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Research Article
Insecticidal Activities of African Nutmeg Solvent Extracts Against Cowpea Seed Bruchid, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae)

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Abstract
Background and Objective: Protection of stored products from reduction in quality and market value by insect pests have been promoted with the use of aromatic and medicinal plants that are eco-friendly and relatively cheap biopesticides. African nutmeg, *Monodora myristica* (*M. myristica*) (Gaertn) Dunal, an African spice was investigated for its insecticidal activities against cowpea seed storage bruchid using solvent of different polarities for extraction. **Materials and Methods:** Extracts of the African nutmeg were evaluated at tropical ambient temperatures of 28±2°C and 75±5% relative humidity for their long-term protection ability against cowpea storage beetle *Callosobruchus maculatus* (*C. maculatus*). Indices used were, seed damage, water absorption capacity and viability of treated seeds. Data was analyzed by one way analysis of variance (ANOVA) using SPSS. **Results:** All test extracts protected cowpea seeds against infestation by *C. maculatus* with 0% seed damage and weight loss during a 3 month storage period at highest concentration of 0.6% v/w. Germination of seeds and water absorption capacity was not significantly affected ($p>0.05$) after treatment. **Conclusion:** The most effective extract was the steam distillate, although other solvents tested (methanol, ethanol, petroleum ether, n-hexane) also extracted the active ingredients in the *M. myristica* seeds for protection against beetle damage.

Key words: Seed damage, solvent extracts, steam distillate, stored products, water absorption capacity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alternative to expensive high proteinous staple diets are the food legumes. These are used as supplements to staple diets because of their high and relatively less expensive protein[1]. A major food legume in developing countries such as Nigerian is the cowpea, which represents the legume of choice for much of the population as the "Poor man's meat"[2]. Availability of such crops will ensure food security for the population but inability to protect or preserve crops from contamination and deterioration arising from microbial, fungal, vertebrate and invertebrate pest infestations leads to acute food shortage[3-5] especially in developing countries. In Nigeria, a major pest of stored grain legume including cowpea is *C. maculatus*. The infestations and attack by *C. maculatus* affects the aesthetic and nutritional quality of the seed as well as reduces seed weight[5]. A single insect can cause between 3 and 5% weight loss in a cowpea seed. In Nigeria, annual cowpea production is around 900,000 t but loss due to *C. maculatus* alone exceeds 2.9 t each year and up to 37-54% of unprotected seeds could be damaged during storage[3,5-9]. In some cases, 20-90% of beans have exit holes made by adult beetles and cowpea seeds with more than three holes from beetle damage have drastic reduced germination[5-6,10-11] although this is somewhat infrequent except when insects are left in a closed container over several months. The role of insecticides in pest management systems is constantly debated due to chemical residues in soil, water resources and food combined with high toxicity, increased incidence of insecticide resistance and non-biodegradability of pesticides[1,12]. Hence, the use of green pesticides that are biodegradable and environmentally friendly are regularly investigated for use as potential replacement for chemical control[13].

Extracts from plant parts using appropriate solvents can concentrate the active component and enhance their insecticidal activity[14,15]. The insecticidal effects of extracts of some aromatic and herbal plants obtained by using solvents of different polarities have been investigated and found effective against several insect species[16-18]. In addition, water extracts and essentials oils of different Mediterranean plants have been shown to have antifungal activities which could be used to protect olive plants[19] as well as neem oil in combination with entomopathogens to control vegetable pests[20].

Moreover, tropical African spices have great reduced risk as pest control compared to risk associated with conventional broad-spectrum insecticides[21]. Powdered seed of the aromatic African nutmeg (*Monodora* sp) has been used locally in local medicine and as a condiment in combination with pepper in soups[22-23]. The physical properties of African nutmeg were elucidated by Omobuwajo *et al.*[24] and this nutmeg has shown various antibacterial, antifungal and insecticidal actions[25-27]. The African nutmeg is available from local growers and used locally thus is readily available and this study tested the efficacy of African nutmeg extracts using solvents with different polarities. The objectives of the study were to determine the efficacy of solvent extracts of *M. myristica* in protecting cowpea seed from *C. maculatus* damage and the effect of these extracts on water absorption and germination of cowpea seeds.

