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## General Entomology

# Efficiency of botanical extracts against *Aphis craccivora* Koch (Hemiptera: Aphididae) nymphs in *Vigna unguiculata* (L.) Walp

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**Abstract.** The present study aimed to evaluate the insecticidal activity of hydroalcoholic plant extracts on *Aphis craccivora* Koch nymphs in cowpea. The experiments were carried out under greenhouse conditions in a randomized block design with five repetitions. Hydrated ethanol was used as a solvent in the botanical extract preparation. Cowpea plants were infested with five female adult aphids, eleven days after planting. After 48 hours, the adults were removed from the plants, leaving the recently bred nymphs. The evaluation of the nymphs' survival was carried out forty-eight 48 hours after the application of the plant extracts. The botanical extracts with more than 50% efficiency were: *Allium tuberosum* leaf, *Caesalpinia ferrea* leaf, *Piper aduncum* leaf, *Carica papaya* seed, *Dieffenbachia picta* leaf, *Cucurbita moschata* seed and the control treatment, *Annona squamosa* seed. The botanical extracts with less than 50% efficiency were: *Allium sativum* bulb, *Anacardium occidentale* leaf, *Borreria verticillata* leaf plus flower, *Coffea arabica* leaf and seed, *Delonix regia* leaf and flower, *Euphorbia tirucalli* leaf, *Leucaena leucocephala* leaf, *Mimosa caesalpiniaefolia* leaf, *Nerium oleander* leaf, *Syzygium cumini* leaf, endocarp and pericarp, *Syzygium malaccense* leaf, *Zingiber officinale* rizome and *Ziziphus joazeiro* leaf. The highest efficiency levels were obtained by *D. picta* and *C. moschata*, rendering these extracts suitable for field trials to further evaluate their efficieny against the cowpea black aphid.

**Keywords:** Agroecology; alternative control; black bean aphid; botanical extracts.

The cowpea plays a strategic role in the Brazilian economy, as it is a staple crop and it is also responsible for the employee retention in the farmland, especially in the North and Northeast regions of the country (Rabelo *et al.* 2015; Rodrigues *et al.* 2015). *Aphis craccivora Koch* (Hemiptera: Aphididae) or common bean black aphid, is considered one of the main pests of the cowpea crop (Rabelo & Bleicher 2014). It causes direct damage by sucking the sap and injecting toxins in the plant, and indirectly through its ability to transmit viruses, besides excreting honey dew, that can be used by the *Capnodium* sp. fungi. *Capnodium* sp. develops in the leaf limb, impairing photosynthesis and plant respiration and further weakening the plant (Abdallah *et al*. 2016).

The cowpea black aphid is fought, mainly, via synthetic chemical insecticides. However, the misuse of agrochemicals may cause human and animal poisoning, pest resistance, death of pollinating agents, water contamination and, eventually, toxic residues in agricultural products (Rother 2018). Therefore, there is a need to integrate alternative control methods, with lesser environmental impacts. Among these, botanical insecticides outstand as an environment friendly and low-cost option (Haas *et al.* 2014).

The use of insecticidal plant extracts or essential oils is a well-known practice, however, with the popularization of organosynthetic insecticides, this practice has fallen into disuse. Some plants, through their evolutionary process, have developed chemical barriers against crop pests, producing secondary metabolites with insecticidal properties. Therefore, botanical insecticides are usually: powdery vegetable matter, aqueous extracts or extracts using other solvents such as alcohol, ether, acetone, among others (Wiesbrook 2004; Isman 2008).

Researches that evaluate the effectiveness of plant-based insecticides are trending, due to environmental problems related to organosynthetic insecticides (Isman 2020; Amoabeng 2020). Plant based insecticides work by slowing down both larval and nymph's development, as well as affecting the feeding ability and oviposition of insects which increases the mortality of such insects (Matos *et al.* 2006; Fernandes & Favero 2014).

Currently, there is no scientific consensus regarding botanical insecticides toxicity to humans and the environment, and it is most likely related to the dose and the plant species. According to Kingsbury (1964), some natural products are toxic to humans and at the high rates needed for some of these natural products to control insects, these products could actually be less safe than the currently used synthetic insecticides that can control insects at very low doses (Suthisut *et al*. 2011).

