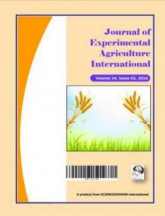

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Toxicity Aqueous Extract of Castor Bean (*Ricinus communis*) to *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae)

Anderson Mathias Holtz[1], José Romário de Carvalho[2*], Mylena da Silva Gomes[1], Vergilio Borghi Neto[1], Ana Beatriz Mamedes Piffer[1] and Ronilda Lana Aguiar[1]

[1]Instituto Federal do Espírito Santo – Campus Itapina, BR 259, Km 70, Caixa Postal 256, CEP. 29717-000, Colatina, ES, Brazil.
[2]Universidade Federal de Viçosa, Avenida P. H. Rolfs, s/no, Campus Universitário, CEP 36570-900, Viçosa, MG, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. Authors AMH, RLA and JRC designed the study, wrote the protocol and first draft of the manuscript. Authors MSG, VBN and ABMP conducted the experiments, managed the literature searches and did a critical review of the manuscript. Author JRC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to evaluate the insecticidal effect of aqueous extract of castor bean (*Ricinus communis*) cake for the control of *Maconellicoccus hirsutus*. The toxicity tests were performed in the laboratory. Subsequently lethal concentrations 50 and 90% ($LC_{50}$and LC_{90}, respectively) were estimated. Tween80® + distilled water was used as surfactants and solvent respectively. The assay was performed in petri dishes containing a *Coffea canephora* leaf disc. The spraying was carried out in Torre de Potter spray tower. The extract was toxic to mealybug and the

**Corresponding author: E-mail: jromario_carvalho@hotmail.com;*

mortality data was adapted to the probit model. Estimates of LC_{50} and LC_{90} were 5.32% and 29.30%, respectively. It was concluded that the aqueous extract of *R. communis* cake is promising for the management of *M. hirsutus*.

Keywords: Insect pests; pink mealybug; Ricinus communis.

1. INTRODUCTION

In Brazil, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcus), commonly known as pink mealybug or hibiscus mealybug, is a quarantine pest of type A2, i.e. it is not distributed throughout the national territory and can cause damages in more than 200 species of plants, as registered in other countries. Brazil offers ideal climatic conditions for its development, being its greatest economic impact on fruit growing, mainly in fields of cacao (*Theobroma cacao*) [1]. While feeding, the mealybugs introduce toxic substances in the plants, causing poor formation of leaves and fruits, apical growth, and may lead to premature senescence of flowers [2].

It is a recent pest in the country, because the first signs of it were only observed during 2010. There is therefore no effective method of controlling the pest and its environmental impacts are therefore also unknown. For the countries that already have this pest, biological control, using parasitoids and predators, has been an alternative. However, due to its rapid dispersal, studies are needed to develop management programs to combat this pest [3].

Research related to the use of extracts and substances obtained from plants are demonstrating a satisfactory efficiency in the control of some pests [4]. These substances act as a defense mechanism for plants to protect themselves from pests and diseases and to help with the adverse environmental conditions [5].

Castor bean [*Ricinus communis* L. (Euforbiaceae)] is an example of an insecticidal plant in which studies have demonstrated its effectiveness in pest control. Lima et al. [6] obtained excellent results by testing the oil extracted from castor oil seeds on *Diaphania nitidalis* (Stoll) (Lepidoptera: Pyralidae) caterpillars. The author reports that on the second day of evaluation, a mortality of 92% was obtained. Santiago et al. [7] studying the aqueous extract of 10% (v / v) *R. communis* green fruits on *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) caterpillars observed reduced activity on biological parameters, such as larval and pupal duration and viability, reduction in pupal weight. Carvalho [8], on the other hand, verified reduced activity of aqueous extracts of the castor bean cake on *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae) caterpillars, whereas for castor oil the results showed high toxicity of the oil for the species.

However, castor oil is widely used in the production of biodiesel. In the process of production, a residue is generated, the cake, which, in the case of *R. communis*, does not yet have a specific use [9]. However, studies have already proven that the insecticidal activity of this byproduct may be due to the presence of ricin, which is a very toxic substance [10]. Therefore, with the need to develop methods for the management of *M. hirsutus*, castor bean cake may be considered as an alternative to the management of this pest.

The objective of this study is to quantify the insecticidal potential of the castor bean cake as an alternative control method of *M. hirsutus*.

2. MATERIALS AND METHODS

The experiment was carried out at the Federal Institute of Education, Science and Technology of Espírito Santo – *Campus* Itapina (IFES-*Campus* Itapina) Colatina, ES, Brazil, in air-conditioned chambers at 25 ± 1ºC, relative humidity 70% ± 10 and 12h photophase. Seeds of *R. communis* were used to study the insecticidal activity.

2.1 Rearing of Pink Mealybug *M. hirsutus*

Samples of *M. hirsutus* were collected in commercial cocoa plantations [*Theobroma cacao* (Malvaceae)] in the municipality of Colatina, ES, Brazil and taken to the laboratory. The breeding technique used was an adaptated from Sanches & Carvalho [11]. Pumpkins were used in the initial state of maturation to feed the *M. hirsutus*. After establishment of the colony, the process of mealybug breeding began. If there was a need to change the pumpkins, new fruits were placed in contact with those already infested for approximately two hours. The approximation of these pumpkins favored the transfer of newly

hatching nymphs from the cochineal to the new fruit due to its mobility in that phase.

