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Bioactivity of plant extracts from caatinga on cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae)[1]

Bioatividade de extratos vegetais da caatinga sobre o caruncho do feijão-caupi *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae)

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HIGHLIGHTS:
- *Plants found in the caatinga biome have effects on* Callosobruchus maculatus.
- *Plant extracts have insecticidal or repellent effects on stored grain pests.*
- *The action of plant extracts on* Callosobruchus maculatus *differs among species found in the Caatinga biome.*

ABSTRACT: Cowpea (*Vigna unguiculata*) is an economically and nutritionally important crop. However, cowpeas are subject to attack by insect pests that reduce the quality and nutritional value of the grains during storage. The use of plant-based products as an alternative control of insect pests has been highlighted for their low toxicity on the environment and human health. This study aimed to evaluate the bioactivity of different plant extracts on the cowpea weevil (*Callosobruchus maculatus*). A completely randomized design was adopted with seven treatments and six replicates. The treatments consisted of extracts from six plants, namely *Morus nigra*, *Anadenanthera macrocarpa*, *Dysphania ambrosioides*, *Moringa oleifera*, *Ziziphus joazeiro*, and *Licania rigida* and saline solution (NaCl) 0.15 M as control. Survival probability, oviposition, and adult emergence were evaluated. The plant extracts showed different effects on *C. maculatus*, with *D. ambrosioides* extract being the most lethal to the bean weevil. *A. macrocarpa* and *D. ambrosioides* extracts showed repellency against the cowpea weevil; *A. macrocarpa*, *D. ambroisoides*, *Z. joazeiro*, and *L. rigida* extracts interfered with the oviposition of females; and *M. oleifera* and *Z. joazeiro* extracts decreased the emergence of male and female *C. maculatus*.

Key words: *Vigna unguiculata*, stored grain pests, insecticidal plants, botanical extracts, repellent effect

RESUMO: O feijão-caupi (*Vigna unguiculata*) é uma cultura econômica e nutricionalmente importante. No entanto, o feijão-caupi está sujeito ao ataque de insetos-praga que reduzem a qualidade e o valor nutricional dos grãos durante o armazenamento. O uso de produtos à base de plantas como alternativa de controle de insetos-praga tem-se destacado por sua baixa toxicidade ao ambiente e a saúde humana. Este trabalho teve como objetivo avaliar a bioatividade de diferentes extratos vegetais sobre o caruncho-do-feijão (*Callosobruchus maculatus*). O delineamento inteiramente casualizado foi adotado, com sete tratamentos e seis repetições. Os tratamentos foram constituídos por seis extratos vegetais *Morus nigra*, *Anadenanthera macrocarpa*, *Dysphania ambrosioides*, *Moringa oleifera*, *Ziziphus joazeiro* e *Licania rigida*) e solução salina (NaCl) 0,15 M como controle. A probabilidade de sobrevivência, oviposição e emergência de adultos foram avaliadas. Os extratos vegetais apresentaram efeitos diferentes sobre o *C. maculatus*, sendo o extrato de *D. ambrosioides* o mais letal para o caruncho do feijão. Os extratos de *A. macrocarpa* e *D. ambrosioides* mostraram repelência ao caruncho-do-feijão. Os extratos de *A. macrocarpa*, *D. ambroisoides*, *Z. joazeiro* e *L. rigida* interferiram na oviposição das fêmeas; e os extratos de *M. oleifera* e *Z. joazeiro* diminuíram a emergência de machos e de fêmeas de *C. maculatus*.

Palavras-chave: *Vigna unguiculata*, pragas de grãos armazenados, plantas inseticidas, extratos botânicos, efeito repelente

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Introduction

Cowpea (*Vigna unguiculata*) is an important legume in tropical and subtropical regions that provides nutritious grains with high concentrations of proteins, carbohydrates, lipids, minerals (Fe, Zn and P), vitamins, thiamine, and riboflavin for human consumption (Lonardi et al., 2019; Alfa et al., 2020).

Various types of biological agents compromise the nutritional quality of cowpea beans in storage. Among them, the cowpea weevil, *Callosobruchus maculatus* F., is capable of causing direct including loss of grain mass and decreased nutritional value and indirect caused by the presence of dead insects, eggs, and excrement in the mass of grains damages, which reduces the commercial value of Cowpea beans (Gad et al., 2021). Generally, the attack of *C. maculatus* starts in the field with a low infestation rate, but the population develops rapidly during storage. Consequently, significant losses in grain mass (4-90%) occur months later (Umeozor, 2005).

