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Chemical composition of essential oil in *Piper nigrum* from Nigeria and its bioactivity against stored-grain insects

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Abstract. Experiments were carried out on the essential oil (EO) and its bio-efficacy against rice weevil (*Sitophilus oryzae*) and cowpea seed beetle (*Callosbruchus maculatus*). The EO from *Piper nigrum* was extracted by using steam distillation. Thirty-nine components were identified by using GC-MS, and the major components are linalool (21.73%), γ-bisabolene (8.75%), and β-caryophyllene (7.35%). The insects were exposed to the EO. At $p < 0.05$, the efficacy depends on the dose and time of exposure. *S. oryzae* is more resistant to the oil than *C. maculates*. The essential oil of *P. nigrum* may be used as a botanical insecticide against insect pests.

Keywords: *Piper nigrum*, essential oil, insecticide, stored-grain insect

1 Introduction

Various synthetic insecticides are widely used to preserve stored grains and other agricultural goods against insect infestation as a regular practice among farmers [1, 2]. These agrochemicals are persistent in the environment and end up as a part of the food chain. Synthetic agrochemicals form part of the ecologically unsafe agricultural chemicals [3]. The havoc caused by synthetic insecticides increases because of the insecticide residues left on food and eventually got into man's body via the food chain. There is a need to regulate the use of these pesticides based on the documented environmental risk of the use of synthetic chemicals [4-6].

There is an increasing demand for biopesticides because of the growing need to regulate the use of these pesticides. Biodegradable plant extracts are environmentally friendly and safe compared with most widely used synthetic chemicals. Therefore, it is necessary to explore these plant extracts to develop safe alternatives to synthetic pesticides [7].

Essential oils (EOs) from plant or their fractions outperform synthetic insecticides regarding local availability, minimal mammalian toxicity, and quick degradation [8, 9]). Several studies are carried out on essential oils' insecticidal activities on store pests [1, 10-13]. These essential oils are potential insecticides; therefore, several plants grown in Africa are investigated for possible pesticide use.

Callosbruchus maculatus and *Sitophilus oryzae* are huge pests that affect various grains, such as dried beans, wheat, corn, and rice, in Africa [14]. The infestation of this pest results in enormous economic damage to farmers. Although several studies were conducted on *P. nigrum,* research shows that the plant's chemical composition varies with location [15-18]. Khan et al. [2] suggested examining the extracts collected from

different locations under various climatic conditions to determine their effectiveness for pest management. It is then vital to know the EO chemical composition of *P. nigrum* from Nigeria and investigate its insecticidal activity against rice weevil and cowpea seed beetle with regard to the exposure time at various doses of the formulation.

2 Material and methods

2.1 Material

Filter paper (Whatman No. 1) and acetone (100 %) were purchased from RIRO Ltd., Nigeria. *Piper nigrum* (black pepper) was obtained from the Agbalata market in Badagary, Lagos, Nigeria. The Federal Research Institute of Nigeria (FRIN), Ibadan, Nigeria, identified the seeds. The seeds were ground to powder after being air-dried.

2.2 Methods

Culture of insects

Rice weevil (*Sitophilus oryzae*) and cowpea seed beetle (*Callosbruchus maculatus*) founding insect cultures were taken from beans and rice that were already infested. The founding insect culture was kept in a 5 L plastic container at 24 °C and 70% humidity.

Essential oil distillation

The *P. nigrum* ground powder was subjected to hydro distillation in a modified Clevenger-type apparatus for six hours. The EO was dried after extraction with sodium sulphate (anhydrous) and kept at 4 °C in an air-tight container.

Gas-chromatography and mass-spectrometry (GC-MS) analysis

An Agilent 6890N instrument equipped with a flame ionisation detector and an HP-5MS (30 m × 0.25 mm × 0.25 μm) capillary column was used for the GC-MS analysis. An Agilent Technologies 5973N mass spectrometer was used to identify the components of the EO. First, the GC was set at an oven temperature of 60 °C and ramped at 10 °C per minute to 180 °C. This heating was followed by another ramping at 20 °C per minute to 280 °C. The sample (1 μL) was injected with a 1:10 split ratio with helium as a carrier gas with a flow rate of 1 mL per minute. Prior to injection, the injector was set at 270 °C. The spectra were measured over 20–550 m/z; the components were classified from retention indices and compared with those found in the literature. The retention indices were also examined in reference to a homologous sequence of n-alkanes (C8–C24). The mass spectra from both columns were classified further by comparing them with those found in the literature. Without the use of correction factors, GC peak areas were used to evaluate component relative percentages.

