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Full Length Research Paper

Organomineral fertilization in growth, physiology and phytomass production of castor oil plant BRS energia

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This work aims to study the effect of castor oil plant cake doses (0.0; 1,100; 2,200 and 3,300 Kg ha^{-1}) associated with P doses (0.0 and 90 Kg ha^{-1}) and K doses (0.0 and 60 Kg ha^{-1}) on initial growth period of castor oil plant BRS Energia. The experiment was conducted under greenhouse's conditions at the Embrapa Algodão, located in Brazil. The experimental design of randomized blocks was used in a 4x2x2 factorial arrangement, with 4 replications, totaling 64 experimental units. The castor oil plant cake application influenced the pH, phosphorus (P), potassium (K), magnesium (Mg) and organic matter (M.O.) values in the soil. The variables of growth, height, stem diameter, number of leaves and leaf area increased linearly with the application of castor oil plant doses, as well as for the variables of phytomass production from root, aerial part and total. The doses of castor oil plant cake influenced the nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) contents. The relative chlorophyll content and transpiration increased in a quadratic manner due to the castor oil plant cake application.

Key words: Castor oil plant cake, organic waste, phosphorus, potassium.

INTRODUCTION

Considered a rustic plant with high productive potential, the castor oil plant or castor bean (*Ricinus communis L.*) is an oleaginous belonging to the Euphorbiaceas family and it has been occupied, a prominent place among the main oleaginous plants cultivated in Brazil (Brito et al., 2017). The castor bean can be a profitable alternative for small farmers in the semi-arid region, but it is necessary to solve some problems related to fertilization and

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Table 1. Chemical characteristics of the soil used in the experiment before and after liming, performed at Embrapa-Algodão.

pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	SB	H + Al	CTC	V	P	M.O.
1:2.5	Sortive Complex (mmolcdm^{-3})							%	mg dm^{-3}	g kg^{-1}
Before the liming										
5.4	5.5	2.7	2.3	1.2	11.7	19.2	30.7	38.1	19.1	3.7
After the liming										
6.5	14.6	10.4	9.2	1.2	35.9	n.d	35.9	100	19.1	3.6

Source: Campina Grande-PB, 2013.

Table 2. Physical characteristics of the soil used in the experiment, performed at Embrapa-Algodão.

Textural Composition			Textural Classification (1)	Density		Total Porosity
Sand	Silt	Clay		Soil	Particles	
--------g kg^{-1}--------				--------g cm^{-3}--------		%
79.35	12.18	8.47	Sandy loam	1.58	2.67	40.67

Physical analysis performed at the Soil Laboratory at Federal University of Campina Grande-PB, 2013.

nutrition management which reflects in the increase of productivity.

Most of Brazilian soils present problems related to fertility (Beltrão et al., 2006; Costa et al., 2013; Torres et al., 2016) making it indispensable to know their ability to supply nutrients for plants, as well as improve fertilizer recommendation, aiming for a more efficient and sustainable production over the years. In this sense, it is important the organic matter maintenance allied to conservationist practices to ensure the immobilized nutrients flow to the soil solution, especially N (Olsen et al., 1997).

Thus, the castor oil plant cake is one of the alternatives for organic fertilization sources in the northeast region which is a by-product from oil extraction and has important characteristics such as high N content, acting as a soil conditioner (Severino, 2005; Marques et al., 2010). The viability of the use in organic fertilization can also be attributed to the low C/N ratio (11:1), making it available through nutrient mineralization instantly. Soil incorporation promotes changes in physical, chemical and biological characteristics which improve the structure, increase the water retention capacity, aeration and soil fertility.

Despite all these favorable characteristics to castor oil plant cake, it has low P content which make necessary the use of sources for these elements associated with castor bean cake, resulting in an organomineral compound. Among the three main macronutrients, P is the one that is required in smaller quantities by plants. However, it is an important element in plant metabolism, because it participates in cell energy transfer, respiration and photosynthesis (Furtini et al., 2001).

Thus, the knowledge about the P amount that should be associated with the castor oil plant cake to formulate an organomineral compound as a fertilizer becomes really important for the increase in nutrients and organic matter in the soil. In this sense, this work aims to study the effect of castor oil plant cake doses associated with P and K doses on the initial growth of the castor bean BRS Energia.

MATERIALS AND METHODS

The experiment was carried out in a protected environment, belonging to the Centro Nacional de Pesquisa do Algodão (CNPA) [National Cotton Research Center] of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) [Brazilian Agricultural Research Company] located in the municipality of Campina Grande-PB, with the geographic coordinates 7°13'50" south latitude and 35°53'52" west longitude and approximate altitude of 550 m at sea level.

Substrate was used, a material from the first 30 cm of the Regolithic entisol (soil of the region) that has medium texture, according to the new classification of Embrapa Solos (2009), from the Estação Experimental da Empresa de Pesquisa Agropecuária (EMEPA) [Experimental Station of the Agricultural Research Company] located in the municipality of Lagoa Seca-PB. A compound soil sample was collected and sent to Laboratório de Solos e Nutrição de Plantas da Embrapa-Algodão [Embrapa Algodão's Laboratory of Soils and Plant Nutrition] for chemical analysis, according to Embrapa (1997), whose chemical and physical characteristics are presented in Tables 1 and 2, respectively.

The treatments were distributed in a randomized block design

Table 3. Chemical composition of castor oil plant cake used in the experiment.

Determinations						
U	M.O.	P.B	Cz	N	P	K
----------%----------						
9.27	80.13	50.68	10.40	8.11	1.72	1.77

U= humidity; M.O.= Organic matter; P.B= Crude Protein; Cz= Ashes; N= Nitrogen; P= Phosphor; K= Potassium. Laboratório de Solos e Nutrição de Plantas da Embrapa-Algodão (Laboratory of Soils and Nutrition of Plants of Embrapa-Algodão).

with factorial arrangement 4x2x2 with four doses of castor oil plant cake (00; 1,100; 2,200; 3,300 kg ha^{-1}). It were determined considering the N content in the chemical composition of the cake to provide 0.0; 89.2; 178.4 and 267.6 kg of N/ha, respectively, also two P doses (00 and 90 kg ha^{-1}) and K doses (00 and 60) both determined based on the P and K content present in the soil, using triple superphosphate and potassium chloride as sources. This was made in four replications, totaling 64 experimental units. Each experimental unit was composed of a plastic vase with 35L of capacity and a soil mass of 55.3 kg. At the base of the vase, a 3 cm layer of fine gravel was placed, which was previously washed with running water to facilitate the drainage in the vase. The castor bean cake doses were calculated based on the N content present in its chemical composition (Table 3).

Based on the soil chemical analysis results, the liming was realized with the application of dolomitic limestone for pH correction. The soil was incubated for a period of 20 days, with irrigation until the humidity was raised to 80% of the field capacity, for limestone reaction and consequently, acidity neutralization. After the incubation, the treatments were applied and also the incorporation of the castor oil plant cake doses together with the P and K doses and soil, was incubated for another period of 15 days. After the soil incubation period, five castor seeds of cv. BRS Energia were treated with fungicide, at 3 cm depth and 15 days after emergence and the thinning was performed, leaving the plant more vigorous per vase.

During the experiment, cultural treatments were carried out in the experiment, such as the removal of invasive plants, which were manually removed weekly. In the same way, it was proceeded with the old leaves that senesced in order to avoid the emergence of diseases in the plants. Irrigation was performed daily through the replacement of water lost during evapotranspiration, leaving the vases close to the field capacity. In order to do so, we adopted the vase weighing method, which four vases of each block were weighed, then was made the average of the weighing and the result used for all vases.

At the end of the experiment, 60 days after seedling emergence, the measument of plant height (ALT), stem diameter (DC) and leaf area (AF) were taken. The plant height was determined with the help of a millimeter ruler, measuring from the lap of the plant to the apex. To determine the stem diameter, a digital caliper was used. It measure in the lap of the plant 1 cm from the ground in a previously marked point. A millimeter ruler was used to determine the leaf area by measuring the leaf length and width and then applying the values to the formula:

$$S = 0,2622 \times P2,4248$$ (Severino, 2004)

where:
S= Leaf Area
P= Main leaf vein length

Specific leaf area (AFE) was determined, which relates the leaf surface to the leaf weight, meaning leaf area availability in each leaf gram indicate the leaf thickness.

$$AFE = AF \ (dm^2 g^{-1})$$

where:
AF= leaf area
PF= dry matter weight of the leaf.

The leaf area ratio (RAF) was determined by the relationship between specific leaf area and leaf weight ratio, in other words, represents the leaf area available to occur in photosynthesis.

$$RAF = AF \ (dm^2 g^{-1})$$

where:
AFE = leaf area
PP = dry matter weight of the plant.

The leaf weight ratio (RPF), which is the dry matter fraction produced by photosynthesis, is not used in respiration nor exported to other parts of the plant, which is retained in the leaves and represent how much the plant invested in its production via photosynthesis to the leaves, being a dimensionless calculation.

$$RMF = MF / MP$$

PF= Dry matter weight of the leaf
MT= Dry matter mass of the total plant.

Sixty days after the seedlings emergence, the aerial part was cut from each vase in 1 cm of distance from the soil, separating the plant in aerial part (stem and leaf) and root, which the sum resulted in the total dry mass, determining the relation aerial part - root. So, the vegetable material was washed in running water and then in distilled water, pre-dried in the greenhouse and packed in perforated paper bag. To complete drying, the material was taken to a forced air circulation oven at 65°C until constant weight, and then weighed in an analytical balance of 0.01 g precision to obtain the dry mass.

After determining the dry mass of the aerial part of the plants, it was Spelling eror in a Wiley mill type and mineralized by sulfuric digestion to determine macronutrients (TEDESCO et al., 1995). The photosynthetic capacity, the internal CO2 concentration, the stomatal conductivity, and the transpiration rate were obtained in saturated light using the Infrared Gas Analyzer (IRGA - Infra RedGasAnalyzer) LI-6400 model (LICOR®, Inc., Lincoln, NE , USA) according to the methodology described by Walker (1987) and Prado and Moraes (1997).

Table 4. Summary of the variance analysis and respective mean squares for the chemical attributes of the soil.

Variation source	GL	Mean squares					
		pH	Ca	Mg	P	K	M.O.
Linear Reg.	1	0.15**	26.50^{ns}	53.95**	7592.23**	0.10**	8.51**
Quadratic Reg.	1	0.01^{ns}	11.81^{ns}	1.44^{ns}	447.85^{ns}	0.16^{ns}	2.32*
Cubic Reg.	1	0.03^{ns}	1.55^{ns}	0.36^{ns}	942.22^{ns}	0.09^{ns}	1.56^{ns}
Blocs (B)	3	0.014	9.98	3.37	75.65	0.07	14.0
Cake (T)	3	0.068*	13.29^{ns}	18.58**	2994.13**	0.12^{ns}	4.13**
Phosphorus (P)	1	0.113*	121.27**	3.61^{ns}	62819.15**	1.05**	14.06^{ns}
Potassium (K)	1	0.213**	6.82^{ns}	4.00^{ns}	43.39^{ns}	13.87**	6.12**
T x P	3	0.048*	18.48^{ns}	3.05^{ns}	1325.22**	0.36*	1.33^{ns}
T x K	3	0.063^{ns}	14.77^{ns}	1.62^{ns}	226.12^{ns}	0.09^{ns}	4.88^{ns}
P x K	1	0.0001^{ns}	7.63^{ns}	0.0006^{ns}	152.21^{ns}	0.22^{ns}	5.40^{ns}
T x P x K	3	0.035^{ns}	11.13^{ns}	3.72^{ns}	155.82^{ns}	0.05^{ns}	7.09^{ns}
Residue	45	0.026	10.56	3.43	330.53	0.126	0.601
CV (%)		2.16	18.71	18.35	38.96	20.56	15.71

Potential of hydrogen (pH), calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and organic matter (M.O.), GL - Degree of freedom; ns - not significant; ** and, * meaning 1 and 5% of probability, respectively, by the F test. Source: Campina Grande, PB, 2013.

The relative chlorophyll index (IRC) was determined using a portable chlorophyllometer, Clorofilog 1030®. Before the readings, the instrument was calibrated according to the recommendations found in the manual. Some care was taken with damaged leaves not to used it as sample, or with pest symptoms and disease attack. The determinations were performed in the morning, shading the device to avoid interference of the solar rays. There were realized two readings per plant, one in the leaf of the middle third and the other in the leaf of the upper third of the plant, the leaves was observed with fully developement, avoiding the leaf vein region.

The results were submitted to variance analysis, by the F-test at 5 and 1% probability levels, the averages were compared by Tukey test at 5% of probability; the quantitative treatments were submitted to regression analyzes of greater significance (Pimentel Gomes., 1990), using the software SAS (Statistical Analysis System).

RESULTS AND DISCUSSION

The summary of the variance analysis with the mean squares and their respective significance by the F test with 5% probability for the parameters as soil fertility, potential of hydrogen (pH), calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and organic matter (MO) as a function of the castor oil plant cake, P and K factors are shown in Table 4.

Among the soil fertility variables in the end of the experiment, it was possible to observe that there was a significant effect of the interaction between the factors cake and P for pH, P and K variables. Silva et al. (2012) verified that the levels of residual P, K, Ca and Mg in the soil, with the exception of Ca, were linearly increasing as a function of castor bean cake doses. However, there was a significant response of the Mg and M.O.

Contents as a function of the isolated effect of the castor bean cake doses plus this result was already expected due to the amount of M.O. present in the castor bean cake. Fernandes et al. (2009), using two doses of castor bean cake (0.0 and 10 t ha^{-1}), obtained a significant increase (23 g kg^{-1}) in the M.O. content of the soil with the application of 10 t ha^{-1} castor bean cake relative to the control sample (12 g kg^{-1}).

The Table 5 shows the averages of the variables studied in the soil (pH, Ca, Mg, P, K and M.O.) in function of P and K doses, where it is possible to verify that there was a significant difference between the P doses studied (0.0 and 90 kg ha^{-1}). higher mean values for pH, Ca, P and K with the applicattion of 90 kg ha^{-1} doses was observed, when compared to the control sample (0.0 kg of P) however, there was no statistical difference between the doses worked on the contents of Mg and M.O. in the soil.

The K application did not influence the Ca, Mg and P contents, but it was observed higher mean values with the applicattion of 60 kg ha^{-1} doses for pH, M.O. and K variables. This behavior was already expected for K due to its supply through fertilization (1.2 mmolc dm^{-3}), considered in the average range for most crops (Ribeiro et al., 1999).

The unfolding of P doses within the castor oil plant cake doses showed that, the pH of the soil at the end of the experiment was strongly influenced by the doses of castor bean cake with the decreasing linear adjustment for the absence of P and quadratic for the 90 kg ha^{-1} dose with high coefficients of determination.

In Table 6, the summaries of variance analyses with

Table 5. Averages of soil chemical attributes.

Treatments	Variables analyzed					
	pH	Ca	Mg	P	K	M.O.
Phosphorus doses						
0 Kg ha^{-1} of P	7.46^{b}	15.99^{b}	9.84^{a}	15.33^{b}	1.60^{b}	4.78^{a}
90 Kg ha^{-1} of P	7.55^{a}	18.75^{a}	10.34^{a}	77.99^{a}	1.85^{a}	5.08^{a}
DMS	0.081	1.636	0.932	9.15	0.178	0.390
Potassium doses						
0 Kg ha^{-1} of K	7.40^{b}	17.O04^{a}	9.85^{a}	45.84^{a}	1.26^{b}	4.65^{b}
60 Kg ha^{-1} of K	7.56^{a}	17.70^{a}	10.33^{a}	47.49^{a}	2.19^{a}	5.22^{a}
DMS	0.081	1.636	0.932	9.15	0.178	0.390

Potential of hydrogen (pH), calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K). The averages in the columns followed by same letters do not differ among themselves by the Tukey test. Source: Campina Grande, PB.

Table 6. Summary of variance analyses and respective mean squares for the variables of growth.

Variation source	GL	Mean squares			
		ALT (cm)	DC (mm)	NF	AF (cm²)
Linear Reg.	1	1524.69**	111.99**	15.75**	3399784.57**
Quadratic Reg.	1	43.06^{ns}	3.16^{ns}	0.39^{ns}	20206.62^{ns}
Cubic Reg.	1	3.93^{ns}	0.84^{ns}	0.15^{ns}	18477.12^{ns}
Blocs (B)	3	7.73	3.52	2.80	806114.05
Cake (T)	3	523.89**	38.66**	5.43**	1146156.10**
Phosphorus (P)	1	1410.94**	79.14**	3.51*	2700106.24**
Potassium (K)	1	93.84*	0.32^{ns}	1.89^{ns}	117940.73^{ns}
Contrasts					
T x P	3	24.62^{ns}	1.60^{ns}	3.68**	35208.96^{ns}
T x K	3	30.09^{ns}	2.90^{ns}	0.39^{ns}	125066.43^{ns}
P x K	1	0.03^{ns}	0.43^{ns}	0.76^{ns}	533.61^{ns}
T x P x K	3	23.76^{ns}	1.65^{ns}	1.68^{ns}	165808.20^{ns}
Residue	45	23.41	1.81	0.840	167287.78
CV (%)		13.42	11.05	12.25	39.20

Plant height (ALT), stem diameter (DC) number of leaves (NF) and leaf area (AF) of castor bean plants, GL - Degree of freedom; ns - not significant; ** and, * meaning 1 and 5% of probability, respectively, by the F test. plant height (ALT), stem diameter (DC) number of leaves (NF) and leaf area (AF) of castor bean plants. Source: BRS Energia,Campina Grande-PB, 2013.

the mean squares and their respective significance by F-test at 5% probability for height (ALT), stem diameter (DC), number of leaves (NF) and leaf area (AF) are presented. The statistical analysis revealed significant effect of the interaction between the castor bean doses with P doses only for the number of leaves. However, there was an isolated effect of castor oil plant cake doses for the variables height, stem diameter, number of leaves and leaf area.

The mean values of growth variables analyzed in function of P and K doses are presented in Table 7, where it can be verified that there was a significant difference between the P doses studied (0.0 and 90 kg ha^{-1}). It can be observed that the average values were higher for the variable height, stem diameter, number of leaves and leaf area when fertilized with 90 kg ha^{-1} dose as compared to the control sample (0.0 kg of P). The summary of the variance analysis by the mean squares and their respective significance by F-test at 5% probability for growth measures (specific leaf area, leaf mass ratio and leaf area ratio) are presented in Table 8.

The statistical tests did not indicate a significant

Table 7. Averages for growth variables.

Treatments	Variables analyzed			
	ALT (cm)	DC (mm)	NF	AF (cm^2)
Phosphorus doses				
0 Kg ha^{-1} of P	31.37[b]	11.09[b]	7.25[b]	837.85[b]
90 Kg ha^{-1} of P	40.76[a]	13.32[a]	7.71[a]	1248.65[a]
DMS	2.43	0.67	0.46	205.04
Potassium doses				
0 Kg ha^{-1} of K	34.85[b]	12.28[a]	7.31[a]	1000.33[a]
60 Kg ha^{-1} of K	37.28[a]	12.13[a]	7.65[a]	1086.18[a]
DMS	2.43	0.67	0.46	205.04

Plant height (ALT), stem diameter (DC) number of leaves (NF) and leaf area (AF) of castor oil plants Cv. BRS Energia, in function of phosphorus (P) and potassium (K) doses. The averages in the columns followed by same letters do not differ among themselves by the Tukey test. Source: Campina Grande-PB, 2013.

Table 8. Summary of variance analyses and respective mean squares for the variables of growth components.

Variation source	GL	Mean squares		
		AFE	RMF	RAF
Linear Reg.	1	167.504[ns]	0.056[ns]	507.880*
Quadratic Reg.	1	5247.191[ns]	0.098**	78.721[ns]
Cubic Reg.	1	17.784[ns]	0.004[ns]	61.881[ns]
Blocs (B)	3	2380.489	0.026	348.504
Cake (T)	3	1810.827[ns]	0.053*	216.161*
Phosphorus (P)	1	0.001[ns]	0.038[ns]	1115.560**
Potassium (K)	1	1721.420[ns]	0.003[ns]	0.462[ns]
Contrasts				
T x P	3	2517.383[ns]	0.031[ns]	35.715[ns]
T x K	3	250.933[ns]	0.003[ns]	171.111[ns]
P x K	1	12. 762[ns]	0.0008[ns]	18.705[ns]
T x P x K	3	3312.121[ns]	0.021[ns]	22.428[ns]
Residue		2204.43	0.016	89.65
CV (%)		43.15	27.73	20.29

Specific leaf area (AFE), leaf mass ratio (RAF) and leaf area ratio (RMF) of castor bean plants CV, GL - Degree of freedom; ns - not significant; ** and, * meaning 1 and 5% of probability, respectively, by the F test. Source: BRS Energia, Campina Grande-PB, 2013.

interaction effect between the factors studied for any of the analyzed variables. However, there was a significant effect of the castor oil plant cake only on leaf mass ratio (RMF) and leaf area ratio (RAF). As for P, there was a significant effect only on the leaf area ratio. And for K, no significant effect was observed on any analyzed variables. The growth analysis expresses the morphophysiological conditions of the plant and quantifies the net production, derived from the photosynthetic process being the performance result of assimilatory system during a certain period of time. This performance is influenced by the biotic and abiotic factors of plant (Larcher, 2006). In this specific case, it was more due to the effects of the castor bean cake doses.

According to the literature, low levels of P slows the initial growth of castor bean plants and causes a considerable reduction in productivity (Machineski et al., 2011), because this is one of the main nutrient for this oleaginous plant by participating in important reactions with emphasis on processes related to energy flow,

Table 9. Average of specific variables of castor oil plants Cv.

Treatments	Variables analyzed		
	AFE	RMF	RAF
Phosphorus doses			
0 Kg ha^{-1} of P	108.803^{a}	0.441^{a}	42.485^{b}
90 Kg ha^{-1} of P	108.793^{a}	0.490^{a}	50.835^{a}
DMS	23.64	0.06	4.76
Potassium doses			
0 Kg ha^{-1} of K	23.612^{a}	0.459^{a}	46.575^{a}
60 Kg ha^{-1} of K	113.984^{a}	0.473^{a}	46.745^{a}
DMS	23.64	0.06	4.76

Leaf area (AFE), leaf mass ratio (RAF) and leaf area ratio (RMF). The averages in the columns followed by same letters do not differ among themselves by the Tukey test . Source: BRS Energia, Campina Grande-PB, 2013.

making up the ATP molecule and other molecules that compose some storage substances in seeds such as oils, proteins and carbohydrates. For K, only the height variable was influenced by its application, the highest average was related to the application of 60 kg ha^{-1} doses. No difference was observed between the K doses studied for the variables stem diameter, number of leaves and leaf area. Unsatisfactory P and K levels slows down the plants initial growth and causes considerable reduction on productivity (Severino et al., 2006; Ma et al., 2012).

No significant interaction effect between the cake and K was observed on any of the analyzed variables of growth, also this similar behavior was observed between the P and K factors. However, there was an isolated effect of P factor for all growth variables analyzed, but only the height variable was influenced K doses. Severino (2005) evaluating the effect of increasing potassium doses on production components of BRS Nordestina cultivar, verified an average primary racemic length of 51.28 cm.

The Table 9 presents the mean values of growth variables analyzed in function of the P and K doses, where it can be verified that there was a significant difference between the P doses studied (0.0 and 90 kg ha^{-1}). It was observed that the mean values were higher only for leaf area ratio (RAF) variable when fertilized with 90 kg ha^{-1} doses, compared to the control sample (0.0 kg of P). According to Rodrigues (1982), the leaf area ratio represents the relative size of the photosynthetic apparatus being quite appropriate to evaluate the genotypic, climatic and plant communities effects, so the RAF trend is to decrease from a certain cycle phase in function of the reduction of the useful leaf area (Alvarez et al., 2000). While for K, the 60 kg ha^{-1} doses did not influence any of the analyzed variables. No difference was observed between the K doses studied for the specific leaf area (AFE), leaf mass ratio (RAF) and leaf area ratio (RMF) variables.

By the summary of the variance analysis (Table 10) it was possible to observe the average squares and their respective significance by F-test at 5% probability for the mineral composition variables of plants. It was observed that there was a significant interaction effect spelling bean cake doses and P doses on the nutritional components (N, K, Ca, Mg) in the dry mass of the aerial part of the plant, except for P and S contents. For interaction between K doses and castor bean cake doses, no significant effect was observed between these factors for any of the analyzed variables. Through the analysis of the isolated effects of the studied factors, it was verified that the castor oil plant cake doses significantly influenced the P content in the dry matter of the aerial part of the castor bean plants, this is mainly due to the fact that the castor cake has cake in its chemical composition.

Silva et al. (2012) working with castor bean cake doses as nutrient source to plants, verified higher accumulation of P content in the dry mass of the aerial part of castor bean plants. The statistical analyzes for the isolated P rates showed significant effect only for the N, P, K and S contents. But for K doses, these influenced only P and K contents in the dry mass of the aerial part of the plant (Table 10).

The average values of the plant mineral composition variables (N, P, K, Ca, Mg and S) are presented in Table 1111 in function of P and K doses, where it is possible to verify that there was a significant difference between the P doses analyzed (0.0 and 90 kg ha^{-1}). The mean values for the variables N, P, K and S were higher than the control group (0.0 kg of P) with the application of 90 kg ha^{-1} doses. However, there was no statistical difference between the doses that studied about Ca and Mg levels

Table 10. Summary of variance analyses and respective mean squares for the the nutritional components of the aerial part of castor oil plant.

Variation source	GL	Mean squares					
		N	P	K	Ca	Mg	S
Linear Reg.	1	0. 552*	0.074^{ns}	0.105	0.756*	0.136*	0.0003^{ns}
Quadratic Reg.	1	0.068^{ns}	0.191**	0.160	0.079^{ns}	0.031^{ns}	0.0047^{ns}
Cubic Reg.	1	0.124^{ns}	0.007^{ns}	0.098	0.020^{ns}	0.364^{ns}	0.0028^{ns}
Blocs (B)	3	0.164	0.0003	0.071	0.093	0.020	0.0013
Cake (T)	3	0.248^{ns}	0.0911*	0.121*	0.285*	0.056*	0.0026^{ns}
Phosphorus (P)	1	1.822**	0.3969**	1.050**	0.122^{ns}	0.037^{ns}	0.0236*
Potassium (K)	1	0.216^{ns}	0.5292**	13.875**	6.825^{ns}	0.011^{ns}	0.00001^{ns}
Contrasts							
T x P	3	1.170**	0.0582^{ns}	0.367**	18.485**	0.026^{ns}	0.0029^{ns}
T x K	3	0.258^{ns}	0.0388^{ns}	0.091^{ns}	14.777^{ns}	0.006^{ns}	0.0047^{ns}
P x K	1	0.573^{ns}	0.0182^{ns}	0.225^{ns}	7.631^{ns}	0.038^{ns}	0.00097^{ns}
T x P x K	3	0.250^{ns}	0.0079^{ns}	0.054**	11.135^{ns}	0.067*	0.0083^{ns}
Residue		0.113	0.026	0.042	0.107	0.021	0.004
CV (%)		20.49	23.50	20.56	11.58	18.65	22.46

Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), GL - Degree of freedom; ns - not significant; ** and, * meaning 1 and 5% of probability, respectively, by the F test. Source: Campina Grande-PB, 2013.

Table 11. Average of nutritional variables.

Treatments	Variables analyzed					
	N	P	K	Ca	Mg	S
Phosphorus doses						
0 Kg ha^{-1} of P	1.473^{b}	0.297^{b}	1.600^{b}	2.793^{a}	0.814^{a}	0.266^{b}
90 Kg ha^{-1} of P	1.810^{a}	0.454^{a}	1.856^{a}	2.880^{a}	0.765^{a}	0.305^{a}
DMS	0.169	0.082	0.178	0.165	0.074	0.032
Potassium doses						
0 Kg ha^{-1} of K	1.584^{a}	0.285^{b}	1.706^{b}	2.802^{a}	0.803^{a}	0.285^{a}
60 Kg ha^{-1} of K	1.700^{a}	0.466^{a}	1.942^{a}	2.881^{a}	0.776^{a}	0.286^{a}
DMS	0.169	0.082	0.102	0.165	0.074	0.032

Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), in function of phosphorus (P) and potassium (K). Source: Campina Grande-PB, 2013.

in the aerial part of the plant. For K doses (Table 11), higher mean values with 60 Kg ha^{-1} doses only for P and K variables were observed. Table 12 presented the summary of the variance analysis by the mean squares and their respective significance by F-test at 5% probability for the physiological variables of photo-synthesis (Fot), transpiration (TRmmol), internal CO_2 concentration (Ci), stomatal conductance (Cond) and relative chlorophyll index (IRC).

Based on the statistical tests results, there was no significant interaction effect between the factors studied (bean cake, phosphorus and potassium) for some of the analyzed variables. However, there was a significant isolated effect of the castor bean on the transpiration variables and relative index of chlorophyll. A significant effect in the photosynthesis and transpiration variables for P was observed. Meanwhile, there was no significant effect between K and any of the variables analyzed.

The average values for physiological variables (photosynthesis, transpiration, internal CO_2 concentration, stomatal conductance and relative chlorophyll index) due to P and K doses are shown in Table 13, where it

Table 12. Summary of variance analyses and respective mean squares for various variables.

Variation source	GL	Fot	Mean squares			
			TRmmol	Ci	Cond	IRC
Linear Reg.	1	47.555^{ns}	0.002^{ns}	2.048^{ns}	0.0002^{ns}	0.0206^{ns}
Quadratic Reg.	1	0.714^{ns}	2.250*	0.010^{ns}	0.021^{ns}	0.0213**
Cubic Reg.	1	8.153^{ns}	2.363^{ns}	119.805^{ns}	0.012^{ns}	0.0069^{ns}
Blocs (B)	3	27.178	9.544	4464.302	0.087	0.008331
Cake (T)	3	191.338^{ns}	1.538*	40.621^{ns}	0.011^{ns}	0.016322**
Phosphorus (P)	1	121.385**	3.285*	208.080^{ns}	0.028^{ns}	0.000014^{ns}
Potassium (K)	1	6.786^{ns}	1.995^{ns}	2457.680^{ns}	0.016^{ns}	0.000002^{ns}
Contrasts						
T x P	3	18.807^{ns}	0.698^{ns}	3851.708^{ns}	0.007^{ns}	0.000656^{ns}
T x K	3	26.246^{ns}	3.713^{ns}	2095.541^{ns}	0.014^{ns}	0.000627^{ns}
P x K	1	12.311^{ns}	3.027^{ns}	1907.507^{ns}	0.022^{ns}	0.011827^{ns}
T x P x K	3	11.363^{ns}	2.686^{ns}	3142.196^{ns}	0.023^{ns}	0.005843^{ns}
Residue		32.208	0.564	1968.878	0.010	0.14713
CV (%)		22.39	19.03	20.88	31.44	11.25

photosynthesis (Fot), transpiration (TRmmol), internal CO_2 concentration (Ci), stomatal conductance (Cond) analyzed with IRGA device, and relative chlorophyll index (IRC). Source: Campina Grande-PB, 2013.

Table 13. Average of various variables.

