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INSECTICIDAL EFFECT OF AFRICAN NUTMEG (*Monodora myristica*) OIL ON *Sitophilus zeamais* and *Tribolium castaneum* IN AFRICAN BREADFRUIT

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Abstract
This study investigated insecticidal effect of African nutmeg (*Monodora myristica* Gaertn) oil against *Sitophilus zeamais* (Motsch) and *Tribolium castaneun* (Herbst) in African breadfruit during storage. Fruits (400g) of African nutmeg were milled into flour. Two hundred grams (200g) of flour was extracted for oil using 500ml of n-hexane. The fruit oil at 0.0, 0.25, 0.50, 0.75, 1.00ml per 1ml ethanol was dosed against 10 adults of either insects infested on 15-20g African breadfruit seeds during post-exposure (24h), contact (7-12 days) and fumigant (72h) toxicity tests at ambient conditions (33-39°C; 42-59% RH) in Lafia, Nasarawa State, Nigeria. The oil caused dose-dependent mortality (%) which increased with exposure time of the insects. *Sitophilus zeamais* (Motsch) had higher mortality than *Tribolium castaneum* (Herbst) within the same oil concentration and exposure time. In a glass vial filled to 70% column with African breadfruit, 1ml oil concentration caused 63% kill of *T. Castaneum* (Herbst), but 90% kill of *S. zeamais* (Motsch) in 7days exposure. The 1ml oil concentration on 72hour fumigation test killed 95% of *S. zeamais* (Motsch) and 62% of *T. castaneum* (Herbst). *Monodora myristica* (Gaertn) oil could replace synthetic insecticides to preserve this grain against the two insect pests under storage.

Keywords: **African nutmeg oil, insecticidal, *Tribolium castaneum* (Herbst), *Sitophilus zeamais* (Mostch), African breadfruit seeds**

Introduction

The current natural disasters, including fire outbreak, flood and drought, and pest infestation pose food insecurity globally. As a result of food insecurity, unsatisfactory feeding persists in most developing countries (UNICEF, 1989; WHO, 2000). Protein-energy malnutrition continues to ravage youths and children in these countries; and this impairs their growth, health status, mental capability and productivity (Osagie and Eka, 1998; Bertman and Kawachi, 2000; Ivanovic *et al*., 2002; Ischara, 2005). Nigeria is one of such developing countries with food insecurity for its people. Animal protein is scarce and too costly to be afforded by the ordinary citizens of Nigeria. Most of the commonly consumed food grains (cereals and legumes) are equally becoming expensive; precisely because of their high demand by the teaming population. Current studies focus on the exploitation of some indigenous, unconventional and underutilized food grains for their food potentials. One of such underutilized food grains is African breadfruit (*Treculia africana* Decne).

African breadfruit thrives in most tropical and subtropical regions. It is a forest tree and produces abundant fruits with numerous seeds. The edible seeds are high in mineral nutrients, and are good source of protein (Osabor *et al*., 2009). It is used as a component of the diets of many Southern Nigerians (Nwabueze, 2004; James and Nwabueze, 2013). The seed flour is a good complement to wheat flour in confectionaries (Giami *et al*., 2004; Nwabueze *et al*., 2008). Unfortunately, African breadfruit seeds are easily attacked and destroyed by insect pests, particularly *Sitophilus zeamais*, *Sitophilus orizae* and *Tribolium custaneum* shortly after harvest. The seeds need to be protected from insect pests. Most seed grains in Nigeria are mainly protected against insect pest attacks with synthetic insecticides. However, many health and environmental experts condemn the use of chemicals for pest management in foods (Lale, 1995; Shaaya *et al.,* 1997). Their arguments were that many insect pests develop resistance to such synthetic chemicals and that most of them degrade to gaseous components that deplete the ozone layer in the atmosphere (WHO, 1995).

