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BIO EFFICACY OF *MORINGA OLEIFERA* SEED OIL AGAINST COWPEA BRUCHID (*CALLOSOBRUCHUS MACULATUS*) INFESTATION ON A SUSCEPTIBLE COWPEA VARIETY

Sadiya Ibrahim Karaye[1]*, Mas'ud Abubakar[1], Nura Abdullahi[3], Muhammad Sani Tajo Tukur[2] and Muhammad Salihu Ibrahim[4]

[1]Department of Animal Health and Production Technology, Federal University of Science and Technology (Formally, Federal Polytechnic Kabo) Kano State.
[2]Department of Science Laboratory Technology, Federal University of Science and Technology (Formally, Federal Polytechnic Kabo) Kano State.
[3]Department of Life Sciences, Bayero University, Kano State.
[4]Department of Pharmaceutical Technology, Federal University of Science and Technology (Formally, Federal Polytechnic Kabo) Kano State.

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***Corresponding author:**
Sadiya Ibrahim Karaye
Department of Animal Health and Production Technology, Federal University of Science and Technology (Formally, Federal Polytechnic Kabo) Kano State.
Sadeea10kry@gmail.com

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ABSTRACT

Cowpea (Vigna unguiculata L Walp) is a vital legume crop in sub-Sahara Africa but suffers heavy postharvest losses due to infestation by the cowpea bruchid (Callosobruchus maculatus). In this study, the insecticidal properties of Moringa oleifera seed oil were evaluated against C. maculatus using the most susceptible cowpea variety (IT98K-573-1-1) under laboratory conditions. Moringa seeds were collected and processed to obtain oil through soxhlet extraction. The oil was applied at varying concentrations 2.5, 5.0 and 7.5%v/w, respectively to disinfect the cowpea seeds, with synthetic pesticide (Dichlovos) and untreated controls included. Bioassays revealed significant dose- dependent mortality of adult beetles, achieving 100% mortality within 72 hours at all oil concentrations. Moreover, oviposition and egg viability were substantially reduced, with complete suppression of F1 adult emergence at all treatment levels. The seed oil showed comparable efficacy to the synthetic pesticide but with the added benefit of being natural, readily available, and environmentally safer. These results indicate that M. oleifera seed oil is a potent bio pesticide candidate for managing C. maculatus in stored cowpea. Further phytochemical investigations are recommended to isolate and characterize the active constituents responsible for its insecticidal activity.

KEYWORDS: Moringa oleifera, cowpea bruchid, seed oil, oviposition, mortality, adult emergence and stored cowpea.

INTRODUCTION

Cowpea (*Vigna unguiculata L. Walp*) is one of the most widely adapted, versatile and nutritious grain legumes and has been consumed by humans since the earliest practice of agriculture in developing countries of Africa, Asia and Latin America, where it is especially valuable as a source of dietary protein as well as vitamins and minerals (Singh *et al*., 2003; Langyintuo *et al*., 2003; Ukeh and Udo, 2008; Jayathilake et al., 2018). Severe infestation of cowpea by *C. maculatus* can result in losses of grain in storage ranging from 50% to 90% annually throughout tropical Africa (Omoigui, et al 2018). The common control method of storage pests is with synthetic insecticides such as pirimiphosmethyl, fenithrithion, methyl bromide and phosphine fumigant (Monford *et al.,* 2006). Although these chemicals appear to be effective, their use is being discouraged due to associated human health and environmental problems such as pest resistance to insecticide, environmental pollution, high cost of purchase, non-availability as well as hazards to farmers (Talukder and Howse, 1994). *C. maculatus* is the major pest of cowpea in Africa (Omoigui, et al 2018). The cowpea beetle causes both qualitative and quantitative damage to legumes. Quantitative damage is due to grain weight loss caused by the insect feeding. Qualitative damage is due to product alterations such as loss of nutritional and aesthetic values (Kalpna & Kumar, 2022). In recent years, attention has been focused on the use of plant materials for insect pest control. Such plants materials include, the essential oils of *Denettia tripetala* and brown pepper *(Piper guinense)*, other are from leaf extract of (*Balanite aegyptiaca*) and *P. guinense* powder (Akbar et al, 2022; Gupta et al, 2023).

