Chemical Composition and Bioactivity of Essential Oil from *Monodora Myristica* against Grain Storage Insects

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Abstract: The essential oil of African Nutmeg (Monodoramyristica) was extracted by steam distillation method, the essential oil was graded into concentrations of (1, 2, 2.5, 3, 4, 5, 7.5, 10 mL/L). Experiments were conducted to study the bioactivity of the essential oil against Callosbruchusmaculatus and Sitophilus oryzae at different exposure time. The chemical components of the oil were analysed by GC-MS. The GC-MS analysis showed a total of Thirty-one (31) components, the major components are trans-13-octadecenoic acid (25.18%), sabinol (20.95%), linalool (9.11%) and n-hexadecanoic acid (7.66%). The results of the contact, repellence and fumigative test showed that the toxicity of the essential oil against the two insects was dose and time dependent (P<0.05). the essential oil of M. myristica induced higher toxicity in C. maculatus than in S. oryzae. Significant variation was observed in repellence, fumigative and contact activities between different concentrations and time of exposure (P<0.05). The result suggested the potential of the essential oil of M.myristica as a botanical insecticide.

Keywords: Monodoramyristica, essential oil, stored-grain insects, insecticidal.

I. INTRODUCTION

Insect pests are a major constraint on crop production, especially in developing countries. Over the past 15 years, interest in botanical insecticides has increased as a result of environmental concerns and insect populations becoming resistant to conventional chemicals. The use of synthetic pesticides has raised a number of both ecological and medicinal problems yet their use has not substantially reduced the pest losses, (Bekele and Hassanali 2001)

Botanical insecticides are naturally occurring insecticides that are derived from plants (Isman 2000). Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non-target organisms (Sharma *et al.*, 2006). There is a lot of hope that botanical pesti-cides will take a long way in fighting the dangers associated with conventional pesticides, however, botanical pesticides also need risk assessment and hazard characterizationin relation to human intake for a given time (Kroes and Walker, 2004). Botanical pesticides are hailed for having a broad spectrum of activitybeing easy toprocess, having a short residual activity and for not accumulating in the environment or in fatty tissues of warm blooded animals, ((Mugisha *et al.*, 2008)). They act in many ways on various types of pests and can be applied to plants or stored products in the same way as other conventional insecticides Many essential oils are Known to possess ovicidal, repellent and insecticidal activities against insects(Won-il *et-al.*, 2003).

However, it is important tonote that botanical pesticides, much as they are derived from plants, do not guaranteesafety to humans and the environment. Some may be quite toxic such as the rotenoids. Toxicological studiesaimed at assessing their safety should be done beforethey are used to avoid possible dangers, Belmain et al., (2001). Some plants have been scientifically tested and have been found to have good pesticidal properties. There is a concern however, as most of the studied plants are from western origin (Java and Dubey, 2005). There is a need to carry out intensive studies on African plants and their possible usage in pesticide compositions. Botanical pesticides, if sufficiently exploited, can play a big role in reducing pollution, health risks and crop losses to pests. Studies have been conducted in accessing the insecticidal activities of various plants, but much emphasis have not been placed on the effects of this essential oil in relation to exposure time as well as the minimal concentration of formulation needed to achieve the desire effect. In this study, the insecticidal activities of essential oil from African Nutmeg (*Monodoramyristica*) against Callosbruchus maculates and Sitophilus oryzae will be evaluated in relation to the exposure period at various concentrations.

II. MATERIALS AND METHODS

2.1 Plant Material

African Nutmeg (*M. myristica*) seeds were obtained from Orisumbare market of Osogbo, Osun State, Nigeria. The seed was identified by the Federal Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The seeds were air-dried and ground to a powder.

2.2 Insects Culture

The insect culture of bean weevil (*C. maculatus*) and rice weevil (*Sitophilus oryzae*) was collected from infested beans

and rice respectively. They were stored in a 5-liter plastic container and stored at a temperature of 24° C and 70° humidity.

