capacity; V: Bases saturation; OC: Organic carbon.

of soybean was evaluated (Tables 2 and 3). The K and $S\text{-}SO_4^{2-}$ contents in soil affected quadratically (K: $\hat{y} = 75.2 + 1.19x - 0.006x^2$, $R^2 = 0.95$ and $S\text{-}SO_4^{2-}$: $\hat{y} = 13.4 + 0.35x - 0.002x^2$, $R^2 = 0.98$) in TH and linearly in TQ (K: $\hat{y} = 19.0 + 0.15x$, $R^2 = 0.98$ and $S\text{-}SO_4^{2-}$: $\hat{y} = 19.9 + 0.05x$, $R^2 = 0.99$) by increasing K_2O doses of "K glycerin" that differed from K inorganic sources (KCl and K_2SO_4) applied (Table 2). In contrast, the K in soybean leaves only was affected linearly (K: $\hat{y} = 7.2 + 0.0171x$, $R^2 = 0.92$) by increasing K_2O doses of "K glycerin" in TQ which differed from K inorganic sources applied (Table 3). Foliar S increased with K_2O doses of "K glycerin" linearly in TH soil (S: $\hat{y} = 0.8 + 0.0046x$, $R^2 = 0.95$) and in TQ soil (S: $\hat{y} = 1.2 + 0.0016x$, $R^2 = 0.89$) and differed from K inorganic sources in both soils (Table 3).

The diagnosis of a nutrient deficiency, it is important to conduct soil testing in advance, to make possible corrections in the fertilization to minimize future losses in yield. Soybean yield (Figure 1) had a relationship with the available K and S content in the soils (Table 2). The exchangeable soil K in TH by initial analysis (Table 1) is above 40 mg K kg^{-1} soil whereas the TQ is below (Rosolem et al., 1993; Scherer, 1998a; Borket and Yamada, 2000). In contrast, the availability of the $S\text{-}SO_4^{-2}$ in the initial analysis (Table 1) is above 9.3 mg $S\text{-}SO_4^{2-}$ kg^{-1} soil (Huda et al., 2004) in both soil cultivated with soybeans. This S and K availability in the soil reflected in the content of such nutrients in the leaves of soybean (Table 3). The contents of K and S in the soil increased with "K glycerin" doses since these nutrients constituents of this waste arising from the production process of biodiesel used, with the K inorganic sources and due to P fertilization of soybean with superphosphate.

The increases in S and K in the soil (Table 2) as reflected in contents of these nutrients in the leaves of soybean (Table 3) provided no increases in grain yield in TH (Figure 1), due to S content in the leaves of soybean being below the 2.5 g kg^{-1} amount (Martinez et al., 1999) and 2.3 g kg^{-1} (Urano et al., 2007). The nutrient with the greatest increase in TQ both in soil (Table 2) as in the leaves of soybean (Table 3) was the K thereby could be responsible for increased yield. The K influences various physiological processes such as photosynthesis, transport of photoassimilates and enzymes activation, which directly affected the yield (Pettigrew, 2008). The K content in the leaves below 14.0 g kg^{-1} (Scherer, 1998a), of 17.0 g kg^1 (Martinez et al., 1999) and 23.1 g kg^{-1} (Urano et al., 2007) related to low yield of soybeans (Figure 1) at the maximum dose of "K glycerin" in TQ.

The effect of K in Ca and Mg uptake, which normally interact with this nutrient (Mascarenhas et al., 1988), decreased the levels of Ca and Mg in the leaves of soybean when cultivated in TQ (Table 3). The difference of Ca and Mg in TQ may be due to competition with K, since they use the same absorption sites (Andreotti et al., 2001), and the increase of K intensifies competition with

Table 3. Effect of K_2O doses in the form of "K Glycerin" (KG) and inorganic sources (KCl and K_2SO_4) on nutrient concentrations in the soybean leaves applied to two types of soil.

Treatment	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg^{-1}						mg kg^{-1}				
Typic Hapludox: KG doses (kg K_2O ha^{-1})											
0	36^a	2.4^a	21^a	10^a	2.2^a	0.9^b	33^a	9^a	78^a	23^a	30^a
40	36^a	2.4^a	20^a	10^a	2.2^a	0.8^b	32^a	8^a	74^a	25^a	28^a
80	38^a	2.5^a	21^a	10^a	2.2^a	1.3^a	31^a	10^a	69^a	21^a	31^a
160	38^a	2.5^a	20^a	11^a	2.2^a	1.5^a	32^a	9^a	66^a	24^a	28^a
KCl (80 kg K_2O ha^{-1})	39^a	2.5^a	21^a	10^a	2.2^a	0.9^b	31^a	9^a	68^a	22^a	30^a
K_2SO_4 (80 kg K_2O ha^{-1})	36^a	2.5^a	21^a	11^a	2.2^a	1.6^a	31^a	12^a	76^a	23^a	30^a
F-test	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
Typic Quartzipsamment: KG doses (kg K_2O ha^{-1})											
0	43^a	3.2^a	7^b	17^a	4.6^a	1.1^b	22^a	12^a	63^a	37^a	28^a
60	41^a	3.1^a	8^b	17^a	4.6^a	1.4^b	22^a	11^a	63^a	37^a	28^a
120	45^a	3.1^a	10^b	17^a	4.2^a	1.5^b	21^a	13^a	69^a	36^a	28^a
240	44^a	3.1^a	11^b	15^a	4.2^a	1.5^b	21^a	12^a	70^a	36^a	28^a
KCl (120 kg K_2O ha^{-1})	45^a	3.1^a	20^a	11^a	2.6^a	1.6^b	22^a	12^a	78^a	39^a	27^a
K_2SO_4 (120 kg K_2O ha^{-1})	42^a	3.0^a	20^a	12^a	2.6^a	1.9^a	22^a	12^a	79^a	39^a	27^a
F-test	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS
CV (%)	7.9	13.4	10.4	13.2	3.7	11.3	12.2	5.4	19.4	17.4	19.2

NS: Non-significant at $p<0.05$; *: significant at $p>0.05$. Means with the same treatment and column sharing the same letters are not significantly different at $p<0.05$.

Ca and Mg (Oliveira et al., 2001). In TH, the high content of K in the initial analysis (Table 1) probably equated to the Ca and Mg competition occurring balanced as opposed to TQ, with higher competition between the exchangeable bases.

Despite the increase in K and S in soil (Table 2) and in leaves of soybean (Table 3), application of the "K glycerin" was not sufficient to modify the CEC and the organic carbonic content in two soils (Table 2). A single application of the "K glycerin" in planting furrow added at a dose of 240 kg ha^{-1} K_2O in TQ a quantity of 28.9 kg ha^{-1} of organic carbonic, being required to maintain the initial stock carbon organic soil a total annual addition of 8,900 kg ha^{-1} carbon in tillage cropping system (Lovato et al., 2004). The increase in organic matter and consequent increase of soil CEC by addition of organic waste was obtained when applied to the total area (Carvalho et al., 2011) and the use of organic waste (poultry manure) did not affect the organic matter in depth up to 0.20 m (Moreti et al., 2007), while the organic matter effect restricted to the surface layers of the soil, which not reflected in the 0 to 0.20 m soil due to the dilution effect on the soil mass (Ciotta et al., 2003; Araújo et al., 2007).

Microbiological analyzes were performed at two different times in order to obtain information regarding the direct application of "K glycerin" in soil microbes (Table 4). The reduction of microbial activity measured by flurescein diacetate hydrolysis (FDA) with increasing K_2O doses (FDA: $\hat{y} = 4.36 - 0.019x + 0.00012x^2$, $R^2 = 0.83$) in the form of "K glycerin" in TH (Table 4) and the source K_2SO_4 may have occurred because the dose was detrimental to soil microbes at the time of soybean emergence (E1). The addition of organic and inorganic fertilizers can cause positive or negative effects on microbial biomass and its activity (Böhme et al., 2005).

It was observed reduction value of the FDA at the time of harvest of soybean (E2) in relation to the time E1 did not differ between the application of "K glycerin" doses and K inorganic sources in TH (Table 4). Already in the soil TQ, microbial activity was not influenced by the levels of "K glycerin" and K inorganic sources at the time E1 and an increase in the time that E2 (FDA: $\hat{y} = 1.98 + 0.002x$, $R^2 = 0.82$) did not differ from K inorganic sources (Table 4). The evaluation times, the behavior was different in relation to TH because TQ on the end of the experimental period the value was greater than the initial fact justified by the non-occurrence of drought stress in the experimental area. This may be the effect of climatic conditions, where there was a moisture stress in the experimental period which may have affected the soil microbes, as adverse environmental conditions affect the soil microbial activity (Nannipieri et al., 2003).

In soybean emergence (E1), with the addition of "K glycerin", microbial basal respiration (Rbasal) increased linearly in TH (Rbasal: $\hat{y} = 1.32 + 0.015x$, $R^2 = 0.85$) and quadratic (Rbasal: $\hat{y} = 1.15 + 0.034x - 0.00011x^2$, $R^2 =$

Table 4. Effect of K_2O doses in the form of “K Glycerin” (KG) and inorganic sources (KCl and K_2SO_4) on microbiological analyzes evaluated in the emergency (E) and harvest (H) of soybean applied to two types of soil.

Treatment	FDA (mg kg^{-1} h^{-1} FDA)		Rbasal (mg kg^{-1} h^{-1} CO_2)		Cmic (mg kg^{-1} soil)		qCO_2 (µg CO_2 µg C_{mic}^{-1} day^{-1})	
	Emergency	Harvest	Emergency	Harvest	Emergency	Harvest	Emergency	Harvest
Typic Hapludox: KG doses (kg K_2O ha^{-1})								
0	4.27^a	0.69^a	1.29^e	0.69^b	374.4^a	389.6^a	0.08^f	0.04^a
40	4.00^a	0.91^a	1.54^d	0.90^a	112.6^e	241.5^b	0.33^c	0.09^a
80	3.39^b	1.14^a	3.09^b	0.95^a	149.7^d	155.5^c	0.49^a	0.14^a
160	4.33^a	1.07^a	3.46^a	1.00^a	187.1^c	137.2^c	0.44^b	0.17^a
KCl (80 kg K_2O ha^{-1})	4.59^a	1.06^a	2.41^c	0.96^a	214.5^c	119.8^c	0.27^d	0.19^a
K_2SO_4 (80 kg K_2O ha^{-1})	3.34^b	1.05^a	2.39^c	1.09^a	273.5^b	125.3^c	0.21^e	0.21^a
F-test	*	NS	*	NS	*	*	*	NS
Typic Quartzipsamment: KG doses (kg K_2O ha^{-1})								
0	1.31^a	2.01^b	1.30^d	2.36^b	145.2^c	266.0^b	0.22^c	0.21^a
60	1.31^a	2.16^b	2.44^c	2.69^b	272.4^b	212.4^d	0.21^c	0.30^a
120	0.83^a	2.08^b	4.14^a	3.23^a	310.6^a	264.7^b	0.32^b	0.29^a
240	0.74^a	2.54^a	3.72^b	3.48^a	144.2^c	244.7^c	0.62^a	0.34^a
KCl (120 kg K_2O ha^{-1})	0.90^a	1.95^b	1.57^d	2.54^b	65.2^d	214.5^d	0.58^a	0.29^a
K_2SO_4 (120 kg K_2O ha^{-1})	1.16^a	2.21^b	2.10^c	2.69^b	86.2^d	296.5^a	0.58^a	0.22^a
F-test	NS	*	*	*	*	*	*	NS
CV (%)	15.1		17.1		9.2		35.6	

NS: Non-significant at $p<0.05$; *: Significant at $p>0.05$. Means with the same treatment and column sharing the same letters are not significantly different at $p<0.05$. FDA: Flurescein diacetate hydrolysis; Rbasal: Soil basal respiration; Cmic: Soil microbial biomass carbonic; qCO_2: Metabolic quotient.

0.94) in TQ, which only differed between the two K inorganic sources in the last soil (Table 4). The application of organic waste (“K glycerin”) Rbasal increased due to addition of organic C and nutrient (Lambais and Carmo, 2008) in both soils. The K inorganic sources (KCl and K_2SO_4) did not stimulate the soil microbes and the “K glycerin” doses were not sufficient to promote higher activity regarding the K mineral sources in the TH soil because the increased soil microbial activity depend on carbon available soil (Araújo and Monteiro, 2006). In soybean harvest (E2), TQ soil only increased the Rbasal with increasing doses of “K glycerin” that differed from K inorganic sources (Table 4). The moisture stress occurred during the experimental period in the cultivation of soybean decreased microbial activity in TH. The higher doses of “K glycerin” apply the TQ may not have been completely decomposed, with a residual effect of C organic in soil. The organic matter added to the soil in the form of organic waste according to the degree of decomposition, may have an immediate or residual soil by means of a slower process of decomposition (Santos et al., 2001).

In soybean emergence (E1), the soil microbial biomass carbonic (Cmic) in TH were reduced quadratic “K glycerin” doses (Cmic: $\hat{y} = 348.94 - 5.30x + 0.027x^2$, $R^2 = 0.90$) that differed from K inorganic sources (Table 4), may have been due to an effect of “K glycerin” that harmed the soil microbes. At harvest of soybean (E2) in TH, due to lack of rainfall during the experimental period, the Cmic values were lower at the time E1, with a quadratic decrease with increasing doses of “K glycerin” (Cmic: $\hat{y} = 388.71 - 4.30x + 0.017x^2$, $R^2 = 0.99$), which differ from the inorganic sources of K (Table 4). In TQ, the behavior of the Cmic was different, showing that “K glycerin” stimulated the growth of microbes quadratically in both evaluation periods E1 (Cmic: $\hat{y} = 145.86 + 2.78x - 0.012x^2$, $R^2 = 0.99$) and E2 (Cmic: $\hat{y} = 216.42 + 0.83x - 0.003x^2$, $R^2 = 0.95$), but the K inorganic sources (KCl and K_2SO_4) caused some effect detrimental microbial growth, with reduced Cmic (Table 4). Environmental factors among them such as soil moisture, can modify the ecology, population dynamics and soil microbial activity due to modification of the microbial habitat (Nannipieri et al., 2003).

The metabolic quotient (qCO_2) valuated in soybean emergence (E1) may verify that the application of “K glycerin” doses and K inorganic sources caused more stress in both soils (Table 4). Already at the time of harvest of soybean (E2), the evaluation can verify the stabilizing trend in both soils due to exhaustion of nutrients, thus achieving a balance in the middle, by reducing stress soil (Moreno et al., 1999). The application “K glycerin” caused no major changes in the soil chemistry unless the concentration of K and S is a component of this organic waste arising from the production process of biodiesel. Microbiological analysis of soil showed a higher when stress was applied and reducing the end of the experimental period. The values of qCO_2 indicate that the use of "K glycerin" does not cause stress in the soil, thus can be used without major environmental consequences related to their direct application to the soil.

The use of "K glycerin" in the field of soybean yield in soils with average K content, resulting in the recommendation of 87 kg ha^{-1} of K_2O to achieve maximum grain yield would result in the removal of 3.5 m^3 ha^{-1} crude glycerin in the biodiesel industry stocks. The area planted with soybeans in Brazil in the harvest of 2012/2013 was just over 28 million ha (Conab, 2013); thus 1% of this area was used to "K glycerin" would remove national stocks a total of 98 million m^3 of glycerin, making this waste not disposed to harming the environment. The "K glycerin" does not cause environmental problems to the place where it was applied as recommended by the available K in soil, however, studies are needed to evaluate its long-term use to understand the dynamics of the crop yield and soil.

Conflict of Interest

The authors have not declared any conflict of interests.

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Water relations and gas exchange in castor bean irrigated with saline water of distinct cationic nature

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Aiming to evaluate the water relations and gas exchange in castor bean cv. BRS Energia under salinity and cationic nature of irrigation water, an experiment was conducted under controlled conditions in an Ultisol Eutrophic of sandy loam texture. Treatments consisted of six combinations of water salinity (S_1– Control (supply water ECw=0.6 dS m^{-1}); S_2 - Na^+, S_3 - Ca^{2+}, S_4 - Na^++ Ca^{2+}, S_5 - K^+ and S_6 - Na^++ Ca^{2+} + Mg^{2+}all prepared with chloride salt of respective cation and having ECw=4.5 dS m^{-1}, distributed in a randomized block design with four replications. The use of saline water of distinct cationic nature affected the water relations of the castor bean, the least deleterious effects were observed in plants irrigated with potassic water The negative effects of the cationic nature of irrigation water were more evident in gas exchange at 40 days after sowing (DAS), the highest values for stomatal condutance, transpiration rate, assimialation of CO_2, instantaneous carboxylation efficiency and intrinsic water use efficiency were observed in case of potassium ion. The castor bean 'BRS Energia' is more sensitive to the presence of sodium ions in the irrigation water, both in terms of water relations as well as for gas exchange.

Key words: *Ricinus communis* L., salt stress, semiarid.

INTRODUCTION

The demand for vegetable oils is growing every year and the global interest in oilchemical industry is high, given its application in diverse forms. In this context, the castor bean (*Ricinus communis* L.) stands out as a distinct crop since oil extracted from its seeds has excellent properties of wide use as an industrial input due to the fact that in many of its applications, it can not be replaced by other vegetable oils (Santos and Kouri, 2007). This crop is

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unique source of ricinoleic acid (over 85% of oil, which may be used for the manufacture of surfactants, coatings, lubrificants, fungicides, pharmaceuticals and cosmetics and a variety of other products (Pinheiro et al., 2008; Babita et al., 2010).

In the semi-arid region of Northeast Brazil, the uneven distribution of rainfall during the year makes irrigation as an indispensable practice for crop production. However, most of the surface reservoirs (small and medium size and ponds) and ground water (wells) has high salt content, and present variability in their ionic composition (Suassuna and Audry, 1990). Use of these water sources may, depending on its constitution, change the physiological and biochemical functions of plants resulting in disturbances in water relations and changes in the absorption and metabolism of essential plant nutrients (Amorim et al., 2010).

Furthermore, the use of water with different ionic compositions may cause varying degrees of stress to plants or alter the physical and chemical properties of the soil (Aquino et al., 2007) however, the degree of severity with which these components influence plant development depends on factors such as plant species, cultivar, growth stage and the ionic composition of the water (Sousa et al., 2012). Among the physiological indices affected by salinity stand outs the CO_2 assimilation rate, transpiration, stomatal conductance and internal CO_2 concentration, which can be inhibited by accumulation of Na^+ and/or Cl^- in chloroplasts, affecting biochemical and photochemical processes involved in photosynthesis (Taiz and Zeiger, 2013).

Associated with this is the interferences of salts in the water relations of the plants that are reflected in osmotic potential, relative water content and saturation water deficit in leaf, in addition to the ion toxicity and imbalance of nutrient absorption causing widespread reduction of growth with serious damage in productivity (Ahmed and Moritani, 2010). Thus, despite the existence of genetic variability for tolerance to salinity, physiological and biochemical mechanisms that contribute to this tolerance are still poorly understood, mainly under different cationic nature of water (Mansour et al., 2003), especially in castor bean crop cv. BRS Energia.

Considering understanding of changes in water relations and physiological responses of plants grown under the influence of abiotic stress, such as saline, can provide important tools to aid in the management of species irrigated with saline waters, this study proposed to evaluate the water relations and gas exchange in the castor bean cv. BRS Energia irrigated with saline water of distinct cationic nature.

MATERIALS AND METHODS

The experiment was carried out during the period of November 2013 to February 2014 under greenhouse conditions at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), located in the municipality of Campina Grande, PB, with geographic coordinates 7°15'18 " S, 35°52'28 " W and mean altitude of 550 m. Treatments consisted of six combinations of water salinity (S_1 - Control; S_2 - Na^+, S_3 - Ca^{2+}, S_4 - Na^++ Ca^{2+}, S_5 - K^+ and S_6 - Na^++ Ca^{2+} + Mg^{2+}), in a randomized block design with four replications each constituted of five plants, totaling 120 experimental units. Plants in the control treatment (S_1) were irrigated with water of electrical conductivity (ECw) of 0.6 dS m^{-1} while in other treatments (S_2, S_3, S_4, S_5 and S_6) were irrigated with ECw = 4.5 dS m^{-1}. For the preparation of water, chloride salt of respective cation was used maintaining equivalent ratio of 1:1 in S_4 (Na^+: Ca^{2+}) and 7:2:1 in S_6 (Na^+: Ca^{2+}: Mg^{2+}), respectively. In Table 1 the characteristics of water used in S_1 (control) treatment are presented. Seeds of castor bean cultivar BRS Energia were used, the plants of genotype present crop cycle of 120-150 days, semi-indehiscent fruits, oil content in seeds on average 48% and productivity of approximately 1.800 kg ha^{-1} (Silva et al., 2009).

Plastic recipients of 100 L capacity (height 50 cm, diameter at bottom 30 cm and superior 33 cm) were used, these were filled with 2 kg of crushed stone (size zero), 54 kg of homogenized soil (without clods) followed by 76 kg of the same soil mixed with humus to attain 1% organic matter, in the total soil volume. The soil used in the study was an Ultisol Eutrophic, collected in a depth of 0 to 30 cm in the district of São José da Mata (Campina Grande-PB). The chemical and physical characteristics of soil (Table 2) were determined according to the methodologies proposed by Claessen (1997). The recipient was adopted as lysimeter having two openings at the bottom to allow drainage of excess water which was collected in plastic bottles allowing estimation of the water consumed by the plant using equation of water balance.

On the basis of soil analysis correction of pH was performed according to Ribeiro et al. (1999) by adding 49.25 g of lime to the soil of each lysimeter (130 kg soil), amount required for neutralization of Al^{3+} and raising Ca^{2+} and Mg^{2+} saturation to 70%. After liming the soil presented the following chemical characteristics: Ca^{2+}; Mg^{2+}; Na^+; K^+; H^+; Al^{3+} and CEC = 1.14 ,1.36, 0.30, 0.14 , 0.11 , 0 and 3.05 $cmol_c$ kg^{-1}, respectively; Organic matter = 1.08 dag kg^{-1}; P = 47.80 mg kg^{-1} and pH soil: water (1: 2.5) = 6.42.

Different irrigation waters were prepared using the chloride salt of respective ion using the water of the local supply system. The amount of salt to be added was determined according to the equation recommended by Richards (1954), taking into account the relationship between ECw and the concentration of salt (10*mmol L^{-1} = 1 dS m^{-1}). The salts used in the preparation of irrigation water had a purity above 99%. Before seeding, volume of water required to reach the field capacity of soil was determined by capillary saturation method followed by free drainage. After the soil was raised to the field capacity by addition of respective water, sowing was performed by placing ten seeds of castor bean cv. BRS Energia in each recipient, at a depth of 5 cm, and distributed equidistantly.Ten days after sowing (DAS) thinning was carried out to maintain one plant per recipient.

The soil was kept at field capacity with daily irrigation, applying volume of respective treatment according to the water consumed by plants, estimated by water balance: volume of water applied minus the volume of water drained in the previous irrigation, plus leaching fraction of 0.10, on the basis of studies conducted previously (Nobre et al., 2013; Lima et al., 2014).

Fertilization was carried out as per recommendations of Novais et al. (1991) using 40.62 g of potassium nitrate and 75 g of monoammonium phosphate, corresponding to 100, 150 and 300 mg kg^{-1} of N , K_2O and P_2O_5, respectively, as top dressing in four applications at intervals of ten days, beginning at 15 DAS. In order to meet the possible micronutrient deficiencies 5 L of solution containing 2.5 g L^{-1} ubyfol (N -15, P_2O_5-15, K_2O -15, Ca -1, Mg -1,4, S -2.7, Zn -0.5, B - 0.05, Fe -0.5, Mn -0.05, Cu -0.5, Mo - 0.02%) was applied by foliar spraying at 30 and 60 DAS.

The phytosanitary treatments were carried out during the assay

Table 1. Chemical characteristics of the supply water used in the control treatment.

Ca^{2+}	Mg^{2+}	Na^+	K^+	HCO_3^-	CO_3^-	Cl^-	Ecw dS m^{-1}	pH	SAR (mmol $L^{-1})^{0,5}$
			($mmol_c$ L^{-1})						
1.19	1.58	2.83	0.10	1.45	0.00	4.22	0.60	7.23	2.41

ECw–electrical conductivity SAR - sodium adsorption ratio.

Table 2. Chemical and physical characteristics of the soil used in the experiment before application treatment.

Chemical characteristics								
pH_{ps}	O.M. dag kg^{-1}	P (mg kg^{-1})	K^+	Na^+	Ca^{+2}	Mg^{+2}	Al^{+3}	H^+
			..........($cmol_c$ kg^{-1})..........					
5.10	0.34	20.09	0.07	0.05	0.40	1.30	0.04	1.74

Physical characteristics								
Size fraction (g kg^{-1})			Textural class	Water content (kPa)		AW	Total porosity m^3m^{-3}	Density (kg dm^{-3})
Sand	Silt	Clay		33.42	1519.5			
				....	dag kg^{-1}	....		
856.10	110.7	33.20	SL	6.72	1.62	5.10	0.49	1.54

pH_{PS} - pH of saturated paste; O.M– organic matter: determined by wet digestion Walkley-Black method; Ca^{2+} and Mg^{2+} extracted with KCl 1 mol L^{-1} at pH 7.0; Na^+ and K^+ extracted with NH_4 OAc 1 mol L^{-1} at pH 7.0; SL – sandy loam; AW – available water.

period, consisting of manual weeding at weekly interval, scarification of surface soil before each irrigation event and staking of plants in the flowering stage, in order to prevent lodging. In addition, insecticide of neonicotinoid chemical group, triazole fungicide chemical group and acaricide belonging to abamectin chemical group were applied at a dose of 5.4; 7.0 to 3.5 g L^{-1}, respectively.

The effects of different treatments on castor bean crop were observed at 20 and 40 DAS, by determining the osmotic potential of leaf (ψs), electrolyte leakage in the membrane (ELM), relative water content (RWC), water saturation deficit (WSD), stomatal conductance (*gs*), transpiration (*E*), CO_2 assimilation rate (*A*), internal CO_2 concentration (CI), instantaneous carboxylation efficiency (EICI) and intrinsic water use efficiency (WUE).To determine the osmotic potential of castor bean, leaves of the plants were collected from middle third part, placed in plastic bags and stored at 5 °C; to extract the cell exudate, samples were placed in tubes for centrifugation at 10000 rpm for 10 minutes; the freezing point of the samples was measured by reading aliquots of 5 mL in osmometer microprocessor (PZL 1000) determining in this way, the osmolality of the sample in H_2O mOsm kg^{-1} being converted in MPa, as per recommendation of Bagatta et al. (2008), by Equation (1):

$$\psi s\ (\mathrm{MPa}) = -C\left(\frac{m0smol}{kg}\right) x\ 2.58\ x 10^{-3} \quad (1)$$

Wherein: ψs (MPa) - leaf osmotic potential; C - sample osmolality observed in the osmometer.

In order to assess the capacity of rupture of cell membrane under saline stress, leakage of electrolyte was determined in the cell membrane. For the purpose, 3^{rd} leaf of the shoot from apex was collected, 10 discs of 113 mm², were washed with distilled water in order to remove other electrolytes adhered to the leaves, after words discs were placed in beaker with 50 ml of double distilled water and closed tightly with foil. The beakers were kept at 25°C for 90 min and proceeded initial determination of electrical conductivity (C); later the beaker was taken to the oven with forced air ventilation and submitted to temperature of 80°C for 90 min, when the measurement of the final electrical conductivity (Cf) was performed. Thus, leakage of electrolyte in the cell membrane was obtained according to Scott Campos and Thu Phan Thi (1997), using Equation (2):

$$\mathrm{ELM} = \frac{\mathrm{Ci}}{\mathrm{Cf}} \mathrm{x}100 \quad (2)$$

Wherein: ELM – electrolyte leakage in the membrane (%); Ci –initial electrical conductivity (dS m^{-1}); Cf- final electrical conductivity (dS m^{-1}).

For assessing the water status of the plant the relative water content (RWC) in the leaf blade was determined; three fully expanded leaves located in the upper third part of the plant were used. The determination of RWC was performed according to the methodology of Weatherley (1950), using Equation (3):

$$\mathrm{RWC}\ (\%) = \frac{(\mathrm{FW} - \mathrm{DM})}{(\mathrm{TM} - \mathrm{DM})} \mathrm{x}100 \quad (3)$$

Wherein: RWC- relative water content (%); FW- fresh weight of leaf (g); TM-turgid mass (g); DM- dry mass (g);

The water saturation deficit (WSD) is an excellent indicator of the water balance of the plant as it represents the amount of water it needs to reach saturation. In this regard, WSD was determined according to the methodology described by Taiz and Zeiger (2013) according to Equation (4):

$$\mathrm{WSD}\ (\%) = \frac{(\mathrm{TM} - \mathrm{FW})}{(\mathrm{TM} - \mathrm{DM})} \mathrm{x}100 \quad (4)$$

Wherein: WSD - water saturation deficit (%); FW - fresh weight of leaf (g); TM - turgid mass (g); DM - dry mass (g).

Stomatal conductance (*gs*) (mol of H_2O m^{-2} s^{-1}), transpiration (*E*) (mmol of H_2O m^{-2} s^{-1}), the CO_2 assimilation rate (*A*) (μmol m^{-2} s^{-1})

Table 3. Summary of analysis of variance and estimated means of different contrasts (ŷ) related to the osmotic potential of leaf (ψs), electrolyte leakage in the membrane (ELM), relative water content (RWC) and water saturation deficit (WSD) of castor bean cv. BRS Energia irrigated with water of different types of salinity, at 20 and 40 days after sowing (DAS).

SV	F test							
	ψs		ELM[1]		RWC		WSD[2]	
	Days after sowing							
	20	40	20	40	20	40	20	40
Block	ns	ns	ns	*	ns	ns	ns	ns
Types of Salinity	*	*	**	*	**	**	**	*
$\hat{y}_1$	*	*	*	*	*	*	*	*
$\hat{y}_2$	ns	ns	*	*	**	ns	**	ns
$\hat{y}_3$	ns	ns	ns	ns	**	ns	**	ns
$\hat{y}_4$	ns	7ns	ns	ns	**	*	**	*
$\hat{y}_5$	ns	ns	*	*	*	*	**	*
CV	3.46	6.18	12.42	11.33	6.46	11.48	12.91	24.53
	Mean estimate							
$\hat{y}_1$	-0.15	-0.23	-5.00	-1.71	11.46	20.01	-11.46	20.01
$\hat{y}_2$	ns	ns	5.96	3.01	-30.14	ns	30.14	-8.25
$\hat{y}_3$	ns	ns	ns	ns	-27.36	ns	27.36	-10.45
$\hat{y}_4$	ns	ns	ns	ns	-30.51	-29.65	-30.51	-29.65
$\hat{y}_5$	ns	ns	5.70	1.62	9.69	23.77	-9.96	23.77

$\hat{y}_1$ (S_1 vs S_2, S_3, S_4, S_5, S_6); $\hat{y}_2$ (S_2 vs S_3); $\hat{y}_3$ (S_2 vs S_6); $\hat{y}_4$ (S_2 vs S_5); $\hat{y}_5$ (S_5 vs S_2, S_3, S_4, S_6); VS – variation source; CV – Coefficient of variation; (*) and (**) Significant at 0.05 and 0.01 probability; (ns) not significant; [1]statistical analysis after data transformation in $\sqrt{X}$; [2]statistical analysis after data transformation in $\sqrt{X+1}$.

and the internal CO_2 concentration (μmol m^{-2} s^{-1}) (CI) were also evaluated in the third leaf from the apex, using portable photosynthesis measurement "LCPro +" equipment from ADC BioScientific Ltda. These data were used to quantified the intrinsic water use efficiency (WUE) (A/E) [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] and the instantaneous efficiency of carboxylation (A/Ci) [(μmol m^{-2} s^{-1}) (μmol mol^{-1})]$^{-1}$(Jaimez et al., 2005).

The data were evaluated by analysis of variance following 'F' test; when significant, mean comparison test (Tukey at 0.05 probability) and the contrasts between the treatment means were performed using the SISVAR-ESAL statistical software. For each treatment apart from mean, standard error was also calculated. The contrasts were defined as follows: $\hat{y}_1$ (S_1 vs S_2, S_3, S_4, S_5, S_6); $\hat{y}_2$ (S_2 vs S_3); $\hat{y}_3$ (S_2 vs S_6); $\hat{y}_4$ (S_2 vs S_5); $\hat{y}_5$ (S_5 vs S_2, S_3, S_4, S_6). Given the normality of residues from the present study observed through the high coefficient of variation values (CV> 20%) (Tables 3 to 5), it was necessary to perform exploratory data analysis with transformation of data in $\sqrt{X}$ and $\sqrt{X+1}$.

RESULTS AND DISCUSSION

Analysis of variance (Table 3) indicated significant effect of salinity on osmotic potential of leaf, electrolyte leakage, relative water content and water saturation deficit of castor bean at 20 and 40 DAS. Comparison of means for the osmotic potential of leaves of castor bean at 20 DAS (Figure 1A), indicated that plants irrigated with water of low electrical condutvity (S_1) reached the highest ψs and differed significantly (p<0.05) from those receiving S_2, S_4, S_5, S_6 and comparing the means of these treatments, it is observed that the plants irrigated with water having sodium (S_2, S_4, S_6) and potassium (S_5) ion in its composition, had the lowest values in the energy status of the water in the leaves.

At 40 DAS significant effect (p<0.05) was observed in leaf osmotic potential (Figure 1B) in plants irrigated with water of lowest salinity (S_1) as compared to the S_2, S_4 and S_5 treatments, however, there were no significant differences compared to plants irrigated with the S_3 and S_6 treatments. According to comparison of means, the lowest values for the leaf osmotic potential occurred in plants S_2 followed by plants of treatments S_5 and S_4.

Comparing the data of 20 DAS with that of 40 DAS, a greater reduction in osmotic potential of leaf in the latter period is observed. This response suggests that the decrease in ψs is a function of exposure time to salt stress, in order to maintain a high water potential in tissues. This decrease in leaf osmotic potential can be considered an adaptive strategy of the species in relation to increased salt concentration in the soil solution enabling the hydration of plant tissues and slowing the damaging processes caused by water deficit (Santos et al., 2012; Coelho et al., 2014).

In assessing the contrasts of the means for the osmotic potential of leaf at 20 and 40 DAS (Table 3) it is noticed that the plants irrigated with ECw = 0.6 dS m^{-1} (S_1) differed significantly in relation to those irrigated with ECw of 4.5 dS m^{-1} (S_2, S_3, S_4, S_5, S_6). In both periods the

Table 4. Summary of analysis of variance and estimated means related to the stomatal conductance (*gs*), transpiration (*E*), assimilation rate of CO_2 (*A*), internal concentration of CO_2 (CI), instantaneous efficiency of carboxylation (EICI) and intrinsic water use efficiency (WUE) of castor bean plants irrigated with water of different types of salinity at 20 and 40 days after sowing.

SV	F test											
	gs[1]		*E*		*A*		CI		EICI		WUE	
	Days after sowing											
	20	40	20	40	20	40	20	40	20[1]	40	20	40
Block	ns	*	*	*	ns	*	**	**	ns	*	*	**
Types of Salinity	ns	*	ns	ns	**	**	**	ns	**	*	*	*
$\hat{y}_1$	ns	*	ns	*	*	**	*	ns	*	*	*	*
$\hat{y}_2$	ns	ns	ns	*	*	*	ns	ns	*	*	ns	*
$\hat{y}_3$	ns	ns	ns	ns	ns	*	*	ns	*	*	ns	*
$\hat{y}_4$	ns	ns	*	*	*	**	*	ns	*	*	ns	*
$\hat{y}_5$	ns	ns	*	ns	*	*	*	ns	*	*	ns	*
CV	21.55	17.37	19.51	13.83	13.81	17.95	6.91	5.28	17.76	10.56	18.91	16.52
	Mean estimate											
$\hat{y}_1$	ns	0.84	ns	0.90	8.61	6.55	-30.00	ns	0.07	0.02	0.84	0.77
$\hat{y}_2$	ns	ns	ns	-0.66	-7.17	-5.71	ns	ns	-0.04	-0.02	ns	-1.44
$\hat{y}_3$	ns	ns	ns	ns	ns	4.79	-17.00	ns	-0.02	-0.02	ns	-1.27
$\hat{y}_4$	ns	ns	-2.80	-0.55	-9.06	-8.64	24.00	ns	-0.06	-0.03	ns	-2.40
$\hat{y}_5$	ns	ns	2.41	ns	6.45	5.51	-22.00	ns	0.05	0.02	ns	1.62

$\hat{y}_1$ (S_1 vs S_2, S_3, S_4, S_5, S_6); $\hat{y}_2$ (S_2vs S_3); $\hat{y}_3$ (S_2vs S_6); $\hat{y}_4$ (S_2vs S_5); $\hat{y}_5$ (S_5vs S_2, S_3, S_4, S_6); VS – variation source; CV – Coefficient of variation; (*) and (**) Significant at 0.05 and 0.01 probability; (ns) not significant; [1]statistical analysis after the transformation of data in $\sqrt{X}$.

data indicated that the plants when irrigated with water of 0.6 dS m^{-1}, increased their Ψs by -0.15 and -0.23 MPa in reference to plants irrigated with ECw of 4.5 dS m^{-1}, respectively, at 20 and 40 DAS. However, analysing the other contrasts there were no significant changes in the Ψs at any time of evaluation. In this context, it can be inferred that the different cations present in the irrigation water affected Ψs in a similar way.

For the electrolyte leakage measured at 20 DAS it is observed through the comparison of means (Figure 1C) that plants under irrigation with water of low salinity (S_1) and predominant in calcium (S_3) had the smallest leakage in membrane (10.52 and 11.34%, respectively) showing minor damage to the integrity of the leaf membrane. This result is justified by the role of calcium in the integrity of cell membrane, being a constituent of the cell wall (pectates), participating in the regulation of functionality of the cell membrane, and activation of several enzyme systems (Mengel and Kirkby, 2000). But when the plants were irrigated with S_5 treatment (potassium), largest electrolyte leakage (20.51%) was observed, though statistically not different from plants that were submitted to S_2 (Na), S_4 (Na+Ca) and S_6 (Na+Ca+Mg), indicating occurrence of injuries to cell membrane. Increasing the concentration of electrolytes in leaf cells may represent a mechanism to prevent desiccation of the tissue due to the reduction of the osmotic component of leaf water potential (Fioreze et al., 2013).

Similar to that observed at 20 DAS, electrolyte leakage obtained at 40 DAS (Figure 1D) differed significantly due to the application of different types of salts, the largest highlighting values (12.84 and 12.81%) were observed in plants irrigated with water prepared using NaCl (S_2) and KCl (S_5). It is noted when plants were irrigated with water having calcium in its composition (S_3) or with low concentration of salt (S_1), the lowest values were obtained for electrolyte leakage. It appears from the results obtained for ELM, the plants irrigated with water consisting of potassium, showed the greatest permeability of the

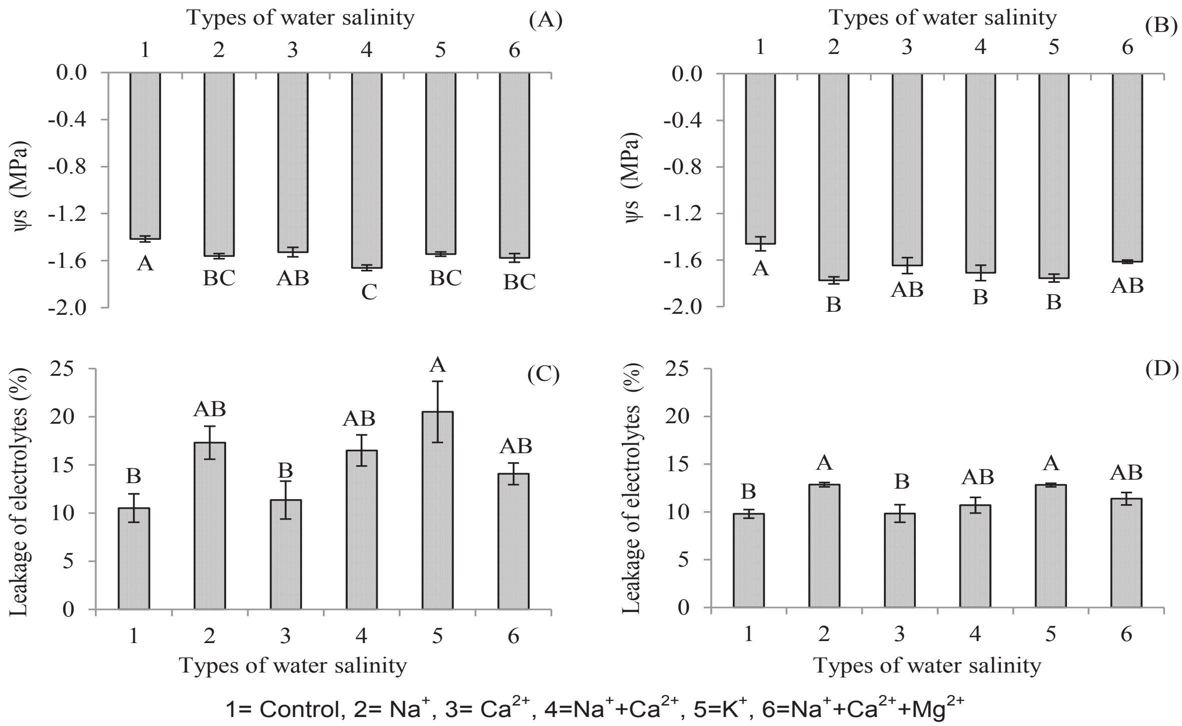


Figure 1. Osmotic potential of leaf - ψs of (A and B), and leakage of electrolytes in the leaves (C and D) of castor bean at 20 and 40 days after sowing, depending on the types of irrigation water salinity.