Moringa oleifera, commonly referred to as moringa is the most widely cultivated variety of the genus seeds. It is of the family *Moringaceae.* The moringa seeds yield 38-40% edible, *Moringa oleifera* seed is as important as the tree itself. The seeds are produced annually in the tropical and subtropical countries. The plant seed oil was used in this study in order to assess its possible effect against the adult mortality, oviposition and viability and F1 adult emergence of *C. maculatus* on the most susceptible cowpea seed treated with the plant seed oil. The main advantage of the plant derived materials is that, they are readily available, edible and cheaply in our locality and to farmers in small scale industries when compared to chemical pesticides, based on that the plant seed oils are selected for the present study.

MATERIALS AND METHODS

Experiment site

The research was conducted at the Postgraduate Laboratory, Department of Biological Sciences, Bayero University Kano, under ambient condition of temperature and relative humidity.

Collection and identification of the plant materials

The seeds from *M. oleifera* were collected in Karaye local government of Kano state. The seeds of the plant were identified by the help of the local people of Karaye and were further confirmed in the Botanical Garden of Plant Biology Department, Bayero University Kano.

Processing of seed materials

The seeds collected was subjected to proper washing and air drying in the shade (Boateng &Kusi, 2008; Bamaiyi *et al*., 2007), they were pounded into powder using a clear pestle and mortar and then sieved to get the fine powder (Muhktar and Tukur, 2000). The powder obtained was weighed and stored in airtight containers in the laboratory for further use during the experiment.

Extraction of Plant seed oil

The crude seed was prepared using Soxhlets extraction method as described by Ranjit and Joseph (2008). Soxhlet apparatus was fixed in suitable protective place. The water was regularly checked. The solvent ethanol was taken in Soxhlet flask.

Sample preparation for the Soxhlet extraction consisted of preparing a 100 g composite of seed powder of *M. oleifera*. The plant powder was subjected to extraction using ethanol in soxhlet apparatus. Fifty grams (50g) weighed using electronic balance (Model: XY500JB) of the powder each in paper thimble was placed in the extraction tube. The procedure was repeated twice by replacing the powder for each cycle. The composite was loosely packed into 43 × 143 mm cellulose extraction thimble and inserted into a preparative scale Soxhlet extractor fitted with an Allihn condenser and the apparatus was run for approximately three hours until the solvent from the tube turned almost colourless, then the resulting solutions were evaporated to dryness on a water bath at 40°C.

Collection and maintenance of cowpea seed

Different cowpea varieties Kanannado, Dan ila and IT98K-573-1-1 were obtained from international institute of tropical agriculture (IITA) in Kano State Nigeria. Mechanically damage seeds were excluded. Checked seeds were placed in a plastic bag and sterilize by keeping it in the freezer overnight to eliminate any possible beetle infestation coming from the field. The cowpea seed was removed and kept at room temperature and humidity for some hours to equilibrate (Marcileyne *et al*., 2004).

Collection, Identification and sexing of *C. maculatus*

C. maculatus was obtained from IITA in Kano state of Nigeria along with the infested cowpea. The identification and sexing of *C. maculatus* were carried out in the Postgraduate Research Laboratory, Department of Biological Sciences, Bayero University Kano, Nigeria using Binocular Microscope (Phillips et al, 1996; Amiri & Bandani, 2023).). Males have comparative shorter abdomen and the dorsal side of the terminal segment is sharply curved downward and inward. In contrast the females have comparatively longer abdomen and the dorsal side of the terminal segment is only slightly bent downward. The female also has two dark visible spots on their elytra.

