n-hexane fraction of *Zingiber officinale and Moringa oleifera* interferes with biological

parameters of *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae)

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Abstract

The development of insecticide resistance, high cost, misuse, dearth of technical expertise, and

restrictive legislation associated with synthetic insecticides have necessitated the development of

alternatives. Lessons from plant-insect interactions demonstrate that plant terpenes are worthy

probes for insecticidal exploration. Hence, this study screened *n*-hexane fractions of *Zingiber*

officinale and *Moringa oleifera* oils as protectant against *Callosobruchus chinensis* and revealed

their chemical profiles using Gas Chromatography – Mass Spectrometry (GC-MS). *M. oleifera*

(LC$_{50}$; 0.007 µl) was found to be more toxic than *Z. officinale* oil (LC$_{50}$; 0.055 µl) to *C. chinensis*.

The oils showed a positive correlation with concentration at 24 h (r = 0.959), 48 h (r = 0.977), 72

h (r = 0.915) and 96 h (r = 0.924). GC-MS revealed 21 and 15 volatile compounds in *Z. officinale*

and *M. oleifera* oils, respectively. The most domimant were 5-(1, 5-dimethyl-4-hexenyl)-2-methyl-

1,3-Cyclohexadiene (13.64 %) and 8-Octadecenoic acid, methyl ester (34.52 %) in *Z. officinale*

and *M. oleifera* oils, respectively. The plant fractions reduced the oviposition potential, egg

hatching rate, and adult emergence of *C. chinensis*. Taken together, the results demonstrate

possible developmental and inhibitory effects of the oils against *C. chinensis* and points to its

possible inclusion in Integrated Pest Management (IPM) practices for *C. chinensis.*

Keywords: botanical insecticide; grain protectant; pulse beetle; bruchid; inhibitory potential

1. Introduction

The geometric increase in human population has made the task of food protection of foremost interest. Globally, post-harvest losses of agricultural produce due to insect infestation is one of the major threats to food protection. In Africa alone, post-harvest losses of legumes (especially peas and beans) due to *Callosobruchus* species have been estimated to be about 45 % (Tapondjou et al., 2002). For example, the pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) begins infestation by laying eggs on cowpeas and pigeon peas in the field and continues its development throughout storage thereby inflicting damage on the grain wholesomeness and marketability (Lale and Kabeh, 2004; Gbaye et al., 2011). Insecticides such as permethrin, phosphine, pirimiphos-methyl, and lindane have been routinely used to protect legumes against *C. chinensis* damage. However, the development of resistance, acceptability, limited availability in certain areas, environmental contamination, and legislative restrictions have necessitated the need for the development of sustainable and eco-friendly alternatives (Adarkwah et al., 2017, Dougoud et al., 2019). At the forefront of these is also a weak and under-resourced capacity to control *C. chinensis* (Chaubley, 2008; Ajayi et al., 2014). Thus, the recent trend in the exploration of plant metabolites (also known as essential oils) as candidates to mitigate against *C. chinensis* attack is unsurprising.

Oil fractions from plants have showed promising bioactivity against insect pests including *C. chinensis* (Ajayi et al., 2017; Oladipo et al., 2019; Ribeiro et al., 2020). Plant oil desirability is due to its non-persistence, biodegradability, and low toxicity (Koul et al., 2008; Oladipupo et al., 2019a). These characteristics explain why essential oils (EOs) are perceived as worthy candidates for inclusion in integrated pest management strategies. Moreover, the ease of registration and

ubiquity in tropics, especially to farmers in under-developed countries, make EOs worthy

candidates for scientific explorations (Oladipupo et al., 2019b). Earlier studies have shown that

the content and concentration of EOs are dictated by plant chemotype and genotype, solvent

polarity, and extraction method (Boursier et al., 2011; Gahukar, 2014, Dougoud et al., 2019). Thus,

chemical characterization of EOs evaluated as candidates for legume protectants is an important

step towards understanding structural-activity relationship and subsequent inclusion/adoption as

constituents of commercialized botanicals.