Treatments	Variables analyzed				
	Fot	TRmmol	Ci	Cond	IRC
Phosphorus doses					
0 Kg ha^{-1} of P	23.621^{b}	3.720^{b}	216.237^{a}	0.350^{a}	0.508^{a}
90 Kg ha^{-1} of P	27.079^{a}	4.173^{a}	214.253^{a}	0.308^{a}	0.507^{a}
DMS	2.857	0.378	22.342	205.04	0.028
Potassium doses					
0 Kg ha^{-1} of K	23.973^{a}	3.770^{a}	219.416^{a}	0.313^{a}	0.508^{a}
60 Kg ha^{-1} of K	26.727^{a}	4.123^{a}	211.066^{a}	0.345^{a}	0.508^{a}
DMS	2.857	0.378	22.342	205.04	0.028

Photosynthesis (Fot), transpiration (TRmmol), internal CO_2 concentration (Ci), stomatal conductance analyzed (Cond) and relative chlorophyll index (IRC) variables in function of phosphorus (P) and potassium (K) doses.The average in the columns followed by same letters do not differ among themselves by the Tukey test. Source: Campina Grande-PB, 2013.

was possible to observe that there was a significant difference between the P doses studied (0.0 and 90 kg ha^{-1}).

The analysis revealed that the average values were higher for photosynthesis and transpiration variables with the application of 90 kg ha^{-1} dose than the control sample (0.0 kg of P) for these variables. However, there was no statistical difference between P doses worked on the other analyzed variables. For K, the statistical analysis did not indicate any significant effect of this element on any of the analyzed variables. The summary of the variance analysis by the mean squares and their respective significance by F-test at 5% probability for the production variables (root dry mass, shoot dry mass and total dry mass) are presented in Table 14.

Among the variables of phytomass production evaluated at the end of the experiment, it was verified that there was no significant interaction effect between the cake and P factors for none of the analyzed variables. Meanwhile, there was an isolated effect of castor bean doses for variables, root dry mass, dry shoot mass and total dry mass. The statistical analyzes also revealed an isolated effect of P rates for these phytomass production variables (Xie et al., 2014). However, no significant effect

Table 14. Summary of the variance analysis and their respective mean squares for growth components.

Variation source	GL	Mean squares		
		MSR (g)	MSPA (g)	MST (g)
Linear Reg.	1	93.48**	1475.16**	2311.35**
Quadratic Reg.	1	15.13*	3.90^{ns}	34.39^{ns}
Cubic Reg.	1	0.67^{ns}	1.41^{ns}	4.04^{ns}
Blocs (B)	3	5.62	113.43	151.43
Cake (T)	3	36.43**	493.49**	783.26**
Phosphorus (P)	1	110.77**	1652.01**	2618.36**
Potassium (K)	1	0.12^{ns}	8.67^{ns}	6.70^{ns}
Contrasts				
T x P	3	2.93^{ns}	21.12^{ns}	37.27^{ns}
T x K	3	6.71^{ns}	64.34^{ns}	105.06^{ns}
P x K	1	0.001^{ns}	105.72^{ns}	105.01^{ns}
T x P x K	3	1.37^{ns}	35.23ns	40.33^{ns}
Residue		2.82	43.07	55.31
CV (%)		32.97	36.15	31.98

Root dry mass (MSR), shoot dry mass (MSPA) and total dry mass (MST) of castor bean CV, GL - Degree of freedom; ns - not significant; ** and, * meaning 1 and 5% of probability, respectively by the F test. Source: BRS Energia, Campina Grande-PB, 2013.

Table 15. Average of the production variables.

Treatments	Variables analyzed		
	MSR (g)	MSPA (g)	MST (g)
Phosphorus doses			
0 Kg ha^{-1} of P	3.78^{b}	13.07^{b}	16.85^{b}
90 Kg ha^{-1} of P	6.41^{a}	23.23^{a}	29.65^{a}
DMS	0.84	3.30	3.74
Potassium doses			
0 Kg ha^{-1} of K	5.05^{a}	17.78^{a}	22.93^{a}
60 Kg ha^{-1} of K	5.14^{a}	18.52^{a}	23.57^{a}
DMS	0.84	3.30	3.74

Root dry mass (MSR), shoot dry mass (MSPA), total dry mass (MST), in function of phosphorus (P) and potassium (K) doses.The averages in columns followed by same letters do not differ among themselves by the Tukey test. Source: Campina Grande-PB, 2013.

of K doses was observed on the variables analyzed, as well as on the interaction between K doses and castor bean cake. For Phosphorus application on the castor bean fertilization, it was observed that there was a significant differences for root dry mass, shoot dry mass and total dry mass of castor bean plants (Table 15). The highest results for this variable were observed when 90 kg ha^{-1} of P_2O_5 was applied. Generally, poor soils in P are responsive to phosphate fertilization. Phosphorus is one of the most important macronutrients for vegetative growth because it participates in the formation of important enzymes involved in the absorption process of N and in the energy consumption in the form of ATP (Marschner, 2005).

Brito Neto et al. (2017) found that P is essential for the good development and increase of castor bean production because, P participates in the formation of fatty acids and seed filling. The results obtained in this work confirm that the supply of adequate doses of phosphorus from the beginning of development

stimulates the root development which is important for the formation of the primordia of the reproductive parts, essential for good formation of fruits and thus increasing the production of this oilseed and other nutrients availability, management of fertilization (form, type and application time), soil sampling form and plant age (Boem et al., 2011; Silva et al., 2012b; Costa et al., 2013).

The average test did not show a significant difference between the K doses for the phytomass production variables (root dry mass, shoot dry mass and total dry mass). The nitrogen fertilization facilitated a faster growth and higher dry matter mass production because N promoted higher root growth, higher photosynthetic efficiency and increased leaf area (Corsi,1993; Brito Neto et al., 2014).

Conclusion

The castor bean cake reduced the pH of the soil and increased the P, Mg, K and M.O. levels from soil; the mineral composition of the castor bean was significantly influenced by the castor bean cake doses with the linear increment for N, P, K, Ca, and Mg contents. The water type did not influence significantly the number of leaves variable for both cultures being themselves.

Physiological variables, transpiration and relative chlorophyll index were influenced by castor bean cake doses. The addition of castor bean cake influenced significantly the root, shoot and total phytomass production of castor bean plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Improving salt tolerance and weight percent reduction in tomato by exploiting physio-agronomic seedling traits

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Salinity being a serious limitation to crop production is an established fact since ages. It has adversely affected the adaptive behavior of our field crops particularly at seed germination and seedling stages. Identification of particular plant traits conferring salinity tolerance is important for inducing genetic variation among the target traits and adjusting the selection pressure for them in field. This experimental study was conducted to explore percent (%) weight loss of roots and shoots at increasing salt stresses (control, 10 dS m^{-1} and 15 dS m^{-1}) along with certain dry weight and cationic ratio tolerance indices. The experiment was conducted in the glasshouse to screen seedlings of 25 tomato genotypes. Principal component analysis and correlation analysis were used to screen the genotypes for variability and salt tolerance. Based on associative interactions for salt tolerance traits and highly negative response towards weight percent (%) reductions, three genotypes were identified as salt tolerant; BEAVER LODGE SLICER, ZARNITZA, and FORME DE COEUR. Two genotypes GLACIER and Rio-GRANDE were highly positive for K^+/Na^+ and Ca^{2+}/Na^+ ratios tolerance indices. Based on these findings, the genotypes BEAVER LODGE SLICER, ZARNITZA and FORME DE COEUR are suggested to be planted in salt affected area. The six genotypes (ANAHU, LO-2707, 17860, UOVO ROSEO, NAGINA and LA-2821) showed significant negative behavior towards weight % reduction, and a little positive towards salt tolerance indices were considered as moderately salt tolerant.

Key words: Weight percent reduction, NaCl, tomato seedling, physio-agronomic, salt tolerance.

INTRODUCTION

Over 800 million hectares of land (>6%), including one third portion of the cultivated land, throughout the world are salt affected (FAO, 2008; Naz et al., 2010; Kosová et al., 2013). Worldwide, out of 230 million ha of total irrigated land, 45 million ha (about 20%) are salt affected (FAO, 2008). Although there is merely 15% land that is irrigated out of total cultivated, but it is producing world's one third food and has productivity twice in contrast to rainfed (FAO, 2008; Kosová et al., 2013). Approximately 2% (32 million ha) of 1500 million ha dryland used for

agricultural purposes is affected with varying degrees of secondary salinity (Munns and Tester, 2008).

Field vegetables like tomato (*Solanum lycopersicum* L.) are prominently found in arid and semiarid climates where salinity is a major problem (Qadir et al., 2006; Azevedo-Neto et al., 2006). In semiarid and arid regions, salts move from basal rocks and accumulate over the upper layer of soil because of prevalent water evaporation (Kosová et al., 2013). Saline areas continue to expand in semiarid and arid regions due to improper cultural practices, use of saline water, insufficient irrigation and excessive fertilization; which consequently promises a decline in crop production over the period (Shahid et al., 2012).

Globally, irrigated and cultivated land areas of arid and semi-arid regions have limited agricultural productivity mainly because of saline conditions (Azevedo-Neto et al., 2006; Nawaz et al., 2010; Shahid et al., 2012; Kosová et al., 2013; Maurya and Gothandam, 2014)) along with other stresses (Abbas et al., 2010; Bhantana and Lazarovitch, 2010; Siringam et al., 2012). Fifty percent of the total irrigated arable land is undergoing the salinization, and this area contributes to one third of the total global food productivity (Munns, 2002; Munns and Tester, 2008). Under saline environment, plant growth reduction has been reported in tomato in a number of studies (Romero-Aranda et al., 2001; Fujita et al., 2006).

Saline soils composition varies due to different types and concentrations of salts where plants show specific responses against particular salts at their different developmental stages (Cuartero et al., 2006). Tomato (*S. lycopersicum* L.) also unevenly tolerates salt stresses at altered growth stages though it has natural sensitivity at its seedling stage (Al-Taisan 2010). Principally, if the seedling stage has been adversely affected with saline conditions, this could limit plant growth and will translate into poor economic yield (Maas, 1986). Although plants differ in their ability to cope with adverse saline conditions (Kosová et al., 2013), there are certain attributes to assess salinity tolerance that is, reduction in rate of plant relative growth (biomass reduction) or as survival of plant (index of salt tolerance), at defined concentrations of salts (Munns, 2002).

Higher salinity causes serious, and in many cases, irreversible damage to the plants. It includes stomatal closure and reduction in leaf expansion due to deficient osmotic conditions, overall drop in photosynthesis and biomass production (Rahnama et al., 2010; James et al., 2011). Both Cl^- and Na^+ ions in excessive forms are a unique cause of leaf scorching and firing that leads to stunted growth of plants (Shannon et al., 2000). Elevated levels of Na^+ may be responsible for shortage of other essential elements and osmolytes such as Ca^+ and K^+, and could disturb K^+-dependent processes which eventually lead to conformational changes in proteins (Mahajan and Tuteja, 2005).

To identify salt tolerant genotypes a selection criterion should be devised that best explains the behavioral retort of genotypes over multiple saline conditions. Formerly, physio-agronomic plant traits; (K^+/Na^+ and Ca^{2+}/Na^+ ratios) (Dasgan et al., 2002; Juan et al., 2005; Ahmadi et al., 2009; Turhan and Seniz, 2012), root fresh and dry weights, shoot fresh and dry weights at early plant stages were preferred as screening criterion for salt tolerance (Ibrahim, 2003). Shoot biomass production under salinity (Kumar et al., 2012; Bolarin et al., 1991; Foolad, 1996) and selectivity of K^+ over Na^+ are some of the best salt tolerance indicators to study cultivated and even wild species of tomato (Cuartero et al., 1992).

This study aims to phenotype the seedlings based on numerically descriptive parameters such as weight % reductions of roots and shoots along with tolerance indices that will be derived from dry weights and inorganic osmolytes (K^+, Na^+ and Ca^{2+}), collectively to find salt tolerant tomato genotypes.

MATERIALS AND METHODS

Plant materials

The germplasm consisting of 25 tomato genotypes was collected from Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (UAF) and Vegetable Research Institute (VRI), Faisalabad, Punjab, Pakistan.

Saline soil preparation, layout and growth conditions

The experiment was conducted in a glasshouse at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (31°26'00.6" N 73°04'19.6" E). Seeds of each genotype were surface sterilized with 2% bleach by dipping for 5 min, and then washing with distilled water. The seedlings were evaluated to record their response against salinity by artificially producing three levels of salinity (S_0 = control, S_{10} = 10 dS m^-1 and S_{15} = 15 dS m^{-1}) in soil media. A homogenous mixture of sand to silt (50:50) ratio was used as control having 1.7 dS m^{-1} salinity, but to prepare other two salinity levels, exact amount of NaCl salt was determined using the formula of U.S. Soil Salinity Lab (1954):

$$NaCl\ salt\ per\ kg\ of\ soil = \frac{TSS \times eq.\ wt.\ of\ NaCl\ salt \times SP}{1000 \times 100}$$

(*TSS* is total soluble salts and *SP* is saturation percentage of soil)

The calculated amounts of salt (one for each S_{10} and S_{15}) were

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mixed separately with the soil media by mechanical ways. Salinity of each level was confirmed after power-driven mixing of soil and salt. Small pots (with capacity of 1 kg soil media, height of 6 inches and diameter of 4 inches) were filled with prepared saline soil. Thirty days old healthy tomato seedlings from October sown nursery were selected for transplantation. Transplanting was done under controlled conditions and 10 plants per pot were maintained. The experiment had three replications in a randomized complete block design with factorial arrangements. A total of 75 (25 genotypes × 3 salinity levels) treatments were prepared for each replication. The experiment was subjected to controlled conditions (humidity 60-70%, temperature 23±3 °C and photoperiod of 12±1 hours) within glasshouse. Irrigations were applied at 60% field capacity of the soil mixture on weekly basis before permanent wilting point (8.2% moisture) arrived. There was no application of fertilizers at all. Data of the following traits was recorded after 60 days of seedling transplantation.

Scoring the seedling tolerance

Fresh and dry weight percent (%) reductions (FWPR and DWPR)

Ten plants were chosen randomly from each treatment within each replication and uprooted carefully after heavy irrigation to minimize any root loss. Plants were washed with tap water, dried with paper towel and cut into shoots and roots with particularity at crown (the root-shoot junction). Fresh roots weight (FRW) and fresh shoots weight (FSW) were measured with a digital balance. Average values of FRW and FSW were calculated for each treatment. Roots and shoots of each plant were first sundried in paper bags for 3 days then placed in an oven (70°C) for 72 h for complete drying. Dry roots weight (DRW) and dry shoots weight (DSW) were recorded. Average values of DRW and DSW were calculated for each treatment. FWPR and DWPR were determined using the following formula as given by El-Goumi et al. (2014):

$$FWPR\ \% = 100 \times [1 - (FW_{salt\ stress}/FW_{control})]$$

$$DWPR\ \% = 100 \times [1 - (DW_{salt\ stress}/DW_{control})]$$

Where:

FW = Fresh weight of roots or shoots, and *DW* = Dry weight of root or shoot

Taking in consideration the aforementioned formula, four types of FWPRs were calculated including two fresh shoot weight % reductions ($FSWPR_{10}$ using observations of S_{10} and S_0, and $FSWPR_{15}$ using observations of S_{15} and S_0) and two fresh root weight % reductions ($FRWPR_{10}$ using observations of S_{10} and S_0, and $FRWPR_{15}$ using observations of S_{15} and S_0). Four types of DWPRs were also calculated including two dry shoot weight % reductions ($DSWPR_{10}$ using observations of S_{10} and S_0, and $DSWPR_{15}$ using observations of S_{15} and S_0) and two dry root weight % reductions ($DRWPR_{10}$ using observations of S_{10} and S_0, and $DRWPR_{15}$ using observations of S_{15} and S_0).

Ratios of K^+/Na^+ and Ca^{2+}/Na^+

Na, K and Ca concentrations were determined from cell sap. Cell sap was extracted from leaves according to Ghanem et al. (2010). Five plants from each treatment within each replication were randomly selected, their leaves were harvested chopped into small pieces separately, and placed in perforated falcon tubes for flash freezing in liquid N. The samples were then thawed at room temperature to rupture cell membranes. The freezing-thawing cycle was repeated three times, and tubes were encased in an intact second falcon tube which was centrifuged at 9000 rpm at 4°C for 10 min. The collected sap was diluted 40 times and concentration of Na^+, K^+ and Ca^{2+} was determined by Sherwood Flame Photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK) and average concentration was calculated for each replication. Ratios of K^+/Na^+ and Ca^{2+}/Na^+ were calculated after concentrations of all three elements were determined from cell sap of same plant. Average values of both of ratios (K^+/Na^+ and Ca^{2+}/Na^+) for each replication were calculated from all five plants. These inorganic osmolytic ratios of tomato seedlings were further used to determine tolerance indices.

Tolerance indices

Tolerance indices being individual genotypic response towards salt treatments were calculated using the formula of LaRosa et al. (1989):

$$Tolerance\ index\ (TI) = 100 + \Sigma^n\ [X\ (T_x/T_0)100]$$

Where:

n = number of salinity levels;
X = NaCl concentration (g L^{-1}) in soil;
T_x= value of seedling trait on stressed plants;
T_0= value of seedling trait on control plants.

Four types of tolerance indices were determined for every genotype including two dry weight tolerance indices (RDWTI for roots and SDWTI for shoots) and two ratio based K^+/Na^+ and Ca^{2+}/Na^+ tolerance indices (K.Na.TI and Ca.Na.TI) following the study of Turhan and Seniz (2012).

Statistical analysis

After taking data for all the seedling traits, it was subjected to analysis of variance (ANOVA) following the study of Steel et al. (1997) to sort out significant differences among genotypes using their interactions with salinity levels subsequent of complete randomized design. Using RStudio software Version 0.98.1102, RStudio, Inc. (R Core Team), principle component analysis (PCA) was performed to obtain more reliable information on how to identify groups of genotypes that have desirable salt tolerance traits for breeding. The graphical data representation of salinity and plant interactions whenever provided by PC-biplot is so informative that it requires only a look to understand the potential salt tolerance of genotypes (Raza et al., 2016). PCA was obtained following the method as given by Husson et al. (2011).

RESULTS AND DISCUSSION

Variability in germplasm and associations among seedling tolerance traits

Hitherto, different crop species were observed under saline conditions for fresh and dry weights of roots and shoots (Liem et al., 1985; Azhar and McNeilly, 1989; Noori and McNeilly, 2000; Akinci et al., 2004) along with salt affected cationic ratios (K^+/Na^+ and Ca^{2+}/Na^+) (Dasgan et al., 2002; Juan et al., 2005; Ahmadi et al.,

Table 1. Mean square table for seedling tolerance traits.

Source of variation	Genotype	Replication	Error
Degree of freedom	24	2	150
Fresh shoot weight % reduction at S_{10} ($FSWPR_{10}$)	3732.80**	3.200	0.600
Fresh shoot weight % reduction at S_{15} ($FSWPR_{15}$)	8430.90**	0.500	1.800
Dry shoot weight % reduction at S_{10} ($DSWPR_{10}$)	21135.30**	27.800	2.900
Dry shoot weight % reduction at S_{15} ($DSWPR_{15}$)	23524.90**	23.000	7.000
Fresh root weight % reduction at S_{10} ($FRWPR_{10}$)	14335.40**	0.600	1.800
Fresh root weight % reduction at S_{15} ($FRWPR_{15}$)	12952.20**	1.300	2.700
Dry root weight % reduction at S_{10} ($DRWPR_{10}$)	189644.00**	206.000	23.000
Dry root weight % reduction at S_{15} ($DRWPR_{15}$)	46445.00**	82.200	9.300
K^+/Na^+ ratio tolerance index (K.Na.TI)	17.96**	111.620	1.080
Ca^{2+}/Na^+ ratio tolerance index (Ca.Na.TI)	131.18**	120.420	1.150
Shoot dry weight tolerance index (SDWTI)	8.84**	108.190	1.050
Root dry weight tolerance index (RDWTI)	4.90**	107.220	1.027

DF indicates degrees of freedom; ** indicates significance at 1% level; S_{10} is salinity level of 10 dSm^{-1}; S_{15} is salinity level of 15 dSm^{-1}.

2009; Turhan and Seniz, 2012), that are maintained by plants. These physio-agronomic plant traits were used as worthy indicators of salt tolerance (Ibrahim, 2003) for screening tomato genotypes at seedling stages. These traits were further employed to get attributes of salt tolerance that is, weight % reductions of roots and shoots at increasing salinity levels (S_{10} and S_{15}) were recorded following El-Goumi et al. (2014) and salt tolerance indices according to Turhan and Seniz (2012).

Genotype as a source of variation was found highly significant ($p < 0.01$) for all weight % reductions (El-Goumi et al., 2014) and tolerance indices (Turhan and Seniz, 2012) (Table 1) giving an indication of a diverse genetic variability that suits in identifying tolerant and susceptible tomato genotypes. Different genotypes failed to respond in a definite and predictable response (Akinci et al., 2004) in terms of weight % reductions and tolerance indices on a given saline media. While comparing means, some genotypes had negative values of weight % reduction which means no loss of mean plant weight due to harsh saline conditions, rather an increase in overall biomass production.

One group of these genotypes including ZARNITZA, BEAVER LODGE SLICER, FORME DE COEUR, GLACIER and LO-2707, which had no weight % reduction of root and shoot at both salinity levels other than control. Second group included the genotypes (ANAHU, Rio-GRANDE, UOVO ROSEO and 17860) that showed significantly increased biomass but at highest salinity level (S_{15}). Third group consisting of NAGINA, ROMA, BL-1079, 6232, NUTYT-701 and LA-1021 genotypes produced a high fresh and dry biomass at both S_{10} and S_{15} level of salinity compared to control. All other genotypes were representative of a group with sharp decrease in plant fresh and dry biomass at saline environments other than control (Li and Stanhellini, 2001; Hajer et al., 2006; Maggio et al., 2007).

The selected trait inter-relationship positively helps in deploying the selection procedure to evaluate resilience. So, correlation analysis of seedling traits had depicted delightful results (Figure 1). It was found that root and shoot dry weight tolerance indices (RDWTI and SDWTI) have a strong negative association with all other traits particularly the weight % reductions (both fresh and dry shoots and roots weight reductions at S_{10} and S_{15} salinity levels that is, $FSWPR_{10}$, $FSWPR_{15}$, $FRWPR_{10}$, $FRWPR_{15}$, $DSWPR_{10}$, $DSWPR_{15}$, $DRWPR_{10}$ and $DRWPR_{15}$. However it does not stand true for K^+/Na^+ ratio tolerance index (K.Na.TI). At certain points, Ca^{2+}/Na^+ ratio tolerance index (Ca.Na.TI) and K^+/Na^+ ratio tolerance index (K.Na.TI) negatively correlated with other traits that is, $FSWPR_{10}$, $DSWPR_{10}$, $DSWPR_{15}$ and RDWTI (Figure 1). All weight % reductions either of roots or shoots regarding S_{10} and S_{15} levels of salinity, are strongly positive in their relationships with each other (Figure 1).

Principle component analysis (PCA) of seedling tolerance traits

The mean data were analyzed by PCA through RStudio software Version 0.98.1102, RStudio, Inc. (R Core Team). Eigen values, % variance and cumulative % variance are presented in supplemental data. Table 2 shows that the first three principal components (PCs) have Eigen values greater than 1. First two PCs contribute a cumulative variance of 69.656%, however, with first three PCs, the cumulative variance contribution was 83.731% (Table 2).

Using RStudio software two data matrices of 25 (genotypes) × 12 (PCs) and 12 (traits/variables) × 12 (PCs) were prepared for the analysis (Tables 3 and 4). Since used traits are genotypic responses with respect to

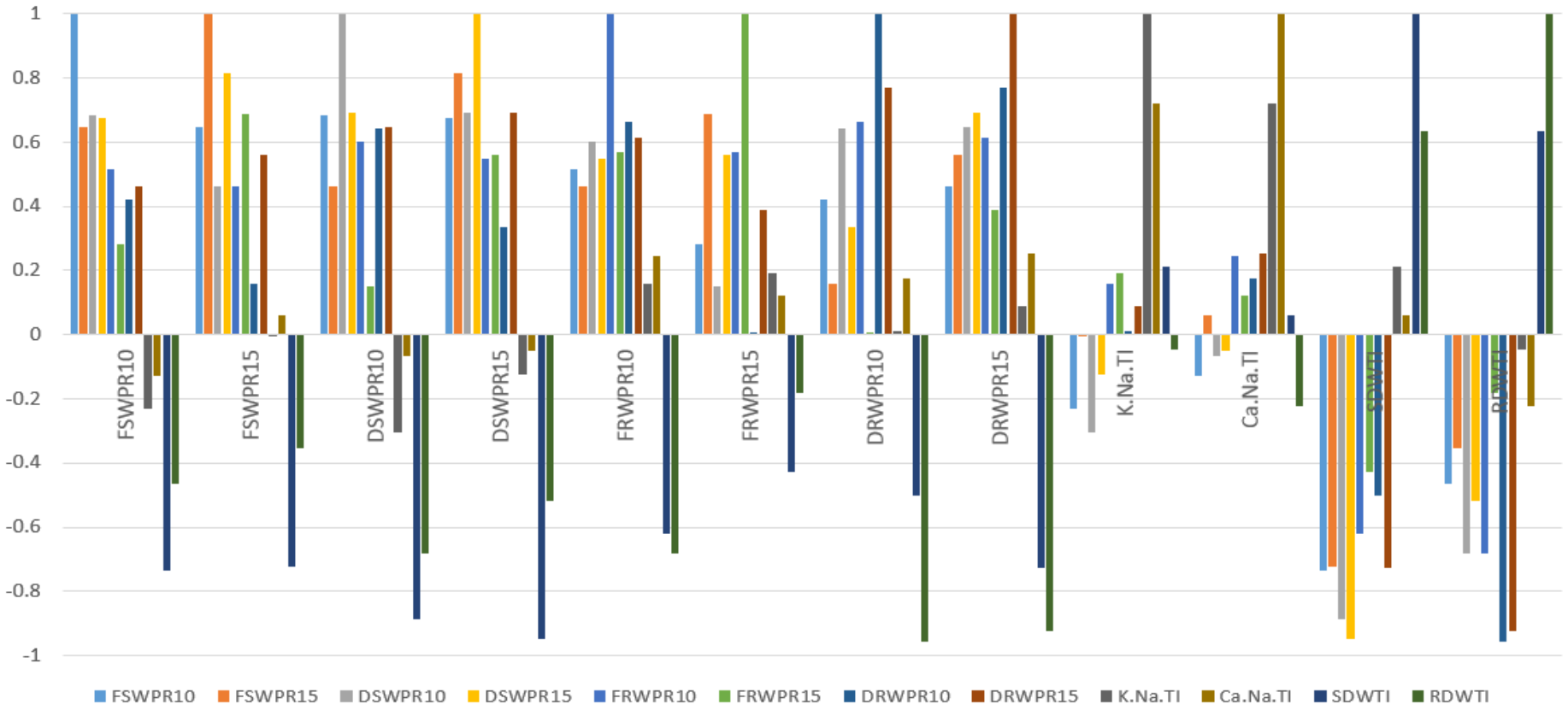

Figure 1. A graphical representation of correlation among 12 seedling salt tolerance traits.
FRWPR10; DRWPR10 and FSWPR10; DSWPR10 (Fresh/dry root and shoot weight % reduction at 10 dSm-1), FRWPR15; DRWPR15 and FSWPR15; DSWPR15 (Fresh/dry root and shoot weight % reduction at 15 dSm-1), K.Na.TI (K+/Na+ tolerance index) and Ca.Na.TI (Ca2+/Na+ tolerance index) SDWTI (Shoot dry weight tolerance index), RDWTI (Root dry weight tolerance index)

cumulative effect of all salinity levels, whatsoever, PCA distributes the overall mean data into individual PC contributive loadings. These loadings are representative of variability produced by all variables in the form of individual PC (Tables 3 and 4).

In fact, each variable contributes in each PC, so a complete data matrix table is formed. This data matrix was used to draw a principal component biplot (PC-biplot) which was a very handy graphical representation of variability within the germplasm (Figure 2). The PC-biplot had shown a complete relationship among observed salt tolerance traits and among genotypes, and particularly the response of individual genotype for all traits, so selection pressure can be easily applied (Figure 2). Principal component analysis was used as one of the most reliable statistical model that best expresses the genotypic performance at given saline conditions (Kaya et al., 2006; Ali et al., 2012).

First of all, PC-biplot had shown variability and association of salt tolerance traits. Each trait was allocated its demonstrative vector in the PC-biplot

Table 2. Eigen values, percent variances and cumulative percent variance for each principal component depending upon 12 seedling tolerance traits.

Principal Component	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Eigen values	6.261	2.098	1.689	0.604	0.504	0.352	0.255	0.109	0.097	0.031	0.000	0.000
% Variance	52.172	17.484	14.075	5.036	4.197	2.931	2.127	0.910	0.812	0.255	0.000	0.000
Cumulative % var.	52.172	69.656	83.731	88.767	92.964	95.896	98.023	98.933	99.745	100.000	100.000	100.000

Table 3. Principal component loadings of 25 tomato genotypes.

S/N	Genotypes	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
1	NUTYT-701	0.0380	1.1561	1.0341	1.3290	0.2246	-0.3601	-0.3135	0.0419	-0.4084	-0.0238	0.0003	0.0001
2	ANAHU	0.0540	0.1881	-0.5588	0.3453	-1.4353	-0.0900	-0.1970	0.2269	-0.1248	0.0570	-0.0007	-0.0004
3	ZARNITZA	5.5399	2.0294	2.3663	0.7076	-1.1320	1.0247	-0.2466	0.1439	0.4966	-0.0830	0.0001	0.0000
4	LA-2821	-1.3137	-1.8764	0.5176	-0.4788	0.7667	0.5362	-0.8446	1.2227	-0.2302	-0.0217	0.0001	0.0000
5	Rio-GRANDE	0.1390	-2.2204	1.3552	-0.4044	0.5166	-0.2363	-1.3852	-0.6773	0.4978	0.2792	0.0001	-0.0001
6	LA-1021	-0.5131	0.8300	1.9295	-0.5300	-0.1472	0.4681	0.0321	-0.1451	-0.1476	0.1046	-0.0006	0.0002
7	ROMA	-1.2780	1.2395	0.3987	-0.5614	0.4717	-0.4239	0.1950	-0.0362	-0.2164	0.1358	0.0004	0.0002
8	FORME DE COEUR	3.6851	0.6069	2.1839	-1.5284	0.9501	0.0472	0.4596	-0.2062	-0.4759	-0.2914	0.0001	-0.0002
9	EARLY ANNIE	-1.7592	1.4341	0.3332	1.4287	0.9540	-0.5395	-0.1426	-0.0388	0.0446	-0.0979	-0.0002	-0.0002
10	NAGINA	0.4045	1.9915	-0.2094	-0.9073	0.5575	-0.3926	0.1530	0.1339	0.0075	0.3459	-0.0007	0.0000
11	BEAVER LODGE SLICER	7.1370	-1.5658	-2.7811	0.2743	-0.1259	-0.6067	-0.6606	-0.1035	-0.4281	-0.1171	-0.0001	0.0002
12	GLACIER	2.4020	-3.7121	1.5325	0.8316	0.0857	-0.8512	1.4182	0.2467	0.3246	0.1076	-0.0003	0.0001
13	UOVO ROSEO	1.6848	1.9397	-1.4809	0.0567	-0.2867	-0.4484	0.1103	0.2736	0.2094	0.2727	0.0004	0.0002
14	17860	0.3058	-0.3843	-0.8380	-0.7285	-0.9457	0.1443	0.2578	-0.3873	-0.4004	0.0728	0.0003	-0.0001
15	LO-2831	-1.4090	-0.5949	-0.0272	-0.1536	-0.5302	0.2396	0.2449	0.0171	-0.0918	0.0745	0.0004	-0.0002
16	BL-1079	-0.7463	0.9814	-0.6517	0.4003	0.4638	-0.2446	0.2325	-0.0539	-0.1439	0.1350	0.0000	-0.0003
17	6232	-1.4204	1.0056	-0.5403	0.1573	0.2600	-0.3002	-0.0662	0.0730	0.2174	-0.0417	0.0002	0.0002
18	17856	-0.7833	-0.9209	-1.3515	1.2149	0.7229	1.9901	0.3923	-0.3032	-0.2571	0.1570	-0.0003	0.0002
19	6233	-0.9946	-0.9159	0.0827	0.5057	0.6979	0.0167	0.0175	-0.2217	0.1803	-0.1669	0.0006	-0.0002
20	PB-017909	-1.9624	-0.0798	-0.4635	-0.0347	-0.1014	-0.2571	-0.2026	-0.1139	0.2083	-0.3292	-0.0013	0.0000
21	LO-2576	-3.0807	-0.1034	0.0226	-0.0362	-0.3835	-0.1900	-0.0109	-0.1927	-0.1021	-0.2077	-0.0002	0.0004
22	LO-2692	-2.4546	-1.5640	0.1931	-0.2575	-0.8621	0.1711	0.0653	-0.0388	-0.1840	0.0536	0.0004	0.0000
23	LO-2707	1.2578	0.3389	-2.5795	-1.1739	0.8042	0.6235	0.3921	0.0782	0.7112	-0.1518	0.0001	-0.0001
24	LO-2752	-2.7535	-0.6232	-0.0094	-0.9817	-1.0706	-0.0874	0.0421	0.0678	0.1708	-0.0704	0.0000	0.0001
25	LO-2831-23	-2.1794	0.8201	-0.4583	0.5250	-0.4553	-0.2334	0.0570	-0.0071	0.1421	-0.1931	0.0008	-0.0001

Table 4. Principal component loadings of 12 seedling tolerance traits.