However, Ribeiro *et al*. (2010) and Lima *et al*. (2014) report that the active principles that act as defenses against herbivorous insects are usually photo-unstable, i.e. they are rapidly degraded in the presence of light, thus, its environmental impact, when compared to synthetic agrochemicals, is low, leaving next to none residues in food.

However, Nevertheless, the low cost associated in the production of botanical insecticides render them as a viable alternative to agricultural pest management. However, for this technology to be efficient there is a need for frequent prospection and evaluation of new bioactive compounds (Ribeiro et al. 2010; Lima et al. 2014).

In previous studies by the research group, the use of the hydroalcoholic extraction method proved the most efficient against the cowpea black aphid and the dilution of ethanol in water did not statistically differ from the absolute control (distilled water).

The aim of this study was to evaluate the insecticidal efficiency of hydroalcoholic extracts obtained from different plant parts on the control of the cowpea black aphid nymphs, under greenhouse conditions.

## MATERIAL AND METHODS

The study was carried out in a greenhouse belonging to the Plant Science Department of the Universidade Federal do Ceará (UFC) – Pici *Campus*; located at 3°40.405' South, and 38°34.534' West, at an altitude of 12 m above sea level, from April to October 2012. The environmental conditions in the greenhouse were: maximum temperature of 33.4 ± 1.40 °C; minimum temperature 26.7 ± 0.6 °C, and; relative humidity of 70 ± 10%.

**Experimental Design.** The design used in each experiment was randomized blocks with seven treatments and five repetitions. The plant material used to obtain the extracts was either collected in the Pici *Campus* surroundings or purchased from local businesses.

**Treatments.** The treatments consisted of distilled water (absolute control), a botanical insecticide of proven efficiency (*Annona squamosa*) (positive control) at a dosage of 0.5% (w/v) (Rabelo & Bleicher 2014), and the hydroalcoholic extracts (5% w/v) (Table 1).

**Plant extracts.** The plant materials for the brewing of the botanical extracts were collected in the Pici campus and some were bought in the local market. The plant material was visually assessed, in which the material that was free from any kind of injury was chosen. The samples (Table 1) were oven dried for 72 h at 45 °C and then ground in a mill. The botanical species were chosen based on the market availability and previous assays that showed potential toxicity to insects.

For the preparation of the *A. squamosa* extract, one gram of the seed powder was weighed and diluted in 100 mL of hydrated ethyl alcohol, resulting in a solution of 1% concentration (g $mL^{-1}$).

For the preparation of the extracts, five grams of the dry ground vegetable parts were weighed and then placed in amber glass containers. Then 50 mL of hydrated ethyl alcohol at 92.8° was added to obtain a solution at 10% concentration (g $mL^{-1}$). The immersion lasted 24 hours. The extracts were immediately used after preparation.

**Insect rearing.** Aphids were collected from bait plants grown in an area nearby the breeding house. With the aid of a fine, wet bristle brush, the adult insects were transferred to cowpea plants, grown in a black plastic pot with a capacity of 1.5 L, with substrate made of 60% soil, 30% humus and 10% vermiculite, inside rearing cages of 0.80 x 0.80 x 0.80 m, screened with white escaline fabric, and daily irrigated. Four days after the aphids introduction the plants were substituted. This was done so that the *A. craccivora* population did not develop winged forms due to nutritional stress.