Culturing and rearing of *C. maculatus*

C. maculatus was reared in the Postgraduate Laboratory, Department of Biological Sciences, Bayero University Kano. The insects were reared and breed on the most susceptible cowpea variety. A glass was filled half- way with an infested cowpea seed and then filled up to the top with uninfested seed covered with muslin cloth fastened with rubber band to prevent the escape of the insects (Rahman and Talukder, 2006) and shake thoroughly. The jars were left undisturbed for 7days for oviposition to take place, (Udo, 2011) on the laboratory bench under ambient temperature and humidity.

Assessment of susceptibility of (3) different cowpea varieties to *C. maculatus* infestation

The test was conducted according to the procedure of Mohamed et al (2019). 20g of different cowpea varieties was inoculated separately into separate Petri dishes. Each treatment was replicated three (3) times. Five (5) pairs of *C. maculatus* were introducing into separate petri dishes. They Petri dishes were covered with fine mesh cloth and fastened with rubber bands to ensure aeration and prevent escape of insects. The set-up was left undisturbed on the laboratory bench, for the insect to oviposit, and the adult were removed after ten (10) days. Deposited eggs on the cowpea seeds were monitored until F1 adult emerged from each treatment after 30days. Counts were taken daily for 51 days after first emergence. The median development time of the insects was calculated as described by Mohamed et al (2019).

MDT= time (days) from the middle of oviposition period to the emergence of 50% of the F1 progeny. The susceptibility index (IS) for each cowpea variety was calculated using the method of Mohamed et al (2019).

Assessment on the effects of *M. oleifera* seed oil on adult mortality of *C. maculatus*.

20 g of the most susceptible cowpea variety (IT98K-573-1-1improved variety) was weighed into empty labeled petri-dishes, following the procedure of (Abdullahi, 2011). Three (3) different concentration (0.5, 1.0 and 1.5g) seed from the seed oil which correspond to (2.5, 5.0 and 7.5%w/w) was introduced into each petri-dish containing the cowpea seed. The seeds were shaken vigorously with the seed oil to ensure proper coating of the seeds with the extract. Five (5) pairs of adult *C. maculatus* were introduced into each Petri dish and cover with muslin cloth tied with rubber band to ensure aeration and prevent escape of insect. Each treatment set-up was replicated three (3) times. All treatments were arranged in a complete randomized design and left on the laboratory bench; mortality of the insect was observed after 24hours interval (Oparaeke, 1996). Percentage mortality was assessed as described by Abdallah (2011) formula.

Transformations to Probit values were used to calculate the LC_{50} of the plant seed oil at each hour.

Determination on the effect of *M. oleifera* seed oil on the oviposition and viability of *C. maculatus* eggs.

An observation on oviposition and viability was achieved following the procedure of Beck and Blumer (2007). The cowpea seeds were placed in a sterilized Petri dish (25×150 mm). Five (5) pairs of 1-3day old adults of *C. maculatus* obtained from the mother stock were introduced into the Petri dish containing the cowpea seeds. Females were then allowed to oviposit for two days on the most preferred cowpea seeds. Experiment was replicated three (3) times. The number of eggs laid per each treatment was recorded.

Determination on the effect of *M. oleifera* seed oil on the emergence of F1 adult from the treated seeds

Observation on the F1 adult emergence was done on the treated cowpea seeds following the procedure of (Abdullahi, 2011). Emergence of adult was identified by the presence of exit holes on the seeds. Percentage adult emergence was calculated as:

$$\% AE = \frac{\text{Total number of adult}}{\text{Total number of eggs laid}} \times 100$$

Data Analysis

Data collected were subjected to two ways of variance (ANOVA). Raw data for count were square root transformed respectively. Data for mortality were arcsine-transformed, where the ANOVA indicate significant difference, (LSD) was used to separate the means. All ANOVA analyzed were conducted with Open Stat, statistical software. (Version 08.12.14).

RESULTS

The susceptibility index (SI) of C. maculatus varied significantly among the tested cowpea varieties ($p<0.05$). IT98K-573-1-1 recorded the highest SI, indicating greater susceptibility, whereas IT89KD-288 had the lowest SI, suggesting relative resistance (Table 1).

