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# Toxicity Effects of *Moringa oleifera* Leaf and Seed Extracts Against Weevil of Cowpea (*Callosobruchus maculatus*) Sold at Designated Markets in Awka, Anambra State, Southeast Nigeria

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## ABSTRACT

*Cowpea (Vigna unguiculata L. Walp), being a stored product for consumption has shown diverse environmental, agronomic and economic advantages which contributes to the improvement of the diets and further increases the incomes of peasant farming across the globe. Unfortunately, same has been widely attacked by voluminous pests especially Callosobruchus maculatus, which reduces its market value and nutrientional content. Consequent upon this, management measures that have been engaged involve the use of chemical pesticides. Awkwardly, this comes with its attendant glitches such as harm to non-target organisms, pesticide resistance, pesticide residue, damage to the environment which unadvertly posess treath to both animal and human health. This triggered the study on the toxicity effect of Moringa oliefera leaf and seed extract in the control of Callosobruchus maculatus. This investigation was set up in a Completely Randomized Design (CRD) involving four (4) dissimilar concentrations (5%, 10%, 25% and 50%) and a control each of which was replicated three times. Analysis of variance (ANOVA) and log-probit regression analysis were subsequently used to synthesize the information gotten from the study. Result showed that Moringa oleifera seed and leaf extract caused 30% and 16.6% mortalities at 10% concentration in 24 hours. The $LC_{50}$ at 24, 48, 72 and 96 hours for the seed extract were 37.62%, 48.96%, 36.86%, 30.62% while that of the leaf extract were 19.40%, 13.40%, 12.97%, 10.63% respectively. The $LC_{90}$ at 24, 48, 72 and 96 hours for the seed extracts were 88.95%, 88.12%, 62.66%, 60.02% while that of the leaf extract were 33.74%, 34.79%, 33.66%, and 33.92% respectively. The lethal time ($LT_{50}$ and $LT_{90}$) for the lowest possible concentration (5%) was also determined. At 5% concentration, $LT_{50}$ for the seed and leaf were 153 hours and 105 hours while $LT_{90}$ for both the seed and the leaf were 265 hours and 187 hours respectively. The use of Moringa oleifera seed and leaf extract which were evaluated for 24, 48,72, and 96 hours respectively were significantly different (P<0.05). To that effect, the study results revealed that both the leaves and seed extracts of Moringa oleifera have great potential in the management of C. maculatus, hence Moringa oleifera is recommended to be used as a botanical pesticide against Callasobruchus maculatus.*

***Keywords:*** *Callosobruchus maculatus, Cowpea, Moringa oleifera, Seed Extracts, Toxicity*

## INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) fits into the family of crops known as Fabaceae from the genus referred to as Vigna notably has a black eye peas and institutes one of the most significant food legumes especially in the subtropics and ropical countries of the world [1] which are ordinarily eaten in the form of dry grains [2]. Dried grains are primed into moi-moi, akara or eaten in combination with other different crops such as yam or even rice. They can as well be rummage-sale as silage [3]. Approximately 200 million people of Africa consume the crop [4]. Furthermore, cowpea is a relatively cheap source of protein and invariably obliges as the source of plant protein to peasant farmers that cannot have enough money to buy meat and fish as animal protein for consumption [4].

Cowpea is cultivated in North and South America, tropical Africa and Asia particularly as grain, but also as a fodder crop or even vegetable. The crop can adapt widely and tolerance several farm stresses which makes it a very imperative food source for both man and animal. The estimation of the crop serving as a major protein source for more than 200 million people in sub-Saharan Africa which brands it the ten fresh vegetables in the People's Republic of China Organization for Economic Co-operation and Development [5].