Earlier studies have evaluated the bioactivity of some plant oils against *C. chinensis.*

Upadhyay et al. (2006) reported ovipositional deterrence by *Capparis decidua* (Forssk.) against *C.*

chinensis. Petroleum ether extract of four vegetable seeds in Cucurbitaceae family were found to

be effective protectant against *C. chinensis* (Mishra et al., 2007). In contact and fumigation tests,

methanolic fractions from 30 plant species were found effective for managing populations of *C.*

chinensis (Isman et al., 2001; Kim et al., 2003). Oladipo et al. (2019) reported that *n*-hexane

fractions of *Xylopia aethiopica* (Dunal) and *Senna occidentalis* (L.) were more toxic to *C.*

chinensis than acetone fractions. Contrastingly, acetone fractions of oils of both plants displayed

higher ovipositional and eclosion deterrence than *n*-hexane fractions (Oladipo et al., 2019).

Moreover, *Moringa oleifera* Lam. and *Zingiber officinale* (Roscoe) oils have demonstrated

insecticide effects against other insect pests. For example, hydrodistillate of *Z. officinale* are toxic

to adult *Tribolium* spp. and larvae of *Plodia interpunctella* (Hubner) (Maedeh et al., 2012;

Martynov et al., 2019). Ethanolic fraction of *Z. officinale* demonstrated higher toxicity against *C.*

chinensis than *M. oleifera* oil (Ajayi et al., 2017).

To the best of our knowledge, the chemical characterization of *n*-hexane fractions of *Z.*

officinale and *M. oleifera,* and bioactivity on aspects of biological parameters of *C. chinensis* have

never been evaluated. Therefore, the aims of this study were to: (1) reveal profile of chemical

components of *n*-hexane fractions of *Z. officinale* and *M. oleifera* using Gas Chromatography

coupled with Mass Spectrometry (GC-MS), (2) evaluate their toxicity against *C. chinensis* over

time (hours), and (3) investigate the inhibitory potentials of these oils on oviposition and adult

emergence of *C. chinensis*.

2. Materials and Methods

2.1 Insects

A *C. chinensis* colony was started from an established laboratory colony maintained at the

insectaries of the postgraduate research laboratory of the Department of Biology, Federal

University of Technology, Akure, Nigeria. The laboratory colony were reared on disinfested

cowpea seeds in a 1-liter glass jars at ambient temperature of 28 ± 2 °C, with 75 ± 5 % RH, and a

photoperiod of 12:12 (L: D) h. Five pairs of newly emerged adults (0–24 h old) of *C. chinensis*

were selected from the laboratory colony and introduced into 250 ml plastic jars containing 200 g

of disinfested cowpea seeds. The plastic jars were covered with muslin cloth to allow for

ventilation and prevent the escape of *C. chinensis*. Adults (0–24 h old) that subsequently emerged

from this were used for the bioassays.

2.2 Plant materials and oil extraction

Rhizomes of *Z. officinale* and seeds of *M. oleifera* were purchased from the central market

(Oja–Oba) in Akure municipal, Ondo state, Nigeria. Purchased specimens were confirmed by

taxonomist and the valid correct names were confirmed at the plant list (available at

http://www.theplantlist.org/). The plants were sorted to remove impurities, air dried on a laboratory

bench, and ground into a fine powder with a marlex grinder (USHA 500-Watt Motor Power). The

powder from each plant were sieved using 16 mesh size sieve. 300 g of each plant powder was

soaked in 900 ml *n*-hexane in round-bottomed glass jar for 72 h and periodically stirred. Each

solution was sieved using muslin cloth and concentrated using rotary evaporator at 40 °C and rotary

speed of 138 to 148 rpm for 3–4 hours. The resulting oil was air-dried to allow for traces of *n*-

hexane to escape. The crude oil from each plant was diluted to obtain concentrations of 1-5 %

(v/w) with the solvents (*n*-hexane).

2.3 Bioassays

2.3.1 Chemical composition analysis of n-hexane fractions of Z. officinale and M. oleifera

Purification of oils were done using liquid-liquid chromatography with sodium sulphate on

separating funnel packed with silica gel. The chemical composition analysis of *n*-hexane fractions

of *Z. officinale* and *M. oleifera* were carried out using a gas chromatograph (model 7890A; Agilent

Technologies, Palo Alto, CA, USA) equipped with an Agilent J & W non-polar HP-5MS capillary

column (30 cm X 0.320 mm; 0.25 µm film thickness), mass spectrometer (5975C VLMSD), and

an injector (7683B series).