Seedling tolerance trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
$FSWPR_{10}$	-0.3000	0.2215	0.0433	-0.3089	-0.5467	-0.5450	-0.0410	-0.3338	-0.2248	-0.0644	0.0005	0.0001
$FSWPR_{15}$	-0.2934	0.0845	0.4289	-0.1739	0.1509	-0.3242	-0.2307	0.6559	0.2849	-0.0049	0.0004	0.0001
$FRWPR_{10}$	-0.3362	0.1649	-0.2099	-0.2000	-0.1782	0.4797	0.0119	0.4023	-0.4774	-0.0237	-0.3508	0.0194
$FRWPR_{15}$	-0.3457	0.1514	0.2361	-0.1727	0.2276	0.1839	0.2755	-0.4064	0.3896	0.1980	-0.5035	0.0279
$DSWPR_{10}$	-0.3157	-0.1895	0.0180	0.4527	-0.5219	0.2172	0.0050	0.0345	0.4215	-0.4008	-0.0001	0.0002
$DSWPR_{15}$	-0.2025	-0.0854	0.5544	0.5400	0.0342	0.0327	-0.1457	-0.1356	-0.4696	0.3005	0.0000	-0.0002
$DRWPR_{10}$	-0.2838	-0.1860	-0.4704	0.1836	-0.0325	-0.2125	0.0026	0.1062	0.1261	0.5665	-0.0277	-0.4845
$DRWPR_{15}$	-0.3485	-0.1707	-0.1184	0.0310	0.5036	-0.1479	-0.0399	-0.1608	-0.2183	-0.5951	-0.0194	-0.3596
SDWTI	0.0239	-0.6012	0.2140	-0.1941	-0.1106	-0.1159	0.6903	0.1730	-0.1499	0.0030	0.0005	-0.0002
RDWTI	-0.0404	-0.6000	0.0829	-0.4246	-0.0817	0.2386	-0.5802	-0.1953	0.0487	0.1025	0.0001	0.0000
K^+/Na^+ TI	0.3705	-0.1704	-0.0567	0.1987	-0.0660	-0.3317	-0.1812	0.0806	-0.0369	-0.1169	-0.7876	0.0440
Ca^{2+}/Na^+ TI	0.3304	0.1905	0.3399	-0.1258	-0.2080	0.1960	0.0162	0.0081	0.0221	-0.0763	-0.0442	-0.7955

$FRWPR_{10}$; $DRWPR_{10}$ and $FSWPR_{10}$; $DSWPR_{10}$ (Fresh/dry root and shoot weight percent reduction at 10 dSm^{-1}), $FRWPR_{15}$; $DRWPR_{15}$ & $FSWPR_{15}$; $DSWPR_{15}$ (Fresh/dry root and shoot weight percent reduction at 15 dSm^{-1}), K.Na.TI (K^+/Na^+ ratio tolerance index), Ca.Na.TI (Ca^{2+}/Na^+ ratio tolerance index). SDWTI (Shoot dry weight tolerance index), RDWTI (Root dry weight tolerance index).

(Figure 2). Traits with longer vectors were representative of more variability (Figure 2), in which SDWTI, DRWPR15 and DSWPR15 have longest vectors depending upon values that can be seen on PC-biplot and their respective loadings (Table 4). These vectors also depicted some sort of relationship among salt tolerance traits. Vectors in same direction were positively correlated while those in opposite direction were negatively correlated. From Figure 2, it was seen that RDWTI was negatively correlated with reductions in root weight that is, FRWPR10, FRWPR15, DRWPR10 and DRWPR15.

Similarly, SDWTI was negatively associated with shoot weight reductions that is, FSWPR10, FSWPR15, DSWPR10 and DSWPR15, while both RDWTI and SDWTI were independent of K^+/Na^+ and Ca^{2+}/Na^+ ratio tolerance indices (K.Na.TI and Ca.Na.TI). All weight % reductions were positively correlated with each other, however, K^+/Na^+ and $Ca^{2+/}Na^+$ ratio tolerance indices were in a positive relation with each other.

Next from PC-biplot, the genotypes similarities were revealed with other genotypes and their response to a particular salt tolerance trait (Figure 2). Genotypes that were nearer to each other were of same group in their overall behavior in the form of observed traits for example, EARLY ANNIE, ROMA, NUTYT-701, 6232, BL-1079, LA-1021 and LO-2831-23 were very close to each other so must be place in a single group (Figure 1). Other group includes ANAHU, LO-2707, 17860, PB-017909, LO-2576, LO-2831, LO-2752, 6233 and 17856. NAGINA and UOVO ROSEO were side by side while Rio-GRANDE, LA-2821 and LO-2692 were adjacent to each other (Figure 2). Some genotypes like BEAVER LODGE SLICER, GLACIER, ZARNITZA Rio-GRANDE, NAGINA, UOVO ROSEO, FORME DE COEUR and LO-2576 had provided more diversity to the tomato germplasm, therefore, were considered a separate group (Figure 2).

Furthermore, PC-biplot depicted individual and group-wise performance of genotypes for a particular salt tolerance trait. Genotypes including GLACIER, Rio-GRANDE on or very immediate to the vectors of Ca^{2+}/K^+ and K^+/Na^+ ratio tolerance indices (Ca.Na.TI and K.Na.TI), and their projections to these vectors were longest inrespective to the origin, so, these genotypes are considered good performer (Yan, 2001) for both traits (Figure 2).

BEAVER LODGE SLICER revealed very good for SDWTI because of its longest vector considering farthest perpendicular (Yan, 2001). ZARNITZA, FORME DE COEUR, UOVO ROSEO, NAGINA and BEAVER LODGE SLICER were highly responsive for RDWTI (Figure 2). These three groups of genotypes were showing highly tolerant behavior for provided saline conditions

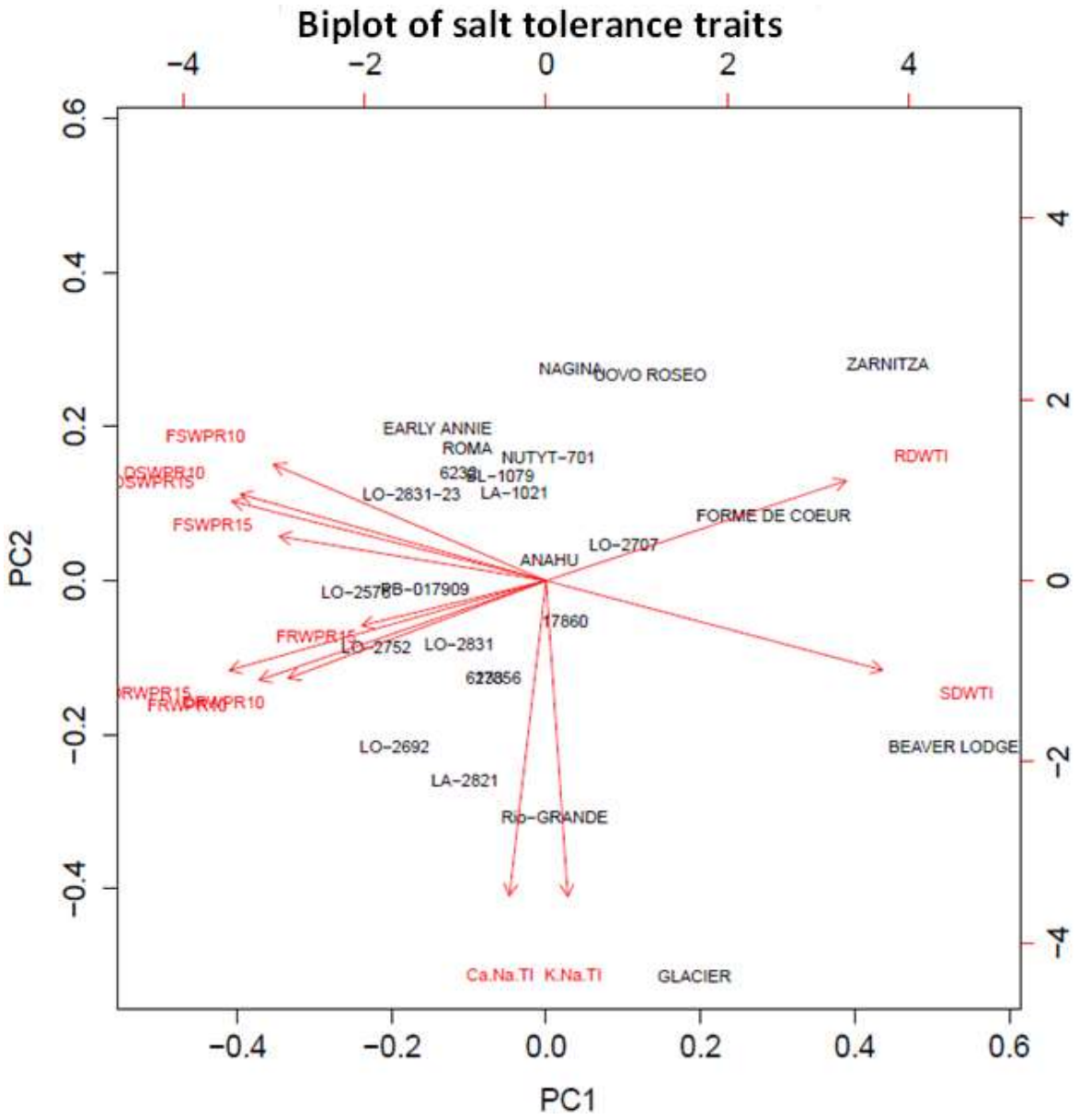

Figure 2. Principle component biplot for salt tolerance traits.
FRWPR10; DRWPR10 and FSWPR10; DSWPR10 (Fresh/dry root and shoot weight % reduction at 10 dSm^{-1}), FRWPR15; DRWPR15 and FSWPR15; DSWPR15 (Fresh/dry root and shoot weight % reduction at 15 dSm^{-1}), K.Na.TI (K^+/Na^+ tolerance index) and Ca.Na.TI (Ca^{2+}/Na^+ tolerance index) SDWTI (Shoot dry weight tolerance index), RDWTI (Root dry weight tolerance index)

Virtually, a group of 7 genotypes consisting of EARLY ANNIE, ROMA, NUTYT-701, 6232, BL-1079, LA-1021 and LO-2831-23 was found with highest shoot weight % reductions at both S_{10} and S_{15} levels of salinity, and were irrespective in their response to all roots weight % reductions (Figure 2).

On the other hand, a different group of seven genotypes including PB-017909, LO-2576, LO-2831, LO-2752, 6233, 17856 and LO-2692 were seen with a highly positive response for root weight % reductions at both S_{10} and S_{15} levels (Figure 2). This group apparently, did not seem to be effectively engaged with shoot weight % reductions. Three genotypes % (ANAHU, LO-2707 and 17860) were found with a slight involvement towards all tolerance indices but highly negative behavior for all weight % reductions, as these were closer to the origin. This group could be said to be a moderately tolerating group to the investigated salinity levels (Figure 2).

Genotypes having higher values for tolerance indices (GLACIER, Rio-GRANDE, LA-2821) and lowest values of

weight % reductions (BEAVER LODGE SLICER, ZARNITZA, FORME DE COEUR) were established as more tolerant than remaining. These genotypes can be allotted to a wide spectrum of soils that constitute harsh saline environment to bred ideotypes having a suitable combination of both traits.

A group of 6 genotypes including ANAHU, LO-2707, 17860, UOVO ROSEO, NAGINA and LA-2821 based on their little positive performances for tolerance indices but significantly negative behavior against weight % reductions were detained under moderately salt tolerant group and could be used for further breeding programs as well to retain diverse genetic base. While genotypes using greater capability for either roots weight % reductions or shoots weight % reductions were actually producing very less biomass, therefore, considered as salt susceptible and cannot be regarded as good choice for future breeding programs (Figure 2).

The differences in behavior of these groups are a result of underlying genes behind individual response. Gene mining approaches could be further applied to identify the mechanism of such genes and that when stimulated themselves produce specific combinations of salt tolerance traits, which result in higher yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of aqueous and oil leaf extracts of *Pterocarpus santalinoides* on the maize weevil, *Sitophilus zeamais* pest of stored maize grains

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The effects of aqueous and oil leaf extracts of *Pterocarpus santalinoides* at concentrations of 5, 10, 15 and 20%v/v, each as proctectant in the control of the maize weevil, *Sitophilus zeamais,* were compared with a conventional insecticide, pirimiphos-methyl at a rate of 0.5 ml / 50 g maize grains of Oba Super II variety in a Completely Randomized Design with four replicates. Parameters assessed, include adult mortality, rate of oviposition and adult emergence, grain damage and weight loss, and seed viability. The data were subjected to analysis of variance and means separated by using Duncan's Multiple Range Test. Mortality of adult *S. zeamais* increased with increased concentration of the extracts as well as with days of exposure. Emergence rate was significantly ($p<0.05$) reduced from 41.82% pt in the control values to 19.35 to 2.14% in *P. santalinoides* aqueous and 9.30 to 0.90% in *P. santalinoides* oil with pirimiphos methyl having the least number of emerged adults (0.60%). Damaged maize seeds varied from 12.32 to 1.98% in *P. santalinoides* aqueous extract and 4.86 to 0.61% in *P. santalinoides* oil extract while 0.59% was recorded in Pirimiphos methyl-treated seed grains. Grain weight loss significantly varied between treatments after two and a half months ($F_{11, 24}= 1499.2$, $p<0.0001$). Highest seed germination percentage (78.60%) was observed in seeds treated with 5%v/v *P. santalinoides* aqueous extract and the control, followed by 72.40% in *P. santalinoides* oil concentrations at 5, 10 and 15%v/v. The findings showed that almost all treatments except un-treated control decreased (weevil reproduction) grain weight loss and grain damage (holes on grains) in maize.

Key words: *Pterocarpus santalinoides*, pirimiphos-methyl, *sitophilus zeamais*, effects, grain damage and weight loss.

INTRODUCTION

Maize is a cereal crop of the grass family Poaceae. It is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Awika, 2011; Mrkovacki et al., 2016). According to Abdulrahaman and Kolawole (2006), some of the local dishes prepared with maize include hot and cold pap, 'tuwo', donkunnu', 'maasa', 'couscous', 'Akple', 'Ukejuka', 'Gwate', 'Nakia', 'Dambualubosa', 'Abari', 'Egbo' and

'Donkwa'. Maize is also made into 'popcorn', 'Ajepasi', 'Aadun', 'Kokoro' and 'Elekute.

Apart from food, maize is also useful as medicines and as raw materials for industries. According to Oladejo and Adetunji (2012), levulinic acid, a chemical derived from maize, is used as ingredient in antifreeze and is capable of replacing the toxic petroleum-based ingredients normally used. Plastic and fabrics are made from corn stocks, ethanol obtained from maize can be used as a biomass fuel and stigmas from female corn-flowers, known as corn silk, can be used as herbal supplements (Oladejo and Adetunji, 2012). Also, the leaves of the maize plant serve as forage for livestock. Maize is used extensively as the main source of calories in animal feeding and feed formulation, and also in making silage after fermentation of corn stocks (Oladejo and Adetunji, 2012).

Despite the vast uses of maize grains and its products, there are problems to its sustainable production. One of the most important limitations to maize production is the effect of pests (both field and storage pests). Some of them include: the bacterium *Pantoea stewartii,* which causes Stewart's Bacterial Wilt,; the pathogenic fungus *Ustilago maydis* that causes smut on maize (Hoopen and Maïga, 2012).

Nevertheless, by far the most important pest of maize is the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). The insect is a major pest of stored maize grains in the tropics and temperate regions of the world (Adedire, 2001; Yeshaneh, 2015). Its infestation causes severe post-harvest losses of staple food crops in Nigeria leading to major economic losses (Oni and Ileke, 2008). Various strategies aimed at checking the menace of pest infestation, especially with the maize weevil, *S. zeamais* in stored grains have been employed. These strategies include physical, biological and chemical control among others.

However, because of serious health and environmental concerns as well as genetic resistance by insect species, pest resurgence and residual toxicity related to the use of these chemicals, most societies have questioned their use and are in search of more favourable/acceptable pest control methods (Adedire, 2002; Adedire et al*.,* 2011; Ekeh et al*.*, 2013). The main advantage of botanical pesticides centers majorly on their eco- friendliness, easily bio-degradable and plant-derived natural products that are toxic to pests and they could be produced from locally available organic materials.

Currently, attention is being given to the use of edible plant materials as grain protectants (Ivbijaro and Agbaje, 1986; Adedire and Lajide, 2003; Akinkurolere et al*.,* 2006; 2009 Adedire et al*.,* 2011). Farmers, especially the peasant ones in developing countries had over the years been using selected indigenous plants materials believed to possess insecticidal properties as crop protectants by mixing them with the stored grains (Edelduok et al., 2015). And one of the plants employed is *Pterocarpus santalinoides*, (French: Ouokisse; Hausa: Gyadar kurmi, Gunduru; Igbo: Nturukpa; Yoruba: Gbengbe) a tree in the family fabaceae*.* The tender leaves of the plant are used as vegetables in soup making while the stem bark is used in making "pepper soup" (Okwu and Ekeke, 2003).

The aqueous extract of the stem bark of *P. santalinoides*, has been established to have effects on *Pseudomonas aeruginosa* which is responsible for such infections as pneumonia, urinary tract infections (UTIs) and bacteraemia (Eze et al., 2012). According to Anowi et al. (2012b), the methanolic extract of leaves of *P. santalinoides* possesses analgesic activity and Okpo et al. (2011) reported the anti-diarrhea property of aqueous extract of the leaves of the plant while Otitoju et al*.* (2014b) observed that the leaves of *P. santalinoides* are used in preparing soups like ogbono and egwusi (melon soup). Also, Adeleke et al. (2009) reported the larvicidal properties of the seed oil of *P. santalinoides.* But there has been paucity of information on the effects of aqueous and oil leaf extracts of *P. santalinoides on* maize weevil, *S. zeamais.*

Hence, the need to study this plant`s oil and aqueous leaf extracts on maize weevil, *S. zeamais* was borne due to its ready availability and non-toxicity to humans, local farmers use of the seed extracts, leaf extracts and stem-bark extracts as grain protectant. However, whether or not this has been effective is subject to investigation. Therefore, the present study investigated the *P. santalinoides* effectiveness by using its aqueous and oil leaf extracts on the maize weevil. *S. zeamais* access the rate of mortality, oviposition, rate of adult emergence, grain damage, weight loss and seed viability of *S. zeamais* with application of varied concentrations of aqueous, and oil leaf extracts of *P. santalinoides,* also compare its effectiveness with that of a synthetic pesticide (*Pirimiphos methyl*).

MATERIALS AND METHODS

Study location

All experiments were conducted at the Entomology Research

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Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka under ambient temperature of 27±2°C and 65±5% relative humidity.

Stock culture of weevils

Weevils were purchased from traders' granary stores in town (Ogige market) as initial stock. They were first mass reared in a separate container (tins, cans covered with muslin cloth at the top). 500 unsexed adult *S. zeamais* weevils were introduced into the maize seeds and the container kept in ideal conditions for reproduction to take place. Jars were placed on a table whose stands were dipped in plastic bowls containing oil to prevent ants from contaminating the culture. Newly emerged weevils were used for the rest of the study (Ojo and Ogunleye, 2013).

Procurement of maize grains

The Oba Super II variety of maize grains was used for this research. One kilogram of the uninfested grains was bought from Ogige Market, Nsukka L.G.A Enugu state and identified at the department of Crop Science, University of Nigeria, Nsukka. The grains were still handpicked to make sure only clean unhampered grains were picked, thereafter the clean seeds were left under the sun in order to eliminate any resident insect pest. The grains were then sieved with a 2 mm- mesh size sieve to remove any dead insect and frass. The maize seeds were then packaged in an air-tight container and kept in a refrigerator, pending usage.

Procurement of *Pterocarpus santalinoides* leaves

The plant material used were leaves of *P. santalinoides* which were collected from a farm in Ugboezeji Abakpa Nike, Enugu, Enugu State, Nigeria and identified to species level at the Department of Plant Science and Biotechnology. The plant materials were dried under room atmosphere for three weeks until a constant weight was maintained. 2 kg of the leaves were then ground into very fine powder using an electric blender and kept in plastic containers with tight lids and stored in a refrigerator at 4±2°C till the time for Soxhlet extraction (Mulungu et al., 2010; Ileke and Oni, 2011). *P. methyl* was bought from Lavans Group Limited, Enugu, Enugu State.

Extraction of the plant material

Aqueous

800 g of the ground plant material was soaked in 5500 ml of water and left for 24 h. The solution was filtered with a muslin cloth of 1.5 mm mesh size. The filtrate was then poured into an evaporation dish and dried under fan at room temperature.

Oil

The soxhlet extraction method was used for the extraction of oil from the plant material. The soxhlet extractor was set up with pre-weighed soxhlet flask and the extraction procedure followed. 400 g of powdered plant material was put into the thimble and the material was continuously shaked for 6 h using 800 ml distilled N- hexane (40 to 60°C) and thereafter left to cool and concentrate at room temperature for 24 h using cold pressing through decanting method at 130 rpm (Ekeh et al., 2013b). At the end of the extraction, the thimble was removed and the solvent distilled off and filtered using whatman filter paper. Crude extract of *P. santalinoides* were later diluted with N-hexane to obtain five different concentrations of 10, 20, 30,40, and 50% v/v with a control (0 %v/v) containing only N-hexane. The oil was transferred to glass jar with cover and kept in a refrigerator until needed.

Phytochemical test of the plant material

The qualitative phytochemical composition of the leaves of *P. santalinoides was* studied following the method of Ndukwe and Ikpeama (2013) and Otitoju et al. (2014b).

Experimental design

50 g of clean maize grains contained in 200 ml plastic plate of about 0.075 m diameter were added five pairs of a day old male and female adult *Sitophilus zeamais* which were obtained from a stock culture. Each of the two treatments of aqueous and oil extracts of the plant material was added to the different plates (12 plates for the aqueous treatment and 12 plates for the oil treatment) at the rates of 10, 20, 30, 40 for the aqueous and oil extracts, and each concentration was replicated three times. A total of 26 plates were used. The experimental control for each of the treatments was set up with maize grains and *S. zeamais* but no plant or oil extract. Each of the plate was covered with muslin cloth of about 0.2mm mesh size to permit air passage and prevent escape of the insects. The set up was allowed for six weeks at temperature of about 30±3°C with daily monitoring. Dead insects in each plate were removed and counted. Oviposition of eggs in the maize grains by *S. zeamais* was also monitored daily and natality was recorded as adult emergence, also number of holes and seed damage was recorded.

Evaluation of extracts for contact toxicity for determination of LC_{50}

Twelve day-old *S. zeamais* were placed per petri-dish and were individually picked and treated by applying 1-2µl drops of each concentration of both extracts on their ventral sides from a micro syringe. The contents of the petri-dishes were provided with maize grains and insects were observed at 24 h. Insects that do not respond to probing with a seeker were considered dead. The concentrations were converted into logarithmic values while mortality values were converted to probits. Probit values were plotted against logarithmic values and a regression line (of best fit) was drawn. The logarithmic dose at the median point when changed to antilogarithm was taken as the effective LC_{50} for each extract (Finney, 1971).

Effects of plant extracts and P. methyl on the mortality of *S. zeamais*

50 g of maize grains were weighed into jars. Using a micro-syringe, 0.5 ml of the concentrations (10, 20, 30 and 40% v/v) of each of the extracts and *P.methyl* was applied to the grains and shaken to allow for coverage. Grains in the control jar were treated with ethanol only. The grains were infested with ten (1 male: 1 female) adult s. *zeamais* per jar and jars covered with a lid of fine mesh to allow for aeration. Mortality was recorded at 24, 48, 72

and 96 h after infestation, with insects considered dead if they did not move when probed with a camel hairbrush. Dead adults were removed at each assessment, counted and recorded. Data on percentage mortality were corrected using Abbott's (1925) formula:

$P_T = P_O - P_C / 100 - P_C$; where P_T = corrected mortality (%), P_O = observed mortality (%), Pc = control mortality (%).

Effects of plant extracts and P. methyl on emergence of S. zeamais, grain damage, weight loss and seed viability

After 10 days, all adult weevils were removed and the jars left undisturbed and monitored (two and half months) until the emergence of F_1 progeny. Data on F_1 adult emergence were assessed from the commencement of adult emergence with emerged adults removed, counted and recorded. The grains were later sieved to remove the dust produced from adult feeding and re-weighed by using a Mettler Weighing balance and the percentage loss in weight determined as follows:

$$\text{Per (\%) weight loss} = \frac{\text{initialwt} - \text{finalwt}}{\text{final weight}} \times 100$$

The number of grains perforated in each of the treated and control jars were counted and percentage seed damage was determined as:

$$\text{\% seed damage} = \frac{\text{Number of perforated grains}}{\text{Total number of grains counted}} \times 100$$

In order to assess the viability of seeds, germination test was conducted using twenty seeds from each jar. The seeds were placed on moist filter paper in plastic Petri dishes kept in an incubator at 25°C and the number of germinated seed was counted and recorded, and percentage seed viability was calculated as:

$$\text{\% viability} = \frac{\text{Number of germinated seed}}{\text{Number of seed sown}} \times 100$$

Statistical analysis

All percentage data were angular transformed prior to statistical analysis, in order to equalize variances. All data were analysed and significant differences were compared at 0.05 significant level using Duncan's Multiple Range Test (DMRT) (Zar, 1984).

RESULTS

Contact toxicity of plant extracts on *S. zeamais*

Contact effect of aqueous extracts of *P. santalinoides* and its oil extract on *S. zeamais* at various concentrations showed an increase in mortality in both extracts from 39.4 to 78.4% in *P. santalinoides* aqueous and to 100% in *P. santalinoides* oil. The lethal concentration (LC_{50}) of P. *santalinoides* aqueous extract was 19.95% while that of *P. santalinoides* oil was 13.18% (Figures 1 and 2).

Effects of plant extracts and *P. methyl* on the mortality of *S. zeamais*

Mortality of adult *S. zeamais* exposed to different rates of extracts of *P. santalinoides* aqueous extract, *P. santalinoides* oil and the conventional insecticide (*P.methyl*) was compared in Table 1. Adult mortality increased with increase in concentration and with days of exposure to both extracts and in *P. methyl*. There was no significant difference ($p < 0.05$) between *P. santalinoides* oil at 20%v/v and *P. methyl* at 72 and 96 h post-treatment. *P. santalinoides* oil at 20%v/v also caused 100% mortality to adult *S. zeamais* even though the synthetic insecticide caused 100% mortality 24 h earlier. All rates of application of both extracts and *P. methyl* were significantly ($p < 0.05$) different from the control in all the days of the trials (Tables 2 and 3).

Effects of plant extracts and *P. methyl* on emergence of *S. zeamais*, grain damage, weight loss and seed viability

The effect of *P. methyl* and different concentrations of extracts of *P. santalinoides* (aqueous and *P. santalinoides* oil) is shown in Table 4. Mean number of emerged F_1 adults decreased with increasing concentrations of both extracts. Emergence was significantly ($p<0.05$) reduced from 41.82 in the control values to 19.35 to 2.14 in *P. santalinoides* aqueous and 9.30 to 0.90 in *P. santalinoides* oil with *P. methyl* having the least number of emerged adults (0.60). However, treatment with *P. methyl* did not significantly ($p > 0.05$) reduce emergence than those with *P. santalinoides* oil at 15, 20 and 20%v/v at *P. santalinoides* aqueous extract. All treatments were significantly better ($p < 0.05$) than the control in reducing adult emergence (Table 4). Damaged maize seeds in treatments of various concentrations of the extracts varied from 12.32 to 1.98% in *P. santalinoides* aqueous and 4.86 to 0.61% in *P. santalinoides* oil while 0.59% was recorded in treatment with *P. methyl*. All treatments proved superior to control, (28.25%) with *P. methyl* and 20 %v/v with *P. santalinoides* oil outstanding (Table 5). The percentage loss in weight of grains due to damage varied among treatments from 0.04% in *pirimiphos - methyl* to 4.57% in the control. 20 % v/v of *P. santalinoides* oil and *pirimiphos –methyl* were significantly ($p<0.05$) better than the other treatments in reducing weight loss (Table 3). Highest germination percentage (78.60) was observed in

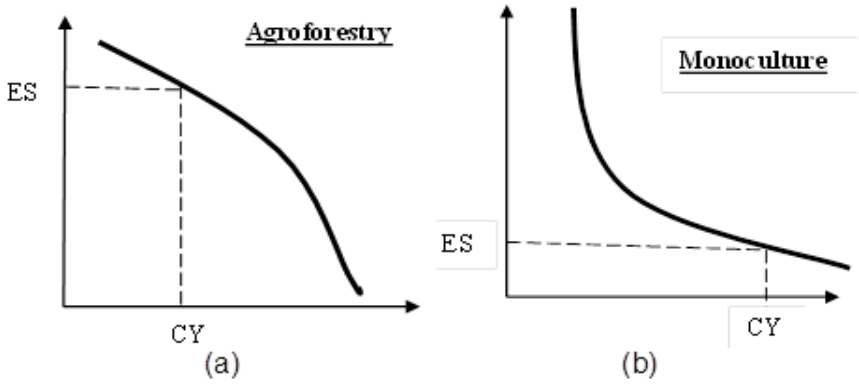

Figure 1. (a) Agroforestry and (b) Monoculture practices (ES = Ecosystem Services, CY = Crop Yields). Source: Adapted from Elmqvist et al. (2011).