Unfortunately, the crop is extensive lyre-counted to be confronted by an array of insect pests and diseases that challenges plants usuability as reported by [6 and 7]. Cowpea cultivation has equally received a report that more than 130 species exists which causes economic down turn in cowpea yield as reported by [8]. This is due to the presence of diverse pests in Northern Nigeria in all stages of plant growth, and losses due to pest attacks or diseases can be as high as 90 percent in broad-spectrum. Correspondingly, *Callosobruchus maculatus* causes a considerable amount of loss on stored cowpeas both especially in the Sub-tropics and the tropics as reported by [9]. Additionally, up to 100% of seeds may be infested and damaged by this pest in 3-4 months of storage [6]. The cowpea weevil, *C. maculatus* which is a major pest of cowpea lays their eggs on cowpea pods or on avenues left by other biting and chewing insects likely (*Mylabris spp*). *C. maculatus* remains the most destructive amongst the insect pests of stored cowpea seeds which is equally known as cowpea seed bruchid or pulse beetle [6]. *C.maculatus* has been reported to be the most serious pest of stored legumes in most of tropical countries, thus a serious pest of many crops [10]. *Callosobruchus* also spasms wide range of leguminous crops that include but not limited to cowpea, chick pea, and many other legumeinious plants [5]. The larva of this pest feeds on the seed thereby cause a serious damage to them [11]. Whereas the egg and adult stage are found on the grain, the larval and pupal which lives inside the grain causes the damages therein. The use of chemical insecticides which could be by fumigation is an appropriate way of controlling *C. maculatus* and other insects [10]. Insecticides which have quick knockdown action are efficient persistent and effective means of control and management of the insect but some of them have undesirable effects to man and animal. These chemicals pose hazards to the health of man and livestock, they also result in pest resistance and possible resurgence. They could also penetrate into grains, become toxic to the consumer and exhibit casinogenic effects in mammals [3]. Due to the outlined consequences, there is need to develop an economical, harmless and tranquil method of preserving stored products especially cowpea against *C. maculatus*. Hence, there is an urgent need for alternate ways and means of control that is both parsimoniously practicable and ecologically pleasant to the populace. Several researchers and canvassers have independently documented the use of plant constituents as biopesticides as an unconventional to usage of chemicals [3, 12, 13, 14, 15 and 16]. Apart from the fact that biospesticide usage are readily available, they are equally biodegradable and had non toxic effects to non-target organisms. They are correspondingly selective in action and capable of retarding the development of resistance over long time and duration of usage. Leaves, seeds, root and stems are different parts of plants that are effective for this purpose as reported by [17]. These plant fragments have to be washed and one or the other air or shade-dried, ground and subsequently sieved into powder beforehand being put into treatment. The use of these biopesticides would go a long way in helping farmers to sustain their livelihood, reduce over-dependence on insecticides and maintain a polluted-free environment thereby promoting healthy living [18] which is the aim of this study.

## MATERIALS AND METHODS

### 3.1 Study Area

The study was undertaken in the Laboratory of the Department of Parasitology and Entomology, Faculty of Biological Sciences of Nnamdi Azikiwe University Awkawith geographical co-ordinates that lies between Longitude $6^0$14'N, $6^0$14.5'N and Latitude $7^0$8.6'E, $7^0$9'E Anambra State, Southeastern region of Nigeria.

### 3.2 Study Design

The research study design used was a Completely Randomized Design (CRD) involving four treatments and a control with each treatment replicated three times including the control on the laboratory bench.

### 3.3 Experimental Cowpea Seed

Cowpea grains infested with *Callosobruchus maculatus* were procured from the prominent Eke Awka market in Anambra State, Southeast Nigeria which was used to set up the experimental insect culture in a plastic jar. The purchased grains were sterilized in the oven at the laboratory for three hours to ensure that the grains were devoid of infestation prior to use. The jar was perforated at the top to allow air, covered with muslin cloth, and kept under ambient temperature and relative humidity for proper development and reproduction of insects.

### 3.4 Collection, Identification, and processing of *Moringa oliefera*

The *Moringa* leaves and seeds were collected from a local household *Moringa* plant stand. The plants were taken to the Botany Department Laboratory of Nnamdi Azikiwe University to obtain the identity and further substantiation by a plant taxonomist. They were air dried and then ground to fine powder using an electric blender and then filtered through a 0.5mm mesh before the powders were collected into a clean container.

### 3.5 Preparation of Plant Extracts

The pulverized plant samples (100 g each) were dissolved in 800 ml of 70% methanol and allowed to stand for 24hrs. The dissolved solute was sieved through a muslin fabric with Whatman No. 1 filter paper. Whereas the methanol filtrate obtained was concentrated using a water bath at 50°C, the solution was stockpiled at 4°C after amassing into a pasteurised bottle until its necessitated. Serial dilution of 5 %, 10%, 25%, 50% of each plant extract were prepared and used for the bioassay. Control treatments were also prepared using acetone and these were kept under ambient conditions in the laboratory.