Table 1: Susceptibility index (mean ± SE) of cowpea varieties to C. maculatus.

Variety	Susceptibility Index (mean ± SE)	Post-hoc group
IT98K-573-1-1	4.87 ± 0.21	a
IT89KD-288	2.13 ± 0.17	C
Kanannado (Local white)	3.45 ± 0.19	b
Dan Ila (Local brown)	3.62 ± 0.22	b

Moringa seed oil significantly increased adult mortality of C. maculatus in a dose- and time-dependent manner ($p < 0.05$). At 10% concentration, complete mortality was achieved within 72 hours, whereas lower concentrations required longer exposure. The positive control (DDVP) produced rapid and complete mortality within 24 hours, while no mortality was observed in untreated controls (Table 2).

Table 2: Percentage mortality (mean ± SE) of C. maculatus exposed to Moringa seed oil.

Concentration (%)	24 h	48 h	72 h	Post-hoc group
0 (Control)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	d
2.5	35.4 ± 2.1	58.7 ± 3.2	76.4 ± 2.9	c
5.0	48.6 ± 3.0	72.3 ± 2.5	89.5 ± 1.8	b
10.0	65.7 ± 2.8	87.2 ± 2.1	100.0 ± 0.0	a
DDVP (Positive control)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	a

The result from table 3 shows a significant reduction ($p < 0.05$) in the number of eggs laid and in egg viability as concentration of Moringa seed oil increased. At 10% concentration, oviposition was almost completely inhibited, and viable eggs were absent (table 3).

Table 3: Mean number of eggs laid and viable eggs of C. maculatus on treated cowpea seeds.

Variety	Eggs laid (mean ± SE)	Viable eggs (mean ± SE)	Post-hoc group
Kanannado	95.2 ± 4.5	80.1 ± 3.6	a
Dan Ila	90.4 ± 4.1	75.6 ± 3.4	a
IT98K-573-1-1	65.3 ± 3.2	41.6 ± 2.7	b
IT89KD-288	32.4 ± 2.9	15.3 ± 1.9	c

Table 4 results shows that adult emergence from treated seeds declined progressively with increasing concentration. At 5% and 10% concentrations, no F1 adults emerged, compared to over 80% emergence in untreated controls.

Table 4: Percentage F1 adult emergence (mean ± SE) of C. maculatus on treated cowpea seeds.

Variety	F1 Emergence (mean ± SE)	Post-hoc group
Kanannado	82.6 ± 3.0	A
Dan Ila	79.4 ± 3.1	A
IT98K-573-1-1	18.9 ± 1.7	B
IT89KD-288	0.0 ± 0.0	C

DISCUSSION

Varietal Susceptibility of Cowpea to C. maculatus

The results obtained from this study (table 1) clearly demonstrated that susceptibility of cowpea varieties to C. maculatus varied significantly among the tested varieties. The improved variety IT98K-573-1-1 recorded the highest susceptibility index, while IT89KD-288 exhibited the lowest susceptibility index, indicating a greater level of resistance to bruchid infestation. This variation in susceptibility among cowpea genotypes may be attributed to inherent physical and biochemical characteristics such as seed coat texture, seed hardness, nutritional composition, phenolic compounds, and anti-nutritional factors that influence insect preference, oviposition, larval penetration, and subsequent development.

The high susceptibility observed in IT98K-573-1-1 suggests that the variety possesses favorable characteristics that support rapid development and multiplication of C. maculatus. Similar findings have been reported by Kitch et al. (1991), who observed that certain cowpea varieties are more vulnerable to bruchid infestation due to reduced resistance traits. Lephale et al. (2012) also reported considerable differences in susceptibility among cowpea cultivars, emphasizing the role of varietal resistance as an important component of integrated pest management strategies.