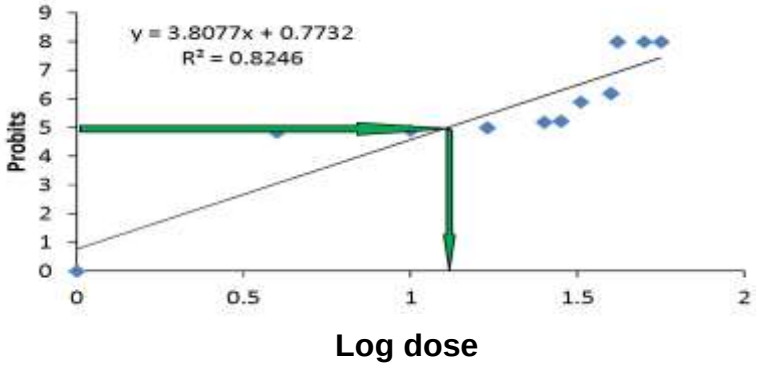

Figure 2. Determination of LC_{50} of oil extract of *Pteracarpus santalinoides* leaves on *S. zeamais.*

Table 1. Percentage proximate composition of stored maize grain.

Nutrient	(%) proximate composition
Moisture	11.60±0.23
Ash	2.95±0.15
Crude fibre	2.50±0.11
Protein	9.35±0.25
Fat	3.95±0.18
Carbohydrate	69.60±0.10

Source: Enyishi *et* al. (2014).

seeds treated with 5%v/v *P. santalinoides* aqueous extract and the control, followed by 72.40% in *P. santalinoides* oil concentrations at 5, 10 and 15 %v/v. Other treatments gave the least germination of 70.25%. There were no significant differences ($p > 0.05$) in seed germination among the treatments and the control.

Table 2. Phytochemical composition of *Pterocarpus santalinoides.*

Phytochemical	Relative presence
Tannins	++
Flavonoids	+++
Oxalate	++++
Steroids	+
Glycosides	-
Phenols	++
Alkaloids	++++
Anthocyanin	+
Carotenoids	+
Saponins	++++

Key: Not present; +, present in very small concentration; ++, present in moderate concentration; +++, present in high concentration; ++++, present in very high concentration.
Source: Ndukwe and Ikpeama (2013) and Otitoju et al. (2014b).

Table 3. Effects of plant extracts and *P. methyl* on mortality of *S. zeamais.*

Insecticidal materials	Conc. %v/v	Percentage mortality over 4 days post treatment			
		24 h	48 h	72 h	96 h
Pterocarpus santalinoides (aqueous)	5	10.4^{g}±2.03	16.6^{f}±2.05	19.0^{de}±3.02	26.5^{d}±3.51
	10	14.0^{fg}±2.34	19.2^{ef}±1.66	23.0^{cd}±1.92	34.0^{d}±2.59
	15	19.0^{ef}±0.00	29.5^{d}±1.51	40.5^{c}±2.83	46.5^{c}±2.89
	20	24.6^{ef}±1.66	36.5^{d}±1.51	46.5^{c}±2.82	54.5^{c}±2.76
Pterocarpus santalinoides (oil)	5	18.0^{ef}±3.02	29.0^{de}±3.02	39.0^{c}±4.14	54.0^{c}±4.39
	10	40.4^{d}±1.51	38.5^{d}±1.51	58.0^{d}±2.41	78.0^{b}±3.17
	15	49.3^{c}±1.44	49.5^{c}±1.44	67.0^{b}±2.59	89.5^{ab}±3.40
	20	68.0^{b}±0.00	74.0^{b}±5.18	96.0^{a}±5.32	100.0^{a}±0.00
Pirimiphos methyl (control)	0.05	89.5^{ab}±3.40	95.00^{ab}±3.02	100.0^{a}±0.00	100.0^{a}±0.00
	0.000	75.0^{a}±1.92	97.5^{a}±4.61	100.0^{a}±0.00	100.0^{a}±0.00

Means followed by common letters in the same column are not significantly different at 5% level.

Table 4. Effects of plant extracts and *Pirimiphos methyl* on emergence of *S. zeamais* and grain damage.

Insecticidal material	% Conc. v/v	Meannumberof emerged adult (±S.E)	Percentage seed damage (±S.E)
P.santolinoides (aqueous)	5	$19.35±0.15^{b}$	$12.32±0.04^{b}$
	10	$17.52±0.21^{c}$	$11.71±0.01^{c}$
	15	$8.19±0.15^{e}$	$4.31±0.04^{e}$
	20	$2.14±0.15^{g}$	$1.98±0.03^{g}$
P.santolinoides (oil)	5	$9.30±0.15^{d}$	$4.86±0.02^{d}$
	10	$6.40±0.15^{f}$	$2.20±0.03^{f}$
	15	$1.93±0.15^{g}$	$1.65±0.02^{h}$
	20	$0.90±0.15^{h}$	$0.61±0.01^{i}$
Pirimiphos methyl (control)	0.5	$0.60±0.15^{h}$	$0.59±0.01^{i}$
	0.00	$41.82±0.15^{a}$	$28.25±0.03^{a}$

Means followed by common letters in the same column are not significantly different at 5% level.

Table 5. Effects of plant extracts and *P. methyl* on percentage weight loss and germination of maize seeds.

Insecticidal	Conc. %v/v	% weight loss (±SE)	% seed germination (±SE)
P. santalinoides (aqueous)	10	3.20^{b}± 0.06	78.65±2.64
	20	2.26^{c}± 0.03	75.14±1.25
	30	2.21^{cd}± 0.08	73.36±2.39
	40	1.42^{e}± 0.10	72.16±2.39
P. santalinoides (oil)	10	2.99^{c}± 0.05	72.40±5.18
	20	2.74^{d}± 0.10	71.40±1.44
	30	1.66^{e}± 0.16	71.40±1.44
	40	0.08^{f}± 0.01	70.00±4.08
Pirimiphos-methyl	0.5 ml	0.04^{g}±0.01	70.25±3.15
Control (solvent)	0.000	4.57^{a}±0.01	79.75±3 .68

LSD (0.05); 0.12; 0.2. Means followed by common letters in the same column are not significantly different at 5% level.

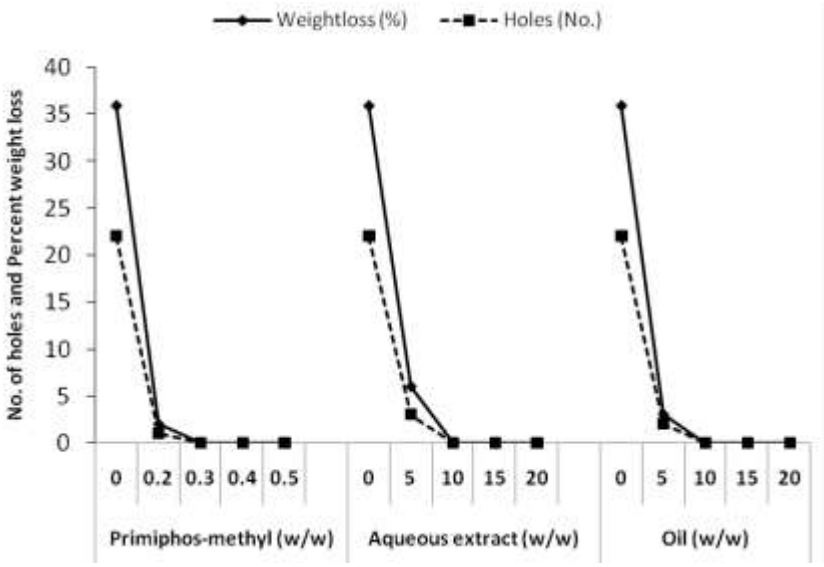

Figure 3. Number of holes and percentage weight loss.

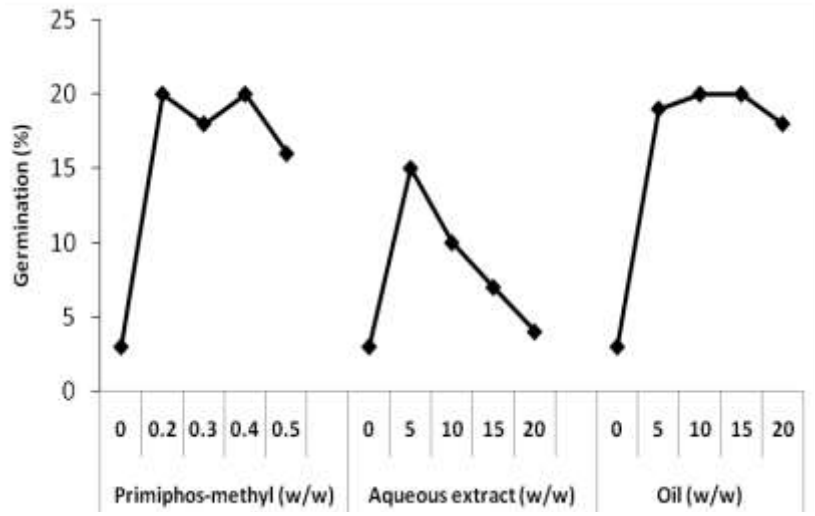

Figure 4. Percentage of germination.

Number of holes

The number of holes on maize seeds was significantly different between treatment levels ($F_{11, 24}$= 631.1, p<0.0001). Number of seeds with holes were significantly different between treated and untreated maize grains (p<0.0001) (Figure 3).

Percent grain weight loss

Grain weight loss significantly varied between treatment levels after two months and a half ($F_{11, 24}$= 1499.2, p<0.0001). After two and half months, percent weight loss of untreated seeds was about 35%. Treated ones did not lose weight (Figure 3).

Percent germination of seeds

Similarly, rate of seed germination significantly varied between treated and untreated seeds ($F_{11, 24}$= 93.53, p<0.0001). Germination rates did not vary significantly

between doses of the *P. santalionis* oil and *P. santalionis* aqueous *leaf* extracts (Figure 4).

DISCUSSION

The results of this study justified the role of *P.s antalinoides* aqueous leaf extract and *P. santalinoides* oil extract in the storage of maize grain against degradation by storage insects. The treatments have been observed to significantly reduce the ability of maize weevils to lay eggs on the protected seeds and thus led to a reduction in the level of damage.

Weevils did not reproduce on treated maize seeds because they did not find these seeds suitable for reproduction to takes place. From the result, it could be concluded that treatments controlled maize weevil reproduction almost 100% for two months and a half. The mechanism of action may be their antifeedant or repellent nature. Antifeedants, or feeding deterrents are chemicals that inhibit feeding or those that disrupt insect feeding by rendering the treated materials unattractive or unpalatable (Saxena et al., 1998). Therefore, according to the result of the analysis of variance, damage, that is, grain weight loss and number of holes of both extracts were significantly reduced or completely prevented by using these botanical products.

Oils are used in insect control because they are relatively efficacious against virtually all life stages of insects (Adedire, 2003; Rajashekar et al., 2014). Topical application caused high mortality to *S. zeamais* suggesting that oils have contact toxicity on the insects. *P. methyl* was very effective in controlling adult *S. zeamais* which agreed with Asawalam and Emosairue (2006) who reported 100% mortality to *S. zeamais* when treated with *P. methyl* in stored maize.

P. santalinoides aqueous and *P. santalinoides* oil extracts may have been potent because of the strong odours emitted thereby disrupting normal respiratory activities of the weevils; resulting in asphyxiation and subsequent death (Adedire and Ajayi, 1996). However, their effectiveness was dependent on dosages and exposure period. Highly significant difference on the emergence of adults *S. zeamais* reared on treated and untreated maize indicated that insecticidal materials tested had significant effects on the developmental stages which in turn affected emergence.

Arannilewa et al. (2003) reported that the oil extract on application covered the outer layer (testa) of the seeds serving as food poison to the adult insects; while some of them penetrated the endosperm and germ layer thereby suppressing oviposition and larval development. Significantly lower number of emerged F_1 progeny relative to control suggests the presence of some active principles in the plants that had contact toxicity and fumigants action on the weevils (Adedire, 2003).

Tahir et al. (2015) did repellency work using four indigenous plant extracts and found that M. longifolia was the most effective repellent while C. longa was least effective repellent against R. dominica. Significant difference in repellency was observed with increasing exposure time and dose rate. The repellency action is contributed to the presence of active metabolites in extract. These metabolites are composed of essential oils which are responsible for the repellent action (Gunarathna and Karunaratne, 2009; Saljoqi et al., 2006; Al-Jabr et al., 2001; Geetha and Roy, 2014) also reported the same trend.

Application of *P. santalinoides* aqueous and *P. santalinoides* oil extracts with pirimiphos – methyl to grains resulted in significant reduction of percentage weight loss. After two and half months, about 70% of untreated maize grains had holes and treated ones did not have holes. Grain weight declined with increasing number of holes and, therefore, weight loss and number of holes were directly related (Pearson's correlation coefficient r=0.99, $P<0.0001$, N=36). Infested maize seeds exhibit holes through which the adults emerge (Sahaf et al., 2008). Many indigenous plants, in powder form, effectively control cowpea seed beetles (Ofuya, 2003). Similar results have been reported earlier. For example, weight loss of wheat was prevented by applying the powder of *A. indica* and *A. boone* (Ileke and Oni, 2011). When maize weevils perforate maize grains, the weight of the grains declines.

In the present experiment, the *P. santalinoides* aqueous leaf and *P. santalinoides* oil extracts have prevented the formation of holes on seeds. This result is also supported by other researches on cowpea bruchids (Swella and Mushobozv, 2007) and common beans (Busungu and Mushobozv, 1991). Malathion treated common beans did not lose weight whereas the untreated ones did. Beans which were treated by Actellic super dust (as in the present study) and coconut oil to prevent *Z. subfasciatus* had the lowest number of holed seeds and the highest weight of seeds as compared to the untreated ones (Busungu and Mushobozv, 1991).

Increase in percent damaged bean seeds and weight loss is because of increasing bruchid number and the degradation of oils with time (Swella and Mushobozv, 2007). Just 2% turmeric powder provided good protection to rice or wheat and reduced grain weight loss (Saxena et al., 1998). Botanical insecticides such as pyrethrum, derris, nicotine, oil of citronella, and other plant extracts have been used for centuries (Singh and Upadhyay, 1993). In the plant powder, 99.1% mortality was recorded in *V. nugundo*, 94% in *N. speciosum*, and 96% in *A. officinuarium.* Adult emergence was registered in *A. indica* and A. *officinarum (*both 18%) followed by *G. superpa* (20%). The lowest grain weight

loss was reported in *A. indica* (18.55%) and *A. officinarum* (18.56%) (Akinnusi, 1986).

The result of an earlier study by Okonkwo and Okoye (1996) showed that percentage weight loss was related to the population of adult *S. zeamais.* Seed viability pre-treated with the extracts showed that the treatments did not negatively affect seed germination. When maize weevils perforate maize grains, seed germination rate declines. Insect pests inflict their damage on stored products mainly by direct feeding (Malek and Parveen, 1989).

Some species feed on the endosperm causing loss of weight and quality, while other species feed on the germ, resulting in poor seed germination and low viability. *P. santalinoides* aqueous and oil extracts prevented the formation of holes on seeds due to their insecticidal properties. In this study, the germination rate of untreated maize grain by *P. santalinoides* aqueous leaf and oil extracts is lower than that of the treated one almost by 80%.

Presence of *S. zeamais* in maize grains led to a reduction in germination with increasing developmental stage of the insects, from 13% at the egg stage to 93% at the adult stage (Santos et al., 1990). This result is also supported by other researches on cowpea bruchids (Swella and Mushobozv, 2007) and on common beans (Busungu and Mushobozv, 1991). This agrees with the report of Adedire et al., (2011) which gave no significant differences in viability of seeds pre-treated with 0.5 and 2.0% of four plant extract concentrations and the control. Results obtained from this study demonstrates active potentials of this plant products as plant-derived insecticides against maize weevil and provide a scientific rationale for the use of these botanicals as alternative to synthetic insecticides in post harvest protection.

The problems posed by broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity (Dayan et al., 2009). The efficacy of the products tested in the present study indicates their potential for replacing synthetic pesticides.

The two plant extracts regardless of dose prevented reproduction. That was a great leap forward. Synthetic insecticides not only do they pollute the environment but they also speed up weevil resistance to synthetic pesticides (Ileleji et al., 2007). On the other hand, pest insects have little chance of developing resistance to botanical products.

Botanical products are receiving more and more attention for pest control. What is needed is refining those using conventional scientific procedures. They have been with grain producers and traders for centuries. For example, Egyptian and Indian farmers used ash and leaves and seeds of neem for the control of stored grain pests (Varma and Dubey, 1999; Ahmed and Koppel, 1985). In eastern Africa, leaves of the wild shrub *O. suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants (Powell, 1989). In Rwanda, farmers store edible beans in a traditional closed structure and whole leaves of *O. canum* are usually added to the stored foodstuff to prevent insect damage within these structures.

Essential oil constituents such as thymol, citronellal and α- terpineol are effective as feeding deterrents against tobacco cutworm, *Spodoptera litura* (Hummel and Isman, 2001). Synergism or additive effects of a combination of monoterpenoids from essential oils have been good against *S. litura* larvae. The *H. spicigera* essential oils showed fumigant toxicity against *S. zeamais*. The mortality rate of *S. zeamais* increased with the concentration and duration of exposure to the essential oils (Wekesa et al., 2011).

Conclusions

So, protecting our food from storage insects is a priority to ensure food security. The treatments decreased weevil reproduction; grain weight loss and grain damage (holes on grains) and increased mortality. No loss of weight and perforation of holes were observed on treated maize grains. However, untreated grains sustained huge weight loss, the greatest number of offspring and holes. As the amount of *P. santalinoides* oil applied increased, the rate of germination was affected unlike that of *P. santalinoides* aqueous extracts, which does not have a negative impact on the rate of germination of maize grains. Generally, *P. santalinoides* extracts treatments were found to be effective against the attack of *S. zeamais.* This provides good arguments for carrying out this study on natural pesticides. Thus, the tested products could serve as potential tools for the management of storage insect pests. Future efforts should focus on product optimization, packaging and marketing.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of seedling fibrous roots on field performance of hybrid coffee varieties

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The objective of this study was to evaluate the effect of number of fibrous roots per seedling on plant growth and yield components of hybrid coffee varieties. A split plot experiment in a randomized complete block design (RCBD) with three replications was used. The main factor consisted of five varieties (N39-2, N39-3, N39-7, KP423-1 and KP423-2) whereas the sub-factor consisted of four types of roots (seedlings with 1-9 fibrous roots; seedlings with 10-17 fibrous roots; seedlings with ≥ 18 fibrous roots and control). Plants were evaluated for vegetative growth and yield components 14 months from the date of planting. The data were subjected to analysis of variance using CoStat software version 6.311 and treatment means were separated based on Tukey's test at P ≤ 0.05. Results indicate that coffee varieties N39-3, KP423-1 and KP423-2 were significantly (P = 0.00) taller than varieties N39-1 and N39-7 while coffee variety N39-2 significantly produced a larger number of fruit clusters per plant (P = 0.00) and higher seed yield (P = 0.00) than the rest of coffee varieties. Results also show that seedlings with at least 18 fibrous roots per seedling highly significantly increased plant height (P = 0.00), stem internode length (P = 0.00), number of fruit bearing primaries per plant (P = 0.00), number of fruit clusters per plant (P = 0.00), number of berries per plant (P = 0.00) and total seed yield (P = 0.00) of hybrid coffee varieties. The interactions between variety N39-3 and seedlings with at least 18 fibrous roots per seedling only significantly increased (P = 0.00) the internode length compared with the interaction between variety N39-3 and seedlings with 10-17 fibrous roots per seedling, and variety KP423-2 and seedlings with 1-9 fibrous roots per seedling. It is concluded that coffee growers should use seedlings with at least 18 fibrous roots per seedling in order to increase plant growth and total seed yield of improved hybrid coffee varieties. Further studies are required to determine propagation technologies which can increase the number of fibrous roots to at least 18 per stem cutting of hybrid coffee varieties.

Key words: Fibrous roots, number of bearing primary branches, number of clusters, plant growth, seed weight, total seed yield.

INTRODUCTION

Seedlings establishment and survival in the field is a function of seedling root and shoot biomass (Johansson et al., 2012; Corpuz et al., 2013). Below and above ground plant growth traits have been used to predict the success of field establishment of seedlings and their subsequent field performance (Wightman, 1999; Davis and Jacobs, 2005; Mohamed, 2013). Root biomass, especially the number of roots and root length, is directly related to establish and survive in the field after transplanting as well as plant height and basal diameter

(Wightman, 1999; Amri et al., 2009); Mohamed, 2013). Research has shown that the ability of crops to absorb water and nutrients is associated with the number and length of roots as well as number of leaves, leaf size, plant height and stem diameter (Hong et al., 2012). Generally, seedlings propagated by stem cuttings have been reported to have shallower roots with branchy stems when established in the field (Longman, 1993). A study by Çiçek et al. (2010) found that seedlings propagated by cuttings of *Fraxinus angustifolia* performed better in the field after transplanting than those propagated by seeds.

Tanzania Coffee Research Institute (TaCRI) released 19 hybrid Arabica coffee varieties that are established early in the field, heavy feeders and produce high yield of up to 3 t/ha against 1.5 t/ha of the traditional coffee varieties (Teri et al., 2011). High plant growth and yield are function of better root and shoot systems with the former enhancing more nutrient and water uptake from the soil (Fitch et al., 2005). There are limited reports on the effect of root systems on growth and yield of improved hybrid coffee varieties developed by TaCRI. The objective of this study was to evaluate the effect of fibrous roots on plant growth and yield components of improved hybrid coffee varieties.

MATERIALS AND METHODS

Description of the study area

The field experiment was set up from July 2014 to December 2016 at TaCRI. The station is located at Lyamungu, Moshi Tanzania at latitude of 03º14.699' S and longitude of 037º14.762'E with a mean altitude of 1268 m a.s.l. The soil is classified as Nitisol with a pH ranging from 4.8 to 5.7 and the climate is classified as tropical with warm-dry (August to February) and rainy (March to June) seasons with an average annual rainfall of about 1250 mm. During the study, the mean temperature from July 2014 December in 2016 was 28.34°C with the lowest temperature of 28.23 in 2014 and highest temperatures of 29.25°C in 2016.

Seedling production

Coffee stem cuttings were raised in a mixture of forest soil and fine sand at ratio of 2:1 (v/v). The number of laterals and fibrous roots of stem cuttings were counted four months from the date of planting to establish four groups of seedlings as recommended by Rouhani et al. (1987). Group 1 consisted of seedlings with 1-9 fibrous roots and 1-4 lateral roots; Group 2 seedlings with 10-17 fibrous and 1-4 lateral roots; Group 3 seedlings with ≥18 fibrous and 1-4 lateral roots; and Group 4 seedlings with unsorted roots as control. The seedlings with grouped roots were transplanted or potted into black polythene tubes with diameter of 15 cm and height of 15 cm. The growth media consisted of top forest soil and fine sand as above supplemented with N-P-K fertilizer (20: 10: 10) at a rate of 33.3 g/m^3. The polytubes were filled two-third with the growth media. The seedlings were placed under a black shade net absorbing 30% of solar radiation. Water soluble fertilizer (poly-feed) containing 19N-19P-19K+Zn, B, Fe, Mn, Cu, Mo at a rate of 150 g/15 L was sprayed on the seedlings using knapsack sprayer three weeks after transplanting.

Land preparation and soil analysis

Land preparation was carried out by ploughing to a depth of 20 cm and harrowing as recommended by Cambrony (1992). Two blocks were established in the experimental area for soil sampling based on land topography and these were categorized as block 1 and 2. Soil samples were collected in three sites in each block using a hand-auger from depths of 0-30 cm and 30-60 cm. Composite samples were prepared to get representative samples in each site as recommended by Nunez et al. (2011) and Bekeko (2014). Separate soil core samples were also collected from the three sites with metal cores forced manually into the soil for determination of bulk density and soil moisture content. The collected soil samples were analysed at Lyamungu Soil Laboratory. Samples were air-dried, ground, sieved through 2 mm sieve as recommended by Nunez et al. (2011). Results show that the soil was sandy loam with a bulk density of 1.27 g/cm^3. Results of chemical properties of the soil are shown in Table 1. Remarks on the level of nutrients were based on FAO (1984) and Landon (1991).

Experimental design

A split-plot experiment in a randomized complete block design replicated three times was used. The main factor was five coffee varieties (N39-2; N39-3; N39-7; KP423-1 and KP423-2) whereas the sub-plot factor consisted of four seedling root groups (Group 1: seedlings with 1 - 9 fibrous roots and 1- 4 lateral roots, Group 2: seedlings with 10-17 fibrous and 1- 4 lateral roots, Group 3: seedlings with ≥ 18 fibrous and 1 - 4 lateral roots and Group 4: seedlings with unsorted roots as control). The treatments were randomized in both main and sub-plots within each replication according to Kuehl (2000) using Gen Stat discovery Edition 3 software.

Seedling planting and management

The seedlings were transplanted in the field in July 2014 at a depth of 60 × 60 cm holes and spacing between and within rows of 2.74 × 2.74 m and between blocks of 3 m as recommended by TaCRI (2011). A border row of the same coffee varieties was planted around the experimental area to overcome border effects on the experimental units as recommended by Tesso et al. (2011). The total number of rows was 20 each comprising 8 plants for each variety and each root characteristic. The total number of experimental units per replication was 160 whereas the plot size was 1,321.2 m^2. Based on soil analysis results, 18 kg of well decomposed farm yard manure per planting hole was incorporated with the top soil one month before planting as recommended by TaCRI (2011) and Marandu et al. (2004).

Fertilizer N.P.K containing 20% N, 10% P and 10% K was

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Table 1. Soil chemical characteristics of area planted with coffee.

Chemical properties	Analysis method	Results
pH	1:2.5 soil: water ratio	5.15[a]
Electric conductivity (dS m^{-1})	1:2.5 soil: water ratio	1.40[a]
CEC ($cmol_{(+)}kg^{-1}$)	Ammonium acetate saturation	4.41[a]
Exchangeable Ca ($cmol_{(+)}kg^{-1}$)	Atomic absorption spectrometry	3.26[b]
Exchangeable Mg ($cmol_{(+)}kg^{-1}$)	Atomic absorption spectrometry	0.45[b]
Exchangeable K ($cmol_{(+)}kg^{-1}$)	Flame photometry	0.43[c]
Exchangeable Na ($cmol_{(+)}kg^{-1}$)	Flame photometry	0.05[a]
Available P (mg kg^{-1})	Bray and Kurtz 1	19.71[c]
Organic Carbon %	Walkley and Black	0.50[a]
Total N%	Semi micro Kjeldahl	0.03[a]
C: N ratio		21.09[b]

a = Very low, b = Low, c = Medium and d = High.
Source: FAO (1984) and Landon (1991).

applied at a rate of 100 g/plant during transplanting. Urea containing 46% N was applied one month from the date of transplanting at rate of 50 g/plant while N:P:K was again applied at a rate of 50 g/plant during the short rains six months from the date of transplanting. Furthermore, N:P:K (20:10:10) at a rate of 100 g per plant was applied four months before flowering as side dressing during the long rains in May 2015. One month after full flowering, calcium ammonium nitrate (CAN) containing 27% nitrogen, 13.5% ammonia, 13.5% nitrate, 4.0% magnesium and 6.0% calcium was applied as side dressing at a rate of 200 g/plant. Water soluble fertilizer (poly-feed) containing 19N-19P-19K+Zn, B, Fe, Mn, Cu, Mo at a rate of 150 g/15 L of water was sprayed using knapsack sprayer twice after 100% fruiting. N.P.K containing 20%N, 10%P and 10% K at a rate of 200 g/plant was applied as side dressing. Irrigation when needed was done by furrow. Agronomic practices such as weeding, insect and pest control were carried out as recommended by TaCRI (2011).

Data collection

Data on all the variables except for days to 50% flowering were collected from six central inner plants in each row as recommended by Tefera et al. (2016).

Plant growth traits

Days to flowering was measured on a plot basis as the number of days from the date of transplanting to when approximately 50% of the plants in a plot produced flowers (Assis et al., 2014; Tefera et al., 2016). The plant height was measured at full fruit bearing (22 months after transplanting) from the base of the stem to the plant apex using graduated ruler (Assis et al., 2014; Tefera et al., 2016). The stem diameter of the main stem was measured at full fruit bearing at 5 cm above the ground using Vernier Calliper (Assis et al., 2014; Tefera et al., 2016). The length of bearing primary branches was measured from the point of attachment to the main stem to the apex using graduated ruler as an average value of four longest bearing primaries per plant (Esther and Adomako, 2010). The length of internodes was measured using graduated ruler as an average value of four internodes per plant (Esther and Adomako, 2010). Total number of bearing primary branches was estimated by counting the total number of bearing primaries per plant at full fruit bearing stage (Esther and Adomako, 2010).

Yield and yield components

Number of clusters or number of fruiting nodes was determined as an average number of clusters per plant from four heavily bearing primaries at the middle across all directions (Etienne and Betrand, 2001). The number of berries or fruits was estimated as an average number of berries per plant counted from the four heavily bearing primaries at the middle across all directions as recommended by Etienne and Betrand (2001). Yield was obtained by harvesting mature red cherries to get fresh weight per plot using gravimetric scale. Transformation of cherry weight to clean coffee weight was done using the conversion factor of 0.16 for Arabica coffee as recommended by ICO (2011). Seed weight was measured using gravimetric scale from 100 cherries taken randomly from each plot during the 7th harvest (peak yield) and then converted to parchment using conversion factor of 0.16 for Arabica (Agbaje et al., 2011).

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using CoStat software version 6.311 and declared significant at P≤0.05 using the following statistical model for the spilt-plot design as described by Kuehl (2002):

$$Yi_{ijk} = \mu + \alpha_i + P_k + d_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}.$$

where μ = the general error mean; $\alpha_{i\,i}$ = the effect of the ith level of factor; P_k = the effect of the kth block; d_{ik} = the whole-plot random error; β_j = effect of the jth level of factor B, $(\alpha\beta)_{ij}$ = the interaction effect between factors A and B; ε_{ijk} = is the sub-plot random error.
The differences between the treatment means were separated based on Tukey's test at a probability of 5%.

RESULTS

Effect of hybrid coffee varieties on plant growth traits

Results indicate that coffee varieties highly significantly (P = 0.00) affected plant height and significantly (P = 0.02) affected the length of bearing primaries (Table 2).

However, the stem diameter (P = 0.56), length of

Table 2. Effect of hybrid coffee varieties on plant growth traits.

Varieties	Plant height (cm)	Main stem diameter (cm)	Plant internode length (cm)	Length of primaries (cm)	Days to 50% flowering
N39-2	201.32[b]	3.61	6.08	106.53[a]	473.00
N39-3	214.43[a]	3.68	6.09	103.60[ab]	473.00
N39-7	198.31[b]	3.56	6.18	98.05[ab]	473.91
KP423-1	207.74[a]	3.55	6.14	98.21[ab]	473.00
KP423-2	217.46[a]	3.59	6.24	87.37[b]	472.82
Mean	207.85	3.60	6.14	98.75	472.95
CV (%)	3.49	6.12	19.38	12.09	0.06
P-values	0.00	0.56	0.99	0.03	0.59

Means followed by the same letter in the same column are not significantly different at P≤0.05 according to Tukey's Test.

Table 3. Effect of number of fibrous roots on plant growth traits.