2.2 Obtaining Aqueous Extract from Castor Pie

The preparation of the cake was through the extraction of the oil from the castor seeds collected at the IFES-*Campus* Itapina. After this procedure, the cake generated from the extraction was submitted to grinding, in knives mills, to obtain a fine powder that will be used in the preparation of the extract.

To obtain the extract, the powder of the ground plant material (30 g) was transferred to an Erlenmeyer flask (100 mL) containing distilled water and Tween® 80 (0.05% v / v) adhesive spreader, to obtain 100 mL of the initial solution at 30% (w / v). Subsequently, it was maintained under homogenization in a cross stirrer (240 rpm) for a period of 24h. After this time, the mixture was filtered with fine sterile tissue, and transferred to a volumetric flask and the volume checked to 100 mL.

2.3 Bioassays

The direct application tests were carried out on the individuals of *M. hirsutus* to evaluate insecticidal action and to estimate the lethal concentration for a medium population (LC_{50}).

2.3.1 Toxicity test of extract to *M. hirsutus*

The treatment consisted of 6 replicates with 12 individuals of the *M. hirsutus* by repetition, totaling 72 insects. The replicates were maintained in Petri dishes (10.0 x 1.2 cm) on coffee leaf discs of about 4 cm in diameter. The discs were fixed to the petri dish with a 0.5 cm layer of agar-agar solution and solid petroleum jelly (Vaseline) around the disc edge to prevent insect escape the arena.

The spray was applied with a Potter Tower® at a pressure of 15 lib/in² and 6 mL of solution for each repetition, corresponding to an average volume of 1.62 mg/cm². Distilled water and Tween® 80 adhesive spreader (0.05% v / v) were used as controls. The insecticidal effect was evaluated 24, 48 and 72 hours after spraying. The experiment was conducted in an air-conditioned room (25 ± 10°C, 70 ± 10% RH and 12-hour photophase). The design was completely randomized, and mortality assessments were conducted after 72 hours.

2.3.2 Estimation of lethal concentration (LC)

Each treatment consisted of 6 replicates and 12 insects per replicate, totaling 72 individuals. The sprays were applied as described above for the toxicity test. The treatments were composed of 4 concentrations of aqueous extract of castor bean cake, spaced on a logarithmic scale (limits of 0.01% to 30% m / v). Distilled water plus Tween 80® (0.05% v / v) was used as the solvent and was used in the control treatment. Mortality was corrected by the formula proposed by Abbott [12]. The experiment was conducted in an air-conditioned room (25 ± 10°C, 70 ± 10% RH and 12h photophase) and evaluated 24, 48 and 72 h after spraying the solutions. With mortality corrected data of 72 h, lethal concentrations were estimated using Probit analysis [12-14].

2.3.3 Data analysis

All data processing and graphical presentation of results were performed in the computational environment R, as proposed by Carvalho et al. [12]. Treatment means were compared by a unilateral t test ($p \leq 0.05$) [12].

3. RESULTS AND DISCUSSION

The toxicity test indicated that the aqueous extract of the *R. communis* cake differed significantly from the control ($t = 14.02$, $P < .0001$) (Fig. 1), with an average mortality of 83.46%. Based on this result, it was proceeded to obtain the estimated lethal concentration.

In the LC_{50} bioassay there was an increase in the percentage of mortality of the *M. hirsutus* proportional to the increase of the concentration of the aqueous extract of castor bean cake. The data was adapted to the Probit model (Table 1 and Fig. 2). The slope of the concentration-mortality curve was 1.73. The lethal concentration required to cause mortality of 50% of the *M. hirsutus* (LC_{50}) population was estimated to be 5.32% and to kill 90% of the population (LC_{90}) was estimated to be 29.30%.

The toxicity of castor bean to insects and mites is due to different compounds, such as ricin that is a protein found exclusively in the endosperm of the seed, and is not detected elsewhere in the plant [15]. Another substance found in castor oil is ricinin, an alkaloid found in all parts of the plant. The insecticidal effect occurs mainly due to this toxin [16]. However, this is in low concentrations and, consequently, has a low

toxic activity. In castor bean seeds, the ricinin content is higher in the outer capsule, medium in the seed shell and small in the endosperm [15]. This toxin has two subunits with different functions, but acting together. One of them has an inhibitory effect on the digestion process by the action of α-amylase enzyme inhibitors, preventing the digestion and absorption of starch and the other has an insecticidal effect due to the action of ribosome inactivating proteins (RIPs), which promotes cell death by inhibition of protein synthesis [17].