2.3 Essential Oil Distillation

The ground powder of *M. myristica* was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. Anhydrous sodium sulphate was used to remove water after extraction. Essential oils were stored in airtight containers in a refrigerator at $4 \,^{\circ}$ C.

2.4 GC-MS Analysis

chromatography and mass spectrometry Gas Gas chromatographic analysis was performed on an Agilent 6890N instrument equipped with a flame ionization detector and HP-5MS ($30m \times 0.25mm \times 0.25\mu m$) capillary column, while the essential oil components were identified on an Agilent Technologies 5973N mass spectrometer. The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and ramped at 10 °C min-1 to 180 °C for 1 min, and then ramped at 20 °C min-1 to 280 °C for 15 min. The injector temperature was maintained at 270 °C. The samples $(1 \ \mu L)$ were injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 mL min-1. Spectra were scanned from 20 to 550 m/z at 2 scans s-1. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8-C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.5 Contact Toxicity

The contact toxicity of the essential oil against bean weevil (C. maculatus) was evaluated on filter paper disc by treating a whatman No.1 filter paper with the essential oil diluted in 100% acetone. A micropipette was used to suck out 2 µL, 4 μ L, 6 μ L, 8 μ L and 10 μ L of the essential oil and was diluted with 2ml of acetone to form concentrations of 1 ml/L, 2 ml/L, 3 ml/L, 4 ml/L, and 5 ml/L respectively. They were each poured and allowed to flow regularly on a disc of filter paper placed in a petri dish. The solvent was allowed to dry after which 10 bean weevils were introduced into the petri dish and then closed. Percentage mortality of insects was observed every 10 minutes. Insects which did not respond to the gentle touch of a small probe were considered dead (Su, 1976). Each experiment was conducted in triplicate. Control experiment was done using only acetone. The same test was repeated for rice weevil (Sitphilusoryzae) but 5 µL, 10 µL, 15 µL, 20 µL of the essential oil and was diluted with 2ml of acetone to

form concentrations of 2,5 ml/L, 5 ml/L, 7.5 ml/L and 10 ml/L respectively to formulate insecticide.

2.6 Vapour Effect

Direct exposure of insects to vapors from essential oils and their chemical components was done with a small, sealed 1.51 glass jar. A micropipette was used to suck out 2 μ L, 4 μ L, 6 μ L, 8 μ L and 10 μ L of the essential oil to form concentrations of1 ml/L, 2 ml/L, 3 ml/L, 4 ml/L, and 5 ml/L of the essential oil and was diluted with 2 mL of acetone respectively to formulate insecticide. They were each poured and allowed to flow regularly on a disc of filter paper (whatman no 1) placed upwardly in the top cover of the glass jar. After this application, 10 bean weevils were introduced into the glass jar. The percentage mortality of insects was observed every 15 minutes. Each experiment was conducted in triplicate. Control experiment was done using only acetone. The test was repeated for rice weevil (S. oryzae). but 5µl, 10µl, 15µl, 20µl of the essential oil and was diluted with 2 ml of acetone to form concentrations of 2,5 mL/L, 5 mL/L, 7.5 mL/L and 10mL/L respectively to formulate insecticide.

2.7 Repellent Effect

The repellent effects of the essential oil against beans weevils (Callosbruchusmaculatus) were evaluated using the area preference method. Tested areas consisting of Whatman No.1 filter paper cut in half. 2µl, 4µl, 6µl, 8µl and 10µl of the essential oil and was diluted with 2ml of acetone to form concentrations of 1 mL/L, 2 mL/L, 3 mL/L, 4mL/L, and 5 mL/L respectively to formulate insecticide while for rice weevil, 5µl, 10µl, 15µl, 20µl of the essential oil and was diluted with 2mL of acetone to form concentrations of 2.5 mL/L, 5 mL/L, 7.5 mL/L and 10mL/L respectively to formulate insecticide. Full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. 10 adult insects of each species were released separately at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in incubator at 27 $\pm 2^{\circ}$ C and 75 $\pm 5\%$ relative humidity.

