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Insecticidal activity of plant extracts and essential oils of bleed water against the bean weevil

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This study was aimed to evaluate the toxicity of semi-purified fractions (FS) (Hexane - FH, ethyl acetate - FAE, ethanol/water - FEA) and the essential oils of the bark and leaves of *Croton urucurana* against the bean weevil (*Callosobruchus maculatus*). The insects were subjected to concentration-mortality bioassays of the FS, to determine the LC_{50}. Subsequently, the effect of the FS was evaluated, through the method of vaporization and multiple choices. The fumigation action was evaluated using the essential oils, and the population growth was assessed using the FAE-leaf. All FS were toxic for *C. maculatus*, however, the lowest LC_{50}as well as a higher mortality in the vaporization method were obtained with the FAE-leaf. The essential oils from the bark caused an insect mortality greater than 80%.The least preferred rate of insects was obtained with the beans treated with FAE-bark while the beans treated with FAE-leaf reduced the population growth of the *C. maculatus*. Thus, we conclude that the semi-purified fractions and the essential oils of bark and leaves of *C. urucurana* interfere with the survival and biology of *C. maculatus*.

Key words: Euphorbiaceae, Plant extracts, repellency, bean weevil.

INTRODUCTION

The weevil *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) is an important pest for stored grains that can cause significant damage to cowpea when left untreated (Gbaye et al., 2011). *C. maculatus* larvae feed on the inside of the grains causing weight losses of up to 80% after six months of storage, where various holes are left by the insects, thereby facilitating the mycotoxin contamination of grain and reducing the commercial value of beans (Aboua et al., 2010; Kedia et al., 2015; Kirado and Srivastava 2010). The control of this pest in storage systems depends primarily on fumigant insecticides such as deltamethrin, malathion, methyl bromide and phosphine (Erler et al., 2009; Manzoomi et al., 2010; Nyamador et al., 2010).

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However, the use of conventional insecticides and fumigant compounds has caused serious side effects such as the selection of specimens resistant to these chemical molecules, toxic waste problems and toxicity for humans and the environment (Aboua et al., 2010; Mollaei et al., 2011).

Therefore, there is a need to develop safer alternatives that can reduce the use of conventional insecticides and fumigants for stored products (Ketoh et al., 2002; Jenkins et al., 2003). There are studies in which it was discovered that products derived from plants degrade quickly in the environment and the majorities majority are less toxic to mammals, while also being more selective to non-target organisms. There are also reports that state that these products can also delay resistance development of the insect plague (Rahman and Talukder, 2006).

The Euphorbiaceae family, has several biological activities, however, it is little studied for its insecticidal activity. Numerous species of the *Croton* genus have various activities, which are anti-inflammatory, anti-bacterial, against gastrointestinal disorders (Peres et al., 1997; Suárez et al., 2003; Anazetti et al., 2004; Fisher et al., 2004; Dalbo et al., 2006; Salatino et al., 2007). Among the medicinal plants, *C. urucurana*, stands out in the treatment of gastric ulcers, rheumatism, wound healing and diarrhea (Peres, 1998). However, there are few reports for the species *C. urucurana* Baillon and its activity asinsecticide, deterrent, repellent or inhibitor to the digestive enzymes of insects. Despite numerous studies reporting on the plant's insecticide potential against Lepidoptera, there are few studies that use *C. maculatus.* Considering the advances in research related to insecticides of botanical origin, the prospects for its use in the control of insect pests, and even the lack of studies using *C. maculatus*, this research was developed in order to evaluate the insecticidal activity of semi-purified fractions (hexane, ethyl acetate and ethanol / water) and essential oils from the bark and leaves of *C. urucurana* against *C. maculatus.*

MATERIALS AND METHODS

Croton urucurana was collected in the municipality of Dourados, Mato Grosso do Sul (MS), Brazil. A voucher specimen of the species was deposited in the Herbarium of the Federal University of Mato Grosso do Sul, in Campo Grande, MS, Brazil (CGMS) under N° 5009. For the preparation of semi-purified fractions (FS) fresh bark and leaves of *C. urucurana* was first fragmented into small pieces and subjected to extraction by maceration with ethanol (w/v, 1:2). After seven days, filtering was carried out and the solid material was disposed, and subsequently the solvent evaporated (± 40°C) under vacuum in a rotary evaporator, yielding the crude ethanolic extract (CEE) from the barks and leaves of *C. urucurana*. Subsequently, the CEE was fractionated by liquid-liquid partition with increasingly polar solvents, hexane and ethyl acetate to give fractions: hexane (FH-leaf), ethyl acetate (FAE-bark and FAE-leaf) and ethanol-water (FEA-leaf). The water content of the fractions was determined from an aliquot of the same fractions, submitted to drying (100°C), until the weight was constant.