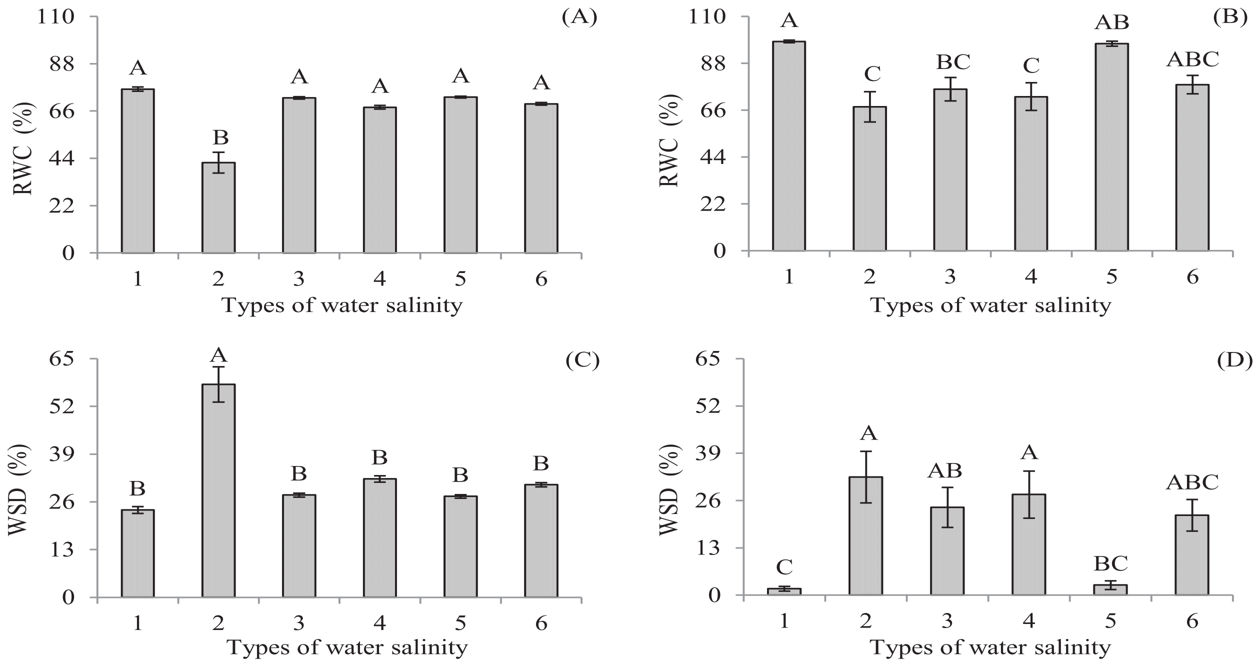
membrane with consequent release of ions, that is, greater loss of integrity and destabilization of the cell membrane. The rupture of cell membrane occurs through the release of electrolytes in higher levels of stress due to increased amounts of reactive oxygen species (superoxide) free radical and lysing enzymes, that result in the disruption and increased permeability of membranes and often irreversible damage to the organelles and molecules present within the cells (Alonso et al., 1997).

The contrasts of means for the leaf electrolyte leakage at 20 and 40 DAS, are presented in Table 3, comparing the data of plants irrigated with ECw of 0.6 dS m^{-1} in relation to those under ECw = 4.5 dS m^{-1}, significant effect was found at both stages. On the basis of estimated means (Table 3) a decrease in electrolyte leakage of 5.0 and 1.17% was observed in plants irrigated with low salinity water (S_1) compared to plants subjected to ECw of 4.5 dS m^{-1}, respectively, at 20 and 40 DAS.

The higher ELM observed in plants under the high level of electrical conductivity, is the result of stress to which the plants were exposed because of high salt concentrations found in irrigation water which caused changes in water status of the plant due to the decrease in osmotic potential of leaf (Figure 1A and B). In a manner similar to that observed for S_1 versus other treatments, S_3 vs. S_2 also presented significant differences observing increase in leakage of electrolyte of 3.01 to 5.96% at 20 and 40 DAS, respectively; however S_2, when confronted with S_6 and S_5 did not influence significantly the ELM in any study period (Table 3).

This response may be related to the decrease in Ψs (Figure 1A and B) due to the excess salts present in irrigation water because the effect of cationic nature of water occurred in similar way. While the S_5 versus S_2, S_3, S_4 and S_6 showed significant effect on the ELM indicating, through the mean estimate (Table 3) that irrigation water with potassium increased by 5.70 and 1.62% ELM compared to the other treatments (S_2, S_3, S_4 and S_6) at 20 and 40 DAS, respectively. The increase in leakage of leaf electrolytes, especially in plants irrigated with potassic water (S_5), may have occurred because of this element does not participate in the composition of any organic compound (structural function) in plant metabolism (Rodrigues et al., 2009), therefore is easily released and/or redistributed to the different organs of the plant.

Analysing the relative water content (RWC) of leaf obtained at 20 DAS (Figure 2A), it is observed that the plants irrigated with sodic water (S_2) showed lower RWC (42%), differing from the other treatments. On the other hand, plants subjected to irrigation treatment under S_1, S_3, S_4 S_5 and S_6 were statistically similar. The reduction in RWC of leaf observed in the plants irrigated with sodium water, can be related to an increase in damage caused to



1= Control, 2= Na^+, 3= Ca^{2+}, 4=Na^++Ca^{2+}, 5=K^+, 6=Na^++Ca^{2+}+Mg^{2+}

Figure 2. Relative water content - RWC (A and B), and water saturation deficit -WSD (C and D) of leaf of castor bean at 20 and 40 days after sowing, depending on the types of irrigation water salinity.

cell membranes (Figure 1C and D), because the increase in the leaching of ions affect the cell water potential and therefore promoted loss of cellular turgor (Mansour and Barbosa, 2000). This restriction in the leaf water status, observed in the present study by reduction in RWC affects the absorption of nutrients by the roots and therefore growth and development of plants are seriously impaired.

Comparison by mean test at 40 DAS (Figure 2B) showed that the castor bean plants irrigated with low salinity water (S_1) and water salinized by potassium (S_5) had a significantly higher RWC, averaging respectively 98.23 and 97.24%, whereas plants irrigated with other treatments (S_2, S_3; S_4 and S_6) had respectively 67.58, 75.85, 72.36 and 78.04%. It should be underscored that the effects of different types of salinity were more evident in plants irrigated with water treatments S_2 and S_3 (Figure 2B). When results are compared, a decrease of 30.50, 21.99, 25.58 and 19.74% occurs in plants irrigated with treatments S_2, S_3; S_4 and $S_{6,}$ when contrasted to those irrigated with potassium water.

As mentioned earlier, decrease in the RWC of leaf in plants under saline stress induced by different types of salt (S_2, S_3, S_4 and S_6) is an adaptive mechanism developed by the plants, even though the absorption of water from the soil occurs in low proportions (Garcia et al., 2009). Further, water deficiency, caused by osmotic effect, characterizes physiological drought and brings about morphological and anatomical changes in the plants with an imbalance in water absorption and in transpiration rates (Santana et al., 2011).

Contrasts of means for RWC presented in Table 3 show that plants irrigated with low ECw (0.6 dS m^{-1}) have significant differences compared to treatment with water at 4.5 dS $m^{-1}$and estimated means at 20 and 40 DAS indicated an increase of 11.46 and 20.01%, when plants were irrigated with water of ECw=0.6 dS m^{-1}. In the case of treatment S_2 versus S_3 and S_2 versus S_6, the differences were significant at 20 DAS and during this period, RWC decreased respectively by 30.14 and 27.36% in plants irrigated with treatment $S_{2.}$

However, when S_2 versus S_5 is analysed, there is significant influence on RWC in both the periods under analysis, the decrease in treatment S_2 being 30.51 and 29.65%, respectively at 20 and 40 DAS. Table 3 shows that the RWC of plants irrigated with S_5 was affected significantly when compared to treatments S_2, S_3, S_4 and S_6 and estimate of mean showed an increase of 9.69 and 23.77%, respectively, at 20 and 40 DAS in plants irrigated with S_5. Results in current study demonstrate a greater decrease in RWC of leaf with the cationic traits of water particularly the plants irrigated with sodic water. Water saturation deficit (WSD) of castor bean plants assessed at 20 DAS was affected significantly ($p<0.05$) by irrigation water of different types of salinity.

Comparison by mean test (Figure 2C) showed that WSD of plants irrigated with water salinized by sodium (S_2) was significantly higher than other treatments (S_1, $S_{3,}$

S_4, S_5 and S_6). Comparison of results in Figure 2A and C reveal inverse relation between RWC and WSD. Increase in WSD may be due to the high osmotic level of the soil solution which affects the availability of water for the plants. Its absorption becomes more difficult, with the occurrence of low turgor pressure and elongation of cells (Jácome et al., 2003).

The behavior of the results of water saturation deficit of the castor bean plant at 40 DAS is different from that at 20 DAS. When means of treatments are compared (Figure 2D), the plants irrigated with low ECw (S_1) and with potassium-induced salinity (S_5) showed the lowest means, differing statistically from those irrigated by treatments S_2, S_3 and S_4. However, there was no significant difference with regard to plants irrigated with S_6. Means of treatments at 40 DAS revealed a smaller WSD in all the treatments (Figure 2D) compared to 20 DAS (Figure 2C) possibly because of osmotic adjustment in the castor bean plants.

Data of estimates of means (Table 3) demonstrate that there was a reduction of 11.46 and 20.01% in WSD in plants under control treatment compared to those under ECw=4.5 dS m^{-1} at 20 and 40 DAS respectively. Lowest WSD in plants at the low water salinity (0.6 dS m^{-1}) may be explained by the high foliar osmotic potential (Figures 1A and B), due to decrease in electrolyte leakage (Figure 1C and D) and higher RWC in leaves (Figures 2A and B) in the two periods under analysis.

Contrast between treatments S_2 versus S_3 (Table 3) demonstrated that, when sodic water was used for irrigation, the plants had 30% higher WSD compared to S_3 (calcium) while at 40 DAS, plants in S_3 had 8.25% increase in WSD when compared to those with S_2. When treatment S_2 is compared to S_6 at 20 DAS, one may note that the plants irrigated with former had a 27.36% increase in WSD compared to those irrigated with S_6, however, at 40 DAS, the plants suffered 10.45% WSD when compared to those with water salinity induced by Na+Ca+Mg.

In the case of treatments S_2 versus S_5, the plants irrigated with sodic water showed an increase of 30.51 and 29.65% in WSD, respectively at 20 and 40 DAS. On the other hand, there was a 9.96% decrease in plants irrigated with S_5 at 20 DAS when compared to treatments (S_2, S_3, S_4 and S_6). A different trend was verified at 40 DAS observed by estimated means (Table 3), namely, that the use of water with potassium caused a 23.77% decrease in WSD in relation to treatments S_2, S_3, S_4 and S_6.

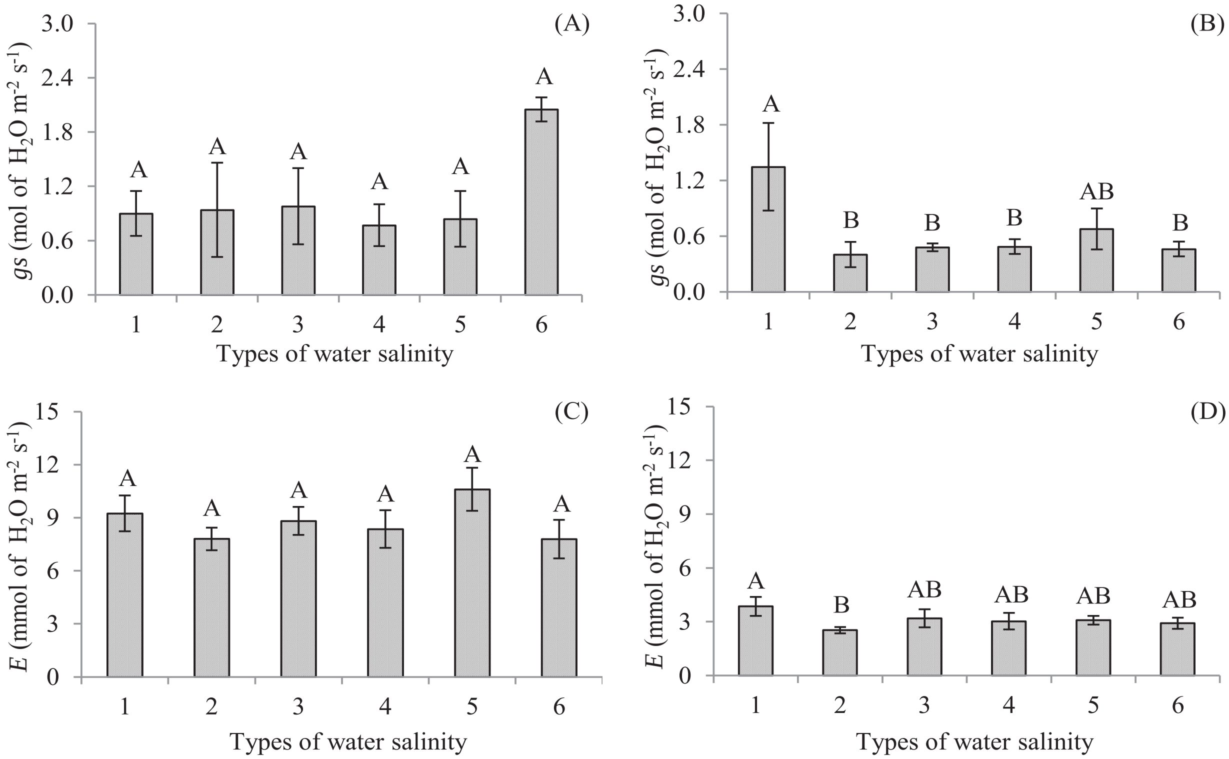
Based on the summary of F test (Table 4), there was a significant effect of the different types of water salinity on the assimilation rate of CO_2, instantaneous efficiency of carboxylation and intrinsic efficiency in the use of water, at 20 and 40 DAS. However, internal concentration of CO_2 and stomatal conductance were affected significantly only at 20 and 40 DAS respectively. The aforementioned table also shows that transpiration rate was not affected at any time during the study period.

At 20 DAS *gs* was not affected due to different types of salt, whereas at 40 DAS irrigation with saline water caused significant changes in stomatal conductance (Figure 3A and B). Comparison of means for *gs* at 40 DAS (Figure 3B) demonstrates that plants irrigated with low salinity water (control) showed *gs* statistically higher than those of treatments S_2, S_3, S_4 and S_6 which did not differ statistically neither among themselves nor compared to S_5. The decrease of stomatal conductance of the plants irrigated with water of the treatments S_2, S_3, S_4 and S_6 respectively with regard to S_1, is related to the change in osmotic potential and, consequently, to the reduction in water availability in their tissues. Stomatal closure probably occurred, with the subsequent decrease of the normal flux of CO_2 towards the carboxylation site. The latter brought about changes between the appropriate equilibrium and the transport of electrons, carbon metabolism and ATP and NADPH consumption which together made inefficient photosystem II and compromised significantly the production of photosynthates (Tezara et al., 2005).

Table 4 shows the results of the contrasts of means with regard to stomatal conductance measured at 20 and 40 DAS. Significant effect for *gs* occurred only at 40 DAS. It should be emphasized that in castor bean plants irrigated with low salinity water (ECw=0.6 dS m^{-1}) *gs* increased by 0.84 mol of H_2O m^{-2} s^{-1} when compared to those irrigated with water at ECw=4.5 dS m^{-1} (S_2, S_3, S_4, S_5 and S_6). Therefore, the most negative result on *gs* is the result of the cationic nature of the irrigation water due to excess of salt in the soil solution which causes changes in the osmotic-water potential directly affecting water absorption by the plants. On the other hand, the lack of significant effect ($p>0.05$) at 20 DAS for all treatments under analysis suggests that stomatal conductance under saline conditions varies according to the development of the plant and depends on the duration of exposure to stress and the age of the plant (Freitas et al., 2013).

Transpiration (*E*) had partially the same behavior as stomatal conductance at 20 DAS (Figure 3C). No significant effect was observed for the different types of salinity in irrigation water and with regard to plants irrigated with low salinity water (control). In other words, irrigation water with Na^+, Ca^{2+}, Na^++Ca^{2+}, K^+, Na^++Ca^{2+}+Mg^{2+} in the initial stages of the development of the castor bean plant did not affect the plant´s physiology with regard to transpiration since as the stomatal conductance was not affected (Figure 3A) but there were significant differences ($p<0.05$) in measures at 40 DAS which suggest that the effect of the different salts (Na^+, Ca^{2+}, Na^++Ca^{2+}, K^+, Na^++Ca^{2+}+Mg^{2+}) on *E* intensified as stress time was prolonged. These results demonstrate that stress effect caused by different types of salinity in castor bean plants may differ during the crop cycle.

The partial closing of the stomata caused by different



1= Control, 2= Na^+, 3= Ca^{2+}, 4=Na^++Ca^{2+}, 5=K^+, 6=Na^++Ca^{2+}+Mg^{2+}

Figure 3. Stomatal conductance –*gs* (A and B), and transpiration – *E* (C and D) of the castor bean plant at 20 and 40 days after sowing, depending on the types of irrigation water salinity.

types of salinity in the water at 40 DAS (Figure 3B) also influenced the rates of transpiration of the castor bean plant during the same evaluation period. When the means of the treatments were compared, it may be seen that castor bean plants irrigated with lower ECw (S_1) had a higher *E* (3.85 mmol H_2O m^{-2} s^{-1}), significantly differing ($p<0.05$) only from those irrigated with treatment S_2. It may also be observed (Figure 3D) that castor bean plants irrigated with water of the treatments S_2, S_3, S_4, S_5 and S_6, had a 34.37,17.15, 21.41, 20.06 and 24.33% reduction in transpiration when compared with plants irrigated with low salinity water. When different treatments were evaluated, there was a greater reduction in the *E* of plants irrigated with water containing only sodium (Figure 3D) in its composition. This is due to decrease in osmotic potential (Figure 1B), and the increase in the water saturation deficit (Figure 2D) and to the decrease in stomatal conductance (Figure 3B) at 40 DAS. Moreover, decrease in transpiration may also have been caused by the toxic effects of the salts absorbed by the plants, by the low capacity of osmotic adjustment of the crops and/or reduction of total potencial of water caused by the increase of saline concentration (Silva et al., 2011).

Contrasts of transpiration means revealed that there was a significant alteration in *E* at 20 DAS when S_2 versus S_5 and S_5 versus the other salts (S_2, S_3, S_4 and S_6) were compared (Table 4). Estimate of mean (Table 4) shows that *E* decreased by 2.80 mmol of H_2O m^{-2} s^{-1} with regard to treatment S_5 when plants were irrigated with S_2 water containing sodium. When mean obtained in S_5 is contrasted by comparing plants irrigated with water containing other types of salt, a reduction of 2.41 mmol of H_2O m^{-2} s^{-1} occured. It has been verified that, at 40 DAS (Table 4), when the plants were irrigated with water of ECw=0.6 dS m^{-1}, there was an increase of 0.90 mmol of H_2O $m^{-2}s^{-1}$ in *E* compared to plants irrigated with ECw = 4.5 dS m^{-1}. According to data of mean estimates (Table 4), there was a reduction of 0.66 and 0.55 mmol of H_2O m^{-2} s^{-1} in plants irrigated with treatment S_2 when compared to those irrigated with S_3 and S_5, respectively. As a general rule, the above-mentioned results showed that water containing sodium ion affects transpiration regardless of its proportion in irrigation water.

Assimilation rate of CO_2 was significantly affected ($p<0.05$) by different types of water salinity at 20 DAS, whilst comparison of means (Figure 4A) showed that CO_2 assimilation rate of plants irrigated with S_1 water of low salinity and with potassium (S_5) was significantly different ($p<0.05$) when compared to plants irrigated with treatments S_2 and S_6. However, no significant difference ($p.>0.05$) was found when *A* of S_1 and S_5 were compared

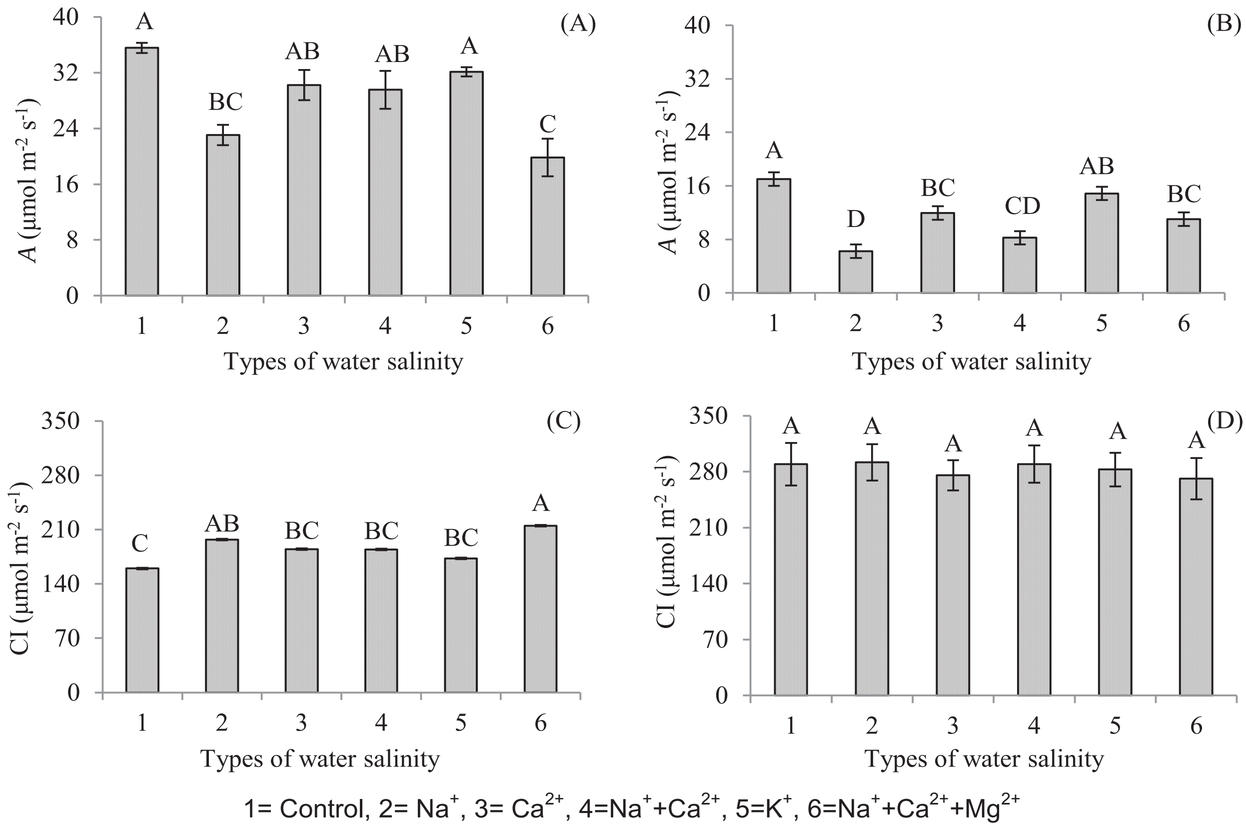


Figure 4. CO_2 assimilation rate –*A* (A and B), and internal concentration of CO_2 –CI (C and D) of the castor bean plant at 20 and 40 days after sowing, depending on the types of irrigation water salinity.

with treatments S_3 and S_4. It may also be perceived (Figure 4A) that castor bean plants at 20 DAS irrigated with water of treatments S_1 and S_5 had the higher CO_2 assimilation rates (35.56 and 32.12 µmol m^{-2} s^{-1}) compared to other treatments (S_2, S_3, S_4 and S_6), following the same trend observed for stomatal conductance. These rates are satisfactory since the castor bean is a C_3 plant in which the CO_2 assimilation rates varies between 10 and 20 µmol m^{-2} s^{-1} (Taiz and Zeiger, 2013). On the other hand, decrease in CO_2 assimilation rate especially in plants irrigated with sodic water may have been caused by the reduction of stomatal conductance (Figure 3A), transpiration (Figure 3C) and increase of CO_2 internal concentration (Figure 4C). This reveals low efficiency in the use of CO_2 introduced into the cell.

In the case of CO_2 assimilation rate at 40 DAS (Figure 4B), comparison of mean test showed that the highest rate (17.02 µmol m^{-2} s^{-1}) occurred in plants irrigated with low ECw (S_1), which was statistically higher than the rates observed in those irrigated with treatments S_2, S_3; S_4 and S_6. However, there was no significant difference in the *A* of plants under treatment S_5 compared to S_1.There was 63.45, 29.84, 51.52 and 35.25% decrease in *A* when means of treatments S_2, S_3, S_4 and S_6 were compared to control. Data demonstrate (Figures 4A and B) that decrease in CO_2 assimilation rate is associated with age and imposition of stress period in the crop. In fact, plants irrigated with sodium water were greatly affected in the two periods under analysis.

The observed behavior of *A* corroborates with data on stomatal conductance (Figure 3B) and transpiration (Figure 3D) and suggests that the decrease in the photosynthesis rate may have been caused by the partial closure of the stomata, associated to the osmotic effects of salinity and ionic toxicity on the metabolism (Bezerra et al., 2005). Further, decrease in CO_2 assimilation rate, caused by the closure of the stomata as a response to the low potential of soil water due to high concentration, is also a determining factor in growth and productivity of crops (Gurgel et al., 2003).

Table 4 shows the contrasts of means for CO_2 assimilation rate. There was no significant difference ($p>0.05$) for S_2 versus S_6 at 20 DAS; estimates of means (Table 4) revealed that plants irrigated with low water conductivity (0.6 dS m^{-1}) had an increase in *A* of 8.61 and 6,55 µmol m^{-2} s^{-1} at 20 and 40 DAS, respectively, when compared to those irrigated with ECw of 4.5 dS m^{-1}(S_2, S_3, S_4, S_5 and S_6). On the other hand, a decrease of 7.17 and 5.71 µmol m^{-2} s^{-1} respectively at 20 and 40 DAS in the CO_2 assimilation rate occurred when the variable was analysed in plants irrigated with sodium water (S_2) and in those irrigated with calcium (S_3).

When the effect of treatment S_2 is compared to that of S_6 (Table 4), an increase of 4.79 μmol m^{-2} s^{-1} occurred in CO_2 assimilation rate at 40 DAS while it decreased 9.06 and 8.64 μmol m^{-2} s^{-1}, respectively at 20 and 40 DAS, in comparison to plants irrigated with potassic (S_5) water. When the behavior of *A* between S_5 and the other saline waters (S_2, S_3, S_4 and S_6) is evaluated, the estimate of mean (Table 4) reveals that CO_2 assimilation rate, at 20 and 40 DAS, in S_5 (potassic water) increased by 6.45 and 5.51 μmol m^{-2} s^{-1}, respectively, with regard to those irrigated with the other cations. It may thus be inferred that the reduction of CO_2 assimilation rate was caused by the decrease in RWC, transpiration and stomatal conductance. The most evident effect in both the periods was observed due to variation in water salinity (ECw from 0.6 to 4.5 dS m^{-1}).

In the case of internal concentration of CO_2 at 20 DAS (Figure 4C), plants irrigated with water S_6 (Na^++Ca^{2+}+Mg^{2+}) had the highest CI (214.90 μmol m^{-2} s^{-1}) and exceeded by 25.63, 14.02, 14.20 and 19.61% ($p<0.05$) of concentration obtained in plants irrigated with treatments S_1, S_3, S_4 and S_5, respectively. Low internal concentration of CO_2 observed in plants under S_1 and S_5 treatments explains the highest values found for the CO_2 assimilation rate (Figure 4A) in these treatments. It should be underscored that a higher internal concentration of CO_2 (S_2, S_3, S_4 and S_6) means that the carbon introduced into the leaf mesophyll cell was not being metabolized by the photosynthesis apparatus due to saline stress to which the plants were exposed. Further, increase in the internal concentration of CO_2 indicated that there was no restriction in the acquisition of CO_2 by the plant. However, when it reached the mesophyll cells, the fixation process during the carboxylation phase was compromised, this may have been caused by the degradation of the photosynthesis apparatus as a response to the process of leaf senescence of the tissues due to stress from excess of salts (Silva et al., 2013).

The internal concentration of CO_2 at 40 DAS was not affected significantly ($p>0.05$) by the treatments under analysis (Figure 4D). Similarity among treatments suggests that, regardless of the type of salinity, the availability of CO_2 to plants was illimited and shows that the decrease in the photosynthesis process was not only due to restricted stomatal opening but also due to damages to the cell structure responsible for the assimilation of CO_2. The latter may be due to the reduction of the osmotic potential and by the accumulation of ions beyond the tolerance limit of the castor bean plants (Fernandes et al., 2010).

Table 4 presents contrasts of means for the internal concentration of CO_2. There was a significant ($p<0.05$) effect of treatments at 20 DAS. Estimates of means in Table 4 showed that plants irrigated with water of ECw= 0.6 dS m^{-1} reduced internal concentration of CO_2 by 30 μmol m^{-2} s^{-1} in comparison to those of ECw=4.5 dS m^{-1}. Increase in CI in plants under ECw=4.5 dS m^{-1} level was probably due to decrease in stomatal conductance, transpiration and CO_2 assimilation rate, perhaps because of damages in the photosynthesis apparatus due to high internal concentration of CO_2. On the other hand, since the internal concentration of CO_2 of plants irrigated with S_2 did not differ significantly from those under S_3 water, it indicated that salinity caused by sodium and calcium had a similar effect on CI.

Different from the above-mentioned results, the estimate of means (Table 4) at 20 DAS shows that water salinized by sodium (S_2) resulted in a decrease of 17 μmol m^{-2} s^{-1} in CI when compared to plants irrigated with S_6 (Na+Ca+Mg). Analysis of the data S_2 versus S_5 registered that castor bean plants irrigated with sodium water (S_2) had an increase of 24 μmol m^{-2} s^{-1} in the CI of CO_2 when compared to treatment S_5. Likewise, the use of irrigation water with potassium (S_5) caused a decrease of 22 μmol m^{-2} s^{-1} in CI when contrasted to plants with the other types of salt (S_2, S_3, S_4 and S_6).

Since instantaneous efficiency of carboxylation is a physiological parameter with close links to the intracellular concentration of CO_2 and to the assimilation rate of CO_2 (Konrad et al., 2005), data showed a significant effect on EICI of the different types of water salinity at 20 DAS (Figure 5A). Comparison of means reveal that plants under treatments S_1 and S_5 had the highest EICI [(0.22 and 0.18 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] and did not differ statistically from rates of plants under treatments S_3 and S_4 [0.16 and 0.16 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$]. On the other hand, rate of EICI of castor bean plants irrigated with S_1 and S_5 differed significantly from rates with treatments S_2 and S_6. The lowest EICI rate [0.09 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] occurred in plants irrigated with S_6 water containing sodium+calcium+ magnesium.

Consequently, increases in the instantaneous efficiency of carboxylation in current analysis, especially in plants with treatments S_1 and S_5, are mainly due to increases in the assimilation rate of CO_2 (Figure 4A) and to decreases in the internal concentration of carbon dioxide (Figure 4C). Further, reduction in EICI rates in treatments S_2 and S_6 may be associated with stomatal and non-stomatal causes due to the osmotic and toxic effects, caused by the accumulation of Na^+ and Cl^- ions in the foliar limbos (Neves, 2008).

The instantaneous efficiency of carboxylation was also significantly affected at 40 DAS by the different types of water salinity. Results of mean test (Figure 5B) showed that the highest rates [(0.06 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$)], with regard to EICI, occurred in water with low salinity (S_1) and potassium (S_5) which differed significantly from treatments S_2 and S_4. However, there were no differences between treatments S_3 and S_6. Above results suggest

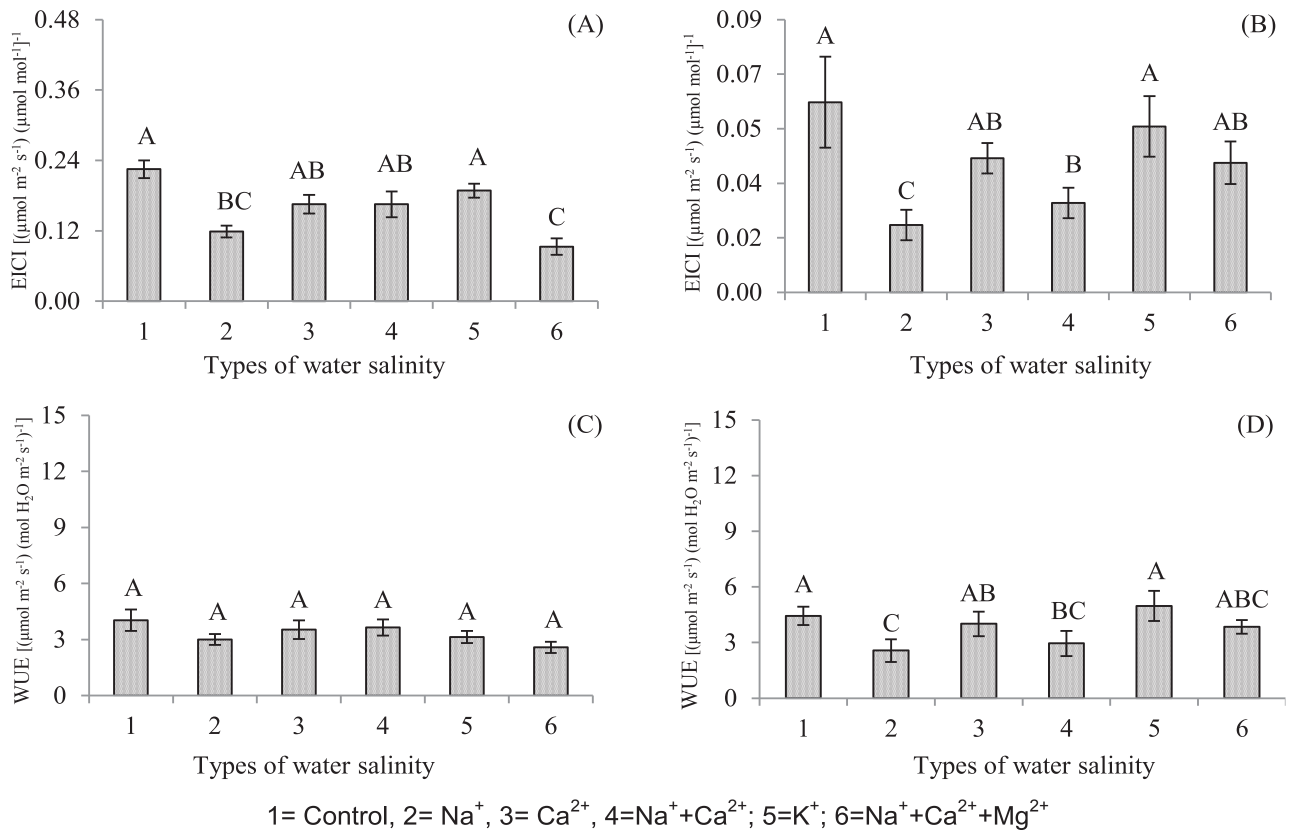


Figure 5. Instantaneous efficiency of carboxylation – EICI (A and B), and intrinsic water use efficiency-WUE (C and D) of the castor bean plant at 20 and 40 days after sowing, depending on the types of irrigation water salinity.

that decrease, which was greater in EICI of plants with treatments S_2 and S_4, is a consequence of the low CO_2 assimilation rates (Figure 4B) with regard to CO_2 in the sub-stomatal chamber (Figure 4D). If the internal concentration of CO_2 increases and there is decrease in the consumption of CO_2 in the chloroplasts due to the reduction in photosynthesis activity, EICI will also decrease.

Based on results of contrast of means for EICI (Table 4), a significant effect occurred among all the treatments evaluated at 20 and 40 DAS. Estimate of means showed that castor bean plants irrigated with water at the low electrical conductivity (0.6 dS m^{-1}) increased EICI by 0.07 and 0.02 [(μmol m^{-2} s^{-1}) (μmol $mol^{-1})^{-1}$] respectively at 20 and 40 DAS when compared to those with ECw of 4.5 dS m^{-1} (S_2, S_3, S_4, S_5 and S_6). Plants irrigated with water salinized with sodium (S_2) EICI decreased by 0.04 and 0.02 [(μmol m^{-2} s^{-1}) (μmol $mol^{-1})^{-1}$] in comparison to plants irrigated with treatment S_3 at 20 and 40 DAS, respectively.

Data from estimate of mean (Table 4) revealed that the use of water rich in sodium decreased EICI by 0.02 and 0.02 [(μmol m^{-2} s^{-1}) (μmol $mol^{-1})^{-1}$] when compared to plants irrigated with treatment S_6 in the two periods analysed (20 and 40 DAS). However, EICI decreases by 0.06 and 0.03 [(μmol m^{-2} s^{-1}) (μmol $mol^{-1})^{-1}$] in plants irrigated with S_2 with regard to mean of plants irrigated with S_5 at 20 and 40 DAS, respectively. On the other hand, EICI increased by 0.05 and 0.02 [(μmol m^{-2} s^{-1}) (μmol $mol^{-1})^{-1}$] when treatment S_5 was compared to the other types of salts in irrigation water (S_2; S_3; S_4 and S_6). As a rule, decrease in EICI, especially in plants irrigated with different types of cations (S_2; S_3; S_4 and S_6) is associated with the reduction of CO_2 assimilation rate and to the increase in the internal concentration of CO_2.

The intrinsic water use efficiency at 20 DAS (Figure 5C) did not show significant effect of the treatments under study. However, plants irrigated with S_1 had the highest WUE rate 4.03 [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} $s^{-1})^{-1}$]. The absence of a significant influence of the different types of salinity on WUE is due to the results of the assimilation rate of CO_2 (Figure 4A) and transpiration (Figure 3A) during the same period of study. Since intrinsic water use efficiency is obtained by the relationship between CO_2 assimilation rate and leaf transpiration, in which rates are related to the amount of carbon fixed by the plant for each unit of water lost (Jaimez et al., 2005), probably the plants which are capable of maintaining a high efficiency in the use of water under saline conditions, have a great tolerance to saline stress. In fact, decrease in water consumption implies a reduction in the absorption of specific ions thereby avoiding toxicity to the plants (Flowers and Flowers, 2005).

Based on the results of the test of comparison of mean for the intrinsic water use efficiency at 40 DAS (Figure 5D), treatments S_1 and S_5 were statistically superior than S_2 and S_4, although they did not differ from plants irrigated with treatments S_3 and S_6. Figure 5D shows that the highest rates of intrinsic WUE [4.43and 4.96 (μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] were obtained in plants irrigated by water with the low salinity (S_1) and containing potassium (S_5), respectively. On the other hand, irrigation with sodic water (S_2), sodium+calcium (S_4) and sodium+calcium+magnesium (S_6) provided lower WUE rates, respectively with mean values of 2.56, 2.56 and 3.84 [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})^{1}]. Decrease in intrinsic water use efficiency in treatments S_2, S_4 and S_6 may be associated to changes in the rates of assimilation of CO_2 and transpiration which possibly occurred because of the low availability of water in the soil, caused by the reduction of osmotic potential due to high concentration of salts. The latter caused the closure of stomata and consequently reduced CO_2 assimilation and transpiration, directly affecting WUE (Willadino and Camara, 2004).

