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Full Length Research Paper

Physiological quality of bean genotypes seeds peanut and xamego treated with fungicides and insecticides

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Bean is one of the most important crops because it is a staple food. In order to control diseases and pests, the use of fungicides and insecticides in the seed treatment are efficient methods. In this sense, the aim of this study was to evaluate the effect of fungicides and insecticides on physiological quality of bean seeds. The experiment was conducted in a laboratory and greenhouse, and seeds of two genotypes of common bean (Peanut and Xamego) were used. The seeds were treated with the following fungicides: Captan, Fluazinam, Metioram and insecticides: Acefate, Imidacloprid and Thiametoxan. The experiment was arranged in a completely randomized design, with replications and seven treatments. Not treated seeds were utilized as control. The bean seeds treatment of the genotype Peanut and Xamego using insecticide and fungicide did not affect the physiological quality of the seeds. Xamego genotypes shows greater vigor in the laboratory, while the Peanut genotype in the greenhouse, and the treatments do not interfere in the initial growth of the plants.

Key words: *Phaseolus vulgaris* L, germination, phytosanitary treatment, seedling emergence, vigor.

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is one of the most important crops in Brazil, which stands out as one of the largest producers in the world with production in 2016 of 3,328.1 tons, equivalent to an increase of 6.8% in relation to 2014

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Table 1. Fungicides, insecticides and dosages used in the treatments of the genotypes of bean seeds.

Treatments	Doses in seeds (g)
Fungicides	
Captan	3.2
Fluazinam	1.0
Metiram	3.0
Insecticides	
Acefate	10.0
Imidacloprid	10.5
Thiametoxan	7.0
Control	0.0

to 2015 harvest while from 2013 to 2014, harvest was 3,302.1 tons which has a planted area of approximately 4,380 hectares (Conab, 2016).

In bean crop, countless pests and diseases negatively interfere in production. Pest and diseases control in seeds, insecticides and fungicides are usually used, some of which may present physiological activity with a tendency to establish vigorous growth and to improve production (Castro et al., 2008). The active ingredients Thiametoxan and Imidacloprid, belonging to the chemical group of neonicotinoids, are systemic substances of insecticidal action (Ávila and Duarte, 2012). The insecticide Acefate belongs to the chemical group of organophosphorus and also presents systemic action.

The insecticides used for seed treatment is an alternative to avoid possible losses due to the soil and aerial plant part pests, which damage young seeds and seedlings (Martins et al., 2009). Therefore, seed treatment protects them against the initial attack of specific pests, being important in the rational use of effective products and prolonged residual action, guaranteeing seedlings stand and initial establishment. Insecticides in most cases can reduce the number of applications after crop emergence (Marchi et al., 2011) which can have protective and physiological effects, helping both the initial growth and the plant development (Dan et al., 2012).

Among the insect pests in stored common bean seeds, the weevil, *Zabrotes subfasciatus* whose eggs are deposited on the grains surface and larvae develops inside the grain, causing cotyledon weight loss, reducing the germination capacity of seed and commercial devaluation (Costa et al., 2014). In addition to controlling insects in the seeds, it is necessary to control fungi. The action of chemical fungicides in the physiological quality of the seed can increase or decrease the seeds germination and vigor (Pereira et al., 2007).

The active ingredient Captan belonging to the chemical group dicarboximide, Fluazinam to the phenylpyridinylamine group and Metiram to dithiocarbamate are systemic fungicidal substances. Some of the results of the present study have shown the efficiency of seed treatment with fungicides applied in the crops (Cardillo et al., 2017; Oliveira et al., 2016). The main storage fungi, *Aspergillus* sp and *Penicillium* sp, contaminate bean seeds, which cause germination losses, produce toxins and reduce the dry weight of the seeds (Torres and Bringel, 2005). With the absence of initial seed control, it is easier to introduce the fungus in the crop area, consequently, causing losses to the farmer.

Thus, the objective was to evaluate the physiological quality of bean seeds due to fungicide and insecticide treatment.

MATERIALS AND METHODS

The experiment was carried out in the Laboratory of Seed Analysis (LAS) and in a greenhouse located at the Center of Agrarian Sciences and Engineering of the Federal University of *Espírito Santo* (CCAE-UFES), in *Alegre*-ES.

Seeds of bean genotypes of the Peanut group and black group (Xamego) from the region's farmers were planted at the *Barro Branco* site in *Rive*, *Alegre*-ES district. The seeds were harvested in July 2017 and immediately under phytosanitary treatment. They were treated with fungicides and insecticides according to the manufacturers recommendations, with the application of the doses presented in Table 1, followed by homogenization.

Each product was added to the bottom of a plastic bag and scattered to a height of approximately 15 cm. Thereafter, 0.20 kg of seeds were added and shaken for 5 min. Seeds were allowed to dry at room temperature for 24 h. Meanwhile, seeds that were not treated chemically were used as control. In the laboratory the following were analyzed:

Germination (%): This was carried out with four replicates in 25 seeds, seeded in a germitest type paper roll, moistened with distilled water three times the mass of the dry paper and kept in growth room at 25°C. The evaluations were performed after five and nine days of sowing, calculating the percentage of normal seedlings (Brasil, 2009) and the results were expressed in percentage of germination.

Abnormal seedlings (%) and germination speed index: This involves concomitant determination using the germination test, alongside counting daily the number of seeds with primary root protrusion ≥ 2 mm (Maguire, 1962).

Length of the shoot of seedlings and root length (cm): They were determined after 9 days of sowing, measuring from the last leaf neck to the apex and from the neck to the tip of the largest root, respectively.

Total dry matter (mg): of the seedling, total dry matter were evaluated nine days after sowing, in an analytical balance (0.0001 g), obtaining dry mass and placing seedlings in paper bags, and putting inside a convection oven at 72°C for 72 h. The results were expressed as mg seedling^{-1}.

Table 2. Germination (%), abnormal seedlings (%), germination speed index of bean seeds of the genotypes Peanut (P) and Xamego (X) treated with fungicides and insecticides under laboratory conditions.

Treatments	Germination (%)		Abnormal seedlings (%)		Germination speed index	
	P	X	P	X	P	X
Captan	96 bB(1)	100 aA	4 cdB	10 bcA	11.48 bB	17.54 bA
Fluazinam	100 aA	100 aA	6 bcB	9 cA	22.65 aB	24.68 aA
Metiram	100 aA	100 aA	7 abB	12 bA	23.83 aA	24.50 aA
Acefate	96 bB	100 aA	10 aA	8 cB	23.08 aA	24.25 aA
Imidacloprid	100 aA	100 aA	5 bcB	10 bcA	23.38 aB	24.75 aA
Tiametoxam	100 aA	100 aA	9 aB	17 aA	11.64 bB	18.91 bA
Control	100 aA	100 aA	2 dA	3 dA	12.52 bA	13.62 cA
CV (%)	0.1		15.19		4.55	

(1)Means followed by the same lowercase letters in the column and uppercase in the row did not differ statistically by the Tukey test at 5% probability for each variable.

In the greenhouse the following were analyzed:

Emergence (%): was analyzed and placed in tubes of 53 cm^3 with commercial substrate Mercplant® sowed in 2.5 cm depth, being watered daily according to the crop needs. Evaluations were carried out daily, counting the emerged seedlings and the results were expressed as emergence percentage.

Emergence speed index: this was determined according to Maguire (1962); height (cm), stem diameter (mm), length of the shoot of seedlings and root length (cm) and total dry mass (mg) of the seedlings were obtained after nine days of sowing.

The obtained data were submitted as a test for normality and homogeneity of variance. The analysis of variance and the means were compared to the Tukey test at a 5% probability level. For all analyzes, the statistical program R was used with the ExpDes package (R Core Team, 2017).

RESULTS AND DISCUSSION

There was a significant interaction between fungicide and insecticides treatments and common bean genotypes (Table 2). The Xamego genotype presented higher values of germination, abnormal seedlings and germination speed index in relation to the Peanut genotype.

Higher germination values were observed for the treatments Fluazinam, Metiram, Imidacloprid, Thiametoxan and control presenting 100% for the Peanut genotype. Fungicides and insecticides did not affect seed germination of the Xamego genotype presenting 100%. Increased number of abnormal seedlings was found for the Xamego genotype (17%) in the treatment with the fungicide Thiametoxan. For germination speed index, higher values were observed for Fluazinam, Metiram, Acefate and Imidacloprid for both genotypes.

These results are important for the use of fungicides and insecticides via seed, since it is a technique capable of eradicating the pathogen from the seed surface and protecting them during germination, especially in infected soils, with decreasing responses as the seeds quality of increases.

In soybean seeds, treatment with Imidacloprid and Thiametoxan insecticides did not affect germination (Tavares et al., 2007; Castro et al., 2008), and in sorghum seeds, there was no reduction in the seed vigor (Vanin et al., 2011). However, in rice seeds with low physiological quality, the seed treatment with rhizobacteria or Thiametoxan increased their physiological potential (Soares et al., 2012). Free radicals provide membrane lipid peroxidation, protein oxidation, and DNA damage. Reactive oxygen species are formed upon the metabolism of xenobiotics to one or more of their reactive intermediates (Delgado, 2006).

The seed treatment using Thiametoxan can favor the absorption and stomatal resistance to lose water, and act as a bioactivator, determining the increase in the percentage of germination, in the emergence, root length and aerial part length, in the dry phytomass; due to transporting through the cells which triggers biochemical reactions. This can significantly improve the seedlings performance under adverse conditions, such as water deficit, soil acidity and salinity, phytotoxic effects of elevated levels of aluminum and temperature, in addition to determining the increase of protein levels and plant enzyme (Almeida et al., 2011; Almeida et al., 2012).

In studies carried out by Castellanos et al. (2017), in bean seeds treated with Thiametoxan during storage, it was verified that doses of the insecticide between 200 and 300 mL 100 kg^{-1} showed that seeds obtained good germination and satisfactory vigor expression, with the

Table 3. Length of the shoot of seedlings (cm), root length (cm) and total dry mass (mg) of bean seeds of genotypes Peanut (P) and Xamego (X) treated with fungicides and insecticides under laboratory and greenhouse conditions.

Treatments	Length of the shoot of seedlings (cm)		Root length (cm)		Total dry mass (mg)	
	P	X	P	X	P	X
Captan	14.52 cA(1)	14.21 dA	15.82 aA	13.08 aB	221.13 dA	105.06 cB
Fluazinam	15.25 bcB	16.82 bA	11.58 bA	13.05 aA	242.51 aA	110.29 bB
Metiram	13.31 dA	13.25 dA	11.41 bB	13.95 aA	225.92 cA	102.37 cB
Acefate	16.16 bB	19.60 aA	11.51 bA	13.90 aA	217.25 eA	110.76 bB
Imidacloprid	17.96 aA	17.20 bB	10.75 bB	14.85 aA	207.15 fA	125.60 aB
Thiametoxan	12.21 eB	14.15 dA	16.11 aA	13.27 aB	236.62 bA	109.88 bB
Control	15.08 cA	15.32 cA	17.95 aA	12.06 aB	208.16 fA	110.20 bB
CV (%)	3.19		13.14		0.93	

(1)Means followed by the same lowercase letters in the column and uppercase in the row did not differ statistically by Tukey test at 5% probability.

potential to be stored under controlled conditions, reducing the germination rate loss over time. This result corroborates with the results found in the present study, in which the insecticides used do not negatively affect seed potential. The occurrence of abnormal seedlings is related to increased seed deterioration (Marcos Filho, 2015). Seeds treated with insecticides and fungicides may be related to deterioration during storage, as well as, the phytotoxic effect of the insecticide product, either applied alone or with fungicide.

The Peanut genotype presented higher values of total dry mass in relation to the Xamego genotype (Table 3). Higher length of shoot of seedlings values of the Peanut genotype were observed for the treatment Imidacloprid (17.96 cm) in relation to the other treatments and Xamego genotype. The Xamego genotype showed a higher value of length of the shoot of seedlings (19.60 cm) for Acefate in relation to the other treatments and Peanut genotype. Larger root lengths were found for Peanut genotype (17.95, 16.11 and 15.82 cm) for the control, Thiametoxan and Captan treatments, respectively, in relation to the other treatments and Xamego genotype. For total dry matter, higher values were observed for Fluazinam for the Peanut genotype (242.51 mg) and Xamego genotype.

The increase in the length of the shoot of seedlings with the use of Thiametoxan can increase depending on the applied dose and the absorption and stomatal resistance to water loss, according to Castro et al. (2007), therefore favoring metabolism and increasing stress resistance. It can also increase, according to Castro and Pereira (2008), the efficiency in the absorption, transport and assimilation of nutrients.

Considering the growth and accumulation of total dry mass, there was a higher accumulation of dry masses in Peanut seedlings, and the products did not interfere with the physiological quality. These phases are associated with an increase in organic synthesis, rubisco activity, the amount of chlorophyll and hormones, leading to increase in the photosynthetic capacity in plants (Taiz and Zeiger, 2017). These results can improve the seedlings development due to the seed treatment, and Thiametoxan acting as a bioactivator in providing a larger aerial plant part (Acevedo and Clavijo, 2008; Lauxen et al., 2010). Pyraclostrobin + Metiram applied in tomatoes promoted an increase in the weight and volume (Guimarães et al., 2014).

The fungicide used in the seed treatment may influence in its physiological quality, and vary depending on the chemical used, causing an increase or decrease in the germination and treated seeds vigor (Pereira et al., 2007). However, Silva et al. (2013) verified positive effects on cowpea beans vigor treated with fungicide. In the insecticide treatment, the group carbamates and organophosphates may have a decrease in the physiological potential, due to the formation of free radicals, and exogenous stress produced by insecticides (Soares and Machado, 2007).

There was a significant interaction (treatments x genotypes) for emergence, emergence speed index, height and stem diameter (Figure 1). The Peanut genotype presented higher values of emergence, emergence speed index, height and stem diameter in relation to the Xamego genotype. Behavior checked when seeds were treated with Captan (100%), Imidacloprid (99%), Thiametoxan (100%) and control (100%) were compared to the other treatments. For the emergency speed index, higher Peanut values were observed for Captan (6.78), Fluazinam (7.92) and Thiametoxan (7.25).

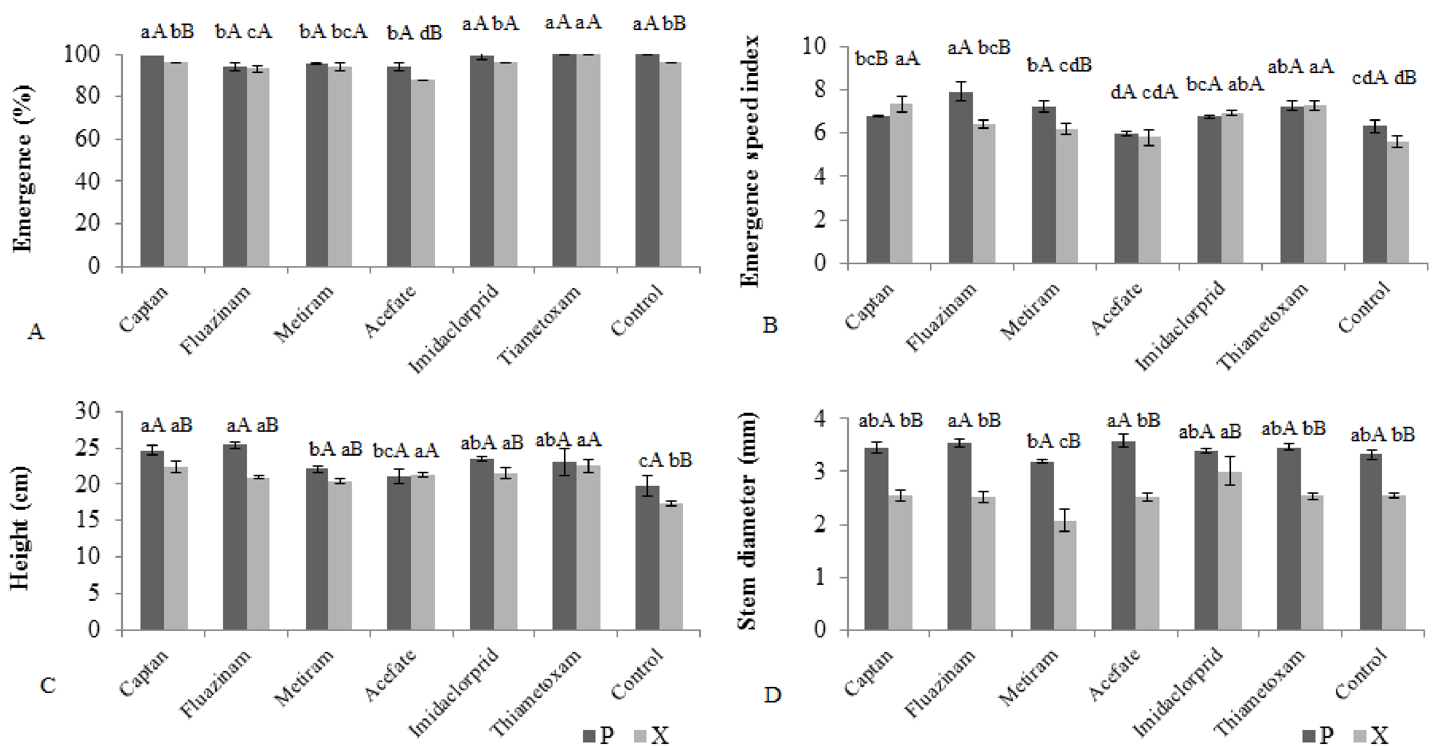

Figure 1. Emergence (%-A), emergence speed index (B), height (cm-C) and stem diameter (mm-D) of bean seeds of the genotypes Peanut (P) and Xamego (X) treated with fungicides and insecticides under greenhouse conditions. Means followed by the same lowercase letters (fungicide and insecticide treatments) and uppercase (seeds of bean genotypes) did not differ statistically by the Tukey test at 5% probability for each variable.

The Xamego genotype presented higher values of emergency and emergence speed index for the treatment Thiametoxan (100%, 7.27, respectively) in relation to the other treatments. The effect of Thiametoxan that presents the neonicotinoid chemical group acts on the germination of the seed producing plants, with greater number of fasciculated roots and of greater extension, at the same time in which a bigger growth of the aerial part is observed, seen in the work that the phytosanitary treatments do not affect the physiological quality of the seeds (Nunes, 2006).

However, seed treatment seeks to control fungi that may potentially cause soil seed deterioration or seedling death, such as *Phytophthora* spp., *Pythium* spp., *Fusarium* spp. and *Aspergillus* spp. (Henning et al., 2010). Bean genotypes showed higher heights for fungicide and insecticides treatments in relation to control. For the stem diameter, the Peanut genotype showed higher values for Fluazinam (3.54 mm) and Acefate (3.58 mm) in relation to the other treatments. Several pesticides, such as organophosphates (Acefate), may exert toxic effects with the induction of oxidative stress (imbalance between production and catalysis of free radicals) and alteration of the antioxidant system (Braguini, 2005).

The Peanut genotype presented higher values of length of the shoot of seedlings, root length and total dry mass in relation to the Xamego genotype (Figure 2). The Peanut genotype presented higher values of length of the shoot of seedlings for the treatments Fluazinam (17.33 cm) and Imidacloprid (18.26 cm) in relation to the other treatments. For root length, higher values of Peanut bean were observed for Fluazinam (15.56 cm), Metiram (15.65 cm) and Imidacloprid (15.78 cm). The Xamego genotype showed higher root length values for the Imidacloprid treatment (17.09 cm) in relation to the other treatments. The Peanut genotype presented the highest values for the control (237.30 mg) and the Xamego genotype for Thiametoxan (130.10 mg) in relation to the other treatments.