MATERIALS AND METHODS

Insect culture: The study was carried out during the cowpea storage seasons (dry season) in Nigerian. Infested cowpea seeds purchased from the Erekesan Market in Akure, Ondo State, South-Western Nigeria, were used to establish a laboratory colony of *C. maculatus*. Beetles were reared subsequently by replacement of damaged cowpea seeds with fresh uninfested brown cowpea seeds in 2 L Kilner jars covered with muslin cloth to allow air circulation. After disinfestation by freezing at -1°C for 48 h, cowpea seeds used for the experiments were later air-dried and healthy seeds free of infestation were selected for use. All experiments were carried out at ambient temperature of 28±2°C and relative humidity of 75±5%.

Extraction processes and soxhlet extractions: African nutmeg seeds were obtained from the same market as the cowpea seeds. They were dried and milled into powder using a Binatone electric blender (BLG-400). One hundred grams of powdered nutmeg was put in a muslin cloth and transferred into a thimble and extracted via soxhlation with each solvent for 2-10 h[17]. Soxhlet extractions were carried out with each of the five solvents of analytical grade: Methanol, acetone, ethanol, petroleum ether and n-hexane[28]. The solvent was evaporated after extraction using a rotary vacuum evaporator (Resona type WB) under reduced pressure. The resulting oily extracts were air-dried at room temperature to remove excess solvent. The concentrated oily extracts were kept in brown bottles until ready for use.

Steam distillation: Steam distillation was carried out for 4 h using AOAC[29]. Briefly 100 g of the powdered material was added to 300 mL distilled water in a 500 mL round bottom flask. This was heated and the steam distillate was collected in a distillation receiver and was stored as before.

Effect of sub-lethal doses of *Monodora myristica* extracts on damage and survival of *C. maculatus* on treated cowpea seed after 12 weeks: Cowpea seeds were treated with the oils in plastic cups. Each cup contained 50 g of the seeds and different oil was added at three rates of 0.1, 0.2 and 0.3 mL (which represent 0.2, 0.4 and 0.6% v/w, respectively). The plastic cups containing the seeds and extracts were shaken manually to ensure uniform coating of all the seeds. All the treated grains were allowed to air dry for 1 h. They were then covered with muslin cloths which were held tightly in place with rubber bands to prevent entry or exit of insects but to allow aeration. Untreated seeds were included as a control. Five copulating pairs of *C. maculatus* were introduced into each cup containing the treated seeds and incubated for 12 weeks at room conditions. All treatments were arranged in a complete randomised design (CRD). The parameters monitored after the incubation period included number of damaged and undamaged seeds, number of exit holes, number of insects alive or dead and seed weight loss (50 g). The percentage seed damage and weight loss was calculated based on the method of Oni[30] and Ileke[31] as:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}} \times 100$$

$$\text{Seed damage (\%)} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times 100$$

Effect of *M. myristica* extracts on germination (viability) of cowpea seeds: An aliquot of 0.5 mL oily extract was added to disinfested 100 g [equivalent to 0.5% (v/w)] of cowpea seeds in 2 L Kilner jars. The seeds and oily extracts were manually agitated to evenly distribute the extracts on the seeds. The jars were then covered with muslin cloths to allow for aeration. Treated and untreated seeds in Kilner jars without insects were left in the laboratory at ambient tropical storage conditions for 12 weeks. For the eventual germination tests, forty seeds were randomly selected from each extract treated seed. Ten seeds/replicate were placed on moistened Whatman No. 1 filter paper in petri dishes with 9 mm inner diameter in a CRD. The untreated seeds were similarly placed in petri dishes. Seeds were inspected after 5 days post planting for germination. The percentage germination was calculated based on the method of Ileke[31] as:

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds planted}} \times 100$$

Effect of *Monodora myristica* extracts on water absorption capacity of cowpea seeds: Twenty grams of cowpea seeds were manually agitated with 0.1 mL (0.5% v/w) each of *M. myristica* oily extracts in petri dishes to ensure uniform distribution. The treatments were each replicated 4 times including a control without an extract. The treated grains were allowed to air dry for 1 h and incubated for 12 weeks in a CRD. They were then soaked in 50 mL distilled water and monitored after 1, 3, 6 and 24 h interval. Seeds were dried with paper towel and reweighed on each occasion to calculate weight change, which was used as an index of water absorption capacity of the seeds[32].