Insecticide formulation

For *C. maculatus*, five different concentrations of EOs were prepared. A volume of 2, 4, 6, 8, and 10 μL of the EO was picked out with a micropipette, then diluted with 2 mL of 100% acetone to obtain concentrations of 1, 2, 3, 4, and 5 $mL \cdot L^{-1}$, respectively. Similarly, four different concentrations of EOs were formulated for rice weevil. A volume of 5, 10, 15, and 20 μL of the EO was diluted with 2 mL of 100% acetone to obtain 2.5, 5, 7.5, and 10 $mL \cdot L^{-1}$ concentrations, respectively.

Contact effect

The essential oil contact activity for the samples prepared against cowpea seed beetle (*C. maculatus*) was assessed on a filter paper disc. The sample was allowed to flow on a filter paper disc placed in a Petri dish on a regular basis. After the solvent was eliminated, ten bean weevils were

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placed in the Petri dish, which was then sealed. Every six hours, the percentage mortality of insects was measured. Insects that did not respond to a mild touch with a little probe were deemed dead. Each experiment was carried out in triplicate, except for the control experiment, which utilised only acetone. A similar test was carried out for *S. oryzae* with the samples prepared for the rice weevil.

Vapour effect

The insects were directly exposed to EOs vapour in a 1.5 L air-tight glass jar. The filter paper was placed upward on the top cover of the jar; then, ten bean weevils were introduced into the jar. Every six hours, the percentage mortality of insects was measured. Each experiment was carried out in triplicate, except for the control experiment, which utilised only acetone. A similar test was carried out for *S. oryzae* with the samples prepared for the rice weevil.

Repellent effect

The area preference method was used to evaluate the EO repellent effects against *C. maculatus*. The filter paper was divided into two halves, and one half was used as the tested area. Afterward, the treated half was attached to the untreated half with a clear adhesive tape, and the re-made filter paper was put in a Petri dish. This procedure was repeated for all the samples. For each sample, ten mature insects from each species were separately placed in the centre of the re-made filter paper; then, each Petri dish was capped and preserved in an incubator at 27 ± 2 °C and relative humidity of 75 ± 5%.

Statistical analysis

The probit analytical method was used to evaluate the data. The LD_{50} and LD_{95} values of the EOs against each stored-product insect species were estimated by using the IBM SPSS software, version 21.0. The percentage mortality estimates for different exposure times were determined by using an analysis of variance (one-way ANOVA).

3 Results and discussion

3.1 Gas chromatography-mass spectroscopy

The GC-MS analysis indicates 39 components in the *P. nigrum* EO, which agrees with what Fan et al. reported [15]. In corroboration with other researchers, the major components (Table 1) are linalool (21.73%), γ-bisabolene (8.75%), and β-caryophyllene (7.35%) [15-17]. Fan et al. reported limonene as the major compound presenting 35.06% of total oil from the fruits of black pepper, followed by β-pinene (12.95%), and linalool (9.55%) [15]. Singh et al. reported 49 compounds representing 99.4% of total oil [19]. The major components identified were β-caryophyllene (24.2%), a sesquiterpene – limonene (16.9%), and sabinene (13%), both monoterpenes. Jirovetz et al. observed that the main components of essential oil from dried fruits of black pepper originating from Cameroon are germacrene D (11.01%), limonene (10.26%), β-pinene (10.02%), α-phellandrene (8.56%), β-caryophyllene (7.29%), α-pinene (6.40%), and cis-β-ocimene (3.19%) [20]. Linalool and β-caryophyllene were some of the major components observed in this study; they were also reported by other researchers. This revelation proves that the component of the *P. nigrum* EO depends on other factors apart from the plant part. The variations in the components of the EO of *P. nigrum* could be a result of the soil composition, climate, and vegetative conditions [21].