For two groups of bean plants in the treatment with Imidacloprid, insecticide presented higher values for the root length, which according to Horii et al. (2007), may be due to the increase of the energy availability from the hydrolysis via pentose phosphate, for the germination and emergence process. Similarly, there was a better

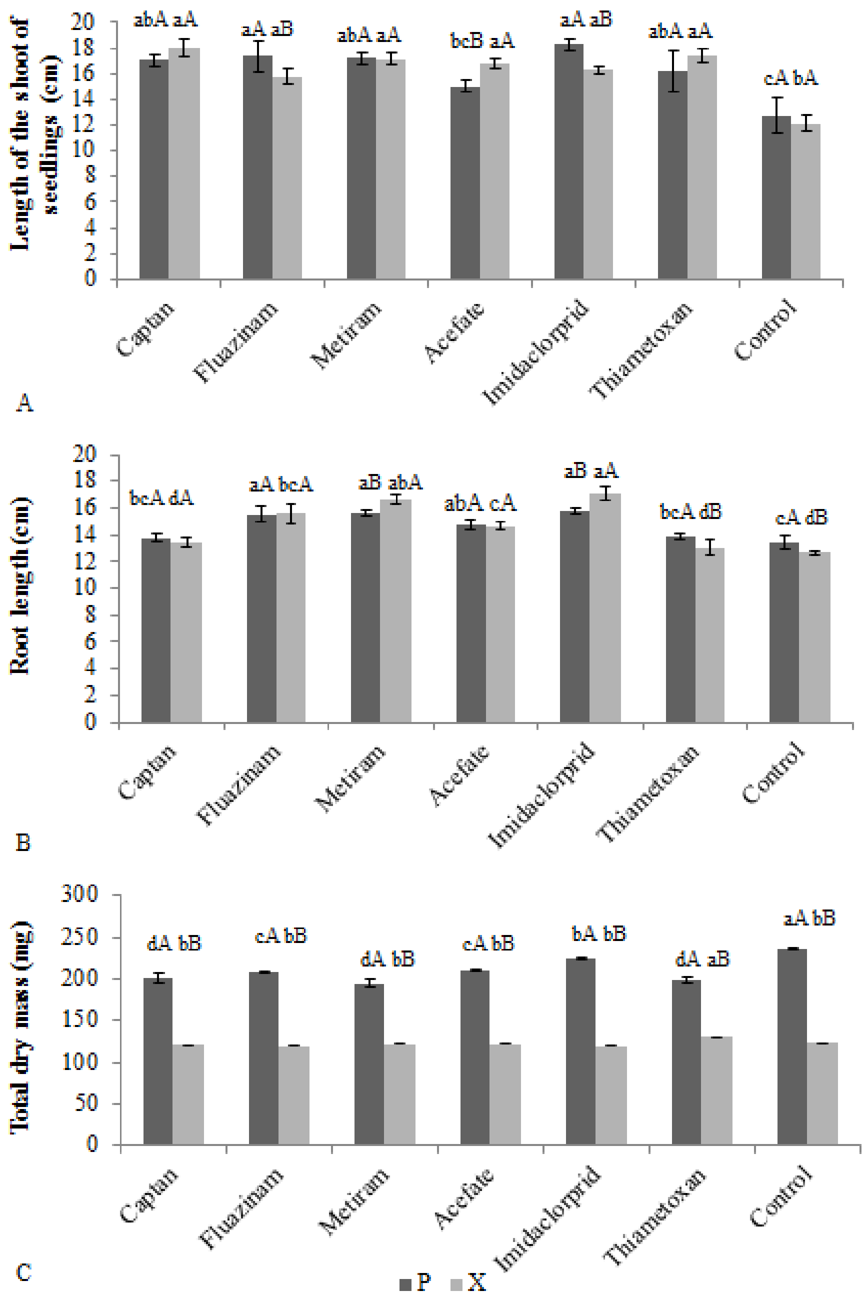

Figure 2. Length of the shoot of seedlings (cm - A), root length (cm - B) and total dry mass (mg - C) of bean seeds of the genotypes Peanut (P) and Xamego (X) treated with fungicides and insecticides under greenhouse conditions. Means followed by the same lowercase letters (fungicide and insecticide treatments) and uppercase (seeds of bean genotypes) did not differ statistically by the Tukey test at 5% probability for each variable.

physiological performance of rice seeds (Almeida et al., 2011), and cotton seeds (Lauxen et al., 2010), when treated with 35 g of active L^{-1} ingredient.

The phytosanitary treatments did not interfere in the physiological quality of the seeds, because the quality of the seeds is related to the capacity of the same to perform its vital functions, such as longevity, germination and vigor. Therefore, the effects on seed quality are generally translated by the increase in the percentage of germination, normal seedlings and vigor, as observed in the work (Kappes et al., 2012).

Conclusions

The bean seeds treatment of the genotype Peanut and Xamego using insecticide and fungicide did not affect the physiological quality of the seeds. Xamego genotypes shows greater vigor in the laboratory, while the peanut genotype in the greenhouse, and the treatments do not interfere in the initial growth of the plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Use of alternative substrates in production of tomato seedlings

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Tomato is one of the most produced vegetables in the whole Brazilian territory, also presenting great income to the family producer. The use of organic or alternative substrates has grown, since its acquisition by the producer is easy. Aiming at these factors, the objective of this work was to evaluate the production of three cultivars, Santa Clara, Santa Cruz Kada Gigante and Santa Adélia tomatoes in seven types of substrates formulated with humus and added bovine manure, chicken manure and carbonized rice husk, thus forming a randomized block design in factorial scheme 7×3. The seeds were sown in styrofoam trays and placed in a greenhouse at Emater-GO experimental station, in the city of Anápolis. Some analyses were carried out to verify the quality of seedlings. The analysis of variance was not significant for the cultivar × substrate interaction, so the analyses proceeded in isolation. The cultivars Santa Clara and Santa Cruz Kada Gigante were superior to cultivating Santa Adélia in all tests. The substrates that contained chicken manure composition were the ones that presented the best results in all analyses.

Key words: Bovine manure, chicken manure, cultivars, humus.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is among the main vegetables consumed in Brazil, both fresh and processed, being the most economically important vegetable (Soares et al., 2012). The production of vegetables has driven advances in production techniques that lead to quality seedlings with reduced costs and higher financial returns (Sediyama et al., 2014).

According to Oviedo (2007), the success of tomato production depends on the high quality of the seedling, being an essential factor, since the initial condition of the plant influences the emergence of seedlings, early production, total production and fruit size. This production is highly dependent on the use of inputs to which the substrate has stood out in importance, due to its wide use in the production of seedlings.

A good substrate is one that provides good moisture conditions, nutrient and water availability, macro and microporosity, cation exchange capacity, good root aggregation and uniformity (Teach et al., 2011; Costa et al., 2015). Several are the materials that can be used as

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substrate. These materials should provide adequate development of the seedlings, allowing good formation of the root system and aerial part of the plant (Trigueiro and Guerrini, 2014).

There is no substrate considered to be ideal, each has its advantages and disadvantages, so its choice depends on the characteristics of the crop and the cost to purchase. Therefore, it is necessary to test different substrates or mixtures of substrates for each oleraceous species (Garay et al., 2014).

The use of organic and/or alternative fertilizers makes it possible to provide a balanced supply of nutrients to the plants, decreases their apparent density, improves their structure and the possibility of root penetration into the substrate, and is more available for use by some family farmers (Cerqueira et al., 2015).

Among the materials frequently used as substrate are: earthworm humus (Oliveira et al., 2013), chicken litter (Brugnara, 2014), bovine manure (Gonçalves et al., 2014), and charred rice (husk et al., 2013).

Tomato cultivation may be more of an income option for family agriculture, because it uses labor intensively and generates higher returns per area of exploitation than annual crops (Costa el al., 2013). The production of alternative substrates becomes an important step in the process of production of seedlings in small properties and reducing production costs.

In view of the aforementioned, the objective of the present study is to evaluate the quality of tomato seedlings production cultivated in seven types of alternative substrates.

MATERIALS AND METHODS

The experiment was conducted at the Emater Experimental Station in Anápolis, GO, whose geographic coordinates are 16° 22 '22 "south latitude and 48° 53' 08" west longitude and 1,012 m altitude. The climate of the region according to the Köppen classification is Aw type, with dry winter and hot and rainy summers (Pereira et al., 2002).

The experimental design was completely randomized, in a 7×3 factorial scheme, with four replications. The substrates comprised of: S1 control (100% humus), S2 (50% humus + 50% bovine manure), S3 (50% humus + 50% chicken manure), S4 (50% humus + 25% chicken manure + 25% bovine manure), S5 (50% humus + 25% chicken manure + 25% charcoal rice husk), S6 (50% humus + 25% bovine manure + carbonized rice husk 25%), and S7 (50% humus + 50% carbonized rice husk). And for three types of tomato cultivars: C1 (Santa Clara 5800), C2 (Santa Cruz Kada Gigante), and C3 (Santa Adélia).

Seeding was performed by placing two seeds in the center of each cell of the tray, at a depth of 0.5 cm. Trays of expanded polyethylene (styrofoam) with a volume of 12.39 cm^3 were used, the dimensions of each cell being 6 × 5 cm, totaling 200 cells.

After sowing, the trays were placed at 0.20 m from the soil to facilitate the drainage of excess irrigation water. The irrigation system used was the micro-sprinkler, where two irrigations were done daily, the first in the morning and the second in the afternoon.

The experiment was conducted in a protected arc-type environment with the following internal dimensions: 6.0 m in length and 4.0 m in width (total area of 24.0 m^2) and 3 m in height, oriented in the East-West direction, protected with polypropylene mesh with 65% shading, on all sides of the metal structure (top and sides).

The thinning was performed 11 days after sowing (DAS) leaving the most vigorous seedling per cell, in which the plots were constituted by 10 seedlings. For the evaluations, eight seedlings per plot were used, and the sides of the trays were considered border.

At 31 days after sowing, the following characteristics were evaluated: number of leaves (NL), count of the fully developed definitive leaves; seedling height (SH), determined with ruler graduated in cm, with the seedlings still in the tray, measuring from the base of the stem to the apex of the last leaf; stem diameter (SD) obtained with a digital caliper (mm) by measuring the diameter of the seedling considering a change in cm above the neck; root length (RL) determined by measuring the roots from the base of the seedling to its end, with a ruler graduated in cm.

To determine fresh shoot mass (MFPA) and roots (MFR) were obtained by separating the seedlings in aerial part and roots. Afterwards, they were washed in running water, the parts were placed in bags of Kraft paper duly identified according to the treatment and taken for drying in an oven with forced air circulation, at a constant temperature of 70°C, for 72 h, and then weighed in an analytical balance with a precision of 0.001 g, for the determination of the dry masses of the area (MSPA) and roots (MSR).

For the calculation of the development quality index (IQD), the methodology of Dickson et al. (1960), considering the dry mass of shoots, roots and total dry mass, height and diameter of the lap of the seedlings, using the following equation:

$$\mathrm{IQD} = \frac{\mathrm{PMST}}{\left(\dfrac{\mathrm{AP}}{\mathrm{DC}}\right) + \left(\dfrac{\mathrm{PMSPA}}{\mathrm{PMSR}}\right)}$$

where IQD = Dickson development index; MST = total dry mass (g); AP = plant height (cm); DC = lap diameter (cm); PMSPA = dry weight of aerial part (g); and PMSRA = root dry mass weight (g).

The obtained data were submitted for analysis of variance by the F test at 5% of probability and when significant means comparison test was done-Scott Knott at 5% of probability. For the variance homogeneity test, Bartlett test and ASSISTAT 7.7 Software were used (Silva and Azevedo, 2016) and the SISVAR Software (Ferreira, 2014) was used for the statistical analyses.

RESULTS AND DISCUSSION

The different types of substrate and the different cultivars were significant by the F test ($p<0.05$), different from the Scott-Knott test ($p<0.05$) for all variables studied. For the interaction between the factors, F test ($p<0.05$) was not significant for all the analyses that were done, showing that their relation does not interfere in the production of tomato seedlings.

It can be observed that the Santa Clara and Santa Cruz tomato cultivars did not differ among all the variables in the study, presenting the best results for the same, the same was not observed in the cultivar Santa Adélia. The values found for the Santa Clara and Santa Cruz were close to the variables (SH), (SD), (NL) and (RL). These cultivars proved to be the best when expected to produce good seedlings (Table 1).

Table 1. Values of seedling height (SH), stem diameter (SD), average leaf number (NL) and root length (RL) for the three tomato cultivars.

Cultivate	SH (cm)	SD (mm)	NL	RL (cm)
Santa Clara	6.88^{A}	1.47^{A}	2.53^{A}	7.89^{A}
Santa Cruz	6.93^{A}	1.49^{A}	2.58^{A}	7.93^{A}
Santa Adélia	3.20^{B}	0.97^{B}	1.34^{B}	6.88^{B}
CV (%)	23.45	23.25	24.87	16.52

*Values followed by the same letter do not differ from each other by the Scott-Knott test at the p<0.05 level.

Table 2. Values of seedling height (SH), stem diameter (SD), mean leaf number (NL), and root length (RL) for the seven substrates.

Substrate	SH (cm)	SD (mm)	NL	RL (cm)
S1	3.75^{C}	0.92^{B}	1.50^{B}	7.76^{A}
S2	5.65^{B}	1.35^{A}	2.28^{A}	6.59^{B}
S3	7.11^{A}	1.58^{A}	2.72^{A}	8.61^{A}
S4	6.77^{A}	1.52^{A}	2.42^{A}	7.63^{A}
S5	6.62^{A}	1.46^{A}	2.43^{A}	7.64^{A}
S6	4.32^{C}	1.07^{B}	1.76^{B}	7.16^{A}
S7	5.44^{B}	1.27^{A}	1.97^{B}	7.59^{A}

*Values followed by the same letter do not differ from each other by the Scott-Knott test at the p<0.05 level. S1 (100% humus), S2 (50% humus + 50% bovine manure), S3 (50% humus + 50% chicken manure), S4 (50% humus + 25% chicken manure + 25% bovine manure), S5 (50% humus + 25% chicken manure + 25% carbonized rice husk), S6 (50% humus + 25% bovine manure + 25% carbonized rice husk) and S7 (50% humus + 50% carbonized rice husk).

Silva et al. (2012) and Cerqueira et al. (2015), when working with production of tomato seedlings of the cultivars Santa Clara and Santa Cruz, respectively, found similar values for height, stem diameter, and average number of leaves, which corroborate this study.

In Table 2, the types of combinations of the substrates that were used in this study influenced the production of tomato seedlings.

For the height variable, it was observed that the substrates S3, S4 and S5, obtained the highest averages when compared with other combinations. The substrate that had only humus was the one that presented the worst result for height. The highest height was found for the substrate S3 that presented seedlings with 7.11 cm of height.

For the diameter, a higher number of substrates presented equal means, in addition to S3, S4, and S5, which obtained values between 1.46 and 1.58 cm, the substrates S2 and S7 also presented the same averages statistically. Regarding the number of leaves, the substrate combinations S1, S6 and S7 were the ones with the worst means. In this variable, the highest average found was for the combination of substrate S3, with an average number of 2.72 leaves.

For the root length, it can be seen that only the substrates S2 showed below the other substrates, with a mean of 6.59 cm of root length. All other combinations of substrates were statistically the same, but the substrate S3 stood out from the others presenting the highest average for the root length.

Some works with production of tomato seedlings also found that the addition of chicken litter and bovine manure to the substrate, promoted an increase in the quality of the seedlings.

Since the chicken litter has one of the highest levels of nitrogen found in its composition, this is the nutrient that is most required throughout the plant formation phase, especially in the initial period (Silva junior et al., 2014; Santos et al., 2015). Brugnara et al. (2014), when analyzing the quality production of passion fruit seedlings, found that the higher addition of chicken litter on the substrates leads to a higher quality of seedlings.

As already seen from the other analyses, the Santa Clara and Santa Cruz tomato cultivars did not differ statistically (Table 3), presenting the highest averages in all the evaluated criteria, being these superior to Santa Adélia.

A good porosity allows the movement of water and air in the substrate, favoring the germination, favoring the increase in the fresh masses of the aerial part, of the root, and their respective dry masses, and presenting good quality development index seedlings.

Table 3. Fresh air mass values (MFA), fresh root mass (MFR), dry mass area (MSA) and root dry mass (MSR), development quality index (IQD), for the three tomato cultivars.

Cultivate	MFA (g)	MFR (g)	MSA (g)	MSR (g)	IQD
Santa Clara	0.65^{A}	0.61^{A}	0.35^{A}	0.31^{A}	0.34^{A}
Santa Cruz	0.71^{A}	0.63^{A}	0.37^{A}	0.28^{A}	0.37^{A}
Santa Adélia	0.51^{B}	0.51^{B}	0.15^{B}	0.18^{B}	0.15^{B}

*Values followed by the same letter do not differ from one another by the Scott-Knott test, at the level of $p<0.05$.

Table 4. Fresh air mass values (MFA), fresh root mass (MFR), dry mass area (MSA) and root dry mass (MSR) development quality index (IQD), for the seven substrates.

Substrate	MFA (g)	MFR (g)	MSA (g)	MSR (g)	IQD
S1	0.42^{B}	0.46^{B}	0.12^{B}	0.17^{B}	0.12^{B}
S2	0.63^{A}	0.49^{B}	0.30^{A}	0.19^{B}	0.30^{A}
S3	0.80^{A}	0.72^{A}	0.47^{A}	0.39^{A}	0.47^{A}
S4	0.70^{A}	0.70^{A}	0.35^{A}	0.33^{A}	0.35^{A}
S5	0.70^{A}	0.69^{A}	0.36^{A}	0.34^{A}	0.36^{A}
S6	0.58^{B}	0.56^{B}	0.17^{B}	0.24^{B}	0.17^{B}
S7	0.54^{B}	0.46^{B}	0.24^{B}	0.15^{B}	0.24^{B}

*Values followed by the same letter do not differ from each other by the Scott-Knott test at the $p<0.05$ level. S1 (100% humus), S2 (50% humus + 50% bovine manure), S3 (50% humus + 50% chicken manure), S4 (50% humus + 25% chicken manure + 25% bovine manure), S5 (50% humus + 25% chicken manure + 25% carbonized rice husk), S6 (50% humus + 25% bovine manure + 25% carbonized rice husk) and S7 (50% humus + 50% carbonized rice husk).

For all the substrates under study (Table 4), the averages were statistically the same for the substrate S2, S3, S4, and S5, except for the studied variable of fresh root mass, where the substrate S2 presented a lower average in relation to the others. Evaluating the production of fresh matter of the aerial part of the root and their respective dry masses, it was verified that the substrates that contained addition of chicken manure and bovine manure provided a better balance between the growth of the aerial part and the roots, forming seedlings with the vigorous root system, associated with greater leaf development, stem diameter, and seedling height.

Costa et al. (2015) verified when working with seedlings of cherry tomatoes in different types of substrates, that the ones that contained chicken bedding were the ones that presented the highest averages in relation to the quality index of seedlings, dry and fresh matter of roots and compared to other types of substrate formulation.

Silva et al. (2012) evaluating the addition of carbonized rice husk on different substrates for the production of tomato cv. Santa Clara observed a quadratic reduction of shoot mass and root mass.

This fact explains that the seedlings produced lower dry mass with the addition of charcoal rice husk on substrates S6 and S7.

The values of fresh and dry mass of seedlings are a criterion for demonstrating how much a seedling has absorbed the nutrients and water that are present in this substrate, thus, the more nutritious the medium in which the seedlings are, the more they will present a greater weight in its mass (Isah et al., 2014).

According to Costa et al. (2011), Dickson quality index serves as an indicator of the quality of seedlings, involving several parameters, such as seedling height, stem diameter, aerial and root dry mass and total dry mass.

The quality index of tomato seedlings presented values between 0.12 and 0.47. For the substrates S2, S3, S4 and S5 obtained the highest values of quality, not differing between them, they were not observed in the other substrates under study.

In tomato seedlings, Cerqueira et al. (2015), studying different alternative substrates, observed that the quality values of the seedlings ranged from 0.0037 to 0.0073, which was not observed in this study.

When analyzing all the variables in question, it can be observed that all the substrates that contained the chicken manure in its composition, presented the best results. Thus, the addition of any other type of compound to the substrates will not have a significant effect on the production of seedlings, since the addition of compounds on substrates will increase its final cost.

Conclusions

The Santa Clara and Santa Cruz cultivars perform better in all variables studied. The use of humus as a base for alternative substrates, with the addition of compounds such as chicken litter and bovine manure in the appropriate proportions (S3, S4, and S5), can produce seedlings with a high quality index, making feasible the use of these compounds in the formulation of substrates for tomatoes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Tree species' growth in a silvipastoral system in Amazon

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This research aimed to evaluate the dendrometric development of four tree species (*Carapa guianensis* Aubl. (Meliaceae); *Dipteryx odorata* (Aubl.) Willd (Fabaceae), *Swietenia macrophylla* King (Meliaceae) and *Handroanthus chrysotrichus* (Mart ex A. DC.) Mattos (Bignoniaceae)) in a silvopastoral system in Santarém, Pará, Brazil. Productive activities in the forest sector create a demand for technical capacity to promote sustainable management, with the silvipastoral system presented as one of the paths to sustainability. Three permanent plots of 1 ha each were established on the 240-hectare Diamantino Farm, and stem DBH 1.30 m from the soil surface, total height and crown diameter data were obtained for all individuals. In addition to the quantitative data, crown regularity and bole tortuosity parameters were measured. *S. macrophylla* achieved better performance in almost all parameters. *C. guianensis* and *D. odorata,* respectively showed greater uniformity in growth. The species, *H. chrysotrichus* had the smallest crown diameter; however it was still able to provide enough shade for animal thermal comfort.

Key words: Dendrometry, forest management, reforestation.

INTRODUCTION

The growth of the Brazilian forestry sector contrasts with the low level of investments in replanting and forest formation. In order to avoid market retention in the medium and long term, it is important to invest in reforestation (Juvenal and Matos, 2002).

In addition to the traditional planting systems, integrated crop-livestock-forest (ICLF) systems represent a new method developed for the cultivation of tree species which aim to diversify production, integrating different productive systems such as agriculture, livestock and forestry, simultaneous or consecutively (Guimarães, 2015). When trees and pastures are in the same system, a silvipastoral model is formed, characterized by the combination of the use of trees, pastures and cattle in the same area concomitantly, being managed in an integrated way aiming to increase productivity per unit

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area (Nair, 1984).