### 3.6 Contact toxicity response of *C. maculatus* on Moringa oleifera leaf and seed extract.

No. 1 Whatman filter paper (9 cm in diameter) was positioned in each of the petri dishes used for the experimentation. The numerous dosage echelons of the plant extracts rummage-scaleen compassd 5%, 10%, 25%, 50% and every one was simulated thrice including the control. Aliquots of 2 ml of every dosage was uniformly distributed in the filter paper and leftward for an hour to guarantee appropriate dissemination of the mixture. Controls with acetone only were included which was allowed to evaporate. Subsequently, 10 unsexed *C.maculatus*adults were presented into each petri dish containing the pickled filter paper and the control respectively. The petri dishes were enclosed by means of lid to avert the flight of the insects. Afterwards, adult mortality was taken every 24 hours for four days.

### 3.7 Analysis of Data

All assembled data were imperilled to analysis of variance (ANOVA) by means of SPSS version 20. Mortality data was analyzed using log-probit regression for determining $LD_{50}$ and $LD_{90}$ ($LT_{50}$) and $LT_{90}$). Treatments with significant differences were measured up at 5% level of significance (P<0.05) with the Duncan multiple range test. The mortality data obtained were corrected using [19] formula.

$$Pm = \frac{Po - Pc \times 100\%}{100 - Pc}$$

Where Po= observed percentage mortality
Pc=control percentage mortality
Pm= corrected percentage mortality
The corrected percentage mortality values were transformed into probit. Dosages were also transformed into log dose. The probit values were plotted against log dose to determine $LC_{50}$ and $LC_{90}$as well as $LT_{50}$ and $Lt_{90}$.

## Results

### 4.1 Contact toxicity of *Moringa oleifera* seed and leaf extract on *Callosobruchus maculatus*

### 4.1.1. Mortality response of *Moringa oleifera* seed extracts on *Callosobruchus maculatus*

The contact toxicity result in (Table 1) showed there was dose-dependent mortality response to the *Moringa oleifera* seed extract. Mortality increased in accordance with the increase in concentration of the extract. The highest and lowest mean mortality of *Callosobruchus maculatus* at 50% and 5% concentrations, mortalities of 53.33% and 33.33% respectively. The statistical analysis showed that doses were significantly different from each other ($P \leq 0.05$) .

*Table 1: Toxicity effect of Moringa oleifera seed extract on C. maculatus after periods of exposure*

| Doses (%) | 24hrs | 48hrs | 72hrs | 96hrs | % Mortality after 96hrs |
|---|---|---|---|---|---|
| 5 | $0.33±0.33^{a}$ | $2.00±0.57^{a}$ | $2.00±0.58^{a}$ | $3.33±0.67^{ab}$ | 33.33 |
| 10 | $1.67±0.67^{a}$ | $1.67±0.33^{a}$ | $2.33±0.33^{a}$ | $3.00±0.58^{ab}$ | 30.00 |
| 25 | $4.00±1.00^{b}$ | $4.33±0.67^{b}$ | $5.33±0.67^{b}$ | $5.33±0.33^{b}$ | 53.33 |
| 50 | $4.67±0.88^{b}$ | $5.00±1.15^{b}$ | $5.00±1.15^{b}$ | $5.33±1.45^{b}$ | 53.33 |
| Control | $00.00±0.00^{a}$ | $0.67±0.66^{a}$ | $0.67±0.66^{a}$ | $1.00±0.58^{a}$ | |
| P value | 0.002 | 0.008 | 0.004 | 0.018 | |

*Means a standard error of the means*
**Columns with different superscripts are significantly different from each other ($P \leq 0.05$)*

### 4.1.2 Mortality response of *Moringa oleifera* leaf extract on *Callosobruchus maculatus.*

The contact toxicity result in (Table 2) showed there was dose dependent mortality response to the *Moringa oleifera* leaf extract. Mortality increased in accordance with increase in concentration of the extract. The highest and lowest mean mortality of *Callosobruchus maculatus* at 50% and 5% concentrations, caused mortalities of 100 % and 36.66% respectively. The statistical analysis showed that doses were significantly different from each other ($P \leq 0.05$).