Every week new plants were infested for the maintenance of

**Table 1.** Botanical species, common name, family and plant part used for the preparation of the botanical extracts.

| Botanical species | Common Name | Acquisition | Family | Plant part |
|---|---|---|---|---|
| *Allium sativum* | Garlic | Purchased | Amaryllidaceae | Bulb |
| *Allium tuberosum* | Chinese chives | Purchased | Amaryllidaceae | Leaf |
| *Anacardium occidentale* | Cashew | Collected | Anacardiaceae | Leaf |
| *Annona squamosa* | Sugar-apple | Collected | Annonaceae | Seed |
| *Spermacoce verticillata* | Shrubby false buttonweed | Purchased | Rubiaceae | (Leaf+flower) |
| *Caesalpinia leiostachy* | Jucá | Collected | Fabaceae | Leaf |
| *Carica papaya* | Papaya | Collected | Caricaceae | Seed |
| *Coffea arabica* | Coffee | Purchased | Rubiaceae | Seed<br>Leaf |
| *Cucurbita moschata* | Pumpkin | Purchased | Cucurbitaceae | Seed |
| *Delonix regia* | Flamboyant | Collected | Fabaceae | Leaf<br>Flower<br>Seed |
| *Dieffenbachia picta* | Dumb cane | Collected | Araceae | Leaf |
| *Euphorbia tirucalli* | Indiantree spurge | Collected | Euphorbiaceae | Leaf |
| *Leucaena leucocephala* | White leadtree | Collected | Fabaceae | Leaf<br>Seed |
| *Mimosa caesalpiniaefolia* | Sabiá | Collected | Fabaceae | Leaf |
| *Nerium oleander* | Oleander | Purchased | Apocynaceae | Leaf |
| *Piper aduncum* | Spiked pepper | Purchased | Piperaceae | Leaf |
| *Syzygium cumini* | Java plum | Collected | Myrtaceae | Leaf<br>Endocarp<br>Pericarp<br>(Endocarp+Pericarp) |
| *Syzygium malaccense* | Malaysian apple | Collected | Myrtaceae | Leaf |
| *Zingiber officinale* | Ginger | Purchased | Zingiberaceae | Rizome |
| *Ziziphus joazeiro* | Juazeiro | Collected | Rhamnaceae | Leaf |


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the insect population. Every six months, the entire population is renewed by natural infestation.

### Bioassays

**Plant model and aphid infestation.** The *Vigna unguiculata* (L.) Walp. plants were grown in the greenhouse. Two seeds of *V. unguiculata* beans were sown in 300 mL plastic cups, drilled at their bottoms to allow drainage, and filled with substrate consisting of sieved sand (60%), tanned manure (30%) and vermiculite (10%). Five days after sowing, seedlings were thinned, leaving one plant per cup. Eleven days after sowing, the plants were used in the experiments, providing food to aphids and this set was then considered a plot.

The level of infestation was based on the following rating scale: high infestation (main branch petiole totally taken up by the insect), moderately high (main branch petiole infested but not fully covered with insects), medium (few insects in the main branch) and low (insects only on petioles).

The application of the thirty treatments, the positive control (*A. squamosa*) and the absolute control (distilled water) was made using a gravity spray gun (ARPREX®, model-5), coupled to an air compressor. Forty-eight hours after the application of the treatments, the evaluation of the surviving nymphs was performed by counting the number of live nymphs.

For the application of the plant extracts, cowpea plants were manually infested with five *A. craccivora* adult females, eleven days after sowing. Forty-eight hours after the infestation, the adult females were eliminated and the plants were separated into blocks based on the level of infestation observed through visual inspection.

The positive control used was *A. squamosa* seed extract. This extract was chosen due to the reported efficiency of more than 95% against *A. craccivora* Koch (Rabelo & Bleicher 2014).

The containers of both the treatments and the positive control were closed and wrapped in aluminum foil to avoid light, given the photoinstability of the active ingredients. After resting for 24 h, the solutions were filtered and diluted, yielding hydroalcoholic solutions at 5% and 0.5% concentration, respectively.

**Statistical Analysis.** The data obtained were then converted through the equation $\sqrt{x + 0,5}$. Treatments were compared through variance analysis, with means submitted to the Tukey test at 5% probability. Abbot's formula (1925) was used for the calculation of the relative efficiency.

## RESULTS AND DISCUSSION

The relative efficiency of the extracts is displayed in Table 2. The extracts that significantly differed from the absolute control were *Allium sativum*, *Allium tuberosum, Anacardium ocidentale, Annona squamosa, Borreria verticillata, Caesalpinia ferrea, Carica papaya, Coffea Arabica, Cucurbita moschata, Dieffenbachia picta, Euphorbia tirucalli, Leucaena leucocephala, Nerium oleander, Piper aduncum, Syzygium cumini* leaf and endocarp + pericarp, *Syzygium malaccense, Ziginber officinale* and *Ziziphus joazeiro* (Table 2).

Statistical differences were observed between the extracts of different structures of the *S. cumini* species, in which the leaf presented higher biocidal potential compared to the endocarp and pericarp. The different structures of *S. cumini*, given the statistical differences among structures, possibly present different concentrations or different secondary metabolites throughout the different plant parts (Cirak *et al.* 2012).