Agroforestry practices encompass a range of products to be achieved, both to meet the demands of wood and its multiple uses in the industry, such as forage, fruit and vegetable production, as well as benefits such as improved physical, chemical and biological, improve the productivity of the components, economic stability and income increase with the diversification of activities, reduction of costs in the medium and long term, and reduction of vulnerability to risks, including susceptibility to pests (Cordeiro et al., 2015). In this area of Brazilian livestock raising, problems caused by pasture degradation, poor pasture formation, inadequate management and maintenance, and extensive stocking of animals are still present, and these are the main agents of the soil compaction process, and also of negative changes in soil and climatic properties (Peron and Evangelista, 2004; Macedo, 2005). In view of this problem, the integration of tree species with pastures in the ILPF system is presented as an option in order to recover the productive capacity of pastures (Rozados-Lorenzo et al., 2007; Paciullo et al., 2011), besides being essential to improve the quality of the pasture, and to contribute to the thermal comfort of the animals, resulting in better meat and milk production and also an increase in the nutritive value of the pasture (Dias-Filho, 2006). In addition, other types of animals such as buffaloes, sheep and goats can also be integrated into the silvipastoral system (Garcia et al., 2011; Campanha and Araújo, 2010). In the Amazon region, there are still few studies that deal with silviculture in forest plantations in ICLF systems, and there is a need for these systems to be developed in agricultural regions in Brazil areas so that in the coming decades, Brazilian agriculture and cattle raising can be promoted as an example of sustainability in the international market, in addition to offering healthy products, in line with new concepts regarding the timber market and land use systems (Silva, 2009). Among some tree species that could be chosen in the implantation of a silvopastoral system, Andiroba - *Carapa guianensis* Aubl. (Meliaceae), is highlighted for its high demand in the municipality of Santarem due to its multiple uses, such as wood and oil supply (Souza et al., 2006). Another multipurpose species that also provides wood, bark and oil, is Cumaru - *Dipteryx odorata (*Aubl.) Willd (Fabaceae). It is a species that adapts well to different types of soil. From the seed, an oil rich in coumarin is extracted, for which there is great demand in the international cosmetics and perfumery market (Carvalho, 2009). Mahogany- *Swietenia macrophylla* King (Meliaceae) is another species of interest to the markets, which is currently banned from being cut in natural forest, and can only be commercialized if its origin is reforestation. It is a species that adapts to hydromorphic, podzolic, medium to high fertility soils (Lamb, 1966; Brasil, 2003). The species, ipê-amarelo- (*Handroanthus chrysotrichus* (Mart. ex A.DC) Mattos) (Bignoniaceae) is also recommended as species for the recovery of degraded areas, landscaping, as well as providing good quality wood for the construction of bridges, boards for general use, frames and other purposes (Lorenzi, 2002). Thus, measuring dendrometric parameters of a stand is important to evaluate the arrangement and estimate the forest production, helping to make a productive comparison of different locations, besides defining guidelines for forest management practices establishing harvest goals (Pinto et al., 2005). In this sense, the objective of this work was to evaluate the dendrometric development of four forest species, seven years in age, in a silvipastoral system.

MATERIALS AND METHODS

Study area

The research was developed at Diamantino Farm (Figure 1), located on the left bank of the Santarem/Curuá-Una Highway, km 11 (02° 30' 57,7"S e 54° 39' 36,2"W) covering an area of 240 ha, with annual average temperature between 25 and 28°C, relative air humidity of about 86% and average annual precipitation of 1920 mm. According to Köeppen's classification, the tropical monsoon subclimate (Ami) of the region presents the traditional climate of Tropical Forests, hot and humid, having a period of 3 months (August to October) with rainfall less than 50 mm monthly, with periods of drought (4 to 6 months) and well defined rainfall, directly influencing the seasonal changes in the water level of the rivers. According to IDESP (2014), the soils of the study area are yellow Oxisols of medium, clayey and very clayey texture in association with concretionary lateritic and dystrophic indiscriminately textured soils and rocky outcrops.

Main characteristics and area history

Diamantino Farm belongs to the Congregation of the Brothers of Santa Cruz, Diocese of Santarém, PA. Historically, there was cutting of timber in the area and introduction of annual crops. Over time, the area became abandoned, and in 2008, the pasture was planted with *Panicum maximum* Jacq, and the forest species were introduced. The planting of the seedlings was done manually, in pits of 40 cm x 40 cm x 40 cm, and spacing of 7 m x 7 m between trees. Organic manure from cattle was used with terra preta (black earth) as fertilizer. Subsequently, after the pasture grew substantially, it was cut using a tractor. After this first cutting, in order to reduce the costs and control pasture growth, cattle were introduced in a rotating system. The silvicultural treatments consisted of pruning and cleaning two to three times a year in order to minimize competition from invasive plants, especially in the first two years. The vegetation of the area is secondary vegetation composed by sparse grasses and shrubs, popularly called "capoeira" vegetation in Brazil. The physical-chemical properties of the soils are shown in Table 1.

Data collection

The 2008 planting area was 17 ha wherein in 2015, three plots of 100 m × 100 m (1 ha) were installed. The trees planted in these plots were *Carapa guianensis* (Aubl.) (Meliaceae), *D. odorata*

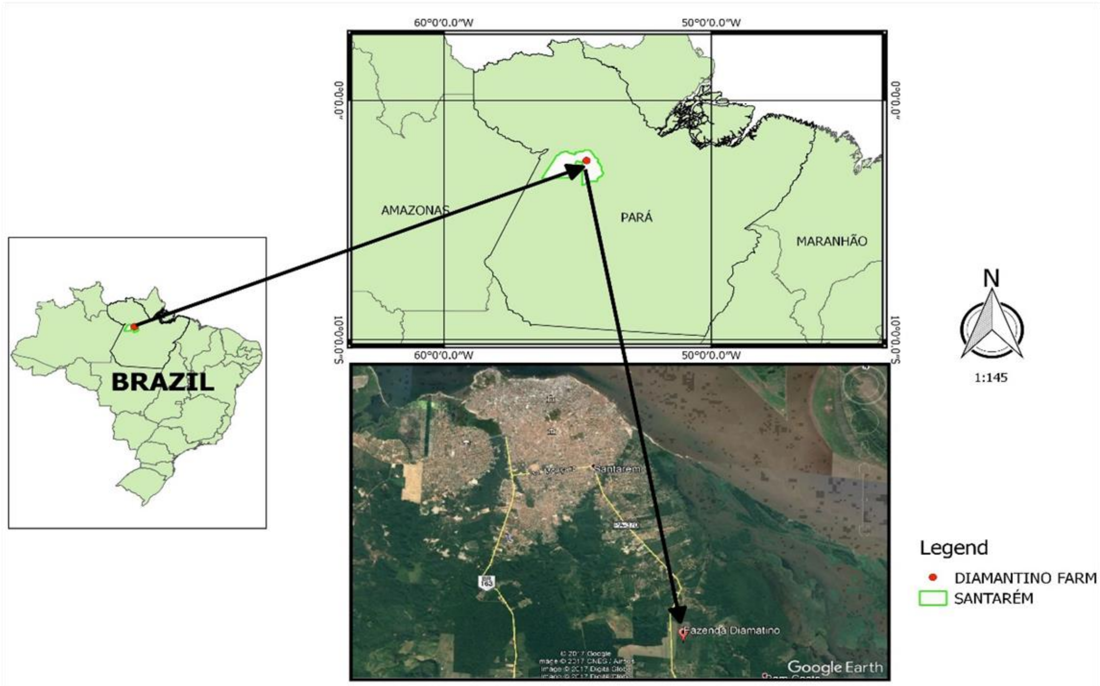

Figure 1. Map of location of the Diamantino Farm in Santarém, Pará, Brazil.

Table 1. Physical-chemical characteristics of soil in the experimental area (0-20 cm).

Depth	OM*	pH	P	K	Na	Ca	Ca+Mg	Al	H+Al	CTC		Saturation	
										Total	Effective	Base	Al
(cm)	(g/kg)	(water)	(mg/dm³)				(cmol$_c$/dm³)			(cmol$_c$/dm³)		V (%)	m (%)
0-20	40-70	5.8	583	22	7	7.2	8.7	0.1	7.10	15.88	8.88	55.28	1.13

*OM = Organic matter.

(Aubl.) Wild (Fabaceae), *H. chrysotrichus* (Mart. Ex A.DC) Mattos) (Bignoniaceae) and *S. macrophylla* King (Meliaceae) among other species (Figure 2).

In total, 133 individuals of *S. macrophylla*, 104 of *D. odorata*, 101 of *H. chrysotrichus*, and 82 *C. guianensis* were recorded. To obtain the dendrometric data, a diametric tape was used to measure the diameter at a height of 1.30 m (DBH), a hypsometer was used to measure total height (HT) and a 30 m line was used to measure crown diameter (CD). In addition, the quality of the trunk (QT) was classified as straight, bifurcated or tortuous, and crown quality (CQ), classified as regular or irregular, was evaluated in order to determine the ability of the forest cover to provide thermal comfort to animals in the silvipastoral system. Measurements were performed in the period from May to July 2015, wherein the data of all the inventoried individuals were collected.

Data processing

The diametric distribution is an important tool applied in the description of the diametric structure of forest populations. It is also possible to describe changes in the structure of the stands, as well as hypsometric relationships and mortality rates, all of which are possible to study in certain periods, stand growth (Schikowski et al., 2016).

The diametric distribution of the planting was analyzed considering a 2 cm diameter class amplitude, followed by application of the non-parametric Komolgorov-Smirnov T test at the level of (α) 0.05% significance. Comparison of each individual of a species with another of the same species was done for all possible comparisons, for example, individual 1 when compared with individual 2, then 3 and 4, and subsequently repeating this pairwise comparison for all possible combinations with the objective of finding the largest difference value (D) between them from the relative frequency of distributions, obtained by dividing the cumulative frequency (F) by the number of observations (n), and subsequently comparing the critical value ($D\boldsymbol{\alpha}$), as presented by Sokal and Rohlf (1995) and used by Werneck et al. (2000), where:

$$D = \left|\frac{F1}{n1} - \frac{F2}{n2}\right|$$

$$D\alpha = K\alpha\sqrt{\frac{n1+n2}{n1.n2}}$$

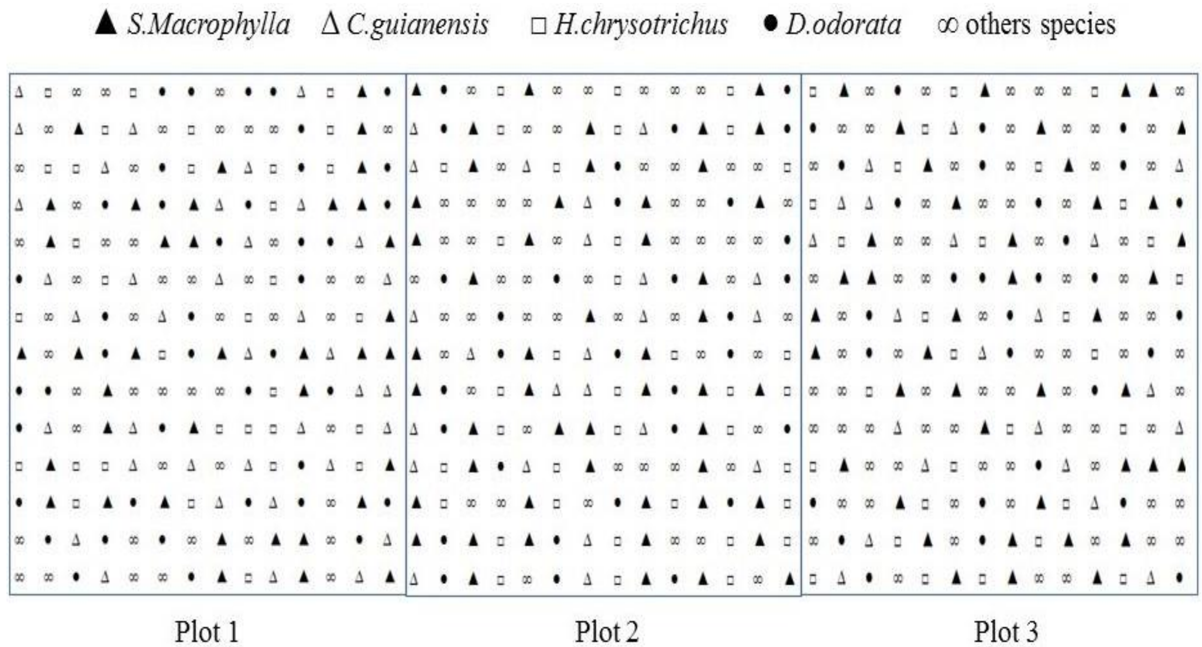

Figure 2. Sketch of the installed plots of the 7-year-old planting in 7 x 7 m spacing, in Diamantino Farm, Santarém, PA.

$$K\alpha = \sqrt{\frac{1}{2}\left[-ln\left(\frac{\alpha}{2}\right)\right]}$$

The qualitative analyses of the stem (straight or tortuous) and canopy (regular or irregular) were described by means of percentage (%). The individual canopy area was calculated using the ellipse area formula that uses the largest longitudinal and perpendicular measurement of the canopy.

A canopy = $\pi[(L1+L2)/2)^2/4]$

Where, A canopy = individual area of canopy (m²); L1 = length of the largest longitudinal measurement of the canopy (m); L2 = length of the largest perpendicular measurement of the canopy (m). All statistical analyses and graphs were done using the software Excel 2010.

RESULTS AND DISCUSSION

Quantitative analysis: Height and diameter

The mean values of the variables measured at 7 years in relation to DBH, total height and their respective annual mean increments, in addition to the crown area, are listed in Table 2. The species that obtained the best performance in all these variables was *S. macrophylla*. At 7 years of age in the silvipastoral system, it reached a maximum height of 14 m and 23.81 cm of DBH. In studies by Matos et al. (1996) and Sousa et al. (2010), performed in other types of Agroforestry Systems of the same age, the maximum height and DBH recorded for *S. macrophylla* was 10.2 m and 12.3 cm, respectively. Castro et al. (2008) in a silvipastoral system of the same age at Embrapa Rondônia, found values of growth in height of 12 m and diameter of 22 cm, suggesting, therefore, that the best performance of the species manifests itself with the presence of the animals in the planting area.

C. guianensis presented superior results in relation to DBH and height when compared with *D. odorata* and *H. chrysotrichus*. Souza et al. (2010), working in the city of Manaus, showed that *C. guianensis* obtained better results when compared with *S. macrophylla* of the present study in all variables, being similar only in height growth (m.year^{-1}); however, the spacing used by the authors was 3 m x 3 m, in an area of 225 m². In order to evaluate the growth of *C. guianensis* in a planting of mixed tree species of the same age, Tonini et al. (2005), reported a similar result of 10.5 cm in DBH, but with an additional 2 m in height as compared to the present study.

Among the averages of height, *H. chrysotrichus* obtained the lowest value (5.64 m). However, for mean DBH (8.88 cm), *H. chrysotrichus* had the third best performance in relation to the other species. The growth of *H. chrysotrichus* occurs slowly, being able to reach a height of 4 m to 35 m in the adult phase (Carvalho, 2006). Lorenzi (2002) reported, for DBH of this species, values between 30 and 130 cm in the adult phase. There are few studies on the behavior of this species in silvipastoral systems. In an agroforestry system in Mato Grosso do Sul, Salomão et al. (2012), estimated a mean height of 7.99 m for this species at the age of seven years in a 1.5 m x 1.5 m spacing model in a consortium between different tree types and agricultural species.

Table 2. Dendrometric performance by species in the full sun trial at 7 years of age.

Species	DBH (cm)	TH (m)	Canopy area (m²)	MAI DBH (cm.year^{-1})	MAI TH (m.year^{-1})
S. macrophylla	16.01	7.99	17.87	2.29	1.14
C. guianensis	10,32	7.00	8.92	1.47	1.0
H. chrysotrichus	8.88	5.64	6.26	1.27	0.81
D. odorata	7.60	6.31	10.71	1.09	0.90

DBH = diameter measured at 1.30 m from the soil, TH = total height, and MAI = mean annual increment.

The Komolgorov-Smirnov non-parametric T Test yielded a higher value (D) between the populations of *D. odorata* and *H. chrysotrichus*, and the diameter distribution of *S. macrophylla* had a higher concentration in the 19 cm DBH class (Figure 3); *H. chrysotrichus* was the only other species that had an individual in this class. However, although it is present in the largest class, *S. macrophylla* was one of the species that presented the greatest heterogeneity as a result of this distribution, which is not a desirable type of behavior for a plantation forest in terms of planning criteria.

D. odorata had superior development to *H. chrysotrichus* in all measurements except DBH. Galeão et al. (2003), in a pure planting of the species with spacing of 6 m x 1.3 m in the same city (Santarém), obtained an average growth rate of 2.91 m in height and 6.41 cm of DBH at 7 years. Souza et al. (2010), in a silvicultural plantation in full sun with multiple arboreal species, in a spacing of 3 m x 3 m obtained averages of 6.61 in height and 6.12 in DBH. These behaviors in different types of systems indicate good performance of the species in a silvipastoral system.

In addition, *C. guianensis* is the species that presented the second lowest coefficient of variation in DBH (CV% 44.65) and after *S. macrophylla*, had a higher frequency of trees in classes 9 and 11. Most *D. odorata* individuals were included in the 9 cm class, and none of the individuals reached a diameter greater than 12 cm. However, although it did not reach larger diameters, it presented the highest homogeneity pattern in DBH with the lowest CV% (44.45), and it was possible to establish objective predictions and targets for the cultivation of this species, based on this parameter. However, the species *H. chrysotrichus* was the most heterogeneous with the highest coefficient of variation in DBH (CV% = 45.12), registering the presence of individuals in all the classes with majority (30.7%) of the population centered around the 7cm diameter class.

It is important to mention the aptitude that other species have in integrating the silvipastoril system. Martínez et al. (2010) and Tonini et al. (2016), present as possibilities, the species, *Schizolobium amazonicum*, common in the Amazon region, and the exotic species, *E. grandis* and *E. urophylla.* Results of the dendrometric performance of these species under the same conditions of age as the system adopted in this work was not available in the current literature. However, in research carried out by Tonini et al. (2016), in a silvipastoril system in West-Central Brazil, the species of *E. grandis* and *E. urophylla* at 3 years of age showed average DBH of 10.4 cm, and height of 10.5 m in 3.5 x 3 m spacing, and this is a result that exceeds the performance of the best species studied in this research. Maneschy et al. (2009), evaluating growth of *S. amazonicum* in an Amazonian silvipastoril system in 5 x 3 m spacing, registered average results of 17.81 and 19.25 m in height, and DBH of 16.83 and 19.72 cm, measured in different years. In the current research, the result of average height and diameter of *S. amazonicum* also presented results significantly higher than the species with better performance in this research, despite being one year younger.

Qualitative analysis of trunk and crown

For trees with a straight trunk (Figure 4), the highest contribution was from *S. macrophylla*, with 93.23% of its individuals, followed by *C. guianensis*, *D. Odorata* and *H. chrysotrichus*. Only 1.92% of the *D. odorata* species were classified as bifurcated. With respect to trunk tortuosity, the species *H. chrysotrichus* (57.42%) and *D. odorata* (36.53%) had the highest proportions of individuals. One of the techniques used to reduce the coefficient of variation (CV %) is selective thinning, a forest activity that has as objective, removal of trees of inferior quality, favoring the growth of others with larger diameters. For this, it is necessary to monitor the growth of the plantation in order to determine the best time for intervention (Maestri and Scolforo, 1997). For the analysis of trunk straightness, more than 72.61% of the total individuals presented straight boles, indicating that there was good performance in relation to the silvicultural treatments in the maintenance stage of the planting. The high degree of tortuosity of the *H. chrysotrichus* trunks is explained by Carvalho (2006), as characteristic of this species.