Plant materials which are cheap, readily available, simple and convenient to use are advocated for use for insect control (Isman *et al*., 2006; Bakkali *et al*., 208; Regnault-Roger *et al*., 2012). Most of these plant materials, including spices do not decompose to any harmful residue nor constitute any environmental health hazard. Many spices of international trade such as English nutmeg, clove, ginger and garlic, *Xylopia*, and their extracts have been evaluated for insecticidal effects against many insect pests of common food grains (Raja-Pakse and Van-Emden, 1997; Ogunwolu *et al*., 1998; Adewoyin *et al*., 2006; Touati *et al*., 2011; Rastegar *et al*., 2011). However, many important indigenous grain legumes, including African breadfruit, of tropical Africa are yet to be preserved with such natural plant materials. There is the need to exploit such spices and their extracts to preserve the seeds of African breadfruit to fully utilize its food potentials. The spice African nutmeg (*Monodera myristica*) commonly used to flavour soups and stews in many homes in Nigeria, and which is proven to have insecticidal effects on many storage insect pests is to be exploited to preserve the seeds of African breadfruit(*Treculia africana* Decne). The objective of this study was therefore to extract the oil of African nutmeg, and then investigate its insecticidal effect on two primary insect pests of *Treculia Africana*; *Sitiophilus zeamais* and *Tribolium castaneum*.

Materials and Methods

***Materials*:** Dry undehulled seeds of African breadfruit were purchased from a peasant farmer at Ogbede-Aku market, Igbo-Etiti Local Government Area (LGA), Enugu State, Nigeria. Dry fruits of African nutmeg were obtained from commercial stockers at the Ogige Main Market, Nsukka, Enugu State. Two storage insect pests of African breadfruit seeds; *Trebolium castaneum* Herbst and *Sitophilus zeamais* Motsch were obtained from the Department of Entomology, University of Agriculture, Abeokuta, Nigeria.

***Extraction of oil from the seeds of African nutmeg*:** The seeds (400g) were cracked manually and the hard dry hull winnowed away. The intact seeds were milled into flour in attrition mill. The flour (200g) was mixed with 500ml of n-hexane and shaken for 30 minutes on a rotary shaker. The suspension was left still for 48 hours and then filtered off the solid residue through a nylon sieve to recover n-hexane-oil mixture which was heated on a rotary evaporator maintained at 60^0C for 30min to recover the hexane and the spice oil (Ashurt, 1991; Moyler, 1988). The spice oil was then stored in Bijou bottle for further use.

***Cleaning and preparation of African breadfruit seeds*:** African bread fruit seeds were cleaned to get rid of infested seeds, dirt, stones and chaffs. The cleaned seeds (500g) were sun-dried for 48 hours, oven-dried at 50^0C for 48 hours and then used for insecticidal tests.

***Rearing of the insect pests*:** *Tribolium castaneum* Herbst were reared on wheat flour mixed with yeast (10:1, w:w), while *Sitophilus zeamais* Motsch were reared on whole breadfruit seeds (12 – 13% moisture), all maintained at room temperature (30 - 39^0C; 42 – 59% RH) in Lafia (03.33^0N and 08.32^0E), Nassarawa State, Nigeria. Adults of both insect species for the fumigant, post-exposure and contact studies were 2 – 4 weeks old before use.

***Post-exposure toxicity*:** The toxicity test of African nutmeg oil was carried out as described by Huang *et al*. (1997). The spice oil concentrations (0.00ml, 0.25ml, 0.5ml, 0.75ml and 1.0ml) diluted with 1ml absolute ethanol was impregnated on filter papers. The filter papers of each oil concentration were fixed to the inner floor of each 35-ml glass vial. Ten adult insects were confined to each filter paper treatments within the 35-ml glass vial for 24 hours. After the 24 hours confinement, the insects were transferred to holding cages {70% filled with culture medium (*Treculia Africana*) seeds; whole for *S. zeamais* and broken seeds for *T. castaneum*)} and kept on laboratory bench at ambient condition for 10 days. Mortalities for the insects were recorded to compare with the control. Each treatment was replicated four times.