The oven temperature was set at 80 °C for two min, increased by 6 °C per min until a

temperature of 240 °C was reached, and held constant at this temperature for 6 min. Helium carries

gas flow, which was maintained at 100 kPa. A 1 µl aliquot of each oil was injected in a mass

spectrometer. The run time for each sample was 36 min. The peak of each chemical component

was expressed based on its retention time and abundance. The identification of components was

achieved by searching the mass spectra database and checking for direct similarities with identified

components in the system (Adams, 2001). The National Institute of Standards and Technology

(NIST) library was also accessed for components' properties and documentation.

2.3.2 Assessment of the toxicity of n-hexane fractions of Z. officinale and M. oleifera

To evaluate the toxicity of *Z. officinale* and *M. oleifera* oils against *C. chinensis*, five test

concentrations (10, 20, 30, 40 and 50 µl v/w) were used. One ml of each oil test concentration was

applied to 20 g of cowpea seeds in 250 ml round-bottomed transparent plastic containers.

Containers were agitated for 5 - 10 min to ensure uniform coating of the seeds with the oils. Five

pairs of 0 – 24 h old *C. chinensis* adults were introduced into each container. Untreated and solvent

(*n*-hexane) controls were similarly set up. All experiments were replicated three times in

Completely Randomized Design (CRD). Adult mortality was recorded for 96 h at 24 h interval.

At the expiration of 96 h; all adult insects, dead or alive, were removed from all replicates.

2.3.3 Ovipositional inhibitory bioassay of n-hexane fractions of Z. officinale and M. oleifera

Number of eggs laid on the seeds from the above set-up in each replicate were recorded.

Observation for adult emergence commenced at 26th day post-infestation. Number of adults that

emerged from each replicate were recorded. Percent reduction in adult emergence of F_1 progeny

was calculated using:

$\% \text{ IR} = \frac{C_n - T_n}{C_n} \times \frac{100}{1}$ (Tapondjou et al., 2002)

Where C_n is the number of emerged insects in the control and T_n is the number of emerged insects

in the treated containers.

2.4 Statistical analysis

Adult mortality data were corrected with mortality obtained in control (untreated) using

Abbott formula (Abbott, 1925). Data transformation was done using square-root (for count data)

and arc-sine (for percentages) transformation (Tukey, 1977). Transformed data were analyzed

using One-way Analysis of Variance (ANOVA). Tukey's post-hoc test was used to separate the

means at $\alpha = 0.05$. Also, factorial analysis was conducted on mortality data to compare all variable

factors for possible interactions using Minitab version 17 (Payton et al., 2006). The median lethal

concentration (LC_{50}) was calculated using Probit analysis (Finney, 1971). The relationship

between concentrations and percentage mortalities, at different exposure periods (24 – 96 h post-

treatment) was determined using regression analysis. The corresponding correlation coefficients

(r) for prediction equations were also established. All analyses, unless otherwise stated, were done

using IBM SPSS software version 20 (IBM SPSS Inc., 2011).

3. Results

3.1 Acquisition and chemical profile of n-hexane fraction of Z. officinale and M. oleifera

The chromatograms showing profiles of the components in *Z. officinale and M. oleifera* oils are shown in **Figures 1** and **2**, respectively. The GC-MS analysis revealed 21 volatile compounds in *Z. officinale* rhizome (**Table 1**). The most dominant were 5-(1, 5-dimethyl-4-hexenyl)-2-methyl-1, 3-Cyclohexadiene (13.64 %) and dec-4-en-3-one-1-(4-Hydroxy-3-methoxyphenyl) (9.57 %). 15 volatile compounds were identified in *M. oleifera* seed (**Table 2**). 8-Octadecenoic acid, methyl ester (34.52 %) and cis-Vaccenic acid (16.11 %) were the dominant components.

