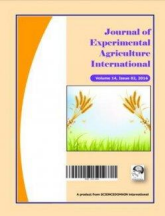

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Effect of Neem Powder (*Azadirachta indica* A. Juss) on the Control of Cowpea Weevils [*Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)] in Cowpea Beans

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Authors' contributions

This work was carried out in collaboration between all authors. Author EPSN carried out the research in the laboratory and wrote the text with the authors ABS and JLGS. Authors ABAA, EMC, PBM and TAP contributed with the statistical analysis and as advisors for the writing and bibliographic revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The cowpea weevils [*Callosobruchus maculatus* (F.)] are the primary pest affecting grain and seeds of stored cowpea beans. The control of this insect comprises expensive methods such as fumigation or spraying of chemicals, which are unfeasible for small farmers. The use of insecticidal plants, such as the neem tree (*Azadirachta indica*), may stand out as a cheaper alternative. This study evaluates the bioactivity of neem powder on the control of weevils in cowpea seeds. We tested four types of powders according to the part of the plant from which it originated: leaves, fruits, bark, and the mixture of these three parts in the same proportion. The bioassay of the action spectrum and the

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insecticidal effects were assessed using four doses of each type of powder: 0.25%, 0.50%, 0.75%, and 1.00% per 20g of beans. The fruit powder repelled weevils at the lower doses used, while leaf powder, bark, and the mixture were neutral. Although neem powder reduced the survival of insects, the reduction was slow, showing mild toxicity. Neem powder may be an alternative for the control of cowpea weevils in storage units. However, the efficiency of the control depends on the part of the plant and dosage used.

Keywords: Vigna unguiculata; alternative control; insecticidal plants; bioactivity.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] comprises an essential food source in the tropics and subtropics, mainly in Africa, Central America, and South America [1,2]. The northern and northeastern region of Brazil leads the national cowpea production, where family farmers cultivate these beans in subsistence agricultural systems. Cowpea is a low-cost food supply, rich in proteins and essential amino acids [3].

Among the phytosanitary problems affecting cowpea, the pest insects both attack the crop in the field and damage stored grains and seeds. The cowpea weevil [*Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)] is the primary storage pest of cowpea, with widespread worldwide occurrence [4,5].

The infestations of weevil in cowpea compromise seeds viability, grains physiology, and its nutritional quality, as well as contaminate the product with excrement. Such problems cause qualitative and quantitative losses through, which reduces beans commercial value. Cowpea weevil causes annual losses between 30 and 50% and sometimes above 90% [6,7,8,9,10].

The control of cowpea weevil has been carried out by fumigation or spraying with chemicals of different toxicological classes. Synthetic insecticides are expensive for small farmers and require equipment and training for their use [11]. The massive use of these products in recent years has driven to many problems, such as the emergence of resistant populations and high amount of insecticides residues in foodstuffs, which harm consumers' health and the environment [7,12].

In addition to the problems mentioned above, many producers, especially in family farms, neglect control the weevil due to lack of financial resources. In this scenario, the use of insecticidal plants stands out as a promising alternative for weevil control since these plants usually have low cost, easy application, biodegradability, and may be available on the producer's property [13,14,15].

Among the promising vegetable species for the control of cowpea weevil, products derived from neem (*Azadirachta indica A. Juss*) stand out because they contain substances, especially Azadirachtin, that act as an insecticide [16]. Neem leaf powder caused increased adult mortality of weevil in cowpea seeds [17], without causing changes in the viability characteristics of the seeds [18,19]. However, there are still few studies evaluating the effect of powders made from different parts of the neem tree on the mortality of cowpea weevil.

Given the above, this work aimed to evaluate the bioactivity of the powder of different parts of the neem plant in the control of adult cowpea weevils in stored seeds of cowpea.

2. MATERIALS AND METHODS

The study was carried out at the Laboratory of Entomology of the Agriculture Sciences Academic Unit (UAGRA) of the Center of Agrifood Science and Technology of the Federal University of Campina Grande (CCTA-UFCG), Campus of Pombal, Paraíba. The experiment occurred under controlled conditions of temperature (32 ± 2°C) and relative humidity (70 ± 5%).

