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Entomotoxic effect of tobacco seed extracted with different solvents against *Callosobruchus maculatus* infesting stored cowpea

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Abstract
Methanol, ethanol, acetone, pet-ether, n-hexane and aqueous extracts from *Nicotiana tabacum* seeds were investigated for their Entomotoxic effect against *Callosobruchus maculatus*. The potency of these extracts as grain protectant against *C. maculatus* was investigated using mortality, oviposition, adult emergence and viability as indices. The ability of the extracts to protect the seeds from damaging after long time of storage was also investigated. The result showed that pet-ether, n-hexane and aqueous extracts were very effective, causing 100% mortality of adult *C. maculatus* by 96 h of exposure to the extracts. While the other extracts (methanol, ethanol, and acetone) showed 80%, 85.20% and 70.15% mortality respectively. The extracts considerably reduced oviposition and adult emergence to the extent that no adult emerged in cowpea seeds treated with pet-ether and n-hexane extracts. All the extracts drastically reduced seed damage during storage for a period of 3 months. No damage was observed in seeds treated with acetone, pet-ether and n-hexane extracts. Most of the treated seeds also germinated after storage for a period of 90 days. The result of this investigation showed that all the extracts from tobacco seeds are very effective in controlling *C. maculatus*.

Keywords: *Callosobruchus maculatus, Nicotiana tabacum* seed, solvents, seed damage, germinability.

Introduction

The major concern among the governments of countries of the world is how to feed the world ever increasing population. In the world at large, statistics have shown that one out of seven people are living without insufficient food. Adamu [1] as well as Oni *et al*. [2] reported that more than 54.6 million Nigerians go to bed without food. This insufficient food among the populations of the world is posting a lot of threat on the national security of many countries of the world. In fact, Oni *et al*., [2] opined that the national security of any country is directly proportional of sufficiency of food among the citizen of the country. Therefore, there could be no national security without food security. Moreover, growth in agriculture sector of the countries of the world has been suggested as the way out to the insufficient food among the populations of the world [3]. In actual fact, Adeyemo *et al*., [4] as well as Oni and Ogungbite [5] stated that the development of any country is directly proportional to the achievement of her agriculture sector. Nevertheless, the negligence of governments of many African countries toward the development of agriculture in their countries has resulted to high food insecurity among their citizens.

In recent years, government of countries as well as individual are giving attention to agriculture but a lot of problems among which insect infestation play vital role have been impeding the large production and storage of agricultural produce and products [6-9]. Dubey *et al*. [10] as well as Rajashekar and Shivanandappa [11] reported that more than 5-10% and 20-30% grains loss in the temperate and tropical regions of the respectively are due to insect infestation. Cowpea being one of the major staple foods highly produced in Nigeria and other West African countries is facing a challenge of infestation by many insects pest. *Callosobruchus maculatus* is a popular pest of cowpea and its multivotine type of reproduction is aggravating its attack on cowpea [3]. This insect capable of infesting cowpea both on the field and in storage where its evil deed is more pronounced. In fact the insect has been reported of its capability in causing more than 50-90% postharvest loss in cowpea [9, 12, 13].

In yesteryears, the control of this ill-famed insect pest and other agricultural insect pests is heavily relied on the use of synthetic chemical insecticides which have been reported of being linked with many cons denting their image among the pest managers of the world as well as general public [14]. Their effect on both human and environmental health has made the government of many developed countries to ban their use [15, 16]. Therefore, the search for alternative methods of controlling insect and related pest becomes of major interest among the world entomologist and pest managers probably because of the public knowledge of the perils associated with chemical insecticides.

Before the discovery of my popular and successful insecticides in early 1930s to late 1940s, plants and their derivatives has been the major weapon in famers armory. Therefore, researches have been shifted toward the use of plant and plant products in the control of insect pests. More so that plants are believed to contain myriads of allelochemicals that could have antifeedant, repellent and other insecticidal property against insects [17-19]. In spite of the effectiveness of many botanical extracts, the solvent used for their extraction are believed to have significant effect on their potency as reported by Ashamo and Ogungbite [20]. So, it is necessary to find the best method of extracting the botanical oils. Tobacco seed (*Nicotiana tabacum*) is a medicinal plant which has been proven insecticidal against many insects but the best solvent for its

extraction has not been investigated has it was done for other popular botanicals like *Azadirachta indica.* Therefore, this present study investigated the entomotoxic effect of tobacco seed extracted with different solvent against *C. maculatus* infesting cowpea in storage.