***Fumigant toxicity*:** Different volumes (0.00, 0.25, 0.50, 0.75 and 1.00ml) of the spice oil were mixed with 10g of Africa breadfruit seeds in 1litre glass vial to give approximately 25, 50, 75 and 100ml oil/kg seed respectively. Ten adults of either insect, *Sitophilus zeamais* Motsch or *Tribolium castaneum* Herbst were introduced into another 500-ml glass vial. This was sealed immediately with nylon sieve using rubber band, and inverted with the mouth fitting with that of the bigger vial containing the treated seeds. Both ends were sealed with masking tape and kept at the laboratory bench over time for mortality assessment. Control samples were equally prepared but with no spice oils.

***Contact toxicity*:** Whole and broken seeds of African breadfruit sealed in polyethylene bags were kept for 8 days in a deep freezer to destroy any hidden pest. After the 8 days, seeds were allowed to equilibrate to ambient temperature and relative humidity. The African nutmeg oil was dissolved at 0.0, 0.25, 0.5, 0.75 or 1ml concentrations in 1ml of hexane and then ad-mixed with 20g of whole or 15g of broken seeds of African breadfruit in 70% filled columns in glass vials (Hao *et al*., 1998). The control had no spice oil. Five hours after treatment, each bottle of treated and control samples were infested with 10 adults of *Tribolium castaneum* or *Sitophilus zeamais* at the ratio of 6 females to 4 males. Insect mortalities were screened and recorded daily for 10 days. All treatments were replicated 4 times

***Data analysis*:** Probit analysis (Finney, 1971) using maximum likelihood programme software was employed in analysing the dosage mortality responses of the insects.

Results and Discussion

Effect of African nutmeg oil on adult T. castaneum in African breadfruit seeds

African nutmeg oil at 0.00, 0.25, 0.50, 0.75 and 1.0mls per 15g – 20g seeds of African breadfruit caused mortalities of *T. castaneum (Herbst)* in the African breadfruit seeds. The results of contact and fumigant toxicity tests of this spice oil at the different concentrations and storage time are shown in Tables 1 and 2. Mortality of *Tribulium castaneum* increased with increasing oil concentrations and storage periods in the 70% filled chambers (Table 1). No *T. casteneum* died within the first two days of contact toxicity test. However, on the 3rd day of the storage test, insect mortality occurred in all the treated samples, except the control (0.0ml). The % cumulative mortality of *T.castaneum* increased significantly ($p< 0.05$) with increased oil concentrations (Table 1) within 10 days storage period. Mortality of *T. castaneum* was significantly higher ($p<0.05$) in all the samples with various *Monodora* oil concentrations, (0.25, 0.50, 0.75 and 1.0ml), than in the control, (0.0ml) as from the 6th day of storage (Table 1). After 10 days of storage, the highest oil concentrations of 1ml in broken seeds had as high, as approximately 2½ times dead adult *T. castaneum* as 0.25ml oil per 15g broken seeds, and 2 times mortality of adult *T. castaneum* compared to 0.50ml oil per 15g broken seeds. The control had only 5% cumulative mortality of the insect on the 9th and 10th day of the contact toxicity test.

Table 1: Cumulative motality (%) of adult *T. castaneum (Herbst)* with dosage in broken Treculia seeds (15g) protected with monodora myristica (Gaertn) oil in 70% filled glass vial

Storage time (days)	Concentration (ml oil /15g broken seeds) levels				
	0.00	**0.25**	**0.50**	**0.75**	**1.00**
1	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
2	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
3	0 ± 0^{c}	3 ± 2^{c}	5 ± 3^{c}	17 ± 3^{b}	40 ± 6^{a}
4	0 ± 0	3 ± 2^{c}	5 ± 3^{c}	25 ± 3^{b}	45 ± 6^{a}
5	0 ± 0^{d}	8 ± 3^{c}	10 ± 4^{c}	32 ± 2^{b}	48 ± 5^{a}
6	0 ± 0^{d}	13 ± 3^{c}	13 ± 5^{c}	33 ± 3^{b}	50 ± 4^{a}
7	0 ± 0^{d}	15 ± 5^{c}	23 ± 4^{b}	53 ± 6^{a}	63 ± 3^{a}
8	0 ± 0^{e}	15 ± 5^{b}	23 ± 4^{b}	53 ± 6^{a}	63 ± 3^{a}
9	5 ± 3^{d}	17 ± 3^{c}	37 ± 3^{b}	57 ± 4^{a}	65 ± 5^{a}
10	5 ± 3^{d}	30 ± 4^{c}	40 ± 4^{b}	65 ± 5^{a}	78 ± 8^{a}