To obtain the essential oils, the fresh barks and leaves of *C. urucurana* were subjected to extraction for four hours by hydrodistillation in a modified apparatus type Clevenger, followed by distillate extraction with hexane (Simionatto et al., 2007). After removal of the solvent, the crude yield was calculated. Insects, *C. maculatus*, were collected in the Property of producers in the region of Bom Jesus (PI), Brazil, and elsewhere in southern Piauí state.These populations were conducted reared in for the laboratory, after which they were multiplied and stored in plastic jars of 19 cm in height by 12 cm in diameter, sealed with fabric of the type *voile,* which allows air circulation and prevents the escape of insects. The substrate used for the maintenance of the populations was cowpea, which was used as a food resource and for the inventory of insects. The maintenance of the populations was performed weekly to avoid the presence of mites and parasitoids.

Bioassays I – Concentration-mortality of (FS) of *C. urucurana*

To conduct For the bioassay the semi-purified fractions were diluted in absolute ethanol at concentrations of 0, 390, 781, 1552, 3125, 6250, 12,000, 25,000 and 50,0000, 100,000, 200,000 and 500,000 ppm. Thereafter, 0.3 ml of each solution was added into a cylindrical and transparent glass vials with a 20 ml capacity of 20 ml. The vials were homogenized in order for the active ingredient to be uniformly distributed over the entire inner surface of the vial, until the complete evaporation of the solvent. The experiment was conducted in a completely randomized design with five repetitions for each treatment. Twenty non-sexed adult insects were exposed to dry residues of the fractions hexane, ethyl acetate and etanol-water of *C. urucurana*. Mortality was assessed after 72 h to calculate the LC_{50} values of the extracts.

Bioassay II - Vaporization test

For this bioassay pots with a capacity of 2.5 liter were used with 250 g of beans treated with LC_{50} of each FS of *C. urucurana*. The containers had a lid that was perforated in two places with3 cm holes for the entry and exit of the steam generated by the compressor. With the aid of an adapted compressor, the FS were applied within the pots where the insects were. After 72 h the containers were opened and the number of dead insects was recorded. The experimental design was completely randomized, with five replications and 100 insects in each container in which the evidence only included beans and insects.

Bioassay III - Multiple choice test

The bioassay was performed with an arena containing five plastic flasks with a capacity of 145 ml interconnected by hoses of 5 cm in diameter to a central flask of 250 ml. The central flask received 100 non-sexed adult insects while the vials at the extremities received 50 g of beans treated with 2 ml of each FS; the concentration used was LC_{50} and zero (0). The experiment was conducted with five replications and the preference of the insects was evaluated after 1, 24 and 48 h.

Bioassay IV – Fumigant effect (Essential oils)

In this bioassay, 100 g of beans were used and were added to pots with a capacity of 150 ml. At the bottom of the lid, a filter paper was placed that was treated with 2 ml of the essential oil obtained from the leaves and bark of *C. urucurana*. Then, 20 non-sexed adult insects were released in every pot, totaling five replications in a completely randomized design. The evidence was treated with

Table 1. LC_{50} values of Semi-purified fractions of *C. urucurana* on *C. maculattus.*

Fractions	Inclination± EPM	LC_{50} (95% CI) (ppm)	X^2	*P*
FH (Sheet)	0.8002 (0,034)	357.70 (30033 - 43075)[c]	113.4232	0.3923
FAE (Shell)	0.8564 (0,034)	175.98 (15057 - 20634)[a]	98.6397	0.7931
FAE (Sheet)	0.8462(0,033)	150.00(13355-24655)[a]	97.6591	0.7888
FEA (Sheet)	0.7253 (0,036)	446.86 (41372 - 490285)[b]	95.9570	0.8447

SEM= Standard Error of Means, LC= Lethal Concentration, 95% CI = 95% Confidence interval, X^2= Chi-square, *P*= probability. Means followed by the same letter in the column do not differ significantly amongst themselves by the Tukey's test ($p < 0.05$) Draw the line after FH (Sheet).