Table 4 demonstrates that at 20 DAS all contrasts except S_1 versus other salts (S_2; S_3; S_4; S_5; S_6) were not significant while at 40 DAS, all were significantly affected. Estimates of mean (Table 4) demonstrated intrinsic WUE increase of 0.84 and 0.77 [(μmol m^{-2} s^{-1}) (mol H_2O $m^{-2}s^{-1}$)$^{-1}$] in castor bean plants irrigated at ECw 0.6 dS m^{-1} compared to those cultivated with saline water of ECw= 4.5 dS m^{-1} at 20 and 40 DAS respectively. Table 4 also shows that intrinsic water use efficiency in S_2 was 1.44 [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] lower than that in S_3. When plants irrigated with sodium water (S_2) were contrasted, there was a reduction of 1.27 [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] in intrinsic WUE in plants irrigated with S_6 (Na+Ca+Mg) water. Estimate of mean (Table 4) demonstrated that, when S_2 was employed in irrigation water, a decrease of 2.40 [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] in intrinsic WUE occurred in comparison to plants with potassium water (S_5). Results show that the use of predominantly sodium water caused a greater deleterious effect on intrinsic WUE. However, plants irrigated with treatment S_5 had an increase of 1.62 [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] with regard to the other types of salts (S_2, S_3, S_4 and S_6). As a rule, among the cations analysed, there was a higher efficiency in plants irrigated with potassium water (S_5). The results obtained corroborate with data for rates of CO_2 assimilation (Figure 4B) and transpiration (Figure 3D) at 40 DAS.

Conclusions

(1) Water and physiological relationships of the castor beancv. BRS Energia plants are more sensitive to variations in electrical conductivity of irrigation water as compared to the cationic nature of water.
(2) Irrigation with distinct cationic nature of water affects the osmotic potential, electrolyte leakage, relative water content and water saturation deficit of castor bean cv. BR Energia in the studied periods, the lowest deleterious effect in plants were observed with potassium water.
(3) The negative effects of cationic nature of irrigation water are more evident in gas exchanges, especially in stomatal conductance and CO_2 assimilation rate at 40 days after sowing.
(4) Among the ions studied, plants irrigated with potassic water show highest values for stomatal conductance, transpiration, CO_2 assimilation rate, instantaneous efficiency of carboxylation and intrinsic efficiency of water use at 40 DAS.
(5) Castor bean cv. BRS Energia plant is more sensitive to sodium ions in irrigation water with regard to water relationships and gas exchanges.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Land suitability evaluation in Wadla Delanta Massif of north central highlands of Ethiopia for rainfed crop production

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Land evaluation, using a scientific procedure, is essential to identify the potential and constraints of a given land for defined use in terms of its fitness and ensure its sustainable use. In view of this, a study was conducted to evaluate physical land suitability for major agricultural crops under rainfed conditions at the Wadla Delanta Massif in the north central highlands of Ethiopia. Four common field crops *Triticum aestivum* L., *Hordeum vulgare* L., *Vicia faba* L. and *Lens culinaris* L. and four land mapping units (LMU1Ac and 2Ac, LMU2Bc, LMU3CcI, and LMU4DcI), identified based on soil types, were considered for this study. Climate, soil and landscape data were also collected. The maximum limitation method was used to decide the degree of suitability of the land. The results showed that among the total area (24025 ha) of the land evaluated, about 65.13, 23.62, and 11.25% of the land is moderately, marginally and not suitable, respectively for all the selected crops. The overall land suitability evaluation showed that LMU1Ac and 2Ac are moderately suitable (S2c,f,w) for all tested field crops, and LMU2Bc is moderately suitable (S2c,f) for barley and marginally suitable (S3c,f,w) for wheat, faba bean and lentil. Land mapping 3CcI is moderately suitable (S2c,r,s) for barley and marginally suitable (S3c,f,r,t) for wheat and faba bean, not suitable for lentil and LMU4DcI is marginally suitable (S3c,r,s.t) for barley and not suitable for others. As a whole, LMU 1 and 2 are suitable for all considered crops with integrated land and soil fertility management, and LMU 3 and 4 are not suitable for crop production and, hence, it is better to shift to other land use types.

Key words: Field crops, land mapping unit, physical land suitability, soil fertility management.

INTRODUCTION

Land is a complex and dynamic combination of factors vis-à-vis geology, topography, hydrology, soil, microclimates and communities of plants and animals that are continually interacting under the influence of climate and people's activities (Hudson, 2005). The variation in land must be identified, characterized and

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information communicated via the most inclusive and cost effective means if people understand the different forms of land use, the hazards due to accelerated changes and degradations that accompany those uses. Information on the constraints and opportunities for the use of land will provide basic tools for better crop management practices and guides decisions on optimal utilization of land resources in a sustainable way (Nayak et al., 2010).

Van Ranst et al. (1996) pointed out that physical land suitability is a prime requisite for land use planning development, since it guides decisions on land utilization type for optimal use of land resources which contributes towards better land management, mitigation of land degradation and designing land use pattern that prevents environmental constraints through isolation of rival land uses. Making effective decisions regarding agricultural land suitability problems are vital to achieve optimum land productivity and ensure environmental sustainability (Oluwatosin, 2005; Kurtener et al., 2008; Teshome et al., 2013). In contrast, the incongruous use of land has resulted in environmental degradation of natural resources that leads to decline in land productivity and deterioration of soil quality for its future use (Menale et al., 2008).

Due to the ever increasing population pressures and heavy reliance of their own land resources, the unreceptive use of land and environmental sustainability of agricultural production systems have become an issue of concern (Gong et al., 2012; Singh, 2012). Land should be used based on its capacity and fitness to meet human needs and to ensure sustainable agricultural service. The sustainability of agriculture, which involves producing quality crops in an environmentally friendly, socially acceptable and economically feasible way, would be achieved if lands could be categorized and utilized based upon their capacity (Amiri and Shariff, 2011).

To obtain optimum benefit out of the land, proper utilization of its resources is inevitable. Various studies (Rossiter, 1996; Ziadat and Al-Bakri, 2006; FAO, 2007; Ritung et al., 2007; Braimoh and Vlek, 2008) addressed that land evaluation is initiated from the need for an ample assessment of land performance when used for specified purposes using a scientific process which involves the execution and clarification of surveys and studies of landforms, soils, climate, vegetation and other aspects of land. Land evaluation employs estimating the land resources and grouping for a defined use in terms of their appropriateness that have vital roles for potential agricultural efficiency and sustainable land use planning. It facilitates the full utilization of the land resources at a regional level with specified land qualities or land characteristics as a land unit, which can be mapped and serially numbered (like land unit 1, land unit 2, land unit 3, and so on).

There are several approaches to land suitability evaluation for which Van Lanen (1991) identified three general types. The first one is qualitative evaluation based mainly on expert judgment, where physical suitability is obtained by qualitative procedure. This approach gives a useful result that generalizes the constraint of an area for specific kind of land use type. The FAO (1976) framework for land suitability evaluation is a typical example. The approach is presented in discretely ranked classes (e.g. S1, S2, S3, N1 and N2). This concept of FAO is mostly applied, and, although it is qualitative, it can be complemented and enhanced by more quantitative methods (Teshome and Verheye, 1995). The second type includes a qualitative evaluation based on parametric methods that assess the suitability of land on a continuous scale. The essentials of these methods can be expressed by a mathematical model. The Storied index (Storie, 1933) is an example. The third method is that based on process-oriented simulation models where land performance is related to individual land characteristics with their net effect assessed using a model of land function. These quantified methods usually require high data input, which make them more expensive.

Of all the different approaches to land evaluation, each has different data needs and different qualities of prediction. There are no rules that indicate when any given approach is adequate, or when there is the need to proceed to a more complex level of analysis (Burrough, 1996). Therefore, in developing countries where inadequate land resources data exist and funds are limited to do detailed data analysis, qualitative physical land suitability evaluation methodology may be used, which may later be complemented with a more complex quantitative methods.

In Ethiopia, limited numbers of investigations were made to assess the land suitability based on their agricultural potentials or their physical land resources. These studies conducted on land evaluation of the country seem to be inadequate in providing Wadla Delanta basic information that can guide land use decisions on proper utilization of resources. For instance, very few studies (Teshome and Verhey, 1994; Mohammed, 2004; Kassa and Mulu, 2012; Abraham and Azalu, 2013; Teshome et al., 2013; Gizachew, 2014) were conducted to evaluate suitability of land for a given use across different parts of the country. In the study area, population pressure is increasing from time to time, while the productivity of land is declining. Agricultural production in the area has, thus, become unsustainable; marginal lands are being converted into cultivated lands. It is imperative to evaluate the potential of such marginal lands to decide their potential, limitations and their suitability. This will ensure sustainable use with better return from the land. Nevertheless, although production of crops has been practiced for many years, the potential and constrains of the land has not been identified scientifically. As a result, productivity has remained extremely low in the study area.

Thus, to fill this gap, the present study was conducted to identify the potentials and constraints of the land for

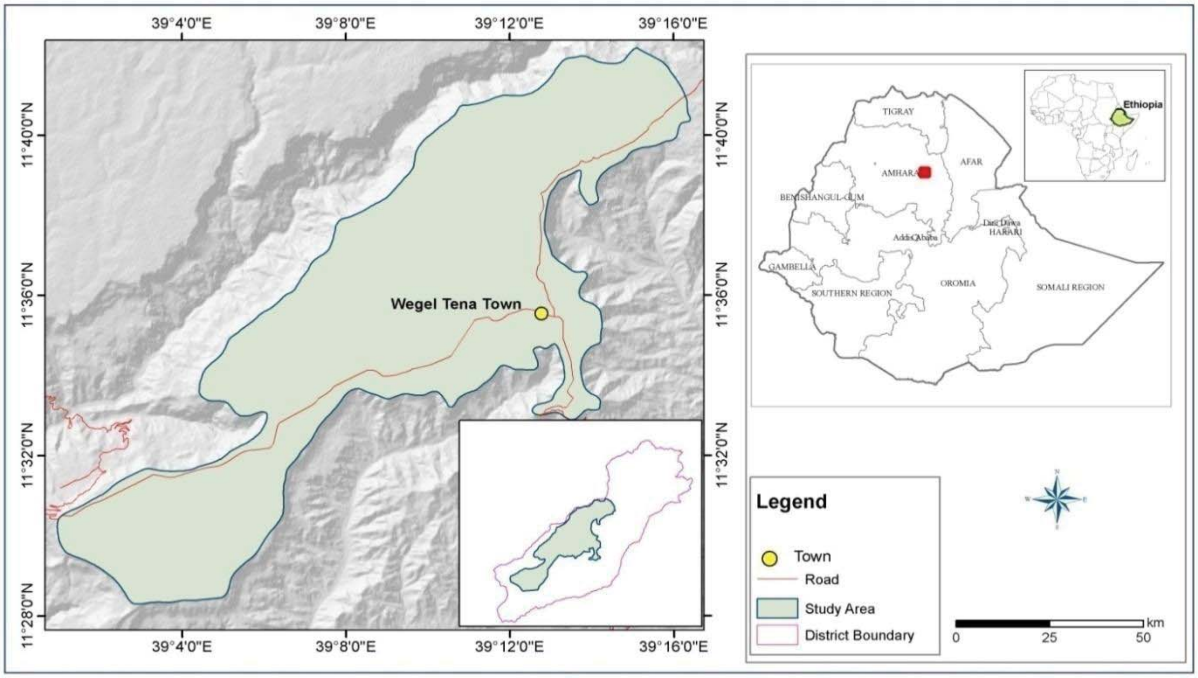

Figure 1. Location map of the study area.

production of major crops in Wadla Delanta Massif of north central highlands of Ethiopia with the intention of providing scientific information that can be used for future sustainable land use planning and development.

MATERIALS AND METHODS

Description of the study area

The study was conducted at the Wadla Delanta Massif in Delanta district, northcentral highlands of Ethiopia (Figure 1). The study area lies between 11° 29' 29.82" to 11° 41' 25.53" latitude and 39° 02' 19.19" to 39° 19' 53.74" longitude with elevation ranges from 2600 to 3500 m above sea level and covering an area of 24,025 ha. It is located at about 499 km north of Addis Ababa and 98 km northwest of Dessie town, South Wello Zone

According to the information obtained from the WAOR (2013), the total area of the District is 105678 ha stretching from lowland to highland, much of it being in the mid-altitude ranges dominantly plateau plains. Average land holding size is one hectare per household (0.75 ha for crop production and 0.25 ha for grazing) and their major sources of traction for ploughing are oxen. Among the total area of the District, 24025 ha was covered by this study along toposequence which was mainly situated in plain areas with altitude ranges from 2600 to 3500 masl in the north, northwest and west from the center of the District town (Wegel Tena).

Geomorphology and topography of the study area

The major landforms of the study area comprise extensive plateaus, chains of hills with mountainous ridge, oval in shape with dendritic drainage pattern, numerous convex hills at the plain area, river-valleys and very deep gorges at the boundary. About two-third of the area, embracing altitude ranges from 2100 to 3500 m, was highly populated. The remaining one third of the District is located mainly along the river valleys on the east, southeast, north and northwest location which range from 1500 to 2100 m. Topography of the highland plateaus, especially those elevated above 3000 m are dominated by chains of hills. According to WAOR (2013) reports, the general classification of the area is about 30% mountainous, 30% plains, 36.5% gorges and 3.5% other land features.

Climate and land use systems of the study area

The traditional agro-ecological classification of the study area falls in all the categories that are basically correlated with elevation. These are Kolla, Woina-dega, Dega and Wurch (Table 1). The climate of the area is characterized by dry seasons (October to February Cold-Dry and March to June Hot-Dry) and wet season (mid-June to September).

The rainfall pattern is bimodal with peak periods from mid July to early September. For fifteen years (1999 to 2013), mean annual rainfall of the study area was about 812 mm of which 60 to 70% is received in summer (*Kiremt)* and 40 to 30% in the spring (*Belg*) seasons. The mean annual minimum and maximum temperatures are 6.8 and 19.6°C, respectively (Figure 2). People are living in the upper topographic position and their farming activities primarily depend on *Belg* rains, whereas the middle and lower topographic positions rely on both the *Kirem* and *Belg* rains. As a result, there is small, erratic and unreliable rainfall and the area is prone to sporadic droughts.

The land use systems in the area are both private (farming) and communal (grazing) land holdings which can be identified through land use patterns. Cultivated and grazing lands are the major land use types in the area which account 22 and 8%, respectively. The

Table 1. Traditional agro-ecological zones (ACZ) of the northern Ethiopian highlands.

Traditional ACZ	*Kolla*	*Woina-dega*	*Dega*	*Wurch*
Elevation (m)	1500-1800	1800-2400	2400-3500	> 3500
Temperature (°C)	18-20	15-18	10-15	< 10
Rainfall (mm)	300-900	500-1500	700-1700	> 900
Dominant crop	Sorghum, maize	Teff, maize, wheat	Barley, wheat	Barley

Source: Adapted from Getahun (1984).

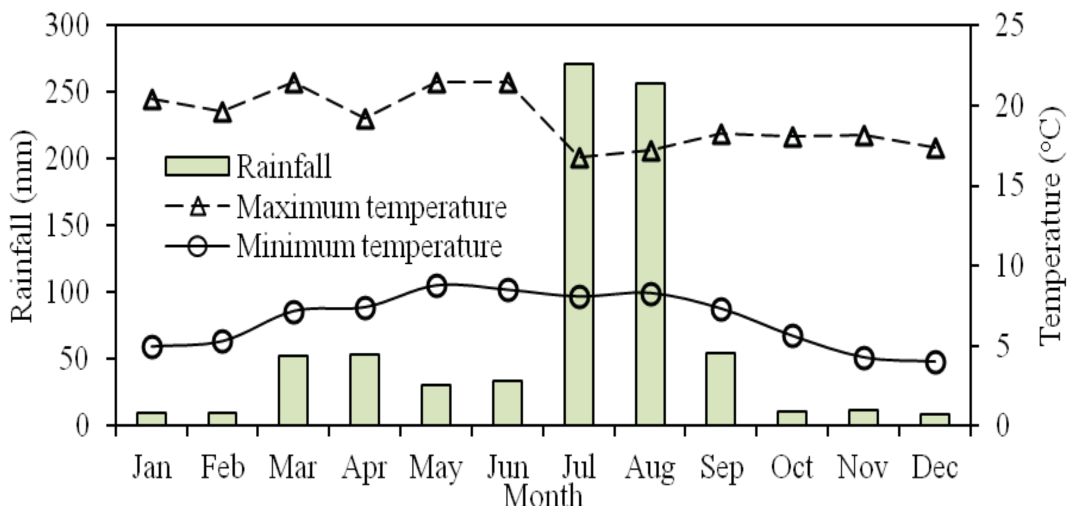

Figure 2. Mean monthly rainfall, maximum and minimum temperatures of the study area.

largest proportion (45%) of the land in the study area is currently unutilized and the remaining (25%) is covered by shrub/bush, and natural and plantation forests. Agriculture is the predominant economic sector which is over 95% of the population engaged in this sector (WAOR, 2013). The farming system is mixed which include livestock and crop production activities and is characterized by subsistence methods. The overall farming system is strongly oriented on the way of crop production to sustain farmers' livelihoods. It is practiced using oxen and horses for land ploughing and threshing. Crop residues and intensive grazing are major livestock feed resources in the area.

The common rainfed crops grown in the area are bread wheat *(Triticum aestivum* L.*)*, food barley *(Hordeum vulgare* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* L.), grass pea (*Lathyrus sativus* L.), chickpea (*Cicer arietinum* L.) *teff* (*Eragrostis tef* L.) and sorghum (*Sorghum bicolor* (L.) Moench. All these crops are managed using traditional agricultural techniques and equipment. Moreover, few types of vegetables, fruits, root crops and spices are also produced. Most of the arable land is under rainfed farming while very small area is irrigated at the valley bottom or around riverbanks to produce vegetables and fruits (WAOR, 2013).

The natural woodland and vegetation of the study area has disappeared due to overgrazing, increasing demand for fuel-wood and conversion into cultivated lands. There are small patches of remnant natural forests found on farm boundaries and around churches. Planted tree species like *Eucalyptus camaldulensis*, C*upressus lustanica, Acacia saligna* and A*cacia decurrens* are common around homesteads and conserved areas. The *Eucalyptus camaldulensis* plantations are replacing the arable/cultivated lands and expanding on backyards, stream banks and gully sides.

Geology and soils of the study area

Geology of the study area is characterized by the trap series of tertiary periods, similar to much of the central Ethiopian highlands (Mohr, 1971). As per Dereje et al. *(*2002), the area is covered by Oligocene rhyolite and very thick ignimbrite units encompassing predominantly of alkaline basalt with numerous inter-bedded flow of trachyte. The granite, gneisses and basalt rock types exist in the area forming part of the basement complex and most of the soils are basaltic parent material. Soils of the study area are greatly influenced by topography with high surface runoff during the main rainy season. There was no scientific studies in the area except FAO/UNDP (1984) small scale soil survey (1:1 000 000 scales) at the national level. The local people have traditionally classified the soils, namely *Walka or Mererie Afer* (Vertisols) in the plain area and *Nechatie or Gracha Af*er (Cambisols and leptosols) in steep slope or mountainous area.

Description and characterization of land mapping units

Land mapping units (LMUs) were selected on the basis of slope, soil depth and textural classes, as well as morphological, physical and chemical characteristics which were considered in terms of soil fertility. As a whole, the study area was classified into five land units, three topographic positions, three soil depths and two textural classes. Twelve representative pedons were opened along the topographic position and classified according to WRB (2006) as Calcic Vertisols, Pellic Vertisols, Haplic Cambisols and Mollic Leptosols (Table 2).

Table 2. Identified mapping units and their area coverage of the study area.

Mapping unit	Slope (%)	Altitude (m)	Depth (cm)	Texture	Area		Soil classification
					ha	%	
LMU1Ac	< 2	2800-2900	>150	Clay	4939.54	20.56	Pellic Vertisols
LMU2Ac	2-4	2900-3000	>150	Clay	10707.94	44.57	Pellic Vertisols
LMU2Bc	4-8	3000-3100	50-100	Clay	2061.35	8.58	Calcic Vertisols
LMU3Ccl	8-16	3100-3300	50-100	Clay loam	3613.36	15.04	Haplic Cambisols
LMU4Dcl	> 16	3300-3450	< 50	Clay loam	2702.81	11.25	Mollic Leptosols
Total					24,025	100.00	

The number and letters indicate the slope (1Ac = < 2%, 2Ac = 2-4%, 2Bc = 4-8%, 3Ccl = 8-16% and 4Dcl = > 16%); the capital letters indicate soil depth (A = > 150, B = 50–100, C = 50–100 and D = < 50 cm), and the last small letters indicate soil texture (c = clay and cl = clay loam).

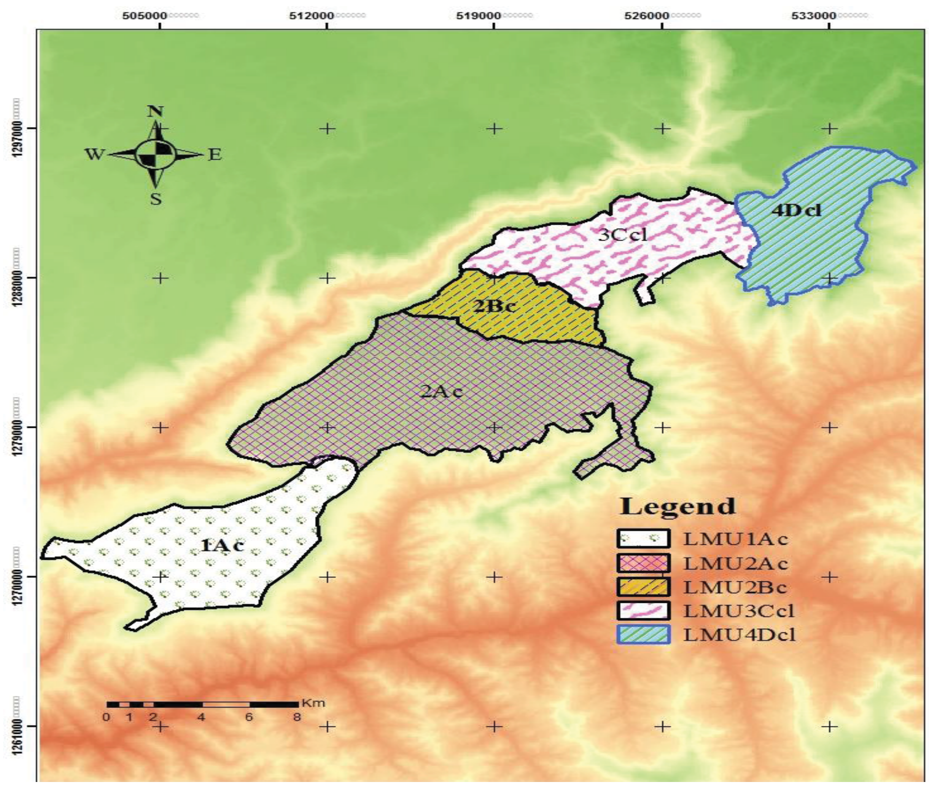

Figure 3. Land mapping units of the study area.

For the qualitative land suitability evaluation, simple limitation method was applied. Based on this method, land suitability classes were determined for *T. aestivum* L., *H. vulgare* L., *V. faba* L. and *L. culinaris* L. crops. For the purpose of land suitability evaluation, the twelve pedons were further categorized into five land mapping units (Figure 3).

Land suitability evaluation procedures for rainfed crop production

Land utilization type and their requirement

In this study, different physical land resources such as soil, climate, hydrology and topography were evaluated based on the simple limitation (matching system) approach between land quality and land characteristics with crop requirements. To obtain information on potential and limitations of the land in the study area for rainfed crop production, four principal crop varieties (*Guna-HAR-2029* for *T. aestivum* L, *Shedho-3381-01* for *Hordeum vulgare* L., *Degaga* for *V. faba* L. and *Chalew* for *L. culinaris* L.) were selected as priority land utilization types (Table 3) and the land use requirements of each crop were established using FAO (1976, 1983), FAO/UNDP (1984b), Sys et al. (1991, 1993) and Teshome and Verhey (1994) procedures.

The average lengths of the crop varieties' cycles for bread wheat (*T. aestivum* L.), food barley (*H. vulgare* L.), faba bean (*V. faba* L.) and lentil (*L. culinaris* L.) were taken as 129, 103, 125 and 119 days, respectively (EARO, 2004; ARAB, 2011). The selection of varieties were made based on their dominance (area coverage) and

Table 3. Improved varieties and some agronomic characteristics of crops in the study area.

Crop type	Varieties name	Year of released	Altitude (m)	Rainfall (mm)	Maturity days
**Food Barley	*Shedho* (3381-01)	2002	2400-33000	550-1100	123-135
*Bread Wheat	*Guna* (HAR-2029)	2001	2200-2700	> 600	110-125
**Faba Bean	*Degaga*	2002	1800-3000	700-900	116-135
***Lentil	*Chalew*	1985	1850-2450	500-1200	111-128

Source: Adapted from Ethiopian Agricultural Research Organization (2004) and Amhara Region Agricultural Bureau (2011). Sources of variety *Adet Agricultural Research Center; **Holetta Agricultural Research Center and ***Debre Zeit Agricultural Research Center.

importance in the area, but low productivity as per the information obtained from discussion held with the agricultural experts of the local district and information obtained from farmers.

Agro-climatic analysis for determination of growing period

The length of growing period of the study area was defined by comparing decadal rainfall with reference evapotranspiration (ETo). The beginning of growing period, end of rains, start and end of the humid period were determined using graphic method as described by Sys et al. (1991b). The growing period starts as the rainfall amount is greater than or equal to half of the reference evapotranspiration (RF ≥ 0.5ETo) during the beginning of the rainy season and the end of the rainy seasons was set when the rainfall amount during the end of season is again less than half of the corresponding reference evapotranspiration (RF < 0.5ETo). The same applies to the start and end of humid period but in reference to ETo.

The agro-climatic requirements (temperature, length of growing period, total growing period of rainfall and occurrence of frost hazard) were considered for the land utilization types. These agro-climatic characteristics were used as input parameters for the computation of length of the growing period (LGP), sowing date and selection of crop varieties. The mean values of the climatic data were used for the computation of the LGP and average values for the LGP of selected parameters were calculated and used for suitability evaluation

The climate data (temperature and rainfall) were obtained from Wegeltena Meteorological Station. Fifteen years (1999 to 2013) data records were used. The data for relative humidity, wind speed and sunshine hours were not available at this station. Henceforth, the reference evapotranspiration (ETo) was estimated by Hargreaves-Samani (1985) model and the results of the analysis are provided in the following equation:

$$ETo = 0.0135 \times \left(KT \times Ra \times \sqrt{TD} \times \left(TC + 17.78\right)\right) \qquad (1)$$

where, ETo, = reference evapotranspiration (mm d^{-1}), TD = Square root differences of the maximum and minimum daily temperature for weekly or monthly periods; Ra = Extraterrestrial radiation (mm d^{-1}) by degree latitude from Hargreaves-Samani table; KT = empirical coefficient (0.162 for "interior" regions and 0.19 for coastal regions) and TC = mean daily temperature. The extraterrestrial radiation (Ra) can be computed in the following equation:

$$\frac{X - Y}{X - Z} = \frac{X_2 - Y_2}{X_2 - Z_2} \qquad (2)$$

where, X and Z = The upper and lower location of degree latitude (°) from the table; Y = site location found between X and Z degree latitude (°); X_2 and Z_2 = the standard tabulated value of the corresponding degree latitude (mm d^{-1}) for each month; and Y_2 = the calculated value (mm d^{-1}) for each month, that is, Ra value. Thermal zoning was based on a range of mean daily temperatures during the growing season and closely related altitude ranges using a 500 m contour (Teshome and Verheye, 1994). LUPRD (1984) revealed that the relationship between temperature and altitude for the whole country of Ethiopia (except southeastern parts and the Ogaden) was given by the equation.

$$T(gp) = 30.2 - [0.00059 \times \text{Altitude}] \qquad r^2 = 0.90 \qquad (3)$$

Where, T(pg) is the mean temperature during the growing period (°C), altitude in meters above sea level (masl).

Finally, the length of growing period (LGP) was defined by counting the number of days between the start of the growing period and end of rains plus the period required to evapotranspiration the assumed 100 mm moisture stored in the soil reserve (time of soil moisture utilization) during the rainy season. The length of period (in days) required for evapotranspiration of the assumed 100 mm water stored in the soil at full rates of evapotranspiration was computed using the simple water balance (SWB) method, which is the difference between rainfall (RF) and reference evapotranspiration (SWB = RF-ETo). The computation of the water storage began with the first month of humid season (RF > ETo) as indicated in FAO (1983) and Sys et al. (1991a).

Characterization of soil and landscape

For soil and landscape evaluation topography (t), wetness (w), physical characteristics (s), fertility characteristics (f) and salinity and alkalinity (n) were considered (Table 4). The main focus of the study was on cultivated lands. Since the land suitability evaluation in the study area was for annual crops of *T. aestivum* L, *H. vulgare* L., *V. faba* L.and *L. culinaris* L., and the reference depth of 100 cm was used. The soil horizons had different textural classes that were calculated for the depth of the rooting zone for the representative pedons using equal sections and weighting factors. Weighted average of the upper 25 cm was used for the evaluation of soil pH, soil OM, total nitrogen, available phosphorus and sum of basic cations, and apparent CEC in the B horizon at a depth of 50 cm was calculated (Sys et al., 1991b, 1993).

The soils were characterized by opening pedons at representative sites on the identified mapping units and soil samples were collected from generic horizons and analyzed following the WRB (2006) guideline. The description of landscape characteristics were recorded during the pedon site characterization and the relevant soil properties were determined in the laboratory following standard procedures and analytical methods for each parameter. These soil data were used for evaluating the soil and landscape suitability for the specified crops (Table 4).

Table 4. Land qualities/characteristics of mapping units at the Wadla Delanta Massif.

Land quality/characteristics	LMU1Ac	LMU2Ac	LMU2Bc	LMU3Ccl	LMU4Dcl
Topography (t)					
Slope gradient (%)	0-2	4-8	4-8	8-16	> 16
Topographic position (Altitude) (m)	< 2900	2900-3000	3000-3100	3100-3200	>3200
Wetness (w)					
Drainage	Poor	Poor	MWI	MW	Well
Flooding	Fo	Fo	Fo	Fo	Fo
Physical characteristics (s)					
Textural class of the soil	Clayey	Clayey	Clayey	Clay	Clay loam
Soil depth (cm)	> 150	>150	50-100	50-100	< 50
Coarse fragments/stoniness	0-3	3-15	3-15	15-35	15-35
Fertility characteristics (f)					
pH -H_2O	6.91-7.57	6.6-7.03	6.95-7.46	6.54-7.09	6.89-7.01
Soil organic matter (%)	1.66-1.97	1.99-2.83	1.58-2.11	1.57-2.40	1.74-2.42
Total Nitrogen (%)	0.08-0.10	0.10-0.16	0.09-0.14	0.08-0.12	0.09-0.14
Available phosphorus (mg kg^{-1})	10.26-13.25	7.24-14.43	9.26-10.49	6.80-10.38	7.77-11.13
Sum of basic cations (cmol(+) kg^{-1}))	27.85-41.28	29.77-41.02	29.77-41.02	27.43-40.67	26.29-40.88
Cation exchange capacity (cmol(+) kg^{-1})	37.49-45.89	35.17-44.79	38.51-53.2	44.14-56.5	45.84-47.20
Base saturation (%)	82.15-89.86	86.10-90.76	74.10-86.73	70.14-89.92	65.53-79.97
Salinity and alkalinity (n)					
Electric conductivity (dS m^{-1})	0.01-0.05	0.02-0.04	0.02-0.04	0.02-0.03	0.01-0.03
Calcium carbonate ($CaCO_3$)	3.93-5.17	3.14-3.72	3.34-4.46	3.49-13.22	0.85-1.38

Fo = none flooding; Dec. = December; Nov. = November; Oct. = October; MW moderately well drained.

Land suitability evaluation (matching) and production of suitability maps

To delineate the watershed and land mapping units with different GIS input data (thematic layers), topographic map, topo map sheet, shuttle radar topography mission (SRTM) image, satellite image and digital elevation model (DEM) were used. The DEM were derived from the SRTM image which generates slope, flow accumulation and drainage network by using ArcGIS 10.2. The land unit map was used as a guide in the field survey and soil sampling. In turn, more detailed soil maps were developed following the reinterpretation of field observation and soil analysis. The global positioning system (GPS) data were used for geo-referencing of the soil pedons. Finally, the land suitability classification was done according to the FAO (1976, 1983) methods and the land suitability maps were produced for the production of the suitability mapping units for the selected land utilization types.

RESULTS AND DISCUSSION

Description and characterization of land mapping units

Land mapping unit 1Ac

This LMU contains Pedons LG05, LC06 and LC07. They have been characterized by very gently sloping with slope of (1 to 2%), altitude ranges from 2800 to 2900 masl, basaltic parent materials, very deep soil depth (> 150 cm), area coverage 20.6%, poorly drained, color ranges from dark grayish brown (10YR 4/2) to very dark grayish brown (10YR 3/2) when dry and from very dark brown (10YR 2/2) to black (10YR 2.5/1) when moist; heavy clayey soils with high shrink and swell potential that would have wide and deep cracks when dry, high gilgai micro relief, common distinct slicken sides, very strong medium prismatic structure, hard to very hard (dry), firm (moist); very sticky and very plastic (wet) consistency; no excessive compaction and restriction of root development, abrupt and wavy boundary.

The LMU has slightly acidic to slightly alkaline in pH-H_2O (6.9-7.6) and non saline soils (EC < 0.5 dS m^{-1}), low in organic matter (1.66 to 1.97%) and total nitrogen (0.08 to 0.10%) contents, medium in available phosphours (10.26 to 13.25 mg kg^{-1}), high to very high in exchangeable Ca (21.09 to 26.25 cmol (+) kg^{-1}) and Mg (7.45 to 8.20 cmol (+) kg^{-1}) and moderate in monovalent cations (Na and K), high to very high in CEC (37.49 to 45.89 cmol (+) kg^{-1}) and PBS (82.15 to 89.86%). The extractable micronutrients (Fe, Mn, Cu and Zn) were also having high values of nutrient contents.

Land mapping unit 2Ac

This unit refers to soil Pedons MG01, MC02 and MG08 which have gently undulating with slope (2-4%), very deep soil depth (> 150 cm), altitude ranges from 2900-3000 masl, basaltic parent materials, color ranges from dark gray (10YR 4/1) to dark grayish brown (10YR 4/2) in dry and from very dark grayish brown (10YR 2.5/2) to bluish black (10YR 2.5/1) in moist, clay to heavy clayey soils with high shrink and swell potential that would have wide and deep cracks when dry, common distinct slickensides, high gilgai microrelief, area coverage 44.6%, poor drained, few boulders sub rounded slightly weathered quartz nature of rocks, strong medium prismatic structure, hard to very hard (dry), firm (moist), sticky to very sticky and plastic to very plastic (wet) consistency, abrupt and smooth boundary.

The mapping unit has slightly acidic to neutral in pH-H_2O (6.6-7.03), non saline soil (EC < 0.5 dS m^{-1}), medium in OM (1.99-2.83%) content, very low to medium in total N (0.01-0.16%), low to medium in available phosphorous (7.24-14.43 mg kg^{-1}), high to very high exchangeable Ca (23.22-26.90 cmol (+) kg^{-1}) and Mg (6.58-7.86 cmol (+) kg^{-1}) and moderate in monovalent cations (Na and K), high to very high in CEC (35.17-44.79 cmol (+) kg^{-1}) and PBS (86.10-90.76%). In the exchange sites, the divalent (Ca and Mg) cations were dominant than the monovalent (Na and K) cations in the study area. All the extractable micronutrients (Fe, Mn, Cu and Zn) have high values above the critical levels.

Land mapping unit 2Bc

This LMU contains with Pedons UC03 and UC09 which is slopping with slope of 5 to 8%, altitude ranges from 3000 to 3100 masl, moderately well drained, area coverage 8.6%, moderately deep (50 to 100 cm), color ranges from brown (10YR 4/3) to dark gray (10YR 4/1) in dry and dark brown (10YR 3/3) to very dark gray (10YR 3/1) in moist, clay in texture with shrink and swell that would have moderately wide and deep cracks when dry, some gilgai microrelief, strong coarse granular structure, slightly hard to hard (dry), firm (moist), sticky and plastic, abrupt and smooth boundary.

The LMU 2Bc was slightly acidic to alkaline in pH-H_2O (6.9 to 7.5) and non saline soils (EC < 0.5 dS m^{-1}), low to high contents of $CaCO_3$ (3.34 to 13.22%), low in soil OM (1.58 to 2.11%) and low to medium in total N (0.09 to 0.14%) contents, medium consents of available phosphorous (9.26 to 10.49 mg kg^{-1}), high to very high continents of exchangeable Ca (19.98 to 27.58 cmol (+) kg^{-1}) and Mg (6.90 to 7.91 cmol (+) kg^{-1}) and moderate in monovalent cations (Na and K), high to very high in CEC (38.51 to 53.20 cmol (+) kg^{-1}) and PBS (74.10 to 86.73%). The extractable micronutrients (Fe, Mn, Cu and Zn) were high that would have above the critical ranges.

Land mapping unit 3Ccl

This LMU refers to soil Pedons UC04 and UC10 which is steeply dissected topography with slope of 8 to 16%, altitude ranges from 3100 to 3300 masl, 15.04% of area coverage, shallow to moderate soil depth (50 to 100 cm), well-drained soil, soil color ranged from dark brown (7.5YR 3/2) to dark gray (10YR 4/1) in dry and from very dark brown (10YR 2/2) to very dark gray (10YR 3/1) in moist, clay loam texture, no excessive compaction and restriction of root development, moderate to strong medium granular structure, hard (dry), friable to firm (moist), slightly sticky to sticky and slightly plastic to plastic (wet) consistency, many very coarse high plane pores, abrupt and smooth boundary.

The LMU 3Ccl was slightly acidic to neutral in pH-H_2O (6.5 to 7.1), non saline soil (EC < 0.5 dS m^{-1}), low in OM (1.57 to 2.40%) and total N (0.08 to 0.12%) contents, low to medium available phosphorous (6.8 to 10.38 mg kg^{-1}), high to very high in exchangeable Ca (21.54 to 28.97 cmol (+) kg^{-1}) and Mg (7.69 to 8.86 cmol (+) kg^{-1}), and moderate in monovalent cations (Na and K), very high in CEC (44.14 to 56.5 cmol (+) kg^{-1}), high to very high in PBS (60.22 to 89.92%) and high status of micronutrients (Fe, Mn, Cu and Zn).

Land mapping unit 4Dcl

The LMU 4Dcl refers to soil Pedon UC12 which is steeply dissected topography with the slope of > 16%, shallow soil depth (< 50 cm), high elevation with 3300 to 3450 masl, area coverage 11.3%, well-drained soil, color ranged from dark grayish brown (2.5Y 4/2) to light reddish brown (2.5YR 6/4) in dry and from reddish brown (2.5YR 5/4) to dark reddish gray (5YR 4/2) in moist, clay loam texture, no excessive compaction and restriction of root development, many boulders sub rounded slightly weathered quartz nature of rocks, moderate fine crumb to strong medium prismatic structure, slightly hard to hard (dry), friable to firm (moist), sticky and plastic (wet) consistency, common medium plane pores and abrupt and smooth boundary.

The LMU 4Dcl has slightly acidic to neutral in pH-H_2O (6.8 to 7.01), non saline soil (EC< 0.5 dS m^{-1}), low in OM (1.74 to 2.42%) and low to medium in total N (0.09 to 0.14%) contents, low to medium in available phosphorous (7.77 to 11.13 mg kg^{-1}), high to very high in exchangeable Ca (21.69 to 21.88 cmol (+) kg^{-1}) and Mg (7.02 to 7.98 cmol (+) kg^{-1}) and moderate in monovalent cations (Na and K), very high in CEC (45.84 to 47.20 cmol (+) kg^{-1}), high in PBS (65.53 to 79.97%) and high status of micronutrients (Fe, Mn, Cu and Zn).