Values are means of 4 determinations ± standard deviation. Means with the same superscript in same row are not significantly (p > 0.05) different

Similarly, bioassay fumigant toxicity of the African nutmeg oil against *T. castaneum* exhibited increasing mortality of the insect with increasing oil concentrations and fumigation time (h) in 1500ml fumigation chambers (Table 2). However, no insect was killed within the first 6 hours. Insect mortality was recorded on the first 12th hours and thereafter, with cumulative mortality increasing with oil concentration. There was no insect mortality in the control, (0ml) throughout the 72 hours period of fumigation study. However, within the oil-treated insect chambers, mortality of *T. castaneum* increased gradually and significantly ($P< 0.05$) as the oil concentration increased (Table 2). Contact and fumigant toxicity study of *Monodora* essential oil exhibited remarkable protection against *T. castaneum.* The toxicity of a number of plant oils and their constituents, monoterpenes had been evaluated against stored product insect pests. Kinganf *et al.* (1983) showed that 16 essential oils exhibited fumigant toxicity against *Acanthascelides obtectus.* A number of essential oils extracted from various spices and medicinal plants of the Mediterranean area were also found to be active against *S. orizae, R. dominica, O. surinamensis* and *T. castaneum* (Pugazhvendan *et al*., 2012; Kim; 2014). The result of this study on contact and fumigant study with African nutmeg against *T. castaneum* agrees with those of Huang *et al.* (1997), Chaubey (2008) and Mondal *et al*. (2010) on contact and fumigant toxicity of essential oil from English nutmeg against the same insects.

Table 2: Fumigant toxicity (%) of *Monodora myristica* (Gaertn) oil against *T. castaneum* Herbst on broken *Treculia africana* Decne seeds at 10% filling in 1500ml fumigation chamber (%)

Spice oil (ml oil/15g broken seeds)	Exposure time (Hours)					
	3	**6**	**12**	**24**	**48**	**72**
0.00	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{c}	0 ± 0^{d}
0.25	$0 \pm 0_{a}$	0 ± 0^{a}	2.5 ± 2.5^{b}	15 ± 6.0^{b}	15 ± 6.0^{b}	20 ± 4.0^{c}
0.50	0 ± 0^{a}	0 ± 0^{a}	7.5 ± 2.5^{ab}	10 ± 4.0^{b}	17 ± 2.5	20 ± 4.0^{c}
0.75	0 ± 0^{a}	0 ± 0^{a}	7.5 ± 2.5^{ab}	17 ± 4.0^{a}	25 ± 8.0^{a}	45 ± 6.5^{b}
1.00	0 ± 0^{a}	0 ± 0^{a}	15 ± 5.0^{a}	32 ± 7.5^{a}	40 ± 4.0^{a}	62 ± 8.5^{a}

Values are means of 4 determinations ± standard deviation Means with the same superscript in the same column are not significantly (p>0.05) different