During storage, *C. maculatus* is controlled using chemical products in the form of fumigants. Though effective, this method of control causes health problems for the applicator, pollution of the environmental, presence of toxic residues in the grains, and selection of resistant insect pests that are not adequately controlled (Paul et al., 2020). To solve these impacts and meet the demands of the increasingly aware society for healthier foods, it is necessary to search for alternatives to synthetic pesticides.

Increasing studies of the use of plant-based insecticides have shown the potential of this strategy for the control of stored grain pests (Pannuti et al., 2012; Langsi et al., 2018). In the Caatinga biome, several plants traditionally used in folk medicine have also shown efficient control of stored grain pests, reducing the emergence of adults, repellency, and mortality of different species of weevils (Almeida et al., 2012; Melo et al., 2015; Okwor et al., 2021).

The objective of this study was to evaluate the insecticidal potential of six plant species found in the Caatinga biome on the survival and reproduction of *C. maculatus* in cowpea beans.

Material and Methods

The study was carried out at the Laboratório de Seletividade de Produtos Químicos (LSPQ) of the Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte, Brazil 5º 12' 31" S, 37º 19' 9" W, and altitute of 37 m, with the weevil species, *C. maculatus*, and six plant species.

Callosobruchus maculatus were obtained from cowpea grain masses from São João do Rio do Peixe region, Paraíba, Brazil. The grain masses infested with woodworm were placed in 1 L plastic containers, covered with thin fabric to allow gas exchange inside the container, and kept in the laboratory at 25 ± 2 °C temperature, 70 ± 5% relative air humidity, and 12 hours photophase.

The species were identified based on characteristics described by Athié & Paula (2002), using stereomicroscope. The insects were bred on cowpea beans purchased from local stores in Mossoró, Rio Grande do Norte, Brazil. Bean grains, used for rearing *C. maculatus*, were kept at -10 ºC for seven days and kept at room temperature for 10 days to reach their hygroscopic balance.

Thereafter, the cowpea grain masses were placed in plastic containers (0.7 L capacity) covered with thin tissue (voil) and infested with 50 newly emerged adults of *C. maculatus*. After seven days, the adults were removed, and infested egg kernels were kept under laboratory conditions previously described. New adults emerged four weeks after performing the grain infestation procedures.

Leaves were collected from six adult plant species in the semiarid region (Caatinga Biome) of the Mossoró municipality, namely *Morus nigra* L. (blackberry), *Anadenanthera macrocarpa* (Benth.) Brenan (angico), *Dysphania ambrosioides* L. (mastruz), *Moringa oleifera* Lam. (moringa), *Ziziphus joazeiro* Mart. (juazeiro), and *Licania rigida* Benth. (oiticica), using pruning shears. Samples of these plants were compared with materials identified and deposited in the Dárdano de Andrade Lima Herbarium of the Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte, Brazil.

The plant materials were individually packaged in plastic bags, identified, and transported to the laboratory. The materials were washed with distilled water, placed in plastic trays, and dehydrated on a bench for two weeks. Subsequently, the materials were ground separately in a blender and sieved until fine powder was obtained. The fine powder was subjected to an extraction process at 10% (w/v) in 0.15 M NaCl solution byconstantly stirring for 16 hours at room temperature of approximately 25 °C. At the end of the process, the material was filtered through gauze and centrifuged at 8000 rpm for 20 min at 4 °C, and the resulting supernatant was called the crude extract (EB) (Silva Filho et al., 2013).

Once the extracts were obtained, the cowpea beans were subjected to the following treatments: T1 = saline solution (NaCl) 0.15M (control); T2 = *Morus nigra*; T3 = *Anadenanthera macrocarpa*; T4 = *Dysphania ambrosioides*; T5 = *Moringa oleifera*; T6 = *Ziziphus joazeiro*; and T7 = *Licania rigida* extracts. The cowpea beans were wrapped in gauze, immersed in saline solution or the extracts of each plant species for 10 s, and dried under ambient conditions for 10 min. The treated grain masses were evaluated for survival, repellency/attractiveness, and insect emergence and reproduction , as described below.