Root characteristics	Plant height (cm)	Main stem diameter (cm)	Plant internode length (cm)	Length of primaries (cm)	Days to 50% flowering
Group one (1-9 roots/seedling)	206.25[b]	3.47[b]	5.71[b]	86.32[b]	472.86[a]
Group two (10-17 roots/seedling)	203.42[b]	3.53[ab]	5.84[b]	102.85[a]	472.93[a]
Group three (≥18 roots/seedling)	218.24[a]	3.75[a]	6.96[a]	105.92[a]	473.00[a]
Group four (control)	203.50[b]	3.62[ab]	6.07[b]	99.94[a]	473.00[a]
Mean	207.85	3.59	6.14	98.75	472.95
CV (%)	5.06	6.68	10.74	10.85	0.04
P-values	0.00	0.02	0.00	0.00	0.21

Means followed by the same letter in the same column are not significantly different at P≤0.05 according to Tukey's Test.

internodes (P = 0.99) and the number of days to 50% flowering (P = 0.59) were not significantly affected by coffee varieties.

Effect of number of roots on plant growth traits of hybrid coffee varieties

The number of fibrous roots per seedling highly significant (P = 0.00) affected coffee plant height, length of primaries and length of internodes, and significantly (P = 0.02) affected stem diameter (Table 3). However, the number of days to 50% flowering was not significantly (P = 0.21) affected by number of fibrous roots per seedling.

Interaction effect of coffee varieties and number of roots on plant growth traits

Interaction effect between varieties and number of roots per seedling significantly (P=0.05) affected internode length (Table 4). However, plant height (P = 0.14), stem diameter (P = 0.19) and length of primaries (P = 0.21) and days to 50% flowering (P = 0.85) were not significantly affected by the interaction between varieties and root characteristics.

Effect of hybrid coffee varieties on yield and yield components

Coffee varieties highly significantly (P = 0.00) affected seed yield and number of fruit clusters per plant and significantly affected the number of bearing primaries (P = 0.02) and number of berries per plant (P = 0.01) (Table 5). However, seed weight was not significantly (P = 0.43) affected by varieties.

Effect of number of roots on yield and yield components

The number of fibrous roots per seedlings highly significant (P = 0.00) affected seed yield, seed weight, number of bearing primaries, number of fruit clusters and number of berries per plant (Table 6).

Interaction effect of hybrid coffee varieties and number of roots on yield and yield components

Interaction between varieties and number of fibrous roots

Table 4. Effect of interaction between hybrid coffee varieties and root characteristics on plant vegetative morphological characteristics.

Varieties × Root characteristics	Plant height (cm)	Main stem diameter (cm)	Length of primaries (cm)	Internode length (cm)	Days to 50% flowering
N39-2 x 1-9 roots/seedling	195.00	3.47	102.86	6.00^{a-c}	473.00
N39-2 x 10-17 roots/seedling	201.44	3.70	114.63	5.92^{a-c}	473.00
N39-2 x ≥ 18 roots/seedling	213.96	3.63	116.46	6.60^{a-c}	473.00
N39-2 x control	194.90	3.64	92.16	5.81^{a-c}	473.00
N39-3 x 1-9 roots/seedling	210.00	3.53	90.63	5.73^{a-c}	473.00
N39-3 x 10-17 roots/seedling	217.70	3.84	109.20	5.47^{bc}	473.00
N39-3 x ≥ 18 roots/seedling	221.66	3.68	112.43	7.62^{a}	473.00
N39-3 x control	208.38	3.67	102.16	5.56^{a-c}	473.00
N39-7 x 1-9 roots/seedling	194.66	3.46	82.96	5.77^{a-c}	472.66
N39-7 x 10-17 roots/seedling	191.49	3.42	102.77	5.57^{a-c}	473.00
N39-7 x ≥ 18 roots/seedling	222.11	4.01	107.00	6.88^{ab}	473.00
N39-7 x control	185.00	3.36	99.50	6.50^{a-c}	473.00
KP423-1 x 1-9 roots/seedling	208.72	3.36	82.10	6.29^{a-c}	473.00
KP423-1 x 10-17 roots/seedling	197.11	3.31	102.70	5.99^{a-c}	473.00
KP423-1 x ≥18 roots/seedling	210.93	3.74	104.36	6.40^{a-c}	473.00
KP423-1 x control	214.22	3.71	103.70	5.88^{a-c}	473.00
KP423-2 x 1-9 roots/seedling	222.91	3.53	73.03	4.77^{c}	472.66
KP423-2 x 10-17 roots/seedling	209.39	3.37	84.96	6.28^{a-c}	472.66
KP423-2 x ≥18 roots/seedling	222.55	3.70	89.33	7.31^{ab}	473.00
KP423-2 x control	214.99	3.70	102.16	6.59^{a-c}	473.00
Mean	207.85	3.59	98.75	6.14	472.95
CV%	5.06	6.68	10.85	6.14	0.04
P-values	0.14	0.19	0.21	0.05	0.85

Means followed by the same letter in the same column are not significantly different at P≤0.05 according to Tukey's Test.

Table 5. Effect of hybrid coffee varieties on yield and yield components.

Varieties	No. of bearing primaries	No. of fruit clusters/plant	No. of berries/plant	100 seed weight (g)	Seed yield (t/ha)
N39-2	38.93^{a}	12.27^{a}	88.15^{a}	13.59^{a}	2.28^{a}
N39-3	37.05^{ab}	10.85^{b}	78.95^{ab}	14.16^{a}	1.90^{ab}
N39-7	34.67^{b}	10.53^{b}	71.57^{ab}	13.18^{a}	1.22^{c}
KP423-1	35.35^{ab}	10.66^{b}	75.18^{ab}	14.54^{a}	1.43^{bc}
KP423-2	34.28^{b}	9.60^{c}	59.63^{b}	14.25^{a}	1.08^{c}
Mean	36.06	10.78	74.69	13.95	1.58
CV (%)	8.09	5.65	18.40	13.18	29.11
P-values	0.02	0.00	0.01	0.43	0.00

Means followed by the same letter in the same column are not significantly different at P≤0.05 according to Tukey's Test.

per seedling highly significantly (P=0.00) affected number of bearing primaries and seed yield, and significantly (P = 0.01) affected 100 seed weight (Table 7). However, the number of fruit clusters per plant (P = 0.89) and number of berries per plant (P = 0.63) were not affected by the interaction between varieties and root characteristics.

DISCUSSION

Effects of varieties on vegetative growth components

The five hybrid coffee varieties differed in their plant and primary branch height where variety N39-3, KP423-1 and

Table 6. Effect of number of roots on yield and yield components.

Number of roots per seedling	No. of bearing primaries	No. of clusters/plant	No. of berries/plant	100 seed weight (g)	Seed yield (t/ha)
Group one (1-9 roots/seedling)	34.69^{b}	10.06^{b}	68.38^{b}	14.74^{a}	1.06^{c}
Group two (10-17 roots/seedling)	35.54^{b}	10.32^{b}	72.77^{b}	13.95^{ab}	1.43^{b}
Group three (≥18 roots/seedling)	38.86^{a}	12.46^{a}	90.98^{a}	13.35^{b}	2.61^{a}
Group four (control)	35.14^{b}	10.28^{b}	66.64^{b}	13.74^{b}	1.24^{bc}
Mean	36.06	10.78	74.69	13.95	1.58
CV (%)	6.60	11.22	17.21	6.81	20.33
P-values	0.00	0.00	0.00	0.00	0.00

Means followed by the same letter in the same column are not significantly different at P≤0.05 according to Tukey's Test.

Table 7. Interaction effects between hybrid coffee varieties and number of roots on yield and yield components.

Varieties x number of roots	No. of bearing primaries	No. of clusters/plant	No. of berries/plant	100 seed weight (g)	Seed yield (t/ha)
N39-2 x1-9 roots/seedling)	45.70^{a}	10.76^{b}	84.20^{a-d}	14.52^{ab}	1.02^{d}
N39-2 x 10-17 roots/seedling	39.00^{a-d}	12.10^{ab}	90.83^{a-c}	13.94^{a-c}	2.77^{ab}
N39-2 x ≥ 18 roots/seedling	35.80^{b-d}	14.66^{a}	111.63^{a}	12.88^{a-c}	3.69^{a}
N39-2 x control	36.60^{b-d}	11.56^{ab}	91.60^{a-c}	13.02^{a-c}	1.64^{cd}
N39-3 x 1-9 roots/seedling	37.50^{b-d}	10.13^{b}	74.26^{a-d}	14.39^{a-c}	1.11^{d}
N39-3 x10-17 roots/seedling	41.00^{a-c}	10.13^{b}	79.43^{a-d}	14.20^{a-c}	1.61^{cd}
N39-3 x ≥ 18 roots/seedling	39.50^{a-d}	12.36^{ab}	95.63^{ab}	14.31^{a-c}	3.72^{a}
N39-3 x control	40.70^{a-d}	10.76^{b}	74.80^{a-d}	13.75^{a-c}	1.14^{d}
N39-7 x 1-9 roots/seedling	37.80^{b-d}	10.03^{b}	71.76^{b-d}	13.75^{a-c}	1.01^{d}
N39-7 x 10-17 roots/seedling	34.0^{cd}	9.66^{b}	65.03^{b-d}	13.76^{a-c}	1.01^{d}
N39-7 x ≥ 18 roots/seedling	42.00^{ab}	12.13^{ab}	77.96^{a-d}	11.38^{c}	1.77^{b-d}
N39-7 x control	39.00^{a-d}	10.30^{b}	71.53^{b-d}	13.86^{abc}	1.08^{d}
KP423-1 x 1-9 roots/seedling	42.00^{ab}	9.83^{b}	54.83^{cd}	15.23^{ab}	1.15^{d}
KP423-1 x 10-17 roots/seedling	37.70^{bcd}	10.73^{b}	73.16^{bcd}	15.30^{ab}	0.91^{d}
KP423-1 x ≥18 roots/seedling	36.40^{b-d}	12.36^{ab}	84.93^{a-d}	14.43^{ab}	2.30^{bc}
KP423-1 x control	36.30^{b-d}	9.73^{b}	52.26^{d}	13.19^{abc}	1.35^{cd}
KP423-2 x 1-9 roots/seedling	37.50^{bcd}	9.53^{b}	70.86^{b-d}	15.83^{a}	1.01^{d}
KP423-2 x 10-17 roots/seedling	36.70^{b-d}	9.00^{b}	78.83^{a-d}	12.55^{bc}	0.83^{d}
KP423-2 x ≥18 roots/seedling	33.30^{d}	10.80^{b}	86.33^{a-d}	13.74^{a-c}	1.54^{cd}
KP423-2 x control	34.30^{cd}	9.07^{b}	81.36^{a-d}	14.91^{ab}	0.94^{d}
Mean	36.06	10.78	78.56	13.95	1.58
CV%	6.6	11.22	15.3	6.81	20.25
P-values	0.00	0.89	0.63	0.01	0.000

Means followed by the same letter in the same column are not significantly different at P≤0.05 according to Tukey's Test.

KP423-2 were the tallest followed by variety N39-2 and N39-7 with shortest stems. High stem height increased amounts of water soluble carbohydrate reserves and enhanced plant growth in wheat genotypes (Ehdaie et al., 2006). Similarly, stem carbohydrate reserves account for 20 and 70% of the final plant weight under non-stressed and water stressed conditions, respectively and 20-40% of the total yield in wheat crop (Liw et al., 2015; Kumar et al., 2017). Stored water soluble carbohydrates also maintained a steady growth and accounts for more than 40% of the stem dry weight in rape seed (Li et al., 2015). Carbohydrate reserves are also associated with plant response to the environmental conditions. Plants with high carbohydrate and mineral nutrient reserves are more resilient to stressful environmental conditions as reported in Robusta coffee (*Coffea canephora*) and hardwood tree Alder (*Alnus* spp) (Rezende et al., 2010; Pijut et al., 2011).

Effects of number of roots on coffee yield and yield components

Results from this study show that the number of fibrous roots per seedling increased yield components and therefore total seed yield of hybrid coffee varieties. The observed higher seed yield from coffee plants with the largest number of fibrous roots is associated with increased nutrient and water uptake. Previous studies have also associated yield with the ability of plants to absorb water and nutrients (Hong et al., 2012). Root traits have been used to predict the success of field establishment of seedlings and their subsequence field performance (Davis and Jacobs, 2005). Similarly, root biomass was significantly and positively correlated with yield in 12 peanut varieties (Hong et al., 2012). Atta et al. (2013) also noted a stronger relationship between root and above ground traits with root traits contributing 30-45% of the total variation in water use efficiency and grain yield in wheat.

CONCLUSION AND RECOMMENDATION

The objective of this study was to evaluate the effect of number of fibrous roots per seedling on plant growth and seed yield of hybrid coffee varieties. Seedlings with at least 18 fibrous roots per seedling increase total seed yield by 146% compared to coffee plants derived from seedlings with 1-9 fibrous roots per seedling, by 110% compared to the control plants and by 83% in comparison to coffee plants derived from seedlings with 10-17 fibrous roots per seedling. It is therefore recommended that farmers should use coffee seedlings with at least 18 fibrous roots per seedling to increase field plant establishment, growth and total seed yield. Further studies are required to determine propagation technologies which can increase the number of roots per seedling to 18 fibrous and 1-4 lateral roots per seedling.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Allelopathic effects of aqueous extract of leaves and roots of *Luetzelburgia auriculata* (Allemão) Ducke on seeds germination and initial growth of lettuce *(Lactuca sativa* L.)

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The secondary metabolism of plants produces allelopathic substances which are able to interfere with germination and growth of other species, when released in the environment. In order to evaluate the allelopathic effects of *Luetzelburgia auriculata* on the germination and early growth of lettuce (*Lactuca sativa*), leaf extracts and roots were tested in lettuce seeds sowing. The *L. auriculata* plant material was collected from adults, subsequently washed, dried, weighed, crushed and thus used to preparing a raw extract. In a completely randomized design, six concentrations (0, 20, 40, 60, 80 and 100%) of the extracts of leaves and roots were tested separately with four replications on lettuce seeds in germitest paper. The experiment was conducted in a germination chamber TE 402 during seven days with photoperiod of 12 h light and a constant temperature of 20°C, to check the germination speed index (GSI), germination percentage (% G) and initial growth lettuce (radicle length and hypocotyl). The data were submitted to a variance analysis applying the F test at 1% probability and using the error bars model. It was observed that the results of the treatments with root extract and leaf extract, when compared to the control test, were negatively affected by the aqueous extracts of *L. auriculata*. The GSI and %G of seeds treated with roots extract were the parameters that presented most significant responses to the allelopathic effects of *L. auriculata* extract at 20% concentration, followed by GSI and %G seeds subjected to 20% leaf extract. Both extracts at other concentrations reduced GSI and %G in 85 and 90%, respectively. The length of the radicle and hypocotyl decreased by 32 and 15% respectively when the extract was used at a lower concentration, and 40% (radicle) and 30% (hypocotyl) in other concentrations. The aqueous extracts of leaves and roots *L. auriculata* caused negative allelopathic interference on the germination and growth of lettuce seedlings.

Key words: Secondary metabolites, Brazilian semiarid region, allelopathy.

INTRODUCTION

In nature living organisms interact naturally. This interaction, called interference, is responsible for the

occurrence of many phenomena that can happen between plants and microorganisms. The competition, indirect interference and allelopathy are examples of such phenomena, which can be generated in individuals of an ecosystem, have been largely investigated from the organisms behavior (donors and recipients) presents in the same environment perspective. It is important to remember that these interactions can have positive, negative or no effects.

The allelopathy phenomenon occurs, according Rice (1984), when an organism releases chemicals into the environment and these substances interact inhibiting or stimulating the growth and/or development of another organism present in the same environment. Allelopathic substances, phytotoxins, allelochemicals or secondary products are the names given to chemicals released by organisms in the environment that affect other community components (Oliveira et al., 2011), such chemicals are released from the plant tissue through volatilization, leaching, root exudate and the decomposition of plant residues and their allelopathic effects can be studied experimentally, by biological tests of plant extracts containing allelochemicals.

There are many species that presents allelopathic features; the effects on other species of these individuals have been studied by applying plant extracts donor (allelopathic) in seeds or seedlings of other plants (receptor). The techniques for preparing such extracts are varied, generally, the extract is obtained firstly from the milling of plant parts (roots, leaves, flowers or fruits), and secondly placed in contact with water or organic extractors (alcohol or ether, for example), and finally filtered and tested on test plants like tomato, lettuce and radish (most sensitive of all species used in such studies) (Medeiros, 1989).

Moreover, *Luetzelburgia auriculata* has drawn attention by the way it occurs in the landscape, usually in coppices, suggesting rusticity, developing in dry, rocky places, maintaining green foliage even in much of the dry season. Considering these features, it has aroused interest in the study of possible allelopathic effects that may be caused by these individuals.

As stated earlier, the use of biological tests is fundamental to the increase of the knowledge of allelopathic effects. According to Chou (2014), the study of allelopathic interactions is useful in the search for natural phytotoxins produced by plants or micro-organisms, and its synthetic derivatives that can be used as natural herbicide to have more specific and less harmful to the environment compounds. Therefore, this study aimed to investigate the allelopathic effects of aqueous extracts of leaves and *L. auriculata* roots (Allemão) Ducke on germination and growth of lettuce seedlings (*Lactuca sativa* L.).

MATERIALS AND METHODS

Leaves and roots were collected manually and randomly of adult individuals of a population of *L. auriculata*, located in Cachoeira Farm in São Porfirio, Várzea-PB city. The plant material was packaged separately in plastic bags and sent to the Plant Mineral Nutrition Laboratory at the Center for Health and Rural Technology Federal University of Campina Grande, Campus of Patos, where two different experiments (leaves and roots) were installed.

In order to obtain the aqueous extracts, leaves and roots separately, were weighed, washed, rinsed with distilled water, minced in broken (fresh still) in a household mixer for 5 and 10 min, respectively, in a proportion of 200 g of plant material to 800 ml of distilled water, and finally allowed to rest for a period of 30 min. Later, the extract was filtered through a sieve with 2.0 mm mesh to give crude extracts (concentration 100%).

Soon after, 25 lettuce seeds were equidistant distributed on two sheets of *germitest*, 25 × 30 cm size overlapped; a third sheet was also placed over the seeds distributed and 250 ml of the extract already diluted was applied to each of the experimental units, totaling a 1000 ml per treatment. Once the application of the extract is finished, paper rolls were confectioned maintaining the seeds, conditioned inside plastic bags (capacity 1.0 kg), and placed in a germination chamber TE 402 for 7 days with a photoperiod adjusted to 12 h light and constant temperature of 20°C.

Each experiment was installed in a completely randomized design with six treatments (concentrations), four replications and twenty-four experimental units; treatment (control) was prepared only with distilled water. Evaluations were made daily, looking at seed germination submitted to treatments with the aqueous extracts of the leaves and roots, separately, adopting as a criterion, the germination radicle protrusion (2.0 mm).

Once the data were collected, the germination speed index (GSI), germination percentage (% G) and checked the radicle length and hypocotyl in seedlings emerged in both experiments were determined. The collected data were transformed into $\sqrt{x + 1}$ and subjected to analysis of variance, applying the F test at the 1% probability level. For the complete presentation of the answers, the error bars of the averages were included.

RESULTS AND DISCUSSION

Aqueous extract of leaves *L. auriculata*

In the analysis of the results, regarding the GSI, %G, radicle length and hypocotyl of lettuce seeds submitted the leaf aqueous extract of *L. auriculata*, it has been found from the determination coefficient value that the most appropriate model to explain the relation between ratio concentrations of the extracts and the parameters analyzed was the regression model until the second order. For the germination speed index, it is possible to checks that the increase in concentrations of aqueous

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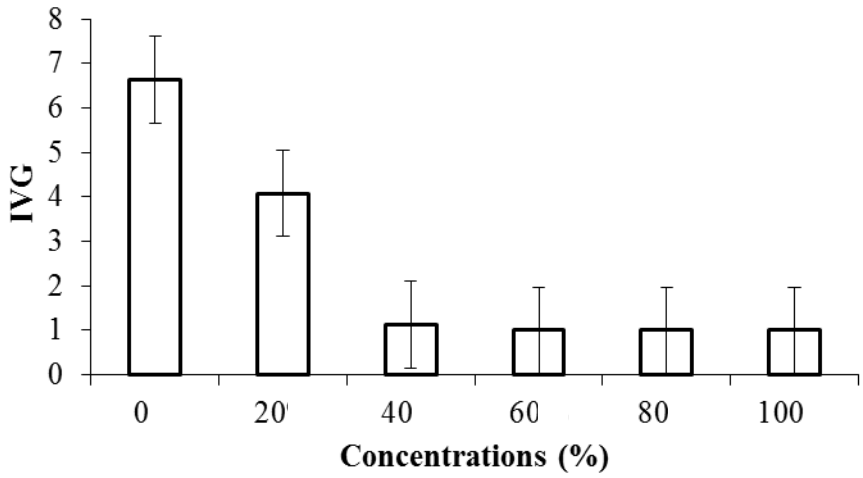

Figure 1. Speed index of lettuce seeds germination under aqueous extract treatment of *L. auriculata* in different concentrations.

extract of *L. auriculata* leaves promoted reduction of this parameter in relation to the attestant test in the order of 43, 72, 88, 95 and 81% of the concentrations of 20, 40, 60, 80 and 100%, respectively (Figure 1). The result suggests that the extract of *L. auriculata* exerted negative effect allelopathic on this parameter.Pelegrini and Cruz-Silva (2012) when using aqueous extracts of fresh leaves of *Coleus barbatus* (A.) Benth on lettuce seeds, observed that the GSI was reduced as the extract concentration increased, occurring what authors call dose dependent response. Borges et al. (2007) also identified this negative relationship between GSI lettuce seed and plants extract. For Aoki et al. (1997), the intensity of allelopathic effects is dependent on the concentration of allelopathic substances present in a species.

Souza and Furtado (2002) observed negative alelochemical effect of rye extracts on lettuce seed GSI as a result of the tested extract concentrations. In a study of aqueous extracts of umbu (*Phytolacca dioica* L.) on lettuce seeds, Borella and Pastorini (2010) observed that the germination rate index also decreased when the seeds were submitted to extracts with concentrations starting from 4%.

Teixeira et al. (2004) observed that there was a 90% reduction in lettuce seed GSI submitted to the aqueous extract of *Crotalaria juncea* and found out that often the extract influences more the speed of germination than any other parameter.

Aquila (2000), using *Ilex paraguariensis* extracts in lettuce seeds also found a loss of seed vigor, so the author says this is an economically important parameter to be evaluated. According to Rodrigues (2012), a late germination may mean losses for the farmer, especially in the case of species with short life cycle.

It is expressed in Figure 2, the percentage of germination of lettuce seeds submitted to different concentrations of aqueous extracts of *L. auriculata* leaves. It can be perceived from the data that a negative effect of the extracts related to the different concentrations did happened. In the treatment where the concentration of the extract was 20%, seed germination was reduced by 25%; the extract where the concentration was 40% had a reduction of 85%, and to the other concentrations, the decrease was also high (approximately 90%).

Conducting laboratory tests, Al-Sherif et al. (2013) have identified that the germination of weed seeds was reduced even when used at lower concentrations (1%) of aqueous allelopathic extract, and totally inhibited when used at higher concentrations (4%). França et al. (2008) found out that neem extracts (*Azadirachta indica*) also was responsible for reducing the percentage of lettuce seed germination and beggartick.

Pelegrini and Cruz-Silva (2012) observed that the use of aqueous extracts of fresh leaves of *Coleus barbatus* (A.) Benth on lettuce seeds significantly inhibited the germination percentage, which presented a considerable difference from the control of this study. Corsato et al. (2010) observed that there was a significant reduction in the percentage of germination of conventional soybeans when applied the highest concentrations of aqueous extract of sunflower (80 and 100%).

In a study on the allelopathic effects on weeds *Annona Crassiflora*, Inoue et al. (2010) reported that extracts ofleaves from the donor species reduced by more than 50% germination of *Brachiaria* seeds. According to Souza Filho and Duarte (2007), the biological activity of an allelochemical depends directly on the response limitof the receiving species, because it is closely related to its sensitivity. In an experiment conducted by Corsato et al. (2010), it was observed that there was a reduction in germination of beggartick seeds, caused by fresh

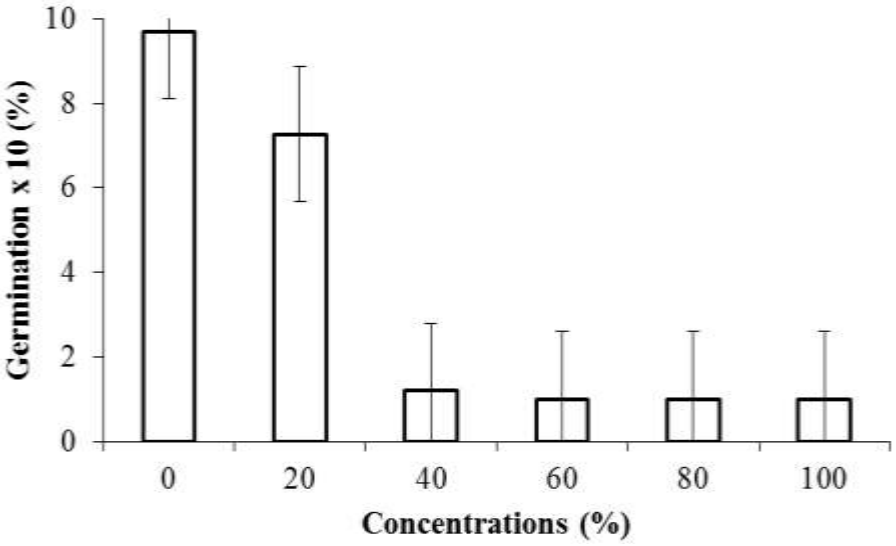

Figure 2. Lettuce germination under different concentrations of aqueous leaves *L. auriculata* extract.

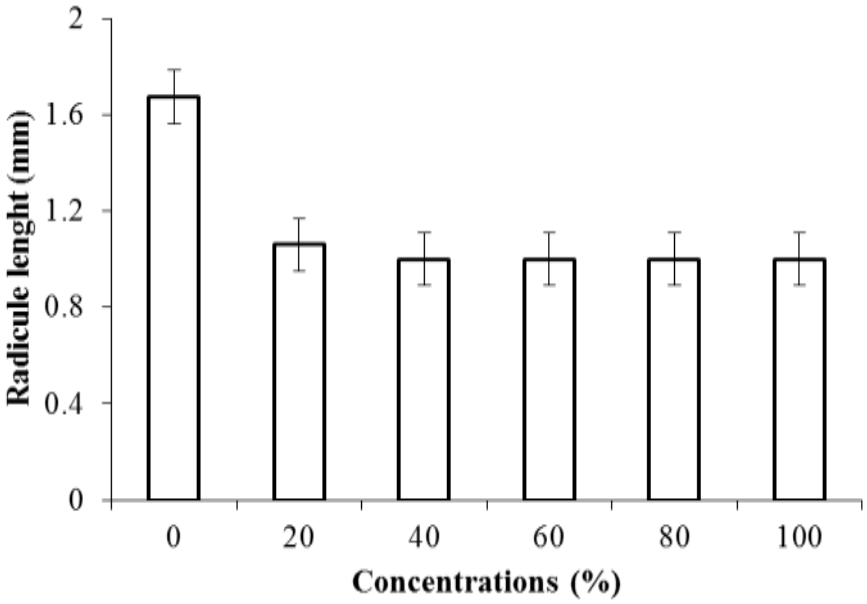

Figure 3. Lettuce radicle lenght under different concentrations of aqueous *L. auriculata* leaves extract.

leaves of sunflower (*Helianthus annus* L.), species considered as allelopathic.

The results obtained in relation to the length of radicle seedling grown under different concentrations of aqueous extracts of *L. auriculata* leaves' is as shown in Figure 3; it displays that the seedlings emerged in the extract with a concentration of 20%, have developing 32% less than the control. Moreover, other treatments have a reduction of radicle length by almost 40%, compared to the control. Thus, results indicate that there was a negative allelopathic effect of the donor species on the development of seedlings. This study corroborates with the results of Tur et al. (2010), which while testing aqueous extracts of fresh leaves of *Duranta repens* on the germination and early growth of lettuce, found that there was significant reduction in root length of this vegetable seedlings. Lima and Moraes (2008) also observed a significant reduction in the growth of radicle,

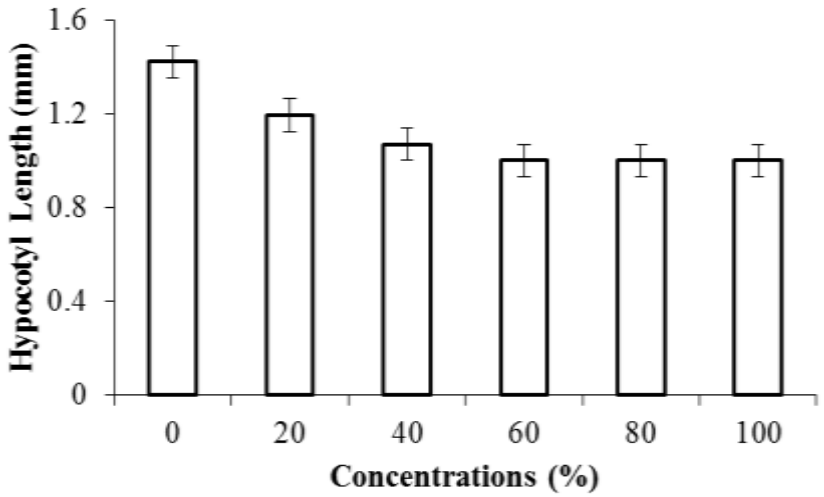

Figure 4. Lettuce hypocotyl length under different aqueous extract concentrations of *L. auriculata* leaves.

while studying the effect of aqueous extract of *Ipomoea fistulosa* on the germination and early growth of lettuce. Peres et al. (2004) observed that the extract obtained from *Adiantopsis radiate* leaves' and *Adiantum tetraphyllum* significantly inhibited the growth of lettuce radicle at all concentrations used (250, 500, and 1000 $mg.L^{-1}$). In these cases, also, it was noticed that the increase in the concentration resulted in higher inhibition and morphological abnormalities apex oxidation radicle and the absence of absorbents structures.

Aumonde et al. (2012), by using extract of *Zantedeschia aethiopica* leaves in lettuce seeds obtained similar results, as there was a negative interference of the extract on the radicle and a more evident effect, as the concentration increased. According to Chung et al. (2001), the effect of the extract on the radicle can be attributed to increased sensitivity of the organ and by direct contact of the extract with the tissue.

Utilizing error bars (Figure 4), the hypocotyl length of lettuce seedlings was analyzed and subjected to the control and seedlings subjected to different concentrations of *L. auriculata* leaves aqueous extract. A reduction of 15% the hypocotyl length of seedlings emerged in the extract concentration with 20 and 30% in seedlings of other treatments (concentrations 40, 60, 80, 100%) was found as the main result.

In a similar study, Maraschin-Silva and Aquila (2006) tested plant extracts of native species on the initial growth of lettuce and found that *Psychotria leiocarpa* provided a negative allelopathic effect on hypocotyl size of lettuce seedlings treated with the extract. Formagio et al. (2012) evaluated the allelopathic effect of *Tropaeolum majus* leaves extract on the initial growth of beggarticks and observed that the hypocotyl length of seedlings was also negatively affected under the treatment when compared to the control treatment. However, negative effects suffered by radicle were more significant than those suffered by the hypocotyl.

Evaluating the allelopathic potential of aqueous extract of oat leaves, Hagemann et al. (2010) observed that *Avena sativa* and *Avena strigosa* caused a reduction in the growth of radicle and hypocotyl on *Lolium multiflorum* and *Euphorbia heterophylla*, respectively.

Oliveira et al. (2012) found that the allelopathic effects of Mulungu aqueous extract also affected the development of lettuce seedlings. While applying leaf extract of *Annona crassiflora* on weed seeds, Inoue et al. (2010) detected that there was a significant inhibition in the development of hypocotyl compared to the control, however, this inhibition was less intense than that affecting the radicle.

