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Effect of *Monodora myristica* seed oil on *Callosobruchus maculatus* fabricius infesting cowpea seeds (coleoptera: chrysomelidae: bruchidae) in the store

O.M Obembe[1], J. Kayode[2*]

Author's Affiliation:
[1,2]Department of Plant Science and Biotechnology, Plant Resources Management Programme, Ekiti State University P.M.B 5363, Ado-Ekiti, Nigeria

***Corresponding Author:**
Joshua Kayode
Department of Plant Science and Biotechnology, Plant Resources Management Programme, Ekiti State University P.M.B 5363, Ado-Ekiti, Nigeria

E-mail:
joshua.kayode@eksu.edu.ng

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Abstract

Monodora myritica seed powder was prepared in the laboratory and was extracted with n-hexane using soxhlet procedure. The seed oil and powder were evaluated on *Callosobruchus maculatus* to study their effects on mortality, oviposition and adult emergence of the weevil. The results showed that the seed oil was the most toxic when compared to the powder. The seed oil caused 100 % mortality when treated at 2.0 % concentration by the 72 and 96 h post-treatment while the powder only caused 100 % mortality with the highest dosage of 2.0 g by 96 h after treatment. The oil and powder significantly reduced oviposition when compared to the controls. The seed oil completely prevented oviposition when treated at 2.0 % concentration and completely prevented adult emergence with 1.5 and 2.0 % seed oil concentration. The results obtained from this study showed that cotyledon oil and powder of *Monodora myristica* were effective in controlling cowpea weevil, *C. maculatus* and could serve as alternative to chemical insecticides for the protection of cowpea seeds against infestation by weevils

Keywords: *Monodora myristica, Callosobruchus maculatus,* oviposition, adult emergence.

INTRODUCTION

Cowpea, *Vigna unguiculata* L. Walp (Fabaceae), is an important grain legume in Nigeria and a good source of protein for the increasing human population and livestock (Murdock and Shade, 1991, Akinkurolere *et al*., 2006). It constitutes the cheapest source of dietary protein for most people in many developing countries where the daily intake of protein from other sources is low (Ogunwolu and Idowu, 1994; Oparaeke *et al*., 1998). In addition to the high protein content of cowpea, it also has high iron content but low in fat. Nigeria alone produces over two million tons of cowpea annually

(Oladiran, 1990) which was estimated to be about 70% of the total word production. However, cowpea seed is highly susceptible to a wide variety of pests both in the field and in the store (Ofuya, 1998).

The major pest affecting the efficient and effective storage of grains in Nigeria and other tropical countries are the insects and vertebrate pests (Adedire, 2001; Akinkurolere *et. al.*, 2006). Cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchidae) is a cosmopolitan insect of cowpea. It is a field-to-store pest, as its infestation of cowpea often begins in the field on the matured dry pod (Huignard *et al*., 1985; Sathyaseelan *et al*., 2008) and the insects are carried to the store after harvest where the population increases rapidly, leading to heavy infestation within a period of 3-4 months (Huignard *et.* al., 1985; Rhaman and Talukder, 2006). The multivotine type of reproduction of *C. maculatus* is a chief factor aggravating its ability to cause notable loss of cowpea seeds after harvest thereby lowering its market value.

The use of conventional insecticides was advocated as an effective means of controlling the destructive activities of the insects and other storage pests. The problem of these conventional insecticides which include environmental pollution, chemical residue in food, potential chronic toxicity, poor knowledge of application, non-availability, destruction of non-target organisms, resurgence of pest or potential pest, cost, ozone layer depletion, potential of chemical insecticides containing chlorofloro carbon and illiteracy of farmers resulting to their inability to read the labels on the insecticide containers, necessitated the search for cheap and ecologically friendly method of controlling pests (Sighamony *et al*., 1986; Adedire and Ajayi, 1996; Obembe and Kayode, 2018).

Thus in an attempt to circumvent the aforementioned problems associated with the use of chemical insecticides, attention is currently being focused on the use of natural compounds of plant origin for stored products protection because they are affordable, readily available, bio-degradable and pose no danger to the environment if used in small quantity (Keita *et al*., 2001).

The purpose of this paper was to examine the effectiveness of *Monodora myristica* seed oil in protecting stored cowpea seeds from infestation and damage by cowpea pests. *Monodora myristica* is a tropical tree of the family Annonaceae. The seed is used as popular spices in West Africa dish. It is also used for medicinal purposes and as well as insect repellent.