For the canopy evaluation, Figure 4 shows that 89.04% of the individuals presented a regular shape, with insignificant differences between the species *C. guianensis* and *D. odorata,* and there was low crown regularity in the *H. chrysotrichus* population. In relation to fruit and seed production, Grogan (2001) reported that

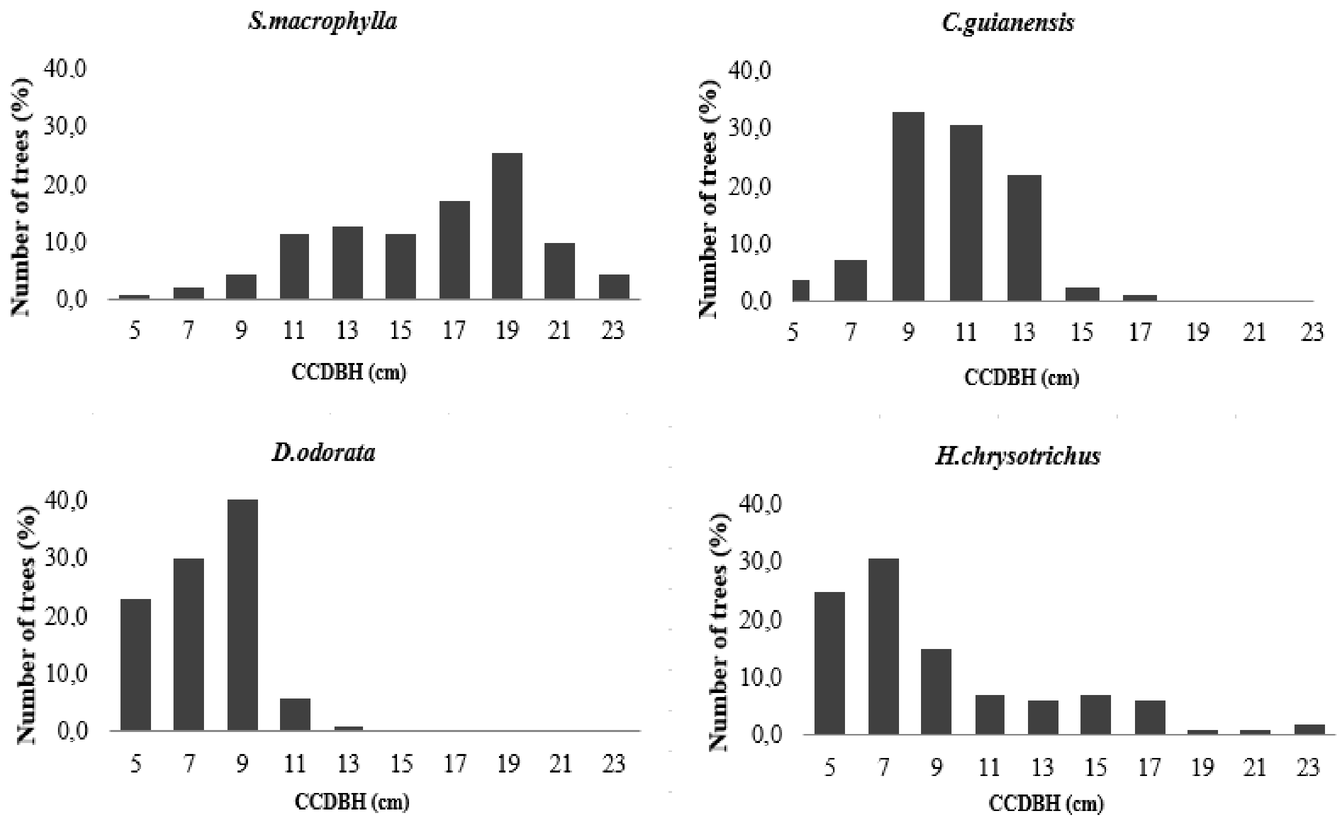

Figure 3. Diametric distribution by species (amplitude 2 cm), Diamantino Farm, Santarém, Pará in which: CCDBH = Center class diameter at breast height.

individuals of *S. macrophylla* with DBH ≥ 30cm produce approximately 50 seed pods, each, having on average, 60 seeds. Thus, if the DBH growth rate presented in this work continues for further six years, this species will be producing seeds for commercialization. According to IBF (2013), one of the benefits of commercializing the seed of this species is its great economic value. It is noted that after *S. macrophylla*, *C. guianensis* is the species that grew the most in diameter, confirming the study by Souza et al. (2006), which concludes that this species, in fertile soils, has rapid development with high increase in volume.

For the canopy cover analysis, an extremely favorable result in relation to the planting prospects is given by Melo et al. (2007), in a study on reforestation that showed a positive correlation between canopy cover and the age of planting; however, the authors suggested that better equations could be obtained if the whole range of ages and site variation are considered.

Considering the silvicultural treatments used in the area (pruning of the branches and crowning), even with the great extension of the property and low number of workers, the planting presented trees with trunks and crowns of satisfactory quality. According to Sena (2013), it is worth noting that in plantation of forestry for commercial purposes, whether seedlings or seeds, some of the factors that should be given special attention are size and shape of the crown, besides the volume and quality of the fruits produced. Figure 5 shows the performance per species in average crown diameter, considering the average of the greatest longitudinal and perpendicular measurement of the crown.

Planting, because it is young, is still in the process of growth, and its capacity of effective canopy cover at the age of seven years covers 2.796 m². That is, of the 2 ha study area, 27.96% is covered by 240 individuals from the plantation, tending to increase its reach until reaching maturity. In this way, the species, through this component, are already providing some types of thermal comfort to the animals.

Silva (2009) underlines the importance of shade for cattle, given the growing concern for animal welfare. On the other hand, if there is excessive shade for grazing, there may be a reduction in storage capacity and a negative influence on soil carbon. The author stated that there is still little information on the size of the shade area adequate to meet the needs of the animals, with values varying from 1.8 to 10 m² per animal. Garcia (2013) emphasized the importance of determining the appropriate shade design for

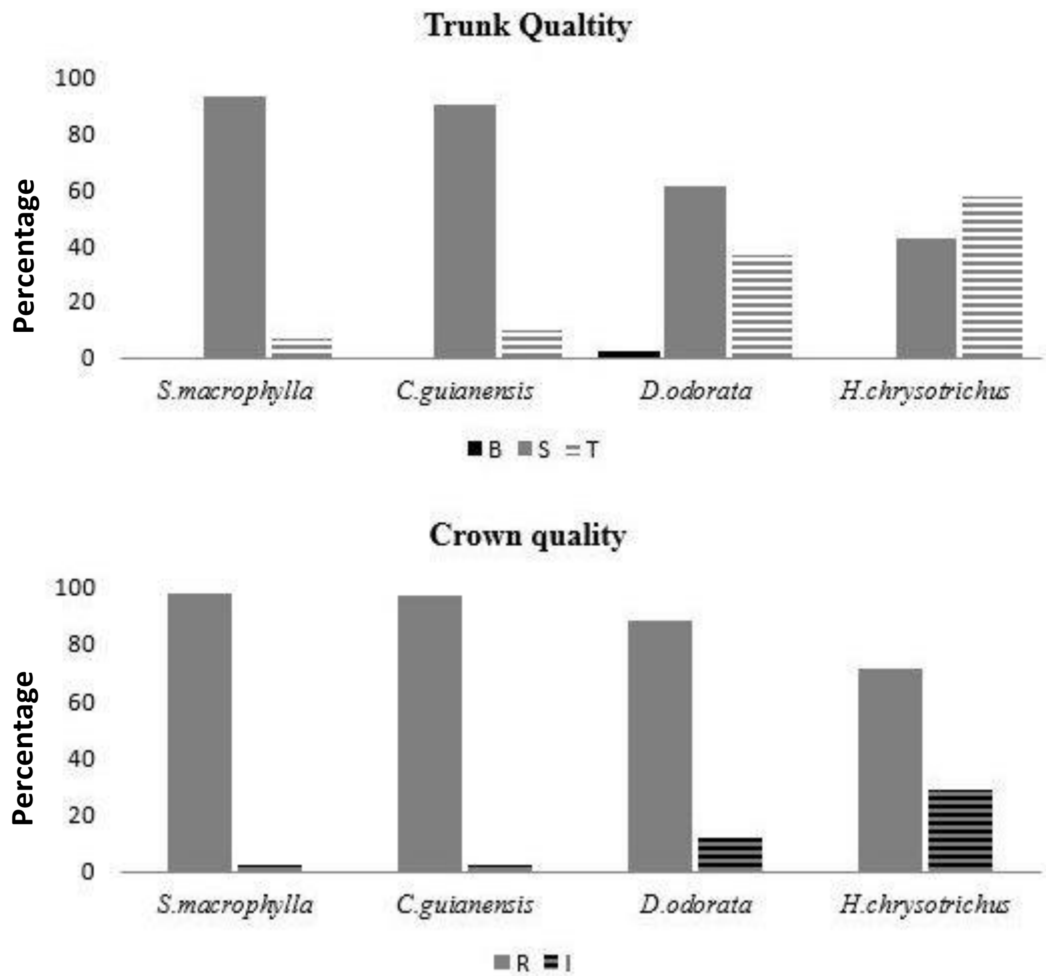

Figure 4. Qualitative evaluation of trunk (TQ) and crown (QC), Diamantino Farm, Santarém, Pará. Where: TQ (B = bifurcated, S = straight, T = tortuous); QC (R = regular, I = irregular).

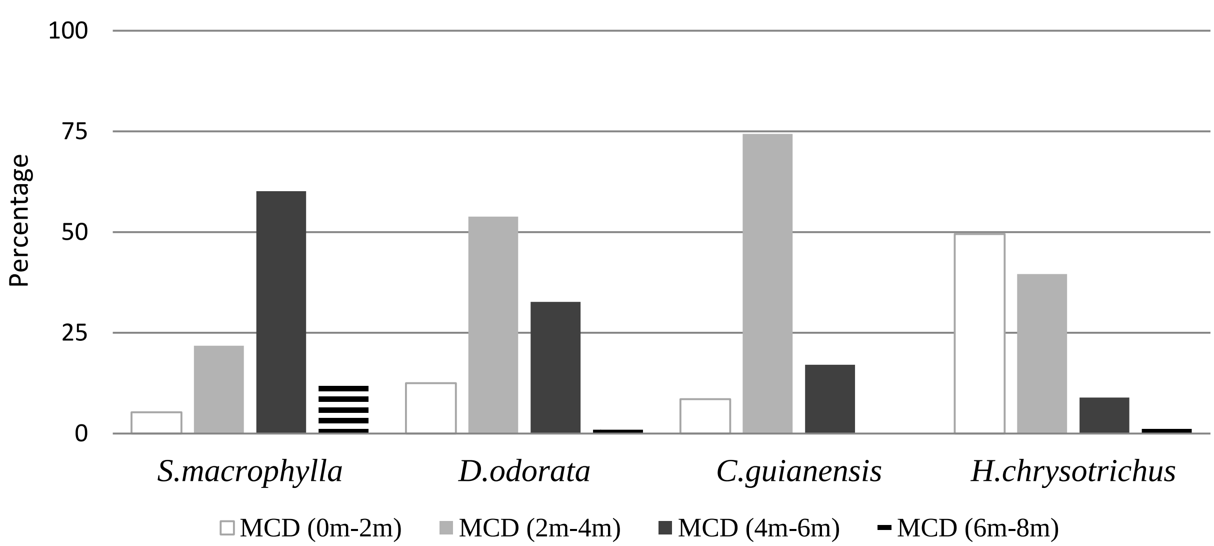

Figure 5. Mean crown diameter (MCD) by species, Diamantino Farm, Santarém, Pará, Brazil.

larger livestock groups in the plantation.

Conclusion

S. macrophylla had the best growth with regards to diameter and height. *C. guianensis* and *D. odorata* presented greater uniformity in diametric distribution. *H. chrysotrichus* provided a smaller crown area, however, sufficiently capable of offering thermal comfort to the animals in the silvipastoral system.

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest.

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Full Length Research Paper

Effects of extracts of *Oxalis barrelieri* L. and *Cymbopogon citratus* stapf, coupled with NaCl sorting on; seed health, germination and seedlings vigor of rice (*Oryzae sativa* L).

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The extracts of *Oxalis barrelieri* and *Cymbopogon citratus* were tested for their antifungal activity against *Bipolaris oryzae* and *Fusarium moniliforme*, and use as seed treatment coupled with NaCl treatment to improve the seed germination and the seedlings vigor of treated rice seeds. The significance of the inhibitory activity of the plant extracts against *B. oryzae* and *F moniliforme* was type of extract and concentration dependent. The ethanolic extract of *C. citratus* at 10000 ppm, showed the highest inhibitory effect, with 58.51 and 55.9% growth inhibition of *F. moniliforme* and *B. oryzae,* respectively. The rice seeds sorted in 20% NaCl solution had a greater mass (quantify), a germination percentage of 12.5% higher as compared to seeds sorted in with water and 19.5% higher than that of unsorted seeds. A moderate positive and significant linear relationship (Rho (78) = 0.427 **, p <0.01) was shown between weight and germination of rice seeds. The ethanol extracts of *O. barrelieri* and *C. citratus* stimulated rice seed germination at comparable degree; 15.15 to 22.06% from the top of paper method and 11.1 to 16.2% from sand method in green house trials. Rice seed treatments at 1% concentration exhibited the best germination and reduced the infestation of *B. oryzae* and *F. moniliforme* by 25.25 and 9.83%, respectively. Stressed seeds treated with plant extracts showed less pronounced decrease (5.08 %) in germination compared to untreated stressed seed (25.5%). Sorting of rice seeds with 20% NaCl and/or treatment with ethanol extracts of *O. barrelieri* and *C. citratus* showed potential green alternative to reduce seed-borne infestation, improvement of rice vigor and seed germination.

Key words: Rice seeds, NaCl sorting, *Cymbopogon citratus*, *Oxalis barrelieri,* Infestation, *Bipolaris oryzae, Fusarium moniliforme,* germination, vigor.

INTRODUCTION

Rice is one of the oldest food crops and constitutes the staple food of more than 60% of people living in the world (Chandrasekaran et al., 2010). Many diseases, including cryptogamic diseases, cause considerable damage in all areas of rice production (Oerke, 2006). Most of these diseases are mainly seed-borne. *Bipolaris oryzae* and

Fusarium moniliforme, responsible for the brown spot disease and the bakanae disease in rice, respectively, are mainly found in rice seeds (Mew and Gonzales, 2002). Losses due to these pathogens range from 4 to 52% for *B. oryzae* (Barnwal et al., 2013) and 4 to 20% for *F. moniliforme* (Reddy and Sathyanarayana, 2001). Seed dressing of rice to overcome these pathogens has many advantages, including the protection of seed and seedlings against pathogens in the early stages of germination and development. It requires less chemicals application than plants treatment in the field. It is ecofriendly with minor effects as environmental pollutant and on beneficial to soil organisms, specific to the target pathogens (Sharma et al., 2015).

On the other hand, pretreatment of seed is considered as a viable technology to improve rapid and uniform emergence, high vigor and better yields in some crops (Hu et al., 2005). Worldwide and particularly in Cameroon, the majority of rice growers keep seeds from season to season. Most often there is no seed treatment prior to seeding because of lack of knowledge of available chemicals. In addition, some fungicides used for seed treatment are increasingly criticized for their adverse effects (Wilson and Tisdell, 2001); thus, in recent years, there have been considerable pressures on agriculture in reducing their use, and finding better alternatives (Tripathi and Dubey, 2004).

The flora is recognized as the most effective producer of the various biologically active compounds and, some higher plant products have been formulated as botanical pesticides and are used on a large scale as eco-friendly measures in the management of agricultural pests (Dubey et al., 2011). Rice seeds treatment with sodium chloride (NaCl) solutions is an empirical practice. However there has been no investigation on the targets and optimal sorting concentrations, beneficial to the seed health and germination percentage. This work was initiated to study the activity of extracts of *Oxalis barrelieri* and *Cymbopogon citratus*, on two rice seed-borne fungi (*B. oryzae and F. moniliforme*), and to assess their effects on the fungal incidence, the germination percent and seedlings vigor of rice seeds sorted in NaCl solutions.

MATERIALS AND METHODS

Rice varieties used

Seeds of red rice from Tonga with rainfed system and Tox 3145-38-2-3 from Ndop with irrigated system; two highly cultivated rice varieties in these localities of the western regions of Cameroon, were used.

Plant material and preparation of plant extracts

C. citratus stapf and *O. barrelieri* L., whole plants were harvested in Yaoundé-Cameroon; They were identified, respectively, in comparison with the specimen of Herbarium Collection No. 18622 SRF / Cam (YA) of the sample of *C. citratus* (De Candolle) *Staff* of the Collector Daniel Dang No. 202 and the specimen of Herbarium Collection No 19798 SRF / Cam (YA) of the sample of *O. barreleri Linn* of the Collector A.J.M Leeuwemberg No 6048.

After harvest, the plant material was dried under shade for one and half month and crushed into fine powder. For aqueous extract, the powder was degreased by dipping 100 g in 600 mL of hexane for 2 h. The mixture was filtered with a fine cloth; the residue obtained was recovered and dried under the hood until the hexane was completely evaporated and further macerated in 600 mL of distilled water for 12 h and filtered through a fine cloth.

The resulting filtrate was centrifuged at 6000 g for 20 min and freeze dried to give a powder (AE). For ethanolic extraction, the procedure was roughly the same as for aqueous extraction, except that the residue was macerated in 70% ethanol and the mixture filtered through Whatman paper, and centrifuged. The filtrate obtained was lyophilized (EE).

Synthetic fungicide used

The synthetic fungicide Banko Plus®, made up of 550 g/L chlorotalonil and 100 g/L of carbendanzime was use a positive control.

Evaluation of the *in vitro* antifungal activities of *C. citratus* and *O. barrelieri* extracts

Strains of *B. oryzae* and *F. moniliforme* had been identified and, isolated from infected rice seeds by standard mycological techniques (Mathur and Kongsdal, 2003) at the Plant Pathology Laboratory of the Institute of Agricultural Research for Development, Nkolbisson, Cameroon. The antifungal activities of the extracts (aqueous and ethanolic) of *C citratus* and *O. barrelieri* were evaluated according to the method described by Grover and Moore (1962) with some modifications. The powders of extracts were dissolved in distilled water and then supplemented at concentrations of 1000, 5000 and 10000 ppm in conical flask containing the Potato Dextrose Agar (PDA) culture media; 5 mg of chloramphenicol was added per liter of culture medium; the whole was autoclaved and poured between 40 to 50°C in the petri dishes. After solidification of the culture medium, 5 mm diameter mycelial disk was removed in the peripheral zones of an aging culture of 6 (*B. oryzae*) and 10 (*F. moniliforme*) days and placed in the center of the petri dish. The dishes thus seeded, were sealed with parafilm and incubated in inverted position at -28°C under a photoperiod of 12 h light aimed at 7 days for *B. oryzae* and 12 days for *F. moniliforme*. Two experiments of three replicates were performed. The mycelial growth diameters were measured and percentage inhibitions (I%) was calculated according to the formula:

$$I\% = [(DC-DT) / DC] \times 100$$

With DC, the mycelial mean diameter growth in the control non-

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supplemented dish and DT, the mycelial mean diameter growth in the supplemented petri dish (treatment).

Treatments of rice seeds

Treatment of rice seeds with NaCl

NaCl solutions (300 mL) were prepared at various concentrations: 0 (distilled water), 10, 20 and 30%. Sixty (60) g of rice seed was first introduced into a beaker containing only distilled water (NaCl 0%), and then stirred with a glass rod for about 1 min and allowed to stand until stabilization of the water movement.

The floating seeds (S) were recovered by decantation, washed with distilled water and dried (Ts0% S). A portion of the immersed seeds or pellet (C) was recovered, rinsed with distilled water and dried (Ts0% C). The remainder of the pellet was introduced into the 10% NaCl solution and stirred for 1 min; after stabilization of the solution, the supernatant was recovered, rinsed and dried (Ts10% S); a small part of the new pellet was retained, rinsed and dried (Ts10% C). The remainder of the new pellet was introduced into 20% NaCl solution and the cycle was repeated (Ts20% S and Ts20% C) up to the concentration of 30% NaCl (Ts30% S and Ts30% C). Hundred (100) seeds (x 4) of each of the dried lots (Ts0% S, Ts0% C, Ts10% S, Ts10% C, Ts20% S, Ts20% C, Ts30% S and Ts30% C), non-treated seeds (Sec) and total seed soaked in distilled water for 1 min (TM0%), were weighed and the mass expressed in grams. The germination of seeds was also evaluated.

Treatment of rice seeds with plant extracts and synthetic fungicide

The seeds of red rice from Tonga were used due to their low germination potential. The immersed seeds sorted by treatment in the 20% NaCl solution were divided into 5 identical sub samples; 4 sub samples were dipped for 24 h in solutions of ethanol extracts of *C. citratus*/*O. Barrelieri*, at respective concentrations of 0, 1, 2 and 4%. One (1) sub sample was soaked in 0.2% of the Banko Plus fungicide used as positive control.

Simultaneously, the floating seeds from the 20% NaCl sorting were immersed in distilled water (Sur). Dry seeds (Sec) as used by the farmers served as negative control. After these treatments, seed sub samples (T^+0, T^+1, T^+2, T^+ 4%, Banko plus, Sec and T^+ Sur) were recovered, dried for 1 h and their germination evaluated.

Germination test

The top of paper method (Rao et al., 2006) was used to evaluate the effect of treatments on rice seeds' germination. The above sub samples were divided in four replicates of 50 seeds each. Three moistened blotter papers were placed in 90 mm diameter petri dishes and 25 seeds were seeded per dish and incubated at room temperature (-28°C) under a photoperiod of 12 h of light for 7 days, after which the percent germination was evaluated by, counting the number of normal and abnormal seedlings and dead seeds.

Evaluation of the effect of extracts on, the vigor and the seed health of rice seeds

The accelerated aging test (stress) as described by Delouche and Baskin (1973) was used to evaluate the vigor of the seeds. Dried seeds samples from each of the various treatments described above were deposited on the wire mesh supports. The mesh supports were subsequently placed in transparent plastic boxes containing 500 mL of distilled water, so as to avoid contact between the seeds and the water. The boxes were hermetically sealed and placed in the oven at a temperature maintained at 44°C for 72 h (Suraj and Thawatchai, 2010). After this stress, seed samples were removed and subjected to the standard germination test.

For the evaluation of the seed health status, seed samples were seeded into the petri dishes containing PDA medium supplemented with 5 g/L of chloramphenicol. Four replicates of 25 seeds each were made, under a photoperiod of 12 h light for 5 days. The infestation incidence of *B. oryzae* and *F. moniliforme* were recorded by counting the number seeds colonized by each of the pathogens and the result expressed as percent infection (I%).