2. Materials and Methods

2.1 *Insect culture*

The starter culture of *C. maculatus* used in this research was obtained from an existing culture in the food storage section of the Department of Plant Science laboratory, Ekiti State University. The insects were reared on clean uninfested cowpea (Ife-brown variety) up to third generation before they are being used. The devoured cowpea seeds were replaced with new uninfested cowpea to maintain the culture. The culture was maintained at ambient temperature of 28±2 °C and relative humidity of 75±5%.

2.2 *Collection of cowpea and plant materials*

The cowpea (Ife brown) variety used was obtained from Agricultural Development Project, Akure, Ondo State Nigeria. The *N. tabacum* seed used in this research was obtained from an open field in the compound of Ekiti Anglican Diocesan High School, Ado Ekiti, Ekiti State, Nigeria. The seeds were air dried and the pericarp of the seeds was cracked and the seeds were removed. The seed were milled into fine powder and was stored in an air-tight container till further used.

2.3 *Preparation of the seed extracts*

The extracts of the seeds were made by cold extraction method. The fine powder of the seed was soaked separately into ethanol, methanol, acetone, pet-ether, n-hexane and water. The extracts were sieved and the solvent was separated from the oil extract using rotary evaporator except the water extract in which the oil extract was not separated from the water. After separation of the oil extracts from the solvents, the oil extracts were kept in different air-tight containers for subsequent use.

2.4 *Bioassay*

0.6 ml which corresponds to 3.0% v/w of each extract from oil extracts were thoroughly mixed with 20 g of clean cowpea seed in Petri-dishes. The cowpea seeds were air-dried for 1 h after which, 20 teneral adult *C. maculatus* were introduced into each Petri-dish containing the treated seeds. A control experiment which contains untreated seeds was also included in the set up. Four replicates per treatment were also prepared and the experiment was setup in a complete random design. Beetle mortality was observed daily for 4 days. The number of dead insects were counted and recorded after every 24 h.

To determine the effect of the oil extracts on the oviposition and adult emergence of *C. maculatus* each of methanol, ethanol, pet-ether, n-hexane, and aqueous (water) extracts at the rate of 3.0% v/w was added to 20 g clean seeds in Petri-dishes. The cowpea seeds and the extracts were thoroughly mixed with a glass rod to ensure uniform coating of the extracts on the seeds. The seeds were air-dried for 1 h in the laboratory after which five pairs of newly emerged adult *C. maculatus* was introduced into each Petri-dish containing the extracts. A control experiment comprising untreated seeds was also set up. Each treatment and the control were replicated 4 times and the experiment was setup in a complete random design. The Petri-dishes were covered with Petri-plates and both live and dead insects were removed after 7 days of introduction. The total number of eggs laid was counted and recorded and the Petri-dishes were left for 30 days to allow for the emergence of the first filial (F1) generation of the insect. The total number of adults that emerged from each replicate was counted and recorded. The percentage adult emergence was calculated thus:

$$\%\ adult\ emergence = \frac{number\ of\ adult\ emerged}{total\ number\ of\ egg\ laid} x \frac{100}{1}$$

To determine the effect of the extracts on ability of *C. maculatus* wholesome and pristine seeds (50 g) were measured into transparent plastic containers and admixed with 3.0% v/w of the different tobacco seed extracts, that is, ethanol, methanol, acetone, pet-ether, n-hexane and water extracts. The cowpea seeds were air-dried for 3 h for the solvent to evaporate. Thereafter, 10 pairs of adult weevils were introduced into each Petri-dish. A control experiment without treatment was also included. Each treatment was replicated four times. The mouths of the plastic containers were covered with muslin cloth held tightly in place with rubber band to prevent the escape of insects and entry of other pests. The containers were kept inside a wooden cage in the laboratory. After a period of 90 days, each replicate was assessed for seed damage and weight loss. The seeds were considered damaged when there were adult emergent holes. Percentage seed damage was calculated thus:

$$\%\ seed\ damage = \frac{number\ of\ seeds\ with\ hole}{total\ number\ of\ seeds} x \frac{100}{1}$$

2.5 *Effect of tobacco seed extracts on germination of cowpea seed*

Pristine and wholesome cowpea seeds were disinfested by putting them in deep freezer for 72 h. The seeds were removed from the deep freezer and air-dried for 1 h in the laboratory. Thereafter, 50 g of the cowpea seeds were measured into transparent plastic containers and 3.0% v/w of each extract was added and thoroughly mixed with glass rod to enhance uniform coating of the extracts on the seeds. The seeds were air-dried in the laboratory for 1 h and the mouths of the containers were covered with muslin cloth held tightly in place with rubber band. Four replicates were prepared for each extract. The control experiment consisted of the seeds that were not treated with any extract. The containers were kept in a wooden cage in the laboratory for 90 days. After 90 days, the containers were removed and the seeds were treated with Apron plus to prevent the growth of fungi. 20 seeds were randomly selected from each treatment and the control and planted on a moistened filter paper in Petri-dishes. After seven days the germinated seeds in each Petri-dish were counted and recorded.