Extracts that presented efficiency lower than 50% might also have toxic substances, but not at a sufficient concentration to control the target pest. However, among these toxic substances there may be some substance with high toxicity, but in low concentration. Thus, it would be interesting to verify the constituents of each plant part, to analyze its toxicity and, thus, to evaluate its potential participation in commercial formulations of pesticides.

Among the extracts that differed from the absolute control, the following presented efficiency below 50%: *A. sativum* bulb, *A. occidentale* leaf, *B. verticillata* leaf + flower, *C. arabica* seed and leaf, *E. tirucalli* leaf, *L. leucocephala* leaf and seed, *N. oleander* leaf, *S. cumini* leaf, *S. malaccense* leaf, *Z. officinale* rhizome and *Z. joazeiro* leaf (Table 2).

The insecticidal activity of *Syzygium* sp. essential oils has been studied against *Sitophilus zeamais* Mots. and was found to be significant and concentration dependent (Lawal *et al.* 2014).

The plants: *A. sativum*, *C. arabica*, *N. oleander*, *Z. officinale* and *Z. joazeiro*, are already used in Brazilian popular medicine (Sousa *et al*. 2004) and, therefore, have chemical constituents that may have biocidal activity on the pest.

*C. arabica* parchment crude extracts have dual effects against *Tribolium castaneum* (H.) adults, i.e., fumigant toxicity and repellent effects and the active compounds responsible for the two activities are different (Phankaen 2017).

*N. oleander* ethanolic extract had toxic effects on the larvae of culicid mosquitoes (El-Akhal, 2015).

Many of the insecticidal properties of ginger are ascribed to the presence of compounds in the rhizomes known as 'oleoresisns' especially those known as gingerols and shogoals (pungent component) (Sekiwa *et al.* 2000).

Regarding *Z. joazeiro*, due to its toxicity for various organisms, saponins can be used as an insecticide, antibiotic, fungicide, in addition to having pharmacological properties (Castejon, 2011).

Besides these, *A. occidentale* also have medicinal properties (Matos 2002). However, in the case of *A. occidentale*, substances with medicinal properties are present in the seed coat instead of the leaves (Matos 2002) which may be the reason for the lack of effectiveness of this plant in the control of the black aphid, since in the present study the part that was used for the preparation of the extract was the leaf.

*Allium sativum* extracts are already used in pest control. In the United States there are even commercial products available for the control of some pests (Prakash & Rao 1997). The application of garlic extract substantially reduced the populations of the cowpea pests *Clavigralla tomentosicollis* Stål (Hemiptera: Coreidae) and *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) (Ahmed *et al.* 2009). Murugesan & Murugesh (2008) tested different botanical extracts, including garlic extract, which showed a significant reduction in the population of *Henosepilachna vigintioctopunctata* (Fabricius) (Hadda beetle) after the third day of spraying. Extracts made from the garlic bulb have organosulphurous compounds, such as diallyl disulphite, with insecticidal activity (Thomas & Callaghan 1999). However, in this study, garlic extract did not show significant efficiency against *A craccivora.*

Both *N. oleander* and *E. tirucalli* leaves are listed as toxic to humans and animals due to their latex (Matos *et al.* 2011). However, in this study, their efficiency was less than 50%. This may have been due to the large exudation of latex (which is the toxic substance) during the fragmentation of the material for drying.

**Table 2.** Botanical species, morphological structures utilized and the effectiveness of botanical extracts on controlling Aphis craccivora nymphs.