3.2. Toxicity of n-hexane fractions of Z. officinale and M. oleifera

There was no mortality in the control experiment at 24 – 72 h for *Z. officinale* oil, and 24 h for *M. oleifera* oil (**Figure 3**). At 24 h, only the highest concentration achieved mortality (3.33 %) of *C. chinensis* for *Z. officinale* oil. The mortality rates of *C. chinensis* ranged from 20 – 40 % at 20 – 50 µl for *M. oleifera* oil. Overall (24 – 96 h), the increase of *M. oleifera* oil concentration led to an increase in the mortality of *C. chinensis*. After 72 h, there was a progression in toxicity of (3.33 – 23.33 %) of *Z. officinale* oil against *C. chinensis*. Statistical analysis revealed no signficant differences (P = 0.141) in mortality between concentrations of *Z. officinale* oil. Mortality rate of *C. chinensis* ranged from 37.04 – 66.67 % for *M. oleifera* oil. The toxicity of *Z. officinale* oil against *C. chinensis* improved after 96 h. However, this was at 50 µl. Relative to *Z. officinale* oil, *M. oleifera* oil exhibited significantly higher toxicity against *C. chinensis* from 24 – 96 h. Thus, median lethal concentration of *M. oleifera* (LC_{50}) was much lower (0.007 µl) than that of *Z.*

193   *officinale* oil (0.055 µl) (**Table 3)**. The slope of the log dose-probit line indicated that *C. chinensis*

194   exposed to *M. oleifera* oil had the shallowest slope (0.96) while *C. chinensis* exposed to

Figure 1. Chromatogram showing the chemical components of *n*-hexane fraction of

Zingiber officinale rhizome

Abundance

TIC: M O R I G A S E E D P O W D E R H E X A N E E . D \ d a t a . m s

4.8e+07
4.6e+07
4.4e+07
4.2e+07
4e+07
3.8e+07
3.6e+07
3.4e+07
3.2e+07
3e+07
2.8e+07
2.6e+07
2.4e+07
2.2e+07
2e+07
1.8e+07
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000

25.826
23.783
25.399
25.005
22.487
20.746
22.298
22.148
12.584
12.330
13.173
12.840
30.309

5.00   10.00   15.00   20.00   25.00   30.00

Time-->

Figure 2. Chromatogram showing the chemical components of *n*-hexane fraction of

Moringa oleifera seed

205 **Table 1.** Relative amount and chemical components of *n*-hexane frcation of *Z. officinale rhizome*

RT (mins)	Name of components	CAS no.	% of total (Rel. amt.)
12.491	1-(1, 5-dimethyl-4-hexenyl)-4-methylBenzene	000644-30-4	9.133
12.812	5-(1, 5-dimethyl-4-hexenyl)-2-methyl-1, 3-Cyclohexadiene [S-(R*, S*)]	000495-60-3	13.635
13.054	α-Farnesene	000502-61-4	6.365
13.406	3-(1, 5-dimethyl-4-hexenyl)-6-methylene-Cyclohexene [S-(R*, S*)] -	020307-83-9	7.000
15.146	(1R, 4R)-1-methyl-4-(6-methylhept-5-en-2-yl)-Cyclohex-2-enol	058334-55-7	1.691
16.285	4-(3-hydroxy-2-methoxyphenyl)-Butan-2-one	303187-89-5	9.241
16.658	cis-Z-α-Bisabolene epoxide	1000131-71-2	2.201
16.828	1-Bromo-3, 7-dimethyl-2, 6-octadiene	035719-26-7	2.112
19.022	α-2-dimethyl-2-(4-methyl-3-pentenyl)-, [1-α-(R*), 2- α]-Cyclopropanemethanol	121959-70-4	2.073
21.255	(*E*)-1-(6, 10-dimethylundeca-5, 9-die n-2-yl)-4-methylBenzene	055968-43-9	1.292
22.543	3, 7,11,15-tetramethyl-1,6,10,14-Hexadecatetraen-3-ol (*E,E*)	001113-21-9	1.191
23.634	methyl ester, 11-Octadecenoic acid	052380-33-1	1.092
24.474	7-epi-cis-Sesquisabinene hydrate	1000374-17-9	1.200
24.712	ethyl ester (*E*)-9-Octadecenoic acid	006114-18-7	1.956
25.916	dec-3-en-5-one-(*E*)-1-(4-Hydroxy-3-methoxyphenyl)	863913-65-9	6.909
26.181	2-Vinyl-4, 6-diamino-S-triazine	003194-70-5	0.727
27.157	dec-4-en-3-one-1-(4-Hydroxy-3-methoxyphenyl)	000555-66-8	9.574
28.501	decan-3-one-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)	039886-76-5	4.579