2.8 Statistical Analysis

The data were subjected to probit analyses using SPSS (2001) for Windows to estimate LD_{50} and LD_{95} values of the essential oils against each stored-product insect species. Percentage mortality values for different exposure times were subjected to analysis of variance (one-way ANOVA) using the same statistical program (SPSS 2001) for probit analysis.

III. RESULTS AND DISCUSSION

The result of the contact test revealed that, all graded concentrations caused mortality in Callosbruchusmaculatus and Sitphilusoryzae. The percentage mean mortality increased as the concentration and time of exposure increased (Table I-II). The concentration of 5mL/L of the *M. myristica* achieved 100% mortality within 4 hours of exposure to *C. maculates* in the contact treatment (Table I)

Similar result was also recorded for the oil of *M. myristica* against *S. oryzae* except that higher concentrations were needed to obtain lethal doses against the insect (Table II). The mortality of *S. oryzae* increases as the time of exposure and concentration increases. All concentration recorded 100% mortality within 60 hours except for a concentration of 10 mL/L which recorded 100% mortality within 48 hours in the contact treatment.

From the result of the fumigative activity of the essential oil of *M. myritica* conducted on *C. maculatus* and *Sitophilus oryzae* (Tables III and IV) it revealed that the mortality of both insects was dose and time dependent. The exposure of *C. maculatus* to all doses of the essential oil recorded 100% mortality within 96 hours except for a dose of 5 mL/L of the essential oil which recorded 100% mortality within 72 hours.

A result trend similar to one obtained with *C. maculatus* was also recorded for the oil of *M. myristica* against *S. oryzae* except that higher concentrations were needed to obtain lethal dose against the insects. While other graded concentration recorded 100% mortality within 5 days of exposure to the essential oil, concentration of 10 mL/L recorded same mortality within 3 days. The mortality varies significantly between the concentrations and period of exposure (P < 0.05).

C. maculatus and *S. oryzae* were observed to be susceptible to the essential oil of *M. myritica* in the repellency test (Table V and VI), the repellency test was dose and time dependent. A 100% repellence was observed in *C. maculatus* within 20 minutes when exposed to 5 mL/L concentration of the essential oil of *M. myristica* while in *S.oryzae*, 100% repellence was observed on exposure of the insect to 10 mL/L of the essential oil. The repellency varies significantly between the concentrations and period of exposure (P< 0.05).

From the Gas chromatography-Mass spectroscopy analysis conducted on the essential oil of *M. myristica*, the analysis of the oil revealed a total of Thirty one (31) components, the major components are trans-13-octadecenoic acid (25.18%), sabinol (20.95%), linalool (9.11%) and n-hexadecanoic acid (7.66%) (TableVII).

For management of insect pests in agriculture, essential oils have been screened for their insecticidal activities against stired grain pest (Mattews, 1993). In the present study, *C. maculates* and *S. oryzae* were found to be significantly repelled by the essential oil of *M. myristica* with all doses employed in the study but at varying time of exposure. The study indicated that a higher dose was needed in the repellence activity against *S. oryzae*. Same trend was reported by Ravi and Gayatri (2007); Jianhua and Shuhui (2010) who in their study revealed that essential oil from *A. altissima* bark had strong repellent activity against *T.castaneum*, O.*surinamensis*, *S. Oryzae* and *L. paeta* adults. The repellence activity could be as a result of the pungent smell from the volatile oil.

Several works have been conducted on the contact activity of different plant extracts on pest. The result of the contact activity revealed that the essential oil of *M. myristica* against was dose and time dependent; this is same as reported by owolabiet-al., 2009. The estimated lethal doses of the essential oil against *C. maculatus* after 6 hours of exposure are LD50 (1.45 ml/L) LD95 (2.80 mL/L) while for *S. oryzae* after 12 hours of exposure are LD₅₀ (10.57 mL/L) LD95 (1 2.68 mL/L). Jianhua and Shuhui (2010) revealed that *A. altissima*bark oil also possessed strong contact toxicity on *S. oryzae* adults which gradually enhanced with increased exposure time and the corrected percentage mortality reached 76.5% after 72 Hours treatment.