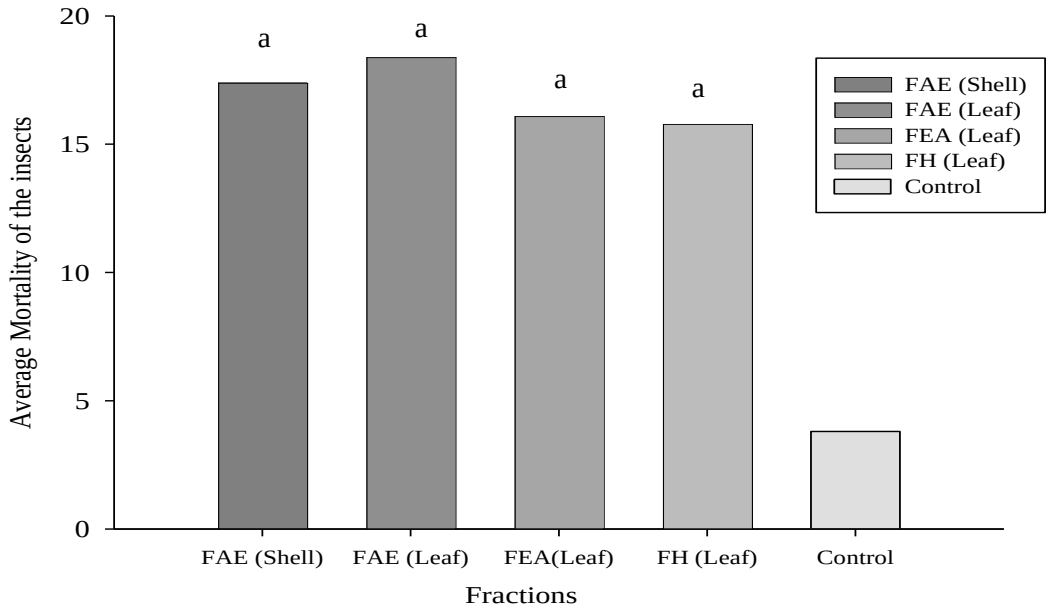

Figure 1. Average mortality of *C. maculatus* treated with semi-purified fractions of *C. urucurana* during the vaporization bioassay. Bars followed by the same letter do not differ between treatments by Tukey's test ($p < 0.05$).

distilled water. Mortality assessments were performed at 24, 48 and 72h after the start of the experiment.

Bioassay V - Instantaneous rate of population growth (FAE-Sheet)

The bioassay instantaneous rate of growth (r_g) was conducted using glass jars with a capacity of 1.5 L by adding 200 g of beans treated with LC_{50} of FAE-leaf and 50 non-sexed adult insects of the population of *C. maculattus*. The jars were sealed with a cap containing a small hole in the center which was closed with a *voile* type fabric to allow gas exchange. The experiment was conducted in a completely randomized design with four replications. After 120 days, after the start of the bioassay, the number of emerged insects, the insects living body mass and the mass of grains, were evaluated. The instantaneous rate of population growth (r_g) was calculated using the equation proposed by Walthall and Stark (1997). Where r_g = ln (Nf / No) /Δt; Nf being the final number of insects; No the initial number of insects and Δt is the variation of time. A positive value of $r_g$indicates population growth, $r_g = 0$ means that the population is stable and a negative value of r_g indicates a declining population until extinction.

Statistical analysis

The mortality results were submitted to the Probit analysis, through the PROC PROBIT procedure of the System of Statistical Analysis Program (SAS Institute, 2000), generating the concentration-mortality curves. The mortality data were corrected for the mortality that occurred in the control treatment. Details of the other bioassays were subjected to avariance analysis and Tukey's average grouping test, where appropriate (PROC GLM, SAS Institute, 2002).