Agro-climatic analysis and suitability evaluation

The Wadla Delanta Massif followed the normal growing

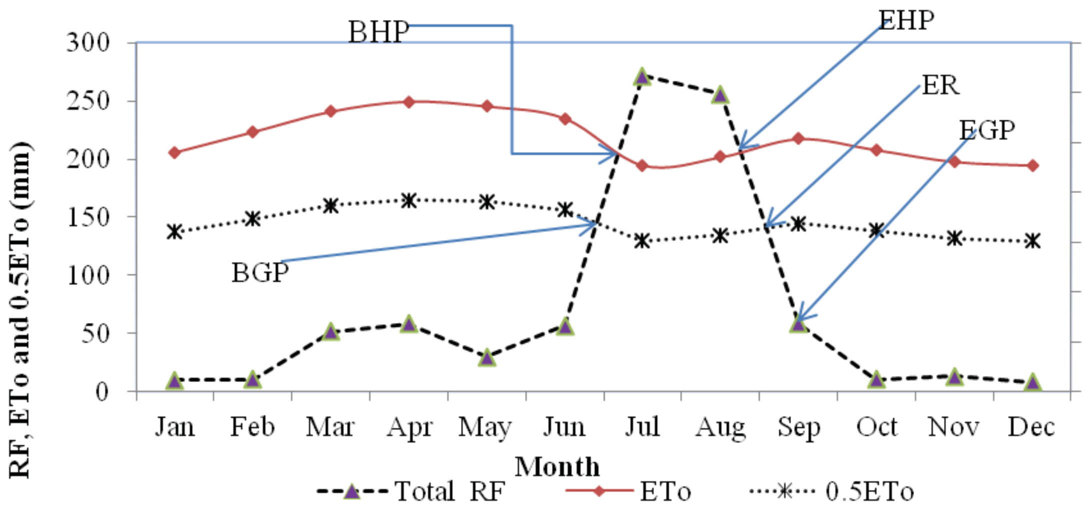

Figure 4. Length of growing period of the study area. BGP = Beginning of growing period, BHP = Beginning of humid period, EHP = End of humid period, ER = End of rains and EGP = End of growing period.

Table 5. Agro-climatic suitability using the simple limitation method.

Climatic characteristics	Factor value	Land utilization type			
		Barley 105[a]	Wheat 118[b]	Faba bean 125[c]	Lentil 120[d]
Mean growing season temperature (°C)	13.2	S2	S2	S2	S2
Total growing season rainfall (mm)	812.2	S2	S1	S2	S3
Length of growing season (day)	133	S1	S1	S1	S1
Occurrence of frost hazard (month)	3	S1	S3	S3	S3
Overall climatic suitability		S2	S3	S3	S3

Varieties Name: a = Shedho (3381-01); b = Guna (HAR-2029); c = Degaga and d = Chalew; Suitability class: S1 = highly suitable; S2 = moderately suitable; S3 = marginally suitable.

periods that agree with FAO procedure which reveals that the humid period (precipitation exceeding the potential evapotranspiration) and the beginning of the growing period was derived from the start of the rainy season. The average reference evapotranspiration (ET_o) of the areas was estimated to be 106.5 mm/month (3.5 mm/day). The mean calculated value of the LGP, in the study area, is 133 days (Figure 4).

The beginning of growing period (BGP) in the study area starts on June 11 (2^{nd} decade) and the humid period (BHP) on June 20 (2^{rd} decade), ends of humid period (EHP) on September 9 (1^{st} decade) and the rain season ends on September 20 (2^{nd} decade). The daily evapotranspiration in September is 3.35 mm/day and the daily evaporation rate for 30 days were required to utilize 100 mm water and generate end of growing period on October 21 (3^{nd} decade). The growing period curve of the study area is presented in Figure 4.

The period launched from the start of growing period to the end of rain which covers around 102 days using the indicated model (Hargreaves-Samani, 1985). Moreover, it required additional number of days intended for evapotranspiration - the assumed 100 mm of water expected to be stored within the soil at the end of rain. Therefore, the LGP is extend up to October 21^{st} (3^{rd} decade), which is a total of 133 days required. This showed that all the selected principal crops with the maximum crop cycle of 133 days can fit into the growing period or can be grown using rainfed agriculture of the study area.

The results of the overall climatic suitability evaluation showed that the agro-climatic situation of the study area is marginally suitable (S3) for selected varieties of *T. aestivum* L. and *V. faba* L. and *L. culinaris* L. whereas it is moderately suitable (S2) for *H. vulgare* L., variety (Table 5 and Appendix Table 1). All the considered varieties are practiced under rainfed conditions. The main limiting factor is the occurrence of frost hazard that appears in three months from October to December. Therefore, the famers might practice early or late sowing dates and choose relative varieties of frost resistance crops.

Table 6. Soil and landscape suitability ratings for physicochemical characteristic requirements for rainfed agricultural crops.

Land quality/characteristics	LMU1				LMU2A				LMU2B				LMU3				LMU4			
	bar	wht	fab	len	bar	wht	fab	len	bar	wht	fab	len	bar	wht	fab	len	bar	wht	fab	len
Topography (t)																				
Slope gradient	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
Topography/altitude	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S2	S3	S2	N	S2	N	S3	N
Wetness (w)																				
Drainage	S1	S2	S2	S1	S1	S2	S2	S1	S1	S2	S2	S1	S1	S1	S1	S1	S1	S1	S1	S1
Flooding	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Physical characteristics (s)																				
Textural class of the soil	S1	S2	S2	S1	S1	S2	S2	S1	S1	S2	S2	S1	S1	S1	S1	S1	S1	S1	S1	S1
Soil depth	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	N	S3
Coarse fragments/ stoniness	S1	S1	S1	S1	S1	S1	S1	S1	S	S1	S1	S1	S2	S2	S3	S3	S2	S2	S3	S3
Calcium carbonate ($CaCO_3$)	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Fertility characteristics (f)																				
pH -H_2O	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Soil organic matter	S1	S2	S2	S2	S1	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
Total Nitrogen	S1	S2	S2	S2	S1	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
Available Phosphorus	S1	S2	S2	S2	S1	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
Sum of basic cations	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Cation exchange capacity	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Percent base saturation (PBS)	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Electric conductivity (EC)	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
Overall	S1	S2	S2	S2	S1	S2	S2	S2	S2	S2	S2	S2	S2	S3	S3	N	S3	N	N	N

Bar = Barley; fab = Faba bean; LMU = Land mapping unit; len = Lentil and wht = Wheat.

Soil and landscape suitability evaluation for agricultural field crops

The results of the ultimate soil and landscape suitability evaluation showed that LMU 1 and 2 are moderately suitable (S2) for all considered crops and having limitations of erosion (e) and wetness (w), whereas LMU 3 is moderately suitable (S2) for barley, marginally suitable (S3) for wheat and faba bean and not suitable (N) for lentil. The main limiting factors are altitude, erosion and surface stoniness. Land mapping unit four was marginally suitable (S3) for *H. vulgare* L. and not suitable (N) for all other selected. The main limiting factors are soil depth, high altitude, erosion, surface stoniness and nutrient deficiencies (Tables 4, 6 and Appendix Tables 2 and 3).

Table 7. Overall land suitability evaluation for LMUs using simple limitation method.

LUT	LMU	Climate suitability	Soil suitability		Level of suitability		Area coverage	
			Physical	Chemical	Actual	Potential	ha	%
Barley	LMU1A	S2(c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	4939.54	20.56
	LMU2A	S2(c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	10707.94	44.57
	LMU2B	S2(c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	2061.35	8.58
	LMU3C	S2(c)	S3(r,s)	S2(f)	S3(c,f,r,s)	S3(c,r,s)	3613.36	15.04
	LMU4D	S2(c)	S3(r,s,t)	S2(f)	S3(c,f,r,s,t)	S3(c,r,s,t)	2702.81	11.25
Wheat	LMU1A	S2 (c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	4939.54	20.56
	LMU2A	S2 (c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	10707.94	44.57
	LMU2B	S2 (c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	2061.35	8.58
	LMU3C	S2 (c)	S3(r,s,t)	S2(f)	S3(c,f,r,s,t)	S3(c,r,s,t)	3613.36	15.04
	LMU4D	S2 (c)	N(r,s,t)	S2(f)	N(c,f,r,s,t)	N(c,r,s,t)	2702.81	11.25
Faba bean	LMU1A	S2 (c)	S2(w)	S2(f)	S2(c,f,w)	S2(c,w)	4939.54	20.56
	LMU2A	S2 (c)	S2(w)	S2(f)	S2 (c,f,w)	S2(c.w)	10707.94	44.57
	LMU2B	S2 (c)	S2(w)	S2(f)	S2 (c,f,w)	S2(c.w)	2061.35	8.58
	LMU3C	S2 (c)	S3(r,s)	S2(f)	S3(c,f,r,s)	S3(c,r,s)	3613.36	15.04
	LMU4D	S2 (c)	N(r,s,t)	S2(f)	N(c,f,r,s,t)	N(c,r,s,t)	2702.81	11.25
Lentil	LMU1A	S2 (c)	S2(w)	S2(f)	S2 (c,f,w)	S2(c,w)	4939.54	20.56
	LMU2A	S2 (c)	S2(w)	S2(f)	S2 (c,f,w)	S2(c,w)	10707.94	44.57
	LMU2B	S2 (c)	S2(w)	S2(f)	S2 (c,f,w)	S2(c,w)	2061.35	8.58
	LMU3C	S2 (c)	N(r,s,t)	S2(f)	N(c,f,r,s,t)	N(c,r,s,t)	3613.36	15.04
	LMU4D	S2 (c)	N(r,s,t)	S2(f)	N(c,f,r,s,t)	N(c,r,s,t)	2702.81	11.25

LMU = Land mapping unit; Limitation factors: c = Climate (occurrence of frost); w = Oxygen availability (drainage); f = Fertility (OM, total N and available P); r = Rooting conditions (depth); s = Physical (stoniness); t = Topography (altitude/elevation).

Overall suitability evaluation

Most of the land characteristics considered in the evaluation, lands currently under rainfed cultivation, range from suitable to moderately suitable for agricultural purposes. As compared to the middle and lower topography, the upper topography soils were found to be well drained, low depths and rocky. On the other hand, some of the middle and all of the lower topographic soils showed that high depth, poorly drained and high water logging condition. Occurrence of frost is the common problem in all topographic positions. The textural classes ranged from clay loam to heavy clayey. The evaluation class for the crops' suitability ranges from moderately suitable (S2) to permanently not suitable (N). This is due to the different condition that the crops require for their developments in the local area in question (Table 7 and Appendix Tables 1 to 3).

The majority of the cultivated land, about 65.13%, is classified as moderately suitable for all considered field crops. As concerning for *T. aestivum* L. and *V. faba* L. crops, about 15.04 is marginally suitable and 8.5% not suitable. For *H. vulgare* L. 80.2% is moderately suitable and 8.5% as marginally suitable, and for *L. culinaris* L. crop, about 23.5% of the land is not suitable. The main limiting factors are altitude, soil depth, erosion and surface stoniness. The results of the analysis provided in Table 7 and Figures 5, 6, 7 and 8).

Conclusion

The overall land suitability assessment of the study showed significant differences among the identified land units for the principal crops. The growing season temperature, elevation and occurrences of frost are the most limiting factors in the area. Some of the limitations are also the results of anthropogenic activities related to inappropriate land uses. Results of the study showed that the lower and middle topographic positions are moderately suitable (S2) for all considered crops with proper land management, whereas the upper topographic position is not suitable for crop production. Therefore, it is better to reserve this part of the land for grazing or other land uses like highland fruits.

Conflict of Interest

The authors have not declared any conflict of interest.

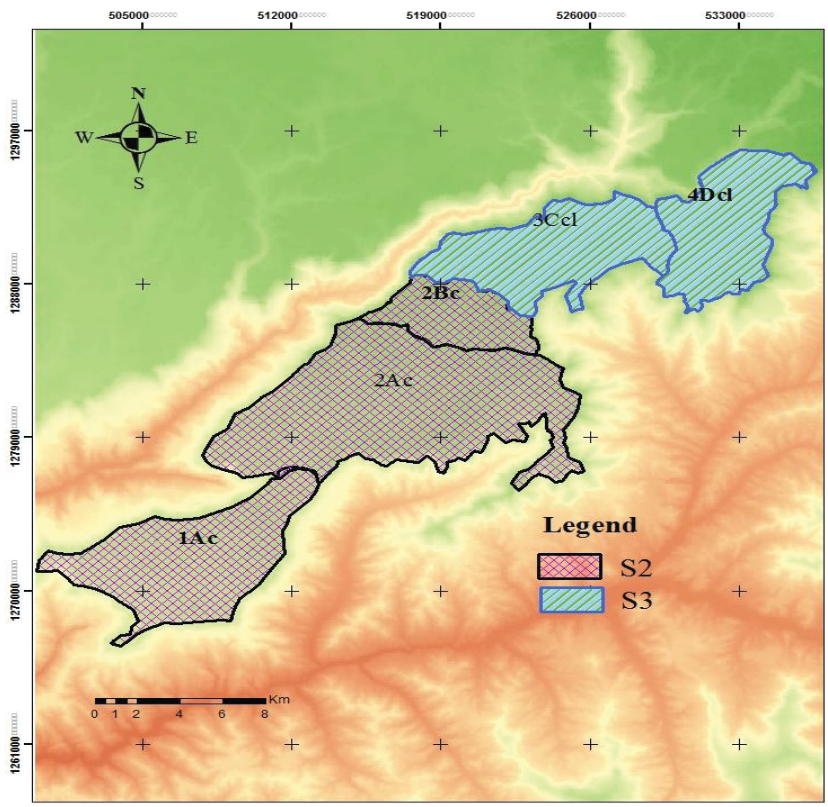

Figure 5. Land suitability evaluation map for barley.

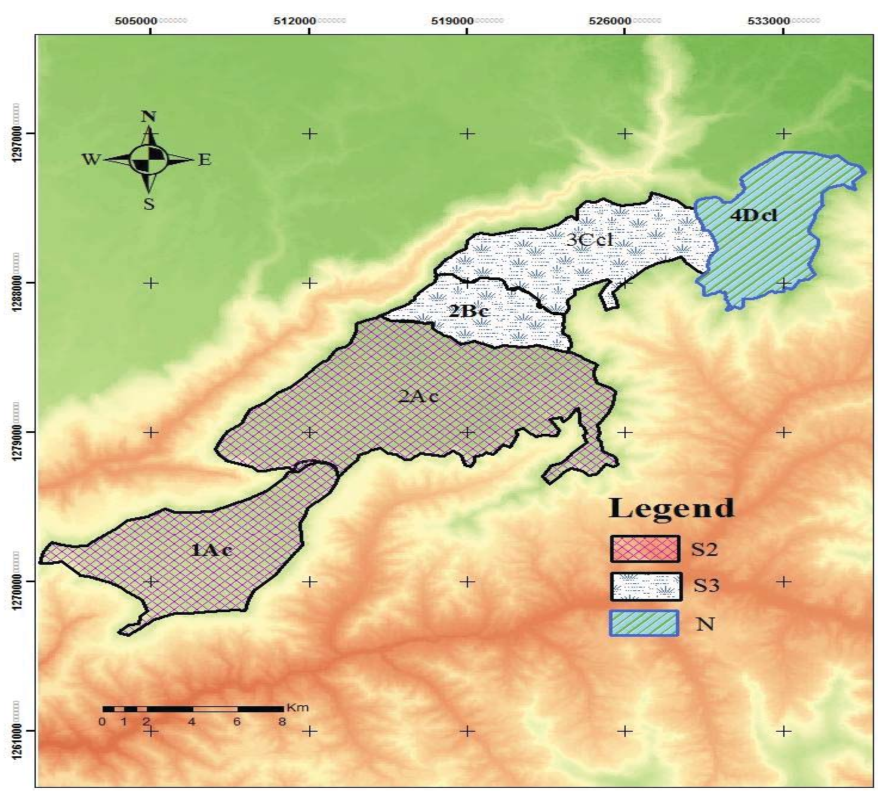

Figure 6. Land suitability evaluation map for wheat.

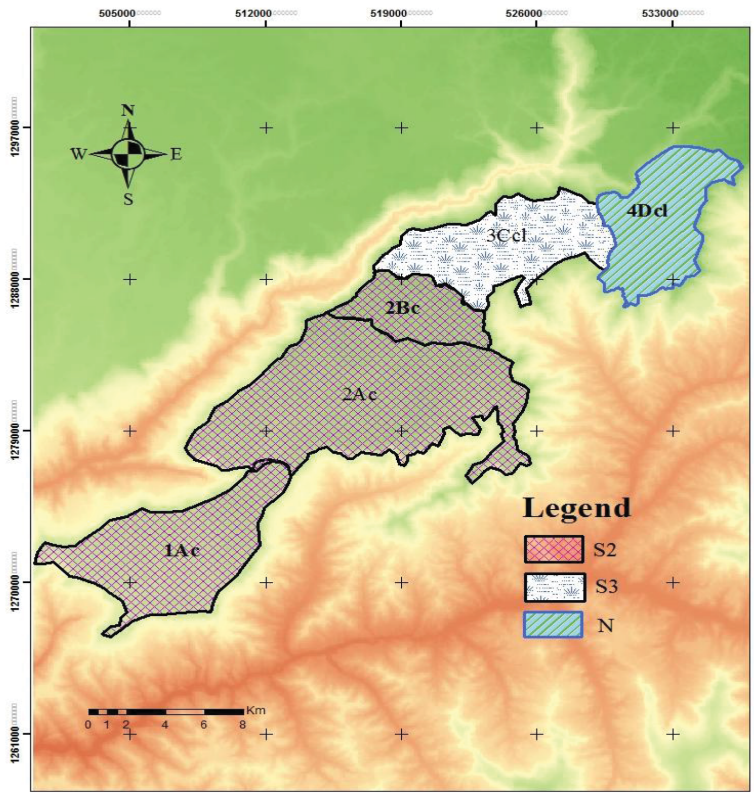

Figure 7. Land suitability evaluation map for faba bean.

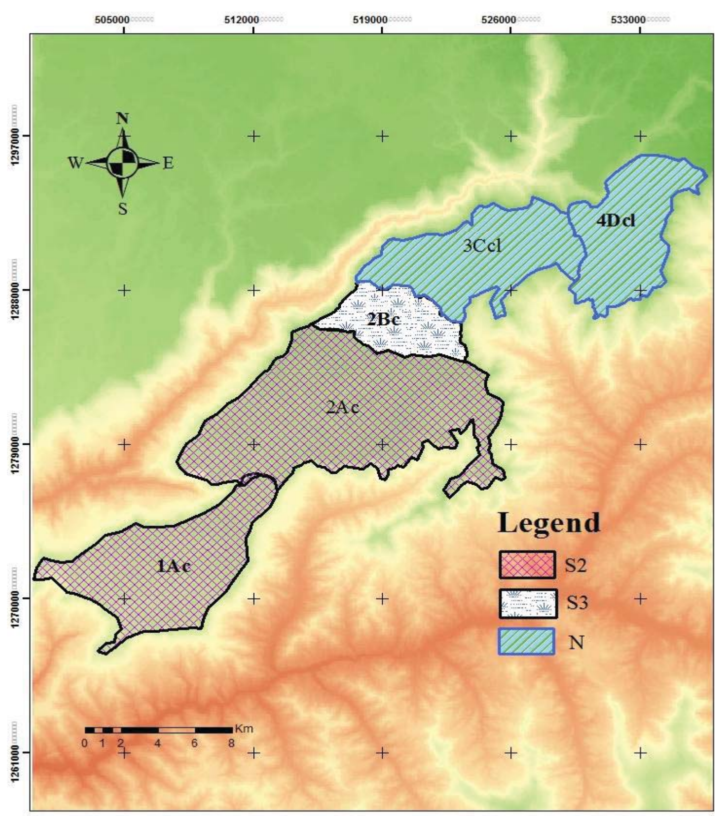

Figure 8. Land suitability evaluation map for lentil.

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APPENDIX

Table 1. Land suitability ratings for agro-climate characteristic requirements for rainfed (barley, wheat, faba bean and lentil) crops

LUT	Rating			Rainfall (mm)	Temperature (°C)	LGP (day)*[1]	Frost hazard (month)
Barley	S1	0	100	400-650	16-18	120-135	None
		1	95	500-750	14-18	110-155	None Oct. to Nov. slight in Dec.
	S2	2	85	300-400 or 750-850	12-14 or 18-20	90-110 or 155-180	None October, slight in Nov. to Dec.
	S3	3	60	200-300 or 850-1000	10 -12 or 20-22.5	75-90 or 180-230	Slight in Oct. to Dec.
	N1	4	40	150-200 or 1000-1250	8-10 or 22.5-28	< 75 or > 230	Any frost in Oct., severe Nov., Dec
	N2		25	< 150 or > 1250	< 8 or > 28	-	-
Wheat	S1	0	100	700 -1000	18-20	130-140	None
		1	95	350 -1250	15-20	120-155	None Oct. to Nov. slight in Dec.
	S2	2	85	250-350 or 1250-1500	12-15 or 20-25	100-120 or 155-180	None October, slight in Nov. to Dec.
	S3	3	60	250-200 or 1500-1750	10-12 or 25-27	80-100 or 180-230	Slight in Oct. to Dec.
	N1	4	40	-	8-10 or 27-30	< 80 or > 230	Any frost in Oct., severe Nov. to Dec
	N2		25	< 200 or > 1750	< 8 or > 30	-	-
Faba bean	S1	0	100	400-500	17.5-20	135-155	None
		1	95	500-600	15-20	130-180	None Oct. to Nov.
	S2	2	85	300-400 or 600-1000	12.5-15 or 20-24	100-130 or 180-265	None Oct. to Nov., slight Dec
	S3	3	60	250-300 or 1000-1200	10-12.5 or 24-27	75-100 or 265-305	None Oct. to Nov., slight Nov. to Dec
	N1	4	40	-	8-10 or 27-30	< 75 or > 305	Slight Oct., sever Nov. to Dec.
	N2		25	< 250 or > 1200	< 8 or > 30	-	Any frost in Oct., severe Nov. to Dec.
Lentil	S1	0	100	450-500	15-18	120-160	None
		1	95	500-700	15-20	150-170	None Oct. to Nov., slight Dec
	S2	2	85	400-450 or 700-800	12-15 or 20-24	100-120 or 160-180	None Oct. to Nov., slight Nov. to Dec
	S3	3	60	350-400 or 800-900	10-12 or 24-27	90-100 or 180-210	Slight Oct., sever Nov. to Dec.
	N1	4	40	300-350 or 900-1000	8-10 or 27-30	75-90 or 210-240	Any frost in Oct., severe Nov. to Dec.
	N2		25	< 300 or > 1000	< 8 or > 30	< 75 or > 240	

Source: Adapted from FAO (1976; 1983), FAO/UNDP (1984), Sys et al. (1991; 1993); Teshome and Verehye (1994). *[1] LGP = Length of growing period.

Table 2. Land suitability ratings for physical characteristic requirements for rainfed (barley, wheat, faba bean and lentil) crops.

LUT	Rating			Slope (%)	Elevation (m)	Drainage	Flooding*[2]	Texture*[1]	Stoniness (vol. %)	Depth (cm)
Barley	S1	0	100	0-4	2000-3000	Good	-	C < 60s, Co, SiCs, SiCL,Si, SiL, CL	0-3	
		1	95	4-8	-	Moderate	F0	C < 60v, SC, C > 60s, L	3-15	> 90
	S2	2	85	8-16	1500-2000 or 3000-3300	Imperfect/ Good	F0	C > 60v, SCL	15-35	50-90
	S3	3	60	16-24	3300-3800	Poor and aeric	F1	SL, LfS	35-55	25-50
	N 1	4	40	24-30	< 1500 or > 3800	Poor but drainable	F2	-	-	10-25
	N2		25	> 30	-	Poor not drainable	F3+	Cm, Si Cm, LcS, fS, cS, S	> 55	< 10
Wheat	S1	0	100	< 2		Good	F0	C < 60s, SiC, Si, SiL, CL	0-3	
		1	95	2.-8	2000-2600	Moderate		C < 60v, SC, C > 60s, L	3-15	> 90
	S2	2	85	8.-16	1500-2000 or 2600-3000	Imperfect	F1	C > 60v, SCL	15-35	50-90
	S3	3	60	16-30	3000-3300	Poor and	F2	SL, LfS	35-55	20-50
	N1	4	40		< 1500 / > 3300	Poor but		-	-	10-20
	N2		25	> 30		Poor not	F3+	Cm, SiCm, LcS, fS, cS	> 55	< 10
Faba bean	S1	0	100	0-4	2100-2400	Good	F0	C < 60s, SiCs, SiCL, CL, Si, SiL	0-3	
		1	95	4 to 8	2000-3000	Moderate	-	C > 60s, SC, C < 60v, L, SCL	3 to 15	> 100
	S2	2	85	8 to 16	1800-2000 or 3000-3200	Imperfect	-	C > 60v, SL, LfS, LS	15-35	75-100
	S3	3	60	16-30	1500-1800 or 3200-3400	Poor and	F1	LcS, fS, S	35-55	50-75
	N 1	4	40	24-30	< 1500 or > 3400	Poor but	-	-	-	20-50
	N2		25	> 30		Poor not	F2+	Cm, SiCm, cS	> 55	< 20
Lentil	S1	0	100	0-4	2100-2400	Good	F0	C < 60s, Co, SiCs, SiCL, CL, Si,	0-3	> 100
		1	95	4 to 8	2000-3000	Well	-	C > 60s, SC,C < 60v, L, SCL	3 to 15	75-100
	S2	2	85	8 to 16	1800-2000 or 3000-3200	Well	-	C > 60v, SL, LfS, LS, L	15-35	50-75
	S3	3	60	16-30	1500-1800 or 3200-3400	Moderately well	F1	LcS, fS, S, SiL	35-55	25-50
	N 1	4	40	-	< 1500 or > 3400	Imperfect	-	CL SiCL	-	10-25
	N2		25	> 30	-	Poor, Very poor	F2+	Cm, SiCm, cS, SC	> 55	< 10

Source: Adapted from FAO (1976, 1983), FAO/UNDP (1984), Sys et al. (1991, 1993); Teshome and Verehye (1994). (*) Textural range: Cm = massive clay; SiCm = massive silty clay; C+60,v = fine clay, vertical structure; C+60,s = fine clay, blocky structure; C-60,v = clay, vertical structure; C-60,s = clay, blocky structure; SiCs = silty clay, blocky structure; SiCL = silty clay loam; CL = clay loam; Si = silt; SiL = silt loam; SL = sandy loam; L = loam; SCL = sandy clay loam; SL = sandy loam; Lfs = loamy fine sand; LS = loamy sand; LcS = loamy coarse sand; fS = fine sand; S = sand; cS = coarse sand; Co = clay, oxisol structure.

Table 3. Land suitability ratings for soil chemical characteristic requirements for rainfed (barley, wheat, faba bean and lentil) crops.

LUT	Rating			pH- H_2O	Soil OC (%)	Total N (%)	Avail. P*1 (mg kg^{-1})	EC (dS m^{-1})	$CaCO_3$ (%)	Gypsum (%)	Cations*2 (cmol (+) kg^{-1})	CEC (cmol (+) kg^{-1})	PBS (%)	EPS (%)
Barley	S1	0	100	7-7.5	> 2.0	-	-	0-8	3 - 20	0-3	> 8	> 24	> 80	0-15
		1	95	6.2-8.0	1.2-2.0	> 0.2	> 10	8 to 12	20-30 or 3-0	3 to 5	5 to 8	24-16	80-50	15-25
	S2	2	85	6.2-5.8 or 8-8.2	0.8-1.2	0.15-0.2	5 to 10	12 to 16	30-40	5 to 10	3.5-5.0	< 16 (-)	50-35	25-35
	S3	3	60	5.8-5.5 or 8.2-8.5	0.4- 0.8	0.1-0.15	3 to 5	16-20	40-60	10 to 20	2.0-3.5	< 16(+)	< 35	35-45
	N	4	40	< 5.5 or v	< 0.4	< 0.1	< 3	20-25	-	-	< 2.	-	-	-
	N2		25	-	-	-	-	> 25	> 60	> 20	-	-	-	> 45
Wheat	S1	0	100	6.5-7.5	> 2.5	-	-	0-1	3-20.	0-3	> 8	> 24	> 80	0-15
		1	95	6.0-8.2	1.5-2.5	> 0.2	> 10	1 to3	20-30 or 0-3	3.-5	5.-8	24-16	80-50	15-25
	S2	2	85	6-5.6 or 8.2-8.3	1.0-1.5	0.15-0.20	5.-10	3 to 5	30-40	5.-10	3.5-5.0	< 16 (-)	50-35	25-35
	S3	3	60	5.6-5.2 or 8.3-8.5	0.5-1.0	0.10-0.15	3.-5	5 to 6	40-60	10.-20	2.0-3.5	< 16(+)	< 35	35-45
	N	4	40	< 5.2 or > 8.5	< 0.5	0.8- 0.10	< 3	6 to 10	-	-	< 2.	-	-	-
	N2		25	-	-	< 0.8	-	> 10	> 60	> 20	-	-	-	> 45
Faba bean	S1	0	100	6.0-7.0	> 2	-	-	0	0-6	0-0.1	> 5	> 24	> 50	0-2
		1	95	5.6-7.6	2-1.2	> 0.2	> 10	0-1	1-6.	0.1-0.5	5-3.5	24-16	50-35	2-5.
	S2	2	85	5.6-5.4 or 7.6-8.0	1.2-0.8	0.15-0.2	5.-10	1-1.5	12.-20	0.5-1.0	3.5-2	< 16 (-)	35-20	5-8.
	S3	3	60	5.4-5.2 or 8.0-8.2	< 0.8	0.1-0.15	3.-5	1.5-2	20-25	1 to 3	< 2	< 16(+)	< 20	8-12.
	N	4	40	< 5.2	-	< 0.1	< 3	-	-	-	-	-	-	-
	N2		25	> 8.2	-	-	-	> 2	> 25	> 3	-	-	-	> 12
Lentil	S1	0	100	6.0-7.0	> 2	-	-	0	0-6	0-0.1	> 5	> 24	> 50	0-2
		1	95	5.5-7.3	2-1.5	> 0.2	> 10	0-1	6-12.	0.1-0.5	5-3.5	24-16	50-35	2 - 5
	S2	2	85	5.3-5.5 or 7.3-7.7	1.5-1.0	0.15-0.2	5 to 10	1-1.5	12-20.	0.5-1.0	3.5-2	< 16 (-)	35-20	5 - 8
	S3	3	60	5.2-5.3 or 7.7-8	1.0-0.8	0.1-0.15	3 to 5	1.5-2	20-25	1 to 3	< 2	< 16(+)	< 20	8 - 12
	N	4	40	5.0-5.2 or 8-8.5	< 0.8	< 0.1	< 3	over 4	-	-	-	-	-	> 15
	N2		25	< 5 or > 8.5	-	-	-	> 2	> 25	> 3	-	-	-	> 12

Source: Adapted from FAO (1976, 1983), FAO/UNDP (1984), Sys et al. (1991, 1993); Teshome and Verehye (1994). *1 = Olsen method analysis; *2 = sum of cations; PBS = % base saturation; EPS = exchangeable sodium percentage.

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Development of cotton leaf curls virus tolerance varieties through interspecific hybridization

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Cotton Leaf Curl Virus (CLCuV) is a major threat in enhancing cotton production in Pakistan. Economic loss due to this disease during last decade is estimated about 75 million rupees. This disease spread in epidemic from 1992 to 1995. The first CLCuV resistant variety was evolved in 1996. Resurgence of this disease occurred in 2001 (Burewala) breaking resistance of all exiting available germplasm of cotton. Interspecific hybridization for leaf curl virus resistance is the only economical and long term approach to tackle this hazardous problem. A total of 3338 genotypes were screened at Cotton Research station Vehari during 2003 to 2004 but none of these genotypes showed resistance to this disease. Two cultivated diploid species viz *Gossypium herbaceum* A1, *Gossypium arboreum* A2, *Gossypium anomalum* B1, *Gossypium capitisviridis* B4, *Gossypium gossypoides* D6, *Gossypium laxum* D8, *Gossypium stocksii* E1, *Gossypium somalense* E2, *Gossypium areysianum* E3 *and Gossypium longicaly x* F1 did not showed the symptoms of this disease through petiole grafting. *G. arboreum* is immune to CLCuV, two artificial allotetraploids of 2(*Gossypium hirsutum* L x *Gossypium. anomalum*).x ^{3}*G. hirs.* and 2 (*G. arboreum* L x *G. anomalum*). x ^{2}*G. hirs*. were manually hybridized under field conditions. These two hybrids were also crossed for gene pyramiding [{.2 (*hirs*. x G. *anom*.) x ^{3}*G. hirs.*} x {^{2}*G. hirs.* x 2 (*G. arbo*. x *G. anom*) x ^{2}*G. hirs* }] x ^{2}*G. hirs*. Exogenous hormones containing 50 mg/l gibberellic acid and 100 mg/l naphthalene acetic acid was applied to control boll shedding. 3:1 ratio was not observed in above said combinations. Some plants were found resistant against CLCuV by using petiole grafting technique. But no resistance was observed. Maximum tolerance was found in this combination, that is, [{.2 (*hirs*. x G. *anom*.) x ^{3}*G. hirs.*} x {^{2}*G. hirs.* x 2 (*G. arbo*. x *G. anom*) x ^{2}*G. hirs* }] x ^{2}*G. hirs*. By using this material CIM-608 has been evolved which is having high tolerance to CLCuV; this will increase cotton production and will be a source of food security.

Key words: Cotton, introgression, cotton leaf curl virus (CLCuV), tolerant.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the backbone of Pakistan's economy accounts for 8.6% of the value

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added in agriculture and about 1.8% to GDP. Total area for cotton cultivation was 3.106 million hectares 10.1% more than last year 2.82 million hectares. Estimated production is 12.7 million bales for 2009-2010, higher by 7.4% over last year production of 11.8 million bales. Yield was 695 kg/ha. However cotton production was 5.0% less than the target of 13.36 million bales mainly due to spread of Cotton Leaf Curl Virus (CLCuV), shortage of irrigation water, high temperature in August resulting high fruit shedding and flare up of sucking pests (Agricultural Statistics of Pakistan, 2009-2010). Cotton is infected by several insects, pests and pathogens inducing different diseases. Among them Cotton Leaf Curl Virus is the most obnoxious disease causing enormous losses to the cotton crop (Khan and Ahmad, 2005). It has caused reduction of 9.45 million bales during the last decade, amounting to rupees 75 billion to the national economy. In 1981, the cotton area affected by this disease was about 35000 acres (Ali et al., 1992). This disease spread in Sindh in 1997, in Khyber Pakhtoon Khawa in 1998 and in Baluchistan in 2001 (Tariq, 2005). Reduction in yield in tolerant varieties was 50% and 85 to 90% in susceptible cultivars (Hussain, 1995; Khan et al., 2001).

A new and mutant strain of CLCuV (Begomovirus) was observed in Burewala region 2001-2002, which is more virulent and named the Burewala Strain of Cotton Virus (BSCV) after being detected in Burewala. In the wake of resurgence of new strain of Burewala virus the resistance was broken down and as a result all the varieties including LRA-5166, CP-15/2 and Cedex (resistant to previous CLCuV became susceptible to BSCV (Briddon, 2003; Mahmood et al., 2003; Tahir et al., 2004).

Different scientists worked on interspecific hybridization for transferring resistant genes for favorable traits from wild diploid species into tetraploid cultivated cotton like (Blank and Leathers, 1963) transferred resistant genes against cotton rust caused by *Puccinia cacabata.* from *Gossypium anomalum* L. and *Gossypium arboreum* L. into *Gossypium hirsutum* L. through interspecific hybridization, induction of polyploidy and back crossing accompanied by continuous screening for resistance. It is worthwhile to combine the genes for Cotton Leaf Curl Virus resistance and other diseases and drought resistance between *G. hirsutum* L. and *G. arboreum* L. cotton (Amin, 1940). Moreover other research workers have attained success in introgression of *G. hirsutum L.* and *G. arboreum* L. (Bao-Liang et al., 2003). Moreover other research workers have attained success in interspecific introgression of *G. hirsutum* L. and *G. arboreum* L. (Bao-Liang et al., 2003). Similarly, introgressed resistant genes B6 found in 'A' genome of *G. arboreum* L. against bacterial blight caused by *Xanthomonas malvacearum*. Into *Gossypium barbadense* L (Knight, 1957; Brinkerhoff, 1970). Breeders also achieved most resistant commercial variety 'Auburn 56'against Root knot nematode (*Meloidogyne incognita*) in U. S. cotton through transgressive segregation (Shepherd, 1974). Sacks and Robinson (2009) also introgressed resistance to *Rotylenchulus reniformis* into the tetraploid 2 (AD1) through crossing a resistant diploid A2-genome *G. arboreum* L. accession (A2-190) with a hexaploid 2 [(AD1) D4] bridging line (G 371) to obtain a tetraploid triple specie hybrid. At present no single variety of *G. hirsutum* L. is resistant to BSCV, whereas *G. arboreum* L. is known to have immunity against Cotton Leaf Curl Virus (Bird, 1973). Keeping in view losses made by CLCuV emphasis should be given to explore the possibility of successful transferring virus resistant genes from Desi cotton (*Gossypium arboreum* L. 2n=26) into cultivated upland cotton (*G. hirsutum* L. 2n = 52) genotypes through conventional breeding.

The Cytogenetics Section of Central Cotton Research Institute, Multan is engaged for the past many years in transferring desirable characters of wild species to the cultivated ones through complex crosses. The objective of this study is to introgress CLCuV resistance from wild species to upland cotton which will ultimately leads to the development of cotton leaf curl virus tolerant varieties. This will be a source of food security all over the world. In screening of available 30 Gossypium species to cotton leaf curl virus observed in field, it was found that the diploid species of cotton viz. *G. herbaceum, G. arboreum, G. anomalum, G. captis viridis, G. gossypioides, G. laxum, G. stocksii, G. areysianum, G. somalense* and *G. longicalyx* showed resistance to Burewala stain of cotton leaf curl virus.

MATERIALS AND METHODS

G. *arboreum* and *G. anomalum* were found resistant rather immune to cotton leaf curl virus. So *G. arboreum* and *G. anomalum* (diploids) were crossed as male with *G. hirsutum* (tetraploid) to transfer the virus resistance from both the diploid species into *G. hirsutum* background. Methods for developing virus resistant material are as follows:

1. *G. anomalum Wawra et. Payer*(2n= 26)-B1 was crossed with *G. hirsutum Linn*(2n=52)(AD)1 as female. The resultant triploid hybrid was treated with 0.2% aqueous solution of colchicine for 72 h using seedling dip method for doubling the chromosomes. The hexaploid was further crossed with *G. hirsutum* to make a pentaploid which was further back crossed four times to get a stable tetraploid.
2. *G. anomalum Wawra et. Payer*(2n= 26)-B1 was crossed with *G. arboreum Linn*(2n=26)A2. The resultant diploid inter-specific hybrid was treated with 0.2% aqueous solution of colchicine for 72 h using seedling dip method for doubling the chromosome. The resultant tetraploid hybrid was crossed and back crossed with *G. hirsutm.*
3. Both the above mentioned species hybrids viz., [{^{4}*hirs*. x 2 *(hirs*. x *G. anom*.)} x {^{2}*hirs*. x 2 (*arbo*. x *anom*.)}] x *hirs*. were also crossed with each other.

The synthesized material was grafted with virus affected petioles of CIM-473 to check its virus resistance against BSCV in green house. Later on these resistant plants were shifted to field for assessment of their resistance against BSCV in field conditions. The number of the plants showing resistance in green house as well as in field was recorded.

The plants showing CLCuV resistance were selected and further

Table 1. Screening of resistant material during 2006-2007.

Material	Total number of plants grafted in green house	Number of plants not showing symptoms and transplanted in the field	BSCV affected plants in the field	Resistant plant	Percentage resistance
4G. *hirs.* x 2 (*hirs*. x G. *anom*.).	303	13	11	2	0.66
2G. *hirs*. x 2(*G.arbo*. x .*anom.*)	774	115	97	18	2.32
[{4G. *hirs.* x 2 (G.*hirs*. x G. *anom*.)} x {2G. *hirs.* x 2(*G.arbo*. x G.*anom*)}] x *G. hirs*	1354	214	173	41	3.0
Total	2431	342	281	61	

Table 2. Screening of material in the field during 2006-2007.

Material	Total number of plants in the field	BSCV affected plants in the field	Resistant plant	Percentage resistance
4G. *hirs.* x 2 (G.*hirs*. x G. *anom*.).	1231	1193	38	3.1
2G. *hirs*. x 2(*G.arbo*. x G.*anom*)	121	85	36	29.7
[{4G. *hisr.* x 2 (*hirs*. x G. *anom.*)} x {2G. *hirs.* x 2(*G.arbo*. x G.*anom*)}] x *G. hirs.*	88	59	29	33.0
Total	1440	1337	103	

selection was done by using progeny row trials. Number of strains have been developed which are having good CLCuV tolerance.

RESULTS

By conventional breeding methods, crosses between the two species of cotton are rarely successful due to abortion of embryo after fertilization. The diploid species that cross directly with upland cotton produce sterile triploid F1 hybrids. Such triploid hybrids have to be treated with Colchicine to produce hexaploids (Joshi and Johri, 1972). We synthesized Triploid hybrid plants of (*G. hirs.* L. x *G. anom.*) L. and 2 (*G. arbo*. x *G. anom*) treated with 0.1% Colchicine solution for seven days using cotton swab method. There was no effect of Colchicine as these plants were old.