Statistical analysis: The data obtained from the insecticidal activity assay were subjected to one way analysis of variance (ANOVA) at 0.05 significance level and means were separated by *post hoc* Tukey's test where significant differences existed (SPSS 22).

RESULTS AND DISCUSSION

The steam distillate at different concentrations gave a better protection against *C. maculatus* infestation than other extracts within the 12 weeks storage period while other extracts were effective in protecting cowpea seeds against infestation at the highest concentration of 0.6% v/w (Table 1). Earlier studies showed that *M. myristica* powder and 50% dilutions of crude extracts protected cowpea seed against *C. maculatus* infestation for more than 4 months[22, 33]. Also, pulverized seed and extracts of different plant materials including African nutmeg had varying degrees of insecticidal effects on *C. maculatus*, *C. chinensis*, *C. rhodesianus* and *Acanthoscelides obtectus* on stored cowpea seeds[15-16,25]. Regarding weight loss, there was a significant difference ($p<0.05$) in weight loss between treated seeds and untreated seeds. The African nutmeg extracts gave long-term protection to stored grains at 0.6% v/w because there was no weight loss, no live adult bruchids, no exit holes and 0% grain damage (Table 1). These results are similar with observations of Emeasor *et al.*[34], that oil of *M. fragrans* gave long-term protection to cowpea seeds at all concentrations. The essential oil of African nutmeg contains active components such as phellandrene, p-cymene and limonene, which have insecticidal properties[35] and these might have been responsible for its contact action as observed in this study (per observation). Furthermore, the insecticidal activity of a closely related species, *M. tenuifolia* has been ascribed to the

Table 1: Effect of *Monodora myristica* seed extracts on insect damage caused by *Callosobruchus maculatus* after 12 weeks

Extracts	Concentration (% v/w)	Weight loss (%)	Number of dead bruchid	Number of live adult bruchid	Number of exit holes	Number of seeds	Number of damaged seeds	Number of undamaged seeds	Seed damage (%)
Methanol	0.2	2.6±0.2^{c}	62.0±3.0^{c}	0.0±0.0^{a}	52.0±3.0^{c}	235.0	50.3±1.6^{c}	184.7±0.3^{b}	25.4±0.4^{c}
	0.4	0.1±0.0^{a}	11.3±1.0^{a}	0.0±0.0^{a}	1.3±0.6^{a}	232.7	1.0±1.0^{a}	231.7±1.7^{bc}	7.1±0.4^{a}
	0.6	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	236.0	0.0±0.0^{a}	236.0±3.0^{b}	0.0±0.0^{a}
Ethanol	0.2	1.0±0.2^{b}	32.0±3.3^{b}	0.0±0.0^{a}	22.0±3.3^{b}	235.7	20.3±3.3^{b}	210.0±8.0^{c}	8.6±1.3^{b}
	0.4	0.0±0.0^{a}	10.0±2.7^{a}	0.0±0.0^{a}	0.0±0.0^{a}	232.7	0.0±0.0^{a}	232.7±3.0^{bc}	0.4±0.0^{a}
	0.6	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	235.7	0.0±0.0^{a}	235.7±3.0^{b}	0.0±0.0^{a}
Acetone	0.2	3.0±0.4^{c}	77.0±11.3^{c}	0.0±0.0^{a}	67.0±11.3^{c}	237.3	65.0±11.0^{c}	172.3±11.3^{b}	27.4±4.7^{c}
	0.4	1.8±0.2^{b}	18.3±2.6^{a}	0.0±0.0^{a}	8.3±2.6^{a}	235.0	8.3±2.0^{b}	226.7±3.7^{b}	1.9±1.1^{a}
	0.6	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	237.3	0.0±0.0^{a}	237.3±0.3^{b}	0.0±0.0^{a}
Petroleum ether	0.2	0.0±0.0^{a}	18.7±2.3^{ab}	0.0±0.0^{a}	8.7±2.3^{ab}	234.3	8.3±2.0^{ab}	226.0±1.6^{cd}	3.6±0.8^{a}
	0.4	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	235.3	0.0±0.0^{a}	235.3±3.7^{bc}	0.0±0.0^{a}
	0.6	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	233.3	0.0±0.0^{a}	233.3±1.3^{b}	0.0±0.0^{a}
Hexane	0.2	0.0±0.0^{a}	13.0±0.6^{ab}	0.0±0.0^{a}	3.0±0.6^{ab}	235.7	3.0±0.6^{ab}	232.7±2.6^{cd}	1.4±0.3^{a}
	0.4	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	235.0	0.0±0.0^{a}	235.0±1.3^{bc}	0.0±0.0^{a}
	0.6	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	236.0	0.0±0.0^{a}	236.0±2.0^{b}	0.0±0.0^{a}
Steam	0.2	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	234.3	0.0±0.0^{a}	234.3±2.3^{d}	0.0±0.0^{a}
Distillate	0.4	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	238.0	0.0±0.0^{a}	238.0±0.7^{c}	0.0±0.0^{a}
	0.6	0.0±0.0^{a}	10.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	237.7	0.0±0.0^{a}	237.7±0.7^{b}	0.0±0.0^{a}
Untreated	0.2	8.4±0.1^{d}	224.3±1.5^{d}	234.3±4.0^{b}	448.7±3.3^{d}	236.3	236.3±0.6^{d}	0.0±0.0^{a}	100.0±0.0^{a}
	0.4	8.3±0.1^{c}	235.0±5.0^{b}	244.7±4.3^{b}	466.3±18.7^{b}	237.0	236.3±0.6^{c}	0.7±0.6^{a}	99.7±0.3^{b}
	0.6	8.5±0.1^{b}	239.7±2.3^{b}	225.3±6.3^{b}	455.0±5.0^{b}	236.3	236.3±0.7^{b}	0.0±0.0^{a}	100.0±0.0^{a}