Garsaball and Natera (2013) stated that the allelopathic effects of aqueous extracts *of Tithonia diversifolia* leaves negatively altered the overall growth of lettuce seedlings, especially the growth of root and hypocotyl.

It is believed that the allelopathic effects of aqueous extracts of *L. auriculata* leaves on lettuce germination may have occurred due to the presence of flavonoids. This substances that in preliminary studies (phytochemical) conducted at the Federal University of Rio Grande do Norte (data not published), were detected in extracts from leaves and roots of *L. auriculata*. According to Alves et al. (2004), flavonoids are bioactive metabolites derived from the secondary metabolism. Chaves et al. (2001) stated that these substances, showing phytotoxic activity, are present in various structures of *Cistus ladanifer* plants and also found in the soil.

Aqueous extract of roots *L. auriculata*

It was found from the value of the coefficient of

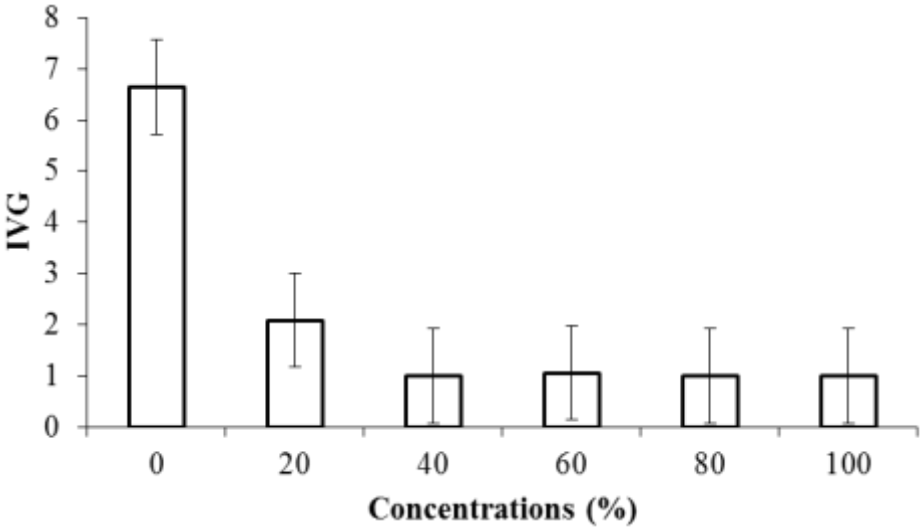

Figure 5. Lettuce germination speed index (GSI) under aqueous *L. auriculata* extract under different concentrations.

determination of each of the parameters considered (GSI,% G, length of the radicle and hypocotyl), that the relationship between the concentrations of the root aqueous extract of *L. auriculata* on lettuce seeds and such parameters had quadratic regression as the most suitable model to explain the relationship.

Lettuce seeds imbibed with the aqueous extract of *L. auriculata* roots 20% had GSI almost 70% lower when compared with the control (without application of aqueous extract), since, increase in extract concentrations made GSI lettuce seeds' decrease, reaching a reduction of 85% to 40-100% concentrations. Thus, these data suggest that the aqueous extract of *L. auriculata* roots exerted negative allelopathic effects on lettuce germination. Gatti et al. (2004) also found similar results (Figure 5), while testing aqueous extract of marcela (*Aristolochia esperanzae* O. Kuntze) on the germination and growth of lettuce. This negative relationship between GSI and concentration of the plant extract is also reported by Borges et al. (2007).

The aqueous root extracts of *L. auriculata* also influenced negatively vigor of lettuce seeds. According to Ferreira and Borghetti (2004), the greater the GSI, the greater the force of seeds. In tests with extracts allelopathic species (40 to 100% concentration), seeds had the GSI six times lower than the control treatment. Similar data were found in Carvalho et al. (2014), where the use of aqueous extracts of six allelopathic species acted decreasing vigor of lettuce seeds.

According to Tur et al. (2010), GSI has shown sensitivity to the allelopathic effects to be an important parameter to be evaluated. Rodrigues (2012) believes that the delay or reduction in the time taken for germination can be reversed as profits or losses in the field, especially when it comes to species that have short life cycle.

It is noticed that there were differences between the extracts and the control treatment, even when the extract was used at lower concentrations of 20%. In a study by Sartor et al. (2009) on allelopathic effect of Loblolly pine extracts on germination and development of *Avena strigosa* seedlings, the authors also observed variations in the receiving species development patterns, being the extract effect perceived in lower concentrations and enhanced as concentration was increased.

It is possible to check on Figure 6, the results of the lettuce seeds germination rate, when submitted to different concentrations of *L. auriculata* roots extract, where a decrease in the rate was found.

The aqueous extract of *L. auriculata* roots, in relation to the control treatment, reduced germination by approximately 60% when used at a concentration of 20%; and at a concentration of 40 to 100%, the germination was reduced to 90%. According to Rodrigues (2012), when the control treatment is above 90%, the result is consistent with the germination recommended by seed producers. According to Corsato et al. (2010), there is a significant reduction in the percentage of germination of the conventional soybean seed when the highest concentrations of the aqueous extract are applied (80 to 100%).

Formagio et al. (2012), in a similar study, found that the seeds of beggartick treated under root extract of *Tropaeolum majus* L., reduced their germination in 32%. In tests with extracts of *Hovenia dulcis* Thunb roots. on *Parapiptadenia rigida* (Benth.) Brena seeds, Araldi (2011), noticed that the viability of the seeds was changed.

For the radicle length of the emerged plants under different concentrations of aqueous extracts of *L.*

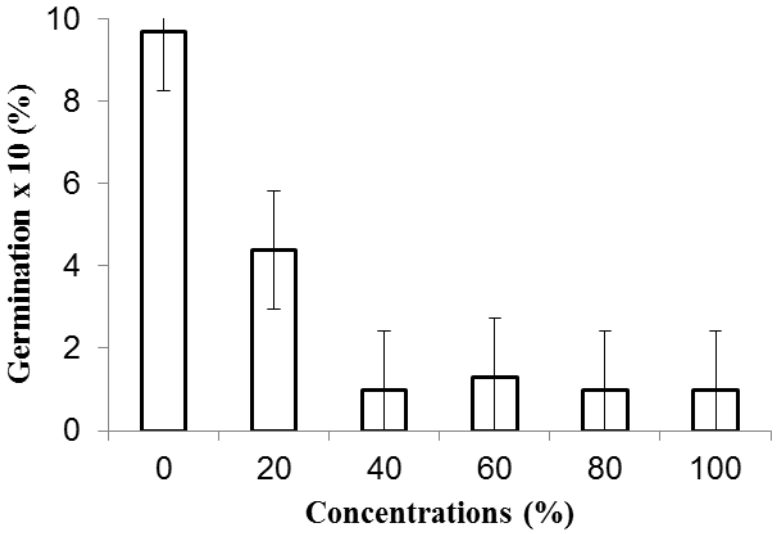

Figure 6. Lettuce germination under different concentrations of aqueous *L. auriculata* roots extract.

auriculata roots, it was observed that, compared to the control, there was a 31% reduction in the length of sprouts from seeds treated with the extract at 20% and almost 40% in those which emerged from other treatments, which suggests that there was an adverse allelopathic effect from the aqueous extract (Figure 7).

The radicle of the emerged plants in the concentrations used was the most affected structure by the aqueous extract of *L. auriculata* root, because 100% showed reduced size, deformation and/or necrosis. Grisi et al. (2011), in a study conducted on the allelopathic effect of *Sapindus saponaria* morphology, found that the reduction in size and necrosis were the most common symptoms in the roots of the seedlings treated with the donor plant extract. In the same study, the authors found that lettuce seedlings showed reduced growth of the primary root, reaching zero values when subjected to the concentration of 7.5%.

Evaluating the initial growth of lettuce under the effect of aqueous extracts of five species of Gleicheniaceae, there was a significant reduction in the length of sprouts of this vegetable. Even plants which have grown, suffered from a toxic effect on its structures growth showing a similar damage found detergents effects in other plants, characterized by reduced size and necrotic aspect of structures (Soares and Vieira, 2000). To Maraschin-Silva and Aquila (2006), many phytotoxins are able to affect the morphology and anatomy of seedlings, which can be evidenced by hardening and darkening of the root apex, fragility and increased branching.

Similar data for the present study were found by Grisi et al. (2013), while verifying that the negative allelopathic effect of aqueous *S. saponaria* roots extract on barnyardgrass and rope-glory was significant on the growth of seedlings of recipients species, particularly, evidenced by the decrease in the length of the radicle. Rosado et al (2009) observed that there was a significant reduction in root growth of lettuce, compared to the control treatment, when submitted to the aqueous exract basil extract.

The hypocotyl length of lettuce seedlings also responded negatively to the allelopathic effect of aqueous *L. auriculata* roots extract; however, showing less intensity compared to results found for the length of the radicle. In the control test, there was a reduction of 15% in structures growth when applied the extract with a concentration of 20%, and almost 30% when applied to the concentration of 40, 60, 80 and 100% of the aqueous extract (Figure 8), respectively. According to Silva and Aquila (2006), in tests with extracts of native species of *Erythroxylum argentinum*, *Divaricata luehea*, *Myrsine guianensis* and *Ocotea puberula* on the initial growth of lettuce, all caused reduction in the size of the hypocotyl-root axis with the hypocotyl presenting a low inhibition.

Silveira et al. (2014) studied the allelopathic effects of aqueous extract *of Araucaria angustifolia* on lettuce initial growth and found that the final growth of seedlings was affected, as the extract concentration increased; however, the length of hypocotyl showed no significant difference for different concetrations of the extract. The allelopathic effect of aqueous extract of *S. saponaria* root on barnyard grass seeds and rope-glory, significantly, reduced the hypocotyl of seedlings of these weed species (Grisi et al., 2013).

In tests with five *Erythroxylum* species extracts ontomato and onion seedlings growth, Taveira et al. (2013) observed that there was a significant reduction in hypocotyl development of the receiving plants. In the

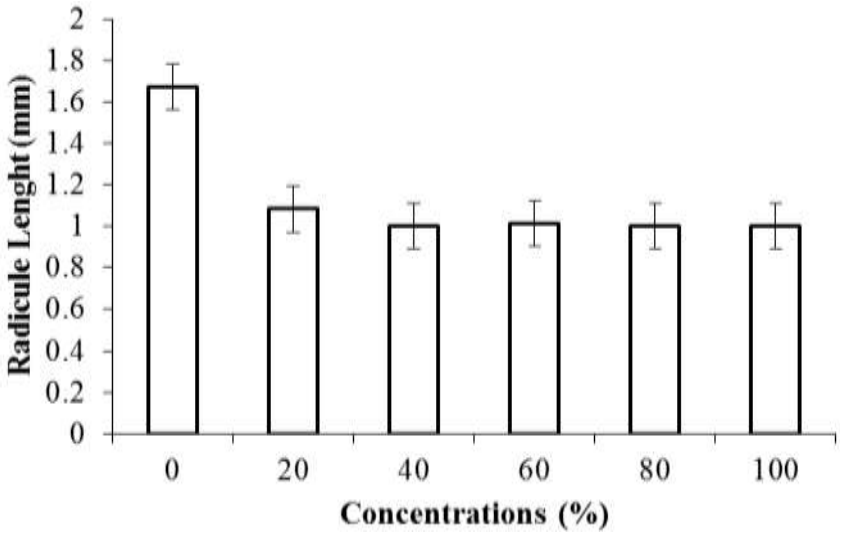

Figure 7. Lettuce radicule seedlings length under different concentrations of aqueous *L. auriculata* extract.

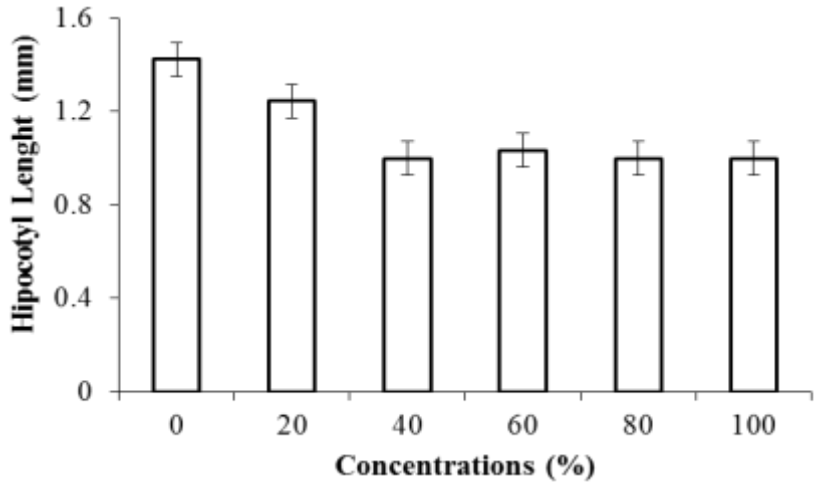

Figure 8. Lettuce hypocotyl length under different aqueous *L. auriculata* extract.

evaluation of allelopathic effects on the aqueous *S. saponaria* extract in onion seedlings development, it was found that the length of hypocotyl also was reduced (Grisi et al., 2011).

Conclusion

The aqueous *L. auriculata* leaves extract caused negative allelopathic interference on the germination and growth of lettuce seedlings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Efficacy of the decoction of cashew leaf (*Spondias mombin* L.) as a natural antiseptic in dairy goat matrices

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Microorganisms resistant to conventional antimicrobial substances are becoming more prevalent. Accordingly, natural alternatives have been sought with the use of extracts from medicinal plants whose purpose is to find new compounds with recognized antimicrobial activity. This work aims to show the efficacy of the cajá (*Spondias mombin* L.) leaf decoction using the microplate dilution technique against *Staphylococcus* sp, a bacterial strain isolated from cases of subclinical mastitis in dairy goat matrices. The 5% probability test was used to compare the means among the studied treatments. Leaf extract from the cajá was effective at concentrations of 1:1 and 1:2 against all strains of *Staphylococcus* coagulase negative, regardless of storage temperatures. All strains were resistant to amoxicillin + clavulanate, ampicillin, cefepime and ceftazidime, being characterized with a multiresistant profile.

Key words: *Staphylococcus* sp. microorganisms, decoction, *Spondias mombin* L.

INTRODUCTION

Inflammatory and non-infectious diseases are traditionally treated with anti-inflammatories, which are usually expensive. Consequently, it is indispensable to find alternative products for microbial control that are economical and ecologically viable. With the need for new alternatives to combat disease, studies have been conducted using plants from the northeastern semiarid as a therapeutic option for this problem. The field man's empirical knowledge regarding medicinal plants and their therapeutic power over some diseases was combined

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with the tests that proved the phyto-therapeutic effectiveness of some of these plants.

The use of medicinal plants as an alternative therapy, with an emphasis on microbial inhibition by natural antiseptic agents, has become a beneficial action for the sertanejo that survives from the caatinga, since conventional alternatives are often inappropriate to socio-environmental conditions. Among these alternative plants is *Spondias mombin* L, which is also known as cajazeira, cajazeira-miúda, taperebá or cajá-mirim.

The cajazeira is a leafy tree with a wide and imposing crown during the phase of flowering and fruiting. As for the taxonomic classification, *S. mombin* L. is of the family Anacardiaceae; Tribe- Spondiadeae; Genus- Spondias L. (Barroso et al., 1999). On the chemical constitution of cajazeira, Diby et al. (2012) identified the presence of alkaloids, flavonoids, polyphenols, quinones, saponins, gallic tannins and terpenes in the stem bark. Regarding its ethnopharmacological characteristics, it has been reported that all parts of the plant have medicinal properties.

The aromatic bark, both astringent and emetic, constitutes a good vomitory in the cases of bilious fevers and palustres, and has an antidiarrheal, anti-disintegrating, antiblenorrhagic and anti-hemorrhoidal reputation (the last property also attributed to the root). The leaves are useful against bilious fever, constipation of the stomach, pains of the stomach etc. In recent years, it has been found that extracts from the leaves and branches of the cajazeira contain gallic tannins with medicinal properties for the control of gram negative and positive bacteria (Sacramento and Souza, 2000). Medeiros (2013) observed flavonoids, catechins, flavanones, xanthones and free steroids in the leaf of the cajá in addition to the presence of tannins.

Therefore, the aim of the present study was to analyze the efficacy of antiseptic on the base of the leaflet (*S. mombin*) against strains of bacterial *Staphylococcus* sp. across different storage times and temperatures.

MATERIALS AND METHODS

Preparation of the decoction of the leaf of cajá

The leaves of *S. mombin* L. were harvested at 07:00 am in April 2017 from a specimen on the campus of the Federal Rural Semi-Arid Federal University (Universidade Federal Rural do Semi-Árido). The samples were packaged in black bags and taken to the UFERSA Veterinary Microbiology Laboratory for decoction processing. Previously, the exsicata was taken to Herbarium Dárdaro de Andrade Lima to be cataloged, where it received the following numbering: *S. mombin* L. – 13953. About 100 g of *S. mombin* leaves were collected and placed in a container with 200 ml of distilled water. This was boiled in a water bath for 15 min for the production of 200 ml of decoction. The material was filtered on filter paper[1] and stored in a sterile, amber glass bottle.

[1] It has an open texture, used in filtrations with fast speed of thick precipitates, with weight: 85 g/m^2 and Porosity: 7.50 microns (Brand: CAAL - CASA AMERICANASão Paulo)

Decoction dish analysis

The decoction was diluted with a serial dilution of 10^{-1}, 10^{-2} and 10^{-3} in distilled water tubes with 9 ml each. Subsequent deposition of these dilutions in Petri dishes with Mueller-Hinton agar medium was analyzed in duplicate. These plates were kept in an oven at 37°C for 24 h to observe possible growth of CFU (colony forming units). This study was conducted in the same manner at 0 and 24 h for 0 and 7 days of the refrigerated decoct, and on the 7th day of the decoction, it was preserved at room temperature (25°C). Ten strains of coagulase-negative *Staphylococcus* (LMV01, LMV02, LMV03, LMV04, LMV05, LMV06, LMV07, LMV08, LMV09 and LMV10) were isolated from milk collected from caprine matrices with subclinical mastitis.

Preparation of the inoculum for in vitro test

The standard inoculum of each microorganism cultured for Mueller-Hinton agar diffusion assay was obtained by sowing BHI broth in the log phase (exponential growth) at the concentration of 0.5 on the MacFarland scale for 18 to 24 h. A sterile cotton swab was introduced into the suspension with the inoculum, which was rotated five times clockwise, squeezing it firmly against the inner wall of the tube above the liquid level so as to remove any excess inoculum in the swab. In the Mueller-Hinton agar dish, the microorganisms were distributed by pressing the swab over the entire sterile surface of the agar to ensure uniform distribution of the inoculum. Each bacterial strain was deposited on two dishes with the agar.

Determination of antimicrobial susceptibility through disc diffusion (Kirby-Bauer)

After inoculum distribution in the Petri dishes with the culture medium, 18 disks of the following antimicrobials were deposited: ampicillin (10 µg), amoxicillin + clavulanic acid (30 µg), aztreonam (30 µg), cephalothin (30 µg), cefepime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ciprofloxacin (05 µg), chlorphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), novobiocin (30 µg), piperacillin + tazobactam (110 µg), polymyxin (300 µg), sulfamethoxazole + trimethoprim (25 µg), vancomycin (30 µg) and tetracycline (30 µg). After this procedure, the dishes were placed into a bacteriological oven at 37ºC for 24 h, after which the halos were read through a millimeter ruler.

Preparation of the microdilution test

The standard inoculum of each microorganism cultured for microdilution plate test was obtained by a second sowing of bacteria in BHI broth in log phase (exponential growth) at the concentration of 0.5 of the MacFarland scale for 18 to 24 h. The study was performed in two 96-well microdilution plates (ALAMAR®, Diadema, São Paulo, Brazil) arranged in 12 columns (1 to 12) and eight lines (A to H). Each microorganism and positive control with chloramphenicol was tested in duplicate. Next, 100 µl of brain and heart infusion broth (BHI) was inserted into each well. Then, 100 µl of the cassava decoction (*S. mombin*) was inserted into the wells relative to the 1:1 concentrations and serial dilution of these wells was then made to the concentrations (1:2, 1:4 and 1:8). In the last well equivalent to that bacterium, 100 µl was discarded so that the wells remained with equal amounts. This was followed by the inoculation of 5 µl of suspension of the BHI microorganisms in each well. In the positive control, 100 µl of previously diluted Chloramphenicol (1 mg/ml) in distilled water was deposited. Chloramphenicol was chosen because it was the best antimicrobial

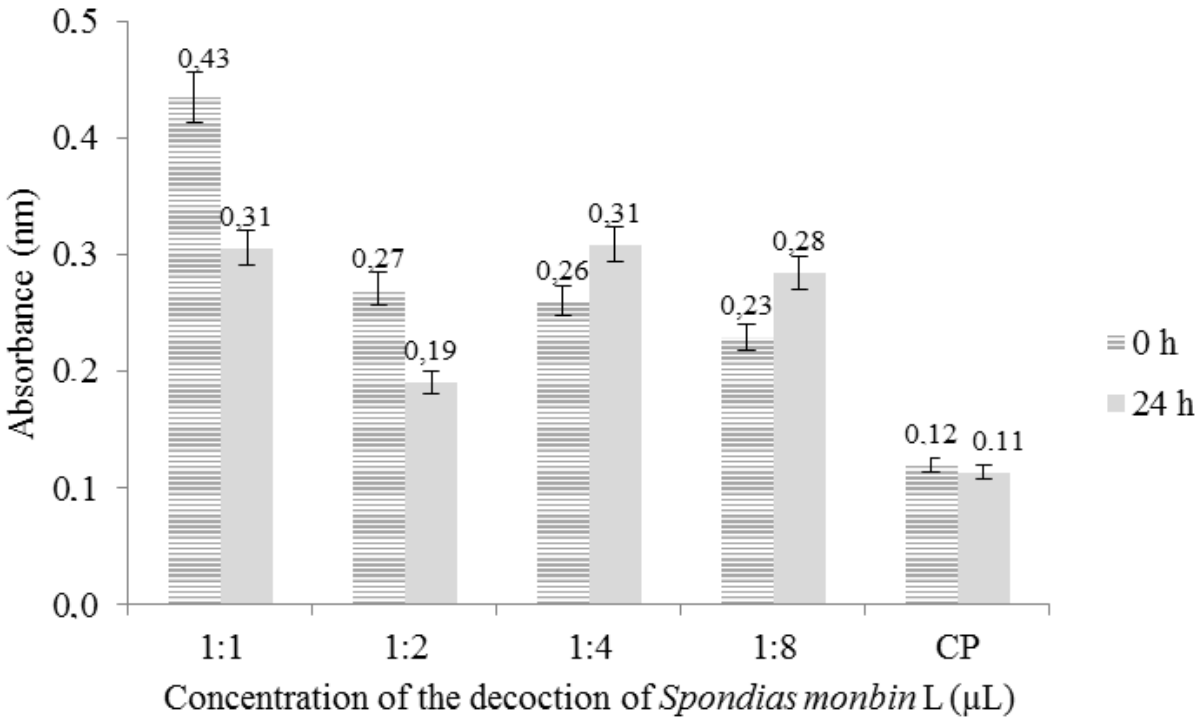

Figure 1. Absorbances according to the concentrations of the extracts of *S. mombin* and the time of culture of the bacteria with 0 and 24 h, using decoct produced on day 0.

in the disc-diffusion test, showing 100% effectiveness against strains of coagulase-negative *Staphylococcus*. After the preparation, the plates passed through the absorbance reader (URIT 660 - MICROPLATE READER). The well readings were performed at 0 and at 24 h on days 0 and 7 of refrigerated decoction, and on the 7th day of the decoction stored at room temperature (25°C).

Statistical analysis

Considering the significance level of 5%, Tukey's test was used to compare the means between treatments and performed with the software application SISVAR 3.01 (Ferreira, 2000)

RESULTS AND DISCUSSION

Plaque analysis for 0 and 7 days decoctions under refrigeration and maintained for 7 days at 25°C showed no bacterial growth. It was observed that the decoction kept for 7 days under refrigeration had concentrations of 1:1 and 1:2 and showed efficacy against the strains in relation to the other concentration results obtained on day 0 where it was analyzed at 0 and 24 h. A decrease in absorbance was observed with differences of 0.12 and 0.08, respectively, in relation to the hours at concentrations of 1:1 and 1:2.

This reduction relates to a smaller quantity of bacteria; thus, indicates the effectiveness of the concentration of decoction used. At concentrations of 1:4 and 1:8, the absorbance level increased (0.05 nm), thus showing bacterial growth in the wells using these concentrations (Figure 1).

Concerning the tests performed with the refrigerated decoction for 7 days, it is possible to verify a remarkable inhibition of the strains in concentrations of 1:1 and 1:2 of the extract, which varies from 0.20 to 0.42 nm and 0.15 to 0.31 nm, at the initial time and 24 h later, respectively (Figure 2). A third analysis was performed with the cajá extract used after the 7th day at room temperature and at the concentration of 1:1, there was a decrease in absorbance of 0.10 nm in relation to 0 h (0.26 nm) and 24 h (0.16 nm) showing that there was significant inhibition by the decoction in relation to the strains tested. At 1:2 concentrations, the mean difference between the hours was much lower, but still inhibition was characterized by a decrease in the absorbance of 24 h (0.22 nm) in relation to 0 h (0.24 nm). The absorbance difference between these two hours was 0.02 nm (Figure 3).

There is no consensus regarding the level of inhibition acceptable for natural products when compared with standard antimicrobials; so much so that some authors consider only antimicrobial-like results, whereas others consider with good potential even those with higher levels of inhibition (Duarte, 2006). Matias (2012) study of the antibacterial activity of *S. mombin* extract and the inhibitory action of extracts at 1:1, 1:2, 1:4 and 1:8 concentrations mainly on coagulase-negative *Staphylococcus*, *Staphylococcus aureus* and *Streptococcus* sp found that only two concentrations

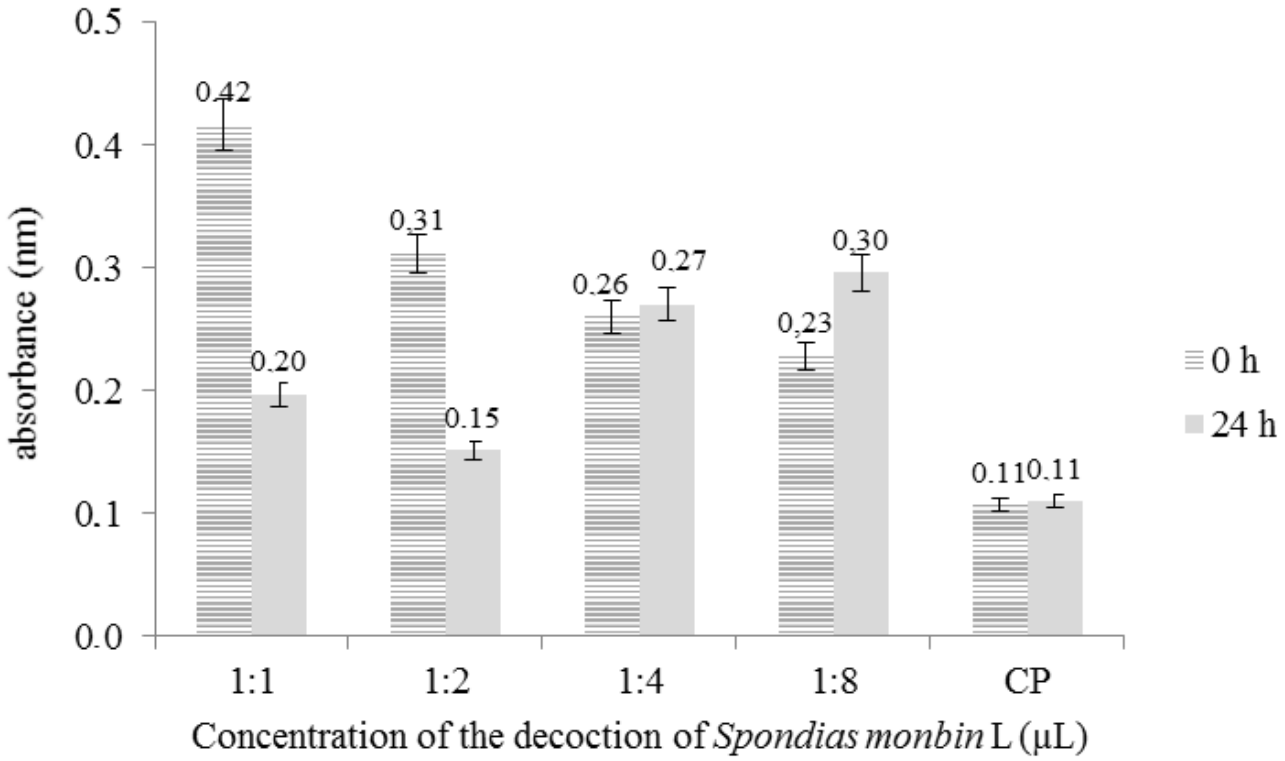

Figure 2. Absorbances according to the concentrations of the extracts of *S. mombin* and the time of culture of the bacteria 0 and 24 h, using decoct maintained at the temperature of 8ºC during 7 days.

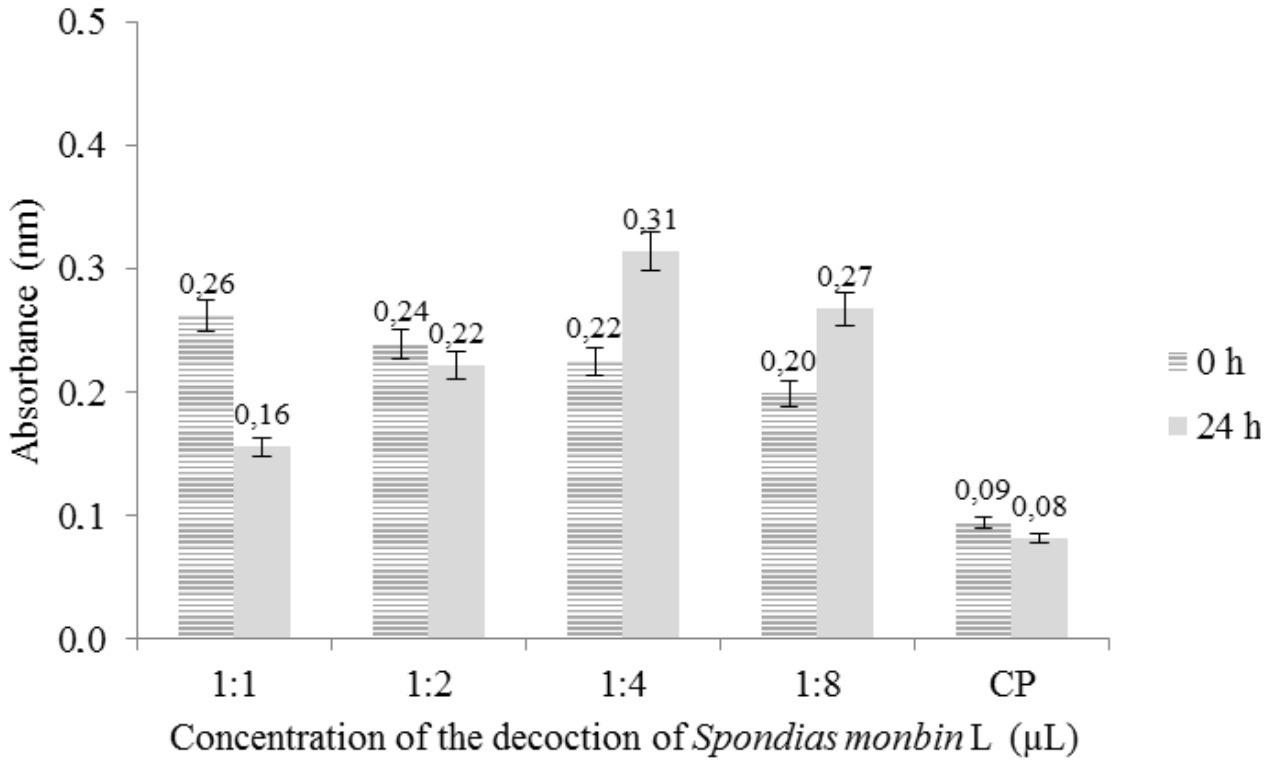

Figure 3. Absorbance as a function of the concentrations of the extracts of *S. mombin* and the time of culture of the bacteria during 24 h using decoct with 7 days maintained at 25ºC.