Table 1. The chemical composition of essential oil of *P. nigrum* from Nigeria

S/N	Compound present	Retention time (min)	% Compound
1	Linalool	4.014	21.73
2	Pinocarveol	4.403	0.17
3	Borneol,heptafluorobutyrate	4.649	0.14
4	Limonen-4-ol	4.975	2.4
5	3-Cyclohexene-1-methanol, α;α,trimethyl	5.444	2.2
6	Benzenamine, N,N,2-trimethyl	5.685	0.66
7	α-Cubebene	7.212	0.31
8	Copaene	7.779	1.67
9	8-Isopropenyl-1, 5-dimethyl-cyclode ca-1,5-diene	8.391	4.48
10	α-Gurjunene	8.494	2.01
11	Caryophyllene	8.963	7.35
12	(⊚,Z)-.alpha.-Farnesene	9.043	2.32
13	γ-Elemene	9.175	1.66
14	β-Arnesene	9.616	6.12
15	Bicyclo[4.4.0]dec-1-ene 2-isopropyl-5-methyl-9-methylene-	10.228	5.88
16	Azulene	10.365	3.43
17	γ-Bisabolene	10.743	8.75
18	β-Sesquiphellandrene	11	3.79
19	Calamenene, cis-T1n3	11.155	0.61
20	γ-Elemene	11.429	1.11
21	Palustrol	11.578	0.4
22	Nerolidol, (E)-	11.939	4.34
23	Caryophyllene oxide	12.116	1.87
24	Cis-Z-.α-Bisabolene epoxide	12.271	1.98
25	1H-Cycloprop[e]azulen-7-ol	12.322	1.23
26	9-Methylbicyclo[3.3.1] nonane	12.625	0.93
27	Naphthalene	12.745	0.62
28	Ylangene	12.866	0.82
29	Γ-Cadinene	13.283	2.52
30	α-Cadinol	13.466	2.99
31	α-Bisabolol	13.695	1.23
32	Tricyclo[3.2.2.0]nonane-2-carboxylic acid	13.913	0.5
33	(2-Isopropenyl-5-methyl-cyclopenty l)-acetonitrile	13.987	0.13
34	Santolina triene	14.29	0.13

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S/N	Compound present	Retention time (min)	% Compound
35	Cedrol	14.376	0.15
36	Isoaromadendrene epoxide	14.92	0.39
37	n-Hexadecanoic acid	17.689	1
38	Oleic Acid	19.738	1.56
39	Octadecanoic acid	19.824	0.44

3.2 Contact

The contact test result shows that the *P. nigrum* EO has insecticidal activity against both insects at varying doses and times. At the 5 $mL \cdot L^{-1}$ formulation, 100% mortality of *C. maculates* and *S. oryzae* was recorded in 48 and 72 h, as shown in Tables 2 and 3. After 24 h of exposure to the EO, the acquired data were subjected to probit analysis; the LD_{50} and LD_{95} for *C. maculates* are 4.37 and 6.07 $mL \cdot L^{-1}$, while those of *S. oryzae* are 4.80 and 12.98$mL \cdot L^{-1}$. This information shows that the essential oil is more toxic to *C. maculates* than to *S. oryzae*. The percentage of mortality increases with the concentration of formulation and exposure time; this mortality change is consistent with what Ashouri and Shayesteh reported [22].

3.3 Fumigating

Methyl bromide is a well-documented environmental pollutant known as one of the causes of ozone layer depletion [23]. Likewise, it is also known as a fumigant in the protection of insect pests. It is imperative to look for a possible alternative to it. Essential oils, owing to their high insecticidal activity, could be a viable alternative to methyl bromide [20]. An application of a 5 $mL \cdot L^{-1}$ EO formulation on *C. maculates* and *S. oryzae* shows 100% mortality in five days in both treatments (Tables 4 and 5). The mortality is dose-dependent. The probit analysis reveals that the LD_{50} and LD_{95} for the fumigating activity of the essential oil on *C. maculates* are 4.36 and 6.95 $mL \cdot L^{-1}$ after 48 h of exposure, while those of *S. oryzae* are 6.23 and 15.86 $mL \cdot L^{-1}$ after the same exposure time. Thus, it can be deduced from the observed fumigant activity that the essential oil of *P. nigrum* has an active vapour that could be deployed as an efficient alternative insecticide. Therefore, further probing these natural fumigants' usage is necessary to control insects in stored products.