Okonkwo and Okoye 1996 reported the effectiveness of *M. myristica* against *C. maculatus*. The result shows that the contact effect of essential oil from *M. myristica* was more effective against *C. maculatus* than *S. oryzae*.

Moreover, similar result trend was recorded for the fumigative activity of the essential oil of *M. myristica* against *C. maculatus* and *S. oryzae*. The activity of the essential oil was also dose and time dependent; however, higher doses were needed to achieve maximum mortality as observed in the contact experiment. The lethal doses of the essential oil against *C. maculatus* after 48 hours of exposure are LD₅₀ (3.60 mL/L) LD₉₅ (7.40 mL/L) while for *S. oryzae* after 48 hours of exposure are LD₅₀ (8.40 mL/L) LD₉₅ (14.54 mL/L). This is same as reported by Negahban and Moharramipour (2007) who found out that the fumigant action of essential oils of *E.intertexta, E. sargentii* and *E. camaldulensis* caused high mortality rate in C. maculatus using lower concentration compared with *S. oryzae* which requires high concentration.

This study has shown that *C. maculatus* was more susceptible to the essential oil of *M. myristica* than *S. oryzae*. To kill the adults of this insect, higher concentrations and exposure times are required than are required for *C. maculatus*. This can be as a result of the volatile constituents entering the softer cuticle of *C. maculatus* that allows easier penetration of the essential oil leading to a faster nerve paralysis in *C. maculatus* resulting in death. Papachristes and Stamopoulos (2002) also reported the higher susceptibility of the *C. maculates* than *T. castaneum*.

From the Gas Chromatography-Mass spectroscopy analysis conducted, a total of Thirtyone (31) components were detected. The major components are trans-13-octadecenoic acid (25.18%), sabinol (20.95%), linalool (9.11%) and nhexadecanoic acid (7.66%). Igweet al., 2005, reported 61 chemical constituents from the hydrodistillation with the major ones as alpha-phell and reneexpoxide (3.20%), carvacrol (2.09%) and delta-cadinene (2.21%). The n-hexane extract contained 39 chemical constituents with the major constituents as hexadecanoic acid (3.96%), (Z, Z) 9, 12octadecadienoic acid (3.77%), propyl oleate (3.45%), thiosulfuric acid (2.98%)and 2hydroxycyclopentanedecanone (2.2%). The chloroform

extract contained 38 components with p-cymene (6.0%), alphaphellendrene epoxide (3.23%), ethyllinoleate (3.79%), linoleic acid (4.36%), oleic acid (14.66%), (Z, Z) 9, 12octadecadienoic acid (7.89%) and 3-hydroxypropyl oleate (4.09%) as the major constituents, while the toluene extract contained 30 chemical constituents with the major ones as pcymene (4.6%), alpha–phellandrene epoxide (2.41%), linoleic acid (23.31%) and (Z, Z) 9, 12–octadecadienoic acid (7.02%). Lamaty*et al.*, 2006 also reported 98%, mainly hydrocarbons among them, α-phellandrene (48.8%), α-pinene (15.9%), limonene (8.7%), myrcene (7.9%) and α-thujene (6.3%). The variation in the composition may be associated with chemotypes for the same or different species or as a result of environmental and physiological differences (Sumangala and Vivek, 2009). Dales (1996) reported that hydrocarbon monoterpenes constituents present in M. myristica might be responsible for itsinsecticidal activity.

Theresults were in agreement with various reports that plant extracts werepotentially used as stored products protectants against insect pests (Akuet al., 1998; Yusuf et al., 1998; Lajideet al., 1998; Ketohet al., 2005; Tapondjouet al., 2002, Ngamoet al., 2007; Mondal and Khaleguazzaman 2006). It has also shown that, the insecticidal activity against the insects was higher at the lowest dose and longest exposure time than at the highest dose and lowest exposure time. This is same as reported by Papachristes and Stamopoulos, 2002.