Emergence test

Pots of about 1 L were filled up to 4/5 with a mixture of black soil and sand, in the ratio 4:1. The seed samples from the treatments described in 'treatment of rice seeds with plant extracts and synthetic fungicide' ($T^+0\%$, $T^+1\%$, $T^+2\%$, T^+ 4%, Banko plus, Dry (Sec) and T^+ Sur) were sown in pots at 2 cm deep.

Each pot contained 15 seeds chosen randomly and, four replicates were made form each treatment. Watering with 20 mL/pot/day was regularly done using tap water. After 12 days of growth, normal seedlings were counted and results expressed in term of percent emergence.

Statistical analysis

The IBM SPSS 23.0 software was used. In order to carry out the parametric tests, the data were transformed whenever necessary, to determine whether there was any significant difference among the average scores of the levels of the independent variables. One, two and three factor ANOVA were applied, values of $P < 0.05$ were considered as significantly different. The nature of the differences between the means of scores was determined by the Student-Newman-Keuls (S-N-K) multiple comparison test, when the hypothesis of equality of variances was assumed, or by the method of Games-Howell, when this assumption was violated (Morgan et al., 2011).

The correlation coefficient of Pearson or Spearman rho, calculated with 99% confidence interval, was used to establish the relationships amongst variables. All the experiments were arranged in a completely randomized design.

RESULTS

Antifungal activity of the plant extracts

Four extracts were obtained: AEc and EEc of *C. citratus* with yield of 5.50 and 4.78%; and AEo and EEo of *O. barrelieri* with yield of 5.82 and 3.54%, respectively. Two-way factor ANOVA was used to evaluate whether the extract and the concentration each had an effect on the inhibition of fungal growth; and whether the extract effect on fungus inhibition was concentration dependent; the results are shown in Table 1.

The extracts exhibited a significant effect $F(3, 24) = 64.89$, $p < 0.05$ and $F(3, 24) = 143.03$, $p < 0.05$, respectively, for the inhibition of *F. moniliforme* and *B. oryzae*. There was a difference in activity between AEc, EEc, AEo, and EEo. EEs were in general more active than EAs. EEc showed the highest inhibitory activity with

Table 1. Analysis of variance for inhibition percentage as a function of concentration and plant extract.

Source	Type III SS	Df	MS	F	Sig	η^2
B. oryzae						
Extract	1971.48	3	657.16	143.03	0.000	0.15
Conc	9783.30	2	4891.65	1064.67	0.000	0.80
Ext*Conc	59.230	6	9.87	2.148	0.008	0.018
Error	110.268	24	4.594			
F. moniliforme						
Extract	1575.86	3	525.28	64.89	0.000	0.165
Conc	8465.80	2	4232.90	522.92	0.000	0.82
Ext*Conc	322.30	6	53.717	6.636	0.000	0.005
Error	194.27	24	8.09	-	-	-

SS: sum square; MS: Mean Square; Conc: Concentration; Ext: Extract; η^2: Eta square; d*f*: degree of freedom.

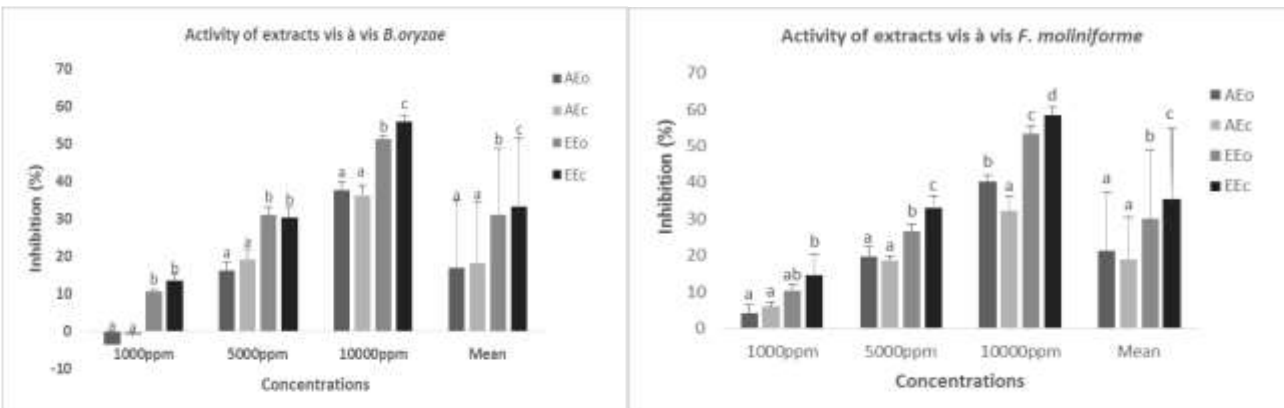


Figure 1. Growth Inhibition of *B.oryzae* and *F. moniliforme* at different concentrations of the extracts of *C. citratus* and *O. barrelieri.*
[a, b,c],At a given concentration, the extracts bearing different letters are significantly different (p <0.05). Data are averages of three experiments of two replicatesAEo: Aqueous extract *O. barrelieri;* EEo: Ethanolic extract *O. barrelieri*; AEc: Aqueous Extract *C. citratus*; EEc: Ethanolic extract *C. citratus.*

58.51% inhibition vis-à-vis *F. moniliforme* and 55.9% inhibition vis-à-vis *B. oryzae*.

The effect of the concentration was significant with F (2, 24) = 522.92, $p < 0.05$ for *F. moniliforme* and F (2, 24) = 1064.67, $p < 0.05$ for *B. oryzae*. The growth inhibition of both pathogens was dose dependent. For example AEo 1000 ppm (4.07%) < AEo5000 ppm (19.62%) < EAo10000ppm (40.36%) against *F. moniliforme*. At 1000 ppm, AEo (-3.33%) and AEc (-1.1%) stimulated the growth of *B. oryzae* (Figure 1).

The extract *concentrations interaction was significant, F (6, 24) = 6.636, $p <0.05$ and F (6.24) = 2.148, $p <0.05$, respectively for *F. moniliforme* and *B. oryzae*. This shows that the effect of the extract on inhibition is concentration dependent and increases with the concentration increase. Nevertheless, it should be noted that, even though the extract *concentration interaction was statically significant for the inhibition of *B. oryzae;* the effect size value (Cohen's f = 0.07) did not meet minimum standard (Cohen's f ≥ 0.10) to be called a "small" effect size (Cohen, 1988).

Effect of rice seed treatments with NaCl solutions on germination and seed weight

One-way ANOVA was used to compare, the means weight and, the means germination of different seed samples treated with the NaCl solutions. A significant difference was found between the seed samples: F (9, 30) = 143.332, $p <0.05$ and F (9, 30) = 74.114, $p < 0.05$ for the percentage of germination and F (9, 30) = 33.64, $p <0.05$ and F (9, 30) = 30.785, $p < 0.05$ for the weight, respectively for the red rice of Tonga and Tox 3145-38-2-3 of Ndop. The nature of the differences between seeds samples were identified and results reported in

Table 2. Percent germination (%) and weight in gram (g) weight and germination percentage of rice seed samples treated with NaCl solutions.

	Red rice		Tox 3145-38-2-3	
Vaiables	**Germination (%)**	**Weight (g)**	**Germination (%)**	**Weight (g)**
Sec	$52^{d} \pm 3.2$	$2.84^{d} \pm 0.03$	$91^{d} \pm 1.15$	$2.84^{d} \pm 0.02$
TM 0%	$53.5^{d} \pm 3$	$3^{de} \pm 0.05$	$92^{d} \pm 1.63$	$2.87^{d} \pm 0.02$
Ts0%C	$59^{e} \pm 4.1$	$3.03^{de} \pm 0.08$	$96^{de} \pm 1.63$	$2.92^{de} \pm 0.04$
Ts0%S	$10^{a} \pm 2.3$	$1.70^{a} \pm 0.09$	$18^{a} \pm 2.82$	$1.32^{a} \pm 0.01$
Ts10%C	$67^{f} \pm 4.7$	$3.05^{de} \pm 0.05$	$98.5^{de} \pm 3$	$2.92^{de} \pm 0.02$
Ts10%S	$31^{b} \pm 2.5$	$2.33^{b} \pm 0.04$	$52^{b} \pm 4.32$	$2.18^{b} \pm 0.02$
Ts20%C	$71.5^{f} \pm 3$	$3.07^{de} \pm 0.1$	$98^{de} \pm 2.3$	$2.96^{de} \pm 0.01$
Ts20%S	$36^{c} \pm 3.6$	$2.71^{c} \pm 0.04$	$71.5^{c} \pm 2.51$	$2.3^{b} \pm 0.04$
Ts30%C	$72^{f} \pm 3.2$	$3.16^{e} \pm 0.03$	$99.59^{e} \pm 1$	$2.97^{e} \pm 0.06$
Ts30%S	$52.5^{d} \pm 1.9$	$2.93^{d} \pm 0.05$	$81.5^{c} \pm 3.41$	$2.54^{c} \pm 0.04$

[a,b,c,d,e,f] Mean values in the same column followed by different letters are significantly different (P <0.05). Data are means four experiments. Ts: Seed treated with NaCl solution; 0%, 10%, 20%, 30%: NaCl Concentrations; C: Pellet (immersed seeds); S: Supernatant (Floating seeds); TM0%: Total Seed immerse in distilled water; Sec: Non-treated seed.

Table 2.

Regardless the rice variety, the weights and the percent germination of the floating (S) seed fractions were significantly low compared to that of their corresponding immersed (C) seeds. Immersed red rice showed a significant ($P < 0.05$) improvement of 7% in germination (T0% C-Sec) when the seeds were sorted with water, and of 19.5% (T20% C- Sec) when sorted in 20% NaCl solution. There was no significant difference between the germination of Tox 3145-38-2-3 treated with 20% NaCl and the counterpart immersed in water (T0%C).

Irrespective of the rice variety and the type of salt treatment applied, the Spearman rho correlation coefficient between weight and germination was calculated. According to Hopkins (1997), positive moderate correlation was found (Rho (78) = 0.427**, $p < 0.01$), indicating a significant linear relationship between the two variables: seed lots of high weight, tend to have a higher percentage of germination.

The correlation between weight and germination was greater and significant when treatments were examined separately; immersed seeds (Rho (78) = 0.606**, $p < 0.01$) and floated seeds (Rho (78) = 0.543**, $P < 0.01$) or by variety; Red rice (Rho (78) = 0.895**, $p < 0.01$) and Tox 3145-38-2-3 (Rho (78) = 0.903**, $p < 0.01$.

Effect of seed treatment and stress on germination

Three way factor ANOVA was conducted to assess whether the extract, the stress, the treatment had an effect on rice seed germination; and if the germination was affected by the interactions between these independent variables; the results as shown in Table 3.

The effect for extract is not significant, $F(1, 84) = 1.87$, $p > 0.05$; the estimated marginal mean germination percentage of seed treated with EEc (57%) is not significantly different from the estimated marginal mean germination percentage of seed treated with EEo (56%).

The main effect of stress (Stress or stress-free) was significant, $F(1, 84) = 142.65$, $p < 0.05$; the average marginal germination percentage of seeds subjected to stress by accelerated aging test (52.143%) was lower and significantly different from that of unstressed seeds (60.857%). A significant difference was found among treatments, $F(6, 84) = 374.95$, $p < 0.05$; regarding the estimated marginal means germination percentage of different treatments, the decreasing order of the effectiveness is as follow $T^{+}1\%$ (75.5%) > $T^{+}2\%$ (70.6%)> $T^{+}4\%$ (68.12%)> $T^{+}0\%$ (60%)> Banko Plus (56%) > Sec (43.7%) > T^{+}Sur (21%). The S-N-K multiple comparison test revealed that the treatments were significantly different from each other, except for $T^{+}2\%$ and $T^{+}4\%$ which were identical. The variance between groups was mainly explained (86.9%) by the effect of treatment.

The extract *stress interaction was significant $F(1, 84) = 5.52$, $p < 0.05$. This shows that the effect of stress thought accelerated aging test on the germination of rice seeds is dependent on the plant extract used. The germination percentage decreases when moving from stress-free to stress state; this decline was significant and decreased when seeds were soaked in EEo (61.2 -50.7%), than when they are treated with EEc (60.5 to 53.5%) (Figure 2). Meanwhile, the effect size associated with this interaction (Cohen's f = 0.045) did not satisfy the minimum standard to be called a “small” effect size (Cohen, 1988).

The effect of extract *treatment interaction was not significant $F(6, 84) = 1.78$, $p > 0.05$; the effect of

Table 3. Analysis of variance for seed germination percentage, as a function of the extract, the stress and the treatment.

Test Between-Subjects Effects/Percentage of germination						
Source/ Dependents variables	**Type III Sum of squares**	**d*f***	**Means square**	**F**	**Sig**	**η^2**
Adjusted Model	394860.000[a]	28	14102.143	946.150	0.000	
Extract	28.000	1	28.000	1.879	0.174	0.0007
Stress	2126.286	1	2126.286	142.658	0.000	0.0551
Treatment	33531.500	6	5588.583	374.953	0.000	0.8691
Extract * Stress	82.286	1	82.286	5.521	0.021	0.0021
Extract * Treatment	159.500	6	26.583	1.784	0.112	0.0041
Stress * Treatment	1380.214	6	230.036	15.434	0.000	0.0357
Extract * Stress * Treatment	20.214	6	3.369	0.226	0.967	0.0005
Error	1252.000	84	14.905	-	-	-
Corrected Total	38580.00	112	-	-	-	-
a R-Squared = 0.997 (adjusted R-Squared = 0.996)						

η^2: Eta square; d*f*: degree of freedom.

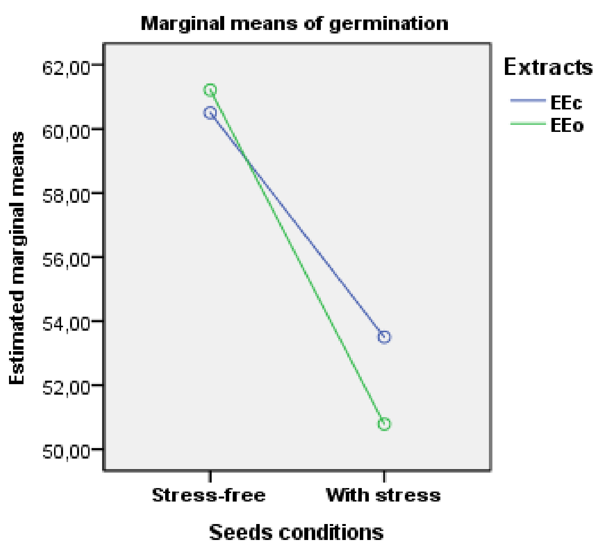


Figure 2. Variation of germination percentage in the presence and absence of stress, according to plant extract. EEo: Ethanolic extract *O. barrelieri*; EEc: Ethanolic extract *C. citratus.*

treatment was not significantly influenced depending on whether the seeds were treated with EEc or EEo. For example, at $T^+2\%$, the germination percentage of EEc and EEo were 71.75 and 69.5%, respectively. The plots of the percentage of germination as a function of the treatments according to EEc and EEo were alike (Figure 3B). For both plant extracts $T^+1\%$ treatment showed the highest percentage of germination, EEc (78%) and EEo (73%).

The effect of the treatment *stress interaction was significant, F (6, 84) = 15.43, p < 0.05; the effect of the treatment on germination was greatly affected depending on whether the seed samples have been stressed or not. In fact, germination decreases in all seed samples when subjected to vigor test via accelerated aging: $T^+0\%$ (63.7 to 56.25%), $T^+1\%$ (78.75 to 72.5%), $T^+2\%$ (72.25 to 69%), $T^+4\%$ (71 to 65.25%), Banko Plus (60 to 52%), Sec (56.5 to 31% and et T^+Sur (23.75 to 19.25%) there was a significant decrease of the germination (25.5%) for non-treated seeds (Sec) (Figure 3A)

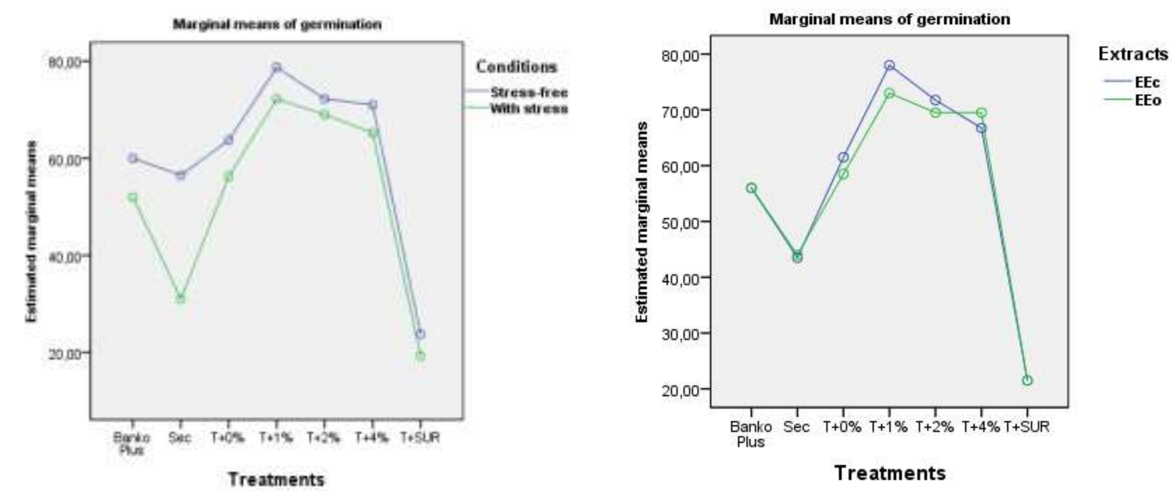
The effect of extract *stress *treatment interaction was not significant, F (6, 84) = 0.226, p > 0.05. The effect of the treatment on germination is not significantly affected by the source of extract (EEc or EEo) and whether seed were stressed or not.

Effects of plant extracts on seed infestation by *B. oryzae* and *F. moniliforme*

Two-way factor ANOVA was realized to assess the effects of the extracts, the treatment and the interaction of both factors on the rice seed incidence of *B. oryzae* and *F. moniliforme*; the results presented are in Table 4.

For *B. oryzae* incidence (24.71 to 25.5%) or *F. moniliforme* (14.57 to 16.42%), the effects for extract and extract *treatment interaction were not significant (p > 0.05). The incidence of both fungi did not significantly differ after seed treatments with EEc or EEo. The plots of the variations in the incidence of *B. oryzae* and *F. moniliforme*, as a function of treatments with respect of EEc and EEo, respectively are alike (Figure 4A and B).

The effect of treatment was significant, F (6, 42) = 22.60, p < 0.05 η^2 = 0.750 vis-à-vis *B. oryzae* incidence and, F (6, 42) = 11.314, p < 0 05 η^2 = 0.607 for *F. moniliforme* seed incidence. The S-N-K test used to detect the nature of differences shows that Banko-Plus reduced significantly the seed incidence of *B. oryzae* from 46.5[a]% of non-treated control to 15.5[c]%; this reduction was statistically same with the treatments $T^+1\%$



(A) (B)

Figure 3. Change in germination percentage of rice seed versus treatments. (A) In the presence and absence of stress; (B) In the presence of plant extracts (EEo and EEc). T^+: immersed seeds (Pellet) treated in 20% NaCl; 0, 1, 2 and 4%: Plant extracts concentrations T^+Sur: floating seeds (Supernatant) treated in 20% NaCl; Banko Plus: Fongicide; Sec: Non treated seed; EEo: Ethanolic extract of *O. barrelieri*; EEc: Ethanolic extract of *C. citratus.*

Table 4. Analysis of variance for the rice seed incidence of *B. oryzae* and *F. moniliforme,* as a function of extract and treatment.