[a]All values are means of triplicate samples followed by standard error of the mean (SEM). Means followed by the same letter (superscripts at the end of each value) within a column are not significantly different, (p>0.5) by Tukey's test

Table 2: Effect of *Monodora myristica* extracts on water absorption of cowpea seeds

	Water absorption (% wt gain/h)			
Extracts 0.5% (v/w)	1	3	6	24
Methanol	16.6±1.3^{b}	19.3±0.2^{ab}	25.0±0.6^{a}	31.0±1.0^{d}
Ethanol	16.6±1.5^{b}	18.4±4.7^{a}	24.1±0.8^{a}	31.7±2.0^{d}
Acetone	16.2±0.3^{b}	19.3±4.1^{ab}	26.8±1.9^{b}	31.3±0.6^{d}
Petroleum ether	16.0±1.0^{b}	19.3±4.5^{ab}	24.2±2.0^{a}	27.1±0.1^{b}
Hexane	15.9±0.9^{b}	22.0±1.9^{b}	25.0±0.8^{a}	28.8±0.8^{c}
Steam distillate	15.8±0.7^{ab}	18.1±0.8^{a}	23.9±0.6^{a}	25.4±0.7^{a}
Untreated	14.6±2.0^{a}	17.7±2.0^{ab}	24.7±1.1^{a}	37.1±3.2^{e}

[a]Each value is the percentage of Mean±Standard error of the mean (SEM) of 4 replicates. Mean in each column followed by the same letter(s) (superscripts at the end of each value) are not significantly different at 5% levels of probability by Tukey's test

presence of high molecular fatty acids, sterols and triterpene alcohols[23]. Eugenol, one of the constituents of *M. myristica,* was effective as a repellent and gave 100% mortality of the insect pests, *S. oryzae*, *T. castaneum*, *Oryzaephilus surinamensis*, *R. dominica* and *C. chinensis* on stored food products[34]. Essential oils of some similar aromatic medicinal plants protected stored grains from damage and showed persistence against some pests such as, *Sitophilus oryzae*, *Rhyzopertha dominica*, *C. chinensis* and *C. maculatus* [36-37].

Rate of absorption of water by treated cowpea seeds varied with extracts and period of submergence (Table 2). The cowpea seeds pre-treated with different extracts of *M. myristica* did not show any negative water absorption capacity when compared with the controls after 3 and 6 h (p>0.05). After 24 h of submergence in water, there were differences in water absorption capacity of treated and untreated seeds and the amount of water absorbed by seeds was directly proportional to period of submergence. Pre-treatment of maize grains with different seed extracts at 1, 2 and 3% did not affect their water absorption capacity[32,37] and as reported for cowpea in this present study.