MATERIALS AND METHODS

Collection of seeds and preparation of seed oil

The fruits of calabash nutmeg, *Monodora myristica* were collected from a wild tree from Ugbo Elu forest in Omuo-Ekiti. The seeds were removed, dried in mild sunlight and later air-dried in the laboratory for 3 weeks. They seeds were subsequently ground in the laboratory into fine powder using an electric Binatone blender (Model BLG400). The powder was stored in black cellophane bag at a temperature of 4^{o}C until needed for the experiment. The oil was extracted from the powder stock of the plant with n-hexane at the temperature between 60^{o}C with soxhlet apparatus, using the method of Adedire *et al*. (2011).

Insect rearing

The initial culture of *Callosobuchus maculatus* was obtained from an infested cowpea. The insects were reared on Ife brown variety of cowpea at a temperature of 28 ± 2^{o}C and relative humidity of 75 ± 5% inside a transparent plastic container covered with muslin cloth held firmly with rubber band. The purpose of the muslin cloth is to prevent the escape of insects as well as prevent the entry of intruding insects. The culture was maintained by replacing the devoured seeds with un-infested ones. The newly emerged adult *C. maculatus* were used in every stage of this research work.

Effect of *Monodora myristica* seed powders on mortality of *C. maculatus*

Twenty grams of clean and dis-infested Ife browm variety of cowpea was weighed into Petri dish (9 cm diameter) and *Monodora myristica* seed powders weighing 0.3, 0.6, 0.9 and 1.2 g were added to the cowpea seeds in each of the Petri dishes. The powder and the cowpea seeds were thoroughly mixed together to enhance uniform spreading of the powder on the seeds. Untreated cowpea seeds were also set up to serve as the control experiment. Ten pairs of newly emerged adult *C. maculatus* (0-24 h old) were introduced into each of the Petri dish and covered with the Petri plate. Each of the treated and the untreated control was replicated four times and laid in Complete Randomized Design (CRD). Beetle mortality was observed at 24 h interval for a period of 96 h. After every 24 h, the number of dead beetles were counted and recorded. The beetles were confirmed dead when there was no response to probing on the abdomen with a sharp pin

Effect of *Monodora myristica* seed oils on mortality of *C. maculatus*

Twenty grams of clean and dis-infested Ife brown variety of cowpea was weighed into 9 cm diameter Petri dish and treated with 0.5, 1.0, 1.5 and 2.0% v/w of the oil. Solvent treated and untreated cowpea seeds serve as the control experiment. The cowpea seeds and the oil were thoroughly mixed with glass rod to enhance uniform coating of the seeds on the extracts. Thereafter, the treated seeds were air-dried for a period of 1 h, after which ten pairs of newly emerged adult *C. maculatus* (0-24 h old) were introduced into the Petri dish containing the treated and the control experiments. Four replicates were prepared for each treatment and the controls and arranged in a Complete Randomized Design. The numbers of dead weevil were counted and recorded at 24 h interval for a period of 96 h. Weevils were confirm dead when there was no response to probing on the abdomen with a sharp pin

Toxicity of *Monodora myristica* seed powders on oviposition and Adult emergence of *C. maculatus*

Twenty grams of clean and pristine Ife brown variety of cowpea seeds were measured into each of 9 cm diameter Petri dishes and *Monodora myristica* seed powders weighing 0.3, 0.6, 0.9 and 1.2 g were added to the cowpea seeds in each Petri dish. The powder and seeds were thoroughly mixed together to enhance uniform spreading of the powder on the seed. Untreated cowpea seeds were also set up to serves as the control experiment. Two pairs of newly emerged (0-24 h old) copulating adult *C. maculatus* were introduced into each Petri dish and covered with the Petri plate. Each treated and untreated control was replicated four times and arranged in Complete Randomized Design (CRD). The set up was left in a wooden cage in the laboratory for 7 days after which the insects were removed and the numbers of eggs were counted and recorded. Thereafter, the experimental set-up was kept undisturbed in the wooden cage till the emergence of adults. The number of adults that emerged were counted and recorded

Toxicity of *Monodora myristica* seed oils on oviposition and adult emergence of *C. maculatus*

Twenty grams of clean, dis-infested and wholesome Ife brown variety of cowpea seeds were weighed into 9 cm diameter Petri dish and treated with 0.5, 1.0, 1.5 and 2.0 % v/w of *Monodora myristica* seed oil. Solvent treated and untreated cowpea seeds were also prepared to serve as the control experiment. The cowpea seeds and the oil were thoroughly mixed with glass rod to enhance uniform coating of the seeds on the oil as earlier discussed above. Thereafter, the treated seeds were air-dried for a period of 1 h, after which 2 pairs of newly hatched (0-24 h old) copulating adult *C. maculatus* were introduced into the Petri dish containing the treated and the control experiment. Four replicates were prepared for each treatment and the controls and laid in a Complete Randomized Design (CRD). The experimental set-up was left in a wooden cage in the laboratory for 7 days after which the insects were removed and the numbers of eggs were counted and recorded. Thereafter, the experimental set-up was kept undisturbed in a wooden cage till the emergence of adults. The number of adults that emerged were counted and recorded.