2431 plants of the material developed by species hybrids were grafted with virus affected of petiole of CIM-473 to check their virus resistance against BSCV in green house. Out of these 342 plants which did not show BSCV symptoms were transplanted in the field. Only 61 plants showed resistant against BSCV till maturity of crop. The results are given in Table 1. The table shows that in the first *Gossypium* species hybrid viz., 4*G. hirs* x 2*(G. hirs x G. anom*), resistance to BSCV was 0.66%, where *G. anomalum* alone resistant to BSCV was used. While in the second combination viz., 2*G. hirs* x 2(*G. arbo x G. anom*), the resistance to BSCV was 2.32%. When two *Gossypium*, that is, *G. arboreum*. and *G. anomalum* resistant to BSCV were used. In the third combination, that is, [{4*G. hirs.* x 2 (*hirs*. x G. *anom*.)} x {2*G. hirs*. x 2(*G.arbo*. x G.*anom*)}] x *G. hirs*. where *G. anomalum* has been used twice and *G. arboreum* once, the resistance against BSCV was 3.0%. Our results are also in agreement with those of (Altman, 1988) that in the development of F_1 hybrids and BC_1 's plants application of exogenous hormone techniques are superior to *in vitro* methods.

The neutral plants neither grafted in greenhouse nor in the field were tested against BSCV. Data given in the Table 2 is also in agreement with that of Table 1. The resistance to BSCV in the first, second hybrid, and in their combination was 3.1, 29.7 and 33.0% respectively. The plants which showed resistance against cotton leaf curl virus were picked, ginned and their fiber quality was evaluated.

The economic and fibre characters of some of the resistant material are given in Tables 3 to 6. Virus resistant plants in the interspecific hybrid 4*G. hirs.* x *2* (*G. arbo*. x *G. anom.)* having very good lint percentage were CP-38/Z55 (45.9), Z52A, CP-37/Z50 (44.2) and Z52 (42.2); while virus resistant plants have extra long fiber length were Z-69 (31.3 mm), CP-38/Z55 (28.3 mm), Z52A (30.5 mm), CP-37/Z50 (30.4 mm) and Z52 (31.0 mm) (Table 4). Neutral plants of interspecific hybrid 4*G. hirs*. x 2 (*G. arbo*. x *G. anom*.) having better lint percentage and fiber length as compared to standard were CP-30 (39.5%, 31.5 mm), CP-31 (46.6%, 28.6 mm), CP-32 (38.6%, 31.6 mm), CP-36 (45.4%, 29.0 mm), CP-37 (45.0%, 29.0 mm) and CP-38 (46.3%, 26.5 mm) as it is shown in Table 4.

Table 3. Performance of resistant plants of ^{4}*G hirs*. × 2 (*G. hir.* × *G. anom*)

Hybrid No.	**Seed cotton yield (g)**	**Lint (%)**	**Fibre length (mm)**	**Fibre fineness (µg/inch)**	**Fibre strength g/tex**
^{4}*G hirs*. x 2 (G. *hir.* x *G. anom.*)					
CP13/Z38	55.0	38.9	26.1	5.0	28.2
Z40	204.3	40.2	29.8	4.5	27.3
^{3}*G hirs*. x 2 (*G.hir.* x *G. anom.*)					
CP13 (2004)	287.4	37.4	26.9	4.6	23.7
^{2}*G hirs*. x 2 (*G. hir.* x *G. anom.*)					
P6 (2003)	37.6	36.9	26.2	4.4	27.1

Table 4. Performance of resistant plants of *G.* ^{4}*hirs*. × *2* (*G. arbo.* × *G. anom.*).

Plant No.	**Seed cotton Yield (g)**	**Lint (%)**	**Fibre length (mm)**	**Fibre fineness (µg/inch)**	**Fibre strength (g/tex)**
Resistant plants of ^{4}***G. hirs. × 2*** **(*G. arbo.* × *G. anom.*)**					
CP-37/Z50	117.4	42.2	30.4	3.3	36.1
Z51	47.4	41.9	29.8	3.2	33.7
Z52	117.8	42.2	31.0	3.9	34.5
Z52A	25.06	44.0	30.5	4.0	31.1
Z54	122.2	42.0	28.2	5.3	27.8
CP-38/Z55	47.6	45.9	28.3	2.9	36.4
Z56	87.0	36.3	29.9	4.3	30.1
AB1/Z61	57.2	39.9	29.7	3.3	31.8
Z67	41.0	34.1	33.3	3.5	34.6
Z69	218.4	39.5	31.3	3.4	35.5
Neutral plants of ^{4}***G. hirs*. × 2 (*G. arbo.* × *G. anom.*)**					
CP-29	184.3	43.2	28.6	5.0	26.0
	96.0	41.5	28.4	4.4	31.2
	225.5	43.9	28.2	5.8	24.9
CP-30	166.6	34.6	31.8	3.5	35.3
	112.2	38.3	30.7	4.2	34.6
	30.4	39.5	31.5	4.3	32.8
CP-31	75.3	43.6	28.1	3.8	28.7
	33.0	46.6	28.8	4.1	29.6
	58.4	41.6	30.2	3.6	32.2
CP-32	292.0	38.8	31.6	3.3	33.0
CP-36	108.2	45.4	29.0	3.0	37.8
CP-37	230.0	45.0	29.	3.8	31.6
	71.0	43.7	31.0	3.2	32.8
CP-38	62.0	46.3	26.5	4.8	25.6
AB1	74.7	41.8	28.8	3.1	32.6
	74.2	39.6	27.8	3.4	30.9
	64.3	40.0	29.4	3.4	30.8
P-22 (2004)	24.0	33.3	31.1	3.2	32.8
CIM-496 (C.S.)	90.3	41.2	28.9	4.8	26.5
CIM-506 (C.S.)	86.1	37.2	28.5	4.7	26.3

Table 5. Performance of of resistant plants of [{[3]*G. hirs*. × 2 (*G. hir*. ×*G. anom.*)} × {[2]*G. hirs*. × 2(*arbo.* × *G. anom.*)}] × *G. hir*.

Plant No.	Seed cotton yield/plant (g)	Lint (%)	Fibre length (mm)	Fibre fineness (µg/inch)	Fibre strength (g/tex)
CP-3/Z2	23	44.1	28.9	3.6	32.2
CP-4/Z3	254	38.4	29.4	3.9	29.1
Z4	114	37.7	33.4	3.5	32.5
Z5	418	41.9	32.3	4.1	29.1
Z6	303	39.0	34.3	3.4	31.9
Z8	116.6	36.7	32.0	3.1	32.5
CP-5/Z15	60.3	35.2	33.8	3.6	29.9
Z16	76.3	35.9	31.8	3.5	29.9
Z18	141.0	35.1	30.0	4.1	29.1
CP-7/Z24	86.0	38.6	30.4	4.5	33.9
Z27	148.2	35.4	30.9	4.4	30.2
Z28	153.4	45.6	28.3	3.8	30.3
Z29	26.4	41.7	27.8	3.9	31.6
CP-8	188.8	39.4	29.0	4.8	27.0
CP-11/Z32	120.4	45.3	31.1	3.4	36.0
Z34	144.3	37.6	32.3	3.6	34.2
Z42A	89.0	40.1	30.0	3.3	34.5
CP-12/Z36	234.6	35.4	30.0	4.5	28.4
Z37	153.0	39.2	30.3	3.3	33.4
CP-17/H3	14902	38.6	29.1	4.3	28.7
H7	83.8	37.8	29.3	4.1	28.7
H12	200.3	44.4	28.1	3.7	29.2
H13	205.0	36.0	29.4	4.4	30.8
H14	353.5	39.5	30.9	4.0	29.8
H15	64.2	41.1	28.5	3.7	29.5
H16	130.0	35.4	30.8	4.0	30.0
CP-17	293.5	39.8	30.7	3.7	30.3
CP-24/Z47	261.8	35.0	31.7	3.2	32.2
P6 (2003)	37.6	36.9	26.2	4.4	27.1
P-22 (2004)	24.0	33.3	31.1	3.2	32.8
CIM-496 (C.S)	90.3	40.2	28.1	4.8	26.5
CIM506 (C.S.)	86.1	37.2	27.8	4.9	26.3

Data in the Table 5 showed that good combinations of economic and fibre characteristics are available in this material synthesized through multiple species hybrids. Virus resistant plants in the multiple species interspecific hybrid [{[3]*G. hirs*. x 2 (*G. hir*. x *G. anom*.)} x {[2]*G. hirs*. X 2(*arbo.* x *G. anom* .)}] x *G. hir***.** having very good lint percentage were CP-7/Z28 (45.6%), CP-11/Z32 (45.3%), CP-17/H12 (44.4%) and CP-3/Z2 (44.1%) while virus resistant plants exhibiting extra long fiber length were CP-3/Z6 (34.3 mm), CP-5/Z15 (33.8 mm), CP-4/Z5 and CP-11/Z34 (32.3 mm), CP-5/Z16 (31.8 mm) and CP-11/Z32 (31.1 mm). Virus resistant plants having good seed cotton yield with good fiber quality traits will be utilized in breeding programme for introgression of cotton leaf curl virus resistance in elite interspecific combinations. The material, synthesized above, showed progressive increase in yield and ginning out turn percentage with improved fibre qualities.

Data in Table 6 revealed that outstanding combinations of different economic and fibre characteristics are present in this material. The cotton leaf curl virus resistant plants were selfed and seeds of this material were sown in the field in plant to progeny row to find out homozygous plants for virus resistance.

Elite strains developed by using interspecific combinations have very good tolerance to cotton leaf curl virus and having desirable fiber traits (Table 7). CIM-608 is the first introgressed strain has been submitted for approval to expert sub-committee in 2012 while Cyto-124 is showing high tolerance to cotton leaf curl virus with

Table 6. Performance of of resistant plants (Neutral plants).

Plant No.	Seed cotton yield/plant (g)	Lint (%)	Fibre length (mm)	Fibre fineness (µg/inch)	Fibre strength (g/tex)
CP-5	61.5	35.8	**31.7**	3.1	37.6
CP-6	166.3	37.3	29.4	3.9	34.6
CP-11	129.0	38.8	30.4	3.5	34.7
CP-17	336.5	35.5	30.6	4.1	29.9
	80.6	39.7	30.3	3.9	31.4
	141.1	38.6	30.1	4.6	30.0
	131.0	38.9	29.9	4.1	31.0
	97.8	40.2	29.4	4.2	28.9
	207.8	37.5	30.7	4.1	31.4
CP-21	59.5	44.9	32.2	2.8	32.6
CP-24	100.8	43.3	29.5	3.2	32.7
	103.0	40.8	30.9	3.6	35.0
CP-25	121.3	50.5	27.5	4.1	29.9

Table 7. Elite strains developed through interspecific hybridization.

Strains No.	Virus (%)	Lint (%)	Fibre length (mm)	Fibre fineness (µg/inch)	Fibre strength (tppsi)
CIM-608	24.9	39.9	28.3	4.8	93.5
Cyto-120	3.6	39.6	29.7	4.5	99.9
Cyto-110	1.4	39.2	31.3	4.2	96.7
Cyto-111	3.8	42.5	29.3	4.9	99.5
Cyto-112	4.2	42.6	28.8	4.8	100.6
Cyto-113	7.6	41.9	28.8	4.9	97.2
Cyto-114	11.9	40.4	29.1	4.8	97.5
Cyto-115	6.4	44.2	28.2	4.8	100.5
Cyto-116	9.2	42.9	29.2	4.6	101.5
Cyto-117	4.7	41.1	30.5	4.4	103.4
Cyto-118	5.2	41.2	30.3	4.4	102.3
Cyto-119	8.2	43.1	29.2	4.8	96.6
Cyto-122	13.4	39.8	29.1	4.4	92.6
Cyto-124	2.7	40.8	29.2	4.8	99.6

very good fiber traits.

DISCUSSION

G. hirsutum L. has low genetic diversity and lack of resistance against Cotton Leaf Curl Virus while wild diploid species of *Gossypium* have potential for resistance against many disasters like insect, pests, diseases and many abiotic factors. Hence there is great need for exploitation of this source for developing resistance against CLCV in cultivated tetraploid species. The primary factor limiting the success of interspecific hybridization in cotton is lack of retention of crossed bolls. Cotton breeders have been trying to obtain hybrids between diploid and tetraploid species for a long time (Gill and Bajaj, 1987) but it has been difficult and sometimes impossible to obtain hybrids under *in situ* conditions because of several incompatibility factors. Weaver (1957) found ovule failure in the cross, *G. hirsutum* L. × *G.arboreum* L. due to deranging effect of the hybrid embryos upon endosperm development, while in reciprocal cross (Weaver, 1958) abnormal endosperm nuclei mitosis and improper embryo differentiation were the main causes. Many workers used ovule and embryo culture for *Gossypium* interspecific hybridization (Stewart and Hsu, 1978; Refaat et al., 1984; Gill and Bajaj, 1987; Umbeck and Stewart, 1985; Thengane et al., 1986; 1987; Mirza et al., 1993) but Louant et al., 1977) was of the opinion that hybrids obtained by those workers could be obtained through standard hybridization. Interspecific hybridization of cotton has been assisted by exogenous hormone application after pollination. Numerous reports from China have documented that exogenous hormone application alone can overcome certain crossing barriers within *Gossypium* (Liang and Sun, 1982; Liang et al., 1978). Gibberellic acid was used as growth regulator to obtain interspecific hybrids between tetraploid *G. hirsutum* L. and diploid *G. arboreum* L. species of cotton (Mofidabadi, 2009).

The plant hormones are known to control pollen tube growth (Kovaleva et al., 2005). Exogenous application of growth hormone has been used to facilitate interspecific crosses in manycrops, that is, cotton (Altman, 1988), wheat (Sitch and Snap, 1987) and tomato (Gordillo et al., 2003). Altman (1988) compared exogenous application with *in vitro* techniques, that is, ovule and embryo culture and found that exogenous hormones used with standard hybridization were superior to *in vitro* methods.

Conclusion

Cotton leaf Curl Virus is menace for cotton production in the world and severally damaged the cotton crop in Pakistan. As there is no resistant genotype in upland cotton to CLCuV, the only way is to introgress this resistance in upland cotton from wild species. By using resistant wild species hybrids the material having very good economic traits with high seed cotton yield has been developed. CIM-608 has been approved for general cultivation in 2013 which will increase seed cotton production and will be a source of food security; Cyto-124 has shown lowest virus for the last two years in national coordinated varietal trial and has been submitted for approval in expert sub-committee. Moreover; this material will be used for insect resistance having good fiber quality.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Socio-economic determinants of tomato retail marketing in Ibadan Southwest Local Government area of Oyo State, Nigeria

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This study examined the socioeconomic determinants of tomato retail marketing in Ibadan southwest Local Government Area, Oyo State, Nigeria. The study was based on the primary data obtained in a cross section survey of 80 randomly selected tomato retail marketers drawn by multi-stage sampling across four (4) markets in the study area. The data were collected by personal administration of questionnaire designed to elicit information on the tomato retail marketers socio-economic characteristics and transportation and storage costs among others. The study data were analysed using descriptive and regression analysis. The result revealed that the modal age group for the tomato retail marketers was 31 to 50 years. Also, the average number per household was found to be of 5 persons with 71.3 and 82.5% being male. 86.3% of the respondents were educated with 5 to 15 years of marketing experience of about 60%. Also, about 81% of the respondents made tomato retail marketing their primary occupation. The regression analysis revealed that Age (X_1), Years of marketing experience (X_2), purchase cost (X_4), labor cost (X_5) and transportation cost (X_6) were statistically significant at p≤0.05, p≤0.05, p≤1, p≤0.05, p≤0.01 and p≤0.1%, respectively out of the six postulated explanatory variables. The coefficient of multiple determination (R^2) was 0.789 which shows that about 79% variations in profit of the tomato retail marketers were caused by variations in the Six (6) postulated explanatory variables. The gross margin analysis results revealed that the gross margin (profit) was ₦38, 076.00 per month. The study therefore concluded that transportation and storage cost were the major factors influencing profitability of retail tomato marketing in the study area. Hence, policy issue aim at encouraging tomato retail marketing will focus on provision of good transportation and storage facilities.

Key words: Socio-economic, gross margin, tomato, retail marketing, Oyo State.

INTRODUCTION

Adejobi et al. (2011) posited that among the wide range of agricultural crops, especially vegetables occupy an important place because of their economic potentials. Among different vegetables grown in Nigeria, tomato

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clearly stands out as the most important both in scale of production and level of consumption. Whereas, other vegetables mostly have restricted demand in Nigeria, as they are consumed exclusively only by the urban rich, demand for tomato is universal, for it is consumed by both the rich and poor. Fadama vegetable production and marketing activities such as handling, transportation and distribution employ a large number of labour force (Adepetu, 2005).

Adepetu (2005) also posited that, tomato production accounts for a total land area of one million hectares cultivated annually making up about 18% of the average daily consumption of vegetables in Nigerian homes. Tomato is cultivated almost throughout Nigeria and the most important areas lie between 7.50 and 130N mostly around urban areas in the Northern and Southern-Western parts of the country. Tomato is an herbaceous plant commonly grown as an annual, though perennial tomato culture is an established practice in many parts of South America.

According to (Purseglov, 1992), tomato is a short lived perennial, grown as an annual, branching herb with hairy weak trailing stems, hairy leaves and variable in shape. Lycopersicon is a relatively small genus within the extremely large and diverse family Solanaceae. The family Solanaceae is an important source of vegetable and desert crops including potato, egg plant, various pepper, the tree tomato and tomato, *Lycopersicon esculentum* among others. The African tomatoes on the other hand were introduced by European merchants and colonizers. Therefore, the African tomatoes were probably descended from varieties bought from Europe (Villareal, 1980).

Adegbola et al. (2012) stated that Nigeria is undeniably the 14th largest producer of tomatoes, second to Egypt in Africa at 1.51 million metric tonnes valued at ₦ 87.0 billion with a cultivated area of 254,430 ha being the biggest producer in Sub-Sahara Africa. Although, most of these tomatoes are locally not processed, leading to increasing import dependency of tomato paste to the tone of 65,809 tons valued at ₦11.7 billion annually. With this potential, it is unbelievable that Nigeria could still be importing tomatoes products of any kind.

Haruna et al. (2012) defined agricultural marketing as the performance of all the activities involved in the flow of agricultural products and services from the initial points of agricultural production until they reach the hands of the ultimate consumers. It is interested in everything that happens to crops after its leaves the farm gate; making decision, taking actions and bearing the responsibility of the action. Agricultural marketing also involves all processes that take place from when the farmer plans to meet specified demands and market prospects to when the producers finally gets it to the consumers. It also recognizes the mutual independence between farmers and marketing middlemen which is the whole essence of marketing in management decision making (Haruna et al., 2012).

Dittoh (1994) defined Agricultural marketing as all activities that aid the movement of commodities from the farms to the consumers and these include assemblage of goods, storage, transportation, processing, grading and financing of all these activities. Olukosi and Isitor (2004) explained that the marketing task involves transferring goods from producers to consumers. It is the marketing function that ensures that consumer acquires the product in the form, places and time desired. As the economy of a nation grows, the gap between consumers and producers widens and the task of marketing becomes more complex (Abbott, 1987). Therefore, this study broadly examines socio-economic determinants of tomato retail marketing in Ibadan Southwest Local Government Area of Oyo State, Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted in Ibadan southwest Local Government of Oyo State. Ibadan southwest. Ibadan South-West is a Local Government Area in Oyo State, Nigeria. Its headquarters are at Oluyole Estate in Ibadan. It has an area of 40 km² and a population of 282,585 at the 2006 census. It is bounded in the West by Ido Local Government, east by Ibadan North and Ibadan South East Local Governments, north by Ibadan North West and Ido Local Governments and South by Oluyole Local Government.

Sources and method of data collection

This study was based on primary data collected by personal administration of questionnaire/interview schedule for farm households in the study area. Information were sought on socio – economic and demographic characteristics such as; age, sex, religion, household size and years of experience.

Multi-stage sampling technique was used to select a cross section of 80 tomato retail marketers from the study area. Four (4) markets were randomly selected out of the markets in the study area. Twenty (20) tomato retail marketers were randomly selected from each of the four markets targeting a total of 80 tomato retail marketers in the study area.

Analytical techniques

Quantitative (econometric) and descriptive techniques were employed to analyse the data collected. Descriptive analyses such as the frequency distribution, ages, mean, mode, standard deviation and standard error were used to analyse the socio-economic and demographic characteristics of the tomato retailers in the study area.

The gross margin model was specified from estimation of total expenses (costs) as well as various returns or revenue within a marketing period.

$$\text{Total Cost (TC)} = TVC + TFC \quad (1)$$

where, TVC = Total variable cost; TFC = Total fixed cost.

$$\text{Total Revenue (TR)} = Q.\ Py \quad (2)$$

where, Q = Quantities of tomatoes sold in a baskets; Py = Unit price of tomatoes in baskets.

Gross Margin (GM) = GI = TVC (3)

where, GI = Gross income; TVC = Total variable cost.

Net Income (NI) = GI –TC (4)

To determine the profitability of tomato marketers, some profit ratios were calculated to show the overall performance of the business thus:

Gross Ratio (GR) =TC/TR (5)

where, TC = Total cost; TR = Total revenue.

Operating Ratio (OR) = TVC/TR (6)

where, TVC = Total variable cost; TR= Total returns.

Fixed Ratio (FR) = TFC/TR (7)

where, TFC = Total fixed cost; TR= Total revenue.

Regression analysis

Regression analysis was used to analyze the determinants of profit margin. An Ordinary Least Square regression model was estimated. The explanatory variables included in the model are age of marketers, years of marketing experience, marital status, start-up capital, labour cost and transportation cost. The empirical regression analysis used is implicitly stated as:

$$\text{Profit} = f(X_1, X_2, X_3, X_4, X_5, X_6) \quad (8)$$

Where X_1 = Age (years); X_2 = Years of marketing experience (years); X_3 = marital status (1=married, 0-otherwise); X_4 = Start-up Capital (₦); X_5 = Cost incurred on labour (₦); X_6 = Cost incurred on Transportation (₦).

RESULTS AND DISCUSSION

Socioeconomic characteristics of tomato retail marketers

The result revealed that the modal age group for the tomato retail marketers was 31 to 50 years. Also, the average number per household was found to be of 5 persons with 71.3 and 82.5% being male. 86.3% of the respondents were educated with 5 to 15 years of marketing experience of about 60%. Also, about 81% of the respondents made tomato retail marketing their primary occupation (Table 1).

Gross margin (profitability) analysis of retail tomato marketing

It is importance to analyze the marketing cost of retail Tomatoes marketing because is a key determinant in the profit margin that accrues to the marketer. Adejobi (2005) defined marketing cost as the difference between the amount paid by the consumers of final products and the total amount received by the producer. This is the cost incurred during the marketing process.

The profitability of marketing of retail tomatoes within a period of one month is indicated in Table 2. The table revealed that variable cost accounted for ₦ 56,170.00 (99.99%) and fixed cost ₦ 54.00 (0.01%) of the total cost of marketing retailed tomatoes in the study area. The results further indicated that the cost of acquisition (86.00%), cost of empty basket (2.04%), transportation cost (2.5%) and cost of loading and offloading (2.2%) were the major variable costs incurred in tomato retail marketing. Based on the computation per basket, the average basket of tomato was 50 kg and average price per basket was ₦1,050.00, total cost of marketing was ₦ 56,224.00 while the total revenue of ₦ 94,300.00 was realized making a net income per month of ₦ 38,076.00. In view of this costs and returns analysis, the retail tomato marketing in Ibadan Southwest was highly profitable because the gross ratio (0.60) was positive and less than one.

Socio-economic determinants of tomato marketing

Table 3 shows the regression analysis revealed that the F-value was 4.01 and was significant at 1% suggesting that the model is fit. The coefficient of multiple determination (R^2) was 0.789 which shows that about 79% variations in profit of the tomato retail marketers were caused by variations in the six postulated explanatory variables. Six explanatory variables were included in the model, out of which five of the coefficients of the variables were significant. These include; Age (X_1), marketing experience (X_2), capital (X_4), Labor cost (X_5) and Transportation cost (X_6) statistically significant at $p<0.05$, $p<0.01$, $p<0.05$, $p<0.01$ and $p<0.1$ % respectively.

Age

The coefficient of this variable was significant at 5% ($p<0.05$) and carries a negative sign; indicating that as the retailer grows older, the lower their profit.

Marketing experience

The coefficient of this variable was significant at 1% ($p<0.01$) and carries a positive sign; indicating that the longer the years of experience of the marketer in sales of Tomatoes, the higher their profit margin. This conforms to the *apriori* expectation in that longer experience enables the marketer to acquire indigenous methods of keeping fresh tomatoes, thereby reducing the age loss due to

Table 1. Demographic and socio-economic characteristics of respondents.

Variable	Frequency	(%)
Age group (years)		5.0
≤30	12	10.0
31 to 40	22	27.5
41 to 50	20	32.5
Above 50	26	
Gender		
Male	14	17.5
Female	66	82.5
Education		
No formal	11	13.8
Primary	22	27.5
Secondary	47	58.8
Marital status		
Single	10	12.5
Married	54	67.5
Widowed	8	10.0
Divorced	8	10.0
Household size		
2 to 3	19	23.7
4 to 5	57	71.3
6 and above	4	5.0
Years of experience		
Below 2	3	3.8
2 to 5	12	15.0
5 to 7	18	22.5
Above 8	47	59.7
Religion		
Christian	27	33.8
Muslim	50	62.5
Others	3	3.8

Source: Field Survey, 2010.

damages and this in turn increases the profit level.

Initial start - up capital

The coefficient of this variable was significant at 5% ($p<0.05$) and carries a positive sign; indicating that the higher the marketing cost, the higher the profit margin of the marketer. This conforms to the apriori expectation in that if high capital is incurred by the marketer, the more the profits that will accrue to the marketers.

Labour cost

The coefficient of this variable was significant at 1% ($p<0.01$) and carries a negative sign; indicating that the lower the labour cost, the higher the profit margin of the marketer. This conforms to the apriori expectation in that if less labour cost is incurred by the marketer, the more the profits that will accrue to the marketer for each sale made.

Transportation cost

The coefficient of this variable was significant at 10% ($p<0.1$) and carries a negative sign; indicating that the lower the transportation cost, the higher the profit margin of the marketer. This conforms to the apriori expectation in that if less transportation cost is incurred by the marketer, the more the profits that will accrue to the

Table 2. Gross margin analysis of retail tomato marketing in naira per basket.

Cost items	Returns ((₦)/month)
Variable cost	
Acquisition cost	48,300
Transportation cost	1,420
Cost of empty basket	2,400
Cost of loading and off-loading	1,250
Taxes	1,400
Labour	1,000
Cost of water	400
Total variable cost (TVC):	**56,170**
Fixed cost (FC)	
Depreciation on rent	54
Total fixed cost (TFC):	54
Total cost: (Naira):	**56,224**
Returns	
Gross income (Q x P. y) (46 x 2,050)	94,300
Net income (GI – TC)	38,076
Return/Naira invested (GI/TC)	1.68
Profitability ratio	
Operating ratio (TVC/GI)	0.6
Fixed ratio (TFC/GI)	0.0006
Gross ratio (TC/GI)	0.6
Total ratio:	1.201

Source: Field Survey (2010).

Table 3. Regression result of the determinants of Tomato Retail Marketing Profit Margin

Independent variables	Coefficient	Significant
Constant	4.283	0.000
Age	-0.001**	.0164
marketing experience	4.712***	.0083
Marital status	0.442	.213
capital	-2.661**	.013
Labour cost	-5.111***	.000
Transportation cost	-4.274*	.086
R^2	.791	
Adjusted R^2	.762	
F-value	4.01	

Source: Field Survey (2010). *,**,*** refer to significant at 10, 5 and 1%, respectively.

marketer for each sale made.

CONCLUSION AND RECOMMENDATION

This study was designed to examine the socioeconomic determinants of tomato retail marketing in Ibadan Southwest Local Government Area of Oyo State, Nigeria. Data from 80 retail tomato marketers were used for this study. The descriptive analysis result revealed that the modal age group for the tomato retail marketers was 31 to 50 years. Also, the average number per household was found to be of 5 persons with 71.3 and 82.5% being male. 86.3% of the respondents were educated with 5 to 15 years of marketing experience of about 60%. Also, about 81% of the respondents made tomato retail marketing their primary occupation. The profitability analysis of marketing of retail tomatoes revealed that variable cost accounted for ₦ 56,170.00 (99.99%) and fixed cost ₦ 54.00 (0.01%) of the total cost of marketing retail tomatoes. Based on the computation per basket, the average basket of tomato was 50 kg and average price per basket was ₦1,050.00, total cost of marketing was ₦56,224.00 while the total revenue of ₦94,300.00 was realized making a net income per month of ₦38,076.00.

In view of this costs and returns analysis, the retail tomato marketing in Ibadan Southwest was highly profitable because the gross ratio (0.60) was positive and less than one. The regression analysis revealed that the F-value was 4.01 and was significant at 1% suggesting that the model is fit. The coefficient of multiple determination (R^2) was 0.789 which shows that about 79% variations in profit of the tomato retail marketers were caused by variations in the six postulated explanatory variables. Six explanatory variables were included in the model, out of which five of the coefficients of the variables were significant. These include; Age (X_1), marketing experience (X_2), Purchase cost (X_4), Labor cost (X_5) and Transportation cost (X_6) statistically significant at $P<0.05$, $P<0.01$, $P<0.05$, $P<0.01$ and $P<0.1\%$, respectively. The findings of this study have revealed that there is need to address the issue of the determinants of retail tomato marketing among marketers for sustainable livelihood. Based on this, the study recommend that policy issue aim at encouraging tomato retail marketing will focus on provision of good transportation and storage facilities.

Conflict of Interest

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

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Full Length Research Paper

Influence of temperature on the reproductive success of a fig wasp and its host plant

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Ambient temperatures influence many aspects of insect behavior and reproduction, and limit their distribution and abundance. Small, delicate insects such as the fig wasps (Agaonidae) that pollinate fig trees rapidly succumb to heat stress when outside figs. We compared survivorship and reproductive success of the fig wasp *Kradibia tentacularis* pollinator of the Asian fig tree *Ficus montana* in three glasshouses maintained at different temperatures during the brief period when foundress females are laying their eggs after their entry into figs (means of 17, 21 and 27°C, respectively). This temperature range had no significant effect on speed of foundress death, or their likelihood of re-emergence. Similarly, fig wasp offspring production were unaffected by temperatures at the time of oviposition in male figs, as was seed production in female figs, although the proportion of failed galls changed marginally. The range of temperatures to which the fig wasps were exposed reflected conditions under a tropical forest canopy and their general lack of responsiveness suggests that the relatively buffered environment within their host figs means that only extreme temperature conditions will influence them once they have entered the plant.

Key words: Agaonidae, climate change, *Ficus*, oviposition, sex ratio, thermal tolerance.

INTRODUCTION

Temperature is probably the abiotic factor with the largest single influence on the distribution and abundance of insects (Andrewartha and Birch, 1954). Predicted changes in climate suggest that many insect species will be exposed to higher median temperatures and extreme temperature events in the future, with consequences for their population dynamics, distribution and evolution (Walters, 2012; Van Velzen et al., 2013). Species involved in obligate mutualisms are likely to be particularly responsive to climate change, because of the strong interdependency between partners (Kiers et al., 2010; Colwell et al., 2012; Gilman et al., 2012).

Fig trees (*Ficus*, Moraceae) are an example of obligate mutualists that have very wide ecological significance; more species vertebrates feed on their fruits than on those of any other plants (Shanahan et al., 2001). The approximately 800 described species of fig trees are found mainly in tropical and subtropical regions, in habitats ranging from rainforests to deserts (Berg and Corner, 2005).They are all pollinated exclusively by host specific fig wasps (Agaonidae), with each fig tree species dependent on one or a small number of fig wasp species.

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Adult female fig wasps enter the figs in order to lay their eggs inside the numerous female flowers the inner surface of these specialized inflorescences. A single wasp larva develops inside each of the galled ovules of female flowers, which will usually also have been pollinated. Monoecious fig trees have figs where fig wasp offspring and seeds develop in the same figs. This contrasts with fig trees that have a dioecious breeding system, where there are distinct male and female plants, characterized by having mature figs that contain either the next generation of fig wasps or seeds, respectively. Fig wasps enter female figs, despite being unable to reproduce there, because of reciprocal mimicry displayed by male and female figs (Grafen and Godfray, 1991). Female fig wasps escape from their natal figs via holes chewed through the fig wall by the males (Suleman et al., 2012). They are small (typically less than 2 mm in length), do not feed, and short lived – most probably die within 24 h of emergence if they fail to find a suitable fig to enter (Kjellberg et al., 2008). Fig wasps lose their wings when entering through the ostiole into a fig, preventing them from dispersing further to other trees.

Temperatures control many aspects of the biology of fig wasps. Variations in temperature have direct impacts on the insects and also act indirectly via their host plants. The fruiting and leafing phenologies of fig trees vary considerably between species, but most respond by initiating and maturing fewer crops at colder times of the year. Studies have shown strong seasonal effects on leaf phenology and fig initiation even under controlled conditions (Suleman et al., 2011). The time that fig crops take to reach maturity (and therefore the generation times of the fig wasps) is highly variable among those fig trees that grow in seasonal environments, with some winter crops taking months to complete development that is completed in weeks during the summer (Bronstein and Patel, 1992; Compton, 1993). Also, for some fig trees, more numerous figs have been recorded in summer thanin winter (Suleman et al., 2013). Emergence times from natal figs differ between diurnal and nocturnal-flying species, and in the day-flying *Elisabethiella baijnathi* the time when this occurs is linked to temperature, with the wasps emerging earlier in the day during summer months (Ware and Compton, 1994). The minimum temperature at which emergence takes place may be particularly significant, because fig wasps maintained below this critical temperature can develop through to pupation, but then eventually die within their galls (Zavodna and Compton, unpublished).

Adult female fig wasps do not eat or drink. Outside of figs, they are rapidly incapacitated when exposed to air temperatures above 30°C (Patiño et al., 1994) and longevity declines above 25°C (Jevanandam et al., 2013). The more rapid deaths of adult female fig wasps in response to higher temperatures are likely to be the result of more rapid dehydration as well as heat stress, though Jevanandam et al. (2013) could not confirm this in their experiments. The darker coloration typical of day flying fig wasps, compared with night-flying species, suggests that they nonetheless display adaptations to reduce water losses (Compton et al., 1991) and among the other insects that inhabit figs, a non-pollinating fig wasp *Walkerella* sp. (Pteromalidae) has a specialist male morph adapted for mating outside the figs that is darker in color and more resistant to desiccation than typical males (Wang et al., 2010). Although darker fig wasps are not necessarily more resistant to dehydration (Warren et al., 2010), thermal tolerances may nonetheless limit the distributions of some fig wasps to sub-sets of the distributions of their host plants, with the day-flying *Ceratosolen galili,* a fig wasp that fails to pollinate its host figs, absent from the western desert areas of the range of *F. sycomorus*, unlike its night-flying congener *C. arabicus* (Compton et al., 1991; Warren et al., 2010). Differing thermal tolerances may also explain seasonal variation in the relative abundance of the pollinator of *F. altissima* and an undescribed congener that utilizes the same host plant, but fails to pollinate it (Peng et al., 2010).

Fig wasps are clearly highly vulnerable to dehydration when in transit between natal and receptive figs, but once they have found and entered receptive figs they are in a more benign environment. Humidities inside healthy figs will be consistently high, and larger figs also lose heat through transpiration, allowing foundress figs wasps to survive in figs where high ambient temperatures would otherwise quickly cause mortalities (Patiño et al., 1994). However, the survivorship of foundresses within figs at a wide range of temperatures does not necessarily mean that the number of flowers they pollinate, or the number of eggs that they manage to lay, are independent of temperature. Under warmer summer conditions, the pollinators of *F. racemosa* lay fewer eggs, but generate the same number of seeds as in the winter (Wang et al., 2005). Wang et al. (2009) argued that this results in contrasting summer and winter temperatures stabilising the mutualism between fig trees and fig wasps, because it results in seasonal variation in the balance between seed and pollinator offspring production. They related the performance of the foundresses to temperature and humidity by removing the females shortly after they had entered receptive figs and monitoring survivorship under varying conditions. As with experiments utilising females that had recently emerged from their natal galls, longevity was found to decline with temperature, suggesting a likely cause for the observed seasonal effects.

Objectives

Here we describe experiments that more directly relate temperature to the longevity, behavior and reproductive success of foundresses within figs maintained at different temperatures. Utilizing a small dioecious fig tree maintained under controlled conditions within three

Table 1. Temperatures experienced by foundress fig wasps during the 48 h after entry into figs.

Temperatures (°C)	Mean + SD	Maximum
Low	17.0 + 0.58	18.1
Medium	21.4 + 2.20	27.4
High	26.7 + 4.79	38.1

different temperature ranges, we addressed the following questions: Do ambient temperatures influence the behavior and survivorship of adult females after they have entered receptive male figs to oviposit? Do the numbers of their offspring vary according to temperatures at the time when eggs were laid? And are the numbers of seeds in female figs similarly modified by temperature at the time that pollinators are active inside receptive figs?

METHODS

Natural history

Ficusmontana (subgenus Sycidium) is a small dioecious shrub growing up to 2 m tall, with a tropical distribution from Thailand and the Malay Peninsula southwards to Sumatra, Java and Borneo (Berg and Corner, 2005). It grows in rainforest understory, in clearings, and in riparian situations (Tarachai et al., 2012) and is pollinated by *Kradibia* (=*Liporrhopalum*) *tentacularis* Grandi, a daytime-flying fig wasp. The two species had been maintained at the experimental gardens of The University of Leeds, UK since 1995 (Moore, 2001). They originated from the Centre for International Forestry Research (CIFOR) Plantation, Bogor, West Java, Indonesia, and Rakata, Krakatau Islands, Indonesia.

The figs of *F. montana* are produced asynchronously by individual plants of both sexes throughout the year, resulting in figs at different developmental stages often being present at the same time (Suleman et al., 2011). Mature figs on male plants contain pollen and the female pollinator offspring that will transport it, whereas mature figs on female plants produce only seeds. *K. tentacularis* females actively pollinate the flowers in female figs, even though they cannot lay eggs there (Raja et al., 2008). The female flowers in its female figs have much longer styles and feathery stigmas that help prevent oviposition, but the mechanism preventing seed set in male figs is unclear (Raja et al., 2008). Figs at the receptive (B) stage (Galil and Eisikowitch, 1968) release volatile blends that are attractive to *K. tentacularis* females. They squeeze through the ostiolar bracts, losing their wings and part of their antennae in the process (Suleman, 2007). Once inside a male fig, they deposit a single egg in each galled ovule (Ghana et al., 2012). Larvae that develop in figs that have not been pollinated are less likely to survive (Tarachai et al., 2008). *K. tentacularis* foundresses often re-emerge from the receptive figs they enter, and walk to receptive figs nearby (Suleman et al., 2013). Offspring sex ratios in *K. tentacularis* are female-biased, but the extent of this bias depends on clutch size, because females lay mainly male eggs initially, then mainly female eggs (Raja et al., 2008a).

Glasshouse conditions

We used *F. montana* growing in three heated glasshouses with contrasting mean and maximum temperatures to provide low, medium and high temperature treatments. Temperatures were monitored during the periods when foundress fig wasps were ovipositing using ‘tiny tag’ data loggers (Meaco measurement and control, Newcastle under Lyme, England) situated within the plants, recording at 30 min intervals. Outside of these experimental periods, the plants and wasps were maintained under medium temperature conditions.

Foundress behavior and survival

Pre-receptive (A phase) figs on male plants were placed in cotton bags to exclude pollinator females. When the figs were receptive (B phase), single foundresses were placed at their ostioles using a fine paint brush. After the foundresses had entered, the bags were replaced to prevent entry by additional pollinators. The foundresses had emerged the same morning from mature (D phase) figs collected from other male plants that had been placed in netting-covered pots, with the wasps allowed to emerge naturally. The experimental figs were opened 3, 6, 12 or 24 h after foundress entry and we recorded how many of the fig wasps were dead (or moribund), alive and active, or had exited the figs. For each treatment, generally one experimental fig per plant were bagged, but this was not always possible.