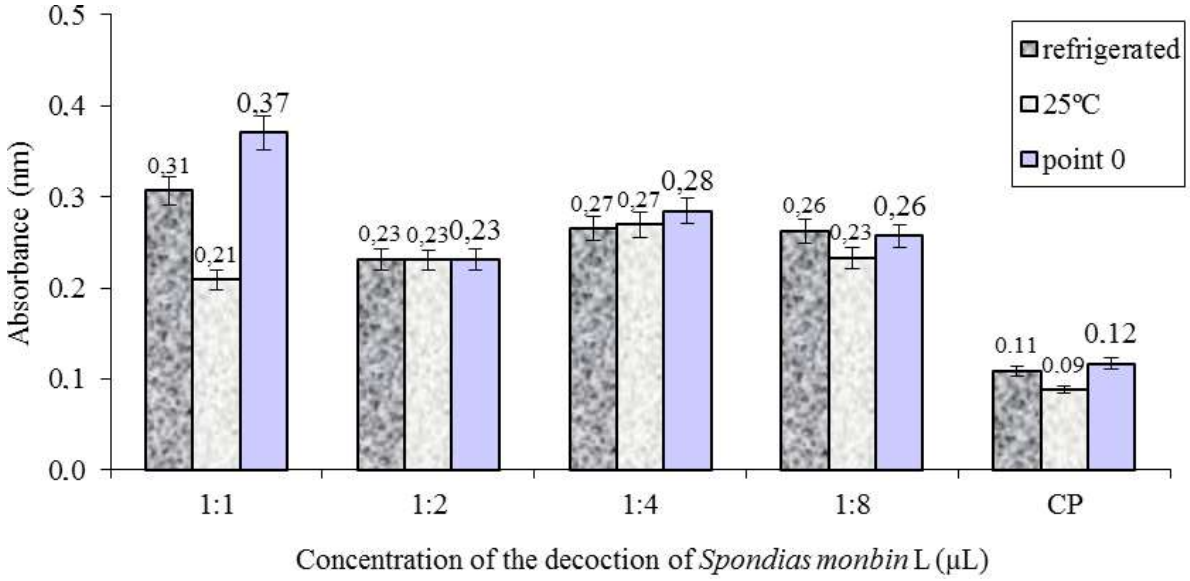

Figure 4. Mean values of the absorbance values in relation to the extract concentrations, at temperatures 0º and 25ºC, compared with the means of the positive control.

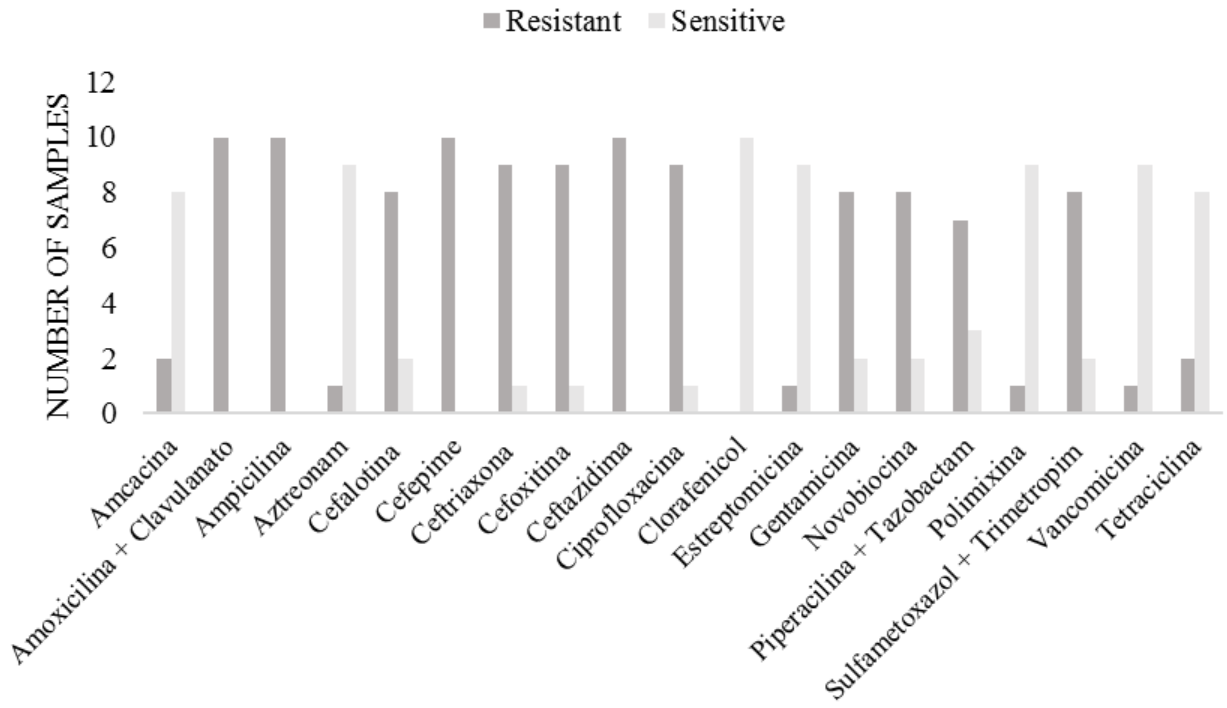

Figure 5. Demonstration of susceptibility and resistance of 10 bacterial strains against traditional antimicrobials.

obtained a positive response regarding the inhibition of the strains.

Comparing the average concentrations between all the extracts, the values of the extract at 25°C with a concentration of 1:1 performed better in relation to the refrigerated decoction, developing the lowest absorbance (0.21 nm). The extracts averages at concentrations of 1:2, 1:4 and 1:8 had no significant statistical changes during inhibition at different temperatures and on the different days they were analyzed (Figure 4).

In Figure 5, it can be observed that 100% of the strains were resistant to amoxicillin + clavulanate, ampicillin, cefepime and ceftazidime, being characterized with a multiresistant profile; that is, they are resistant to four or

more antibiotics. Regarding the other strains, there was a variance between resistance and sensitivity against antibiotics and 100% showed sensitivity to chloramphenicol, which was therefore used as a positive control in the microplate analysis.

The results found in this research corroborate those found by Neves et al. (2010) and Cavalcante et al. (2013), who demonstrated that ampicillin is among the antimicrobials that show the highest resistance indices to *Staphylococcus* sp. However, the results of the present research are somewhat contradictory to those found by Langoni et al. (2006) and Moroni et al. (2005) concerning resistance of the isolates of *Staphylococcus* sp. to amoxicillin and who cited that the sensitivity to ampicillin varied.

According to Rice and Bonomo (2005), the mechanisms of resistance may be intrinsic to the microorganism or acquired by transmission of genetic material or mutation. In this study, it was observed that the resistance of the bacterial strains was against β-lactam antibiotics (amoxicillin + clavulanate, ampicillin, cefepime and ceftazidime). Concerning this, Fluit et al. (2001) believed that mutations in penicillin-binding proteins (PBPs) lead to a decrease in the binding affinity of the antibiotic to the site of action. *S. aureus* and *Staphylococcus* sp. of negative coagulase acquired the chromosomal gene, mecA, encoding PBP, which makes them resistant to β-lactams. The high resistance of this gene causes the inhibition of all β-lactams and maintains active cell wall synthesis, even in the presence of lethal concentrations of the antibiotic (Dzidic et al., 2008; Hawkey, 1998).

Conclusions

All strains were resistant to amoxicillin + clavulanate, ampicillin, cefepime and ceftazidime, being characterized with a multiresistant profile. This indicates that the extract of the leaf of cajá can be used as an alternative, since it was effective in the concentrations of 1:1 and 1:2 against all strains of coagulase negative *Staphylococcus*, regardless of storage temperatures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Plant extracts enhancers of defense response in ponkan mandarin Seedlings against *Alternaria alternate* f. spp*. citri* infection

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The objective of this study was to determine the elicitor potential of plant extracts from *Caatinga biome* by enzyme activity and epidemiological components of *Alternaria* brown spot *(Alternaria alternate* f. spp. *citri).* These were prepared ethanolic, dichloromethanic, and aqueous extracts from 14 plants (*Anadenanthera macrocarpa*, *Schinopsis brasiliensis*, *Maytenus rigida*, *Caesalpinia pyramidalis*, *C. ferrea*, *Peltophorum dubium*, *Capariscyn ophallophora, Ziziphus joazeiro*, *Mimosa hostilis*, *Momordica charantia*, *Erythrina velutina*, *Cleome hassleriana*, *Sideroxylum obtusifolium Spondias tuberosa*) after dilution, (1mg/mL) they were sprayed on *Citrus reticulate* 'Ponkan'seedlings and after 0, 24, 48, 72 and 96 h the leaves were collected for determination of polyphenol oxidase and peroxidase activity. For epidemiological disease components, leaves were collected 72 h after spraying, deposited on Petri dishes with moist paper, inoculated with a spore suspension of *A. alternata* f. spp. *citri* ($2x10^5$ conidia mL^{-1}) and evaluated for 12 days initial and final severity, area below the progress curve of disease and protection. The extracts of *A. macrocarpa* and *M. rigida* potentiated the polyphenol oxidase and peroxidase activity which had great potential in the management of the disease, reducing the final severity and area under the disease progress curve, providing high levels of control.

Key words: *Citrus reticulata, alternaria* brown spot, elicitor.

INTRODUCTION

The national citrus production places Brazil was in a prominent status in world market, being the top producer with 19 million tons of oranges harvested in 2011/2012 (Agrianual, 2012). The hand labor and foreign currency generated by the exportation of concentrated orange juice production makes the citrus species very important

socioeconomically for the country (Struiving et al., 2013). Despite having sweet orange [*Citrus sinensis* (L.) Osbeck] as the main spp produced, several spp of tangerines and some of their hybrids such as tangelos, willow leaf and tangors stands out in Brazilian orchards when the target is the National fruit market in natura (Azevedo et al., 2010). However, these orchards are constantly targets of *Alternaria* brown spot (ABS) infections, initiated by *A. alternate* f. spp. *citri*. which considered the main fungal disease of this specie (Peres et al., 2003), and caused serious damage in the Brazilian orchards resulting in the reduction of some susceptible varieties planted, mainly in the state of Sao Paulo, where many growers are eradicating their orchards due to the drastic increase on production costs, as a consequence of the high number of fungicide applications, discouraging planting in new areas (Azevedo et al., 2010).

The search for alternative strategies to control pests and diseases in agriculture has been the strategy used to reduce the use of pesticides with high toxicity, reconciling the safe food production, environmental preservation (Farooq et al., 2011) and the economic viability of commercial plantations. In other to do that, the use of medicinal plants represent a rich source of natural compounds to be explored for the identification of new defense principles (Belting, 2009).

Plant extracts have secondary metabolites which represent biologically active substances. Several authors have demonstrated the potential of medicinal plants in the control of plant pathogens, both by its direct fungi toxic action, and the ability to stimulate the accumulation of molecules with elicitor features capable of inducing defense responses (Bonaldo et al., 2004; Celoto et al, 2008; Bulhões et al., 2012). These resistance mechanisms may include the accumulation of phenolic compounds, phytoalexins and pathogenesis-related proteins such as β-1,3glucanase, chitinase, peroxidase, phenylalanine ammonia lyase and polyphenol oxidase (Barros et al., 2010).

Hence, this study aimed to evaluate the potential of native plant extracts from *Caatinga biome* as elicitors of defense response by enzymatic quantification in ponkan mandarin seedlings and its efficiency to reduce the severity of infection by *Alternaria alternate* f .spp. *citri* in detached leaves.

MATERIALS AND METHODS

14 leaves of native plant species (Table 1) of the Brazilian semi-arid region in Boa Vista-PB (7º 15'28"S, 36°14'7"W) were collected and taken to the Phytopathology Laboratory of the Universidade Federal da Paraíba, Campus II, Areia-PB and to the Phytochemicals Laboratory of the Universidade deTrásdos Montes e Alto Douro - UTAD, in Vila Real, Portugal.

The leaves were collected, washed and dried at 25±2°C and then placed in a forced air circulation chamber at a temperature of 65°C until constant weight, grounded in a blade-grinder to obtain powder. The material was properly identified and stored until further use. For determination of enzyme activity and efficiency in the control of ABS, an extraction was performed from plant material with water, ethanol and dichloromethane to determine solvent with increasingly polarity, more suitable for better extraction of bioactive compounds. The ethanolic extract and dichloromethane were made from dried plant suspension material (powder) in ethanol (90%) and dichloromethane in a ratio of 1: 3 for 24 h with periodic agitation. The material was filtered through filter paper and the liquid obtained was subjected to evaporation in a rotary evaporator held under constant agitation of 60°C until complete evaporation, yielding crude extract, which was placed in test tubes, identified and stored at -20°C until further analysis. For aqueous extract, the vegetable powder was suspended in distilled water for 24 h and kept under constant agitation at 35°C for further filtering, concentrate obtaining was then freeze-dried.

In a preliminary test, dichloromethane caused death of pathogen, even without the plant extract which caused damage in the leaves of the sprayed plants, masking the results, and for this reason, they were not tested for enzymatic activity and protection. Experiment was carried out in a greenhouse at the Department of Plant and Environmental Sciences and the enzymatic assays were performed at the Laboratory of Biomass of the Department of Biological Sciences, at Center for Agrarian Sciences of the Federal University of Paraiba, Areia - PB.

A randomized block design was used with three blocks by 28 treatments distributed in factorial arrangement (14 x 2 x 5) + (2 x 5), 14 herbal extracts (Table 1) with two extraction solutions (water and ethanol) in five periods (0, 24, 48, 72 and 96 h after spraying) and two controls (water and acibenzolar-S-methyl - ASM [0.2 mg / ml]) in five periods (0, 24, 48, 72 and 96 h after spray). The experimental unit consisted of three seedlings of *Citrus reticulata*, Ponkan variety grafted onto *Citrus aurantifolia*. Each seedling received 20 mL of solution at 1.0 $mg.mL^{-1}$. For enzymatic activity two leaves of each seedling were collected at 0, 24, 48, 72 and 96 h after the application of the extracts. These samples were stored individually in aluminum foil, frozen in liquid nitrogen (N2) and stored in an Ultra freezer at -20°C for experimental analysis, performed in triplicate.

0.25 g of citrus leaves were weighed which were mechanically homogenized in a mortar with 4.0 mL of 100 mM sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 12,000 g for 20 min at 4°C and the supernatant obtained was considered as an enzymatic extract for determination of the peroxidase and polyphenol activity. Peroxidase activity was determined by direct spectrophotometric method with modifications by the measurement of guiacol conversion in tetraguaiacol at 470 nm (Lusso, 1999). The mixture for the reaction consisted of, 0.25 mL protein extract, 0.25 mL guaiacol and 0.25 mL hydrogen peroxide in 0.75 mL 0.1 M phosphate buffer (pH 6.0). The peroxidase activity was expressed as specific activity (absorbance units' min^{-1} mg^{-1} protein).

The polyphenol oxidase activity was determined by Duangmal (1999) methodology with modifications. The assay consisted in measuring the oxidation of catechol converted into quinone, bei

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Table 1. Botanicals used for obtaining extracts.

Common name	Scientific name	Family
Angico	*Anadenanther amacrocarpa* (Benth.)	Leguminoseae
Catingueira	*Caesalpinia pyramidalis*Tul.	Leguminoseae
Canafístula	*Peltophorum dubium* (Spreng) Taub.	Leguminoseae
Jurema	*Mimosa hostilis* Benth.	Leguminoseae
Pau-ferro	*Caesalpinea ferrea*(Benth.) Ducke	Leguminoseae
Mulungú	*Erythrina velutina* Willd.	Leguminoseae
Baraúna	*Schinopsis brasiliensis* Engl.	Anacardiaceae
Umbuzeiro	*Spondias tuberosa* L.	Anacardiaceae
Bom-nome	*Maytenus rigida* Mart.	Celastraceae
Feijão-bravo	*Cappariscyno phallophora* L	Capparaceae
Juazeiro	*Ziziphus joazeiro* Mart.	Rhamnaceae
Melão-de-São-Caetano	*Momordica charantia* L.	Cucurbitaceae
Mussambê	*Cleomehass leriana* L.	Capparidaceae
Quixabeira	*Sideroxylum obtusifolium* (Roem. & Schult.) Penn.	Sapotaceae

this reaction mediated by enzyme under study. The substrate was prepared with catechol in concentration of 20 mM and dissolved in 100 mM sodium phosphate buffer (pH 6.8). Reaction was developed from the mixture of 0.25 mL substrate, 0.5 mL enzyme extract and 0.75 mL reaction buffer in a temperature of 30°C for 15 min, being interrupted by the addition of 0.05 mL of 5 N HCl after this period. A spectrophotometric was made at 420 nm and the results were expressed in min^{-1} mg^{-1} protein absorbance.

Total proteins were determined by Bradford method. 50 µL from the patterns solutions or sample extracts were pipetted separately with 50 µL of distilled water and 1 mL of Bradford reagent being gently shaken and allowed to rest for 10 min. Spectrophotometric were made at a wavelength of 595 nm. On the pH of the reaction (5.0), the interaction between the high molecular weight protein and the reagent dye Coomassie Brilliant Blue (G-250) present on the Bradford dye reagent causes equilibrium shift to the anionic form that strongly absorbs at 595 nm.

For evaluation of plant protection efficiency against ABS mediated by plant extracts, Ponkan mandarin seedlings and bare-root of one year old were used, commercially available in Lagoa Seca -PB (07°10'15"S and 35°51'13"W), transplanted to plastic bags with 1L of capacity and acclimated in a greenhouse for 15 days with daily watering. The extracts applications were made by spraying at a concentration of $1mg.mL^{-1}$. After 72 h of the first spray, mandarin leaves were collected, identified by treatment, packed in cool boxes and taken to the Phytopathology Laboratory of UFPB.

Fifteen leaves from each treatment were immersed in a sodium hypochlorite solution at 0.5% for 5 min and subsequently washed in distilled water. Three leaves were placed in petri dishes containing moist filter paper with sterile distilled water. Subsequently, leaves spore suspension of *A. alternate f. sp. citri* at a concentration of $2x10^5$ conidios mL^{-1} were given. After inoculation, leaves were maintained at room temperature (25±5°C) under light for 72 h before the start of the evaluations.

The disease severity was evaluated for 15 days, in which the initial severity was related to the first day of assessment, starting from the onset symptoms (3 days after inoculation). Evaluations were made from a proposed grade scale by Martelli (2011). Area under disease progress curve was calculated by Shaner and Finney (1977) using the following equation;

$$AUDPC = \sum n\,[(Yi + Yi + 1)/2 * (ti + 1 - ti)\,]$$

Where Y is the severity of the disease at i^{th} observation, ti is the time in days on i^{th} observation and n is the total number of observations. A randomized experimental design was used with blocks in a factorial arrangement (15 x 2 + 2 witnesses) with 14 extracts and two extracting solutions, two additional witnesses (water and ASM) and five replications. The experimental unit was composed of three leaves.

Data analysis

For the effect of enzyme activity comparison and epidemiological variables with initial and final severity, area under the disease and protecting progress curve, were made contrasts between extracts and additional treatments (Water and ASM), using the Dunnett test (α = 0.05) (SAS Institute, 2002) and the data normality was evaluated by Cochran.

RESULTS AND DISCUSSION

To determine the content of soluble proteins, the calibration equation y = 0.0237 + 0,0451x obtained from different standard concentrations were used. The polyphenol-oxidase activity was not significantly different among treatments in period zero for two extractant solutions (water and ethanol), since the leaves were collected shortly after application, there was no immediate enzyme activity. Although the acibenzolar-S-methyl is considered as a resistance inducer, but do not promote increase in the polyphenol oxidase activity when compared to control (water) in all periods (Table 2).

The use of plant extracts in Ponkan mandarin seedlings provided a highly variable response to the tested species over time. Within 24 h after treatment application, the aqueous extracts of *Peltophorum dubim*, *Capparis*

Table 2. Polyphenol oxidase activity in response to application of aqueous and ethanolic plant extracts native of the *Caatinga bioma* at concentration of 1.0 mg/mL.

Treatment	Periods (hours after application)									
	Aqueous extracts					Ethanolic extracts				
	0	24	48	72	96	0	24	48	72	96
Water	0.25^{ns}	0.91^{B2}	0.43^{B}	1.97^{A}	0.63^{B}	0.25^{ns}	0.91^{B}	0.43^{B}	1.97^{B}	0.63^{B}
ASM	0.25^{ns}	0.89^{b}	0.27^{b}	0.73^{b}	0.60^{b}	0.25^{ns}	0.90^{b}	0.27^{b}	0.77^{b}	0.60^{b}
Am[1]	0.25^{ns}	0.41^{Cc}	0.15^{Bb}	0.58^{Cc}	0.56^{Cb}	0.25	2.53^{Aa}	0.25^{Bb}	3.37^{Aa}	0.63^{Bb}
Mr	0.25^{ns}	0.84^{Bb}	0.54^{Bb}	0.69^{Cb}	0.58^{Bb}	0.25	1.42^{Aa}	0.70^{Bb}	0.64^{Cc}	0.55^{Cb}
Sb	0.25^{ns}	0.81^{Bb}	0.31^{Bb}	0.53^{Cc}	0.56^{Cb}	0.25	0.27^{Cc}	0.13^{Bb}	0.57^{Cc}	0.63^{Bb}
Pd	0.2^{ns}	1.12^{Aa}	0.34^{Bb}	0.61^{Cc}	0.51^{Cc}	0.25	0.55^{Cc}	0.79^{Bb}	0.67^{Cb}	0.57^{Cb}
Cp	0.25^{ns}	0.80^{Cb}	0.04^{Bb}	0.69^{Cb}	0.43^{Cc}	0.25	0.81^{Bb}	0.42^{Bb}	0.57^{Cc}	0.43^{Cc}
Cc	0.25^{ns}	2.24^{Aa}	0.41^{Bb}	0.55^{Cc}	0.55^{Cb}	0.25	0.42^{Cc}	0.53^{Bb}	0.63^{Cc}	0.59^{Bb}
Mh	0.25^{ns}	1.11^{Aa}	0.13^{Bb}	0.50^{Cc}	0.56^{Cb}	0.25	0.12^{Cc}	0.13^{Bb}	0.66^{Cc}	0.58^{Bb}
Zj	0.25^{ns}	0.36^{Cc}	3.08^{Aa}	0.72^{Cb}	0.54^{Cb}	0.25	0.05^{Cc}	6.01^{Aa}	0.68^{Cb}	0.51^{Cc}
Ev	0.25^{ns}	0.45^{Cc}	0.45^{Bb}	0.63^{Cc}	0.52^{Cc}	0.25	0.30^{Cc}	3.08^{Ba}	0.81^{Ca}	0.77^{Aa}
Ch	0.25^{ns}	0.58^{Cc}	0.38^{Bb}	0.75^{Cb}	0.50^{Cc}	0.25	1.24^{Aa}	0.19^{Bb}	0.61^{Cc}	0.52^{Cc}
Mc	0.25^{ns}	0.16^{Cc}	0.12^{Bb}	0.76^{Cb}	0.55^{Cb}	0.25	0.55^{Cc}	0.15^{Bb}	0.741^{Cb}	0.547^{Cb}
Cf	0.25^{ns}	0.61^{Cc}	0.41^{Bb}	0.60^{Cc}	0.51^{Cc}	0.25	0.44^{Cc}	0.27^{Bb}	0.670^{Cb}	0.552^{Cb}
So	0.25^{ns}	0.60^{Cc}	0.17^{Bb}	0.41^{Cc}	0.71^{Aa}	0.25	0.19^{Cc}	1.14^{Bb}	1.379^{Ca}	0.491^{Cc}
St	0.25^{ns}	0.75^{Cc}	0.37^{Bb}	0.84^{Ca}	0.51^{Cc}	0.25	0.25^{Cc}	0.28^{Bb}	0.549^{Cc}	0.536^{Cc}

[1]Am=*Anadenanther amacrocarpa*; Mr=*Maytenus rigida*; Sb=*Schinopsis brasiliensis*; Pd=*Peltophorum dubium*; Cp=*Caesalpinia pyramidalis*; Cc=*Cappariscyno phallophora*;Zj=*Zizipus joazeiro*; Mh=*Mimosa hostilis*; Mc=*Momordica charantia*; Ev=*Erythrina velutina*; Ch=*Cleomehass leriana*; Cf= *Caesalpinia ferrea*; So=*Sideroxylum obtusifolium*; ST=*Spondias tuberosa*; ASM=Acibenzolar-S-metil. [2]Equal capital letters do not differ from the treatment water and the equal lowercase letters do not differ from ASM treatment.

cynophallophora, *Mimosa hostilis* and ethanolic extracts of *M. hostilis*, *Maytenus rigida* promote the highest activities of polyphenol oxidase, being superior to the controls with water and ASM. These same extracts had their activity reduced within 48 h and only *Z. joazeiro* extract, in both extraction, enhanced the enzyme activity in this period, showing a later activity, however, most intense.

For aqueous extract within 72 h, the seedlings treated with water obtained the highest average for the polyphenol oxidase activity, differing from the other treatments, while for the ethanol extracts, *Anadenanthera macrocarpa* promoted a higher activity of this enzyme. In the last assessment period (96 h) the *Sideroxylum obtusifolium* aqueous extract and ethanolic of *Erythrina velutina* provided the highest average (Table 2).

Within 96 h, the plants treated with different extracts had low peroxidase activity, however, the aqueous extract of *Caesalpinia pyramidalis* and ethanolic of *C. cynophallophora* provided the highest average, differentiating from the controls. The peak of peroxidase activity was checked after 48 h in plants treated with the aqueous extract of *M. rigida*, averaging 7, 86 UA per mg of protein per min, and the ethanolic extract of *M. hostilis* (2.74 AU/mg protein/min).

The *Schinopsis brasiliensins* aqueous extracts, *Spondias tuberosa* and ethanolic of *A. macrocarpa*, *M. rigida* and *C. cynophallophora* presented higher peroxidase activity than plants treated with water and ASM already at 24 days after application. The enzymatic activity for the aqueous *S. brasiliensis* extract and ethanolic of *A. macrocarpa* decreased after 48 h, however, returned to high activity, differing from the control treatments (water and ASM) within 72 h (Table 3).

Within 96 h, the plants treated with different extracts had low peroxidase activity, however, the aqueous extract of *C. pyramidalis* and ethanolic of *C. cynophallophora* provided the highest average, differentiating from the controls. The peak of peroxidase activity was checked after 48 h in plants treated with aqueous extract of *M. rigida*, averaging 7,86UA per mg of protein per min, and the ethanolic extract of *M. hostilis* (2.74 AU/mg protein/min). The application of plant extracts influenced the epidemiological variables evaluated in detached leaves of Ponkan mandarin seedlings positively reducing infection and in some cases negatively, as occurred with the aqueous extract of *C. pyramidalis* which caused an increase in the initial disease severity, higher than the control with water and ASM, while there was no interference of ethanolic extracts for this variable (Table 4).

The aqueous extracts of *M. rigida, S. brasiliensis, M. hostilis, Ziziphus joazeiro, Momordica charantia* and *S. tuberosas* significantly reduced the final severity and

Table 3. Peroxidase activity (UA/mg of protein per min) in response to application of aqueous and ethanolic plants extracts native of *Caatinga bioma* at concentration of 1.0 mg/mL.

Treatment	Periods (hours after application)									
	Aqueous extracts					Ethanolic extracts				
	0	24	48	72	96	0	24	48	72	96
Water	0.24^{ns}	0.51^{B2}	0.40^{B}	0.493^{B}	0.254^{B}	0.24^{ns}	0.51^{B}	0.40^{B}	0.49^{B}	0.25^{B}
ASM	0.24^{ns}	0.20^{b}	0.74^{b}	0.182^{b}	0.336^{b}	0.24^{ns}	0.20^{b}	0.74^{b}	0.18^{b}	0.34^{b}
Am[1]	0.24^{ns}	0.28^{Bb}	0.69^{Bb}	0.100^{Cb}	0.145^{Cc}	0.24	0.79^{Aa}	2.74^{Bb}	1.17^{Aa}	0.20^{Bc}
Mr	0.24^{ns}	0.73^{Ba}	7.86^{Aa}	0.119^{Cb}	0.138^{Cc}	0.24	0.99^{Aa}	0.95^{Bb}	0.19^{Cb}	0.06^{Cc}
Sb	0.24^{ns}	0.88^{Aa}	0.88^{Bb}	1.155^{Aa}	0.286^{Bb}	0.24	0.14^{Cb}	0.64^{Bb}	0.20^{Cb}	0.34^{Ab}
Pd	0.24^{ns}	0.06^{Cb}	0.29^{Bb}	0.722^{Aa}	0.149^{Cc}	0.24	0.09^{Cb}	0.76^{Bb}	0.18^{Cb}	0.15^{Cc}
Cp	0.24^{ns}	0.24^{Cb}	0.55^{Bb}	0.282^{Ca}	0.435^{Aa}	0.24	0.73^{Ba}	0.63^{Bb}	0.15^{Cb}	0.18^{Cc}
Cc	0.24^{ns}	0.54^{Ba}	0.28^{Bb}	0.170^{Cb}	0.222^{Bc}	0.24	0.91^{Aa}	1.87^{Aa}	0.21^{Cb}	0.43^{Aa}
Mh	0.24^{ns}	0.31^{Bb}	0.21^{Bb}	0.120^{Cb}	0.359^{Ab}	0.24	0.10^{Cb}	0.25^{Bb}	0.21^{Cb}	0.12^{Cc}
Zj	0.24^{ns}	0.55^{Ba}	0.32^{Bb}	0.492^{Ba}	0.317^{Ab}	0.24	0.11^{Cb}	0.31^{Bb}	0.22^{Cb}	0.14^{Cc}
Ev	0.24^{ns}	0.13^{Cb}	1.29^{Bb}	0.177^{Cb}	0.203^{Bc}	0.24	0.21^{Cb}	0.32^{Bb}	0.24^{Cb}	0.36^{Ab}
Ch	0.24^{ns}	0.14^{Cb}	1.85^{Bb}	0.264^{Cb}	0.229^{Bc}	0.24	0.57^{Ba}	0.30^{Bb}	0.16^{Cb}	0.11^{Cc}
Mc	0.24^{ns}	0.09^{Cb}	0.60^{Bb}	0.133^{Cb}	0.249^{Bc}	0.24	0.07^{Cb}	0.32^{Bb}	0.25^{Cb}	0.20^{Bc}
Cf	0.24^{ns}	0.29^{Bb}	0.33^{Bb}	0.093^{Cb}	0.205^{Bc}	0.24	0.09^{Cb}	0.24^{Bb}	0.29^{Ca}	0.18^{Cc}
So	0.24^{ns}	0.55^{Ba}	2.90^{Bb}	1.183^{Aa}	0.349^{Ab}	0.24	0.30^{Bb}	0.63^{Bb}	0.19^{Cb}	0.09^{Cc}
St	0.24^{ns}	0.77^{Aa}	0.534^{Bb}	0.158^{Cb}	0.072^{Cc}	0.24	0.31^{Bb}	0.94^{Bb}	0.69^{Aa}	0.28^{Bb}

[1]Am=*Anadenanther amacrocarpa*; Mr=*Maytenus rigida*; Sb=*Schinopsis brasiliensis*; Pd=*Peltophorum dubium*; Cp=*Caesalpinia pyramidalis*; Cc=*Cappariscyno phallophora*;Zj=*Zizipus joazeiro*; Mh=*Mimosa hostilis*; Mc=*Momordica charantia*; Ev=*Erythrina velutina*; Ch=*Cleomehass leriana*; Cf= *Caesalpinia ferrea*; So=*Sideroxylum obtusifolium*; ST=*Spondias tuberosa*; ASM=Acibenzolar-S-metil. [2]Equal capital letters do not differ from the treatment water and the equal lowercase letters do not differ from ASM treatment.

