



Toxicity of Jatropha and Neem Oil Combination on Pink Hibiscus Mealybug

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Authors' contributions

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

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ABSTRACT

Maconellicoccus hirsutus (pink hibiscus mealybug) is a polyphagous pest species reported in about 350 species of host plants worldwide. The chemical control method is still the most used for the control of agricultural pests. In view of this, we aim to evaluate the potential of the concentration of oils extracted from the species *Azadirachta indica* (Neem) and *Jatropha curcas* (Jatropha) on *M. hirsutus*, aiming at a management alternative for the pest. The experiments were carried out in air-conditioned chambers at a temperature of $25 \pm 1^\circ\text{C}$, relative humidity of $70 \pm 10\%$ and a photophase of 12h. The concentration 3% (v v⁻¹) (defined in preliminary dilution tests) was used in the tests, with 11 interaction ratios between the oils. Mortality was assessed daily up to 72 hours after spraying. The application was carried out in two ways: indirect - on the food and walking

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surface; and direct - about insects. The indirect application caused higher levels of mortality in relation to the direct application. However, there was no significant difference between the concentrations for the indirect application. In the form of direct application, the combination of 60:40% neem oil and jatropha oil was the treatment that provided the highest mortality of the *M. hirsutus* (86.21%). The combinations between neem and jatropha oils demonstrated the potential to control *M. hirsutus* in both routes of application, being an alternative for the management of this pest.

Keywords: *Maconellicoccus hirsutus*; *Azadirachta indica*; *Jatropha curcas*; alternative pest management: combination of fixed oils.

1. INTRODUCTION

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), was originally described in India, in 1908, and also introduced that year in Egypt, spreading to many parts of Tropical Africa. Currently, it can be found in Australia and the Pacific Islands, Asia, the Middle East, Africa, the West Indies and North South America [1]. The incidence of this pest generates an economic impact, not only because of the direct damage caused to plants, but because it is a cosmopolitan pest, thus limiting exports and the commercial mobilization of agricultural products [2]. The eating habit of *M. hirsutus* consists of feeding on phloem tissues, preferentially attacking meristems and young stems, flowers and plant fruits [3]. During feeding, the pest releases toxins along with saliva to destroy tissues near the site and facilitate the absorption of sap, which causes the wrinkling of plant tissues [4].

As it is a recent pest in Brazil, there are few studies on methods of controlling it. For the cocoa crop, for example, deltamethrin is the only insecticide with the registration [5]. Although the use of agrochemicals is relatively successful in the control of agricultural pests, the intensive use of the same active ingredient or mechanism of action can provoke the resurgence of the target pest, as well as the appearance of resistant populations and new pests, since most of these products have a broad biological spectrum and persistence in the environment, in addition, these products can affect populations of natural enemies of the pest species and human health [6,7].

Thus, research related to the use of extracts and substances obtained from plants is showing satisfactory efficiency in pest control, as they have secondary metabolites that have proven insecticidal activity and are easily degraded in

the environment, thus reducing damage to ecosystems [8]. Among these plants with insecticidal properties, *Jatropha curcas* L. (Euphorbiaceae), has shown significant success rates in research. Ingle et al. [9] found mortality levels of up to 100% when using extract of *Jatropha* seeds on *Plutella xylostella* L. (Lepidoptera: Plutellidae), the same study also presented considerable mortality rates of up to 60% on individuals of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Another plant that can be cited with proven insecticidal properties is the neem *Azadirachta indica* A. Juss (Meliaceae). Neem has azadiractin as its main compound extracted from its fruits, which interferes with the functioning of animals' endocrine glands [10]. Studies report that the aqueous extract of the seeds of *A. indica* has insecticidal activity with 96.5% mortality in pupae of *Liriomyza sativae* (Diptera: Agromyzidae) [11]. Vieira and Peres [12] also found mortality levels of 98.5% after spraying neem extract at a concentration of 2% over *Brevicoryne brassicae* L. (Hemiptera: Aphididae).

Although the studies described prove the insecticidal potential of jatropha and neem, their efficiency applies separately, and studies addressing the interaction between species of insecticidal plants are still scarce. Thus, the objective of this work was to evaluate the potential of using the interaction of oils extracted from the species *J. curcas* (jatropha) and *A. indica* (neem) applied directly and indirectly on *M. hirsutus*.

2. MATERIALS AND METHODS

The experiment was carried out at the Agricultural Entomology and Acarology Laboratory of the Federal Institute of Education, Science and Technology of Espírito Santo - Campus Itapina (IFES-Campus Itapina), in air-conditioned chambers at a temperature of 25 ±

1°C, relative humidity of $70 \pm 10\%$ and 12h photophase.