Reproductive success

Figs on male and female plants were bagged and entered by single foundresses, as before. The bags were then returned around the figs to prevent entry of further pollinators, and subsequently to prevent oviposition by non-pollinating fig wasps, that lay their eggs from outside the figs. After 48 h (when all foundresses had died), the plants from the three temperature regimes were all housed together in the medium temperature greenhouse, where they remained for several weeks until the figs had matured. The contents of the figs (total female and male flowers, seeds, failed empty galled ovules (‘bladders’) and pollinator offspring were then recorded.

Analysis

Numbers of galls, empty galls and unpollinated flowers in male figs, and seed set in female figs, were expressed as proportions of the female flowers present in the figs. Temperature effects were analysed by mixed-effects logistic regression, with temperature included as a continuous explanatory variable. To account for the non-independence of data from flowers in the same figs and figs on the same plant, plant and fig were included as random effects. Three male figs which produced no offspring (1 in each temperature group) were excluded from the analysis. Analysis was carried out in Stata 11.2 (StataCorp, College Station, Texas).

RESULTS

Glasshouse conditions

Maximum temperatures during the periods when foundress behavior and survival were monitored inside the figs were 22.7, 27.9 and 34.5°C in the low, medium and high temperature regimes respectively. Temperatures during the 48 h after pollinators were introduced into figs and offspring plus seed production were monitored are summarized in Table 1.

Table 2. The condition and behaviour of single foundress fig wasps introduced into receptive figs maintained at three different temperatures.

Temperature	Condition	Time (Hours after entry)			
		3	6	12	24
	Active	5	4	0	0
Low	Moribund	1	3	0	1
	Re-emerged	2	1	8	7
	Active	7	6	0	0
Medium	Moribund	1	0	0	0
	Re-emerged	0	2	8	8
	Active	7	3	0	0
High	Moribund	0	1	1	1
	Re-emerged	1	4	7	7

Table 3. The contents of mature male figs that had earlier been maintained at three different temperatures for 48 h after entry by a single foundress fig wasp into the figs when they were receptive.

Temperature	N Plants	N figs	Contents (Mean ± SD)			
			Total female flowers	Proportion successful galls	Proportion empty galls	Proportion unpollinated flowers
Low	6	24	97.1± 25.7	0.42± 0.25	0.37± 0.21	0.21± 0.14
Medium	4	29	108.4± 25.9	0.55± 0.20	0.30± 0.15	0.16± 0.14
High	4	25	114.8± 28.0	0.58± 0.21	0.23± 0.19	0.19± 0.18

Foundress behavior and survival

No active foundress females remained in the figs 12 h after single individuals had been introduced, irrespective of temperature (Table 2). Some females died within the figs, but the majority re-emerged to seek out oviposition opportunities in other figs. The overall proportion of wasps that were dead or moribund varied from 1 of 32 wasps at medium temperatures to 5 of 32 wasps at low temperatures, but there was no significant variation across temperatures (Fisher's exact test, p=0.29).

Reproductive success

The proportion of female flowers in male *F. montana* figs that *K. tentacularis* females managed to exploit successfully varied between about 40 and 60% in different temperature treatments (Table 3), but there was considerable variation between individual figs within treatments and no significant relationship between the proportion of galls (galls/total flowers) and temperature treatment (Wald χ^2 = 1.77, df = 1, p = 0.18). Empty galls in *F. montana* figs are likely to be ovules where pollinators laid eggs, but their larvae failed to develop successfully (Ghana et al., 2012). The proportion of unsuccessful galls (empty galls/total flowers) decreased as temperature increased, but not significantly (Wald χ^2=3.44, df=1, p=0.064). In female figs, where *K. tentacularis* cannot lay eggs (Table 4), there was no significant relationship between the proportion of seeds (seeds/total flowers) and temperature treatment (Wald χ^2 =0.13, df=1, p=0.72).

DISCUSSION

Climate warming is expected to have deleterious effects on many plants and animals, including fig trees and the insects that pollinate them and Jevanandam et al. (2013) have shown that a 3°C temperature increase could have a significant impact on the survivorship of female fig wasps when they are dispersing between trees. Whereas female fig wasps are clearly highly susceptible to elevated temperatures after they have emerged from natal figs, or when they are removed from figs where they were ovipositing (Wang et al., 2009), we found that *L. tentacularis* females are more resilient while inside receptive figs, during the period when they are laying their eggs and pollinating the flowers of *F. montana*. A ten degree temperature range failed to elicit significant changes in foundress death rates or behavior, nor did it alter the numbers of offspring they produced or the numbers of seeds that they pollinated. There was however a small, but not significant increase in the

Table 4. The contents of mature female figs that had earlier been maintained at three different temperatures for 48 h after entry by a single foundress fig wasp into the figs when they were receptive.

Temperature	N plants	N figs	Contents (Mean ± SD)		
			Total flowers	Proportion seeds	Proportion unpollinated
Low	3	30	113.7 ± 22.8	0.71± 0.14	0.29± 0.14
Medium	3	25	107.8± 29.2	0.71± 0.19	0.29± 0.19
High	3	27	93.8 ± 23.0	0.67± 0.20	0.33± 0.20

numbers of failed galls recorded at higher temperatures. Failed galls can be a major source of pollinator offspring mortalities inside *F. Montana* figs (Tarachai et al., 2008; Ghana et al., 2012). The reasons for gall failure are unclear, but may reflect inadequate nutrition for larval development.

Our results narrow down the situations during the life cycle of fig wasps when heat stress is likely to be significant, but do not reduce the likely significance of increasing temperatures for the mutualism. Our experimental temperature range reflected temperatures that *F. montana* figs are likely to experience, but not extremes of temperature (Fetcher et al., 1985). Even if brief, extreme events when the temperatures inside figs rise above 30°C are highly likely to be detrimental to the fig wasps (Patino et al., 1994). Some fig wasp species are more likely to be subject to heat stress than others, because habitat and the location of figs on plants will influence the temperatures to which female fig wasps will be exposed once they enter the figs. Understory plants, for example, experience less diurnal variation in temperatures than in the rainforest canopy, or plants growing in gaps or forest edges (Bazzaz and Pickett, 1980; Fetcher et al., 1985), and figs located on tree trunks may be less exposed that those located among the leaves.

Longer term, persistence of fig tree and fig wasp populations is linked to habitat fragmentation (Mawdsley et al., 1998) and the dispersal abilities of the pollinators (Bronstein et al., 1990). Host-finding among fig wasps is surprisingly effective, and some fig wasps can disperse more than 100 km between natal trees and receptive figs (Ahmed et al., 2009), but the longer that females are in the air, the more susceptible they will be to dehydration and the effects of increased ambient temperatures. Some species of fig wasps disperse during the day, others at night, with fig wasps that disperse at night more common in tropical than sub-tropical/temperate latitudes. Any increases in temperature are more likely to have a negative impact on day-flying species, and night-fliers may even benefit, if low temperatures limit when they can fly.

Conflicts of Interest

The authors have no potential conflicts of interest to declare. SGC holds joint appointments at the University of Leeds, UK and Rhodes University, Grahamstown, South Africa.

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Studies on garlic production in Egypt using conventional and organic agricultural conditions

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Balady and Chinese-Sids40 are the main garlic cultivars grown in Egypt. Effect of conventional *vs.* organic agricultural practices on premature and mature garlic bulb yield was studied for these cultivars. Organic agriculture increased yield of garlic bulbs and was more effective in Balady than in Chinese-Sids40 cultivar. Effect of cultivar type, maturity stage and agricultural methods on allicin content in garlic bulbs was studied using high performance liquid chromatography. Cultivar type and agricultural methods showed more difference in allicin content in cured mature bulb stage than in fresh premature bulb stage. In case of cured mature bulbs, Chinese-Sids40 had higher allicin content (3.02 mg/g FW) than the Balady (2.59 mg/g FW). Effect of storage temperature on allicin content in conventionally- and organically-grown garlic bulbs was studied. Allicin content was higher in organically-grown than conventionally-grown garlic stored at room temperature after six months. At 0°C, allicin content was higher in conventionally-grown than organically-grown garlic. The decrease in allicin content can be explained by the prolonged dormancy period and hindered sprouting that suppressed the metabolic activity.

Key words: Garlic; *Allium sativum*, Balady, Chinese-Sids40, organic agriculture, allicin, storage.

INTRODUCTION

Little research has been performed to clarify how different agricultural production methods can affect human health though consumption of food (Woese et al., 1997). Conventional agricultural, using agrochemicals, allowed the world to increase the food production to satisfy the growing human needs. However, extensive abuse of the agrochemicals increased the costs of agricultural production and lead to successive environmental problems causing agricultural un-sustainability. Therefore, there is an increasing interest in organic agriculture using natural fertilizers and biological pest control. During the ten years; 1994-2003, global organic production has increased 20% annually. Currently, the largest global organic producers are Australia, European Union and United States, while the major importers of organic agricultural products are the United States, European Union and Japan (Worthington, 1998). Intake of organic food has some advantages such as intake of higher content of phenolic compounds and some vitamins, and a lower content of nitrates and pesticides (Lima and Vianello, 2011). Garlic (*Allium sativum* L. (Alliaceae)) is an important crop in Egypt used

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medicinally for treatment of memory loss, hypertension and microbial infection (AbouZid and Mohamed, 2011). Garlic yield and quality vary greatly with cultivar, location, soil type, agricultural methods and harvest date. The term “biological elasticity” describes garlic’s ability to be adapted to these factors over time. Domestic ecotypes, that are fully adapted to local conditions, are recommended for their usage as initial breeding material (Baghalian et al., 2006). Garlic can be grown successfully on a wide range of soil types. Generally, it grows best on fertile soil. The amount of fertilizers needed vary with the soil type, the amount of organic matter present, the previous crop grown and the climatic conditions during the growing season.

Garlic is sold in markets in two forms; premature garlic (fresh bulbs) and mature garlic (cured bulbs). Mature garlic is harvested when the aerial parts start to wither. It is then subjected to the curing process which means hanging the garlic plant, while the stem and roots are still on, till the outer skins become dry and crispy (Kamenetsky, 2007). The curing helps the neck cells constrict and minimize weight loss through dehydration. Premature garlic bulbs are harvested about one month before mature bulbs harvesting, to ensure the maximum size of bulbs.

This study aimed to investigate the best agronomic conditions to be adopted during garlic production in Egypt. Allicin content was determined in garlic cloves produced using different cultivars, agricultural methods and maturity stages. The effect of storage period and temperature on allicin content in cured mature garlic cloves was also investigated.

MATERIALS AND METHODS

Plant material

Two garlic varieties were used in this study; *Allium sativum* variety sativum (Balady) and variety ophioscordon (Chinese-Sids40). These varieties were identified by Prof. Mamdouh Abdallah, Horticultural Department, Faculty of Agriculture, Ain Shams University, Egypt. Samples were harvested, cured for 21 days and stored in a well-ventilated dry air room away from direct sunlight till extracted.

Effect of agricultural practice

Two field experiments were conducted for two successive seasons 2004/2005 and 2005/2006 at Sids Experimental Research Station, Agricultural Research Centre, Beni-Suef, Egypt. In early September of each season, the field was cleaned, ploughed, leveled and divided into plots (10.5 m^2) that contained 5 rows (3.5 m length, 0.6 m width and 70 plants each). Each experiment included four treatments; the two garlic cultivars (Balady and Chinese-Sids40) and the two agricultural practices (conventional and organic). The treatments were arranged in a completely randomized block design with three replicates. Conventional agricultural plots were fertilized with phosphorus, nitrogen and potassium fertilizers. Phosphorus fertilizer was applied using calcium super phosphate (15.5% P_2O_5) at a rate of 75 kg/feddan (1 feddan = 4200 m²) during soil preparation. Nitrogen fertilizer as applied using ammonium nitrate (33.5% N) at a rate of 100 kg nitrogen/feddan, equally divided after 30 and 60 days from planting. Potassium fertilizer was applied using potassium sulfate (48% K_2O) at a rate of 72 kg K_2O/feddan equally divided after 30 and 60 days from planting. Organic agricultural plots were fertilized as follows: Phosphorus fertilizer was applied using 250 kg rock phosphate/feddan, nitrogen fertilizer was applied using 70 kg nitrogen/feddan from chicken and farm yard manure (1:3 w/w), potassium fertilizer was applied using 250 kg rock potassium/feddan, and commercial bio-fertilizer was applied using rhizocatertin, phosphorin and potassin at a rate of 3 kg/feddan from each inoculants immediately before planting and one month later. Garlic planting was carried out during the last week of September in both seasons of study. Uniformed cloves were hand-planted on both sides of the ridges at 10 cm apart.

Bulb yield determination

Premature garlic harvesting was done 150 days after planting. At fresh premature garlic harvest date, all plants in the second row of each plot were harvested and cleaned; root and shoot were removed. Fresh bulbs were immediately weighed and converted to record as total yield (kg/plot). Mature garlic harvesting was done one month later, about 180 days after planting, when the aerial parts start to senesce. Marketable yield in the third and fourth rows of each plot were harvested and cured for 3 weeks. The dry root and aerial parts were turned and removed. The cured mature bulbs were weighed in kg and converted to record as total dry yield (kg/plot).

Determination of allicin content

Freshly peeled garlic cloves (2 g) were chopped and blended for one min in 20 ml water. The resulting homogenate was allowed to stand for 5 min at room temperature to ensure maximum production of thiosulfinates. The homogenate was filtered and extracted with chloroform. The chloroform extract was separated, dried over anhydrous sodium sulfate and evaporated at 40°C. The residue was re-dissolved in 3 ml aqueous methanol (50%), filtered through 0.2 µm filter and directly injected into HPLC. The HPLC system consisted of reversed phase (C18), 250 × 2.6 mm, 5 µm particle size column. The mobile phase consisted of methanol:water:formic acid (40:60:0.1) at a flow rate of 1.2 ml/min. The eluate was monitored at a wavelength of 254 nm. Allicin was isolated from garlic extract using preparative silica gel thin layer chromatography using toluene:ethyl acetate (100:30) as developing solvent then identified by spectroscopic data. The resulting spectra were compared with reported data (Cruz-Villalon, 2001; Lawson et al., 1991; Jansen et al., 1987). Pure compound was used to prepare calibration curve.

Effect of storage on allicin content

Random samples, 4 kg of mature bulbs from each plot of Chinese-Sids40 were taken after curing and divided into two samples; one stored at room temperature and the other at 0°C. Allicin content in conventionally-grown and organically-grown cured mature garlic bulbs of Chinese-Sids40 was determined periodically during six months.

Statistical analysis

Bulb yield data were statistically analyzed as a complete randomized block design. A regular analysis of variance was

Table 1. Effects of cultivars and agricultural method on bulb yield and estimated yield/feddan of fresh premature garlic bulb.

Treatment		Premature bulb fresh yield (kg/plot)			Premature estimated yield (ton/feddan)		
		1st season	2nd season	Average	1st season	2nd season	Average
Cultivar	Balady	45.16	43.57	44.36	18.66	17.51	17.79
	Chinese-Sids40	48.68	47.47	48.08	19.47	18.99	19.23
Agricultural method	Organic	49.61	48.61	49.11	19.84	19.45	19.64
	Conventional	44.23	42.43	43.33	17.69	17.06	17.38
	Balady-organic	48.56	47.07	47.81	19.43	18.83	19.13
Cultivar × Agricultural Method	Balady- conventional	41.76	40.07	40.91	16.70	16.20	17.45
	Chinese-organic	50.65	50.15	50.40	20.26	20.06	20.16
	Chinese- conventional	46.71	44.80	45.75	18.68	17.92	18.30

Table 2. Effects of cultivar and agricultural practice on bulb dry weight and estimated yield of cured mature garlic bulbs.

Treatment		Bulb dry weight (g)			Cured mature estimated yield (ton/feddan)		
		1st season	2nd season	Average	1st season	2nd season	Average
Cultivar	Balady	47.42	52.24	49.29	6.51	6.98	6.75
	Chinese-Sids40	49.07	47.15	48.11	6.56	6.09	6.33
Agricultural method	Organic	51.44	52.90	52.17	6.93	7.63	6.98
	Conventional	45.05	46.48	45.77	6.16	6.04	6.10
	Balady-organic	50.86	55.19	53.03	7.05	7.58	7.32
Cultivar × agricultural method	Balady- conventional	43.98	49.28	46.63	5.98	6.38	6.18
	Chinese-organic	52.02	50.61	51.32	6.80	6.48	6.64
	Chinese- conventional	46.11	43.68	44.90	6.33	5.70	6.02

carried according to Snedecor and Cochran (1980). Statistical significance was calculated according to multiple range test of Duncan. Means were compared using the least significant difference test (L.S.D.) at 0.05 level of significance.

RESULTS AND DISCUSSION

Effect of conventional *vs.* organic agricultural practices on bulb yield for the premature and mature bulbs in Chinese-Sids40 is presented in Tables 1 and 2. Organic agriculture resulted in increasing yield of garlic bulbs. Total fresh bulb yield/plot increased 13.3% in organic over conventional agriculture. This result is in agreement with that reported by Abdallah (2000). Table 1 show that organic agriculture was generally more effective in Balady than in Chinese-Sids40. Balady fresh bulb yield/plot increased 16.9% using organic agriculture over conventional agriculture, compared to 10.2% increase for Chinese-Sids40 as average for the two seasons.

Garlic plant prefers very old manure over chemical fertilizers. This may be explained by the poor and inefficient root system of garlic. Our results are contraindicated with that reported by Dahama (1999) who showed that organic farms give lower yields (1-15%) than conventional farms under sandy soil conditions. The organic concept is: feed the soil, the soil will feed the plant and healthy soil will give healthy plant. Therefore, organic treatment in clay soil conditions, like in Nile valley, provides healthier soil than reclaimed sandy soil. Garlic is characterized by its high content of organosulfur compounds (Lawson, 1996). These compounds are known to have pharmacological activity at doses representing typical levels of garlic consumption. These compounds are categorized under two main groups: γ–glutamyl-*S*-alk(en)yl cysteines and *S*-alk(en)yl cysteine sulfoxides (Lawson, 1998).

During sprouting, γ–glutamyl-*S*-alk(en)yl cysteines are converted by γ–glutamyl transpeptidase enzyme to *S*-alk(en)yl cysteines which in turn rapidly converted by oxidase enzyme to *S*-alk(en)yl cysteine sulfoxides. Upon crushing of mature garlic cloves, the cysteine sulfoxides are converted to thiosulfinates (e.g. allicin) by the action of alliinase enzyme (Block et al., 1992). Thiosulfinates are very unstable, self-reacting compounds that give rise to further rearrangements, depending on the temperature, pH and solvent conditions. In order to preserve allicin for routine use as an external standard, it was proposed that the purified compound could be

Table 3. Effects of cultivar (C), maturity stage (M) and agricultural method (A) on allicin content in garlic bulbs.

Maturity stage	Agricultural method	Premature bulb fresh yield		
		Sids40	Balady	Mean
Premature	Organic	1.21	0.99	1.10
	Conventional	1.31	0.96	1.14
	Mean	1.26	0.98	1.12
Mature	Organic	2.47	2.16	2.31
	Conventional	3.57	3.01	3.29
	Mean	3.02	2.59	2.80
	Organic*	1.84	1.58	1.71
	Conventional**	2.44	1.99	2.21
	Mean	2.14	1.78	

* Average data of premature and mature stages produced under organic agriculture. ** Average data of premature and mature stages produced under conventional agriculture. LSD (0.05): C: 0.024, C x M: 0.034; M: 0.024, C x A: 0.034; A: 0.024, A x M: 0.034; C x M x A: 0.047.

adsorbed on silica gel, and that the adsorbate was stable for 3 months at -24°C (Jansen et al., 1987). However, Jaeger and Koch (1992) reported that silicate adsorbents did not protect the sensitive allicin against its spontaneous decomposition and those preparations, obtained by this way, related to only traces of allicin. The presence of solvents that make hydrogen bonds with the oxygen atom of the thiosulfinate greatly improves their stability, so thiosulfinates are more stable in polar solvents. This explains the high stability of allicin in water compared to hexane, although it is slightly soluble in water (1-2%), moderately soluble in hexane and highly soluble in organic solvents more polar than hexane (Lawson, 1993).

Effect of cultivar type, maturity stage and agricultural methods on allicin content in garlic bulbs was studied using HPLC. Data are shown in Table 3. Significant difference in allicin content was found among the two cultivars and maturity stages studied and agricultural production methods tested. Cultivar type and agricultural methods showed more difference in allicin content in case of cured mature bulb stage than in fresh premature bulb stage. In case of cured mature bulbs, Chinese-Sids40 has higher allicin content (3.02 mg/g FW) than the Balady (2.59 mg/g FW).

Garlic cultivars grown worldwide can be classified within two basic subspecies; *A. sativum* L. var. *ophioscordon* (hardneck or topset garlic) and *A. sativum* L. var. *sativum* (softneck or artichoke garlic). Hardneck varieties are characterized by large clove size, small number of cloves per bulb and a tall solid flowering scape protruding through the center of the bulb with bulbils at its top. A small proportion of the scape remains with the marketed bulb. Softneck varieties are characterized by small clove size, large number of cloves per bulb and absence of a scape. Lee and Harnely (2005) showed that hardneck garlic (similar to Chinese-Sids40) contains higher alliin content than softneck garlic (similar to Balady). Alliin is responsible for allicin formation once the garlic cloves are crushed. Thiosulfinates content was determined in 34 different garlic varieties, belonging to the major subspecies; softneck and hardneck garlic grown on the same land (Lawson, 1993). No significant difference was found in the levels of allicin or other thiosulfinates between the two subspecies. Only a 1.6 fold range of variation was found among the 34 varieties. However, Camargo et al. (2005) showed that the allicin content can vary significantly between cultivars, keeping constant the variability attributed to the climate conditions and dormancy state of the cloves. Baghalian et al. (2000) reported that there is no significant variation attributed to climatic factors suggesting that there should be an inherent advantage in Iranian-grown garlic. Hardneck garlic was found to have greater methiin, alliin and total free amino acids content compared to softneck garlic, upon comparing the two subspecies for their cysteine sulfoxides and free amino acid content (Lee and Harnly, 2005).

Effect of storage conditions on allicin content in garlic bulbs of Chinese-Sids40 was studied. Results are shown in Table 4. During the dormancy period, the first three months of storage period, allicin content decreased in garlic bulbs stored at room temperature and 0°C. During the next three months, sprouting-initiation period, allicin content started to increase. This may be explained by the suppressed metabolic activity during the dormancy period followed by its promotion during sprouting. Allicin content was higher in organically than conventionally-grown garlic stored at room temperature. On the other hand, allicin content was higher in conventionally than organically-grown garlic stored at 0°C. It is known that the dormancy period is prolonged at low temperature and sprouting is initiated once garlic is taken out of this temperature (Brewester and Radinowitch, 1990). Organically-grown

Table 4. Effects of storage (P), temperature (T) and agricultural method (A) on allicin content in mature cured garlic bulbs.

Storage temperature	Agricultural method	Storage period (month)				Mean
		0	2	4	6	
0°C	Organic	2.47	1.30	2.67	2.75	2.30
	Conventional	3.57	2.04	3.47	3.74	3.21
	Mean	3.02	1.67	3.07	3.25	2.75
Room temperature	Organic	2.47	2.06	3.16	3.68	2.84
	Conventional	3.57	1.58	2.21	1.95	2.33
	Mean	3.02	1.82	2.69	2.81	2.59
	Organic*	2.47	1.68	2.92	3.22	2.57
	Conventional**	3.57	1.81	2.84	2.85	2.77
	Mean	3.02	1.74	2.88	3.03	

*Average data of premature and mature stages produced under organic agriculture. **Average data of premature and mature stages produced under conventional agriculture. LSD (0.05): P: 0.038, P x T: 0.054; T: 0.027, P x A: 0.054; A: 0.027, T x A: 0.038; P x T x A: 0.076.

garlic seems to be unable to easily break the prolonged dormancy period, therefore sprouting is somewhat hindered during the first six months of storage at low temperature.

In conclusion, organic agriculture is superior to conventional agriculture in bulb yield and allicin content for fresh premature garlic bulb. Chinese-Sids40 is superior to Balady in bulb yield and allicin content. Organic agriculture improved bulb yield in Balady more than it does in case of Chinese-Sids40. Conventional agriculture showed higher allicin content that organic agriculture in cured mature garlic bulb. Organic agriculture, contrary to conventional agriculture, has shown higher allicin content in garlic bulbs stored at room temperature than that stored at 0°C.

Conflicts of Interest

The authors declare no conflicts of interest.

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Foliar tissue, grain yield and economic return by surface application of gypsum and different number of soybean plants in precision seed drill

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The gypsum has elevated calcium and sulfur in the soil, and there are reports of increased grain yield of soybean with reduced number of plants, being necessary in conjunction with economic return on investment. This study investigated the effect of application superficial of gypsum and different numbers of plants in precision seed drill in the nutritional aspect of the leaf tissue, grain yield of soybean cultivated in clayey Rhodic Hapludox and economic return on investment. Used in the experiment was a randomized block design in scheme tracks (4 × 4), with four doses of gypsum (0, 800, 1600 and 2400 kg ha^{-1}) applied at the V4 stage of soybean Vmax RR (SYN 7059RR) and four variations in the number of plants (12, 14, 16 and 18 plants per meter), with four replications. Samples of leaf tissue were collected in the flowering stage of soybean in the 2011/2012 crop, and then determined the levels of Ca, Mg, K, P, S, Cu, Zn, Mn and Fe. At maturity the yield assessment was performed. The number of plants per meter with precision seed drill and superficial application of gypsum at the V4 stage do not affect the content of macro-and micronutrients leaf tissue of soybean cultivar SYN 7059RR. The application of gypsum at the V4 stage and reducing the number of plants per meter statistically do not affect the grain yield. Recommend lower number of plant (12 plants m^{-1}) on soybean cultivar for SYN 7059RR. The economic returns using higher amount of seed (14, 16 and 18 plants m^{-1}) is -283, -260 and -271% with investment of US$ 13.76, 29.24 and 44.72. Use of gypsum focused costs to only two cultures (soybeans and wheat) during the crop season provides residual soil of 104.43 kg ha^{-1} of S. Application 800 kg ha^{-1} gypsum provides US$ 14.56 profit with 44% economic return to payment half investment (US$ 33.32).

Key words: No-till, fertilization, plant population, seeding rate, investment return.

INTRODUCTION

The no-tillage system became indispensable practice in Brazil, reducing the impacts of intensive agricultural activity on the environment and increasing the competitiveness of the agricultural commodities in the international market by grains demands, increasing grain yield and reducing need to use pastures and forests. No-till system requires proper management to maintain soil fertility and provide adequate nutrition to plants (Caires et al., 2011) and adequate number of plants (Tourino et al., 2002; Mauad et al., 2010) to increase grain yield.

One factor that may limit soybean grain yield is soil acidity in surface and subsurface (Gelain et al., 2011). The limestone application is effective in controlling surface acidity, but shows little mobility in the ground, so it has less action in the subsurface layers. Alternatively can be use the gypsum that although minimally alter the pH, it is efficient to reduce the exchangeable Al^{3+} toxicity to plants, reducing the activity of this element in the soil solution, especially in subsurface layers, in addition to providing nutrients for plants by sulfur (S) and calcium (Ca) (Neis et al., 2010; Elrashidi et al., 2010).

The gypsum can increase crop yields due to increased Ca and sulfate (SO_4^{-2}) available to plants (Caires et al., 2002, 2004). Caires et al. (2003) studied the application of limestone and gypsum on the surface and embedded reported that gypsum improved the environment for root growth in the subsoil, but did not cause improvement in the production of soybeans in long time by no-till. Different crop responses to gypsum have been observed in several field studies gypsum application increased corn production (Farina et al., 2000; Caires et al., 2004) but did not increase statistically grain yield of soybean (Oliveira and Pavan, 1996; Caires et al., 2003). However, in Brazilian cerrado (low pH surface and subsurface) in in Red Latosol the application gypsum has promoted increased yield of soybeans in low time by no-till (Broch et al., 2011), by providing S to plants, which plays a fundamental role as a component of some amino acids found in high content in soybean (Novais et al., 2007), intensifying the demand of S in legumes to accumulate protein (Brochi et al., 2011). In fact, Sávio et al. (2011), Motta et al. (2013) and Pauletti et al. (2014) identify responses with lower doses of gypsum in soybean (800 at 1500 kg ha^{-1}).

Moreover, among the cultural practices used to obtain higher production of plant species has to choose the best arrangement of plants is important per favoring weed control and increases the efficiency for the utilization of environmental resources such as light, water and nutrients (Albuquerque et al., 2012), reducing pressure by the increase in crop area expansion. Thus, cultural management or precision seeding aims to obtain optimum plant population and optimal spatial distribution of plants between and within-row, maximizing crop performance at no additional cost (Coelho et al., 2002).

Crops with elevated plant population increase cost with seeds, can lead to lodging of plants rather than providing increased yield. Low populations favor the development of weeds per increasing distance between plants and can result in lower yield (Vasquez et al., 2008). So much so that studies with population of soybean plants have shown no effect on grain yield, which is associated with phenotypic plasticity of the crop. The plants compensate for the reduction in the number of plants, by increasing the individual legumes production, contributing to increased tolerance of this variation (Mauad et al., 2010).

Density of plants effects highlight the importance of uniformity of plants from increase number of plants to achieve greater yield potential. Gypsum efficiency in correcting soil acidity with problems in subsuperficial layers and increase nutrients. Therefore, need for economic evaluation to use new and different technologies together in the crop system. One of fundamental importance to assess the economic level of the input in order to avoid over or under dosing consequently ensure economic return. Thus, it is appropriate to identify the return on investment for each technology used and highlight the point of greatest return on investment.

In addition, use of technologies such as gypsum fertilizer and management of soybean identify adequate number of plants per meter can interfere grain yield, being necessary to know the economic return on investment with these interferences in the crop system. In this sense, the objective of this study was to evaluate the effect of surface application doses of gypsum and different numbers of plants in precision seed drill in nutritional aspect of leaf tissue, grain yield of soybean in clayey Rhodic Hapludox and economic return on investment.

MATERIALS AND METHODS

Description of study

The study was conducted in Guaíra, western Paraná with the following coordinates 24° 21 'S and 54° 12' W, with an altitude of 266 m. The area is grown in the crop sequence and tillage system 25 years ago, in the summer using soybean and wheat in the winter. The soil was classified of clayey Rhodic Hapludox (Eutroferric Red Latosol in the Brazilian classification) (Embrapa, 2013a), and the particle size and chemical soil characteristics are presented in Table 1. This soil develops high Ca/Mg (4.9) and mid-level S to soil with clay >400 g kg^{-1} in 0 to 0.20 m depth, being recommended application of S to increase S levels and for maintenance fertilizer (Embrapa, 2013b).

According to Koppen's classification, the climate of the region is of type Cfa, subtropical with rains well distributed throughout the year and hot summers (Caviglione et al., 2000). The rainfall recorded during the conduct of the experiment, between October 2011 and February 2012 was 997 mm (Figure 1), yet the greater volume of rainfall concentrated in the month of November and the

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Table 1. Granulometric and chemical attributes of clayey Rhodic Hapludox collected in layer of 0 - 0.2 m deep. Guaíra, Paraná, Brazil, 2011.

pH(1)	Ca	P (4)	Ca^{+2}(2)	Mg^{+2}(2)	K^{+}(4)	Al^{+3}(2)	H+Al(3)	SB	CTC
	g dm^{-3}	mg dm^{-3}	------------------ $cmol_c\ dm^{-3}$ ------------------						
5.40	19.09	9.70	7.85	1.60	0.71	0.00	4.28	10.16	14.44

Cu (4)	Zn (4)	Fe (4)	Mn (4)	S (5)	V	Clay (6)	Silt (6)	Sand (6)
------------------ mg dm^{-3} ------------------					%	------------------ g kg^{-1} ------------------		
12.30	4.50	28.00	274	10.00	70.36	660	200	140

(1)pH in $CaCl_2$, the ratio 1:2.5, (2)Extractor KCl 1 mol L^{-1}, (3)Extractor calcium acetate 0.5 mol L^{-1} pH 7.0, (4)puller Mehlich-1, (5)Extractor Ca $(H_2PO_4)_2$ 500 mg L^{-1} of P in HOAc 2 mol L^{-1} (Embrapa, 2009), (6)hydrometer method (Embrapa, 1997).

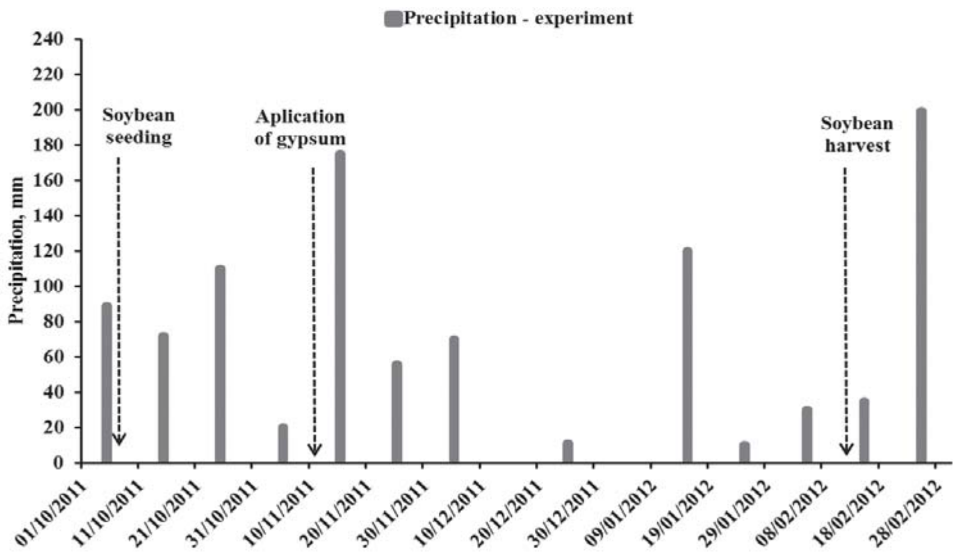

Figure 1. Precipitation (mm) in the experimental area during the period of driving between 10/01/2011 to 03/01/2012 in cumulative 15 days.

end of February, after harvest and lower values in the months of December 2011 and January 2012.

Experimental design, treatments and conduction

The experimental design was randomized blocks, in scheme of tracks (4 × 4), with four repetitions, totaling 64 experimental plots. The first factor was composed of increasing agricultural gypsum doses (0, 800, 1,600 and 2,400 kg ha^{-1} agricultural gypsum with 17% of Ca, 15% $S\text{-}SO_4^{-2}$, 5% S). The second factor was constituted of the variation in the number of plants per meter (12, 14, 16 and 18 plants of soybean per meter). The application of gypsum was conducted to haul in 11 November 2011, during the vegetative stage V4 soybean culture. Gypsum application used low doses to provide S to cultures in crop system (soybean and wheat), thus, first dose limit by uniform distribution by equipment's, that is 800 to 1000 kg ha^{-1} (Raij, 2008).

The cultivation of soybeans was conducted after the wheat harvest in no-tillage system. The sowing of culture was onducted on 7 October 2011, using the transgenic cultivar Vmax RR (SYN 7059RR), 197 g by thousand seed weight (Syngenta, 2015) and 90% germination index recommended for the region, as agroclimatic zoning for the state of Paraná (Mapa, 2011). The plots had five meters in length, and width with six lines of culture, being the spacing between rows of 0.45 m. Thus, each installment had a total area of 13.50 m^2 and floor area of 5.40 m^2, disregarding 0.5 m on each side that make up the length of parcel and a line of culture on each side that make up the width of the parcel.

In the treatment of the seeds was used the fungicide Maxim XL (25 g L^{-1} of fludioxonil and 10 g L^{-1} of metalaxyl-M) at the dosage of 100 ml for 100 kg of soya beans. For the chemical fertilization of sowing was used 250 kg ha^{-1} of the commercial formulation with 20% phosphorus and 20% potassium. Monitoring of pests, diseases and weeds and the need for control was carried out in accordance with the recommendations for the culture of soybean (Embrapa, 2010).

Measurements and field management

In the culture of soybean foliar tissue samples were collected in full bloom, as recommended procedures regarding time and leaves sampled by Malavolta et al. (1997), for the determination of the levels of Ca, Mg, K, P, S, Cu, Zn, Mn and Fe (Embrapa, 2009). At the point of harvest, was conducted the collection of aerial part of plants of the crop of soybeans, thrashing in thresher Winning B-150 for the obtaining of the beans, which were heavy for determination of yield, with subsequent standardization of 14% of the samples for moisture in soybean culture.

Economic return on investment

Investment, grain yield return and economic return on investment calculation was performed through the costs to acquire used amount of seed or gypsum, together with income grain yield related to the treatment of 12 plants per meter or absence gypsum application. Cost of gypsum was divided equally between soybean and wheat used in crop system. Also calculated S residual after two crops (soybean and wheat) in crop system and was considered export of S by cultures.

Equations used for available investment, grain yield return and economic return with different number of plants:

i) Costs seeds (US$ ha^{-1}) = “Use seeds (kg ha^{-1})”(1) * “Costs seeds (US$ kg^{-1})”(2)
(1) considered 90% of emergence and 197 g for mass 1000 grains;
(2) Costs seed in fev 2015.
ii) Costs seeds 12/14/16 (US$ ha^{-1}) = Costs seeds ’14/16/18 plants’ (US$ ha^{-1}) - Costs seeds ’12 plants’ (US$ ha^{-1})
iii) Variation grain soybean 14/16/18 (kg ha^{-1}) = “Grain yield ’14/16/18 plants’ (kg ha^{-1})” - “Grain yield ‘12 plants’ (kg ha^{-1})”
iv) Income soybean (US$ ha^{-1}) = “Variation grain soybean 14/16/18 (kg ha^{-1})” * “Soybean price (US$ kg^{-1})”
v) Profit or Prejudice (US$ ha^{-1}) = "Income soybean (US$ ha^{-1})"- | "Costs seed (US$ ha^{-1})” |;
vi) Investment return = ("Prejudice (US$ ha^{-1})" - | "Costs seed (US$ ha^{-1})” |) / | "Costs seed (US$ ha^{-1})” |
vii) Investment return (%) = ("Prejudice (US$ ha^{-1})" - | "Costs seed (US$ ha^{-1})” |) / | "Costs seed (US$ ha^{-1})” | * 100.

Equations used for available investment (costs gypsum), grain return and economic return with doses of gypsum:

i) Costs doses applied (US$ ha^{-1}) = “Doses of gypsum (kg ha^{-1})” * “Costs product applied (US$ kg^{-1})” (1)
ii) Total Costs gypsum (US$ ha^{-1}) = “Costs doses applied (US$ ha^{-1})” * “Costs application (US$ ha^{-1})” (1)
(1) Costs gypsum and application in fev 2015.
iii) Costs gypsum ‘soybean crop’ (US$ ha^{-1}) = “Total Costs gypsum ‘doses’ (US$ ha^{-1})” - “Total Costs gypsum ‘absence gypsum’ (US$ ha^{-1})”
iv) Variation grain soybean ‘doses gypsum’ (kg ha^{-1}) = “Grain yield ‘doses gypsum’ (kg ha^{-1})” - “Grain yield ‘absence gypsum’ (kg ha^{-1})”
v) Income soybean (US$ ha^{-1}) = “Variation grain soybean ‘doses gypsum’ (kg ha^{-1})” * “Soybean price (US$ kg^{-1})”
vi) Profit or Prejudice (US$ ha^{-1}) = " Income soybean (US$ ha^{-1})"- | Costs gypsum ‘soybean crop’ (US$ ha^{-1}) |;
vii) Investment return = ("Profit or Prejudice (US$ ha^{-1})" - | Costs gypsum ‘soybean crop’ (US$ ha^{-1}) |) / | Costs gypsum ‘soybean crop’ (US$ ha^{-1}) |
viii) Investment return (%) = ("Profit or Prejudice (US$ ha^{-1})" - | Costs gypsum ‘soybean crop’ (US$ ha^{-1}) |) | Costs gypsum ‘soybean crop’ (US$ ha^{-1}) |* 100.

Equation used to describe S residual by gypsum in soil after two crops (soybean and wheat) in crop system:

S residual gypsum (kg ha^{-1}) = “Input S (kg ha^{-1})” – “S grain exported soybean (kg ha^{-1})” – “S grain exported second crop - wheat (kg ha^{-1})”

Statistical analysis

The data were subjected to analysis of variance at the 5% level of significance and in the event of a significant effect for the effect of gypsum and number of plants, regression analysis was performed using the SAEG 8.0 Program (Saeg, 1999). After, describing the economic return on investment for the effect of gypsum and number of plants with grain yield and costs.

