Multidisciplinary

A study on insecticidal activity of neem tree (*Azadirachta indica*) leaf-extract against cowpea weevils (*Callosobrucus maculatus*)

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Abstract

The incessant problems encountered with over reliance on synthetic insecticides have necessitated the search for alternative control strategies which are safe, effective and affordable. This study investigated the bioactivity of neem (*Azadirachta indica* A. Juss.) leaf- extract against the storage of cowpea (*Callosobruchus maculates* F.), under laboratory conditions. The weevils were reared on cowpea seeds treated with aqueous leaf extracts at concentrations; 25ppm, 50ppm, 75ppm and 100ppm to compare toxicity, anti-oviposition effect. After 24hours of exposure, observed mortality was 2.33±0.58, 3.33±0.58, 4.33± 0.58 and 5.33±0.58. It was observed that, the female weevils reared with the treated extract laid significantly fewer eggs compared to the one in the control. The action was dose dependent (the higher the concentration the less the eggs laid). The extract did not alter the sex ratio of the insect pest. The results from this study showed that, neem leaf extract is effective in controlling *C. maculates* and could, therefore, serve as an alternative to synthetic insecticides in controlling the storage pest of cowpea.

Keywords: neem, cowpea, weevil and anti-oviposition

Introduction

Cowpea, *Vigna unguiculata* (L.) Walp., was described by [9] and [11] as a staple food for many people in several parts of Africa and is valued as a nutritional supplement to cereals. They further concluded that, it consists of 23-25% protein, 1.9% fat, 6.3% fibre and 67% carbohydrate in addition, [12] stated that, cowpea contain calcium, iron, vitamins and carotene.

In 2017, Africa produced 96% of the world's cowpea, where Nigeria produced (46%) and Niger (26%) predominating world's production. The major storage constraints in Nigeria and other tropical countries are the insect and vertebrate pests [1]. Among these, the cowpea weevil, *Callosobruchus maculatus* (F.), is the most important pest and it is capable of consuming 50- 90% yield in storage annually. It was reported that, in Nigeria, the dry weight loss due to *C. maculatus* exceed 2,900 t annually [3]. The continuous applications of synthetic insecticides such liquid or powder forms as measure of insect pest management [21]. Although is effective, their constant use has obstructed natural competitors and controlled the eruptions of some insect species, it resulted in the development of resistance, had adverse effects on non-target organisms, and caused environmental and human health concerns [13]. Botanical insecticides are becoming a more popular alternative to synthetic insecticides [2]. Neem contains several active ingredients, and they act in different ways under different circumstances. These compounds bear no resemblance to the chemicals in today's synthetic insecticides [16].

Almost every part of the tree (root, trunk bark, flowers, fruits and seeds) is known to have some uses [5]. *A. indica* elicit a variety of effects in insect such as anti-feedant, growth retardation, reduced fecundity, molting disorders, morphogenic defects and changes of behaviour and large alteration of fecundity [8, 20]. Petroleum ether of *A. indica* was observed and showed high mortality against the larvae of culex species at all the concentration used [17].

In view of the role of cowpea weevil, *Callosobruchus maculatus* as a pest cowpea and toxicidal hazards of chemical insecticides on the non-target organisms and the environment, the present study has been planned to determine the insecticidal activity of leaf-extracts of *A. indica*, by assessing their effects on the development of the pest.

Materials and methods

Study area

This study was conducted in Aliero, Kebbi State. Aliero Local Government shares common borders with Gwandu Local Government Area on the North-east; Jega Local Government Area on the South-east, on the East is Tambuwal Local Government Area of Sokoto State, while the North-east is bordered by Birnin Kebbi Local Government Area. The topography is fast and slightly undulating with compact, stony brown soil. Aliero has savannah vegetation, and is located in the North Western Nigeria on Latitude of 1130S, 12.440N, and Longitude of 360W, 420E. The dry season in Aliero is from November to April and raining season from May to October. The main occupation of the people in Aliero Area is opinion farming and rearing of domestic animals, which serve as their main source of income.

Insect rearing

Adult cowpea weevils were collected from naturally infested cowpea seeds bought at the Aliero market, in Kebbi State, Nigeria. The insects were reared on uninfected cowpea seeds

in the zoology Laboratory of the Department of Environmental and Animal Biology, Kebbi State University of Science and Technology under laboratory condition and 12:12 light: dark photoperiod. Weevil rearing continued to F2 generation to ensure a pure culture before newly emerged adults was used for experiments.

Preparation of plant samples and leaf extraction

Young neem leaves were collected within Kebbi State University of Science and Technology, Aleiro (KSUSTA) and were air-dried on a shaded platform to prevent degradation by direct sunlight. The dried leaves were chopped into small bits and milled mechanically into fine powder.

Aqueous extraction was also made from another 500 g of the material by boiling the leaf powder in water for a few seconds. The filtrate was then transferred into a rotary evaporator to remove the solvent; the concentrated extract was air-dried and kept in a fridge until needed for experiments.

Toxicity test

Prior to the test, healthy but susceptible cowpea seeds were stored in a deep freezer for 72 h to ensure elimination of arthropods present in the pack. Disinfested cowpea seeds (10 g) were treated in triplicate Petri dishes with 2 ml each of 25, 50, 75, and 100 concentration aqueous extract, untreated seeds will serve as positive and negative control experiments, respectively**.** The cowpea seeds were thoroughly mixed with the extracts to ensure uniform coating and air-dried, allowing the solvent to evaporate completely, before ten ≤ 36 h old adult *C. maculatus* were introduced into each Petri dish. All treatments were arranged using a completely randomized design and each Petri dish were monitored for insect mortality at 1, 6, 12, 18 and 24 h post-exposure.