2.6 *Data Analysis*

Data obtained were converted to percentage. Arcsin transformation was carried out on the percentage values. The data were later subjected to one-way analysis of variance (ANOVA) and means were separated using Tukey's test.

3. Result

3.1 *Effect of N. tabacum seed extracts on mortality of C. maculatus*

Bruchid mortality in tobacco seed extract-treated cowpea seeds differ significantly ($P < 0.05$) from the untreated (control) as

revealed by table 1. Adult mortality increased with increase in time of exposure of the bruchids to the extracts. 100% mortality was recorded in cowpea seeds treated with pet-ether, n-hexane and aqueous by 96 h post treatment (Table 1). All extracts showed bruchid mortality ranging from 70.15% to 100%.

3.2 *Effect of N. tabacum seed extracts on oviposition and adult emergence of C. maculatus*

All the tobacco seed extracts tested in this study reduced oviposition by *C. maculatus* (Table 2). Oviposition was significantly lower ($P < 0.05$) in the extract-treated seeds compared to those of untreated (control) seeds. However, there was no significant difference ($P < 0.05$) in number of eggs laid on ethanol, pet-ether, n-hexane and aqueous extracts of tobacco seed. There was no *C. maculatus* emerging in cowpea seeds treated with pet-ether and n-hexane extracts. Percentage adult emergence was drastically reduced in seeds treated with ethanol, aqueous, methanol and acetone extracts with 5.00%, 10.90%, 13.15% and 15.02% respectively.

3.3 *Effect of N. tabacum seed extracts on long term storage of cowpea seeds*

The *N. tabacum* seed extracts of acetone, pet-ether and n-hexane completely prevented infestation and damage by *C. maculatus* in the treated cowpea seeds as revealed in Table 3. There was neither seed damage nor weight loss recorded in the treated cowpea seeds for the three extracts mentioned above. There was 78.02% damage in the control experiment as revealed by emergent holes of the weevils. This is as a result of feeding activity of the developmental stage (larva) of the weevils. The weight of the untreated seed was significantly reduced compared to the treated seeds due to the aforementioned reasons.

Table 1: Mortality of adult C. maculatus exposed to extract of N. tabacum seed extracted with different solvents

Treatments	% mortality in hours			
	24	48	72	96
Untreated	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	3.75±2.20^{d}
Methanol	14.25±4.05^{b}	40.23±1.39^{bc}	60.15±2.20^{d}	80.00±0.00^{c}
Ethanol	15.35±2.39^{b}	55.00±0.00^{b}	75.34±3.15^{c}	85.20±1.44^{b}
Acetone	12.15±3.23^{c}	35.15±1.10^{c}	55.15±3.12^{d}	70.15±1.15^{bc}
Pet-ether	20.12±4.52^{a}	59.13±1.14^{ab}	88.20±1.30^{ab}	100.00±0.00^{a}
n-hexane	22.15±1.24^{a}	68.25±2.31^{a}	93.75±4.20^{a}	100.00±0.00^{a}
water	25.35±2.39^{a}	60.25±3.24^{ab}	90.00±1.25^{b}	100.00±0.00^{a}

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p>0.05$ using Tukey's test.

3.4 *Effect of N. tabacum seed extracts on germination of cowpea seeds*

There was significant difference in percentage germination of the treated and the untreated (control) cowpea seed. The percentage germination of all extract-treated seeds after 7 days was generally high. The untreated cowpea seeds had the highest germination of 100% followed by seeds treated with ethanol, methanol, water, n-hexane, pet-ether and acetone extracts with 92.34, 92.25, 90.00, 88.25, 86.15, and 80.25% respectively.

Table2: Effect of cashew seed extracts on oviposition and adult emergence of C. maculatus

Treatments	No of eggs	% adult emergence
Untreated	31.25±1.24^{c}	92.50±2.34^{d}
Methanol	13.75±1.04^{b}	13.15±1.20^{bc}
Ethanol	12.35±1.32^{a}	5.00±1.50^{b}
Acetone	15.15±1.44^{b}	15.02±1.50^{bc}
Pet-ether	10.85±1.00^{a}	0.00±0.00^{a}
n-hexane	10.75±1.34^{a}	0.00±0.00^{a}
water	12.55±2.02^{a}	10.90±4.23^{c}

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p>0.05$ using Tukey's test.