29.952	dodec -4-en-3-one-1-(4-Hydroxy-3-methoxyphenyl)	036700-45-5	6.015
30.200	decane-3, 5-diyldiacetate-(3R, 5S)-1-(4-Hydroxy-3-methoxyphenyl)	143615-75-2	2.618
34.301	tetradec-4-en-3-one-1-(4-Hydroxy-3-methoxyphenyl)	036752-54-2	9.396

*RT – Retention Time

*CAS no. – Chemical Abstracts Service Registry Number

* Rel. amt. – Relative amount

Z. officinale had the steepest slope (2.69) indicating heterogeneous and homogeneous

response to both oils, respectively. Regression analysis revealed significant (P < 0.05) positive

correlation between mortality and oil concentrations at all exposure periods: 24 h (r = 0.959), 48

h (r = 0.977), 72 h (r = 0.915) and 96 h (r = 0.924) (**Table 4**) of *C. chinensis* exposed to *Z. officinale*

and *M. oleifera* oils. Likewise, there were significant impact of the interactions of time X

concentration (T X C) ($F_{10,192}$ = 5.95, P < 0.0001).

3.3 Oviposition inhibitory assay

There was a significant increase (P < 0.05) in the number of eggs laid by *C. chinensis* on

the untreated control and solvent control (**Figure 5**) compared to the plant fractions. The egg

counts recorded on seeds treated with *Z. officinale* oil at 20 µl (80.67), 30 µl (80.33) and 40 µl

(80.00) are comparable with one another. On the other hand, the egg counts on the seeds treated

with *M. oleifera* oil differed significantly (p < 0.05), one from another at the concentrations

investigated. Taken together, the lowest number of eggs was observed on cowpea seeds treated

with of *M. oleifera* oil at 50 µl (34.67).

Table 2. Relative amount and chemical components of *n*-hexane fraction of *M. oleifera* seed

RT (mins)	Name of components	CAS no.	% of total (Relative amount)
12.330	1-(1, 5-dimethyl-4-hexenyl)-4-methylBenzene	000644-30-4	5.060
12.584	5-(1, 5-dimethy l-4-hexenyl)-2-methyl-1, 3-Cyclohexadiene	000495-60-3	5.866
12.840	β.-Bisabolene	000495-61-4	3.506
13.173	3-(1, 5-dimethyl-4-hex enyl)-6-methylene- Cyclohexene	020307-83-9	3.714
20.746	Hexadecanoic acid, methyl ester	000112-39-0	7.195
22.148	n-Hexadecanoic acid	000057-10-3	0.954
22.298	Palmitoleic acid	000373-49-9	0.999
22.487	n-Hexadecanoic acid	000057-10-3	0.674
23.783	8-Octadecenoic acid, methyl ester	002345-29-1	34.515
25.005	Oleic acid	000112-80-1	6.002
25.399	cis-Vaccenic acid	000506-17-2	16.114
25.754	cis-13-Octadecenoic acid	013126-39-1	8.625
25.791	9-Octadecenoic acid	002027-47-6	0.395
25.826	9-Octadecenoic acid, (E) -	000112-79-8	0.952
30.309	Docosanoic acid, methyl ester	000929-77-1	5.429

*RT - Retention Time

*CAS no. - Chemical Abstracts Service Registry Number

Figure 3. Percentage mortality of *C. chinensis* exposed to *n*-hexane fractions of *Z. officinale*

and *M. oleifera*. 0 = control group. Each bar corresponds to the mean ± SE of four replicates.

Table 3. Median Lethal Concentration (LC_{50} in ml/20 g of cowpea) of *n*-hexane fractions of *Z.*

officinale and *M. oleifera* on *C. chinensis*.

Plant Material	Slope (±SE)	Intercept (±SE)	χ^2	df	LC_{50} (95 % FL)
Z. officinale	2.14 (0.54)	2.69 (0.82)	5.68	1	0.055 (0.041 – 0.106)
M. oleifera	0.96 (0.44)	2.08 (0.71)	2.53	1	0.007 (0.000 – 0.014)

233   χ^2: Chi-square; SE: Standard error; FL: Fiducial limits; LC: Lethal concentration; df:

234   degree of freedom.