The steam distillate and methanol extract did not adversely affect seed germination, whereas other extracts reduced germination (Fig. 1). Similarly, dilution of crude extracts of *M. myristica* at 20 and 50% did not affect the germination ability of treated cowpea seeds after 3 months[22] rather germination of treated seeds like wheat, broad bean and pulses with plant oils increased with use of higher concentrations of extracts[37-40]. This study revealed that, African nutmeg treated seeds gave a low percentage germination (22.5-60%) compared to (82.5%) untreated seeds but are still higher than the 6.7% germination with low powder concentrations (2 g/100 g seeds) recorded by Adedire and Akinkurolere[32]. In this study, methanol and steam distillate

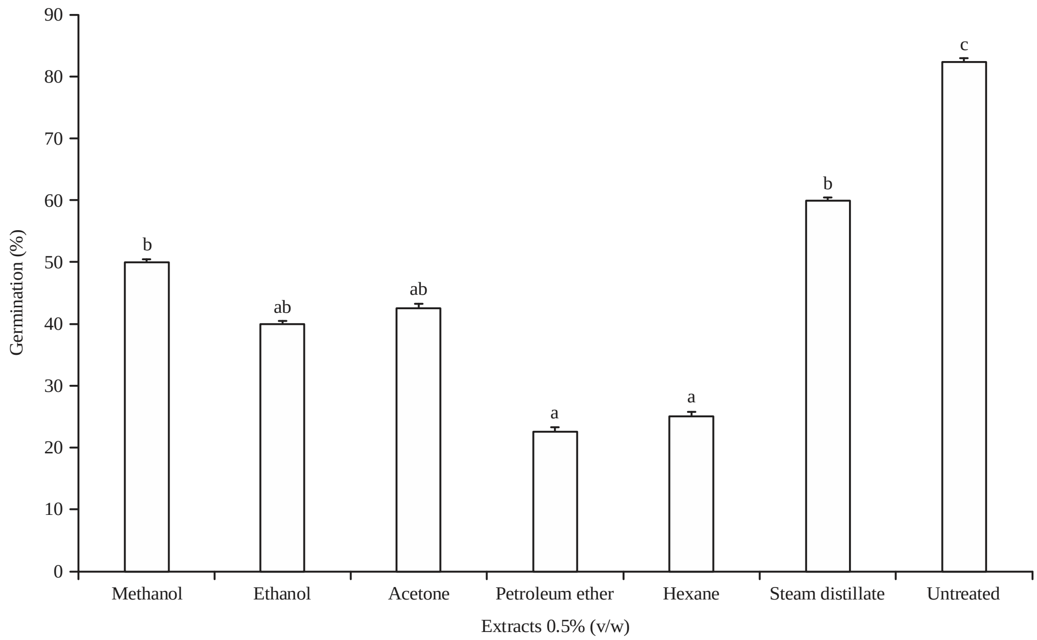

Fig. 1: Effect of *Monodora myristica* extracts on viability of treated seeds

Each value is a mean of 4 replicates±standard error of the mean (SEM) of 4 replicates. Same letter(s) are not significantly different at 5% levels of probability by Tukey's test

extracts did not adversely inhibit viability of the cowpea seeds at 0.5% v/w which indicates that germination ability could be enhanced if higher concentrations of extracts are used as observed by earlier workers that germination ability of seeds treated with African nutmeg increased with increase in concentrations of extracts[22,32]. Steam distillate treated seeds still had 60% germination which is still an improvement over total germination loss caused by beetle infestations. Moreover, there was no negative water absorption capacity of cowpea seeds pre-treated with different extracts of *M. myristica* when compared with the controls after 1-6 h suggesting the suitability as anti-insecticidal agents against bruchid infestations.

CONCLUSION

This study revealed the potential of *M. myristica* steam distillate as an alternative non-chemical control for management of stored cowpea against bruchids infestations. Steam distillate is also safer and produces fewer environmental contaminants relative to chemical control.

SIGNIFICANCE STATEMENTS

In this study revealed the potential of *M. myristica* steam distillate. This study contribute in using eco-friendly and cheap biopesticides in pest management and hence, reduction in crop loss due to infestations. This help to reduce food shortages in developing economies and ensure sustainable food security.

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