Effect of African nutmeg oil on S. zeamais in African breadfruit seeds

African nutmeg oil also exhibited insecticidal effect on *S. zeamais* in whole African breadfruit seeds (13±1% moisture content) during contact and fumigation tests (Tables 3 and 4). The spice oil caused remarkable insect mortality in the seed samples within the ten days experimental period. Cumulative mortality of *S. zeamais* increased with increasing oil concentration throughout the contact and fumigation periods. The 0.25ml o*f Monodora myristica* oil/20g of African breadfruit seeds caused 32% and 42% cumulative mortality of *S. zeamais* within the 4 and 6 days of the contact toxicity test, while, 0.75ml of the same oil caused 67% and 80% cumulative mortality of the same insect on same days respectively. The highest oil dosage of 1.0ml/20g seeds caused 75% and 87% cumulative mortality of the same *S. zeamais* within the 4 and 6 days of the contact toxicity respectively. On the 7th day of the contact toxicity study, the test sample with highest oil concentration of 1.0ml oil per 20g seeds in 70% filled glass vial recorded 90% cumulative mortality of *S. zeamais*, leaving only 10% of *S. Zeamais* insect weakly surviving (Table 3). The percentage cumulative mortality of *S. zeamais* for samples with 0.75ml and 1.0ml oil at each storage day were significantly (P<0.05) comparable but differed significantly (p>0.05) from the mortality rates of samples with 0m1, 0.25ml and 0.5ml oil treatment (Table 3). The control had 5% insect mortality on the second day of storage and maintained this value throughout the remaining period of storage. It is evident that the 5% mortality recorded with the control was due to some physiological failure of the insect species rather than of any oil treatment (Conti *et al*., 2010; Lins *et al*., 2019).

Table 3: Mortality (%) of adult *S. zeamais* in whole *Treculia africana* seeds (15g) treated with *monodora myristica* oil in 70% filled glass vial

	Storage time (Day)						
Oil concentration (ml/20g whole seeds)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
0.00	0 ± 0^{c}	5 ± 2.5^{d}	5 ± 2.5^{d}	5 ± 2.5^{c}	5 ± 2.5	5 ± 2.5^{d}	5 ± 2.5^{c}
0.25	5 ± 2.5^{bc}	20 ± 5.0^{cd}	32 ± 2.5^{c}	32 ± 2.5^{b}	35 ± 2.5^{c}	42 ± 4.5^{c}	52 ± 4.5^{c}
0.50	10 ± 4.0^{b}	37 ± 2.5^{bc}	45 ± 5^{b}	45 ± 5^{b}	60 ± 8^{b}	65 ± 5^{b}	77 ± 4.5^{b}
0.75	32 ± 2.5^{a}	55 ± 5.0^{ab}	72 ± 4.5^{a}	67 ± 7.5^{a}	70 ± 7.0^{ab}	80 ± 4.0^{a}	85 ± 5.0^{ab}
1.00	35 ± 2.5^{a}	65 ± 10.0^{a}	70 ± 7.0^{a}	75 ± 2.5^{a}	75 ± 5.0^{a}	87 ± 4.5^{a}	90 ± 4.0^{a}

Values are means ± standard deviation of 4 determinations. Means with the same superscripted letters in the same column are not significantly (p>0.05) different

No insect mortality was recorded in all the treatments within the first 12 hours of the fumigation study (Table 4). However, with the passage of time, the *S. zeamais* started dying in the oil-treated African breadfruit seed samples. Percentage cumulative mortality of *S. zeamais* increased significantly (P<0.05) with increasing oil concentrations and fumigation time. The percentage (%) cumulative mortality of the insect in the control differed significantly (p<0.05) from those of the treated samples. Also, samples with the highest 1.0ml *Monodora myristica* oil had significantly (p<0.05) highest percentage cumulative insect mortality than every other treatments. It is evident that *Monodora myristica* oil significantly (P<0.05) suppressed survival of *S. zeamais* through fumigation. Unfortunately, higher mortality of adults of both *T. castaneun* and *S. zeamais* occurred in the fumigation study with short duration than with the contact study which had longer duration. No obvious reason could be advanced to explain this discrepancy. However, it could be probably due to different batches of insects used and also due to the different time at which the experiment was performed.