237   **Table 4.** Relationship between concentration of *n*-hexane fractions of *Z. officinale* and *M. oleifera*

238   and mortality of *C. chinensis*

Exposure periods	Correlation coefficient (r)	Regression equation
24	0.959	Y = 0.617 + 0.071X
48	0.977	Y = 1.876 + 0.072X
72	0.915	Y = 1.960 + 0.100X
96	0.924	Y = 2.875 + 0.115X

Figure 5. Mean Percentage (a) reduction in adult emergence, and (b) inhibition of *C. chinensis*

from eggs laid on cowpeas treated with *n*-hexane fractions of *Z. officinale* and *M. oleifera*. SC =

solvent control. Each bar corresponds to the mean ± SE of four replicates.

3.3 Variable factors and toxicity of n-hexane fractions of Z. officinale and M. oleifera to C.

Chinensis

General Linear Model (GLM) revealed significant differences in the effect of time (T)

$(F_{3,192} = 191.35, P < 0.0001)$ and concentration (C) $(F_{5,192} = 198.89, P < 0.0001)$ on the mortality.

Mean percent reduction in *C. chinensis* emergence ranged from 44.95 – 90.71 %, while

inhibition ranged from 18.63 – 83.38 % for *Z. officinale* oil. For *M. oleifera* oil, the range of percent

reduction in emergence and inhibition were 34.01 – 90.71 % and 18.63 – 91.67 %, respectively.

3.4 Developmental inhibitory effects of n-hexane fractions of Z. officinale and M. oleifera

There was similarity in the trend of adult emergence (**Figure 5a**) and mean inhibitory

(**Figure 5b**) effects of *Z. officinale* and *M. oleifera* oils. Significantly higher ($P < 0.05$) number of

C. chinensis emerged from the control. However, for both oils, a dose-dependent relationship was

observed for adults' emergence and percent inhibition.

4. Discussion

The application of insecticides (commonly pyrethroids and organophosphates) for the

control of the notorious pest, *C. chinensis*, remains the most popular among farmers and other

insect pest control managers. However, due to continued exacerbation of insecticide resistance,

high cost, misuse, dearth of technical expertise, and restrictive legislation associated with synthetic

insecticides, the continued use cannot be further encouraged. Thus, there is a sought interest in the

provision of effective and sustainable insect pest control agents. As alternatives, lessons from plant

- insect interactions demonstrate that plant terpenes are worthy probes for insecticidal exploration

(Berenbaum et al., 1986; Li et al., 2002). Interestingly, the use of oils from plant materials have

shown promising results (Ajayi and Adedire, 2003; Tapondjou et al., 2002, Ajayi et al., 2014;

Oladipupo et al., 2019b; Ribeiro et al., 2020). In consonance with this, this research work screened

n-hexane fractions of *Z. officinale* and *M. oleifera* as protectants against *C. chinensis*. The results

demonstrate that the constituent of the plant materials contain chemical compounds with possible

inhibitory, neurotoxic, and insecticidal properties.

The present study reports the insecticidal activity of *Z. officinale* and *M. oleifera* oils against *C.*

chinensis with *M. oleifera* showing higher toxicity. However, both oils showed a concentration

dependent response. Fractions obtained from plant materials have been found to cause severe

damage including mortality in *Callosobruchus* species (Mishra *et al*. 2007; Ajayi *et al*. 2014).

Chaubley (2008) reported that oils from *Piper nigrum*, *Anethum graveolens*, *Cuminum cyminum*

and Nigella sativa caused larvae and adult mortality of *C. chinensis*. In this study, the mortality

rate was found to increase with an increase in concentration, and the LC_{50} values decreased at

different graded exposure periods indicating that the response of the bruchid to *n*-hexane fractions

of *M. oleifera* and *Z. officinale* was concentration and time dependent. The superior toxicity

displayed by *M. oleifera* over *Z. officinale* is consistent with the findings of Ajayi et al. (2007).