The effects of each plant extract on insect survival were evaluated in an experiment conducted in a completely randomized design with seven treatments and six replicates. Each experimental unit consisted of plastic containers (200 mL) containing 20 g of grains treated according to each treatment and infested with 10 unsexed adult insects 1-2 days old. The containers were sealed with voil-type fabric to allow air circulation and conditioned in BOD regulated at 27 ± 2 °C temperature, 75 ± 5% relative humidity, and 12 hours photophase.

Adult survival was evaluated 1, 6, 12, 24, 48, 72 and 96 hours after the start of the experiment. A leak-proof acrylic cage was used for the evaluations and had the following dimensions: 40 cm length × 20 cm width × 20 cm height. It had a 10 cm diameter front opening to facilitate handling of materials and was closed with organza fabric to prevent escape of insects. The beans were carefully distributed on plastic trays and placed in the cage. Dead insects that were unresponsive to frequent mechanical stimulation with a fine brush every 2 min and insects unable to move for a distance at least equal to the length of the body were considered. After counting the number of dead insects, all live insects and bean grains were returned to their respective experimental units.

Insect longevity and lethal time 50 (LT_{50}) were analyzed using R software (R Development Core Team, 2010) with the aid of Survival package (Therneau & Lumley, 2010). Treatments with similar effects (toxicity and mortality rate) were grouped based on contrast and eventually subjected to Weibull distribution analysis, with the LT_{50} calculated for each group.

The experimental design used for repellency activity was completely randomized with six replicates. The bioassay was performed using arenas assembled in five plastic containers (10 cm diameter and 8 cm height). Each arena consisted of a central container connected to other containers and arranged diagonally through plastic tubes (1 cm diameter and 10 cm length) (Mazzonetto & Vendramin, 2003). A preliminary test was performed to confirm efficiency of the arena system. Four containers were filled with untreated cowpea beans and a uniform distribution of insects between containers was observed.

Each plant extract was tested separately in the arenas by placing 100 g of treated and untreated cowpea beans alternately in each container . In the central container, 60 *C. maculatus* females (3-6 days old) were released, and the number of insects per container was counted after 24 and 48 hours.

To determine the repellent effect of the plant extracts, the repellency index (RI) was calculated as follows: $RI = 2G/(G + P)$. Where: G is the percentage of insects in containers treated with plant extracts, and P is the percentage of insects in untreated containers (control). The RI values ranged between 0 and 2, with RI = 1 indicating neutral activity, RI > 1 indicating attraction, and RI < 1 indicating repellency. The safety margin for this classification was the standard deviation (SD) of each treatment added or subtracted from the value of 1.00 (indicative of neutrality) (Mazzonetto & Vendramin, 2003). Thus, each treatment was considered repellent or attractive when the RI was outside the 1.00 ± SD range. The repellency index results were subjected to analysis of variance using GraphPad Prism® software, and means were compared using t-test at $p \leq 0.05$.

To determine the effects of the extracts on emergence and reproduction of *C. maculatus* adults, a completely randomized experimental design with seven treatments and six replicates was used. Forty-two couples (2-6 days old) were selected for breeding. Then, each couple was confined in plastic containers of 100 mL, containing 20 g of previously treated cowpea beans under test and with the solution related to the control. The containers were sealed with voil-type fabric to allow air circulation and conditioned in B.O.D. regulated at a temperature of 27 ± 2 °C, relative air humidity of 75 ± 5%, and photophase of 12 hours. The weevils were kept in plastic containers until their death and eggs were counted. After 30 days, the number of males and females that emerged in each container was quantified with binocular microscope.

The percentages of emerged adults (PEA) and sex ratio (Rsex) were determined using the formulas PEA = 100 (no. eggs/no. emerged adults) and Rsex = no females/(no males + no females) (Silveira Neto et al., 1976). The results were subjected to analysis of variance at $p \leq 0.05$ and means were compared using Tukey test at $p \leq 0.05$ through SISVAR 5.6 software (Ferreira, 2014).

Results and Discussion

Significant differences between treatments were observed for the repellency index, survival probability, number of eggs, and number of *C. maculatus* females and males in cowpea beans (Table 1).

The results confirmed that the extracts had toxic effects on the weevils when compared to the control, with a notable difference in the LT_{50} (lethal time $_{50}$) values for the different plant extracts tested (Figure 1). *D. ambrosioides* extract (Group 4) was the most toxic and showed the highest lethal action speed, with an LT_{50} of approximately three days after exposure to the treatment. The extracts of *A. macrocarpa* and *M. nigra* (Group 3), followed by *M. oleifera*, *Z. joazeiro*, and *L. rigida* (Group 2) took longer to reduce the insect population to 50.0% at approximately 10 and 20 days, respectively. These results were significantly different from those of the control group (Group 1).