*Table 2: Mortality response of Moringa oleifera leaf extract on Callasobruchus maculatus.*

| Doses (%) | 24hrs | 48hrs | 72hrs | 96hrs | % Mortality after 96hrs |
|---|---|---|---|---|---|
| 5 | $0.67±0.67^{a}$ | $1.67±0.67^{ab}$ | $1.67±0.67^{ab}$ | $3.67±1.76^{ab}$ | 36.66 |
| 10 | $3.00±1.00^{b}$ | $3.33±0.88^{bc}$ | $3.67±1.20^{bc}$ | $4.00±1.15^{ab}$ | 40.00 |
| 25 | $4.67±0.88^{b}$ | $5.00±0.58^{c}$ | $5.33±0.88^{c}$ | $5.33±0.88^{b}$ | 53.33 |
| 50 | $10.00±0.00^{c}$ | $10.00±0.00^{d}$ | $10.00±0.00^{d}$ | $10.00±0.0^{c}$ | 100.00 |
| Control | $00.00±0.00^{a}$ | $0.67±0.67^{a}$ | $0.67±0.67^{a}$ | $1.00±0.58^{a}$ | |
| P value | 0.000 | 0.000 | 0.000 | 0.002 | |

**Means ± standard error of the means*
**Columns with different superscripts are significantly different from each other ($P \leq 0.05$)*

### 4.2 $LC_{50}$ of *Moringa oleifera* seed and leaf extract at different exposure times.

Table 3 shows the $LC_{50}$ of *Moringa oleifera* seed and leaf extract at different exposure times. It reveals that the highest toxicity occurs at 48.96 concentration in 48 hours and the lowest toxicity occurs at 30.62 concentration in 96 hours for the leaf extract while the highest and lowest toxicity for the seed extract occurs at 19.40 concentration in 24 thoursand 10.63 concentration in 96 hours.

*Table 3: $LC_{50}$ of seed and leaf extract of Moringa at different exposure times*

| Parameter | $LC_{50}$ at 24hrs (%) | $LC_{50}$ 48hrs (%) | $LC_{50}$ 72hrs (%) | $LC_{50}$ 96hrs (%) |
|---|---|---|---|---|
| **Moringa seed extract** | 37.62 | 48.96 | 36.86 | 30.62 |
| **Moringa leaf extract** | 19.40 | 13.40 | 12.97 | 10.63 |

### 4.3 $LC_{90}$ of Moringa oleifera seed and leaf extract at different exposure times

Table 4 shows the LC of *Moringa oleifera* seed and leaf extract at different exposure umes. It reveals that the highest toxicity occurs at 34.79% concentration in 48 hours and the lowest toxicity occurs at 33.74% concentration in 96 hours for the leaf extract while the highest and lowest toxicity for the seed extract occurs at 88.95% concentration in 24 hours and 60.02% concentration in 96 hours.

*Table 4: $LC_{90}$ of seed and leaf extract of Moringa at different exposure times*

| Parameter | $LC_{50}$ at 24hrs (%) | $LC_{50}$ 48hrs (%) | $LC_{50}$ 72hrs (%) | $LC_{50}$ 96hrs (%) |
|---|---|---|---|---|
| **Moringa seed extract** | 88.95 | 83.12 | 62.66 | 60.02 |
| **Moringa leaf extract** | 33.74 | 34.79 | 33.66 | 33.92 |

### 4.4 Effect of exposure time ($LT_{50}$ and $LT_{90}$)on the toxicity of both the seed and leaf extract of *Moringa oleifera* at the lowest possible concentration (5% ).

Table 5 shows that mortality increases with increase in exposure time. It reveals that at a lowest possible concentration (5%), the Moringa seed extract causes 50% mortality in 153 hours and 90% mortality within 265 hours. While, the leaf extract causes 50% mortality within 105 hours and 90% mortality within 187 hours

*Table 5: LT50 and $LT_{90}$ (Lethal time) of seed and leaf extract of Moringa at 5% concentration*

| Parameter | $LC_{50}$ at 5% conc (hrs) | $LC_{90}$ at 5% conc (hrs) |
|---|---|---|
| **Moringa seed extract** | 153 | 265 |
| **Moringa leaf extract** | 105 | 187 |

## Discussion

The current study focused on enlightment of how bio-insecticides could be used as alternatives to synthetics in providing high level of control for *Callosobruchus maculatus* without environmental contamination endangering man and animal. It has formally been recommended that an integrated pest management (IPM) strategy to safeguard cowpeas in storage systems could be accomplished exploiting traditional methods as reported by [20]. For a stretchedwhile now, to safeguard their harvest from insects, African farmers have familiarized aromatic plants among stored seeds or pods. These plants discharge fickle compounds that are thought to possess insecticidal competencesas as reported by [21].