Pérez et al. [24] reported that the EOs from the analysed three piper species display varying inhibition levels against *S. zeamais*. As the oil dose increases, the percentage of mortality also increases. This finding is similar to what was reported in our work. However, the result from the probit analysis (LD_{50} and LD_{95}) shows that the contact activity exhibits more toxicity against both insects than the fumigating activity.

Table 2. Percentage contact mortality of *P. nigrum* EO against *C. maculatus* (concentration 1–5 $mL \cdot L^{-1}$)

Exposure time (h)	Conc. ($mL \cdot L^{-1}$)							
	1	2	3	4	5	Control	LD_{50}	LD_{95}
12	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	10 ± 0.00^{b}	20 ± 5.8^{c}	20 ± 5.8^{c}	0.0 ± 0.0^{a}	7.7	22.3
24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	10 ± 0.00^{b}	40 ± 5.8^{c}	70 ± 10.0^{d}	0.0 ± 0.0^{a}	4.3	10.8
36	20 ± 0.0^{b}	30 ± 5.8^{c}	30 ± 5.8^{c}	60 ± 5.8^{d}	80 ± 5.8^{e}	0.0 ± 0.0^{a}	3.1	6.1

Exposure time (h)	Conc. (mL·L⁻¹)						LD_{50}	LD_{95}
	1	2	3	4	5	Control		
48	50 ± 0.0^{b}	60 ± 2.8^{c}	70 ± 10.0^{d}	80 ± 10.0^{e}	100 ± 0.0^{f}	0.0 ± 0.0^{a}	1.2	8.2
72	80 ± 10.0^{b}	80 ± 5.8	80 ± 5.8^{b}	100 ± 0.0^{c}	100 ± 0.0^{c}	0.0 ± 0.0^{a}	0.5	1.0

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at $p < 0.05$.

Table 3. Percentage contact mortality of *P. nigrum* EO against *S. oryzae* (concentration 2.5–10 mL·L⁻¹)

Exposure time (h)	Conc. (mL·L⁻¹)					LD50	LD95
	2.5	5	7.5	10	Control		
12	0.0 ± 0.0^{a}	20 ± 0.0^{b}	50 ± 5.8^{c}	70 ± 5.8^{d}	0.0 ± 0.0^{a}	7.6	16.3
24	30 ± 0.0^{b}	50 ± 5.8^{c}	80 ± 10.0^{d}	80 ± 10.0^{d}	0.0 ± 0.0^{a}	4.3	11.5
36	50 ± 0.0^{b}	60 ± 5.8^{c}	70 ± 5.8^{d}	100 ± 0.0^{e}	0.0 ± 0.0^{a}	3.0	9.4
48	70 ± 0.0^{b}	80 ± 5.8^{c}	100 ± 0.0^{d}	100 ± 0.0^{d}	0.0 ± 0.0^{a}	1.9	6.6
72	100 ± 0.0^{b}	100 ± 0.0^{b}	100 ± 0.0^{b}	100 ± 0.0^{b}	0.0 ± 0.0^{a}	1.5	2.9

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at $p < 0.05$.

Table 4. The percentage fumigative mortality of *P. nigrum* EO against *C. maculatus* (concentration 1–5 mL·L⁻¹)

Exposure time (h)	Conc. (mL·L⁻¹)						LD50	LD95
	1	2	3	4	5	Control		
1	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	20 ± 0.0^{b}	30 ± 5.8^{c}	0.0 ± 0.0^{a}	5.9	10.6
2	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	40.0 ± 5.8^{b}	40 ± 5.8^{b}	60 ± 5.8^{c}	0.0 ± 0.0^{a}	4.2	9.3
3	30 ± 5.8^{b}	40 ± 5.8^{c}	50 ± 5.8^{d}	60 ± 5.8^{e}	70 ± 5.8^{f}	0.0 ± 0.0^{a}	2.6	7.3
4	50 ± 10.0^{b}	60 ± 5.8^{c}	70 ± 10.0^{d}	80 ± 5.8^{e}	80 ± 5.8^{e}	0.0 ± 0.0^{a}	1.1	6.1
5	70 ± 10.0^{b}	70 ± 5.8^{b}	80 ± 5.8^{c}	100 ± 0.0^{d}	100 ± 0.0^{d}	0.0 ± 0.0^{a}	0.8	4.6
6	80 ± 0.0^{b}	100 ± 0.0^{c}	100 ± 0.0^{c}	100 ± 0.0^{c}	100 ± 0.0^{c}	0.0 ± 0.0^{a}	0.7	1.2

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at $p < 0.05$.