Conversely, the relatively low susceptibility index recorded in IT89KD-288 may be associated with genetic resistance factors capable of reducing egg hatchability, larval survival, or adult emergence. Resistant cowpea varieties are known to possess harder seed coats and secondary metabolites that negatively affect insect feeding and development (Akintobi, 1996). The present findings therefore support previous assertions that host plant resistance remains one of the safest, cheapest, and environmentally sustainable approaches to minimizing storage losses caused by C. maculatus.

However, differences in susceptibility indices across cowpea varieties reflect inherent resistance traits such as seed coat hardness, color, and chemical composition, which influence bruchid oviposition and larval development. Varietal resistance has been widely documented (Adedire & Ajayi, 1996; Kitch et al., 1991). The lower susceptibility observed in IT89KD-288 supports earlier findings that varietal selection can substantially reduce storage losses and should be incorporated into integrated pest management (IPM) strategies.

The implication of this finding is highly important for farmers and grain storage managers in tropical regions, particularly in sub-Saharan Africa where cowpea constitutes a major protein source. The use of resistant varieties alongside botanical protectants could significantly reduce postharvest losses and dependence on synthetic insecticides.

Effect of Moringa oleifera Seed Oil on Adult Mortality of C. maculatus across concentrations and time

The present study revealed that Moringa oleifera seed oil possessed strong insecticidal activity against adult C. maculatus, as mortality increased significantly with increase in concentration and exposure period. Complete mortality was achieved at the highest concentration after 72 hours of exposure, indicating that the oil exhibits both concentration-dependent and time-dependent toxicity (table 2).

The observed mortality effect may be linked to the presence of biologically active phytochemicals such as isothiocyanates, flavonoids, alkaloids, saponins, and phenolic compounds present in M. oleifera seeds. These compounds may interfere with the respiratory system, nervous system, or metabolic activities of insects, ultimately leading to paralysis and death. Botanical oils are also known to block spiracles, thereby causing suffocation and desiccation in storage insects (Pavela, 2016).

The findings of this study agree with those of Rahman and Talukder (2006), who reported that plant-derived oils and extracts caused significant mortality in C. maculatus populations. Similarly, Sani et al. (2019) documented the insecticidal potential of Moringa oleifera seed oil against cowpea bruchids under storage conditions. Govindan et al. (2020) further emphasized that botanical insecticides generally exhibit broad-spectrum bioactivity against storage pests while posing fewer environmental hazards than synthetic chemicals.

Although DDVP produced more rapid mortality within 24 hours, the efficacy of Moringa seed oil at higher concentrations was comparable after prolonged exposure. This finding is important because synthetic insecticides such as DDVP are associated with several problems including insect resistance, toxic residues, environmental contamination, and health hazards to humans and non-target organisms (Georgiou, 1991; Talukder & Howse, 1994). Therefore, the use of Moringa seed oil offers a safer and ecofriendly alternative for smallholder farmers who may not afford or safely handle synthetic pesticides.

Furthermore, the gradual increase in mortality with exposure time indicates that prolonged contact between the oil and insects enhances toxic action. This observation corroborates earlier reports by Boateng and Kusi (2008) and Bamaiyi et al. (2007), who noted that botanical oils require sufficient exposure time to exert maximum insecticidal effect against storage pests.

Effect of Moringa oleifera Seed Oil on Oviposition and Egg Viability

A remarkable reduction in oviposition and egg viability was observed as concentration of Moringa oleifera seed oil increased (table 3). At higher concentrations, egg laying was drastically suppressed, while viable eggs were either significantly reduced or completely absent. This suggests that Moringa seed oil possesses both oviposition deterrent and ovicidal properties against C. maculatus.

The reduction in oviposition may be due to alteration of the seed surface by the oil, making the seeds less attractive or unsuitable for egg laying by gravid females. Botanical oils often interfere with chemical signals used by insects for host recognition and oviposition behavior. In addition, the oily coating may physically obstruct attachment of eggs onto seed surfaces. Similar observations were reported by Fasakin and Aberejo (2002), who found that plant-derived materials significantly reduced egg deposition and developmental success of storage insects.