Table I The percentage mortality for the contact activity of *M.myristica* essential oil against C.maculatus.

Exposure Time (hrs)	Conc (mL/L)						
	1	2	3	4	5	Control	
2	$0.00{\pm}0.00^{a}$	0.0±0.00 ^a	$0.0{\pm}0.00^{a}$	16±5.80 ^b	50±5.80°	$0.0{\pm}0.00^{a}$	
4	$0.0{\pm}0.00^{a}$	33±5.80°	33±5.80°	66.7 ± 5.80^{d}	100±0.00 ^e	$0.0{\pm}0.00^{a}$	
6	$33.0 \pm 5.80^{\circ}$	66.7 ± 5.8^{d}	100±0.00 ^e	100±0.15 ^e	100±0.00 ^e	$0.0{\pm}0.00^{a}$	
8	66.7±5.80 °	100±10.0 ^e	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e	0.0 ± 0.00^{a}	
10	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e	$100{\pm}0.00^{e}$	100±0.00 ^e	$0.0{\pm}0.00^{a}$	

The result shows the mean \pm SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

Table II The percentage mortality for the contact activity of *M.myristica* essential oil against S.oryzae.

Exposure	Conc (mL/L)					
Time (hrs)	2.5	5	7.5	10	Control	
12	$0.0{\pm}0.00^{a}$	$0.0{\pm}0.00^{a}$	$0.0{\pm}0.00^{a}$	33.3±5.80°	0.0±0.00 ^a	
24	$0.0{\pm}0.00^{a}$	$0.0{\pm}0.00^{a}$	33.3±5.80°	66.7 ± 5.80^{d}	0.0 ± 0.00^{a}	
36	$0.0{\pm}0.00^{a}$	33.3±5.80°	$50{\pm}5.80^{cd}$	83.3 ± 5.80^{d}	0.0 ± 0.00^{a}	
48	83.3 ± 5.80^{d}	83.3 ± 5.80^{d}	100±0.00 ^e	100±0.00 ^e	0.0 ± 0.00^{a}	
60	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e	0.0 ± 0.00^{a}	

The result shows the mean \pm SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

Table III The percentage mortality for the fumigant activity of M.myristica essential oil against C.maculatus

Exposure	Conc (mL/L)						
Time (hrs)	1	2	3	4	5	Control	
24	0.0 ± 0.00^{a}	$0.0{\pm}0.00^{a}$	0.0±0.00 ^a	25±5.80 ^b	50±5.80°	$0.0{\pm}0.00^{a}$	
48	12 ± 5.80^{b}	$25 \pm 5.80^{\circ}$	45 ± 5.80^{d}	$50{\pm}5.80^{d}$	75±10.0 ^e	$0.0{\pm}0.00^{a}$	
72	$50\pm5.80^{\circ}$	$62.5{\pm}5.80^{cd}$	65 ± 5.80^{d}	$70{\pm}10.0^{d}$	100±0.00 ^e	$0.0{\pm}0.00^{a}$	
96	100±0.00 ^e	0.0 ± 0.00^{a}					

The result shows the mean \pm SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

Exposure	Conc (mL/L)					
Time(Days)	2.5	5	7.5	10	Control	
1	$0.0{\pm}0.00^{a}$	$0.0{\pm}0.00^{a}$	$0.0{\pm}0.00^{a}$	33.3±5.80 ^b	0.0 ± 0.00^{a}	
2	$0.0{\pm}0.00^{a}$	33.3±5.80 ^b	33.3±5.80 ^b	66.7±5.80°	$0.0{\pm}0.00^{a}$	
3	33.3 ± 5.80^{b}	$66.7 \pm 5.80^{\circ}$	$83.3{\pm}5.80^{d}$	100±0.00 ^e	$0.0{\pm}0.00^{a}$	
4	$66.7 \pm 5.80^{\circ}$	83.3 ± 5.80^{d}	100±5.80 ^e	$100{\pm}0.00^{e}$	$0.0{\pm}0.00^{a}$	
5	100±0.00 ^e	100±0.00 ^e	100±5.80 ^e	100±0.00 ^e	0.0 ± 0.00^{a}	

Table IV The percentage mortality for the fumigant activity of *M.myristica* essential oil a *S.oryzae*.