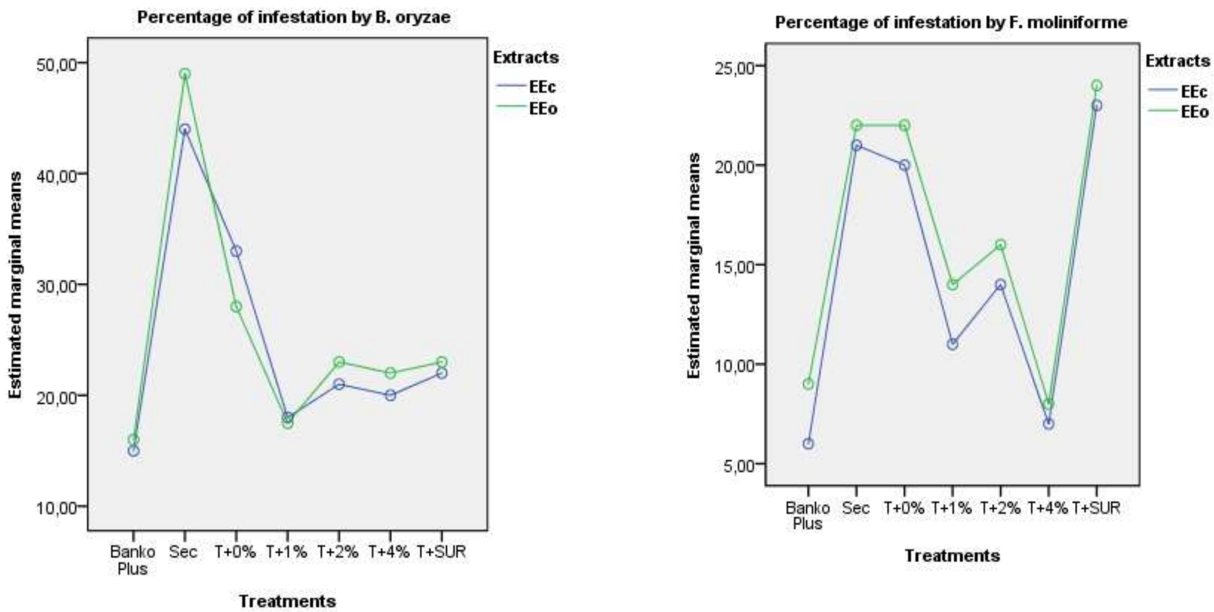
Source	Type III SS	Df	MS	F	Sig	η^2
B. oryzae						
Extract	8.64	1	8.643	0.22	0.642	0.0012
Treatment	5331.8	6	8881.6	22.60	0.000	0.750
Extract*Treatment	111.8	6	18.64	0.474	0.0824	0.015
Error	1651	42	3931	-	-	-
F. moniliforme						
Extract	48.28	1	48.28	1.53	0.223	0.137
Treatment	2140	6	356.66	11.31	0.000	0.607
Extract*Treatment	9.71	6	1.619	0.051	0.999	0.0027
Error	1324	42	31.524	-	-	-

SS: sum square; MS: Mean Square; η^2: Eta square; d*f*: degree of freedom.

(17.75^c %), $T^+4\%$ (21^c %), $T^+2\%$ (22^c %) and T^+Sur (22.5^c %). The immersion ($T^+0\%$) reduced the seed incidence of *B. oryzae* to 30.5^b% as compared to Sec with 46.5^a %. *F. moniliforme* incidence was highest with T^+Sur (23.5^a %) which was statistically identical to Sec (21.5^{ab} %) and $T^+0\%$ (21^{ab} %); the incidence of *F. moniliforme* on seeds treated with Banko Plus (7.5^c %), $T^+4\%$ (7.5^c %) and $T^+2\%$ (15^{bc} %) were lower and similar statistically.

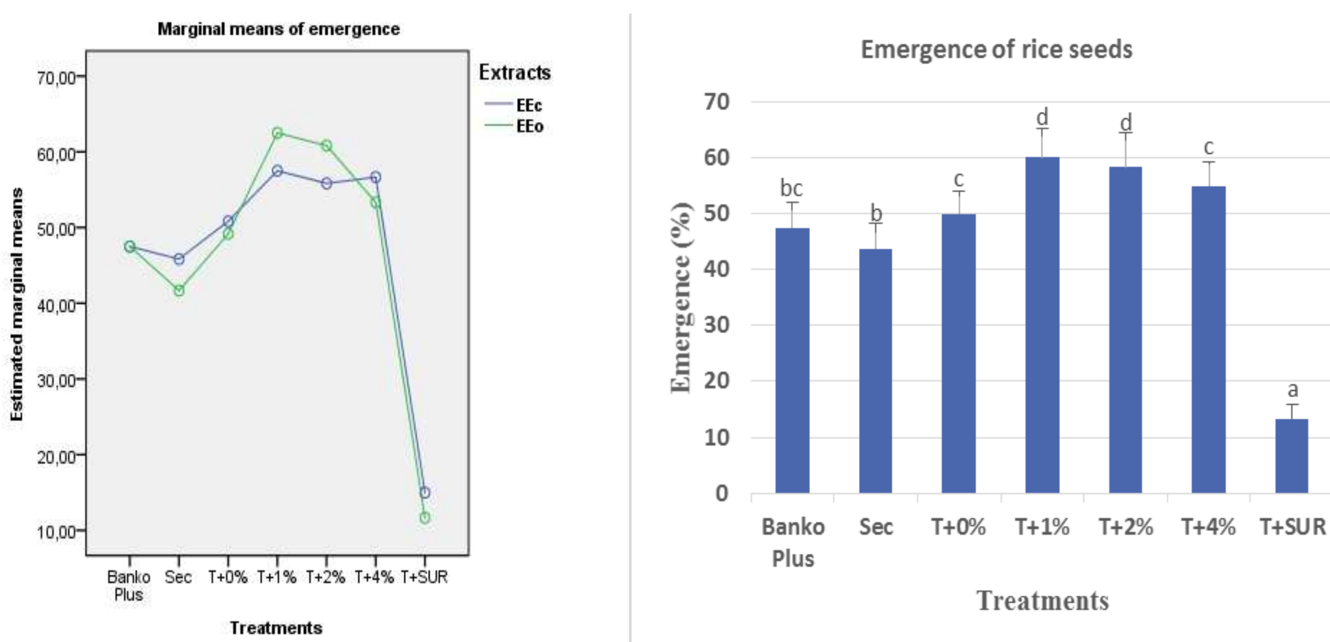
Effect plant extracts on seed emergence

A 7 (Treatments) x 2 (Extracts) between-subjects factorial ANOVA was calculated to compare the emergence for subjects, who had one of the seven treatments and subjects who were treated either with EEc or EEo. It was found that the main effects for extract F (1, 42) = 2.335 p =0.134, η^2 = 0.015) and extract *treatment interaction F (6,42) = 2.262, p = 0.56, η^2 = 0.090) were not statistically significant ($p > 0.05$); the marginal mean of emergence of seed treatments with EEc (47.02%) and that of seed treatments with EEo (46.66%) are about the same (Figure 5A). The treatment main effect was significant F (6, 42) = 15.48, $p < 0.05$ η^2 = 0.612, the major differences among the treatments and their standard deviations are represented in the Figure 5B. The highest emergence was recorded with treatment $T^+1\%$ (59.99%) and the lowest with T^+Sur (13.33%).



(A) (B)

Figure 4. Variation in the incidence of *B. oryzae* (A) and *F. moniliforme* (B), after rice seed treatments with plant extracts (EEo and EEc). T^{+}: immersed seeds (Pellet) treated in 20% NaCl; 0%, 1%, 2%, 4%: Plant extracts concentrations T^{+}Sur: floating seeds in 20% NaCl; Banko Plus: Fongicide; Sec: Non treated seed; EEo: Ethanolic extract of *O. barrelieri*; EEc: Ethanolic extract of *C. citratus.*



(A) (B)

Figure 5. Emergence of rice versus treatments: (A) when comparing the plants extracts used (EEo and EEc); (B) when comparing treatments; [a,b,c,d] mean values followed by different letters are significantly different ($p < 0.05$). T^{+}: immersed seeds (Pellet) treated in 20% NaCl; 0, 1, 2 and 4%: Plant extracts concentrations T^{+}Sur: floating seeds (Supernatant) treated in 20% NaCl; Banko Plus: Fongicide; Sec: Non treated seed; EEo: Ethanolic extract of *O. barrelieri*; EEc: Ethanolic extract of *C. citratus*

Table 5. Inter-correlations between percentage of germination, seed incidence of *B. oryzae,* and *F. moniliforme,* and the emergence (N=56).

Variables	1	2	3	4	Means	SD
1. Germination *in vitro*	- -	- 0.127	- 0.441**	+ 0.931**	60.85	17.29
2. Infestation of *B. oryzae*	- -	- -	+ 0.448**	- 0.106	25.10	11.36
3. Infestation by *F. moniliforme*	- -	- -	- -	-0.426**	15.50	8.00
4. Emergence	- -	- -	- -	- -	46.841	15.47
***p* < 0.01						

1. Germination *in vitro*; 2. Infestation of *B. oryzae;* 3. Infestation by *F. moniliforme;* 4. Emergence; SD: standard deviation.

Pearson correlations were computed to examine the associations among the four variables; percentage of germination, seed incidence of *B. oryzae,* and *F. moniliforme,* and the emergence. Data in Table 5 showed that four of the six pairs of variables are significantly correlated. The strongest positive correlation, which would be considered to be nearly perfect was found between the germination and the emergence *r* (54) = 0.931**, p < 0.01 (Hopkins, 1997),

The correlation between the seed incidence of *F. moniliforme* and *B. oryzae,* was also positive and statistically significant *r* (54) = 0.448**, p < 0.01. The germination and the emergence were each negatively correlated with the infestation of both pathogens but the correlation with the seed incidence of *B. oryzae* was not significant (p > 0.01), and the correlation with seed incidence of *F. moniliforme* was of medium size effects according to Hopkins (1997)

DISCUSSION

The extracts of *O. barrelieri* and *C. citratus*, were tested against two rice seed-borne fungi (*B. oryzae and F. moniliforme*), and their effects as seed treatments evaluated on the seed fungal incidence, the vigor, the percent germination and emergence of rice seeds sorted in NaCl solutions.

In comparison with the findings of Nguefack et al. (2013), in which same methods were used for the evaluation of the antifungal potential, the activities of EEc and AEc against *B. oryzae,* were near but somewhat less important; this slight difference could be explained by the fact that our extraction period last 12 h against 24 h in theirs; the extraction time may have an influence on the content and/or the chemical composition of the secondary metabolites responsible for the biological (antifungal) effects. Very little work has been done on the phytoprotectory potential of *O barrelieri*; Its activities vis-à-vis *B. oryzae* and *F. moniliforme* are reported here for the first time; Nevertheless, Dakole et al. (2016) showed in their work that AEo and EEo completely inhibited the growth of *Fusarium oxysporum f.sp lycopersici* at 50000 and 25000 µg / ml and that of *Phytophtora infestans* at 25000 and 12500 µg/ml, respectively; they suggested the presence of phenols, flavonoids and saponins in these extracts being responsible of this inhibitory effect.

The strong antifungal activity of ethanol extracts thought to be mostly due to its richness in phenolic compounds (Quy et al., 2014) compared to aqueous extracts which contain, among other, considerable quantities of primary metabolites, including polypeptides and carbohydrates (Padmalochana and Rajan, 2014). These primary metabolites may constitute a source of nutrients for fungal growth. The observed stimulatory activity found at low concentration of AE_O and AE_C on *B. oryzae* could however, be exploited in the growing of endophytic fungi for better production of secondary metabolites; in fact *B. oryzae* is capable to produce ophiobolin A and ophiobolin B (Xiao et *al*, 1991) that could be exploited as herbicide.

In order to avoid the negative effect of the saline solution on the germination as found by Khan et al. (2014); seeds were immediately and abundantly rinsed in distilled water after immersion for 1 min in different salt solution. Some authors have found parallel results regarding to the use of aqueous and saline solutions for the sorting of rice seeds. Notably, Pham et al. (2003); found that rice seed cleaning with water reduce unfilled grains by 5.83 to 8.73% in dry season, and discolored grains by 8.32 to 8.65% in rainy season. In addition, they showed that discolored and spotted rice seeds could be cleaned by treatment in 15% sodium chloride leading to an improvement in the phytosanitary status and germination (4.08 to 14.08%) of seeds; spotted and discolored seeds thus sorted being very often associated with high incidence of pathogens among others, *B. Oryzae* and *F. moniliforme* which are liable to deteriorate the seed (Ibrahim and Abo El-Dahab, 2014). The simple cleaning of rice seeds with water, as traditionally often used to subtract failing rice grains, seems limited; the association of NaCl at the right concentration is necessary; this processing means being simple, easy to implement and above all fairly Eco-friendly.

Contrary to the findings of Pham et al. (2003), who worked on the sorting of rice seeds by arbitrarily, choosing the 15% brine concentrations, the method used in this work describes a simple and correct scientific approach with end result; the selection of the best NaCl concentration to eliminate the impurities and sort the good qualities grains with maximum germination rate, no matter the state or origin of the grains.

The positive and significant correlation found between the mass of grain and the germination percentage of red and Tox rice varieties corroborate the results obtained by Roy et al. (1996), who showed that the rate of germination and the vigor increase with an increase in the size (weight) of rice seeds; suggesting therefore sorting of heavy grains for a good field establishment. Teng et al. (1992) support this argument by stating that, the best selection criterion for the strength of rice seed is its mass.

The high germination of seeds treated with plant extracts (68.12 to 75.5%) compared to floating seeds (21%) and untreated seeds (43.75%), could be partially associated with high incidence in *F. moniliforme* and *B. oryzae* on these later seeds. In fact, a study carried out by Imolehin (1983), on seed borne fungi of rice and on their incidence on germination revealed an existence of a strong significant and negative correlation (r = - 0.74) between infestation by these fungi and germination. This seems more true, because we have also found a negative correlation between germination (*in vitro* and in green house trials) and infestation by each of the two pathogens; however, this correlation was average (r = - 0.441) with respect to *F. moniliforme* and was not significant (-0.127) with regard to *B. oryzae*.

The drastic drop of the order of 25.5% for the set of dry seeds (non-treated), moderate (7.5%) for seeds soaked in water (T0%) and lesser (5.33%) for set of seeds treated with plant extracts after subjecting the seeds to vigor test via accelerated aging; is in accordance with conclusions drawn by Veselova and Veselovsky (2003) that, accelerated aging is manifested by a reduction in the germination percentage and, seeds that succeed in germinating tend to give raise to brittle seedlings. These results also reveal evidence that, simple soaking of rice seeds in water has contributed in consolidating its vigor under stress conditions, as observed by Ken-Ichi and Jun-Ichi (2013).

The plant extracts used in this research could therefore be little more involved in the stimulation of germination than increase in vigor; this being realized through the stimulation of α-amylase activity, the liberation of free soluble sugars and increase in the seeds respiration frequency (Wang et al., 2016) as a result of, the action of their secondary metabolites.

Conclusion

Sorting of rice seeds in 20% NaCl, coupled with their treatment in ethanol extracts of *O. barrelieri*/*C. citratus* render seeds more homogenous, maintain their vigor, improve on their health status and germination. This could contribute in; avoiding reseeding, improving the uniformity of growth and maturity stages and finally obtain better yields and good quality seeds for the following campaign.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Gene expression and antioxidant enzymatic activity in passion fruit exposed to aluminum

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The objective of this study was to investigate changes in enzymatic activity and gene expression related to antioxidant defense in two passion fruit cultivars (FB100 and FB200) exposed to aluminum. The specific activity of superoxide dismutase (SOD), catalase and ascorbate peroxidase were analyzed, in addition to their relative expression pattern. The characterization of gene expression was achieved by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique followed by densitometric analysis. Plants were irrigated every two days with a nutrient solution: pH 6; pH 4; 0.2 and 2.0 mM Al (pH 4). The leaves were collected at the times 0 h, 2 days, 5 days and 10 days. The analysis of the potential oxidative stress induced by aluminum demonstrated increased expression of catalase and superoxide dismutase gene, and only on the cultivar FB100 this increase was accompanied by an increase of SOD enzymatic activity, which indicates its higher efficiency on the removal of reactive oxygen species (ROS) compared to cultivar FB200.

Key words: oxidative stress, *Passiflora edulis*, aluminum, semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR), differential expression.

INTRODUCTION

Aluminum (Al) present in soil solution is one of the main agents responsible for reduced productivity in plants (Panda and Matsumoto, 2007). Recent studies have shown that this effect is explained by the inhibition of root growth as well as the reduction of water absorption. Also, how morphological, biochemical and physiological changes occur in different plants was investigated (Sevik and Cetin, 2015; Kravkaz-Kuscu et al., 2018). Meanwhile, Al is the most abundant metal in the earth's crust, and at micromolar concentrations, it causes morphological, biochemical and physiological changes in plants. Toxicity caused by aluminum is exacerbated for plants grown in acidic soils, especially at pH below 5. In acidic solutions, Al is present as Al^{3+}, characteristically toxic to plants (Giannakoula et al., 2008). Over 70% of the world's soil is contaminated with acid, alkali and heavy metals. However, acid soil is the factor that most limits the production of staple foods in the world. Approximately

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50% of arable land is negatively impacted by Al toxicity due to acid soil (Panda et al., 2009). The Al toxicity has been further aggravated by the use of fertilizers and acid rains (Singh et al., 2017).

Aluminum induces the formation of reactive oxygen species (ROS). Evidence that supports this hypothesis is guided on correlations established between exposure to Al and increased ROS, higher activity of some enzymes and/or concentration of metabolites (Darkó et al., 2004). ROS accumulation causes oxidative damage and alters cell functions. Plants have developed defense systems against ROS, including the synthesis of enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Ahmad et al., 2010). The increased activity of antioxidant enzymes is also reported as being a mechanism for tolerance to Al in some varieties of corn and wheat (Boscolo et al., 2003; Ma et al., 2007). Changes in the activity of antioxidant enzymes are an important metabolic process that can influence on tolerance to aluminum by higher plants (Ezaki et al., 2013). Such changes depend on the plant species, the intensity and duration of stress.

Studies indicate increased gene expression encoding antioxidant defense system proteins upon exposure to Al. In *Arabidopsis thaliana* it was observed that aluminum induces gene expression related to oxidative stress, which constitutes an important component of plant responses to toxic levels of aluminum (Corrales et al., 2008) by causing the activation of antioxidant enzymes. The semi-quantitative PCR technique can be used as a tool to study gene expression. This technique is based on comparing the levels of amplification in PCR's with different numbers of cycles, becoming a rapid and reliable way of quantifying specific mRNA present in small quantity in the sample (Rey et al., 2000). The implications of Al toxicity for oxidative stress have been widely investigated in roots of different plant species (Boscolo et al., 2003). However, little information is available about the damage induced by Al in foliar tissues. In addition, few studies have linked the pattern of gene expression and antioxidant enzyme activity related to tolerance to aluminum. The objective of the study was to investigate possible changes in enzyme activity and gene expression, in transcriptional and translational level, respectively, on passion fruit leaves exposed to Al.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was conducted in a greenhouse located in the Goiabeiras campus at Federal University of Espírito Santo/UFES (20°18'52" S and 40°19'06" W). In the experiment were used seeds of *Passiflora edulis* SIMS *cv.* FB 100 (“Maguary”) and FB200 (“Yellow Master”). Plants were watered every two days with 200 ml of a modified nutrient solution (Hoagland and Arnon, 1950) half strength, at 28°C (± 2) and natural photoperiod. Stress induction began 45 days after transplanting and lasted 10 days. The treatments were: control (pH 6); pH (pH 4); 0.2 mM Al^{3+} (pH 4) and 2 mM of Al^{3+} (pH 4). Al was added as $AlCl_3$. The leaves were collected at the times 0 h, 2, 5 and 10 days, after starting treatment with Al, instantly frozen in liquid nitrogen and stored at -80°C.

Enzymatic activity

0.2 g of tissue leaf with 2% (w/v) polyvinylpolypyrrolidone (PVPP) were macerated. Then, was added 1500 µl of extraction solution containing: 750 µl of 100 mM potassium phosphate buffer (pH 6.8), 15 µl of 10 mM EDTA, 150 µl of 100 mM ascorbic acid and 585 µl of ultrapure water. Cell residue was removed by centrifugation for 15 min at 13,000 g at 4°C. This extract was used in the analysis of enzymatic activity and quantification of total soluble protein. For determining total soluble protein contents, the method proposed by Bradford (1976) was used. The crude catalase activity was calculated based on the molar extinction coefficient of H_2O_2 (0.036 µmol cm^{-1}) (Anderson et al., 1995). To determine the crude ascorbate peroxidase activity, the molar extinction coefficient of ascorbate of 0.0028 $µmol^{-1}$ cm was used (Nakano and Asada, 1981). The crude superoxide dismutase activity an enzyme reaction medium was determined according to Beauchamp and Fridovich (1971). The specific activity of CAT, APX and SOD was determined by dividing the crude enzymatic activity by the content of total soluble proteins. The analyses were performed with 5 replicates.

RNA extraction

RNA extraction of collected leaves was performed at time zero, 5 and 10 days. Approximately 100 mg of powdered plant tissue with liquid nitrogen was stored at -80°C. A repetition scheme was organized in 3 pools, each containing 4 different samples. For total RNA extraction, TRIzol Reagent (Invitrogen®) was used, according to manufacturer's recommendations. The resulting RNA was quantified in a NanoDrop 3300 Termo Scientific spectrophotometer. RNA samples were treated with DNAse I AmpGrade (Invitrogen®).

Semi-quantitative RT-PCR

Extracted RNA was used for RT-PCR (Reverse Transcription Polymerase Chain Reaction) having as control the constitutive 18S ribosomal gene. The first complementary DNA strand (cDNA) was synthesized with the kit GoScript™ (Promega®), according to the manufacturer's recommendations. The cDNA samples were quantified using NanoDrop 3300 (Termo Scientific). For the initial amplification of DNA from cDNA fragments obtained, primers were used for each gene that would be analyzed. The primers used for gene expression of *P. edulis* were as follows: 18s 5’-TGACGGAAGAATTAGGGTTCG-3’ 5’-GACTTGCCCTCCAATGGATC-3’ (Azevedo et al., 2003), Cat 5´-CTGCTGGAAACTATCCTGAGTG-3´ 5´-ATTGACCTCTTCATCCCTGTG-3´ (Balestrasse et al., 2008) and Sod 5´-CTACGTCGCCAACTACAACAAG-3’ 5´-GTAGTACGCATGCTCCCAGAC-3’ (Baek and Skinner, 2005).