Cowpea weevils used in the bioassays were reared in the Laboratory of Entomology following the methodology of Freire et al. [20]. The insects were kept in glass cages with a capacity of 1.5 liters (21.0 x 10.5 x 10.5 cm), top coated with anti aphid screen, containing cowpea 'Canapu' seeds.

Leaves, fruits, and bark were collected from neem plants in the CCTA-UFCG (6°48'16"S; 37°49'15"W; 144 m of altitude). The material was packed in kraft paper bags and dried in a forced air circulation oven at 40°C for 48 h. After that,

the different parts of the plant were crushed separately in a food processor and sieved (0.5 mm mesh) until the production of powder with uniform granulometry.

The action spectrum bioassay was carried out to verify the behavior index of cowpea weevils relative to the presence of neem powder. We used multiple-choice experimental arenas consisting of six plastic containers with 10 cm in diameter and 4 cm high (Fig. 1) [21]. The set comprised a central container symmetrically interconnected by 0.5 cm diameter plastic tubes to another five diagonally arranged containers. We released 50 non-sexed adult insects in the central container. The peripheral containers contained 20 g of cowpea 'Canapu' with the powders at 0.0% (control), 0.25%, 0.50%, 0.75%, and 1.00% dosages. Each assay tested one type of powder, leaves, fruits, bark, and leaf+fruit+bark (proportion 1:1:1), in three replicates.

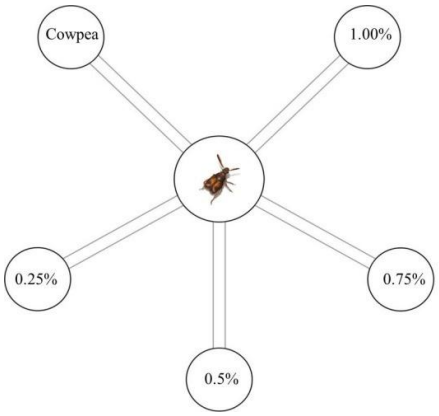

Fig. 1. Experimental arenas used in the action spectrum bioassay

We counted the live and dead insects in each container after 24, 48, and 72 hours from the begging of the experiment. At the end of each count, the dead insects were removed from the container and discarded. The Behavior Index (BI) was used to compare treatments according to the following equation: BI = (% of insects in the test-plant - % of insects in control) / (% of insects in the test-plant + % of insects in control). When BI lies between -1.00 and -0.10 the plant is a repellent, a BI between -0.10 and +0.10 indicates a neutral effect and a BI between +0.10 and +1.00 an attractive effect [21].

Evaluations of the insecticidal effect of the powders followed the same experimental design described above (four types of powder at the four concentrations, and one control). Each treatment was performed in 4 replicates. The insects were exposed to the treatments in round plastic arenas of 500 mL (120 mm diameter and 78 mm height) containing 20 g of cowpea inside. The upper part of the containers was perforated for air circulation. Twenty adult insects were released in each container, evaluating mortality and behavior every 24 hours until all insects died.

For the analysis of the insecticidal action of the powder, we elaborated curves showing the mortality of the insects over time by the Kaplan-Meier method with application of the non-parametric Log-Rank Test to compare the curves and the mean lethal time for the death of 50% of the insects was estimated using non-linear regression models in the GraphPad Prism®6 software [22].

3. RESULTS AND DISCUSSION

The neem fruit powder repelled weevils in cowpea seeds under all doses (Fig. 2B). The use of products with repellent effects to control cowpea weevils comprises a primary technique in the management of this pest. Considering that the attractive odor of an alcohol (2-Ethylhexanol) present in cowpea mediates the preference of cowpea weevils [23], the neem fruit powder may act confusing the insect perception or emitting an unattractive odor.

Several studies report repellent effects against cowpea weevil cause by some plant species of the Caatinga Brazilian ecoregion such as *Amburana cearensis* A. C. Smith, *Croton sonderianus* Müll. Arg., *Cleome spinosa* Jacq., *Mimosa tenuiflora* Benth., *Anadenanthera macrocarpa* (Benth.) Brenan, *Aspidosperma pyrifolium* Mart., *Senna occidentalis* (L.) H.S. Irwin & R.C. Barneby, *Hyptis suaveolens* (L.) Poit., and *Ziziphus joazeiro* Mart. [15], showing the potential of these plant products as an alternative control of this pest.