RESULTS

The data from the experiments revealed that the toxicity of the treatments decreased in the following order: that is, Ethyl acetate (Leaf); Ethyl acetate (Bark); Hexane (Leaf); Ethanol Water (Leaf) in accordance with LC_{50} values (Table 1); since the ethyl acetate fractions (Leaf) and (Bark) are of greater toxicity against *C. macullatus*, with lower LC_{50} values. When it was analyzed, the mortality results in the bioassays of the vaporization (Figure 1)

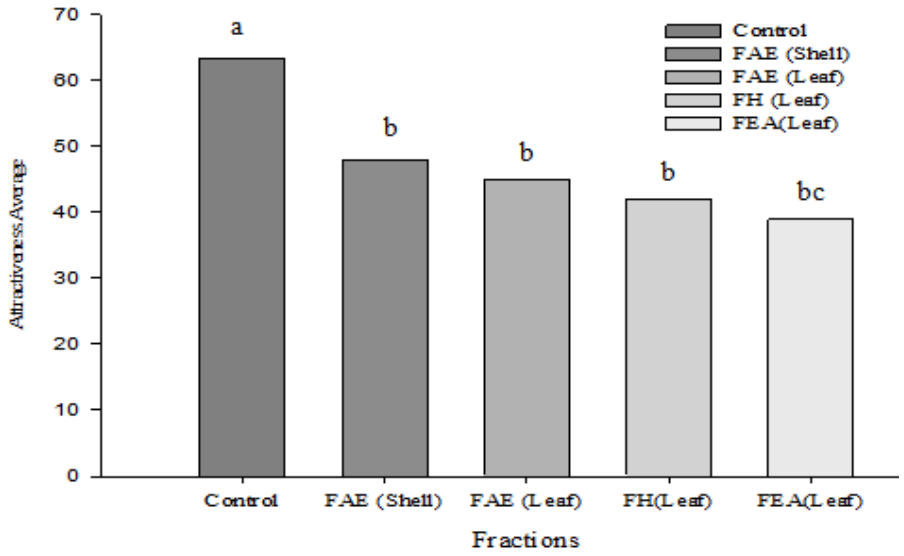

Figure 2. C. Attractiveness Average of *C. maculatus* to the grains treated with semi-purified fractions of *C. urucurana*. Means followed by the same letter do not differ significantly between treatments by Tukey's test ($p < 0.05$).

Table 2. Mortality effect of *C. maculatus* subjected to essences oils of bark and leaves from*C. urucurana*.

Treatments	Insect mortality
Control	00.8[c]
OE bark	17.1[a]
OE leaf	12.8[b]
CV%	29.26
F Average	713.6** 10.23

Means followed by the same letter in the column do not differ significantly between treatments by Tukey's test ($p < 0.05$).

revealed that all FS differ significantly from the control, however there were no significant differences between treatments. The mortality of *C. maculatus* occasioned by FS confirms the insecticide potential of *C. urucurana*.

In the multiple choice test (Figure 2), insects frequented all treatments, however, there was a difference in the repellency of FS when compared to the control. (Figure 2). The results indicate a probable existence of repellency of the tested FS, suggesting that the FEA - Leaf posses one or more allelochemical, which is able to ward off insects on contact with the beans treated with FS. The essential oils from the leaves and bark have shown promising results in the mortality of *C. maculattus*, especially for oils extracted from the bark, which shows that we must consider the plant organ in the study (Table 2). Based on the obtained results, it can be concluded that the essential oils obtained from the bark and leaf of *C. urucurana* showed as potential as a fumigant, mainly when obtained from the bark, where there was an insect mortality percentage higher than 80%. The population growth rate of insects subjected to grains treated with FAE-leaf was different when compared to the control, revealing a reduction of the multiplication of the *C. maculatus* species showing a lower growth of insects, for a period of ninety days (Table 3). The results also showed a lower intake of dry biomass of beans and a lower body mass of insects when treated with FAE-leaf. Based on the results obtained, it can be seen that the number of emerged insects was reduced when compared to the control (by 50%) when in contact with the dry residue of the FAE-leaf from the *C. urucurana* to 150,000 ppm.

DISCUSSION

The findings of this study indicated that the pesticide potential of *C. urucurana* showing that it has chemical metabolites is able to influence the tested parameters.

Table 3. Instantaneous rate of population growth, consumption of biomass of beans, body mass and number of adult insects emerged from *C. maculattus*, for 120 days, subjected to FAE-leaf of *C. urucurana*.

Treatment µg i.a.cm^2	Instantaneous rate of growth r_g	Dry biomass consumption of beans (g)[1]	Body mass (g)	Number of adults emerged for 120 days
Control	0.035766[a]	675.42[a]	3.5[a]	1308.6[a]
FAE(Leaf)	0.030454[b]	473.44[b]	1.3[b]	784.2[b]
CV%	9.0	13.86	39.14	31.18
Averages	0.03311	574.43	2.4	1046.4

Means followed by the same letter in the column do not differ significantly between treatments by Tukey's test ($p < 0.05$).