Moreover, earlier attempt to describe the toxicity of *M. oleifera* against insect pests appear

widespread in literature. For example, the presence of the fatty acids 8-Octadecenoic acid, methyl

ester and cis-Vaccenic acid in *M. oleifera* has been implicated to cause mortality (Ashfaq *et al.*

2012; Leone *et al*. 2015). These fatty acid cause severe asphyxiation, thereby making breathing

impossible in insect (Olayemi and Alabi 1994; Ajayi et al., 2018). Compared to *Z. officinale,* the

proportion of fatty acids in *M. oleifera* is about 30 % more. So, this may demonstrate a scientific

rationale for the superior toxicity of *M. oleifera*. *Z. officinale* showed delayed toxicity in this study.

This contrasts with the findings of Chaubey (2013). Since the bioactivity of a plant metabolite

often varies with respect to edaphic and environmental conditions (Gahukar, 2014, Dougoud et al.,

2019), the absence of the phytochemical constituent analysis in the study complicates straight

forward comparison.

In general, essential oils are sought after because of their biodegradability (high volatility

as a funcion of low-persistence in the enviroment). Volatility is a function of hydrogenated to

oxygenated ratio with hydrogenated compounds being more volatile (Ahn et al., 2008; Regnault-

Roger et al., 2008). Interestingly, the GC-MS results showed that both *Z. officinale* and *M. oleifera*

oils have a higher proportion of hydrogenated compunds. These hydrogenated compounds

(broadly termed monoterpenoids) have been described to disrupt metabolic pathways, alter

biological parameters, and cause death in insects, including *C. chinensis* (Kéita et al., 2001; Jiang

et al., 2012; Ribeiro et al., 2020). Thus, the toxicity associated with the oils may be related to their

components. Nonetheless, a straight forward assumption that the dominant compounds may have

been solely responsible for the observed toxicity should be made with caution as it has been shown

that that is not often the case (Veras et al., 2012; Tak and Isman, 2015).

However, essential oils do not only affect insects by causing mortality, but also interferes

with life-history traits and biochemical functions. For example, the oils inhibited ovipositional

ability of *C. chinensis*, and retarded subsequent morph into adults. Similar observations have been

made on eggs of the grain moth and *C. chinensis* (Ileke, 2013; Chaubey, 2013). Interestingly,

recent findings have shown that essential oils, including *M. oleifera*, not only represent safe

protectants against insect attack, but these oils can also boost the nutritional quality by increasing

the amino acid index, protein efficiency ratio, biological value and net protein value (Ilesanmi and

Gungula, 2016). More so, synthetic insecticides such as organophosphates routinely used as grain

protectants depletes the nutritional constituents of grains in storage, rendering them unsuitable for

consumption (Akami et al., 2017).

In conclusion, the findings of this study demonstrate that *n*-hexane fraction of *M. oleifera* are more

toxic to *C. chinensis* than *Z. offcinale*. More so, both plant oils can reduce egg hatchability, retard

C. chinensis development and induced mortality. Taken together, the results demonstrate possible

developmental and inhibitory effects of the oils against *C. chinensis* and points to its possible

inclusion in Integrated Pest Management (IPM) practices for *C. chinensis.* The ubiquity of these

plant material to local farmers can fast-track adoptions in localities where there is widespread

insecticide development, restrictive legislation, and lack of funds. However, further study focusing

on the mode of action of these oils is merited, before subsequent incorporation into management

system for grain (cowpea) protection.

Conflict of interest

The authors declare no conflicts of interest.

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Highlights:

- *n*-hexane fractions from *Moringa oleifera* seeds are more potent against *Callosobruchus chinensis* than that from *Zingiber officinale* rhizome.

- Both oils can disrupt biological parameters and induce mortality of *C. chinensis*.

- *M. oleifera* can be useful as grain protectant as it is ubiquitous in sub-saharan Africa, including Nigeria, and has been documented to improve grains' protein quality in storage.

453 **Author contributions**

454 **Oluwasegun J. Jegede**: Conceptualization, Methodology, Investigation, Data curation, Data

455 analysis, Resources, Writing – review & editing. **Seun O. Oladipupo:** Data curation, Data

456 analysis, Writing – original draft, review & editing. **Olufunmilayo E. Oladipo:**

457 Conceptualization, Methodology, Validation, Supervision, Writing – review & editing.