The powders made from leaves, bark and the mixture had a neutral effect in most doses, with no potential for insect repellency or attraction, especially at lower doses (Fig. 2).

Boeke et al. [24], treating cowpea with leaf powder of neem in the proportion 5g/kg, found an attractive effect on the weevil, which opposes our neutral result.

Schumacher et al. [25] state that botanical bioactivity on insects can have attractive and insecticide effects at the same time, while others can be repellent and do not cause an insecticidal effect. However, an ideal product should repel and kill the insects, because the repellent effect decreases the oviposition and consequently the number of insects that will hatch, and still cause a substantial decrease in the pest population through the insecticidal action.

The evaluation of insecticidal action of neem powder resulted in significant differences ($P < 0.01$) in the comparison between the mortality curves of all doses with the control treatment, even though in some doses the observed difference was unexpressive (Table 1; Fig. 3).

The leaves powder at 1.0% (10 g/kg) provided the total death of insects in 144 hours (6 days), the shortest time recorded but not showing immediate action of toxicity (Fig. 3). In the control treatments, the longest survival time was 288 hours (12 days). In the insecticidal activity of neem powder on cowpea weevils, the mortality time is dose-dependent, the highest being 10 g/kg. The neem powder efficiency on the mortality of this pest was observed by Silva et al. [26] with the use of 150 g/kg. Tofel et al. [27], using a dose of 83.27 g/kg, recorded mortality of 50% of the weevil population in 3 days.

The powder of the leaves from *Solanum melongena* and *Capsicum annuum* promoted the death of all weevils in 120 hours (5 days). The researches look for products that cause insect mortality as soon as possible so that population decrease occurs and hinders oviposition [20].

For the mortality of 50% of the insect population (TL50), the use of different parts of neem in the powder caused similar results, with the highlight only for the treatment with the mixture (Leaves + Fruits + Bark) that had a faster action at the concentration of 0.75% (7.5 g/kg) with the time of 60 hours (2.5 days), while the control had TL50 of 114 hours (5 days) (Fig. 3). Thus, showing that the use of powder from other parts of neem, besides the leaves, may contribute to the management of cowpea weevil, as the protection by powders of seeds and roots [28].

The neem tree, through the use powders from leaves, fruits, and bark comprises an alternative for the management of cowpea weevil in storages, but the plant part and the doses used are decisive for efficiency in the control.

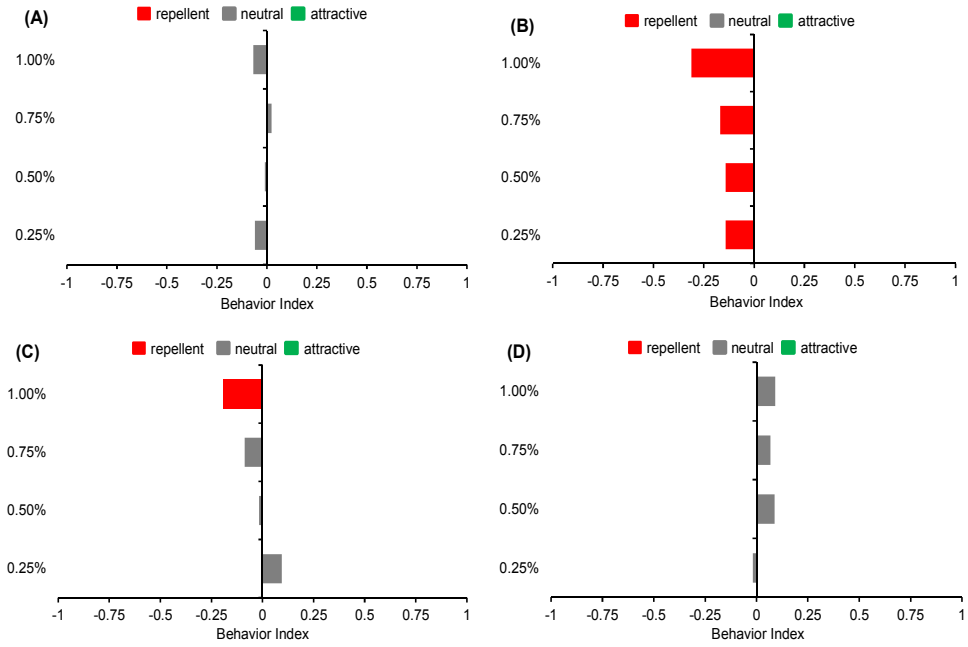

Fig. 2. Behavior of cowpea weevil (*Callosobruchus maculatus*) on cowpea seeds with increasing doses of the powder made from the following parts of the neem tree (*Azadirachta indica*). (A) Leaves; (B) Fruits; (C) Bark; (D) Mixture (Leaves + Fruit + Bark)