The result shows the mean \pm SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

Table V The percentage repellency activity of *M.myristica* essential oil against *C.maculatus*.

Exposure			Conc (mL/L)		
Time(Mins)	1	2	3	4	5
10	37.5±1.2°	37.5±1.15°	50±1.20 ^{cd}	62.5±0.15 ^c	75±0.20 ^d
20	50±1.15°	62.5 ± 2.20^{d}	62.5 ± 2.15^{d}	87.5±2.30 ^e	$100{\pm}0.00^{ef}$
30	$75{\pm}1.20^{d}$	$100{\pm}0.00^{e}$	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e
40	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e	100±0.00 ^e	$100{\pm}0.00^{e}$

The result shows the mean \pm SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

Table VI The percentage repellency activity of Monodoramyristica) essential oil against S. oryzae

Exposure	Conc (mL/L)					
Time(Mins)	2.5	5	7.5	10		
20	$0.00{\pm}0.00^{a}$	$0.00{\pm}0.00^{a}$	18±5.80 ^b	33±5.80°		
40	27 ± 5.80^{b}	33±5.80 ^b	$57\pm5.80^{\circ}$	75 ± 5.80^{d}		
60	55±5.80°	$57{\pm}5.80^{\circ}$	$89{\pm}5.80^{d}$	100±0.00 ^e		
80	$85{\pm}5.80^{d}$	$95{\pm}5.80^{d}$	100±0.00 ^e	100±0.00 ^e		

The result shows the mean \pm SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

Table VII The percentage composition of the chemical components presents in *M. myristica* essential oil.

COMPOUND PRESENT	RETENTION TIME (MINS)	% COMPOSITION
Linalool	3.224	9.11
Cis-beta-Terpineol	3.539	1.16
2-Cyclohexen-1-ol, 1-methyl-4-(1-m ethylethyl)-, trans	3.922	0.52
trans-3,5-Dimethylcyclohexene	4.620	0.86
3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl	5.021	2.73
Sabinol-cis	5.479	17.87
Sabinol	5.753	3.08
Cyclopentylcyclohexane	6.314	1.63
3-(2-Hydroxy-cyclopentylidene)-2-methyl-propionic acid	6.806	0.72
Phenol, 2-methyl-5-(1-methylethyl)	8.185	1.31
3-Methyl-4-isopropylphen	8.402	1.97
4-Hydroxy-3-methylacetophenone	8.505	1.36

Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,	10.113	0.30
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,	10.199	0.51
Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	10.394	0.81
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)	10.560	1.25
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)	10.623	0.67
Caryophyllene oxide	11.910	1.09
Cadinol	12.883	1.21
alphaCadinol	13.146	1.14
o-Anisic acid, 2-pentadecyl ester	14.765	0.54
n-Hexadecanoic acid	17.512	7.66
Camphene	18.433	0.75
trans-13-Octadecenoic acid	19.715	25.18
Octadecanoic acid	19.818	6.62
Cholesta-3,5-diene	25.935	0.47
9-Oxabicyclo[6.1.0]nonane, cis-	29.723	0.16
9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	30.838	2.58
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	30.987	0.91
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	31.136	0.49
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	34.689	5.32

Retention indexes on an SPB-5 column in reference to n-alkanes; Peak areas relative to total peak area; -, not detected; MS, NIST MS library, and the literature; Rretention index; ST, authentic standard compound; * correct isomer not identifed.

IV. CONCLUSIONS

The application of this essential oil as seed protectant is inexpensive, effective, easily adaptable, convenient to use and can be an alternative to the synthetic toxicant. This locally source oil if incorporated into the agricultural sectors of developing countries could minimize the severe damage caused by insect pest.

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COMPETING INTEREST

Authors have declared that there are no competing interests exist.

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