RESULTS AND DISCUSSION

Leaf tissue

The addition of gypsum to the soil to haul in soybean, specifically in the vegetative stage until the dose of 2400 kg ha^{-1}, as well as different numbers of plants did not influence foliar concentrations of macro and micronutrients in soybean (Table 2 and 3). In order that the average levels found were 6.65, 23.98, 2.30, 13.30 g kg^{-1} and 28.22, 33.08, 72.78 and 171.09 mg kg^{-1} of P, K, S, Ca, Cu, Zn, Mn and Fe, respectively, considered adequate (Embrapa, 2010).

The average content of 2.40 g kg^{-1} of Mg seen in Table 1, face down sufficiency range between 2.5 and 10 g kg^{-1} of Embrapa (2010), may be related to elevated levels of Ca and K in soil (Table 1), mainly caused by the imbalance between cations in the soil, which may adversely affect the development of plants (Marschner, 2012). Prochnow et al (2010) and Salvador et al. (2011) highlighted the importance of the relationship between Ca:Mg:K in Brazilian agriculture, and Oliveira Júnior et al. (2013) guides to evaluate the relationship between these cations together with bands of sufficiency to recommend the application of lime and fertilizer. Also noteworthy, the average leaf P content of 6.87 g kg^{-1}, as to Embrapa (2010), the P content in leaf sufficient in soybeans is in the range from 2.5 to 5.0 g kg^{-1}. Thus, it can be seen that the leaf P content was found high above the sufficiency range. However, in relation to the effect of treatments on leaf P content, Nogueira and Melo (2003) and Quaggio et al. (1998) also found no increase in foliar P concentration with application of gypsum preceding the cultivation of annual crops. For K, Ca and S levels of 23.98, 13.30 and 2.30 g kg^{-1} were found in leaf tissue of soybean, respectively (Table 2), the point was not that we found a significant effect levels foliar Ca and S, which are added to the soil with the application of gypsum. This fact is probably due to the sufficient level of these nutrients in the soil (Raij, 2008), as shown in Table 1. Moreover, Souza et al. (2012) found an increase in the accumulation of nutrients in the soybean shoot with the use of gypsum, but the elevation of levels of compaction reduced the accumulation of nutrients, and have found that the bulk density remained high for the development of roots, even with the other Poacea in prior to the deployment of soybean cultivation.

Generally, use of lower doses to supply S to cultures, tested in this work, becomes more interesting because the Mg leaching, provided by surface layer of gypsum, accumulates in 40 to 80 cm layers of soil (Zambrosi et al., 2007). Thus, lower doses of gypsum allows reuse Mg leaching, because gypsum facilitates root growth in subsurface layers, recycling Mg by these layers

Table 2. F values, coefficient of variation (CV) and phosphorus (P), potassium (K), sulfur (S), calcium (Ca) and magnesium (Mg) in the leaf tissue of soybean, arising from the use of different number of plants per meter with precision plants drill (Number plants) and surface application of gypsum in soybean. Guaíra, Paraná, Brazil, 2012.

Treatments	P		K		S		Ca		Mg	
	----- g kg^{-1} -----									
Number plants m^{-1}										
12	6.80		23.43		2.24		13.07		2.50	
14	6.43		26.10		2.20		13.95		2.39	
16	6.67		21.84		2.41		12.47		2.48	
18	6.69		24.56		2.35		13.72		2.23	
Gypsum										
--- kg ha^{-1} ---										
0	6.87		24.84		2.34		14.85		2.37	
800	6.90		26.29		2.19		12.25		2.39	
1600	6.22		24.04		2.27		12.72		2.22	
2400	6.60		20.77		2.39		13.38		2.61	
	Medium value									
	6.65		23.98		2.30		13.30		2.40	
	F value									
Nº plants	0.21	ns	1.66	ns	1.02	ns	0.62	ns	1.75	ns
Gypsum	1.34	ns	0.62	ns	0.10	ns	0.50	ns	0.49	ns
Nº plants x Gypsum	1.12	ns	0.58	ns	1.67	ns	0.97	ns	1.39	ns
$C.V._{Nº\ plants}$ (%)	14.00		22.56		30.84		22.91		18.65	
$C.V._{Gypsum}$ (%)	19.91		50.70		42.97		47.72		37.14	
$C.V._{Nº\ plants\ x\ Gypsum}$ (%)	13.19		31.72		25.72		15.56		22.53	

ns: not significant at the 5% level of probability by F test.

(Zambrosi et al., 2007).

However, Caires et al. (1998) and Soratto and Crusciol (2008) observed an increase in the sulfur content in the leaves in the use of gypsum before planting. In studies with low fertility soils, interference has been observed in chemical soil with gypsum application, being favorable to the development of soybean plants. Zapparoli et al. (2013) observed a reduction of Al^{+3}, Ca and P increased with gypsum application in Typic aluminic sandy texture, as well as increase in the dry matter of the area.

Thus, it is advisable to carefully evaluate the use of gypsum in soils with high fertility, especially in relation to Ca and S, it becomes unnecessary investment, especially when Ca levels are elevated (Table 1), may interfere with the balance between Ca:Mg:K, impairing the uptake of cations by roots of soybean (Fonseca and Meurer, 1997; Watanabe et al., 2005; Novais et al., 2007). Nava et al. (2012) evaluated a cultivar of apple sensitive to calcium deficiency in soil with high fertility, found that the annual use of gypsum for eight years has magnesium deficiency in plants.

It can also be used to increase Ca/Mg ratio, for low ratio affect the absorption of Ca, with priority Mg absorption, which enhances vegetative growth of plants, however, with lower Ca does not confer increased tolerance of plants to water adversity. Elrashidi et al. (2010) observed that the physiological effects of large amount additions of Ca^{2+} and $S\text{-}SO_4^{2-}$ in the region of nutrient uptake by the roots may reduce crop yields after gypsum application. Caires et al. (2011b) found the following response decreasing order of crops the gypsum application: wheat>maize>soybean, and demand for Ca^{2+} and $S\text{-}SO_4^{2-}$ followed the reverse order: soybean>maize>wheat.

The number of plants did not influence the nutrient content in the leaves of soybean (Table 2 and 3). So that morphological changes in the number of plants for planting soybeans have been common, restricted to the reduction in plant height (Marchiori, 1999), increase in the number of branches (Marchiori, 1999; Heiffig, 2002), increase in the number of pods per plant (Tourino et al., 2002) and number of seeds per pod (Tourino et al., 2002; Heiffig, 2002), providing similar yield, regardless of the number of plants.

Grain yield

By analyzing the data in Table 3, there was significant interaction between seed number and of gypsum with

Table 3. F values, coefficient of variation (CV) and copper (Cu), zinc (Zn), manganese (Mn), iron (Fe) in leaf tissue and grain yield of soybean from the use of different number of plants per meter with seeder precision (Number plants) and surface application of gypsum in soybean. Guaíra, Paraná, Brazil, 2012.

Treatments	Cu		Zn		Mn		Fe		Grain yield	
	-------------------- mg kg^{-1}						--		--- kg ha^{-1} ---	
Number plants m^{-1}										
12	33.07		32.06		70.42		161.11		2710.80	
14	23.39		34.85		72.07		170.34		2679.93	
16	30.14		34.84		81.19		173.71		2663.27	
18	26.26		30.57		67.46		179.22		2625.30	
Gypsum --- kg ha^{-1} ---										
0	33.07		32.10		76.55		169.52		2640.43	
800	23.21		37.91		81.50		180.55		2768.82	
1600	29.02		27.48		64.86		176.39		2704.93	
2400	27.56		34.82		68.24		157.91		2565.12	
			Medium value							
	28.22		33.08		72.78		171.09		2669.83	
			F value							
Nº plants	2.97	ns	1.25	ns	2.39	ns	0.97	ns	0.17	ns
Gypsum	0.49	ns	2.05	ns	3.17	ns	0.42	ns	1.01	ns
Nº plants x Gypsum	1.96	ns	0.50	ns	0.71	ns	2.00	ns	2.20	*
C.V.$_{Nº\ plants}$ (%)	35.15		22.92		20.79		17.97		12.94	
C.V.$_{Gypsum}$ (%)	82.56		37.39		23.53		35.61		13.01	
C.V.$_{Nº\ plants\ x\ Gypsum}$ (%)	47.17		42.38		23.98		17.75		10.94	

*: significant at 1% level of probability by F test, however the regression equations were not significant; ^{ns}: not significant at 5% level of probability by F test.

respect to yield variable indicating the use of a linear equation, but there was no significant for any of the possible equations to represent the effect the number of plants and effect of gypsum on yield of soybean. Accordingly, Neis et al. (2010) also observed no increase in soybean yield with application of gypsum, similar fact was detected by Oliveira and Pavan (1996) and Caires et al. (2003) with other annual crops.

Regarding the use of gypsum, there was no benefit from its application to soybean yield, a fact that should be associated with no presence of Al^{+3} in the 0 to 0.02 m layer, and soil fertility limitation not present development of culture (Table 1). Moreover, opportunities to use gypsum as providing Ca in corn, with linear response in the yield of the application of gypsum even in fertile soils has been verified (Ferreira et al., 2013). Caires et al. (2011) also noted an increase in corn yield and increase of P and S in the leaf tissue both in corn and in soybeans when using gypsum dystrophic Oxisol. Another situation in response to gypsum was to reduce the alkalinity of the soil, to minimize the availability of sodium and chlorine ions in Cambissolo Saline Sodic (Santos et al., 2013).

On the other hand, same test in this research with adequate levels by Ca^{+2} and absence Al^{+3} in clayey Rhodic Hapludox soil, soybean has not responded to high doses of gypsum probably due affinity divalent cations by the roots (Caires et al., 2011b). Differently from beans, wheat and corn, and with responses at doses up to 18000 kg ha^{-1} gypsum (Nuernberg et al., 2005). However, there is the possibility of using gypsum to replace nutrients in conditions where there is no pronounced subsurface acidity problems at lower doses, particularly as a source of S (Caires et al., 2011a, b).

Three recent research

Sávio et al. (2011) identified increase of Ca and S in leaf tissue and increase of 21% in grain yield of soybean with 1095 kg ha^{-1} of gypsum and maximum number of pods with dose of 751 kg ha^{-1} of gypsum by studying doses of 500, 1000 and 1500 kg ha^{-1} compared absence of gypsum (dose 0) in Oxisol dystrophic come from degraded pasture with pH 4.5, 26 of V%, 0.4, 0.3 and 0.24 cmolc dm^{-3} de Ca, Mg and K, respectively.

By observing the average yield values with 12 plants per meter was obtained 2710.8 kg ha^{-1} of grain and 18 plants per meter was obtained 2625.30 kg ha^{-1},

Table 4. Investment by use seed in treatments with costs of seeds, grain return with grain yield and economic return to different number of plants. Guaíra, Paraná, Brazil, 2012.

Treatments (Factor 1)	Investment				Grain return				Economic return		
Number of plants	Use of seed(1)	Costs seed	Costs seed ha^{-1}(2)	Costs seed(3)	Grain yield(4)	Variation: grain soybean(3)	Soybean price(5)	Income soybean(3)	Profit or Prejudice(3,6)	Investment return(3,7)	
plants m^{-1}	kg ha^{-1}	US$ kg^{-1}	US$ ha^{-1}	US$ ha^{-1}	kg ha^{-1}	kg ha^{-1}	US$ kg^{-1}	US$ ha^{-1}	US$ ha^{-1}	-------	%
12	53.00	1.72	91.16	0.00	2710.80	0.00	0.37	0.00	0.00	0	0
14	61.00	1.72	104.92	- 13.76	2679.93	- 30.87	0.37	-11.51	- 25.27	- 2.83	- 283
16	70.00	1.72	120.40	- 29.24	2663.27	- 47.53	0.37	-17.73	- 46.97	- 2.60	- 260
18	79.00	1.72	135.88	- 44.72	2625.30	- 85.50	0.37	-31.89	- 76.61	- 2.71	- 271

(1) considered 90% of emergence, and 197 g for mass 1000 grains (Syngenta, 2015); (2) Costs seed (Copagril, 2015); (3) Other treatments compared treatment "12 plants"; (4) obtained in experiment; (5) by Chicago Board (Cmegroup, 2015); (6) Profit or Prejudice = "Income soybean US$ ha^{-1} "- | "Costs seed US$ ha^{-1}" |; (7) Investment return = ("Prejudice US$ ha^{-1} " - | "Costs seed US$ ha^{-1}" |) / | "Costs seed US$ ha^{-1}" | or Investment return (%) = ("Prejudice US$ ha^{-1} " - | "Costs seed US$ ha^{-1}" |) / | "Costs seed US$ ha^{-1}" | * 100.

particularly statistically similar (Table 3). Within this context, Oz (2008) reports that higher plant population did not affect grain yield in soybean. Indeed, several studies have highlighted the lack of effect of the number of plants on yield of soybean, by varying the number of plants per meter between 9.9 to 15.3 (Vasquez et al., 2008) and 5.4 to 16 (Souza et al., 2010), probably due to favorable changes in yield components (Marchiori, 1999; Heiffig, 2002; Tourino et al., 2002).

A similar yield of the number of plants assessed can be explained by the greater number of pods and seeds per pod for treatments with fewer compensating for lower plants number of plants (Mauad et al., 2010). Indeed, Heiffig et al. (2006) also observed no significant effect by altering the number of plants in soybean.

Moreover, Peixoto et al. (2000) and Tourinho et al. (2012) observed an increase in yield due to the increase in the number of plants with achieving proper operation and uniform seeding. In work done by Cortez et al. (2011) found that the use of number of plants between 15 and 20 plants per meter did not alter the yield of soybean, allowing used fewer plants to reduce spending on seeds for sowing. For Hörbe et al. (2013), the plant arrangement is a cultural practice that affects the yield of crops, the most important being the regular distribution of seeds, eliminating spaces between plants, the number of plants, especially in precision agriculture systems.

Economic return of investment

According to Table 4, the statistical difference between the grain yield of soybeans to increase from 12 to 18 plants per meter was not detected, even occurring grain yield 2710.80 and 2625.30 kg ha^{-1}, respectively. However, when assessing the costs to acquire the seeds together with the income provided with different number of plants, it was found prejudice with more plants. Over 12 plants per meter showed a negative economic results, with values of $ -25.27, -46.97 and -76.61 for the use of 14, 16 and 18 plants per meter, respectively; in fact, to evaluate the economic return on investment when purchasing higher amount of seeds, the values were between -283, -260 and -271% for their treatments. The use of seeds to achieve 12 plants per meter is more efficient, since by increasing the number of plants the farmer will have prejudice to the other number of plants tested, interfering in the economic performance for soybean cultivar SYN 7059RR.

Other researchers, Tourinho et al. (2012), Heiffig et al. (2006), Oz (2008), Vasquez et al. (2008) and Souza et al. (2010) highlight the need for uniformity of plants in the area of cultivation, the distribution of plants is more important to increase grain yield of soybean than change number of plants. In addition, higher cost was observed to acquire higher amount of seeds affect income. In the economic return on investment was demonstrated that culture with fewer plants, together with the use of high quality seeds and sowing in suitable soil moisture conditions (Hörbe et al., 2013) has more uniform cultivation of culture (Tourinho et al., 2012) and better

Table 5. Investment, cost by doses of gypsum applied, cost just soybean crop (divided by two crops: soybean and wheat), grain return with grain yield, economic return with profit or prejudice with investment and investment return to soybean crop. Guaíra, Paraná, Brazil, 2012.

Treatments (Factor 2)	Investment – Costs gypsum					Grain return				Economic return		
Doses of gypsum	Product applied(1)	Doses applied	Application	Total	Soybean crop(2)	Grain yield(3)	Variation: grain soybean(4)	Soybean price(5)	Variation: income soybean(4)	Profit or Prejudice(4,6)	Investment return(4,7)	
kg ha^{-1}	US$ kg^{-1}	US$ ha^{-1}	US$ ha^{-1}	US$ ha^{-1}	US$ ha^{-1}	kg ha^{-1}	kg ha^{-1}	US$ kg^{-1}	US$ ha^{-1}	US$ ha^{-1}	-------	%
0	0.061	0	0	0	0	2640.43	0	0.37	0.00	0.00	0	0
800	0.061	48.80	17.85	66.65	- 33.32	2768.82	+ 128.39	0.37	+ 47.89	+ 14.56	+ 0.44	+ 44
1600	0.061	97.60	17.85	115.45	- 57.72	2704.93	+ 64.5	0.37	+ 24.06	- 36.67	- 0.58	- 58
2400	0.061	146.40	17.85	164.25	- 82.12	2565.12	- 75.31	0.37	- 28.09	- 110.22	- 1.34	- 134

(1) Costs seed (Copagril, 2015); (2) total costs by gypsum divided in two crops (soybean and wheat); (3) obtained in experiment; (4) Other treatments compared treatment "absence gypsum"; (5) by Chicago Board (Cmegroup, 2015); (6) Profit or Prejudice US$ ha^{-1}= " Income soybean US$"- | Costs gypsum 'soybean crop' US$ |; (7) Investment return = ("Profit or Prejudice US$ ha^{-1}" - | Costs gypsum 'soybean crop' US$ |) / | Costs gypsum 'soybean crop' US$ | or Investment return (%) = ("Profit or Prejudice US$ ha^{-1}" - | Costs gypsum 'soybean crop' US$ ha^{-1} |) / | Costs gypsum 'soybean crop' US$ ha^{-1} |* 100.

performance of individual plants (Marchiori, 1999; Heiffig, 2002; Tourino et al., 2002; Vasquez et al., 2008; Mauad et al., 2010) providing similar grain yield to use of higher amount plants per meter, especially, greater economic return on investment in seeds.

Table 5 show costs gysum to different doses, grain yield and gypsum price. Prado and Fernandes (2010) pointed out that the dimension of the economic return is sustained in crop yield, production costs and price of the product employed. Costs including product and application cost, with total costs of US$ 66.65, 115.45 and 164.25 by 800, 1600 and 2400 kg ha^{-1}, respectively. Costs gypsum by soybean crop US$ 33.32, 57.12 and 82.12 superior to absence gypsum in the sequence, because divided by soybean and wheat. Although, grain yield soybean increase US$ ha^{-1} +47.89, +24.06 and -28.09.

Look just income soybean grain yield, treatments with 800 and 1600 kg ha^{-1} of gypsum highlighted from the others (Table 5). But, look to economic return, just treatment 800 kg ha^{-1} provides profit US$ +14.56, thus +44% by economic return investment (Table 5) to payment half investment by soybean (US$ 33.32). Others treatments, prejudice US$ -36.67 and -110.22 and -58% and -134% by economic return investment to 1600 and 2400 kg ha^{-1} gypsum.

Others researchers to study economic return has been published in the literature. Prado and Fernandes (2010) also studied economics of slag of siderurgy application in the cultivation of cane sugar, highlighting doses with greater economic return. Fiorin et al. (2011) detected superiority of 9.2 to 13.7% when using precision farming system as application of appropriate amount of inputs into variable rate with the use of conventional system of fixed rate on soybeans.

Soybean culture exported 5.26, 5.14 and 4.87 kg ha^{-1} S in grain (related grain yield in treatments) with 800, 1600 and 2400 kg ha^{-1} by gypsum application, respectively (Table 6). Estimate grain yield with wheat 2503 kg ha^{-1} exported 5.51 kg ha^{-1} of S in grain (Seab, 2015; Rampim, 2014). High doses of gypsum (1600 and 2400 kg ha^{-1}) supply S in soil (80 and 120 kg ha^{-1}) and S residual (69.35 and 109.62 kg ha^{-1}) after two cultures (soybean-grain yield and wheat-estimate). Otherwise, absence gypsum application export 10.52 kg ha^{-1} for both cultures, and didn't have reposition fertilization. This situation reduce S disponible in soil, that's approximate limit to medium class by S level in soil (10 mg dm^{-3} S-SO_4^{-2}).

High yield of soybean and wheat grains, need adequate nutrients levels in the soil, so the export S in soil with the middle class can reduce potential yield of next crops. Gypsum can be used to reset the nutrients exported S and Ca, and in this case, reset especially S (medium level in soil). Nutrients exported by crops can be reset using 800 kg ha^{-1} gypsum in V4 stage of soybean, offering 20.23 kg ha^{-1} of residual S in soil to the next crop season (soybean + wheat), related by economic return

Table 6. Input of S in treatments by gypsum, export of S in the soybean crop and estimate in second crop with wheat and residue of S by gypsum in soil before both crops. Guaíra, Paraná, Brazil, 2012.

Input - treatments by gypsum		First crop - Soybean			Estimate: Second crop - Wheat			Two crop - one year[5]	S residual by gypsum in soil[6]
Doses of gypsum[1]	Input S[1]	Grain Yield[2,4]	S exported[3]	S grain exported	Grain yield[4]	S exported[3]	S grain exported	S grain exported	
-------------------- kg ha^{-1}	--------------------		1000kg $(1000kg)^{-1}$	kg $(1000kg)^{-1}$	kg ha^{-1}	1000kg $(1000kg)^{-1}$	--------- kg $(1000kg)^{-1}$	---------	kg ha^{-1}
0	0.00	2640.43	0.0019	5.02	2503.00	0.0022	5.51	10.52	-10.52
800	40.00	2768.82	0.0019	5.26	2503.00	0.0022	5.51	10.77	20.23
1600	80.00	2704.93	0.0019	5.14	2503.00	0.0022	5.51	10.65	69.35
2400	120.00	2565.12	0.0019	4.87	2503.00	0.0022	5.51	10.38	109.62

[1] Gypsum: 15% S-SO_4^{-2} e 5% S; [3] obtained in experiment; [3] obtained in farm (Rampim, 2014); [4] grain yield average 2009-2014 in Parana State is 2958 kg ha^{-1} and 2503 kg ha^{-1} to soybean (just to campared) and wheat (used to estimate S exported), respectively (Seab, 2015); [5] S exported in two crops (soybean and wheat); [6] S residue after two crops used to paid the investment (soybean and wheat) and deficit S in treatment absence gypsum; S residual gypsum (kg ha^{-1}) = "Input S (kg ha^{-1})" – "S grain exported soybean kg ha^{-1}" – "S grain exported second crop - wheat kg ha^{-1}"

(Table 5) by absorbing nutrients quickly solubilized gypsum with reduce mobilization to subsuperface layers (Caires et al., 1998; Soratto and Crusciol, 2008; Caires et al., 2011; Nava et al., 2012; Michalovicz et al., 2014). In this dose of gypsum, grain yield and low costs income US$ +47.89 and profit US$ 14.56 (Table 5).

Similarly in recent research, in soils with inferiority fertility, identify low doses by gypsum increase grain yield. Motta et al. (2013) also worked with doses of gypsum (0 to 120 kg ha^{-1} S; 5% S in product), in order to provide S to soybean in Oxisoil, which found that the use of doses of 120 kg ha^{-1} S with gypsum in soil with 0.26 $cmol_c$ dm^{-3} K and 4 $cmol_c$ dm^{-3} Ca, raises K and Ca in soybean leaf tissue in absence potassium fertilization, however without interfering in these levels nutrients by applying 60 kg ha^{-1} K_2O, nevertheless increase S both conditions, with and without potassium fertilization in no-till system. Other study, gypsum favored grain yield of corn, wheat and soybeans, when there was water deficiency. However, high doses of gypsum (12000 kg ha^{-1}) damaged the grain yield of soybeans by Mg deficiency induction in adequate water condition in Oxisol dystrophic typical, due to increase Ca/Mg ratio in soil, with added Ca; however, doses of 1500 and 3000 kg ha^{-1} of gypsum favored grain yield independent of weather conditions for soybean and corn in the crop rotation system (Pauletti et al., 2014).

In other research, Raij (2008) identified application of soil conditioner, it is necessary doses above 800 to 1000 kg ha^{-1} of gypsum, because lower doses do not provide uniform distribution of the throw by agricultural machines. In this condition, low dose of gypsum (800 kg ha^{-1}) increase economic return and limit to adequate application.

General considerations

For the number of plants can be indicated using 12 seeds per meter, because we obtain an equivalent yield, being relevant to reduce production costs in the soybean system and elevate economic return of investment. However, it must be ensured operational efficiency during deployment of culture as a condition of adequate soil moisture for seed starting germination process as well as the culture does not deploy with excess moisture in the soil because it reduces soil layer deposited on the seed, also run the sowing operation at low speed, adequate depth of seed, seed quality, pay attention to the position of deposition of fertilizer in the seed, together with the seed distribution system regulated and/or selection of discs appropriate for each seed lot.

The broadcast application of 800 kg ha^{-1} gypsum on soybeans in V4 stage possible to take advantage the nutrients released in the surface layers in the first crop, due to the high solubility (Raij, 2008), reducing intensive mobilization of Ca^{+2} and S-SO_4^{-2} the soil at high dose (Caires et al., 1998; Caires et al., 2002, 2004). Like this, use gypsum to provide nutrients Ca and S (Caires et al., 2003; Soratto and Crusciol, 2008; Sávio et al., 2011; Zapparoli et al., 2013; Motta et al., 2013; Pauletti et al., 2014) with increase grain yield of soybean (Caires et al., 2011; Sávio et al., 2011; Broch et al., 2011; Ferreira et al., 2013; Santos et al., 2013; Motta et al., 2013; Pauletti et al., 2014), introducing more advantages to the crop system, due to the economic return on investment with product.

Conclusion

The number of plants per meter with precision seed drill and superficial application of gypsum at the V4 stage do not affect the content of macro-and micronutrients leaf tissue of soybean cultivar SYN 7059RR. The application of gypsum at the V4 stage and reducing the number of plants per meter statistically do not affect the grain yield. Economic returns using higher amount of seed (14, 16 and 18 plants m^{-1}) is -283, -260 and -271% with investment of US$ 13.76, 29.24 and 44.72. Recommend lower number of plant (12 plants m^{-1}) on soybean cultivar for SYN 7059RR. Soybean seeding in clayey Rhodic Hapludox with 10 mg dm^{-3} S-SO_4^{-2} level by absence gypsum reduce 10.52 kg ha^{-1} of S by exported grain. Application of 800 kg ha^{-1} gypsum provides US$ 14.56 profit with 44% economic return to payment half investment by soybean (US$ 33.32). Use of 800, 1600 and 2400 kg ha^{-1} gypsum focused only on two cultures (soybeans and wheat) during the crop season provides residual soil of 20.23, 69.35 and 109.62 kg ha^{-1} S, respectively.

Conflict of Interest

The authors have not declared any conflict of interest.

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Spacial and temporal characterization of water quality in the Cuiabá River Basin of Central Brazil

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Multivariate statistical techniques were applied to evaluate the temporal and spatial variation of water quality in the Cuiabá River basin that could be affected by urban effluent discharge and diffuse pollution. After data treatment, 75.74% of the total variance was explained in the 28 parameters determined in water samples, collected at 13 stations from July 2010 to May 2011. The first component (29.08%) was represented by dissolved ions from natural processes and samples collected during rainy season, and the second component (15.42%) was related mainly to agricultural and urban effluent discharges and with samples collected during dry season (positive loadings). Hierarchical cluster analysis revealed the existence of five groups, of which three were characterized as regions of low, moderate, and high impact in the basin respectively. The other two sites, Marzagão and Porto Cercado, were considered unique groups. Marzagão is located in the headwaters of the Cuiabá River and is not subject to flow regulation caused by a reservoir, and Porto Cercado is within the flood plain of Pantanal. These data will contribute to the optimization of monitoring programs and to the understanding of other similar watersheds.

Key words: Principal component analysis, environmental monitoring, pollution, watershed, hierarchical cluster analysis.

INTRODUCTION

Decreasing quality and quantity of water resources have become a global concern, even in countries with high water potential, such as Brazil (Colletti et al., 2010). The high concentration of the urban population in Brazil has caused numerous environmental problems for water resources, including contamination of rivers by domestic and industrial sewage, flooding caused by improper occupation of flood plain, inadequate management of urban drainage, and lack of collection and adequate disposal of urban waste (Tucci et al., 2000).

The Cuiabá River, located in Central-western portion of Brazil, is a waterway of major importance for multiple uses, such as water supply and wastewater dilution. This river is one of the main tributaries of Northern Pantanal of

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Mato Grosso State, the largest floodplain in the world (Zeilhofer et al., 2006). Water use conflicts, changes in aquatic biota and degradation of the water quality of the aquatic environment of this river have previously been described (Figueiredo and Salomão, 2009; Laabs et al., 2002; Zeiholffer et al., 2006).

Water quality is a concern because water from this river supplies approximately one third of the population of the state and has a significant impact on the complex, rich Pantanal ecosystem. To monitor water quality, a systematic monitoring program is required to obtain accu-rate estimates of variations in surface water quality due to variations in physical, chemical, and biological para-meters caused by seasonal effects and land use in the basin (Simeonov et al., 2003; Toledo and Nicolella, 2002). However, large amounts of data obtained in systematic studies can be difficult to interpret. Matrices with multiple dimensions are usually generated, but the most relevant environmental informa-tion is included in only a small number of variables, while the remaining variables add little to the interpretation of the results in terms of quality (Vidal et al., 2000; Simeonov et al., 2003; Toledo and Nicolella, 2002; Andrade et al., 2007b; Felipe-Sotelo, 2007).

Multivariable statistic analysis provides an alternative approach to understand the water quality of study region and identify the pollution source apportionments (Wu et al., 2009). Among these tools, factor analysis and hierarchical cluster analysis have become increasingly widespread due to the diffusion of chemometric (science of extracting information from chemical systems by data-driven means) knowledge and the availability of software that significantly reduces the labor of calculation (Mas et al., 2010).

Several studies have addressed the evaluation of water quality data using multivariate analysis in water bodies around the world (Brondnjak-Voncina et al., 2002; Felipe-Sotelo et al., 2007; Mendiguchía et al., 2004; Kazi et. al., 2009; Pejman et al., 2009) including Brazil (Silva and Sacomani, 2001; Zeilhofer et al., 2006; Palácio et al., 2009, Alexandre et al., 2010; Pereira-Filho et al., 2010; Palácio et al., 2011). This study aimed to evaluate the water quality variables responsible for the spatial and temporal variation of water quality as well as the similarities and dissimilarities among stations on the River Cuiabá in the hydrological year 2010/2011 that could be affected by urban effluent discharge and diffuse pollution.

MATERIALS AND METHODS

Study area

This study was performed in the Cuiabá River basin, a sub-basin of the upper Paraguay River basin. In the section that was investigated, which has a size of approximately 680 km, the river passes through several cities, including the main municipalities in the basin, Cuiabá, the capital city of Mato Grosso, and Várzea Grande, which has a population of more than 800 million inhabitants. Before passing the urban areas of Cuiabá and Várzea Grande, the river runs through areas of livestock, agriculture, protected areas, and small towns of low population density. In the urban area of Cuiabá, approximately 70% of the domestic effluent is discharged into the river without any treatment. After Cuiabá, there are areas of livestock and agriculture and point sources of domestic sewage discharge from the urban areas of St. Antônio do Leverger (approximately 18,500 inhabitants) and Barão de Melgaço (approximately 7,600 inhabitants). At the end of its route, the Cuiabá River enters the Pantanal region.

Water samples were collected from 13 sampling stations (Table 1) at which water quality has been monitored by the Environmental Department of Mato Grosso State (SEMA/MT) since 1995. These monitoring stations are located on stretches of the high, medium, and low Cuiabá River (Figure 1).

Sample collection and treatment

We performed six samplings campaigns, with samples taken in triplicate bimonthly between July 2010 and May 2011. This period corresponded to an annual hydrological cycle with sampling occurring in: rainy (October to April) and dry seasons (May to September). Total precipitation in the period ranged from 17.2 mm in July - September 2013, 1484 mm in October 2010 - March 2011, and 188 mm April - June 2011. Water samples were collected approximately 20 cm below the water surface using polyethylene bottles with capacities of one litre (this sample was preserved with addition of solution of 50% sulfuric acid until pH < 2) and two litre (this sample was not chemically preserved). Samples for bacteriological analyses were collected using sterilized 100 ml plastic bags. After sampling, the samples were transported under refrigerated conditions to the laboratory of SEMA-MT, where the analyses were performed within 24 h. The sampling methodology was based on the Standard Methods of Examination of Water and Wastewater (American Public Health Associaton, 2005) and on the Water Sample Collection Guide (CETESB, 1988).

Analyses

A total of 31 physical, chemical, and microbiological environmental parameters (air temperature, water temperature, pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids, total dissolved solids (TDS), total suspended solids (TSS), true color, turbidity, hardness, chlorine, sulfate, fluoride, total alkalinity, phosphate, total phosphorus, sodium, potassium, magnesium, calcium, nitrite, nitrate, ammonia nitrogen, total nitrogen, salinity, electrical conductivity (EC), total coliforms, and *Escherichia coli*) were determined by employing methods recommended in the Standard Methods for the Examination of Water and Wastewater (American Public Health Associaton, 2005).

The field measurements for the parameters pH, water temperature, EC, DO, salinity, TDS, and ORP were conducted with an HI 9828 HANNA Multi-parameter Meter equipped with a pH/ORP/DO/EC/Temperature HI 769828 sensor with a 20 m cable. Analyses were performed using ultrapure water produced with a Milli-Q Advantage Ultrapure Water Purification System. All reagents used were analytical grade (J.T. Baker®, Chemis® and Synth® brands). The spectrophotometric readings for the parameters COD, total nitrogen, total phosphorus, orthophosphate, nitrate nitrogen, nitrite nitrogen, and sulfate were obtained with a DR 5000TM UV-Vis Hach spectrophotometer.

The color measurements were acquired with an Aquacolor Cor PoliControl portable colorimeter. Cation analyses (sodium, potassium, magnesium, calcium, and ammonia nitrogen) were performed using an ICS-1000 Dionex ion chromatograph with an

Table 1. Locations of the sampling sites in the Cuiabá River basin.

Station	Station Name	Municipality	Altitude (m)	Latitude	Longitude
1	Marzagão	Nobres	238	14°32'31.33" S	55°50'50.5" W
2	Downstream of Nobres	Nobres	189	14°45'11.10" S	56°19'38.8" W
3	Bridge in Rosário Oeste	Rosário Oeste	186	14°49'58.19" S	56°24'51.00" W
4	Acorizal	Acorizal	173	15°12'16.22" S	56°22'0.60" W
5	Passagem da Conceição	Cuiabá	153	15°33'53.52" S	56°8'29.83" W
6	Downstream of Córrego Mané Pinto	Cuiabá	156	15°36'58.1" S	56°6'22.53" W
7	Downstream of Córrego Barbado	Cuiabá	147	15°38'25.66" S	56°4'35.18" W
8	Downstream of Córrego São Gonçalo	Cuiabá	147	15°39'0.21" S	56°4'11.61" W
9	Downstream of Córrego Ribeirão dos Cocais	Cuiabá	146	15°46'51.03" S	56°8'34.59" W
10	Santo Antônio do Leverger	Santo Antônio do Leverger	144	15°52'13.40" S	56°04´36.32" W
11	Poço Beach	Santo Antônio do Leverger	142	15°54'48.22" S	56°1'47.27" W
12	Downstream of Barão de Melgaço	Barão de Melgaço	138	16°11'43.19" S	55°58'7.27" W
13	Downstream of Porto Cercado	Poconé	122	16°31'13.17" S	56°22'31.91" W

analytical column (Dionex CS16, 3 mm, coupled to pre-column CG16, 3 mm), a 25 ml loop, 20 mM sulfuric acid eluent solution (H_2SO_4, J.T. Baker brand), an eluent flow rate of 0.36 ml min^{-1}, and a 2 mm CSRS suppressor 300 (Cation Self-Regenerating Suppressor). Unpreserved samples were filtered (0.45 µm pore size) before injection (1 ml) into the ion chromatograph. Microbiological analyses were performed according to the enzyme substrate method, using Colilert® culture medium and IDEXX® cards.

Multivariate analysis

Data treatment

The censored data, below the method's limits of detection (LOD) were replaced by values corresponding to half the LOD (LOD/2) according to procedures proposed elsewhere (Farham et al., 2002) and used by others (Navarro et al., 2006; Terrado et al., 2007; Felipe-Sotelo et al., 2008). After replacing the censored values, an analysis of the missing data was performed to evaluate the implementation of corrective actions to enable factor analysis, such as deletions of variables or cases.

To detect atypical observations, the data were converted into standard scores (Z scores) from the univariate perspective, identifying the observations with scores higher than 3. For multivariate detection, the Mahalanobis D^2 was calculated, identifying those cases with values (D^2/df) above 3.0, where *df* is the number of present variables (Hair et al., 2009).

A Pearson's correlation matrix was elaborated to verify whether the data matrix had a sufficient number of correlations to justify the application of principal component analysis. Bartlett's test of sphericity and the adequacy test applied to the Kaiser-Meyer-Olkin (KMO) model were also performed. The sphericity test examines the matrix as a whole and demonstrates that the correlation matrix presents significant correlations among the variables. In the sphericity test, values that have significance ($p < 0.05$) indicate the existence of a number of correlations among variables, thus permitting the continuation of the analysis (Hair et al., 2009). The KMO test shows normalized values (between 0 and 1) and the proportion of variance that the variables have in common or the proportion due to common factors. Values less than 0.5 indicate that the method of factor analysis is inappropriate for the treatment of the data (Dziuban and Shirkey, 1974). The descriptive statistical as well as multivariate analyses were performed by employing (Statistical Package for Social Sciences (SPSS) version 19.0.

Factor analysis – principal component analysis (PCA)

After processing the data, PCA was performed. For the PCA, in this study, there were no necessity to standardize the data. The analysis was performed by transforming the correlation matrix, by estimation, into a factor matrix containing factor loadings for each variable in each factor obtained. The loads of each variable on the factors were then interpreted to identify the latent structure of the variables (Hair et al., 2009).

The latent root criterion was chosen to define the number of factors to be extracted, and all factors with eigenvalues greater than 1 were considered significant. This criterion is more reliable when the number of factors to be extracted is between 20 and 50, as in the present study (Hair et al, 2009). In this study, the VARIMAX method (orthogonal rotation) was applied to normalized data using the evaluation of the spatial and temporal variability of water quality (Andrade et al., 2007b; Palácio, 2011). Statistical software was used to obtain the rotated component matrices as well as the scores, loadings, and eigenvalues (eingenvectors).

Hierarchical cluster analysis (HCA)

In this study, the HCA aimed to observe the similarities and

Table 2. Statistical description of the chemical, physical, and biological parameters determined in the 13 stations along the Cuiabá River basin.

Variable	N	Mean	Minimum	Maximum	Standard deviation
Air temperature	201	28 .6	19	39	4.7
Water temperature	201	27.3	23.7	30.6	1.6
pH	201	7.2	5.6	8.1	0.4
Salinity	201	0.04	0.02	0.013	0.02
ORP	201	72.5	-8.9	240	54.6
Conductivity	201	79	51	276	34
DO	201	6.6	1.1	9.3	1.33
BOD	201	1.2	0.1	7	0.8
Total Solids	201	136	25	3121	219
TDS	201	39	26	138	17
Color	201	33.5	4.2	134.1	23.8
Turbidity	201	71.6	1.3	337	68.4
Hardness	201	32	5.7	168.3	20.9
Chloride	201	0.72	0.1	2.54	0.49
Sulfate	201	2.84	0.26	18.97	2.36
Alkalinity	201	36	21	154	21
Phosphate	201	0.13	0	0.3	0.14
Total Phosphorus	201	0.22	0.01	2.36	0.45
Sodium	201	1.56	0.68	4.19	0.5
Potassium	201	6.42	0.64	26.29	3.69
Magnesium	201	0.55	0.13	1.52	0.16
Calcium	201	7.6	4.07	30.71	3.95
Nitrite	201	0.17	0	0.6	0.22
Nitrate	201	0.34	0	3.4	0.45
Ammonia Nitrogen	201	0.11	0.02	0.58	0.06
Total Nitrogen	201	0.72	0	5	0.75
Total Coliforms	201	11910	161	24192	9195
Escherichia coli	201	1994	5	24192	4283

dissimilarities among sampling stations, with the goal of clustering in each group the collection stations with similar characteristics based on the water quality data to determine which characteristics are consequences of natural environmental conditions and of land use and occupation in the Cuiabá River basin (Figure 4). To perform cluster analysis, data were standardized into standard scores (Z scores). The squared Euclidean distance was used in the HCA. Average linkage clustering and Ward's method were used as hierarchical cluster algorithms (Hair et al., 2009; Palácio et al., 2011).

To define the optimal number of clusters, the percentage variation for heterogeneity (Palácio et al., 2009; Alexandre et al., 2010; Palácio et al., 2011), with the determination of the clustering coefficient in SPSS, was adopted as a stopping rule because large percentage variations in the coefficient are used to identify stages of cluster combinations that are significantly different (Hair, 2009).