This fact may be related to the phenolic compounds with insecticidal effect present in the species of the genus *Croton*, such as tannins (Peres et al., 1997, 1998). These phenolic compounds can easily bind with the protein to form a protein-tannin complex that reduces the growth and survival of the insects, since they inactivate the digestive enzymes and hence, inhibit digestion (Mello and Silva-Filho, 2002). Furthermore, these compounds interact with proteins to render these class substances that are very toxic to insects, fungi and bacteria (Shirley 2001; Silva et al., 2009).

According to the results obtained in this study, the effect of FS from the bark and leaves of *C. urucurana* were similar to those observed for the mortality of *Anagasta kuehniella* (Silva et al., 2009) and *Dysdercus maurus* (Silva et al., 2012), confirming the need for the continuity of phytochemical studies as well as for the efficiency of molecules in the control of bean pests. When the vaporization test was re-viewed, all of FS from the *C. urucurana* showed a high percentage of mortality, a fact that highlights the toxicity of FS when applied directly on to where the insects are. This makes the investigations promising, while demonstrating the mechanism of the action of the involved metabolites in which the insect metabolic route can act. It is worth mentioning that although 100% of the FS showed satisfactory results of the *C. maculatus* mortality rates, certain criteria should be taken into account to evaluate the efficiency of the FS such as the vegetable organ and the concentration used, where in this study, in the vaporization test, the FAE-leaf proved the most efficient, considering that it caused higher mortality with lower concentrations.

The tested essential oils have a high toxicity against *C. maculatus* causing significant insect mortality when compared to the control, especially the essential oils obtained from the most active bark, with approximately 80% mortality rate. Randau et al. (2004),studying some of the *Croton* species, state that terpenoid can be associated with the insecticide activity and are found in all parts of the plant, with predominance of these substances in the leaves and roots. Therefore, the result of this study shows that the active chemical constituents may be present in FAE-leaf and essential oils of the bark. Essential oils are substances of botanical with insecticide activity (Lee et al, 2008; Chu et al., 2011). Due to its high volatility at ambient temperature, it has fumigant activity that may be important for the control of insect pests of stored products.

The results obtained for the multiple-choice test indicate that FS has a reasonable level of repellency when compared with the control, but highlights that the FEA-leaf which, in absolute terms, showed a lower number of insects that visited the grains impregnated with this fraction. The repellent effect is an important property to be considered in controlling pests in stored products, because the higher the repellency, the lower the infestation. With that, there is a reduction or suppression of the posture, and hence a smaller number of emerged insects. Therefore, natural products can successfully be used by farmers to protect stored grain from insect infestation. Many herbs have been used in developing countries to protect grains and legumes from stored product pests, such as *Lamiaceae*, which showed similar results to the present study (Ngamo et al., 2007; Li et al., 2013). The FAE-leaf reduced multiplication of insects, indicating that the present alleochemical interferes with the life cycle, in which each generation is affected negatively in their development during 90 days exposure to the residue of the FS. Similar results were obtained by (Carvalho et al., 2014), to evaluate the population growth rate of *Zabrotes subfasciatus* in beans treated with the crude extract of *C. Urucurana,* causing population reductions of 50% in 90 days. This confirms that the botanical species has allelochemicals that must be isolated and identified in order to find the bioactive molecule responsible for the negative effects on the insects as well as the site of action of these molecules. The use of plant extracts with repellent or insecticidal properties for stored grain is a traditional method common in rural areas all over the world (Regnault-Roger et al., 2012; Kedia et al., 2015). Tropical ecosystems (such as Caatinga-Cerrado) are particularly rich in plants that are used by local communities to treat diseases, thus, indicating the potential to discover new compounds (Albuquerque et al., 2008). And the species studied in this work has the potential to be used for the control of *C. maculatus.*

Conclusions

The semi-purified fractions of *C. Urucurana,* containing FAE-leaf, exhibit toxicity to *C. Maculatus,* when subjected to the lowest of LC_{50}. Through the Vaporization test, all FS exhibit significant mortality to *C. macullatus*. The essential oil obtained from the bark has increased efficiency to control *C. macullatus.* FAE-leaf reduces the multiplication of insects when evaluated for a period of ninety days. The results suggest that *C. urucurana*, has properties that cause lethal and sublethal effects on *C. maculatus*.

Conflicts of Interests

The author has not declared any conflict of interests.

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