Table 1. Significant variation between mortality curves of cowpea weevil between control treatment and use of neem parts powder

Parts	Comparison with control mortality curve	DF	Chi-square	P value
Leaves	0.25%	1	16.03	<0.0001**
	0.50%	1	19.89	<0.0001**
	0.75%	1	14.31	0.0002**
	1.0%	1	36.21	<0.0001**
Fruits	0.25%	1	22.85	< 0.0001**
	0.50%	1	9.119	0.0025**
	0.75%	1	13.54	0.0002**
	1.0%	1	10.82	0.0010**
Bark	0.25%	1	11.10	0.0009**
	0.50%	1	7.472	0.0063**
	0.75%	1	8.645	0.0033**
	1.0%	1	14.46	0.0001**
Mixture (leaves + fruits + bark)	0.25%	1	17.86	< 0.0001**
	0.50%	1	21.60	< 0.0001**
	0.75%	1	32.55	< 0.0001**
	1.0%	1	18.01	< 0.0001**

*DF: Degree of freedom. **significant value at the 1% probability level by the non-parametric Log-Rank Test*

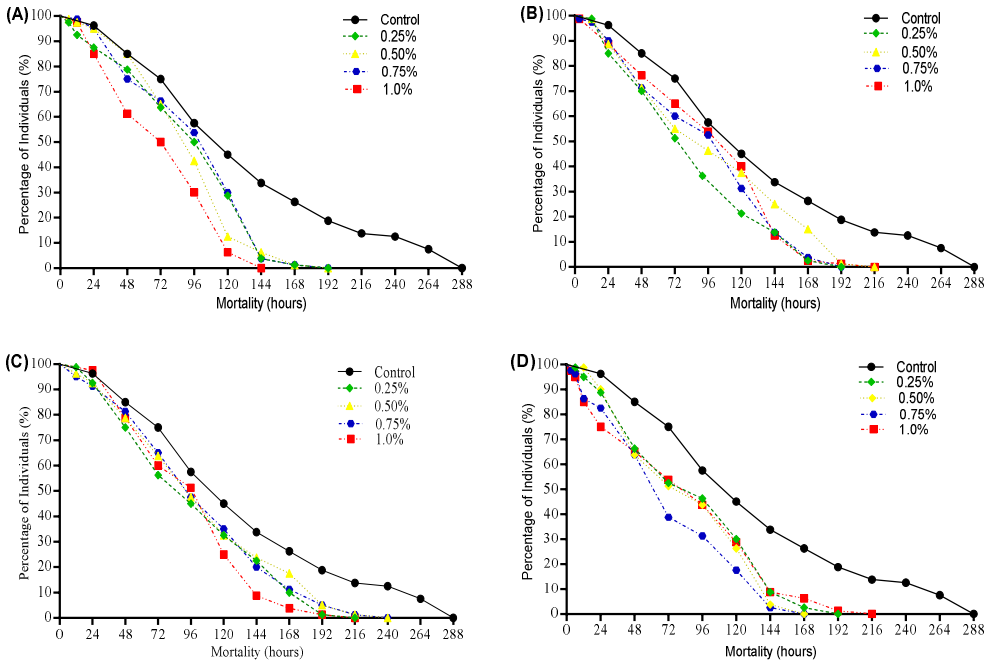

Fig. 3. Mortality curves of cowpea weevil (*Callosobruchus maculatus*) on cowpea beans treated with neem powder tree (*Azadirachta indica*) in increasing doses. (A) Leaves; (B) Fruits; (C) Bark; (D) Mixture (Leaves + Fruits + Bark)

4. CONCLUSION

The powder from neem fruit repels cowpea weevils, and the powder from fruit, leaves, and bark has an insecticide action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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