| Botanical species | Organ used | Statistical difference | Relative efficiency (%) |
|---|---|---|---|
| Distilled water | - | - | 0.06 |
| *Allium sativum* | Bulb | D[1/] | 25.98 |
| *Allium tuberosum* | Leaf | D | 64.96 |
| *Anacardium occidentale* | Leaf | D | 36.41 |
| *Annona squamosa* | Seed | D | 96.07-99.6 |
| *Borreria verticillata* | (Leaf+flower) | D | 39.86 |
| *Caesalpinia ferrea* | Leaf | D | 51.71 |
| *Carica papaya* | Seed | D | 63.58 |
| *Coffea arabica* | Seed<br>Leaf | D | 40.15<br>33.78 |
| *Cucurbita moschata* | Seed | D | 91.14 |
| *Delonix regia* | Leaf<br>Flower<br>Seed | ND<br>ND<br>ND | 6.24<br>9.47<br>6.91 |
| *Dieffenbachia picta* | Leaf | D | 84.30 |
| *Euphorbia tirucalli* | Leaf | D | 37.99 |
| *Leucaena leucocephala* | Leaf<br>Seed | D | 11.09<br>32.79 |
| *Mimosa caesalpiniaefolia* | Leaf | ND | 18.51 |
| *Nerium oleander* | Leaf | D | 43.46 |
| *Piper aduncum* | Leaf | D | 61.31 |
| *Syzygium cumini* | Leaf<br>Endocarp<br>Pericarp<br>(Endocarp+Pericarp) | D<br>ND<br>ND<br>D | 44.63<br>20.63<br>16.27<br>25.83 |
| *Syzygium malaccense* | Leaf | D | 27.53 |
| *Zingiber officinale* | Rizome | D | 45.47 |
| *Ziziphus joazeiro* | Leaf | D | 42.42 |

1/ = The treatments statistically either differ (D) or do not differ (ND) from the absolute control treatment according to the Tukey test at 5% level of probability.

The use of *L. leucocephala* leaves presented good results in the control of whitefly nymphs [*Bemisia tabaci* (Genn.)] (Hemiptera: Aleyrodidae), 75% efficiency in some treatments, thus, being considered a promising botanical insecticide (Cavalcante *et al.* 2006). However, for the control of *A. craccivora*, extracts from both the leaves and the seeds presented efficiency below 50%. Therefore, it can be deduced that the insecticidal activity of the *L. leucocephala* leaf extract is species specific.

The botanical extracts with insecticidal efficiency above 50% and below 80% were: *A. tuberosum* leaf, *C. ferrea* leaf, *P. aduncum* leaf and *C. papaya* seed. None of these plant species has a consecrated use in popular medicine. There is no literature on the efficiency of *A. tuberosum*, *C. ferrea* and *C. papaya* for the control of pests, but in the present study, at a concentration of 5%, these plant species showed potential in fighting *A. craccivora*. The use of *P. aduncum* oil presented promising results against *Tenebrio molitor* L. larvae (Coleoptera: Tenebrionidae) (Fazolin *et al.* 2007). At an interval of 48 hours, leaf and root extracts with 30 mg $mL^{-1}$ concentration induced mortality of *Aetalion* sp. (Hemiptera: Aetalionidae) 72% and 80%, respectively (Silva *et al.* 2007). This indicates that the plant has potential for use in pest control, both with the use of essential oil, leaf and root extract.

On the other hand, the ethanolic extract of *D. picta* leaf is in the range between 80% and 90% of death-related efficiency of *A. craccivora* nymphs. Over 90% efficiency was obtained from *C. moschata* seed extracts, comparable to the positive control treatment, *A. squamosa* seed (Table 2). *D. picta* is highly toxic to both humans and animals (Matos *et al.* 2011), but there are no studies on its toxicity on insects. No literature was found on the use of *C. moschata* as an insecticide, however, at a concentration of 5%, the seed of this plant species proved to be very promising in fighting *A. craccivora*. The extracts of *D. picta* and *C. moschata*, which showed high efficiency under greenhouse conditions, should be studied also under field conditions to make it feasible the recommendation of using the extracts in the farm.

Only the ethanolic extracts of: *Delonix regia* leaf, flower and seed; *Mimosa caesalpiniaefolia* leaf; and *Syzygium cumini* endocarp and pericarp did not differ statistically from the absolute control (distilled water). Therefore, these extracts do not hold potential to be used against *A. craccivora*. However, the effectiveness of these extracts should be evaluated against other pests.

For the extracts with efficiency above 80%, it should be taken into account the availability of the used parts of these vegetables for their direct use in agriculture. *D. picta*, being an ornamental plant, is not widely available for immediate use. Therefore, it is necessary to study the viability of planting these species on farm. As the used parts of *C. moschata* are the seeds, there isn't also a large volume of seed available for immediate use.

The insecticidal activity was found in the extracts from different morphological structures of the plants.

The highest mortality rates were found by using, *D. picta* and *C. moschata.*

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