Table 4: Fumigant toxicity (%) effect of *Monodora myristica (*Gaertn*)* oil on *Sitophilus zeamais (*Motsch*)* in whole *Treculia africana* Decne seeds at 10% filling in 1500-ml fumigation chamber

Oil concentration (ml oil/15g seeds)	**Exposure time (Hours)**					
	3	**6**	**12**	**24**	**48**	**72**
0.00	0 ± 00	0 ± 0	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{d}	0 ± 0^{d}
0.25	0 ± 00	0 ± 0	10 ± 4.0^{b}	20 ± 4.2^{b}	25 ± 6.0^{c}	47 ± 4.0^{c}
0.50	0 ± 00	0 ± 0	12 ± 4.8^{b}	35 ± 2.5^{a}	40 ± 4.1^{b}	60 ± 1.2^{b}
0.75	0 ± 00	0 ± 0	5 ± 1.0^{bc}	20 ± 4.2^{b}	52 ± 4.5^{b}	65 ± 8.8^{b}
1.00	0 ± 00	0 ± 0	27 ± 2.5^{a}	30 ± 0.0^{a}	70 ± 4.2^{a}	95 ± 2.5^{a}

Values are means of 4 determinations ± standard deviation. Means with the same superscript in the same column are not significantly (p>0.05) different

The mean knock down (%) of adult *T. Castaneum* (Herbst) and *S. zeamais* (Motsch) after 24 hours of post-exposure to various concentrations (0.0, 0.25, 0.5, 0.75 and 1.0ml) of *Monodora myristica* (Gertn) (African nutmeg) oil on fixed filter papers confined in 25ml glass vial is shown in Table 5. The knock down effect of the spice oil on both insects increased significantly (P<0.05) with increasing oil concentrations. However, at each concentration of the oil, percentage knock down of the adult *S. zeamais* was higher than that of *T. castaneum*. At 0.25ml, *Monodora myristica* oil concentration, only 17.5% of *T. castaneum* were killed, while, as high as

47.5% of *S. zeamais* were killed; but at the highest 1.0ml oil concentration, 72.5% *T. castaneum* and 82.5% *S. zeamais* were killed. The control with no spice oil did not record any insect mortality. The insect *T. castaneum* was more resistant to *Monodora myristica* oil than *S. zeamais*; although both were susceptible to the oil. In a similar study with English nutmeg oil, the adult *S. zeamais* were about five times more susceptible than *T. castaneum* to contact, fumigant and exposure activities of English nutmeg oil (Huang *et al*., 1997; Ukeha *et al.*, 2009). It is interesting to note that eugenol is one of the principal constituents of English nutmeg (Chaubey, 2008; Mondal *et al*., 2010). However, there appears to be other important bioactive compounds, besides eugenol, present in English nutmeg oil responsible for its insecticidal activity. Similar bioactive compounds are also suspected to be present and responsible for insecticidal activity of African nutmeg oil, although this aspect was not investigated in this study. Further studies are needed to elucidate the very bioactive constituents responsible for its insecticidal properties.

Table 5: Effect of *Monodora myristica (*Gaertn*)* oil on survival of *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (*Motsch*) after 24 hours of post-exposure

Monodora oil Concentration (ml)	knock down(%) of adult insects	
	T. castaneum	*S. Zeamais*
0.00	0 ± 0^{d}	0 ± 0^{d}
0.25	17.5 ± 0.82^{c}	47.5 ± 0.82^{c}
0.50	37.5 ± 0.82^{b}	55 ± 0.87^{bc}
0.75	70 ± 0.25^{a}	62.5 ± 0.82^{b}
1.00	72.5 ± 0.82^{a}	82.5 ± 0.43^{a}

Values are means of 4 determinations ± standard deviation. Means with the same superscripted letters in the same column are not significantly (p>0.05) different

Conclusion

African nutmeg is an indigenous spice used as flavouring ingredient in many African dishes, and has in this study protected African breadfruit against two storage insects; *T. castaneum* and *S. zeamais*, and has proven to be effective against the insect pests during the storage study. It has a good prospect to replace many synthetic insecticides in preservation of food grains, and recommended for further storage study at commercial levels and at longer storage periods for broader application.

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