2.1 Rearing *M. hirsutus*

The breeding technique adopted was an adaptation by Sanches and Carvalho [13], using pumpkins in an early stage of maturity to feed the mealybug. In the initial infestation of pumpkins, individuals were collected in the field of infested host plants. After the establishment of the initial colony, the process of multiplication of mealybugs occurred. When it was necessary to change pumpkins, new pumpkins were brought into contact with those infested. The approach of the fruits favors the transfer of newly hatched nymphs from the mealybug to the new fruit due to its high mobility at this stage.

2.2 Preparation of Oil Suspensions and Combinations

For the preparation of oil suspensions, neem and jatropha seeds were collected in the productive areas of the IFES-Campus Itapina. After this procedure, neem and jatropha seeds were subjected to oil extraction by cold pressing. The concentration used in the said tests was 3%, defined in dilution tests carried out previously (results not shown). The list of combinations used in the treatments are shown in Table 1. using 100 mL of distilled water + Tween® 80 adhesive spreader ($0.05\% \text{ v v}^{-1}$) as solvent. Then, the mixture remained under stirring for 4 hours at room temperature, with the aid of a magnetic stirrer.

2.3 Direct Application Testing

Each treatment previously determined was composed of 10 repetitions, containing 10 adult mealybugs per repetition, totaling 100 insects per treatment. The repetitions were kept in Petri dishes (10.0 x 1.2 cm), on discs of cocoa leaf (4 cm in diameter). These discs were fixed to the Petri dish with a 0.5 cm layer of agar solution. At the top, solid petroleum jelly was used around the disc to prevent the insects from escaping. For spraying, an airbrush with a pressure of 15 psi and 2 mL of suspension from each combination was used for each repetition. The solvent [distilled water and the adhesive spreader Tween® 80 ($0.05\% \text{ v v}^{-1}$)] was used as a negative control. Mealybug mortality was assessed daily until 72 hours after spraying.

2.4 Indirect Application Testing

The indirect application test was carried out under the same conditions as the previous test. However, in this experiment the cocoa discs were immersed for 5 seconds in the treatments. Subsequently, they were placed on filter paper to dry, for 45 min, to eliminate excess moisture. After this procedure, the discs were placed in the Petri dishes, as previously described for the direct application test. Ten repetitions per treatment were used, and the evaluations were carried out according to the previous test.

2.5 Statistical Analysis

The 72-hour accumulated mortality data were subjected to analysis of variance according to a completely randomized design with a factorial arrangement (routes of application x oil combinations). In case of significance, the means were submitted to the Scott-Knott cluster test ($p < 0.05$) to compare the treatments (oil combinations) and the F test ($p < 0.05$) to compare the routes of application. To perform the analyses, the ExpDes.pt package [14] of the computational application R version 3.4 [15] was used.

3. RESULTS AND DISCUSSION

The mortality of *M. hirsutus* was affected by the interaction between the routes of application and combinations of Neem and Jatropha oils ($P > .05$). The indirect application form caused higher percentages of mortality in relation to the direct application, however there was no significant difference between the concentrations in this application route. In the form of direct application, the combination of 60:40% neem oil and physic nut were the treatment that provided higher mortality to *M. hirsutus* (86.21%) (Table 2).

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ by the F and Scott-Knott test, respectively, at the level of 5% probability

The higher percentages of mortality were observed in the form of indirect application could be attributed to the fact that the cocoa leaf discs were completely immersed in the solutions, which could act on individuals in different ways, either by ingestion (through the digestive

Table 1. Proportions of neem (*Azadirachta indica*) and jatropha (*Jatropha curcas*) oils used in the experiment in percentage (%), for 100 mL suspension [distilled water and Tween® 80 adhesive (0.05% v v⁻¹)]

Neem (%)	Jatropha (%)
0	100
10	90
20	80
30	70
40	60
50	50
60	40
70	30
80	20
90	10
100	0

Table 2. Mortality *Maconellicoccus hirsutus* treated with different combinations neem oil and jatropha in different application routes

Treatments	Application routes			
	Direct		Indirect	
Control	7.00	A d	9,00	A b
Neem (100%)	69.37	B b	100,00	A a
Jatropha (100%)	56.72	B b	97,57	A a
Neem (10%):Jatropha (90%)	40.92	B c	100,00	A a
Neem (20%):Jatropha (80%)	34.64	B c	99,17	A a
Neem (30%):Jatropha (70%)	41.96	B c	100,00	A a
Neem (40%):Jatropha (60%)	60.88	B b	93,57	A a
Neem (50%):Jatropha (50%)	37.34	B c	95,06	A a
Neem (60%):Jatropha (40%)	86.21	A a	97,03	A a
Neem (70%):Jatropha (30%)	51.97	B b	95,83	A a
Neem (80%):Jatropha (20%)	40.97	B c	97,27	A a
Neem (90%):Jatropha (10%)	15.32	B d	100,00	A a
CV(%)	23.82			