The tested plant extracts were toxic and could be used as protectants against *C. maculatus*, and the variations observed

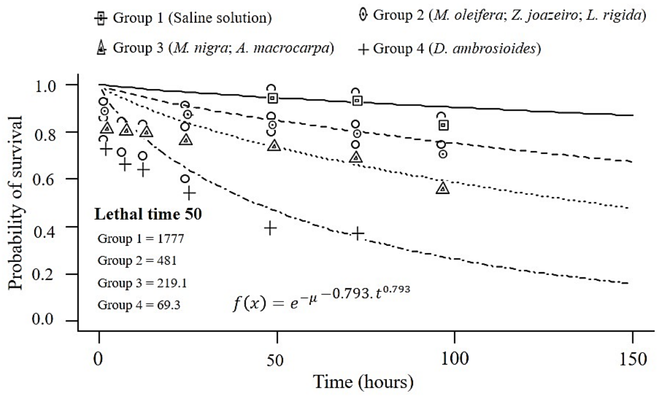


Figure 1. Probability of survival of *C. maculatus* in cowpea beans treated with extracts of different plant species and saline solution

Table 1. F values for repellency index (RI), survival probability (SP), number of eggs (NE), number of females (NF), number of males (NM), percentage of emerged adults (PEA) and sex ratio (SR) of *C. maculatus* in cowpea beans treated with extracts of different plant species

SV	DF	RI	DF	SP	NE	NF	NM	PEA	SR
Treatments	5	0.020*	6	3.094**	2.229**	4.734**	5.716**	1.865^{ns}	0.868^{ns}
CV (%)	-	35.66	-	67	37.54’	39.42	49.52	29.74	21.96

** - p < 0.01; * - p < 0.05; ^{ns}- p > 0.05; SV - Sources of variation; DF - Degrees of freedom between treatments were possibly due to the presence of substances with different toxic actions in the plant extracts. In this case, contact action was observed for adult insects of *C. maculatus* treated with the extracts, possibly owing to the presence of secondary metabolites, such as tannins, alkaloids, flavonoids, and saponins, in the extracts of *A. macrocarpa*, *M. nigra*, *M. oleifera*, *Z. joazeiro*, *L. rigida*, and *D. ambrosioides* (Medeiros et al., 2020; Nascimento et al., 2016; Saraiva et al., 2018; Santos et al., 2019; Lemos et al., 2020).

Dysphania ambrosioides contain secondary metabolites of phytochemicals, such as tannins, coumarins, phenols, steroids, triterpenoids, alkaloids, anthocyanins, flavonoids, cymol, and ascaridol, which have repellent or toxic effects on insect pests of stored grains (Tapondjou et al., 2002; Almeida et al., 2012; Langsi et al., 2018). The volatile compound, ascaridol, can act on the nervous system of insects, causing their death (Tapondjou et al., 2002).

Studies have demonstrated that the use of *D. ambrosioides* under different forms of application is effective against storage insect pests, including *C. maculatus*. Denloye et al. (2010) studied the toxicity of products from *Dysphania* (Syn: *Chenopodium*) *ambrosioides* in Nigeria against storage insect pests and observed that *D.* (Syn: *Chenopodium*) *ambrosioides* used in the form of dry powder, ethanolic extract, or essential oil has toxic effects on *C. maculatus*, causing mortality within 48 h of application. Mkenda et al. (2015) studied the contact and fumigant toxicities of five pesticidal plants against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored cowpea (*Vigna unguiculata*) and observed that the leaf powder of *D. ambrosioides* caused 100% mortality of *C. maculatus* adults at 10% concentration within 24 hours.

The plant extracts applied to cowpea beans affected the foraging behavior of *C. maculatus* females. Grains treated with extracts from *D. ambrosioides* and *A. macrocarpa* showed greater repellent effects on *C. maculatus* females compared to the control, with repellency indices of 0.82 and 0.63, respectively. Grains treated with *M. nigra*, *M. oleifera*, *L. rigida*, and *Z. joazeiro* plant extracts presented repellency indices of 0.94, 0.95, 0.98 and 0.94, respectively, which were not different from the control and showed no repellent effect on *C. maculatus* females (Table 2).