RESULTS AND DISCUSSION

Descriptive statistics and data correlation

After data treatment, the original matrix (234 cases x 31 variables) was reduced (201 cases x 28 variables). Chemical oxygen demand, fluoride and suspended solids were removed to obtain a complete matrix that could be subjected to principal component analysis. Variables exhibiting high values of standard deviation, such as turbidity, total residue and total coliforms (Table 2), displayed variability during the sampling period, which was mainly attributed to the variation in the precipitation during the study period. These variables are influenced by the amount of solid material that is carried to the river in the runoff process. Other variables such as temperature, pH, salinity, DO, BOD, and ammonia nitrogen presented very similar mean and median values, indicating an apparent symmetry of the data distribution, which can also be observed from the low values of asymmetry.

A large number of significant correlations ($p = 0.05$) among the variables were identified (Table 3), but due to the large number of observations, the critical value of r was low (0.196 for $\alpha = 0.05$). Because the significance test for r proves only that the significant correlations differ

Table 3. Binary correlations between the studied variables determined in the Cuiabá River basin.

Variables	Air temp	Water temp	pH	Salinity	ORP	Conductivity	DO	BOD	TS	TDS	Color	Turbidity	Hardness	Cl	Sulfate	Alkalinity	Phosphate	Total phosphorus	Na	K	Mg	Ca	Nitrite	Nitrate	Ammonia nitrogen	Total nitrogen	Total coliforms	*Escherichia coli*
Air temperature	1																											
Water temperature	**0.2**	1																										
pH	0.09	**-0.4**	1																									
Salinity	0.07	**-0.2**	**0.31**	1																								
ORP	**0.34**	**0.36**	**-0.19**	-0.08	1																							
Conductivity	0.05	**-0.2**	**0.31**	**0.98***	-0.11	1																						
DO	-0.03	**-0.64**	**0.41**	0.08	**-0.2**	0.09	1																					
BOD	**-0.27**	0.07	**-0.27**	-0.09	**-0.24**	-0.1	**-0.29**	1																				
TS	**-0.18**	0.02	-0.08	0.07	-0.02	0.06	-0.03	**0.15**	1																			
TDS	0.05	**-0.19**	**0.31**	**0.99***	-0.1	**0.99***	0.1	-0.11	0.06	1																		
Color	-0.13	**0.44**	**-0.41**	**-0.22**	0.11	**-0.24**	**-0.46**	0.09	0.11	**-0.23**	1																	
Turbidity	**-0.25**	**0.37**	**-0.46**	**-0.21**	**0.33**	**-0.22**	**-0.28**	0.04	**0.23**	**-0.21**	**0.64***	1																
Hardness	0.12	**-0.34**	**0.23**	**0.67***	**-0.21**	**0.69***	**0.27**	0.01	**0.16**	**0.69***	**-0.33**	**-0.31**	1															
Chloride	**-0.32**	-0.02	**-0.3**	-0.06	**-0.47**	-0.05	-0.11	**0.18**	0.1	-0.05	**0.38**	**0.24**	0.05	1														
Sulfate	**-0.25**	**0.59***	**-0.32**	-0.2	**0.24**	**-0.2**	**-0.5**	0.07	0.12	**-0.19**	**0.59***	**0.64***	**-0.41**	**0.15**	1													
Alkalinity	0.09	**-0.28**	**0.38**	**0.93***	-0.07	**0.95***	**0.24**	**-0.15**	0.01	**0.95***	**-0.35**	**-0.3**	**0.68***	-0.14	**-0.3**	1												
Phosphate	**0.39**	**-0.62**	**0.51***	**0.2**	-0.13	**0.2**	**0.59***	**-0.24**	**-0.22**	**0.2**	**-0.71**	**-0.74**	**0.36**	**-0.31**	**-0.76**	**0.35**	1											
Total Phosphorus	**-0.34**	-0.11	-0.07	0.05	**-0.16**	0.06	**-0.16**	**0.27**	**0.3**	0.06	0.01	0.06	**0.23**	**0.16**	0.04	-0.01	**-0.2**	1										
Sodium	**-0.28**	**0.23**	**-0.26**	**-0.18**	-0.1	**-0.19**	**-0.35**	**0.39**	**0.15**	**-0.18**	**0.44**	**0.42**	**-0.18**	**0.47**	**0.45**	**-0.28**	**-0.51**	**0.36**	1									
Potassium	0.09	**-0.25**	**0.18**	**0.91***	-0.13	**0.92***	**0.24**	-0.13	0.02	**0.93***	**-0.32**	**-0.25**	**0.67***	-0.04	**-0.3**	**0.91***	**0.28**	-0.03	**-0.27**	1								
Magnesium	0.08	**0.28**	**-0.3**	-0.08	-0.07	-0.09	**-0.56**	**0.16**	0.06	-0.09	**0.46**	**0.21**	-0.1	**0.43**	**0.31**	**-0.17**	**-0.26**	0.02	**0.17**	**-0.14**	1							
Calcium	0.12	**-0.25**	**0.36**	**0.97***	-0.08	**0.98***	**0.18**	**-0.15**	0.03	**0.99***	**-0.32**	**-0.28**	**0.71***	-0.13	**-0.27**	**0.96***	**0.31**	0.03	**-0.27**	**0.93***	**-0.14**	1						
Nitrite	**0.56***	**-0.21**	**0.31**	0.12	0.05	0.13	**0.4**	**-0.21**	**-0.19**	0.14	**-0.63**	**-0.63**	**0.37**	**-0.29**	**-0.64**	**0.26**	**0.82***	-0.12	**-0.39**	**0.21**	**-0.23**	**0.23**	1					
Nitrate	**-0.17**	**-0.51**	**0.32**	-0.04	-0.11	-0.04	**0.3**	-0.07	-0.08	-0.06	**-0.18**	**-0.16**	-0.03	-0.07	**-0.14**	0.02	**0.33**	**-0.14**	-0.13	-0.06	-0.1	-0.03	-0.05	1				
Ammonia Nitrogen	**-0.14**	**0.25**	-0.06	0.03	0.03	0.03	**-0.22**	**0.32**	-0.01	0.05	0.01	0.06	-0.11	0.06	**0.15**	-0.03	**-0.2**	0	**0.16**	-0.02	0.1	0	-0.1	-0.14	1			
Total Nitrogen	-0.11	-0.03	0.11	-0.07	-0.05	-0.08	0.05	0.09	0	-0.08	-0.02	0.11	-0.08	0.05	0.1	-0.09	-0.07	-0.02	0.06	-0.1	0.05	-0.09	**-0.17**	**0.17**	**0.18**	1		
Total Coliforms	**-0.36**	0.02	-0.03	**-0.16**	-0.04	-0.14	0.02	0.12	0.06	-0.14	0.08	**0.27**	**-0.17**	**0.19**	**0.21**	**-0.14**	**-0.31**	-0.02	**0.21**	**-0.15**	-0.11	**-0.19**	**-0.37**	0.04	**0.15**	**0.17**	1	
Escherichia coli	**-0.27**	**0.14**	0.03	0	-0.11	0	-0.08	**0.25**	0	0.01	0.07	**0.14**	-0.13	**0.19**	**0.17**	-0.07	**-0.22**	-0.07	**0.16**	-0.09	0.09	-0.06	**-0.18**	-0.07	**0.52***	0.1	**0.47**	1

* Correlations > 0.5 (absolute value). Significant values are indicated in bold characters (significance level = 95%, $p < 0.05$).ORP: oxidation-reduction potential, DO: dissolved oxygen, BOD: biochemical oxygen demand, TDS: total dissolved solids. TS: total solids. Cl: chloride. Na: sodium. K: potassium. Mg: magnesium. Ca: calcium.

from zero, it is important to focus on correlations that are greater than 0.5 ($p = 4.4 \times 10^{-5}$), as indicated by Helena et al. (2000) and Andrade et al. (2007b).

According to the Brazilian legislation (CONAMA Resolution nº 357/2005), in raining season there were values in parameters like turbidity, total phosphorous and *Escherichia coli* above the maximum limits, mainly in the sampling sites near the urban area. Sewage effluents from industrial and domestic sources and surface runoff of the waste could explain the increase of these para-me-ters values. Dissolved oxygen presented lower values during rainy season, only in the sampling sites Barão de Melgaço and Porto Cercado due to the increasing the organic matter near the Pantanal Floodplain.

Among the observed correlations, there were associations between salinity and conductivity (r = 0.98), salinity and total dissolved solids (r = 0.99), and total dissolved solids and conductivity (r = 0.99); these variables are strongly correlated because they represent the presence of salts in the water. These salts may be natural due to the geological and soil conditions in the region, particularly in the upper course of the river. The salts may also have anthropogenic origins, such as the silting process in the water bodies or the diffuse pollution that occurs in the basin. Similar results were found in a study developed in the basin of the upper Acarajú, Ceará (Andrade et al., 2007b). From this correlation matrix, KMO (value of 0.773) and Bartlett's sphericity (approximate chi-square of 6526.300, significance of 0.000) tests revealed that there were sufficient correlations to apply the factor analysis.

Factor analysis – PCA

Considering the latent root criterion, 7 components were retained, which were composed of 19 of 28 variables from the original data matrix. The 7 components combined explained approximately 75.74% of the total variance.

In this matrix (Table 4), factor loadings represent

Table 4. Stopping rule for the HCA – Ward's Algorithm Method.

Stage	Stopping rule			
	Hierarchical Process		Clustering coefficient	
	Number of Groups		Value	Percentage increase for the next stage
	Before clustering	After clustering		
5	9	8	213.499	29.41
6	8	7	276.29	28.16
7	**7**	**6**	**354.093**	**30.57**
8	**6**	**5**	**462.337**	25.72
9	5	4	581.266	29.88
10	4	3	754.94	27.91
11	3	2	965.667	34.21
12	2	1	1296	-

represent the degree of association (correlation) of each variable with each factor. The 1st component explains the greatest amount of variance, and this factor is composed of 7 variables containing high loads (greater than 0.50). Factor loadings of ± 0.50 or greater are considered practically significant (significance level α = 0.05 and power level of 80% for sample size equal to or greater than 120), and loads greater than 0.70 are considered indicative of defined structure (in this case, the factor explains 50% of the variance of the variable).

The 1st and 2nd components together explain approximately 44.49% of the variance of the data. The 1st component (PC1) was assigned to the variables TDS, conductivity, salinity, calcium, alkalinity, hardness, and potassium. This 1st component is basically a component of dissolved salts, particularly potassium and calcium salts. The occurrence of similar variables in the 1st component and the correlation of these variables with the natural process of weathering of the geological components of the soil have also been reported in other studies (Singh et al., 2004; Andrade et al., 2007b).

The 2nd component (PC2) of this study was attributed to the parameters turbidity, sulfate, and color, which were positively correlated with the factor, and to the parameters phosphate and nitrite, which were negatively correlated. Samples collected during the rainy season are related to the positive values of component 1 (Figure 1) and to variables with negative loadings in component 2 (PC2), while samples collected during the dry season are related to the variables with positive loadings in component 2 (PC2). Observing the sample distribution as a function of the collection station in Figure 2, the highest scores recorded for the 1st component are related to Station 1 (Marzagão). This station is located in the municipality of Nobres, a region with geological records (the Araras, Raizama, and Puga Formations) that are composed mainly of sediments and rocks rich in calcium and magnesium carbonates, such as sandstones (Figueiredo and Salomão, 2009). When incorporated in the water, these compounds increase the concentration of calcium ions and carbonates, increasing the hardness and alkalinity and, therefore, the conductivity and salinity.

During the rainy season, there is an increase in turbidity and color due to surface runoff. This runoff is aggravated by the removal of riparian vegetation in many parts of the basin and by localized processes of siltation. Large amounts of sediments and organic matter reach the tributaries and flow into the Cuiabá River, which results in increased color and turbidity. The transport of sediment in the Cuiabá River basin has already been reported, and the increase in sediment transport was attributed to urbanization, with sediment deposition in the surroundings of St. Antônio do Leverger, at the beginning of the Pantanal Plain (Figueiredo and Salomão, 2009).

Sulfate can be derived from anthropogenic activities, mainly from fertilizers and water and wastewater treatment, which occur closer to the urban areas of Cuiabá and Várzea Grande. The 3rd component, which accounted for 9.60% of the explained variance, can be attributed to the discharge of domestic and industrial effluents and the leaching of organic matter from livestock areas in the sub-basin because this component was composed of the variables *E. coli* and ammonia nitrogen. These variables presented high values, mainly at the Marzagão Station (Station 1), the stations in the urban areas of Cuiabá and Várzea Grande (Stations 7, 8, and 9), and the St. Antônio do Leverger station (Station 10).

The other components each explain approximately 3 to 7% of the total variance. The 4th component is positively related to the variable chloride and negatively related to the variable ORP. The influence of chloride is related to domestic effluent discharges in urban areas (Helena et al., 2000; Andrade et al., 2007a). The increase in the concentration of chlorides (highly reactive chemical species) decreases the ORP (which indicates which half of the chemical species is reduced). The 5th component can be explained by the presence of limestone rich in

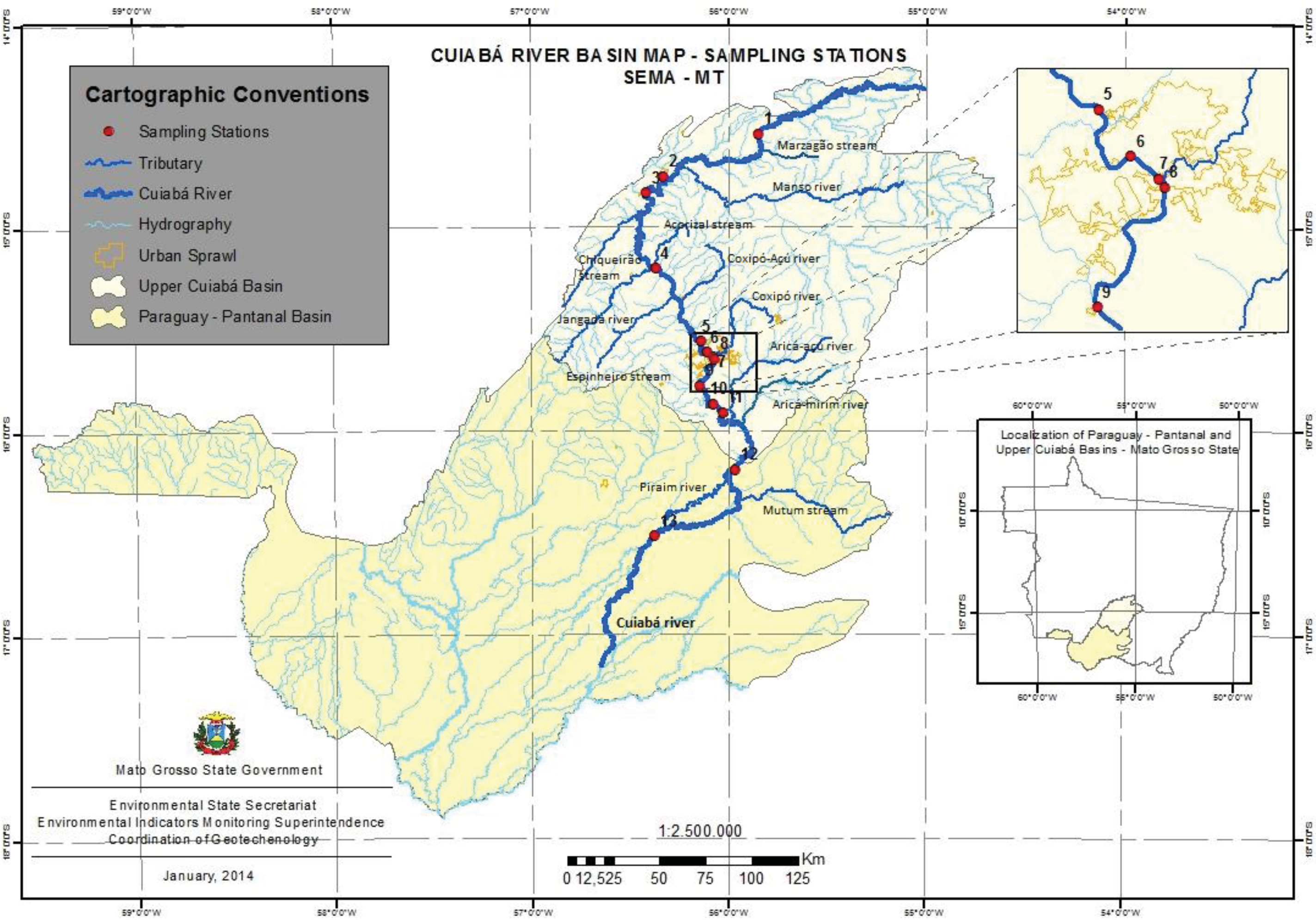

Figure 1. Location of the sampling stations in the Cuiabá River basin.

magnesium, particularly in Província Serrana, where the upper course of the Cuiabá River is located (Figueiredo and Salomão, 2009). The 6th and 7th components are represented by the variables total phosphorus and nitrate. Both variables are indicative of agricultural and urban pollution processes (Andrade et al., 2007a; Campanha et al., 2010; Pereira-Filho et al., 2010). However, because they are different components, they may reveal different aspects of this pollution, as nitrates more closely represents point sources, while total phosphorus represents diffuse sources or a different combination of both sources.

The results obtained here to explain the influence of organic pollution near the urban stations of Cuiabá River and the improvement of the water quality downstream from the urban area of Cuiabá and Várzea Grande are similar to those of a previous study (Zeilhofer et al., 2006). Considering the 17 parameters that were employed in our PCA matrix, the weight of organic pollution (discharge of domestic and industrial effluents) was minimized due to the influence of dissolved salts and surface runoff. The absence of the parameter COD also explains some of the differences among the results of our study and those of previous studies (Zeilhofer et al., 2006).

HCA

After analyzing the diagrams obtained, the dendrogram obtained with Ward's method presented a greater segregation of clusters in the middle stages and was therefore selected for the HCA. Evaluating the percentage difference in the clustering coefficient to estimate the number of clusters formed (stopping rule), the highest percentage difference was observed to occur between stages 11 and 12 (Table 5). However, solutions of a cluster resulting from the union of 2 very heterogeneous groups presented high values for the stopping rule that are unacceptable (Hair et al., 2009). Thus, the increase

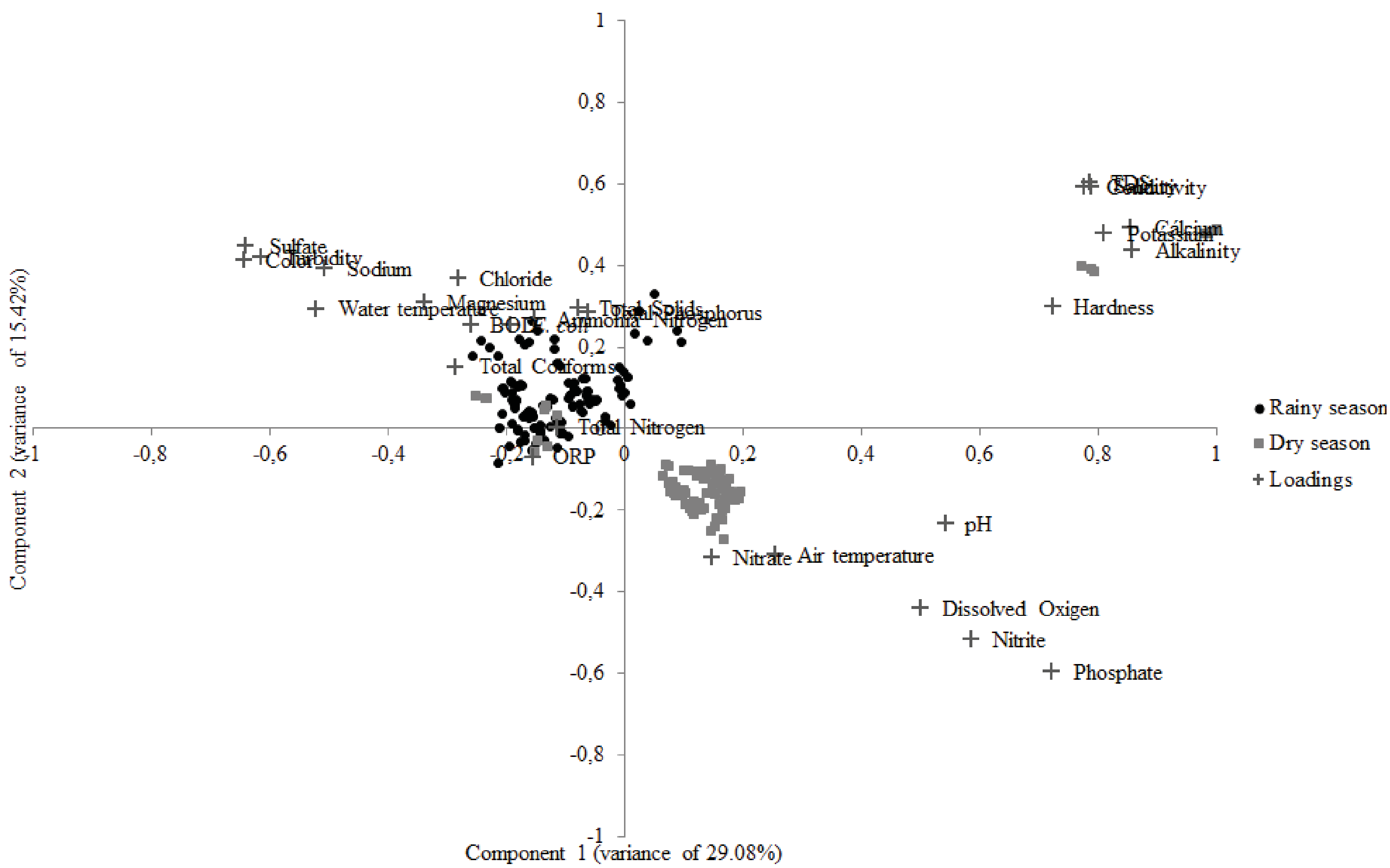

Figure 2. PCA biplot (scores and variables), classified according to seasonality.

registered between stages 7 and 8 was considered to be the highest, with a variation of 30.57%. The cutoff mark was the distance at approximately 225 in the dendrogram obtained by Ward's method (Figure 3), resulting in 5 groups.

Station 1 (Marzagão) differs from the other stations in the dendrogram (Figure 4). There were significant variations in the values of turbidity, color, and total phosphorus, as well as in the concentrations of dissolved salts, as indicated by PCA. These variations, which are most likely attributable to seasonality, contribute to the characterization of a unique station (Figure 5).

The beginning of the operation of the APM-Manso water reservoir in 2001 regulated the water flow in the Cuiabá River. Station 1, which is located upstream from this reservoir, is not affected by this regulation, which has a strong influence on the water quality parameters at sites located downstream from the reservoir, mainly Stations 2-6. At these locations (Stations 2-6), the values of the analyzed parameters were low in comparison to those of other stations. These results are a reflection of the land use and occupation in this stretch, which still has significant conservation of riparian forests and a low degree of anthropization along the banks.

Proximity to point sources of effluent discharge influenced the water quality parameters at Stations 7 and 8, which are located downstream from 3 polluted tributaries that serve as a flow channel for the untreated effluents from Urban Agglomeration. There are also considerable industrial effluent discharges as well as solid waste disposal along this stretch, which suggests that among the uses of this water body, the dilution of effluent is prominent (Zeilhofer et al., 2006). It can be explained by the influence observed in PCA related to component 3 due to high values of *E. coli* and ammonia nitrogen measured in these stations.

Stations 9 to 12 are located along a stretch of the Cuiabá River located downstream from the Urban Agglomeration. These stations presented high concentrations of organic matter but lower concentrations than those recorded in the urban area, which may indicate autodepuration of the organic load coming from the Urban Agglomeration of Cuiabá and Várzea Grande. This phenomenon may occur due to a reduction in the quantity of point sources of effluent discharge in this stretch as well as the small contribution from diffuse sources of pollution, as there are few human activities with significant impact potential in this region.

Station 13 (Porto Cercado) differs from the other stations in this cluster solution because it is located within

Table 5. Explained variance and component matrix of PCA (Varimax rotation) applied for 28 chemical, physical, and biological parameters determined in the 13 stations along the Cuiabá River basin.

Variable	Components							Communalities
	1	2	3	4	5	6	7	
TDS**	**0.994** *	-0.058	0.03	-0.002	0.001	0.012	0	0.992
Conductivity	**0.990***	-0.064	0.022	0.004	0.006	0.013	0.016	0.986
Salinity	**0.982***	-0.053	0.016	-0.02	0.025	0.015	0.029	0.97
Calcium	**0.980***	-0.161	-0.028	-0.051	-0.019	-0.019	0.018	0.99
Alkalinity	**0.948***	-0.185	-0.03	-0.045	-0.073	-0.048	0.039	0.945
Potassium	**0.933***	-0.144	-0.049	0.045	-0.064	-0.056	-0.074	0.908
Total hardness	**0.701***	-0.341	-0.128	0.15	-0.012	0.236	-0.089	0.711
Turbidity	-0.155	**0.839***	-0.017	-0.066	-0.087	0.067	-0.091	0.753
Color	-0.19	**0.769***	-0.091	0.177	0.273	-0.042	-0.115	0.756
Sulfate	-0.153	**0.816***	0.128	-0.139	0.153	0.035	0.025	0.756
Ammonia nitrogen	0.026	0.035	**0.817***	-0.078	0.149	0.052	0.039	0.701
Escherichia coli	-0.006	0.15	**0.797***	0.184	-0.192	-0.137	-0.011	0.747
Chloride	-0.029	0.293	0.055	**0.834***	0.119	0.052	-0.127	0.819
Magnesium	-0.058	0.298	00.029	0.349	**0.735***	-0.098	0.017	0.765
Total phosphorus	0.047	00.022	-0.049	0.107	0.017	**0.834***	-0.054	0.715
Nitrate	-0.067	-00.126	-0.201	0.127	-0.169	-0.116	**0.753***	0.687
Phosphate	0.154	**-0.880***	-0.198	-0.007	-0.082	-0.221	0.164	0.92
Nitrite	0.091	**-0.846***	-0.087	-0.169	0.067	-0.153	-0.226	0.839
ORP**	-0.08	0.24	-0.079	**-.779***	0.051	-0.148	-0.138	0.721
BOD**	-0.127	-0.011	0.471	0.22	0.225	0.538	0.011	0.627
Total solids	0.09	0.206	-0.101	-0.046	-0.096	0.575	-0.026	0.404
Total nitrogen	-0.072	0.101	0.236	-0.06	0.102	0.039	0.659	0.521
Dissolved oxygen	0.093	-0.461	-0.224	0.112	-0.65	-0.157	0.162	0.758
Total coliforms	-0.124	0.333	0.439	0.156	-0.541	-0.054	0.043	0.641
pH	0.277	-0.412	0.011	-0.103	-0.22	-0.125	0.471	0.543
Air temperature	0.043	-0.36	-0.194	-0.382	0.425	-0.382	-0.254	0.705
Water temperature	-0.171	0.471	0.284	-0.359	0.413	-0.093	-0.403	0.803
Sodium	-0.184	0.436	0.182	0.277	0.107	0.425	-0.084	0.533
Sum of squares (eigenvalues)	8.142	4.316	2.687	1.876	1.709	1.383	1.094	
Percentage of trace* **	29.078	15.416	9.596	6.702	6.105	4.938	3.909	**75.742**
Percentage of accumulated trace	29.078	44.494	54.09	60.792	66.897	71.835	75.744	

*Factor loads that indicate a definite structure (value > 70); **ORP: oxidation-reduction potential, BOD: biochemical oxygen demand, TDS: total dissolved solids, ***Trace = 28.0 (sum of eigenvalues).

the floodplain in the Pantanal of Mato Grosso. Among the results presented for this station, low concentrations of DO and low levels of coliforms are highlighted. The increase in organic matter is significant, particularly during the floods in this plain, due to sedimentation of solid particles caused by the decrease in slope, which occurs downstream of the town of Santo Antonio do Leverger (Figueiredo and Salomão, 2009).

The stretches indicated by the HCA are consistent with the spatial gradient indicated in another study (Figueiredo and Salomão, 2009), in which 4 distinct stretches were indicated: I) from Cuiabazinho Springs to Manso Junction (where Station 1 is located); II) after Manso until the beginning of the urban areas of Cuiabá and Várzea Grande (Stations 2 and 6); III) urban areas (Stations 7 and 8); and IV) Pantanal (Station 13). However, the present study suggests a better segmentation of the stretch that begins after the urban area along the Cuiabá River. This study considers a stretch that begins downstream from the urban areas of Cuiabá and Várzea Grange and extends to the urban area of Barão de Melgaço (Stations 9-12) and a stretch that begins within

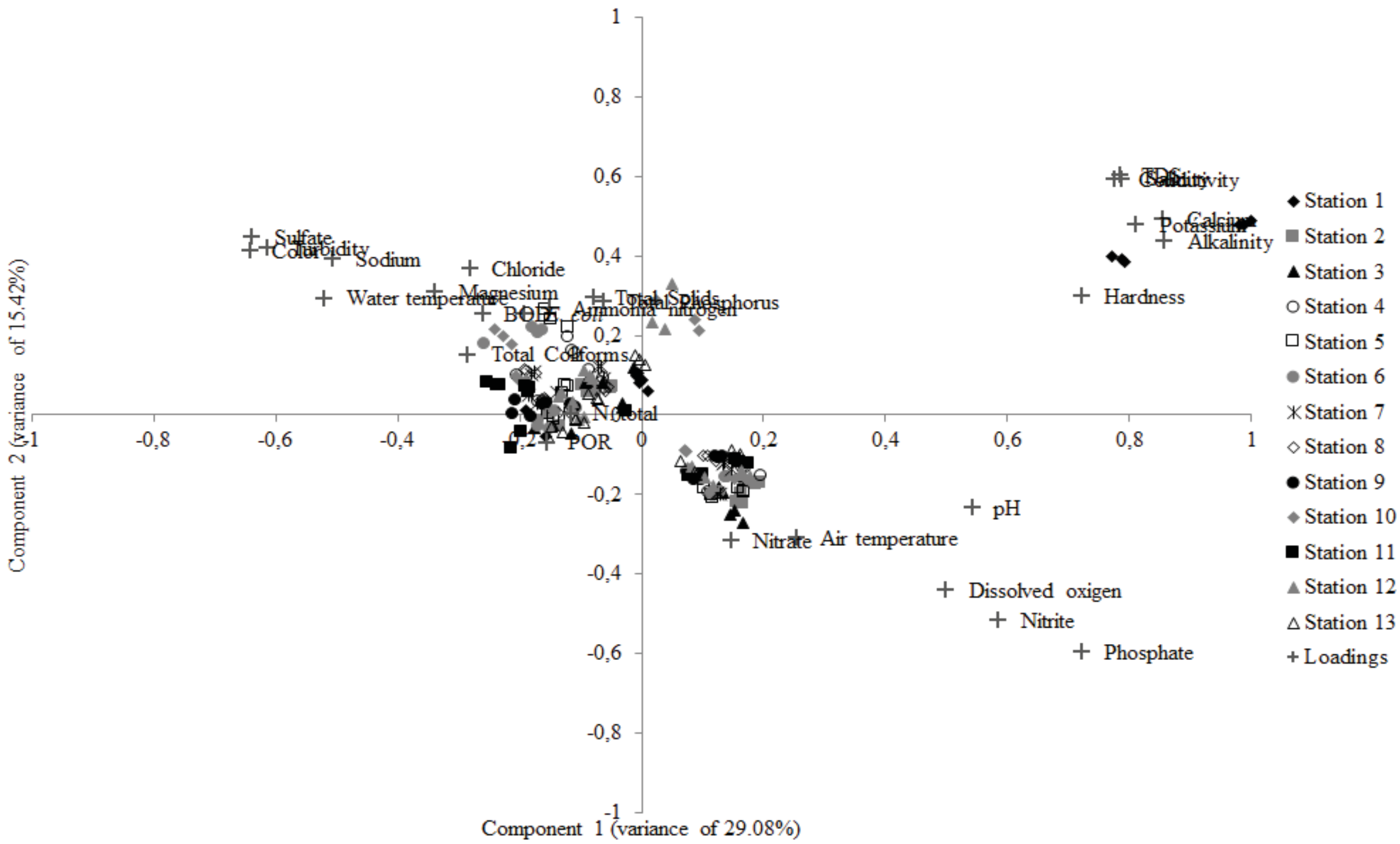

Figure 3. PCA biplot (scores and variables), classified according to the collecting station.

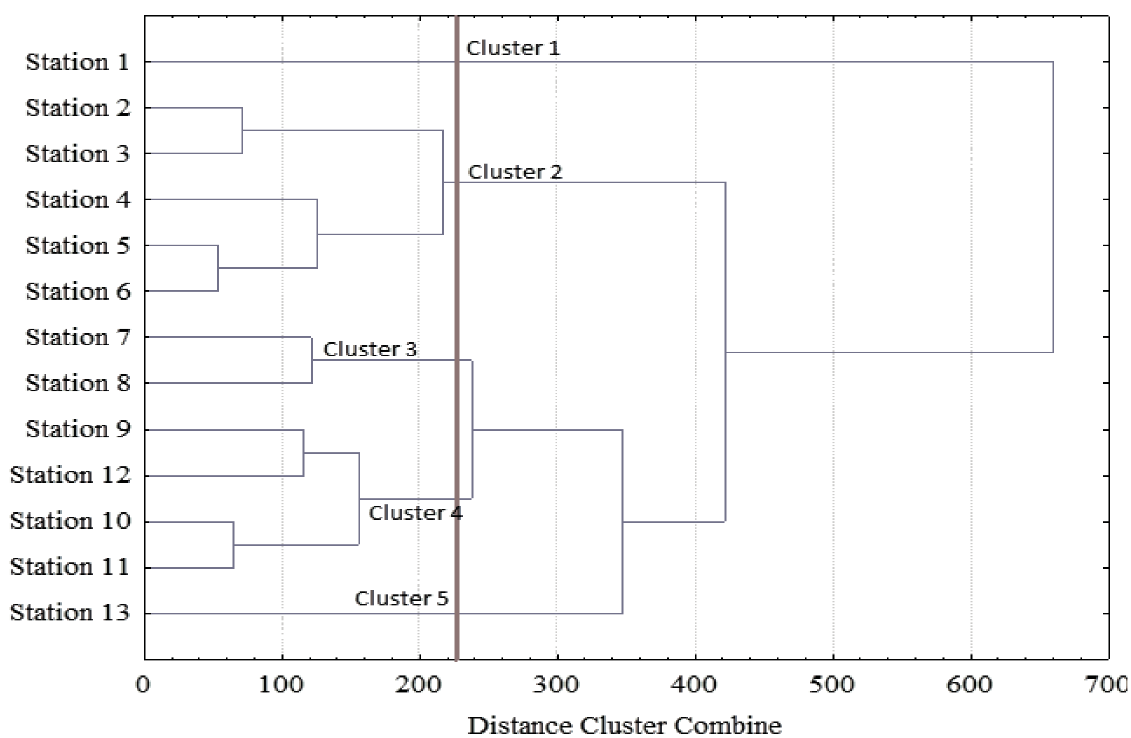

Figure 4. Dendrogram of the 13 sampling stations studied in the Cuiabá River basin.

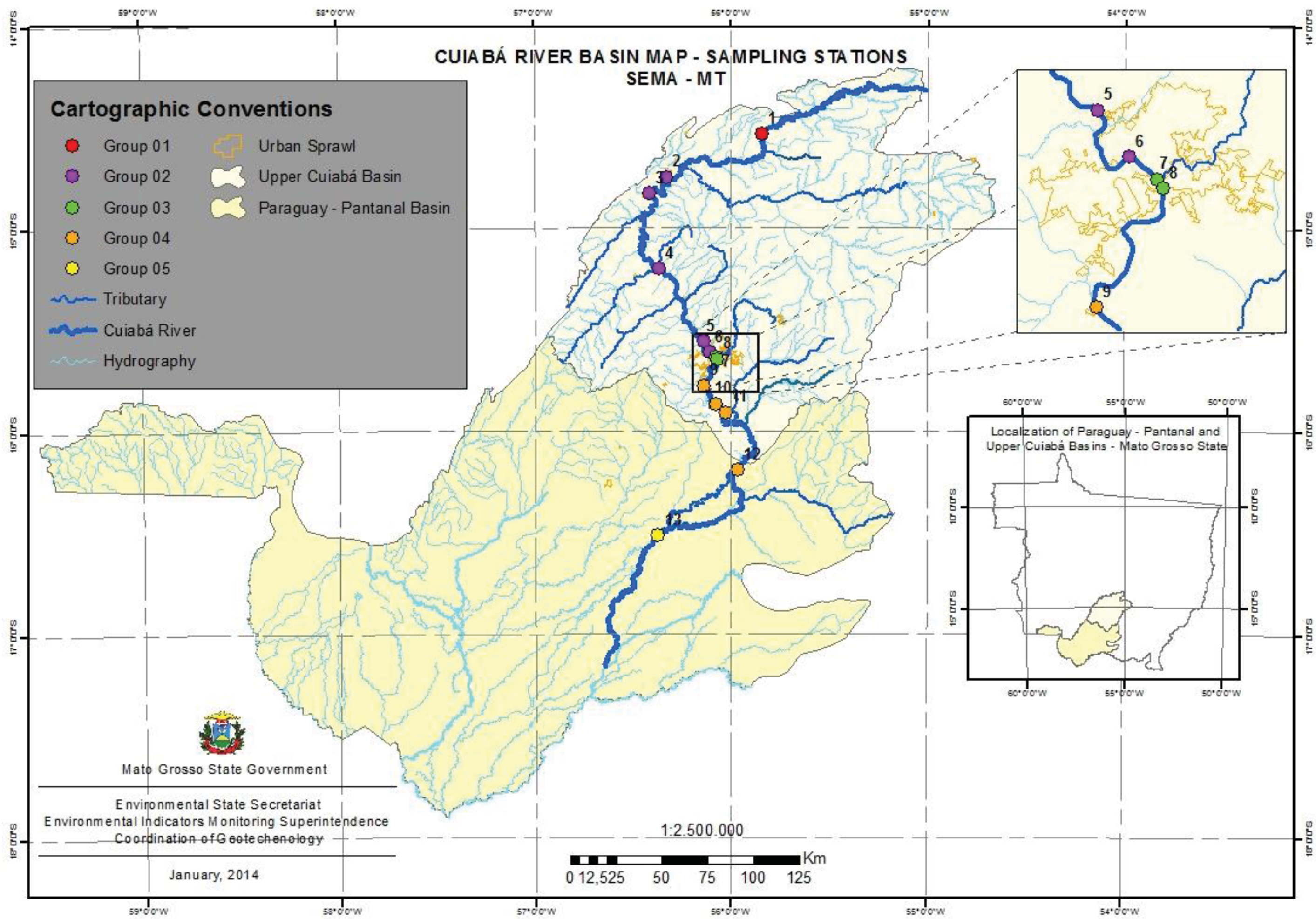

Figure 5. Sampling stations classified according dendogram in the Cuiabá River basin.

the floodplain due to the peculiar behavior of the analytical parameters presented at Station 13. These results will contribute to improved management of the environmental monitoring of the water quality of the studied basin by the Department of Environment of Mato Grosso State (SEMA-MT) and may be useful for a better understanding of other watersheds.

Conclusions

PCA identified 7 components composed of 19 variables that are responsible for the variation in the data from the Cuiabá River basin and that together accounted for 75.74% of the data variation. The 1st axis (29.08%), composed of 7 variables, represents the component dissolved ions, thus reflecting the natural process of weathering of geological components of the soil, particularly in the stretch of the upper Cuiabá River that is characterized by regions containing limestone rocks. The 2nd component (15.42%), composed of 5 variables, was defined by a component associated with human activities that produce diffuse pollution (agricultural and urban) and point sources of effluent discharge. Samples of this sub-basin presented a wide variation in color and turbidity parameters, influenced mainly by the carrying of sediments, which occurs principally during the rainy season, aggravated by the processes of human occupation in the basin.

HCA revealed the existence of 5 clusters of stations that present homogeneous characteristics. Stations 1 and 13 (Marzagão and Porto Cercado) present a high degree of dissimilarity when compared to the other stations and are considered unique clusters. Three other homogeneous groups were identified: II) after the mouth of the Manso River until the beginning of the urban areas of Cuiabá and Várzea Grande (Stations 2-6); III) the urban area (Stations 7 and 8); and IV) after the urban area until the vicinity of Pantanal (Stations 9-12); these groups characterize regions of low, high, and moderate pollution in the basin, respectively. The water quality in Cuiabá River is influenced mainly by natural phenomena, but processes guided by urban agglomerations and diffusion pollution in basin are increasing and contributing for quality degradation. This kind of information is important to improve water management by environmental state agency and regulate the occupation and wastewater discharge in basin.

Conflict of Interest

The authors have not declared any conflict of interest.

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