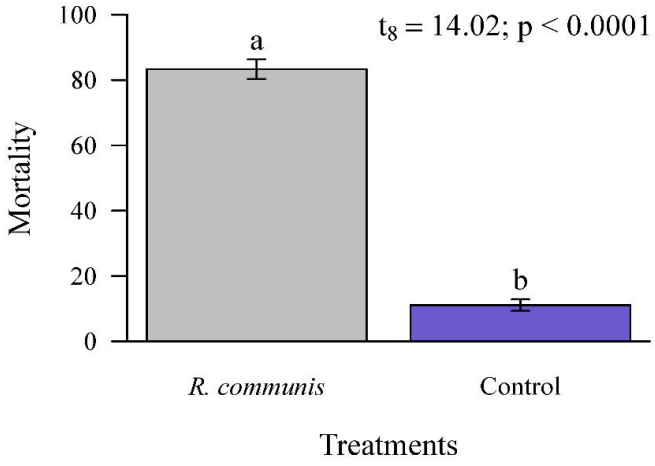

Fig. 1. Mortality of *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) treated with aqueous extract of *Ricinus communis* (Euphorbiaceae) at 30% (m / v)

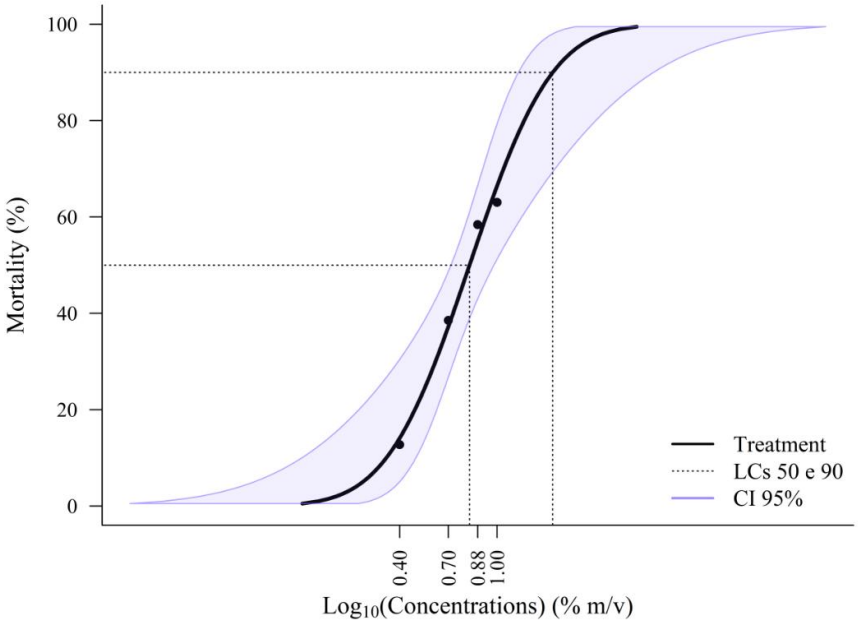

Fig. 2. Concentration-mortality curve and respective LC50 and CL90 of aqueous extract of *Ricinus communis* on *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) (Temp.: 25 ± 1°C, RH 70 ± 10% and 12h of photophase)

Table 1. Concentration-mortality curve parameters and respective LC50 and CL90 of aqueous extract of *Ricinus communis* on *Maconellicoccus hirsutus*(Green) (Hemiptera: Pseudococcidae) (Temp.: 25 ± 1°C, RH 70 ± 10% and 12h of photophase)

n[1]	Slope ± SE[2]	LC_{50}[3] (CI[4] a 95%) (g/100 ml)	LC_{90}[3] (CI[4] a 95%) (g/100 ml)	DF[5]	$\chi^{2(6)}$	*P*[7]
200	1.73±0.52	5.32 (1.00-8.82)	29.30 (21.40-73.43)	2	0.5769	> .05

[1] Number of insects used in the test; [2]Slope ± standard error; [3] Lethal concentration; [4] CI confidence interval at 95% probability; [5] Degrees of freedom; [6] Chi-square test; and [7] p-value

However, as the insect under study feeds directly from the sap of the plant, because it is a hemipteran, these compounds hardly act through the gastrointestinal tract. Due to the spraying process, the insects were subjected to dermal and possibly respiratory contact. In this context, the insect could be absorbing these compounds via the integument or respiratory system through the spiracle cavity. *M. hirsutus* is different from other mealybugs because its body is protected by a pulverulent carapace [18], which could facilitate the absorption of toxins by the dermal route.

However, no studies on the action or ricin absorption and, or ricinin in cutaneous and respiratory systems of the insects were reported.

Another factor that should be considered is the amount of toxins presents in castor bean cake and if there is seasonal variability due to the harvest and storage time, and the effect on the toxicity of these substances. A study carried out with *Moringa oleifera* (Moringaceae) demonstrated increased toxicity over time of storage on the mite *Tetranychus urticae* (Acari: Tetranychidae) [19]. These results justify the need for further chromatographic and storage studies in order to better understand the toxicity mechanisms of the castor bean extract and possibly produce a commercial product that is safe for farmers and the environment.

4. CONCLUSION

It is concluded that the extract of castor bean cake is promising for the management of *M. hirsutus*. However, studies in semi-field and field conditions are should be conducted to optimize and validate the results of the present study.

In addition, studies to elucidate the toxicological mechanisms of ricin and ricinine in hemipteran insects may provide us with promising molecules for the control of insect pests.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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