From the cDNA fragments obtained, specific primers were designed for each gene using Primer 3 and Gene Runner software. Primers designed: Cat F 5´-GTCAACCCGCAAACCACAA-3´ R 5´-ACACCCATAGGCACCGTCT-3´ Tm 54°C and Sod F 5´-TCCAACCCGATAACTGTG -3’ R 5´-TCTGGTGACGAAGGGAGC -3’ Tm 53°C. Each of the primers was subjected to reaction with 32, 25, 20, and 15 cycles of denaturation, annealing and extension. The amplified products were subjected to 2% agarose gel run, stained with GelRedTM (BIOTIUM^{TM}), visualized by UV transilluminator and DNA bands analyzed using ImageJ software. The DNA fragments were sequenced to confirm their identity, by alignment with sequences deposited in GeneBank (NCBI) using the

BLAST program (Basic Local Alignment Search Tool - NCBI). Sequencing was performed using automated DNA sequencer Applied Biosystems model 310.

Statistical analysis

The results related to parametric data were submitted to analysis of variance (ANOVA) and the means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

Aluminum at toxic levels can induce damage caused by oxidative stress, as a result of increased production and accumulation of ROS (Pereira et al., 2010). They control vital processes in plant organisms, however as toxic molecules can cause injuries on the cells, such as the oxidation of lipid membranes, proteins and nucleic acids, as well as interference in physiological processes such as photosynthesis and respiration (Scandalios, 2005). Among the enzymes of the antioxidant system, catalase convert H_2O_2 into molecular oxygen and water, but have low affinity to the substrate. The catalase enzyme did not have its activity changed by the treatments (Figure 1A and B). Boscolo et al. (2003) also reported that there were no changes in CAT activity in corn exposed to aluminum toxicity. This same CAT response was observed in *Oryza sativa* L. under light stress (Aumonde et al., 2013).

The radical considered more reactive to catalase is O_2^-, which can easily move across membranes, thus representing the main inhibitor of this enzyme (Scandalios, 2005). SOD catalyzes the degradation reaction from O_2^- to H_2O_2, in contrast, high levels of this product inhibit its catalytic action. There was an increase in SOD activity in low aluminum concentration only in the cv. FB100, and when the plants were exposed to 2 mM Al there was a return of SOD activity to basal levels equating to control (Figure 1E and F). Pereira et al. (2010) observed increase in SOD activity in *Cucumis sativus* at low aluminum concentration. However, at high concentrations (2 mM), these authors observed decrease in SOD activity, a result similar to that found in this study. The decrease in SOD activity in *P. edulis* may indicate excess peroxide in the leaf tissue, since there was no increase in CAT and APX activity. Peroxide is able to inactivate enzymes by oxidation of the thiol groups. In addition, peroxide is a very stable ROS and its increased synthesis has been shown as a response to various types of stresses (Apel and Hirt, 2004).

APX, as well as CAT, also convert peroxide into O_2 and water, but do so using ascorbate as an electron donor, representing an alternative way of destruction of H_2O_2 (Pereira et al., 2011). In this study, both cultivars maintained APX activity similar to control (Figure 1C and D). González-Santana et al. (2012) also observed that there was no change in APX activity in leaves and roots of *Conostegia xalapensis* exposed to aluminum. Similarly, Panda et al. (2010) showed that APX activity showed no difference in *Pisum sativum* root exposed to aluminum.

Studies show that Al can induce gene expression against its toxic effect; these genes are mostly responsible for the production of enzymes related to antioxidant defense system of plants (Corrales et al., 2008). The DNA sequence obtained from cDNA amplification with primer 18S showed similarity of 99% to 18S ribosomal RNA sequences for 48 species of plants. The cDNA sequence obtained from the primer *Cat* (329pb) showed a high percentage of homology with genes from another species: 80% with *Cat1* gene and *Cat3* of *Brassica juncea*. The cDNA sequence obtained from the specific *Sod* primer (172pb) had homology with the *MnSOD* gene of the species: 88% with *Eichhornia crassipes* and 85% with *Prunus persica*. This homology comparison proves that the primers designed for this study were actually *Cat* and *Sod* gene primers, confirming that the quantified material corresponds to the sequences of the enzymes investigated.

The CAT enzyme is directly involved in controlling damage caused by H_2O_2. The study of gene expression verified that PCR of 25 cycles, the level of transcription of *Cat* cv. FB100 and cv. FB200 was found to be elevated with 2 mM Al, at 5 and 10 days (Figure 2). On the PCR of 15 cycles, *Cat* amplification was reduced, preventing the densitometric analysis. *Cat* transcription levels indicate that the FB100 cultivar is more efficient in the removal of ROS, since at the higher concentration of Al there was increase in production of *Cat* transcripts, at 5 days. In contrast, in the FB200 cultivar that increase in *Cat* transcripts occurred after 10 days, which demonstrates that the cultivar FB100 presented a faster EROS removal response. Induction of gene transcription after exposure to stress factors has also been reported by Balestrasse et al. (2008) where elevated levels of *Cat* transcripts occurred in soybean nodules under cadmium stress. For *Ulva fasciata*, Sung et al. (2009) reported an increase in the amplification of *Cat* genes after imposed to salt stress.

The results obtained with relative expression of *Sod* cv. FB100, with 25 cycles, show that amplification was greater in the plants treated with aluminum (Al 0.2 mM and 2 mM) in the 5-day samples, as for 10-day samples only the treatment with 2 mM Al exhibited higher levels of transcripts. For cv. FB200, the level of expression was increased in the treated plants with lower aluminum concentration (0.2 mM) only at 10 days. On the PCRs of 20 and 15 cycles, densitometric analysis was not possible by reduced amplification (Figure 3). *Sod* gene expression was increased by treatment with aluminum using concentration of 2 mM Al at 5 and 10 days in the cv.FB100, and this differential expression was accompanied by an increase in the enzymatic activity of SOD in the same cultivar. Panda et al. (2010) reported an

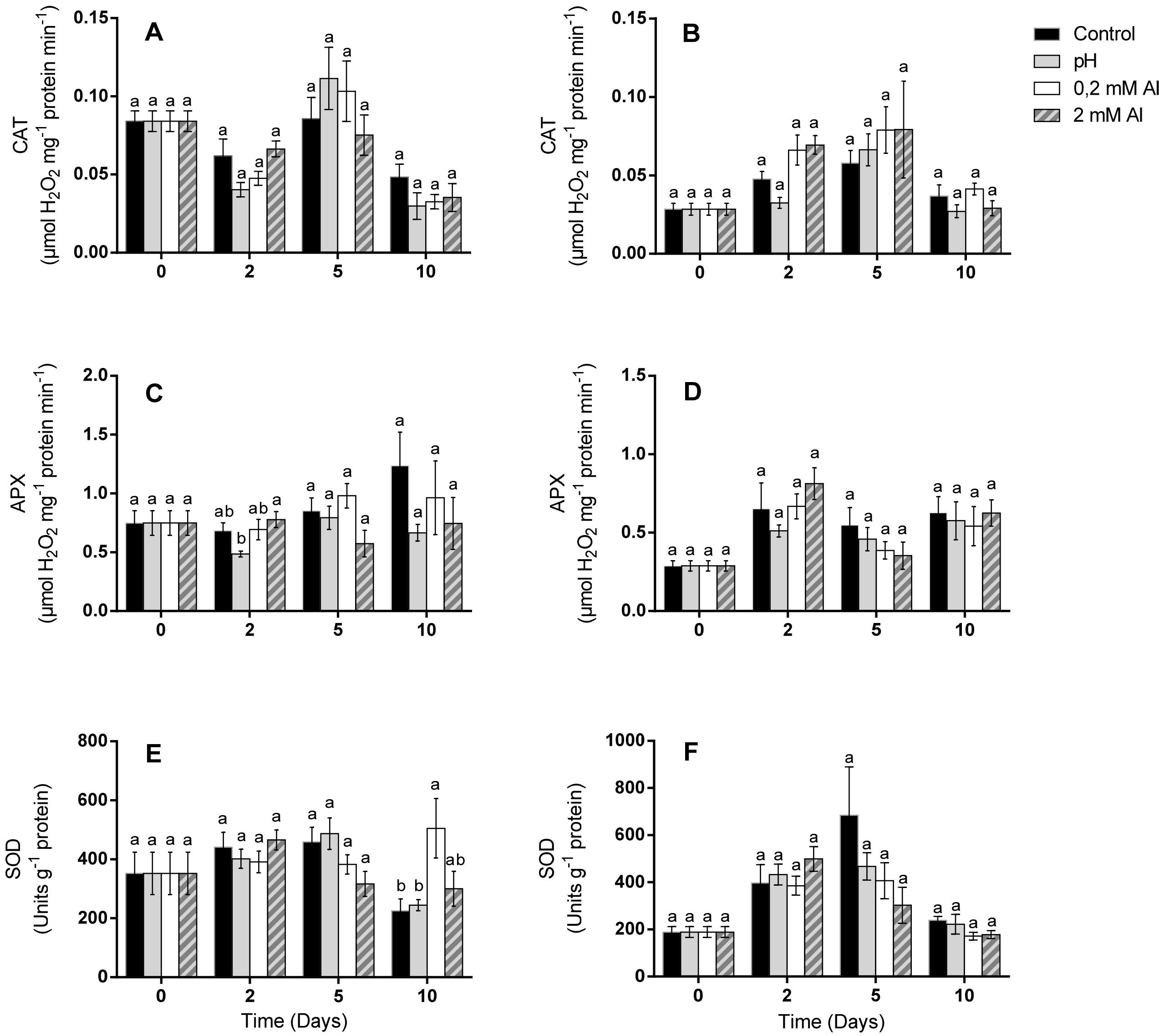

Figure 1. CAT enzymatic activity cv FB100(A) and cv FB200 (B); APX cv FB100(C) and cv FB200 (D); SOD cv FB100(E) and cv FB200 (F) in *P. edulis* leaves. The results represent the mean ± SE. Different lowercase letters indicate statistical significance by Tukey test ($p < 0.05$).

increase in expression level of different types of *Sod* in *Pisum sativum*. Bhoomika et al. (2013) identified that both *FeSod* and *MnSod* participate in stress tolerance by aluminum toxicity in rice cultivar tolerant to this type of stress. *Sod* are the first line of defense against ROS. As the O_2^- radical is produced in any intracellular location where there is transport of electrons, the activation of O_2 can occur in different cellular compartments. Tolerant cultivars tend to increase their antioxidant enzyme activity as well as the induction of genes that encode such enzymes (Panda et al., 2010; Pereira et al., 2010). Thus, elevated levels of *Sod* and *Cat* transcripts accompanied by the increase in the enzymatic activity of SOD, only on cv. FB100 may indicate that it presents more efficient antioxidant defense system, which gives it greater tolerance to aluminum stress when compared to cv. FB200.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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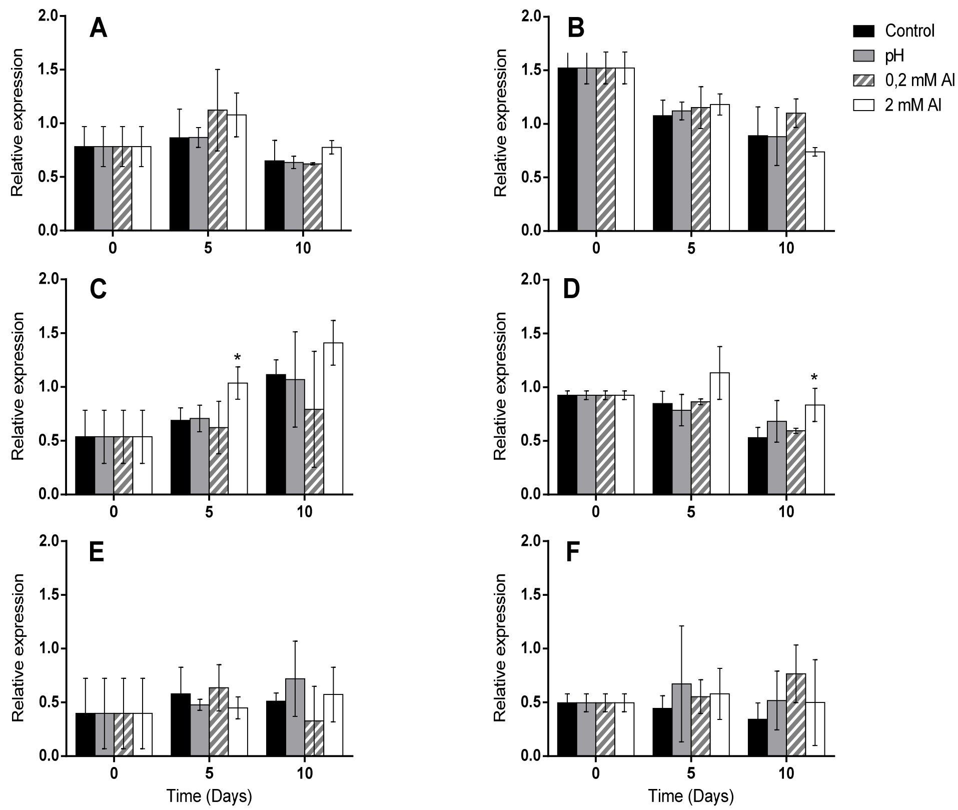

Figure 2. *Cat* relative expression of *P.edulis* PCR with 32 cycles cv FB100 (A) and cv FB200 (B); 25 cycles cv FB100 (C) and cv FB200 (D) and 20 cycles cv FB100 (E) and cv FB200 (F). The results represent the mean ± SD. Asterisk means the value is significantly different from the control.

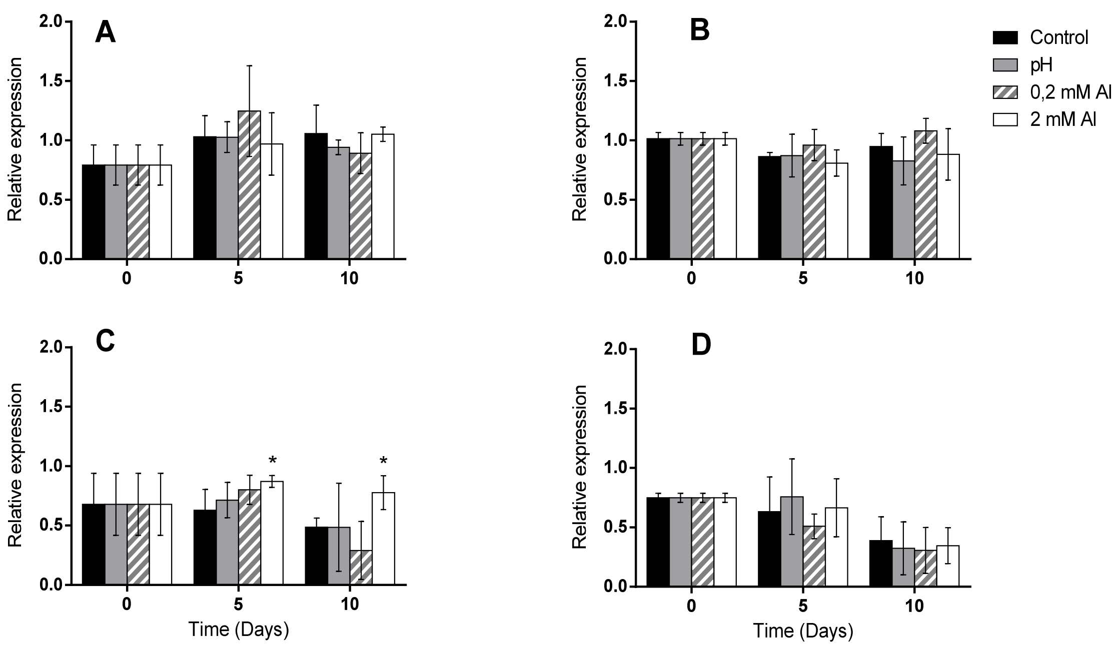

Figure 3. *Sod* relative expression of *P.edulis* PCR: 32 cycles cv FB100 (A) and cv FB200 (B); 25 cycles cv FB100 (C) and cv FB200 (D). The results represent the mean ± SD. Asterisk means the value is significantly different from the control.

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Full Length Research Paper

Assessment of entomotoxic effects of powder and oil from leaves and seeds of *Hura crepitans* (L.) in the control of *Callosobruchus maculatus* (F.)

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Entomotoxic effects of powder and oil from the leaves and seed of *Hura crepitans* (L.) on *Callosobruchus maculatus* (F.) was investigated at the Pest Management Laboratory in the Department of Crop, Soil and Pest Management, the Federal University of Technology Akure, Ondo State, Nigeria under prevailing laboratory conditions of 28±3°C temperature and 65 ± 5% relative humidity. The powders from the leaves and seeds of *H. crepitans* were applied singly at the rates of 1, 2 and 3 g per 20 seeds weighed into sterile Petri dishes and infested with 5 pairs of one day old *C. maculatus* adults in three replicates. The oils were applied at the rates of 1, 2.5 and 5 ml per 1 kg of seeds respectively in 3 L plastic containers by injecting the oils into 1 kg of seeds with a syringe and 20 seeds selected randomly from each container into Petri dishes in three replications including the controls. The oil-treated seeds were infested with 5 pairs of a day old adults of *C. maculatus*. Data were collected on number of dead *C. maculatus* adults, number of eggs laid, number of seeds with eggs, seeds without eggs, number of emerged aduls, number of seeds with holes, seeds without holes and weight loss after 12, 24 and 48 h of application of powders and oils. The results from the study showed that *H. crepitans* powders and oils caused mortality in adult *C. maculatus*. The mortality (80%) of adults *C. maculatus* at 48 h after application was significantly higher (P<0.05) in cowpea seeds treated with 2 g of seed powder. Application of *H. crepitans* significantly reduced (P<0.05) oviposition (4.58) at 2 g of the seed powder applied on cowpea seeds. The number of emerged adults (0.71) was significantly lower (P<0.05) on cowpea seeds treated with seed powder and seed oil at higher rates. Weight loss (0.08) was significantly lower in cowpea seeds treated with 2.5 and 5 ml of seed oil. Therefore, based on these results, *H. crepitans* could be explored as an alternative biopesticide to synthetic insecticide in the protection of stored cowpea against *C. maculatus* (F.).

Key words: *Hura crepitans, Callosobruchus maculatus,* entomotoxic, assessment.

INTRODUCTION

Food insecurity is a fundamental problem confronting developing countries of the world. Although, there are concerted efforts to boost food production in many countries, over 800 million people still suffer from malnutrition (FAO, 2005). Apart from inadequate food production, the problem of hunger and malnutrition is further aggravated by inability of developing countries to store harvested crops for a long period without losing a sizeable proportion of them to pests mainly micro-organisms and insects (Adedire and Lajide, 2001).

Cowpea (*Vigna unguiculata*) is a major staple food crop and essential source of protein in sub-Saharan Africa, especially in the dry savanna regions of West Africa where animal protein is rarely available. The seeds are a major source of plant proteins and vitamins for man, feed for animals, and also a source of cash income and the young leaves and immature pods are eaten as vegetables (Dugje, 2009). It is estimated that cowpea supplies about 40% of the daily protein requirements to most of the people in Nigeria (Odeyemi and Daramola, 2000). It has been suggested as a viable substitute for the costly fish, eggs and other animal proteins in the diet of children (Ileke et al., 2013). In Nigeria, cowpea is commonly consumed amongst others; in the form of bean pudding, bean cake, baked beans, fried beans and bean soup and in addition to dietary fiber, cowpeas contain many health-promoting components such as vitamins, minerals and phytochemicals, which include phenolic compounds (Cai, 2003). The consumption of leguminous plant such as cowpeas has been linked to reduced risk of free radical mediated diseases such as diabetes, obesity and coronary heart diseases (Bazzano, 2001).

In storage, the bruchid, *Callosobruchus maculatus* causes the major losses and they are field-to-store agricultural insect pests of Africa and Asia that presently range throughout the tropical and subtropical world (Beck and Blumer, 2011). *C. maculatus* causes substantial quantitative and qualitative loses by perforation, thus reducing the degree of usefulness and making the seeds unfit either for planting or for human consumption (Ali, 2004; Ofuya, 2003). In Nigeria, over 90% of the damage to cowpea seed storage attributed to bruchids is caused by C. *maculatus* alone (Caswell, 1981) while it has been estimated that 3% of the annual production in Nigeria in 1961/1962 was lost due to attack by *C. maculatus* (Tanzubil, 1991). Survey of cowpea in markets and village stores in West Africa indicate that 20 to 90% of seeds may be holed by storage beetles (Alebeek, 1996; Ogunkoya and Ofuya, 2001). There is therefore no doubt that serious depredation of cowpea seeds by *C. maculatus* can be a threat to food security as well as poverty alleviation in West Africa (Ofuya, 2003). Sub-Saharan Africa is therefore known to provide a favorable environment for this insect pest (Lephale, 2012). Protection of cowpea in the storage against *C. maculatus* had been through the use of synthetic chemicals. However, attempts to preserve the seeds through the use of these synthetic chemical insecticides sometimes result in poisoning of the cowpea plant and environment and with negative effects on non-target organisms including humans (FAO, 1992; Shetty, 1995). Most especially, the non-educated farmers in the sub-sharan Africa are poisoned when these chemicals are misused (Ofuya, 2003).