DATA ANALYSIS

All data were subjected to one way analysis of variance at 5% significance level and were separated using Duncan's Multiple Range test.

RESULTS

Effect of *Monodora myristica* powders on mortality of *C. maculatus*

All dosages of powders used showed weevil mortality significantly different from the control. Adult mortality of *C. maculatus* increased with increased in time of exposure. The highest mortality of 100 % was recorded in cowpea seeds treated with 1.2 g of *Monodora myristica* seed powders by 96 h after treatment, and it is significantly different from 55.20%, 58.25% and 78.25%, in seeds treated with 0.3, 0.6 and 0.9 g of *Monodora myristica* powders respectively. Weevil mortality in powder treated grains differ significantly ($p < 0.05$) from weevil mortality in untreated grain (Table 1

Effect of *Monodora myristica* seed oil on mortality of *C. maculatus*

Weevil mortality in oil treated cowpea seeds differ significantly ($p < 0.05$) from weevil mortality in untreated and solvent treated seeds (Table 2). Adult mortality increased with increased in period of exposure. Weevils exposed to 2.0% v/w oil concentrations showed 100 % mortality by 72 and 96 h post-treatment. There was no significant difference in weevil mortality in the untreated and solvent treated seeds.

Table 1: Percentage mortality of *C. maculatus* treated with *Monodora myristica* seed powders

Dosage (g)	Percentage mortality at hours post-treatment*			
	2 4	2 4	72	96
0.3	12.00±2.26[d]	12.00±2.26[d]	48.75±1.25[c]	55.20±2.39[c]
0.6	24.20±1.25[c]	38.15±3.23[c]	45.25±2.23[c]	58.25±1.20[c]
0.9	34.00±2.04[b]	52.75±1.25[b]	74.20± 4.08[b]	78.25±0.28[b]
1.2	40.15±2.39[a]	70.23±2.39[a]	88.35±3.12[a]	100.00±0.00[a]
Untreated	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[d]	0.00±0.00[d]

*Each value is a mean of ± standard error of 4 replicates. Means within the same column followed by the same letter(s) are not significantly different at p>0.05 using New Duncan multiple range test

Table 2: Percentage mortality of *C. maculatus* treated with *Monodora myristica* seed oil

Conc. (%v/w)	Percentage mortality at hours post-treatment*			
	2 4	2 4	72	96
0.5	25.00±3.22[d]	48.75±4.39[c]	58.25±3.25[d]	58.22±3.25[d]
1.0	35.34±2.28[c]	52.15±1.25[c]	68.25±3.2[c]	75.25±2.39[c]
1.5	55.28±4.25[b]	68.75±3.23[b]	82.00±1.25[b]	92.75±1.44[b]
2.0	72.20±1.25[a]	84.33±1.25[a]	100.00±0.00[a]	100.00±0.00[a]
Untreated	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[f]	0.00±0.00[f]
n-hexane	4.25±1.22[e]	6.12±1.25[d]	9.25±1.30[e]	9.25±1.30[e]

*Each value is a mean of ± standard error of 4 replicates. Means within the same column followed by the same letter(s) are not significantly different at p>0.05 using New Duncan multiple range test

Table 3: Effect of *Monodora myristica* seed powder on oviposition and adult emergences of *C. maculates*

Dosage (g)	mean number of egg laid*	Percentage adult emergence*
0.3	20.25±0.75[b]	30.25±1.33[b]
0.6	15.25±1.2b[c]	25 .00±0.00[c]
0.9	15.75±3.75[c]	8.15±0.65[d]
1.2	12.25±1.22[c]	0.00±0.00[e]
Untreated	66.25±0.85[a]	75.75±2.20[a]

*Each value is a mean of ± standard error of 4 replicates. Means within the same column followed by the same letter(s) are not significantly different at p>0.05 using New Duncan multiple range test

Toxicity of *Monodora myristica* seed powders on oviposition and adult emergence of *C. maculatus*
All dosages of *Monodora myristica* seed powders effectively reduced oviposition by adult female *C. maculatus* (Table 3). Oviposition in powder-treated seeds was significantly lower ($p < 0.05$) than that of untreated seeds. The ability of the powder to reduce oviposition increased with increased in dosages of the powder. The percentage adult emergence in the untreated seeds was significantly higher than percentage emergence in the treated seeds. There was no adult emergence in seeds treated with 1.2 g of the seed powder and this is not significantly different from that of 0.9 g (Table 3).