Anti-oviposition test

Ten grams of disinfested cowpea seeds were subjected to different treatments as described for toxicity test and ten (2♀: 8♂) ≤ 36 h old adult weevils were introduced into each Petri dish. The weight of treated seeds inside each Petri dish (i.e. weight of seeds + weight of coating) was determined before introducing the weevils. All treatments were arranged in Completely Randomized Block Design and replicated three times. The Petri dishes were monitored daily and insect mortality were recorded. Oviposition females glue eggs on the surface of cowpea seeds and the number of eggs laid on each seed as well as total number of eggs per Petri dish were counted carefully using a magnifying lens. Percent oviposition deterrence (OD) and oviposition activity index (OAI) [15] were calculated thus; Percent oviposition deterrence (%OD) is calculated as follows:

$$\% \text{ OD} = \frac{\text{No. of eggs laid on control - No. of eggs laid on treated seeds}}{\text{No. of eggs laid on control}}$$

Oviposition activity index (OAI):

$$\text{OAI} = \frac{\text{No. of eggs laid on treated-No. of eggs laid on control seed}}{\text{No. of eggs laid on treated-No. of eggs laid on control}}$$

Statistical analysis

All the treatments obtained was arranged by using completely Randomized Block Design and replicated three times.

Results

Toxicidal effect of neem extracts on *C. mamulatus*

The results obtained from table one, shows that at 25% concentration of the extracts within the first 1hr no mortality was recorded, however after 6hrs one of the insects was observed dead, after 24hrs an average number of 2.33±0.58 mortality was observed.

At 50% concentration of the extracts, the results were almost similar with the one obtained at 25% of the extracts, the only difference was that after 24hrs, the mortality was a little bit higher (3.33±058) than the one obtained at 25%.

The results obtained at 75% concentration of the extracts at first 1hr, no mortality was recorded, it is after 6hrs that (1.00±1.00) mortality was observed. The highest mortality in this concentration was recorded after 24hrs (4.33±0.58)

At 100% concentration of the extracts, 6hrs after treatment (2.00±1.00) mortality was recorded. However, at the same concentration, out of the ten insects introduced, an average of (5.33±0.58) was observed dead. Table 1 the mortality percentage observed after insects were exposed to different concentrations of the extracts was dose (conc.) and time related. As the concentration and time of application increases, the mortality of the insects also increased. When the insects were exposed to different concentrations of the extracts, female fecundity was also monitored laid. For clarity the results are on table 3.

Table 1: Mortality of cowpea weevils after exposure to different concentration of aqeous neem leaf extracts

	Period (h) post exposure				
No. of % leaf extract (PPM)	**1hr**	**6hr**	**12hr**	**18hr**	**24hr**
25	0.00±0.00	1.00±1.73^{a}	1.00±1.73^{a}	1.00±1.73^{a}	2.33±0.58^{a}
50	0.00±0.00	1.00±1.00^{a}	0.67±1.15^{a}	1.33±0.58^{a}	3.33±0.58^{a}
75	0.00±0.00	1.00±1.00^{a}	1.33±0.58^{a}	1.67±0.58^{a}	4.33±0.58^{b}
100	0.00±0.00	2.00±1.00^{a}	2.33±1.15^{a}	2.67±0.58^{a}	5.33±0.58^{c}
Control	0.00±0.00	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{d}

Note: Data obtained in the form of mean± standard deviation at P≥0.05

Table 2: Percentage mortality of insects after application

Concentration (%)	No. of insect introduced	No. of dead after application	Percentage (%) mortality
25	10	2.33	23.3
50	10	3.33	33.3
75	10	4.33	43.3
100	10	5.33	53.3
Control	10	0.00	0.00

Note: Data obtained in the form of mean± standard deviation at P≥0.05

Table 3: Oviposition test after application of the extract

Extract Concentration % (PPM)	No. of eggs laid
25	27.50 ± 2.121^{a}
50	20.00 ± 1.414^{b}
75	18.00 ± 1.414^{c}
100	15.50 ± 2.121^{d}
Control	32.00 ± 1.414^{e}

Note: Data obtained in the form of mean± standard deviation at P≥0.05

Discussion

The results obtained in this study have shown that the leaf extracts could be used to protect Cowpea seeds in storage with a significant reduction in yield loss. This outcome is lending credence to previous numerous workers [1, 6, 7, 10] who established insecticidal properties of different plants and advocated the use of botanical insecticides in place of synthetic chemicals.

The higher efficacy of leaf extracts can be attributed to the fact that the volatile solvent was able to extract more active ingredient such as azadirachtin from the neem leaves. Also, the active ingredients of neem increase insecticidal efficacy with increasing concentration and time of insects exposure is in agreement with the report of [14, 19].

The toxicity is determined by the proportion of active fraction in a given volume of plant extract and this would invariably increase with extract concentration; a longer period of exposure would also increase the risk of poisoning.

Conclusion

The results obtained from the present study confirmed that neem leaf extracts are effective in controlling the storage pest of Cowpea. This could serve as an alternative to synthetic insecticides thereby reducing the environmental risk associated with the commercial products.

Further Research should carried out on various plants in order investigate the insecticidal properties found on it.

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