Currently, research efforts are being focused on the use of plant-derived biopesticides such as plant powders and oils, which are relatively cheaper and ecologically more tolerable than conventional chemical insecticides (Tamo, 2012). Most plant materials have repellant, anti-feedant and insecticidal properties which also interfere with normal activities of the pests preventing their multiplication and the plant materials are cheap, easily available and easy to use (Onu and Aliyu, 1995). Edible oils, which are produced in commercial quantities, are often used for this purpose. However, competitions for commercial edible plant oils which are obtainable from just about a dozen species of plants (Ihekoronye and Ngoddy, 1985) have necessitated the search for oils from underutilized tropical plants such as *Hura crepitans* L. (Sandbox) (Euphorbiaceae). *H. crepitans* is a widely occurring self-regenerating ornamental plant in the tropics (Keay et al., 1989). Results of determination of chemical composition of seeds of *H. crepitans* showed that the seed has high crude fat content and is very rich in magnesium (Muhammed et al., 2013).

Oleic acid is the most abundant fatty acid and contains more unsaturated fatty acids. Oil extracted from the dry seeds is used as a purgative. The juice of *H. crepitans* contains a volatile colorless liquid called “Hurin’’ which some researchers claimed could be used for the treatment of elephantiasis and leprosy. Fagbemi and Adebowale (2000) had reported that sandbox seed contains 36.03% protein and 49.7% fat and that most of the fatty acids are essential fatty acids, which may possess some insecticidal properties. This study, therefore, examines the effects of *H. crepitans* leaves and seeds powder and oil in protecting stored cowpea seeds from infestation and damage by *C. maculatus.*

MATERIALS AND METHODS

Study area

The experiments were conducted in the pest management laboratory of the Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure (FUTA) located in the southwestern part of Nigeria. Experiments were conducted under ambient laboratory conditions of 28 ± 3°C temperature and 65 ± 5% relative humidity.

Collection of materials

Adult insects used were obtained from infested cowpea seeds

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collected from Oja-oba in Akure, Nigeria, while the cowpea cultivar, Oloyin used for the experiment was obtained from a food store at the south gate of FUTA and kept in the freezer at a low temperature of 10°C in order to disinfest the seeds of any infesting insects.

Culturing of *C. maculatus*

After three days of freezing, the seeds were air dried and then infested with *C. maculatus* obtained from the infested cowpea seeds. The culturing was done in a container with perforated lid by weighing 200 g of clean uninfested cowpea seeds replicated three times and infesting each container with 50 pairs of adult *C. maculatus* in order to allow for oviposition to occur and to obtain fresh *C. maculatus.* The setup was left on the shelf for the emergence of fresh *C. maculatus* which were used for the experiments. The sexes of *C. maculatus* were determined by examining elytra pattern, female *C. maculatus* are usually maculated, dark colored and possessed four elytra spots, and in contrast males are plain, pale brown in color and less distinctly spotted.

Preparation of powders and extraction of oils from leaves and seeds of *H. crepitans* L.

The leaves and dried pods of *H. crepitans* were obtained from the front of the School of Agriculture and Agricultural Technology of the Federal University of Technology, Akure, Nigeria. The leaves were air dried for 2 weeks before being processed into fine powder with Philips blender, while the seeds were carefully removed from the pods and de-shelled. The creamy white cotyledons of sandbox seeds were sun-dried and pulverized in the blender into a fine powder.

The seeds and leaves oils were extracted in a Soxhlet apparatus using n-hexane as solvent. The resulting *H. crepitans* seeds and leaves oils were air-dried to allow for the escape of the solvent and then kept in a plastic container with tightly fitted lid and kept in a deep freezer. It was used within one month of preparation.

Setting up of the experiments

The powders from the leaves and seeds of *H. crepitans* were applied at the rate of 1, 2 and 3 g per 20 seeds. The seeds were weighed into Petridishes and infested with 5 pairs of a day old adult of *C. maculatus* in three replications. The oils from the leaves and seeds of *H. crepitans* were applied at the rate of 1, 2.5 and 5 ml per 1 kg of seeds respectively in 3 L plastic containers by injecting the oils into 1 kg of seeds with a syringe, mixed evenly and 20 seeds selected randomly from each container into Petri dishes and infested with 5 pairs of a day old *C. maculatus* in three replications. Control experiment was also setup (Oni, 2009).

Data collection

The number of dead adults of *C. maculatus* was taken after 12, 24 and 48 h of application of powders and oils. Data on the number of eggs laid was taken after 7 days of oviposition, number of seeds with eggs and seeds without eggs were also taken. The number of emerged adults, number of seeds with holes and seeds without holes was also recorded.

In calculating weight loss, the contents of each petri dish were sieved to remove dust, frass and any insect present within the seeds. The seeds were re-weighed and the weight loss was determined as the difference between the initial and final weights of seeds in each replicate.

Table 1. Mean percentage mortality of adults exposed to different treatments and rates after 12, 24 and 48 h of application.

Treatments	Rates	12 h	24 h	48 h
Control	0	6.67^{b}	10.00^{b}	26.67^{b}
	1 g	6.67^{b}	10.00^{b}	26.67^{b}
LP	2 g	6.67^{b}	13.33^{b}	30.67^{a}
	3 g	23.33^{a}	23.33^{a}	36.00^{a}
	1 g	13.33^{b}	13.33^{b}	36.67^{b}
SP	2 g	30.00^{a}	36.67^{ab}	53.33^{ab}
	3 g	33.33^{a}	53.33^{a}	80.00^{a}
	1 ml	0.00^{b}	6.67^{b}	16.67^{b}
LO	2.5 ml	10.00^{a}	23.33^{a}	30.00^{a}
	5 ml	10.00^{a}	20.00^{a}	43.33^{a}
	1 ml	0.00^{b}	3.33^{b}	20.00^{a}
SO	2.5 ml	3.33^{b}	20.00^{a}	30.00^{a}
	5 ml	16.67^{a}	20.00^{a}	30.00^{a}

Means followed by the same letter along the column were not significantly different at 5% using Tukey's Test. SO: Seeds oil; LO: leaves oil; SP: seeds powder; LP: leaves powder.

Data analysis

The experiment was laid out in a completely randomized design (CRD) with three replications. Analysis of variance (ANOVA) of the data was done using SPSS Statistical Package Version 15 (SPSS, 2015). Prior to analysis, all data in count were appropriately transformed employing square root transformation, while those in percentages transformed using Arcsine transformation. Where differences existed, means were separated using Tukey's test at 5% level of significance.

RESULTS

Effects of the treatments application on measured parameter

The results in Table 1 show the entomocidal effects of powders and oils at different rates of application on the adults of *C. maculatus*. The percentage mortality caused by the application of leaves powder was highest at the rate of 3 g after 12, 14 and 48 h of treatment application and showed significant differences at P<0.05 from the other rates. Cowpea seeds treated with 3 g of seed powder, however, caused the highest mortality of adults (80%) at 12, 24 and 48 h after application followed by 2 g of seed powder and was significantly different at P<0.05 from seeds treated at 1 g of seed powder. Adults mortality was highest with seeds treated with 2.5 and 5 ml of leaves oil and seeds oil which also showed significant difference at P<0.05 from that obtained when seeds were treated with 1 ml of leaves oil and seeds oil. The mortality was highest for the adult *C. maculatus* 48 h

Table 2. Mean number of eggs laid, seeds with eggs and seeds without eggs on cowpea seeds treated with powder and oil at different rates of application.

Treatments	Rate	Eggs laid	Seeds with eggs	Seeds without eggs
Control	0	12.33[a]	4.49[a]	0.88[a]
	1 g	12.37[a]	4.53[a]	0.71[a]
LP	2 g	10.46[a]	4.49[a]	0.88[a]
	3 g	11.69[a]	4.53[a]	0.71[a]
	1 g	10.18[a]	4.53[a]	0.71[a]
SP	2 g	4.58[b]	3.75[a]	2.56[a]
	3 g	5.66[b]	3.72[a]	2.32[a]
	1 ml	12.29[a]	4.49[a]	0.88[a]
LO	2.5 ml	14.28[a]	4.53[a]	0.71[a]
	5 ml	11.24[a]	4.29[a]	1.32[a]
	1 ml	8.97[a]	4.20[a]	1.44[a]
SO	2.5 ml	11.50[a]	4.53[a]	0.71[a]
	5 ml	11.43[a]	4.53[a]	0.71[a]

Means followed by the same letter along the column were not significantly different at 5% using Tukey's Test. SO: Seeds oil; LO: leaves oil; SP: seeds powder; LP: leaves powder.

Table 3. Mean number of emerged adult, seeds with holes, seeds without holes and weight loss on cowpea seeds treated with powder and oil at different rates of application.

Treatments	Rates	Emerged adults	Seed with holes	Seed without holes	Weight loss
Control	0	5.96[a]	4.01[a]	2.15[a]	0.85[a]
	1 g	6.78[a]	3.44[a]	2.59[a]	1.04[a]
LP	2 g	5.96[a]	3.94[a]	2.34[a]	0.66[a]
	3 g	6.54[a]	3.79[a]	2.49[a]	0.91[a]
	1 g	0.71[c]	0.71[b]	4.53[a]	0.22[a]
SP	2 g	0.71[c]	0.71[b]	4.53[a]	0.24[a]
	3 g	0.71[c]	0.71[b]	4.53[a]	0.11[a]
	1 ml	4.95[b]	4.00[a]	2.00[ab]	0.98[a]
LO	2.5 ml	7.21[a]	4.53[a]	0.71[b]	1.39[a]
	5 ml	2.58[b]	3.15[a]	3.11[a]	0.38[a]
	1 ml	0.71[c]	0.71[b]	4.53[a]	0.10[b]
SO	2.5	0.71[c]	0.71[b]	4.53[a]	0.08[b]
	5 ml	0.71[c]	0.71[b]	4.53[a]	0.11[b]

Means followed by the same letter along the column were not significantly different at 5% using Tukey's Test. SO: Seeds oil; LO: leaves oil; SP: seeds powder; LP: leaves powder.

after treatments application.

Table 2 shows the effects of powder and oil at different rates of application on measured parameters. The mean number of eggs laid (4.58) was least in the seeds treated at 2 g of seed powder followed by 3 g application of the same treatment. They are both significantly different ($P<0.05$) from the other treatments at various application rates. Although, highest number of eggs was laid (14.28) on seeds treated with 2.5 ml leaf oil, it was not different significantly ($P>0.05$) from that treated with 2.5 ml seeds oil. The values for the seeds with eggs and without eggs showed no significant difference for all the treatments and at various rates of application.

Table 3 shows the effects of powder and oil at different rates of application on adult emergence, seeds with holes, seeds without holes and weight loss parameters. Numbers of emerged adults (0.71) was significantly lower on cowpea seeds treated with seed powder and seed oil

Table 4. Mean number of eggs laid, seeds with eggs, seeds without eggs, emerged adults, seeds with holes, seeds without holes and weight loss on cowpea seeds treated with powder and oil.

Treatments	NEL	SWE	SWTE	EA	SWH	SWTH	WL
LP	11.71^{a}	4.51^{a}	0.79^{b}	6.31^{a}	3.80^{a}	2.39^{b}	0.86^{a}
SP	8.18^{b}	4.12^{b}	1.62^{a}	2.02^{c}	1.53^{b}	3.93^{a}	0.36^{b}
LO	12.54^{a}	4.45a^{b}	0.95^{b}	5.17^{b}	3.92^{a}	1.99^{b}	0.90^{a}
SO	11.05a^{b}	4.44a^{b}	0.93^{b}	2.02^{c}	1.53^{b}	3.93^{a}	0.29^{b}

Means followed by the same letter along the column were not significantly different at 5% using Tukey's Test. NEL: Number of eggs laid; SWE: seeds with eggs; SWTE: seeds without eggs; EA: emerged adults; SWH: seeds with holes; SWTH: seeds without holes; WL: weight loss.

at all rates. The cowpea seeds treated with 2.5 ml of leaf oil showed the highest mean value of emerged adults (7.21) and were significantly higher than the mean value from the seeds treated with 5 ml of leaf oil. Significantly, fewer adults emerged from the seeds treated with the seed powder and seed oil and were statistically different from the other treatments. The numbers of seeds with holes was significantly lower (P<0.05) in cowpea seeds treated with seed powder and seed oil at all rates. Cowpea seeds treated with 2.5 ml of leaf oil had the highest mean value of seeds with holes which was higher than those of seed powder and oil. The number of seeds without holes was significantly different at P<0.05 in cowpea seeds treated with leaf oil while other treatments showed no significant difference at P>0.05.

The weight loss (0.08) was significantly lower in cowpea seeds treated with 2.5 ml of seed oil and was significantly different at P<0.05 from that of control. The cowpea seeds treated with 2.5 ml leaf oil showed the highest mean value of weight loss (1.39). There was no significant difference at P>0.05 in cowpea seeds treated with leaf powder, seed powder and leaf oil for the weight loss.

The results in Table 4 show that the number of eggs laid (8.18) was significantly lower on cowpea seeds treated with seed powder and was significantly different at P<0.05 from other treatments. The highest numbers of eggs (12.54) were laid on seeds treated with leaf oil. Though was not significantly different (P>0.05) from those on seeds treated with leaf powder and seed oil. The least number of seeds with eggs (4.12) was observed on seeds treated with seed powder and was not significantly different (P>0.05) from other treatments except those treated with leaf powder. Highest value was obtained in seeds without eggs (1.62) for seed powder treated seeds which showed significant difference (P<0.05) among other treatments. Significant differences (P<0.05) existed in the number of emerged adults for all the treatments, with significantly higher value observed in seeds treated with leaf powder followed by those treated with leaf oil. Similar pattern of results were also observed in seeds with holes and seeds without holes. Significantly higher (P<0.05) weight losses were recorded on seeds treated with leaf powder and leaf oil and were different statistically from values obtained for seed powder and seed oil treated seeds.

Table 5 shows the effects of powder and oil at different rates of application on the measured parameters. The number of eggs laid (7.52) was significantly lower on cowpea seeds treated with 2 g of powder and was significantly different (P<0.05) from seed treated with 2.5 ml of oil. Although, highest number of eggs were laid (12.89) on seeds treated with 2.5 ml of oil there were no significant differences (P>0.05) in other rates of application. There were no significant differences (P>0.05) in the values observed in seeds with eggs and seed without eggs in all the treatments applied. The number of emerged adults (1.65) was lowest in cowpea seeds treated with 5 ml of oil, while the highest mean value for the emerged adults (5.96) was obtained on the control and were significantly different from each other. However, there were significant differences (P<0.05) in the values obtained for seeds with holes and seeds without holes. Highest number of seeds with holes (4.01) was recorded in the control while the least (1.93) was on the seeds treated with 5 ml of oil and were significantly different at P<0.05. The converse was observed for the seeds with holes. Although, there were no significant differences (P>0.05) observed in the weight loss at all rates of treatments application, highest weight loss (0.85) was recorded on the control while the least (0.24) was obtained from the seeds treated with oil at 5 ml application rate.

DISCUSSION

The findings from this study showed clearly that powder and oil from seed and leaf of *H. crepitans* possessed entomotoxic properties. Fagbemi and Adebowale (2000) had reported that sandbox seed contains 36.03% protein and 49.7% fat and that most of the fatty acids are essential fatty acids, which may possess some insecticidal properties. The effects of the oil on oviposition in this study could be due to blocking of spiracles thus causing respiratory impairment, which probably affected metabolism and consequently other systems of the body of the bruchids (Osisiogu and

Table 5. Mean number of eggs laid, seeds with eggs and seeds without eggs, emerged adults, seeds with holes, seeds without holes and weight loss on cowpea seeds treated at different rates of powder and oil application.

Rates	NEL	SWE	SWTE	EA	SWH	SWTH	WL
0 g	12.32^{ab}	4.49^{a}	0.88^{a}	5.96^{a}	4.01^{a}	2.15^{c}	0.85^{a}
1 g	11.27^{ab}	4.53^{a}	0.71^{a}	3.75^{b}	2.07^{b}	3.56^{ab}	0.63^{a}
2 g	7.52^{b}	4.12^{a}	1.72^{a}	3.33^{bc}	2.32^{b}	3.43^{ab}	0.45^{a}
3 g	8.68^{ab}	4.12^{a}	1.52^{a}	3.62^{b}	2.25^{b}	3.51^{ab}	0.51^{a}
1 ml	10.63^{ab}	4.34^{a}	1.01^{a}	2.83^{bc}	2.35^{b}	3.26^{abc}	0.54^{a}
2.5 ml	12.89^{a}	4.53^{a}	0.71^{a}	3.96^{b}	2.61^{b}	2.62^{bc}	0.74^{a}
5 ml	11.34^{ab}	4.41^{a}	1.01^{a}	1.65^{c}	1.93^{b}	3.82^{a}	0.24^{a}

Means followed by the same letter along the column were not significantly different at 5% using Tukey's Test. NEL: Number of eggs laid; SWE: seeds with eggs; SWTE: seeds without eggs; EA: emerged adults; SWH: seeds with holes; SWTH: seeds without holes; WL: weight loss.

Agbakwuru, 1987; Onolemhemhem and Oigiangbe, 1991; Cockfield, 1992; Lale and Abdulrahaman, 1999; Ileke et al., 2013).

This present study also showed that *H. crepitans* significantly reduced oviposition as the rate of the seed powder applied on cowpea seeds increased. In a previous study, Idoko (2011) had reported a dose-dependent action of powder and oil of *Eugenia aromatica*. Apart from these findings, the few eggs laid were unable to stick to the cowpea seeds due to the obstruction effects of the seed powder which appeared like dust particles in the plates and on the seeds and the oil also rendered the cowpea seeds unacceptable to the bruchids as they showed oviposition preference for untreated cowpeas. Similar observation was reported by Adebayo and Ibikunle (2014). Oil-induced reduction or complete inhibition of oviposition of female bruchids and mortality of the developmental stages has been reported by a number of workers (Boughdad et al., 1987; Don Pedro, 1989; Lale and Abdulrahman, 1999). Numbers of emerged adults was significantly lower on cowpea seeds treated with seed powder and seed oil at all rates, because of adult mortality and reduced oviposition which consequently reduced adult's emergence at all rates applied. The reproductive potential of *C. maculatus,* which were exposed to *H. crepitans* seed powder and seed oil reduced significantly. Weight loss was significantly reduced in cowpea seeds treated with 2.5 and 5 ml of seed oil. This could be as a result of inability of the beetle to infest the seeds through egg laying or hatched larvae were unable to develop into adult in the seeds thereby prevented the holing of the protected seeds (Janzen, 1977).

Mortality at 48 h after application was significantly higher in cowpea seeds treated with 3 g of seed powder, the mortality effect of the oil on the beetles could be a result of the toxic components of the seed powder such as oleic acid and probably cyanolipids, which may be in the seeds, because *H. crepitans* is a member of the family Euphorbiaceae. The results obtained from this study also suggested that the entomotoxic effects of the parts studied increased with the rates of application and time of exposure. In a similar study, Adebayo (2015) reported an increase in the mortality of adult *C. maculatus* with increased rates of groundnut oil and as the time of exposure increased. The effectiveness of powders from the leaf and seed of *H. crepitans* could be due to hindrances to the movement of the adult insects which might cause reduced oviposition. This agreed with the observations of Adebayo and Ibikunle (2014) when worked on ash of rice husk and powdered clay as seed protectants against *C. maculatus*. The results on *H. crepitans* revealed that seed powder and seed oil have entomotoxic properties as an insecticide in stored products protection. This agreed with the analysis of *H. crepitans* seed oil carried out by Njoku et al. (1996), it was reported that *H. crepitans* seed contains about 50% fat and that it is very rich in fatty acids mainly as palmitic, stearic, oleic and linoleic acid. The observations from the study revealed the seed powder and oil to be more effective when compared with the leaves powder and oil. This could be as a result of the high concentrations of the active ingredients in the seeds. Idoko (2011) and Ileke et al. (2013) had reported similar results while working with oils of *Capsicum* spp. and *Alstonia boonei*

Conclusion

From the results of this study, it was discovered that the *H. crepitans* seed oil and seed powder were highly effective in causing adult mortality, reducing oviposition, adult emergence and reduced weight loss caused by *C. maculatus* while leaves oil and leaves powder were least effective. *Huran crepitans* possess toxic effects which could be used against stored product insects including storage bruchids. The extracts of *H. crepitans* could serve as alternatives to conventional synthetic insecticides for the control of stored pests due to their adverse effects such as ozone depletion, high mammalian toxicity, insect resistance and resurgence and other health hazards. The effectiveness of the parts used increased with the rates of application. The 3 g of powders and 5 ml of the oils were most effective in exerting entomocidal properties on the beetles.

RECOMMENDATIONS

Based on the results from the study, it is recommended that:

1. *H. crepitans* seed oil and seed powder should further be explored as an insecticide in the protection of cowpea against *C. maculatus*.
2. Research should be conducted to determine methods of increasing the effectiveness of the plant based insecticide through appropriate formulations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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