AUDPC compared to control (water) and did not differ from ASM treatment, providing therefore, the higher control rates (Table 4). Plants treated with the ethanolic extract of *M. hostilis* behaved differently from the one treated with aqueous extract, with less protective potential, initial severity and AUDPC equal to control (water) and also, the same happened with *Peltophorum dubium*, *Cleome hassleriana* and *Sideroxylum obtusifolium* extracts. The remaining ethanolic extracts significantly reduced the final severity and AUDPC, representing high rates of protection with values up to 99.12%, not differing from the control provided by the treatment with ASM. The difference between treatments with water and ASM were significant (Table 5), indicating the elicitor efficiency in the protection against *A. alternate* f. spp. *citri.*

Many studies shows the potentiating effect of Acibenzolar-S-methyl enzymes involved in plant defense such as peroxidase and polyphenol oxidase (Baysal et al., 2003; Cavalcanti et al., 2006; Andrade et al., 2013), however, the majority are performed with infected plants. In this case, it is difficult to define if treatment with ASM or if the infective process caused the activation of these enzymes. It is considered that, the polyphenol oxidase is associated with infected tissues, thus playing an important role in plant defense mechanism or senescence (Agrios, 2005). Lucas (2012) found an increased polyphenol oxidase activity in tomato (*Solanum licopersicum L.*), just six days after application of ASM and essential oil of lemon grass (*Citrus aurantifolia)* in non-inoculated plants which observed lower activity in these treated plants inoculated with *A. alternata.* Song et al. (2011) has shown that the increased polyphenoloxidase, peroxidase and phenylalanine ammonia lyase activity in plant tissues are an important factor in the induction of resistance against pathogens.

The polyphenol oxidases are enzymes which oxidize mono- and o-diphenols to o-diquinones (Vaughn et al., 1988), which have antimicrobial activity (Mohammadi, 2002) and participates in the lignification process during tissue invasion by pathogens (Li, 2002), justifying their enhanced activity during the infectious process. Further, peroxidases are also reported in association with the formation of reactive oxygen intermediates (Skelly, 2013) and the oxidative burst is a plant defense response after pathogen recognition, leading to hypersensitivity reaction, promoting the gradual establishment of systemic acquired resistance (SAR) (Resende et al., 2003; Alvarez et al., 1998). However, this enzyme is also synthesized by mechanical stress such injury (Rodrigues et al., 2011) or environmental such water and salt stress (Debouba et al., 2006) can be variable depending on the species, cultivar, age and part of the plant (Amiot et al., 1995) which explains the mutability action of this enzyme

Table 4. Contrasts between controls treatments (Water and ASM) and aquous and ethanolic plants extracts native of *Caatinga bioma* of the initial (IS) and final (FS) severity area under the disease progress curve (AUDPC and protection levels on detached leaves of mandarin seedlings Ponkan.

Treatments	IS[1]	FS	AUDPC	Protection	IS	FS	AUDPC	Protection
	Aquous extracts				Ethanolic extracts			
Water	0.40^{B3}	2.60^{A}	1.10^{A}	0.00^{B}	0.40^{ns}	2.60^{A}	1.10^{A}	0.00^{B}
ASM	0.00^{b}	0.00^{b}	0.00^{b}	98.20^{a}	0.00^{ns}	0.00^{b}	0.00^{b}	98.20^{a}
Am[2]	0.00^{Bb}	0.02^{Bb}	0.01^{Bb}	99.12^{Aa}	0.33	2.13^{Aa}	0.90^{Aa}	23.86^{Ab}
Mr	0.00^{Bb}	0.06^{Bb}	0.03^{Bb}	97.37^{Aa}	0.00	0.49^{Bb}	0.18^{Bb}	84.21^{Aa}
Sb	0.00^{Bb}	0.20^{Bb}	0.10^{Bb}	91.23^{Aa}	0.00	0.02^{Bb}	0.01^{Bb}	99.12^{Aa}
Pd	0.12^{Bb}	1.71^{Aa}	0.80^{Aa}	30.12^{Ab}	0.04	0.84^{Ba}	0.40^{Ba}	64.91^{Aa}
Cp	0.53^{Aa}	3.97^{Aa}	1.72^{Ba}	0.00^{Bb}	0.00	0.67^{Bb}	0.33^{Bb}	70.76^{Aa}
Sb	0.00^{Bb}	0.92^{Ba}	0.41^{Ba}	64.04^{Aa}	0.04	0.62^{Bb}	0.29^{Bb}	74.56^{Aa}
Mh	0.00^{Bb}	0.00^{Bb}	0.00^{Bb}	100.0^{Aa}	0.06	0.34^{Bb}	0.14^{Bb}	88.01^{Aa}
Zj	0.00^{Bb}	0.02^{Bb}	0.01^{Bb}	99.12^{Aa}	0.04	0.28^{Bb}	0.12^{Bb}	89.47^{Aa}
Ev	0.14^{Bb}	1.39^{Ba}	0.62^{Ba}	45.99^{Ab}	0.00	0.27^{Bb}	0.05^{Bb}	95.47^{Aa}
Ch	0.12^{Bb}	2.01^{Aa}	0.88^{Aa}	23.57^{Ab}	0.14	1.09^{Ba}	0.57^{Ba}	50.29^{Ab}
Mc	0.06^{Bb}	0.20^{Bb}	0.03^{Bb}	97.75^{Aa}	0.00	0.04^{Bb}	0.02^{Bb}	98.25^{Aa}
Cf	0.00^{Bb}	0.08^{Bb}	0.04^{Bb}	96.49^{Aa}	0.00	0.02^{Bb}	0.01^{Bb}	99.12^{Aa}
So	0.08^{Bb}	2.23^{Aa}	1.08^{Aa}	17.75^{Bb}	0.12	4.53^{Ba}	2.42^{Ba}	00.00^{Bb}
St	0.06^{Bb}	0.74^{Bb}	0.35^{Bb}	69.30^{Aa}	0.02	0.06^{Bb}	0.02^{Bb}	98.25^{Aa}
CV	13.4	16.8	11.5	19.1	13.4	16.8	11.5	19.1

[1]Am=*Anadenanther amacrocarpa*; Mr=*Maytenus rigida*; Sb=*Schinopsis brasiliensis*; Pd=*Peltophorum dubium*; Cp=*Caesalpinia pyramidalis*; Cc=*Cappariscyno phallophora*;Zj=*Zizipus joazeiro*; Mh=*Mimosa hostilis*; Mc=*Momordica charantia*; Ev=*Erythrina velutina*; Ch=*Cleomehass leriana*; Cf= *Caesalpinia ferrea*; So=*Sideroxylum obtusifolium*; ST=*Spondias tuberosa*; ASM=Acibenzolar-S-metil. [2]Equal capital letters do not differ from the treatment water and the equal lowercase letters do not differ from ASM treatment.

Table 5. Contrasts between controls (Water and ASM) of the initial (IS) and final (FS) severity, Area under the Disease Progress Curve (AUDPC) and protection levels in detached leaves of mandarin seedlings Ponkan.

Treatments	IS[1]	FS	AUDPC	Protection
ÁWater	0.40^{ns}	2.60^{a2}	1.10^{a}	0.00^{b}
ASM	0.00	0.00^{b}	0.00^{b}	98.20^{a}

[1] SI= Initial Severity; FS=Final Severity; AUDPC = Area Under the Disease Progress Curve.[2] Equal capital letters do not differ from the treatment water and the equal lowercase letters do not differ from ASM treatment.

compared to spraying with plant extracts. Peroxidases are related to the processes of growth and cell differentiation, and morphogenetic changes in response to physical, chemical and biological stress. Increased activity of this enzyme in plants under these conditions can be a determinant factor for the adaptive capacity of these plants; this activity can be identified as a biochemical marker of stress (Piza et al., 2003). In a practical way, peroxidases are related to increased hydrogen peroxide production (Hameed et al., 2008) and are central in the plant antioxidant defense responses (Hameed et al., 2009).

Carvalho et al. (2013) in experiments with plant extracts on the alternative control of *A. alternataf. spp. citri* in Tangor Murcot fruits found significant decrease of up to 62% in the rate of spots development with aqueous extract of *A. columbrina,* which belongs to the same genus *A. macrocarpa*, showed the greatest potential for protection (99.12%) in detached leaves, being superior to the treatments with commercial fungicides chlorothalonil by copper oxychloride and Azoxystrobin, which provided protection of 37 to 39% respectively. However, these same authors also reported a direct effect of the extract on the pathogen development, not configuring, therefore, a protection only by induced-resistance.

The results presented here shows the importance of plant extracts and derivatives as sources of efficient bio-active compounds in the management of plant diseases through direct effect on pathogens or as potential sources of a new defense response elicitors and as an alternative to chemical control, which have been indiscriminately used. According to Ootani (2013) exploration of the biological activity of secondary compounds present in the crude extract or essential oil of medicinal plants can be, with resistant induction, another potential alternative form of disease control in cultivated plants.

Conclusion

The extracts of *A. macrocarpa* and *M. rigida* potentiated the polyphenol oxidase and peroxidase activity and had great potential in the management of the disease, reducing the final severity and area under the disease progress curve and providing high levels of control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of lime and fertilizer on soil properties and maize yields in acid soils of Western Kenya

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Many soils in Western Kenya are acidic and deficient in nitrogen and phosphorus. Acidity hinders crop responses to fertilizers applied to remedy nutrient deficiencies. The common liming materials used to ameliorate acidity are Calcium Oxide (CaO) and Calcium Carbonate ($CaCO_3$) in powdery formulations. Broadcasting these materials by hand followed by incorporation is recommended on smallholder farms to enhance their effectiveness but this is laborious. Granular lime which is easier to handle was recently introduced but there is little information on its effectiveness. This study therefore tested the effects of $CaCO_3$, CaO and granulated lime, applied alone or in combination with fertilizer (Diammonium phosphate (DAP) + calcium ammonium nitrate (CAN)), on maize yield for three seasons, 2015 long rains (LR), 2015 short rains (SR) and 2016 LR at four sites: Butere, Emuhaya, Mumias and Kakamega North in Western Kenya. $CaCO_3$ and CaO were applied at 2 t ha^{-1} once in the 2015 LR while granular lime was applied at a ratio of 1:1 with DAP per season. There was no significant effect of lime type on maize yields. Maize did not respond to lime without fertilizer. Application of lime, irrespective of the type, with fertilizer, did not give yields that were significantly different from those of fertilizers alone except at Butere in the 2015 LR when application of CaO and $CaCO_3$ with fertilizer significantly out yielded those with fertilizer applied alone. Similar results were obtained with granular lime in the 2015 SR at Emuhaya. It was concluded that except for Butere, where maize did not respond to fertilizer alone, the other sites are not sufficiently acid to permit the solubility of Al to toxic levels for maize. More attention should therefore be focused on N and P replenishment at these sites than liming. At Butere, soil acidity is a problem and lime should be applied together with fertilizers.

Key words: Aluminum toxicity, lime, maize, nitrogen, phosphorus.

INTRODUCTION

Acid soils are widespread in Western Kenya and cover a large area of arable land (Kanyanjua, 2002; Kisinyo et al., 2015). In these acidic conditions, there is a complex interaction of growth-limiting factors. Plant growth may be

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restricted by one or more of the following: Al or Mn toxicity; Ca, Mg, P, or Mo deficiency; and reduced mineralization, nitrification, nodulation, and mycorrhizal infection (Fageria and Baligar, 2003; Dinkecha and Tsegaye, 2017). In addition, these soils, consisting of mainly the Acrisols, Nitisols and Ferralsols are highly weathered, with widespread N and P deficiencies. Smithson and Sanchez (2000) estimated that 80% of the soils across farms are severely N and P deficient. Under these conditions, yield of maize, the staple food crop is very low, averaging 1.0 t ha^{-1} against a potential of about 6 t ha^{-1} if the soils were well managed by replenishing the essential nutrients (Ojiem et al., 2004). Efforts to ameliorate the deleterious effects of soil acidity must therefore be accompanied by measures to replenish soil N and P. Use of inorganic fertilizers is recognized as an effective way for overcoming nitrogen and phosphorus deficiencies. However, in acid soils, response to fertilizers may not occur because of constraints imposed by soil acidity. Liming is the most dominant and most effective practice to control soil acidity (Fageria and Baligar, 2008; Goulding, 2016). Most plants grow well at soil pH range of 5.5 to 6.5 and liming is aimed to maintain the pH at this range. Liming increases soil pH, Ca concentration, cation exchange capacity (CEC) and base saturation, simultaneously lowering the Al concentration and increasing P availability (Jafer and Hailu, 2017). All these chemical changes, provided they are within a favorable range, improve grain yields and crop sustainability (Merino-Gergichevich et al., 2010; Nduwumuremyi, 2013). Currently, a variety of liming materials are available to farmers in Western Kenya. These materials differ in place of origin, amount of neutralizing power, and nutrients or other elements associated with the liming agent. These characteristics may influence the effectiveness of the liming material (Brady and Weil, 2002). The common liming materials on the Kenyan market are calcium oxide (CaO) and ground limestone composed mostly of calcium carbonate ($CaCO_3$), both of which are in powdery. This formulation increases surface area for quicker reaction with the soils (Bhargava and Subramanian, 2017). For maximum effectiveness, lime should be uniformly spread and incorporated into the soil. Incorporation can be achieved through disking or harrowing followed by rolling but these implementations are not usually available on smallholder farms. Spreading lime by hand is therefore common on smallholder farms but this is laborious and normally not recommended when the weather is windy. To overcome these challenges, granular lime was recently introduced to the Kenyan market (by MEA Ltd, a fertilizer blending Company in Kenya). Granular lime offers some advantages in handling over $CaCO_3$ and CaO. It spreads more uniformly, and it can be blended with fertilizers at low rates for row application (Warncke and Pierce, 1997). Granular lime is however more expensive than $CaCO_3$ and CaO and there is therefore need to determine whether granular lime is more effective than $CaCO_3$ and CaO in order to make it cost effective. The objectives of this study were to evaluate effects of lime types (calcium oxide, calcium carbonate and granular lime) applied alone or in combination with fertilizers containing N and P on maize yields, and assess effects of the three different lime types on soil properties

MATERIALS AND METHODS

Study sites

The study was conducted in 4 sub-counties in Western Kenya: Kakamega North, Mumias, Butere and Emuhaya for three consecutive seasons, 2015 long rains (LR), 2015 short rains (SR), and 2016 LR. Mumias has an average temperature of 21.6°C with an average annual of rainfall 1743 mm. The average temperature in Emuhaya is 20.5°C with an average annual rainfall of 1860 mm. The average temperature and annual rainfall in Butere is 21.3°C and 1830 mm, respectively. The temperature in Kakamega averages 20.4°C. The annual average rainfall at this site is 1971 mm. The rainfall at all these sites is distributed over two main cropping seasons, the long rainy season from March to July and the short rainy season from September to December. The soils in Mumias and Butere are Acrisols with a clay loam texture while those at Emuhaya and Kakamega North are Nitisols with a loamy texture. The sites were selected on the basis of having a soil pH of less than 5.5. Farming in the region is largely undertaken by smallholder farmers, practicing a mixture of cash crops and livestock enterprises. Maize and beans are the most common food crops grown in the area mainly as intercrops with little or no fertilizer and lime inputs.

Soil sampling and analysis

Soils from the study sites were sampled before the onset of the trials and characterized for relevant chemical properties using standard methods (Anderson and Ingram, 1993; Okalebo et al., 2002). The pH of soil samples was measured from a soil suspension solution prepared with 1:2.5 soil: water ratios using conventional glass electrode meter. Exchangeable acidity was extracted using unbuffered 1M KCl. Further, 25 ml of 1M KCl was added to 10 g of air-dry soil and shaken for 10 min on a reciprocal shaker and then allowed to stand for 30 min. The contents were filtered and the soil leached with 5 successive 25 ml aliquots of 1M KCl. The filtrate was titrated with 0.1M NaOH to determine the exchangeable acidity (H^+ and Al^{3+}) in the extract. The basic cations (Ca, Mg and K) were extracted using ammonium acetate at soil pH 7. Exchangeable Ca and Mg in the extract were determined using atomic absorption spectrophotometry, and exchangeable K by flame photometry. Organic C was determined by Walkley and Black sulphuric acid-dichromate digestion followed by back titration with ferrous ammonium sulphate. Total N and P were determined by digesting 0.3 g of the soil/OM sample in a mixture of Se, $LiSO_4$, H_2O_2 and concentrated H_2SO_4. The N and P contents in the digests were determined calorimetrically. Available P was determined by the Mehlich double acid method. A 2.5 g of air-dried soil sample was weighed into a 100 ml shaking bottle and 20 ml of the extracting solution (a mixture of 0.05 M HCl and 0.0125 M H_2SO_4) added. The mixture was shaken for 5 min and filtered through a Whatman No. 42 filter paper. A 5 ml of the extract was transferred to a 25 ml flask and diluted to the mark. Phosphorus concentration in the filtrate was determined calorimetrically by the ascorbic method at 880 nm using a spectrophotometer. Soils were again sampled at the end of the 2015 SR and 2016 LR seasons and analyzed for pH and exchangeable acidity only. However, this time

Table 1. Initial soil properties at the study sites.

Parameter	Sites			
	Butere	Emuhaya	Kakamega North	Mumias
pH	5.21	5.48	5.04	5.01
Exchangeable acidity (me/100 g)	0.40	0.10	0.30	0.30
Total N (g kg^{-1})	1.50	1.30	1.10	1.50
Organic C (g kg^{-1})	14.3	12.90	11.00	14.70
Available P (ppm)	5.00	10.00	10.00	15.00
Ca (Cmol/kg)	1.20	1.20	0.16	1.50
Mg (Cmol/kg)	1.90	2.09	1.10	1.29
K (Cmol/kg)	0.14	0.10	0.16	0.26

only one farm was sampled per site.

Experimental design and agronomic procedures

This trial was established on farmers' fields in each of the sites. A randomized complete block design was used with each farm treated as a replicate. Six replications were used per site (sub-county). The eight treatments consisted of three types of lime, applied alone or with fertilizers (Diammonium phosphate (DAP) and calcium ammonium nitrate (CAN). In addition, a treatment consisting of fertilizer alone (DAPS + CAN) and a control with no fertilizer or lime input was included. A summary of the treatments is as follows: Control (No lime, No fertilizer); 2 tons ha^{-1} $CaCO_3$; 2 tons ha^{-1} CaO; 2 tons ha^{-1} $CaCO_3$+26 kg P+60 kg N; 2 tons ha^{-1} CaO ha^{-1}+26 kg P+60 kg N; DAP 26 kg P (DAP) + 60 kg N (CAN); Granulated lime (only); and Granulated lime + fertilizer.

The liming materials were burnt lime material with 92.5% calcium carbonate ($CaCO_3$) equivalent, and CaO from Homa Lime Company Limited and granulated lime (64% $CaCO_3$, 2.5% MgO and trace elements) from MEA Ltd. The $CaCO_3$ and CaO were applied once at the recommended rate of 2 tons ha^{-1} in the first season (2015 LR) only while granular lime was applied in each season starting with the 2015 SR. The granular lime was applied as a blend with basal fertilizer (DAP), where applicable, in the ratio 1:1. DAP was applied every season (where applicable) at the recommended rate (26 kg P ha^{-1}) and CAN top dressed at 60 kg Nha^{-1} (where applicable) every season. After ploughing, plots of 4.5 m by 5.0 m were demarcated and guard rows between them maintained at 1.0 m apart. Two lime types ($CaCO_3$ and CaO) was evenly broadcasted by hand and thoroughly mixed with the soil using a hoe, in appropriate plots, at least 30 days before planting to allow for adequate reaction time with the soil. Planting was done at the onset of rainy season using recommended agronomic practices. Maize hybrid H 520, a variety recommended in the study areas was planted at a spacing of 25 cm by 75 cm, within and between rows, respectively. One and two seeds were sown in alternate holes and thinned to one per hill, 2 weeks post-emergence. Hand weeding and management of pests and diseases was carried out when necessary. To avoid contamination of inputs from the neighboring plots, each plot was individually tilled using a hoe. Harvesting was done at the end of each season and grain yield determined.

Data analysis

All the data collected was subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the SAS statistical software (version 9.2). Means were separated by least significance difference of means (LSD) at the $p < 0.05$ level of significance.

RESULTS AND DISCUSSION

Initial soil properties

The initial soil properties at the study sites are shown in Table 1. The soil pH ranged from 5.01 (Mumias) to 5.48 (Emuhaya) and would be rated as moderately acidic and therefore likely to encounter challenges associated with acidic soils such as Al toxicity, deficiencies of bases and available P, which are encountered at soil pH <5.5. However, all the sites were low in exchangeable acidity suggesting that Al toxicity may not be a serious problem. The soil available P at all the sites was <20 ppm, which is considered the critical value of available P for maize using the Mehlich method that was used in this study (Landon, 1991). Hence, P deficiency is likely to limit maize yields at these sites. In addition, N was also deficient at all the sites (<0.2%). The low levels of soil N and available P at these sites is consistent with other reported studies in the area and is partly attributed to mining of soil P and N through crop harvest on small-holder farms where the recommended N and P fertilizer rates to replenish the removed nutrients through crop harvests are rarely applied (Smaling et al., 1993; Okalebo et al., 2006). The sites were low in organic Carbon (C) (<2%) indicating low levels of organic matter (Landon, 1991). Exchangeable bases (Ca, Mg and K) were also generally low as would be expected of acid soils because of leaching (Obiri-Nyarko, 2012).

Effect of treatments on soil pH and exchangeable acidity

All treatments with lime application generally increased the soil pH when compared with control in both 2015 and 2016 cropping seasons (Table 2) as expected. However, only granulated lime applied without fertilizer attained

Table 2. Effect of lime and fertilizer on soil pH.

Treatment	pH 2015	pH 2016	∆ pH	t-value	p-value
Control	4.92	5.21	0.28	-1.65	0.20
$CaCO_3$	5.26	5.53	0.28	-1.55	0.22
$CaCO_3$ + fertilizer	5.35	5.41	0.06	-0.46	0.62
CaO	5.23	5.42	0.19	-3.38	0.04
CaO + fertilizer	5.27	5.49	0.22	-1.85	0.16
DAP + CAN	5.21	5.19	-0.02	-0.13	0.91
Granulated lime	5.46	5.28	0.18	0.76	0.50
Granulated lime + fertilizer	5.45	5.23	0.22	0.94	0.42
LSD (0.05)	0.447	0.307	-	-	-

Note: Fertilizer= (26 kg P (DAP) + 60 kg N (CAN)); CaO=2 tons ha-1 Calcium Oxide; CaCO3=2 tons ha-1 Calcium Carbonate

Table 3. Effect of lime and fertilizer on soil exchangeable acidity (cmol/kg).

Treatment	2016	2017	∆ Exchangeable acidity	t-value	p-value
Control	0.25	0.15	0.10	1.4	0.25
$CaCO_3$	0.13	0.13	0.00	0.0	1.00
$CaCO_3$ + fertilizer	0.10	0.13	-0.03	-0.29	0.79
CaO	0.18	0.15	0.03	1.00	0.39
CaO + fertilizer	0.12	0.13	-0.01	0	1.00
DAP + CAN	0.25	0.15	0.10	1.40	0.25
Granulated lime	0.10	0.18	-0.08	-0.68	0.55
Granulated lime + fertilizer	0.15	0.15	0	0	1.00
LSD (0.05)	0.16	0.16	-	-	-

statistical significance in 2015 and $CaCO_3$ alone in 2016. The rise in pH of soil is associated with the presence of basic cations (Ca^{2+}) and anions (CO_3^{-2}) in lime that are able to exchange H^+ from exchange sites to form H_2O + CO_2. Cations occupy the space left behind by H^+ on the exchange leading to the rise in pH (Fageria et al., 2007). Similar increases in pH have been reported by several authors (Whalen et al., 2002; Moreira and Fageria, 2010; Buni, 2014). None of the treatments raised the pH above the critical level of 5.5 in both years. This indicates that the lime rate that was applied was inadequate to overcome the pH buffering capacity of these soils. The change of pH from 2015 to 2016 was significant only for CaO where the pH increased by 0.19 units. This suggests that the residual effects of the applied liming materials are likely to be low, due to the low rate of lime used in this study. Similar results were reported by Kisinyo et al. (2014) in Western Kenya with the same lime rate of 2 t ha^{-1}. Residual effects, lasting up to four years were however observed at a higher lime rate of 6 t ha^{-1} in the same study. Similarly Quaggio et al. (1995) affirm that the residual effects of liming materials were primarily related to the rates than to the chemical components of liming materials.

The effect of lime types, applied alone or in combination with fertilizer on exchangeable acidity is presented in Table 3. There was no significant effect of treatments on exchangeable acidity likely due to its low levels in these soils and high variability among the sampled sites. Similar results were reported by Opala (2017) on Ferralsols of Maseno. The change of exchangeable acidity from 2015 to 2016 was also not significant for all treatments.

Effect of lime and fertilizer on maize yields

The maize grain yields varied across sites and seasons. The yields in the long rains seasons were generally higher than those in the short rains seasons (Tables 4 and 5). The yield ranged from 1.35 t ha^{-1} (control) at Mumias to 6.15 t ha^{-1} ($CaCO_3$+ fertilizer) at Butere in the 2015 LR and 0.90 t ha^{-1} (granular lime alone) to 7.15 t ha^{-1} (CaO + fertilizer) at Butere in the 2016 LR. In the 2015 SR, the highest yields (4.35 t ha^{-1}) were obtained with granular lime applied with fertilizer at Emuhaya and the lowest was by 0.55 t ha^{-1} (control) at Kakamega North. In general, application of lime without fertilizers containing N and P did not significantly increase yields at all the sites in all the seasons. However, all sites, except Butere responded to application of N and P fertilizers when applied without lime. At Butere, maize responded to

Table 4. Effect of lime and fertilizer inputs on maize yields (t ha^{-1}) at four sites in western Kenya in 2015 rain seasons.

Treatment	2015 Long rains				2015 Short rains			
	Butere	Emuhaya	Kakamega N	Mumias	Butere	Emuhaya	Kakamega N	Mumias
1. Control	1.73	1.80	2.22	1.35	0.70	1.38	0.55	2.01
2. CaO	2.96	1.64	2.54	2.25	1.55	1.28	0.38	1.72
3. CaO + fertilizer	6.15	4.08	5.26	4.48	2.23	3.48	0.83	3.97
4. $CaCO_3$	2.32	1.54	2.84	1.75	1.5	1.10	0.57	1.12
5. $CaCO_3$ + fertilizer	5.88	4.50	5.52	3.50	2.60	2.35	0.94	4.31
6. Granular lime	-	-	-	-	0.75	1.83	0.66	2.05
7.Granular lime + fertilizer	-	-	-	-	1.65	4.35	0.80	3.31
8. .Fertilizer	1.83	4.20	5.58	3.33	1.75	2.68	0.64	3.89
LSD	1.46	1.92	1.26	1.64	1.57	1.29	0.71	3.20

In the 2015 LR season, granulated lime treatments were not applied in the experiment.

Table 5. Effect of lime and fertilizer inputs on maize yields (t ha^{-1}) at four sites in western Kenya in the 2016 long rains.

Treatment	Sites			
	Butere	Emuhaya	Kakamega N	Mumias
1. Control	1.65	2.05	2.87	1.05
2. CaO	2.15	4.50	2.60	1.90
3. CaO + fertilizer	2.80	7.15	5.77	5.95
4. $CaCO_3$	2.35	2.85	2.47	1.90
5. $CaCO_3$ + fertilizer	2.75	6.80	6.47	5.20
6. Granular lime	0.90	3.90	3.47	1.95
7. Granular lime + fertilizer	3.75	5.55	5.97	4.40
8. Fertilizer	2.15	6.80	6.10	4.65
LSD (0.05)	2.20	2.10	1.91	1.68

fertilizer only in the presence of lime suggesting that soil acidity was a constraint at this site. The response observed with application of the fertilizers (with or without lime) confirms that both N and P are deficient at these sites and fall under the category of responsive soils (Kihara et al., 2016). Similar responses of maize to N and P fertilizers have been reported by several studies in the region (Okalebo et al., 2006; Ademba et al., 2015; Nziguheba et al., 2016; Kihara et al., 2016) Fertilizers containing these nutrients must therefore be applied, the acidity status of the soils notwithstanding. The application of lime, irrespective of the type, with fertilizer, did not give yields that were significantly different from those of fertilizer applied without lime at two sites (Emuhaya and Kakamega North) in all seasons. However, at Butere application of lime in the form of CaO and $CaCO_3$ with fertilizer gave yields that significantly exceeded those with fertilizer (DAP + CAN) applied without lime in the 2015 LR (Table 4). Similar results were observed with granular lime with fertilizer in the 2015 SR at Emuhaya (Table 4). The general lack of significant responses to lime in Kakamega North, Mumias and Emuhaya may be due to the low levels of exchangeable Al in the soils. Aluminum toxicity is therefore not likely to have been a major problem because the exchangeable acidity of the soil was below the critical level for soils to have acidity problem according to Mohammed et al. (2016). Economic considerations may therefore militate against the use of lime at these three sites because the use of lime resulted in extra costs that were not offset by increased yields. In Butere however use of lime in the form of CaO or $CaCO_3$ could be economically feasible and should be preferred to granular lime which was more expensive yet it was not superior in terms of increasing maize yields.

Conclusions and Recommendations

The maize grain yields varied across sites and seasons. There is need therefore to tailor soil fertility management practices to site-specific conditions to sustainably increase crop productivity. There was no significant effect of lime type on maize yields at all the sites. Maize responded to the fertilizers containing N and P but not to

application of lime without fertilizer at all sites. Application of lime, irrespective of the type, with fertilizer, did not give yields that were significantly different from those of fertilizers alone at all sites except at Butere in the 2015 long rain season when application of CaO and $CaCO_3$ with fertilizer gave significantly higher yields than those with fertilizer applied alone. Similar results were obtained with granular lime in the 2015 short rain season at Emuhaya. Soils in Mumias, Kakamega North and Emuhaya are either not sufficiently acid to permit the solubility of Al to toxic levels for maize or have inherently low levels of Al and that more attention should be focused on replenishing N and P at these sites. However, in Butere, soil acidity is a problem and lime should be applied together with fertilizers. The type of lime to be used should however be based on economic considerations since all the three types of lime tested were equally effective.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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