Toxicity of *Monodora myristica* seed oils on oviposition and adult emergence of *C. maculatus*
All concentrations of *Monodora myristica* seed oils used effectively reduced oviposition (Table 4). Oviposition in oil-treated seeds was significantly lower ($p < 0.05$) than those of untreated and solvent-treated seeds. Oviposition was totally prevented in seeds treated with 2.0% oil concentration while there was no adult emergence in seeds treated with 1.5 and 2.0% v/w concentrations. The ability of the oils to reduce oviposition increased with increased in concentration of the oils.

Table 4: Effect of *Monodora myristica* seed oil on oviposition and adult emergence of *C. maculates*

Dosage (g)	mean number of egg laid*	Percentage adult emergence*
0.5	10.25 ± 3.25^{b}	45.25 ± 6.12^{c}
1.0	6.15 ± 3.25^{c}	15.00 ± 2.61^{d}
1.5	4.00 ± 2.22^{c}	0.00 ± 0.00^{e}
2.0	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
Untreated	64.15 ± 0.75^{a}	75.75 ± 2.20^{a}
N-hexane	58.20 ± 2.25^{a}	65.27 ± 4.12^{b}

*Each value is a mean of ± standard error of 4 replicates. Means within the same column followed by the same letter (s) are not significantly different at $p>0.05$ using New Duncan multiple range test

DISCUSSION

Results from this study showed that seed powder and oil of *Monodora myristica* were toxic to *Callosobruchus maculatus*, causing high beetle mortality and suppressing oviposition and adult emergence. Some researchers who have earlier evaluated plant oils and powders as botanical insecticides and grain protectants had found them very effective against storage beetles (Oparaeke *et al*., 1998; Oparaeke and Bunmi, 2006). The findings of the present investigation are similar to those of other researchers, who have previously reported that oils from botanicals such as cashew kernel and melon reduced the life span, ovipostion and adult emergence of weevils, (Adedire *et al*., 2011; Obembe and Kayode, 2018). Oil of *Azadirachta indica*, *Eucalptus citriodora* and sun flower acted as surface protectants against *C. maculatus* by suppressing seed damage rate, oviposition and adult emergence. It was also observed that different oils of coconut, palm kernel and castor acted as surface protectants of cowpea seeds and maize grains to control infestation by *C. maculatus* and *Sitophilus zeamais* respectively. The toxic effect of the oil on oviposition in this investigation could be due to respiratory impairment which must have adversely affected the metabolic process and consequently other systems of the body of the weevils (Osisiogu and Agbakwuru 1978; Onolemhemhem and Oigiangbe, 1991). The oil also must have inhibited locomotion, reducing the ability of the insects to move freely and thereby affecting mating and consequent oviposition (Adedire *et al.,* 2011).

The results obtained from this study are also similar to the observation of Obembe and Kayode (2018), who obtained 100 % mortality of *Callosobruchus maculatus* in cowpea seeds treated with oil of melon, *Citrillus vulgaris*. Plant oils and powders are commonly used in insect control because they are relatively toxic to virtually all life stages of insects (Adedire *et. al.*, 2003). Powders of some plants are effective in reducing oviposition and adult emergence of the bruchids (Lale and Abudulrahman, 1999).

In this study, *Monodora myristica* powder and oil effectively reduced oviposition and adult emergence of *C. maculatus* especially when treated at higher dosage. Even when eggs were laid, adult emergence was drastically reduced and in some cases totally prevented. Toxicity of the oil in this study could be attributed to the presence of high molecular weight fatty acid in the seed oil. The mechanism of action may be due to repellency, contact toxicity or change in the surface tension, which could cause protoplasm coagulation (Bhaduri *et al*., 1990). It may also be partially attributed to interference with normal respiration by blocking the spiracles (Adedire and Akinkurolere *et al*., 2005; Rhaman and Talukder 2006; Akinkurolere *et al*., 2006; Adedire *et al*., 2011). Plant oil may contain insecticidal and repellent compounds, including fatty acid and other compounds which must have affected the normal functioning of the insect (Don-Pedro, 1990).

The insecticidal activity of *M. myristica* seed oil could be linked to the presence of secondary plant compounds such as free fatty acid, diacylglycerols, sterols, monoacylglycerols and some polar lipids (Adedire *et al*., 2002). The results obtained from this research confirmed that *M. myristica* seed powder and oil are effective in controlling *C. maculatus* and the most effective is the seed oil. Thus, the seed powder and oil could serve as alternative to chemical insecticides. They are cheap and pose no danger to human and animals.

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