system, before penetration into plant tissue) or by contact, crossing the integument and through the airways, which would facilitate the intoxication process, since *M. hirsutus* has a thin layer of powdery secretion around its body that protects it in the case of spraying [2,16]. However, some authors argue that the action by contact is faster than the mode of action by ingestion. This is possibly due to the fact that acting on the target organism, the mode of action by ingestion depends on the process of digestion for absorption and action on the vital systems of the pest [17]. The way in which the oils act on the pink scale can also be related to the secondary metabolites present in the seeds that have a fundamental role in the toxicity on individuals, since they have antibiotic, antifungal, antiviral properties, and also proteins of defense mechanisms that can express insecticidal activity [7].

Azadiractin is the active ingredient in *A. indica* responsible for the insecticidal activity of this plant, acting through the physiological processes of the insects in the regulating hormones of metamorphosis, compromising the normal development of insects both in the larval and pupal stages, which can result in mortality of individuals [11,18], in addition to this component, neem still has triterpenoids such as nimbine and salanine, however these compounds have little effectiveness in controlling insects [19]. Sami et al. [20] by exposing adult insects and larvae of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) to raw neem powder, showed a significant reduction in the weight of insects and an increase in the mortality rate. This deregulation in the development and mortality action of individuals could still be associated with the role of neem in the cell's biosynthetic

machinery, thus deregulating various individuals' metabolic pathways.

The species *J. curcas* has fatty acids such as oleic, linoleic, palmitic and stearic in its composition [21] and the amounts of these components may vary according to the place of cultivation, variety planted, management adopted in the cultivation. However, some studies attribute the toxicity of *J. curcas* mainly to two components present in the seeds: curcine, a ribosome inactivating protein (RIP) and diterpene esters. Curcine is a toxic protein that has a combined anti-food and insecticidal activity. The anti-food activity is due to the inhibition of the enzyme α -amylase in the body, preventing the absorption and digestion of starch by insects, while the insecticidal activity consists of the action of RIP to cause death of the cells of the gastrointestinal tract after ingestion [22]. The activity of the diterpene esters is broad and can affect insects in different ways. Viegas Júnior [23], in a study, stated that diterpenes isolated from the Euphorbiaceae family have appetite suppressant activity, in addition, some isolated compounds may show growth inhibition, neurotoxic effects, reproductive unfeasibility, among other effects in insects. Other substances, for example, linoleic acid, according to some authors, have their toxicity factor related to the octopaminergic receptors of insects, interfering in their development [24,25]. Ingle et al. [26] found significant results when testing crude methanolic extracts of leaf, bark, seed, seed coat and root of *J. curcas* in third instar larvae of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) reaching percentages of mortality of 60% in leaf extract, 20% in seeds, seed and root coating at a concentration of 5%.

When observing the combinations of oils and oil isolated in the direct application route, we found that these results suggested a synergistic action between these oils, where one intensifies the action of the other, depending on the combination. Pierattini et al. [27] using three essential oils [*Foeniculum vulgare* Miller (Apiaceae), *Pistacia lentiscus* L. (Anacardiaceae) and *Ocimum basilicum* L. (Lamiaceae)], found a synergistic effect between oil combinations, especially for those containing *F. vulgare*, that obtained greater efficacy in the mortality of target individuals. Still in this sense, the combination of 90:10% neem oil and jatropha was the treatment with the lowest mortality (15.32%), in direct application, equaling the results presented by the witness. The interactions with the lowest

proportions for the two types of oil were generally those with the lowest mortality rates. A similar result occurred in the combination of 50:50% of neem oil and jatropha, suggesting that in these proportions the antagonistic effect that some plants/substances might have on each other may occur. Andrade-Ochoa et al. [28] when testing the larvicidal and pupicidal effect on *Culex quinquefasciatus* Say (Diptera: Culicidae) of some essential oils and investigating the synergistic and antagonistic effects of the binary interactions of the oils, found an antagonistic effect only between the limonene-pinene combination, associated with the difference of activity between the essential oil of *Citrus aurantifolia* (Rutaceae) and limonene against pupae of the insects, decreasing their efficiency in the mortality rates of individuals.

4. CONCLUSION

It is concluded that the combinations between neem and jatropha oils demonstrated potentiality in the management of pink hibiscus mealybugs via direct or indirect application. However, studies to elucidate the toxicological molecules of the oils that act on the body and the physiology of *M. hirsutus* must be conducted in order to obtain greater efficiency in the applications.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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