Table 2. Foraging behavior and repellent effect of plant extracts on *C. maculatus*. The temperature of 25 ± 2 °C, relative air humidity of 60 ± 10% and 12 hours of photophase

Treatments	Attract adults (%)	RI (M ± SD)	Classification
M. nigra	47.2 a*	0.94 ± 0.06	N
Control	52.7 a		
A. macrocarpa	32.2 b	0.63 ± 0.10	R
Control	67.7 a		
D. ambrodoides	41.1 b	0.82 ± 0.08	R
Control	58.8 a		
M. oleifera	47.7 a	0.95 ± 0.04	N
Control	52.2 a		
Z. joazeiro	46.6 a	0.94 ± 0.11	N
Control	53.3 a		
L. rigida	47.2 a	0.98 ± 0.27	N
Control	52.2 a		

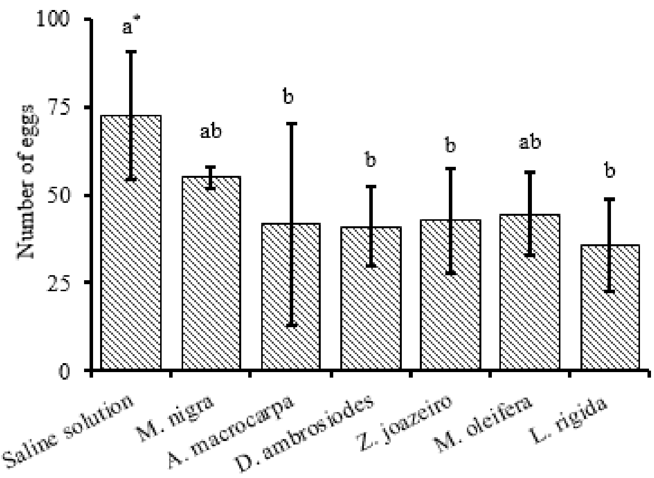
*Means followed by different letters within each treatment are significantly different at $p \leq 0.05$, using t-test. RI, repellency index; M, mean; SD, standard deviation; Classification, R, repellency; N, neutral

In this study, *D. ambrosioides* and *A. macrocarpa* plant extracts showed protective action towards cowpea beans, preventing *C. maculatus* females from using the beans as oviposition substrates. These results match those of Melo et al. (2015), who studied the repellency and bioactivity of powder extracts of Caatinga biome plants against *C. maculatus* and observed that powder extracts from the leaves and stems of *A. macrocarpa* showed a repellent effect on females of *C. maculatus* in treated *V. unguiculata* grains. *D. ambrosioides* have demonstrated repellent effect greater than 60% on *Sitophilus zeamais* in the form of essential oil at the dose of 8.0 μL kg^{-1} (Langsi et al., 2018). The plant species *A. macrocarpa* and *D. ambrosioides* interfered with the foraging behavior of females for oviposition, proving to be alternatives to synthetic chemical insecticides in the management of *C. maculatus* in stored grains.

The repellent activity of plant products can be specific to certain species, which may explain the absence of repellent activities in the other plant extracts studied.

The plant extracts significantly reduced the number of eggs laid by *C. maculatus*. A 50.0% reduction (72.66 to 35.7) in the number of *C. maculatus* eggs was observed in the mass of grains treated with *L. rigida* plant extract compared to the control. The grains treated using extracts of *A. macrocarpa*, *Z. joazeiro*, *D. ambrosioides*, and *L. rigida* were oviposited less by *C. maculatus* when compared to the other treatments, ranging between 35.7 and 42.8 eggs per female. The extracts of *M. nigra* and *M. oleifera* presented mean values of 55.0 and 44.7 eggs per female, respectively (Figure 2).