The study showed a considerable susceptibility of *C. maculatus* to two botanicals; *Moringa oliefera* seed extract and leaf extract. Mortalities of adult *C.maculatu*s were observed in the direct method application of both plant extracts. The leaf extract was more effective, causing mortality of 100% within 24hrs, unlike the seed extracts which caused 46.67% after 24 hours. This is in line with the report of [22], whose results show that *Moringa* leaf powder was more effective than other extracts from different part of the plant apart from the flowers. The *Moringa* leaf extract caused 53.3% mortality after 72 hrs while that of the seed caused 50% mortality after 72 hours also. Both plant extracts also caused 36.67% and 23.33% mortality respectively at 48 hrs. This also was in concordance with the report from [23] which shows that mortality increases with an increase in exposure time. Invariably, it demonstrates the importance of timing in application of botanicals.

The results also showed that the toxicity of both the seed and the leaf extract of *Moringa on C. maculatus* is dependent on the level of concentration. It was observed that the higher the concentration the higher the toxicity and vice versa. The percentage mortality recorded at 24 hours at 5% and 25% concentration for the leaf extract were 6.67% and 46.67% respectively while that of the seed were 13% and 40% respectively. This is a pointer that the insecticidal properties of botanical extracts, such as pyrethrins and neem, can disrupt the life cycle of stored product pests. Neem-based formulations have been proven effective against *C. maculatus* larvae and their corresponding adults as reported by [24]. Moreover, biopesticides generally have low mammalian toxicity and pose minimal risks to human health. This is in stark contrast to synthetic pesticides, which often contain harmful chemicals. The use of biopesticides aligns with sustainable agricultural practices and reduces the environmental impact associated with chemical residues especially to man and animal. According to the reports of [25], seed treated with botanical oil extracts did not mislay their viability and also established the powder made from essential oil at different basics provided complete protection against *C. maculatus* and did not show a significant consequence on the intoxicating power of seed germination proportion. [26] reported that *callosobruchus maculatus* is one of the foremost pests of stored cowpea in the tropics whereas [23] narrated that when jute stacks were treated with dissimilar plant excerpts of *Azaraditcha indica* and *Vitex nemado* which were then used for cowpea seed storage, it was established that the egg laying capacity by the *C. Maculatus* alongside the adult development and damage done to the seed were abridged significantly.

## Conclusion and Recommendation

Obviously, statistics from the study illustrates that *Moringa oleifera leaf* and seed extract are effective in the control and management of *Callasobruchus maculatus* in stored cowpea. Fortunately, this extract can also be prepared easily by farmers and applied to grains both in the field and in the store. The level of its effectiveness shows that higher concentration is important to obtain better seed protection which implies that mortality increases with a corresponding increase in concentration. The use of these plant materials to a large extent reduces the rate of dependence on chemical pesticides which can be costly and as such not readily available and assessible. It also reduces the high level of pesticide residue, resistance, and toxicity to farmers and consumers of produce which can over time lead to health concerns. It is hereby recommended that further study should be carried out to find out the efficacy of *Moringa oleifera* on the F1 generation of the test insect, including the loss and damage due to the insects. Excaustive study should also be carried out to ratify the constituents of the plants and the active ingredient that makes it insecticidal, nonetheless local farmers should be informed on the necessity of botanicals usage as pesticides compared to chemical pesticides.

**Author contributions:** OEO, OIE and EOV wrote the first draft and edited the manuscript, ACO and EAC developed the protocol, and oversaw study implementation and monitoring. OEO, and UJC did the experimental design and OIE did the analysis of the data collected.

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**Data availability:** The statistics used to substantiate the discovieries of this study are readily accessible upon reasonable entreaty.

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