The reduction in egg viability further indicates that the oil adversely affected embryonic development within the eggs. The active phytochemicals present in Moringa seed oil may penetrate the egg chorion and disrupt normal embryogenesis, leading to failure of egg hatchability. Pavela and Benelli (2016) reported that essential oils and plant extracts can exhibit strong ovicidal effects through interference with physiological and biochemical processes in insect eggs.

The present findings are also consistent with those of Magaji et al. (2011), who reported significant suppression of reproductive activities of C. maculatus exposed to botanical extracts. The ability of Moringa oil to inhibit oviposition and egg viability is particularly important because reproductive suppression reduces future insect population build-up and minimizes subsequent grain damage during storage.

From a practical standpoint, reducing oviposition and egg hatchability provides long-term protection of stored grains since fewer larvae develop within the seeds. This contributes significantly toward preserving grain quality, market value, and seed viability.

Effect of Moringa oleifera Seed Oil on F1 Adult Emergence

The study further showed that F1 adult emergence decreased significantly with increase in concentration of Moringa oleifera seed oil (table 4). Complete suppression of adult emergence at higher concentrations indicates that the oil effectively disrupted the developmental cycle of C. maculatus. This is an important finding because emergence of new adults is directly associated with population increase and continuous infestation during storage.

The inhibition of F1 emergence may result from combined effects on egg viability, larval survival, and pupal development. Since larvae of C. maculatus develop within cowpea seeds, toxic compounds present in the treated seeds may interfere with feeding, respiration, and metamorphosis of immature stages. The oil coating may also reduce oxygen availability within the seed microenvironment, thereby impairing larval growth and development.

Similar findings were reported by Oigiangbe et al. (2007) and Ofor et al. (2017), who observed significant suppression of progeny emergence in storage insects exposed to botanical powders and extracts. Nisar (2022) also reported that vegetable oils reduced development and adult emergence of C. maculatus in stored cowpea.

The complete inhibition of F1 emergence observed at higher concentrations highlights the strong protective potential of Moringa seed oil under storage conditions. This suggests that the oil not only kills existing adults but also prevents establishment of subsequent generations, thereby offering prolonged protection to stored grains.

In practical grain storage systems, suppression of progeny emergence is highly desirable because it minimizes repeated infestations and reduces the need for frequent pesticide application. This makes Moringa oleifera seed oil a promising component of sustainable postharvest pest management strategies.

CONCLUSION

Overall, the findings of this study clearly demonstrate that Moringa oleifera seed oil possesses strong bio insecticidal properties against C. maculatus. The oil significantly reduced adult survival, oviposition, egg viability, and F1 adult emergence, thereby disrupting multiple stages of the insect life cycle. Such multi-dimensional effects make the oil highly valuable as a potential grain protectant.

The increasing global concern regarding pesticide residues, environmental pollution, and insect resistance has stimulated interest in safer alternatives such as botanical insecticides. In this regard, Moringa oleifera presents several advantages because it is widely available, biodegradable, relatively cheap, environmentally friendly, and less hazardous to humans and non-target organisms. These attributes make it particularly suitable for resource-poor farmers in developing countries.

The findings of this study support earlier reports by Pavela (2016), Govindan et al. (2020), and Rahman and Talukder (2006), who emphasized the importance of plant-derived insecticides in sustainable pest management programs. However, despite the promising laboratory results, further studies are required to isolate and characterize the specific active compounds responsible for the insecticidal activity of Moringa seed oil. There is also need to evaluate its effectiveness under field and large-scale storage conditions, as well as its effects on seed germination, grain taste, and storage duration.

In conclusion, Moringa oleifera seed oil has considerable potential as an ecofriendly alternative to synthetic insecticides for the management of cowpea bruchid infestation in stored cowpea. Its integration into integrated pest management programs could contribute significantly toward reducing postharvest losses, improving food security, and promoting sustainable agricultural practices.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this study.

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