The attraction of *C. maculatus* to legume seeds is mediated by semiochemicals (Ajayi et al., 2015). Thus, it can be inferred that possible volatilization of the chemical components present in the tested plant species negatively affected cowpea weevil oviposition. The results obtained in this study corroborate those obtained by other authors. Pannuti et al. (2012) evaluated the use of vegetable powders in the control of *C. maculatus* and found that bean grains treated with *D. ambrosioides* powder affected the oviposition of *C. maculatus*. Melo et al. (2014) evaluated the repellency and bioactivity of Caatinga biome



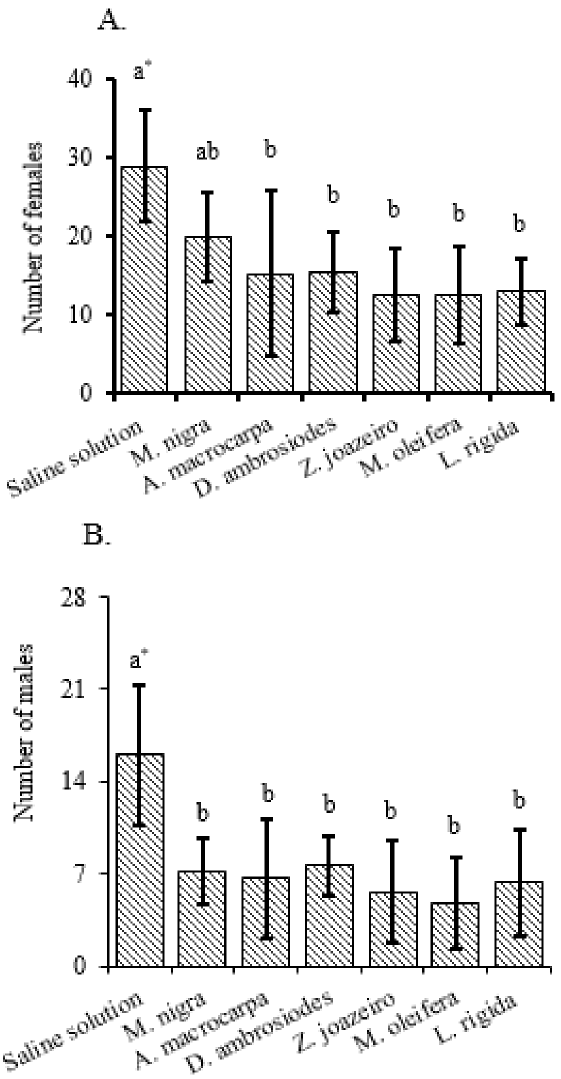
* Means in each column followed by different letters are significantly different at $p \leq 0.05$ using the Tukey test

Figure 2. Number of *C. maculatus* eggs in cowpea grains treated with extracts of plant species and saline solution

plant powders against *C. maculatus* and reported the use of *A. macrocarpa* and *Z. joazeiro* powders and interfered with oviposition of *C. maculatus* on cowpea beans.

However, no effect of plant extracts on the sex ratio and percentage of emerged adults of *C. maculatus* was observed (Table 1). However, the number of females and males that emerged varied between the treatments. Cowpea grains treated with extracts of *M. oleifera* and *Z. joazeiro* showed the lowest emergence of females (12.6 and 12.6%) and males (4.83 and 5.66% (Figures 3A and B).

These results suggest that some of the biological variables of *C. maculatus*, such as oviposition or growth and development of the progeny throughout its phases, were possibly influenced by the presence of secondary metabolites in the plant extracts. Thus, saponins present in *Z. joazeiro* (Nascimento et al., 2016) and *M. oleifera* (Ahmadua et al., 2021) probably influence the number of males and females of *C. maculatus*. Saponins act on insects by interfering with reproduction, causing developmental changes specifically in molting process and reducing food intake (Silva et al., 2012; Pineda-Cortel et al., 2019).



* Means in each column followed by different letters are significantly different at p ≤ 0.05 using the Tukey test

Figure 3. Number of females (A) and males (B) of *C. maculatus* in cowpea grains treated with extracts of plant species and saline solution

The toxic effects on plant species observed in this study demonstrate the potential of plant extracts to control store grain pests.

Conclusions

1. The aqueous extract of *Dysphania ambrosioides* was the most lethal, with greater action of LT_{50} on the survival of *Callosobruchus maculatus*.

2. The aqueous extracts of *Anadenanthera macrocarpa* and *D. ambrosioides* showed repellency against *C. maculatus* when used on the surface of cowpea beans.

3. Oviposition of *C. maculatus* on cowpea beans was reduced by the aqueous extracts of *A. macrocarpa*, *D. ambroisoides*, *Ziziphus joazeiro*, and *Licania rigida*.

4. Emergence of males and females of *C. maculatus* was negatively affected by the aqueous extracts of *Moringa oleifera* and *Z. joazeiro*.

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