Table 3: Effect of N. tabacums seed extracts on long term storage of cowpea seeds

Treatments	Number of seeds	Number of damaged seeds	% seed damage	Weight loss
Untreated	192.25	150.00±1.20^{d}	78.02±1.40^{d}	20.85±2.45^{d}
Methanol	190.20	27.75±1.14^{c}	14.59±1.25^{c}	8.20±2.30^{bc}
Ethanol	194.35	18.25±2.20^{b}	9.38±1.04^{b}	5.15±1.40^{b}
Acetone	192.00	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Pet-ether	193.00	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
n-hexane	190.50	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
water	191.00	15.35±1.44^{b}	8.04±2.39^{b}	5.00±0.00^{b}

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p > 0.05$ using Tukey's test.

Table 4: Germination of cowpea seeds after treatment with 3.0% v/w tobacco seed extracts and preserved for 90 days

Treatments	% germination
Untreated	100.00±0.00d
Methanol	92.25±1.44bc
Ethanol	92.34±2.20bc
Acetone	80.25±2.39a
Pet-ether	86.15±1.25ab
n-hexane	88.25±2.14ab
water	90.00±0.00bc

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at $p>0.05$ using Tukey's test.

4. Discussion

The use of plant base insecticides is gaining more popularity among world entomologist and pest managers probably because of public awareness of dangers associated with many chemical insecticides. However, the effectiveness of many botanical insecticides is directly or directly dependent on the method of extraction, part of plants and type of solvent used for their extraction [20-22]. The polarity of different solvent may causes differences in their ability to extract the active compound contained in botanicals.

In this research, the result obtained showed that the insecticidal efficacy of extract of *N. tabacum* seed varied with type of solvent used for its extraction and period of exposure. Significant differences existed between the extracts. Regardless of the solvent used for the extraction of the plant, mortality of the beetle increased with increase in period of exposure. Only Pet-ether, n-hexane and water extract of *N. tabacum* seed achieved complete insect mortality within 96hours of application. The high mortality rate recorded by the extracts could be due to in ability of the insects to feed on the protected cowpeas. Also, the death of the insects could be due to the fume from the extracts to disrupt the normal respiratory pathway of the insect as suggested by Ashamo *et al*. [23] and this could lead to suffocation and asphyxiation of the insects. The result of this result acquiesced with the findings of Ileke and Olotuah [24] in which the extract of *Anacardium occidentale* caused high mortality rate of *C. maculatus* on cowpea. However, the result obtained disagreed with the findings of Ashamo and Ogungbite [20] in which ethanolic extracts of some botanicals appeared more effective than other solvent used. This variation could be due to the fact that botanical powders vary in their thickness and the thickness has indirect effect on extent to which solvent can extract oil compound from them (p. comm).

The result of this work revealed that the extracts significantly reduced the oviposition rate of the insect compared with the control experiment. The reduction in oviposition rate of the insect could be due to inability of insects to mate many times before death. The result also showed that the extracts have obvious effect on post embryonic survival of the insect. The extracts significantly reduced or prevented the emergence of adult *C. maculatus*. The ability of the extracts to reduced or prevented the emergence of the adult insects could be due to the in ability of the insect larval to fully cast off their exoskeleton that remained connected to the posterior part of their abdomen and subsequently led to their death [25]. The secondary metabolites present in these plants could be responsible for the inability of the adult insect to emerge as opined by Murdue-huntz and Nisbet [26] as well as Yang et al. [27] that secondary metabolites in botanicals are found to disrupt growth and reduced larva survival as well as disruption of life cycle of insects. The result obtained indicated that the extracts significantly reduced or prevented the ability of the beetle to cause seed damage as well as weight loss. This inability of the insect to cause seed damage and weight loss could be due to reduced or no emergence of adult beetle. The result agreed with the findings of Ashamo *et al*., [23] in which the extract of *Newbouldia laevis* significantly reduced or prevented the emergence of *C. maculatus*. However, in spite of the effectiveness of the extracts, the extracts have no adverse effect on the germinability of the protected cowpea as more than 70% of the protected cowpea grown after 90 days of protection.

5. Conclusion

The extracts of *N. tabacum* seed showed high insecticidal potency as they reduce the survival rate of the beetle, reduce the oviposition and emergence rate and as well reduced or prevented the seed damage and weight loss. The effectiveness of the extracts in term of mortality base on the type of solvent used could be arranged thus: n-hexane = water = Pet-ether > ethanol > methanol > acetone. These extracts, most especially that of water which are cheap, ecologically friendly and readily available could serve as alternative to chemical insecticides for use by resources-poor farmers.

6. References

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