Table 5. Percentage fumigative mortality activity of *P. nigrum* EO against *S. oryzae* (concentration 2.5–10 mL·L⁻¹)

Exposure time (h)	Conc. (mL·L⁻¹)					LD50	LD95
	2.5	5	7.5	10	Control		
1	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	30 ± 5.8^{b}	70 ± 5.8^{c}	0.0 ± 0.0^{a}	8.706	13.159
2	30 ± 5.8^{b}	40 ± 5.8^{c}	50 ± 10.0^{d}	80 ± 10.0^{e}	0.0 ± 0.0^{a}	5.551	10.724
3	50 ± 5.8^{b}	60 ± 5.8^{c}	70 ± 5.8^{d}	100 ± 0.0^{e}	0.0 ± 0.0^{a}	3.009	9.391
4	70 ± 10.0^{b}	70 ± 5.8^{b}	100 ± 0.0^{c}	100 ± 0.0^{c}	0.0 ± 0.0^{a}	1.937	7.322
5	100 ± 0.0^{b}	100 ± 0.0^{b}	100 ± 0.0^{b}	100 ± 0.0^{b}	0.0 ± 0.0^{a}	1.502	2.895

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Exposure time (h)	Conc. (mL·L⁻¹) 2.5	5	7.5	10	Control	LD50	LD95
6	100 ± 0.0[b]	100 ± 0.0[b]	100 ± 0.0[b]	100 ± 0.0[b]	0.0 ± 0.0 [a]	1.502	2.895

Note: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at $p < 0.05$.

3.4 Repellant

Tables 6 and 7 show the essential oil average repellancy values of *P. nigrum* at different concentrations and exposure times against *C. maculates* and *S. oryzae*. All formulations were repellent to *C. maculates* and *S. oryzae*. The 5 mL·L⁻¹ formulation entirely evokes repellent action against *C. maculates* in 20 min and *S. oryzae* in 50 min. When the formulation concentration increases, the percentage of repellency also increases. The formulation has a higher repellency against *C. maculates* than against *S. oryzae*, and this repellency is similar to what was reported by Park [25].

Table 6. Percentage repellant activity of *P. nigrum* EO against *C. maculatus* (concentration 1–5 mL·L⁻¹)

Exposure Time (Mins)	Conc (mL/L) 1	2	3	4	5
10	33.3±5.8[a]	33.3±5.8[a]	33.3±5.8[a]	66.7±5.8[b]	75.0±0.0[c]
20	33.3±5.8[a]	75.0±0.0[b]	75.0±0.0[b]	75.0±5.8[b]	100±0.0[c]
30	66.7±5.8[a]	75.0±0.0[b]	100±0.0[c]	100±0.0[c]	100±0.0[c]
40	75.0±0.0[a]	100±0.0[b]	100±0.0[b]	100±0.0[b]	100±0.0[b]
50	75.0±5.8[a]	100±0.0[b]	100±0.0[b]	100±0.0[b]	100±0.0[b]

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

Table 7. Percentage repellant activity of *P. nigrum* EO against *S. oryzae* (concentration 2.5–10 mL·L⁻¹)

Exposure Time (h)	Conc (mL/L) 2.5	5	7.5	10
10	33.3±5.8[a]	33.3±5.8[a]	33.3±5.8[a]	66.7±5.8[b]
20	66.7±5.8[a]	66.7±5.8[a]	75.0±10.0[b]	75.0±5.8[b]
30	75.0±5.8[a]	75.0±5.8[a]	75.0±5.8[a]	100±0.0[b]
40	75.0±5.8[a]	75.0±5.8[a]	75.0±10.0[a]	100±0.0[b]
50	75.0±5.8[a]	100±0.0[b]	100±0.0[b]	100±0.0[b]

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

4 Conclusion

In this study, linalool is the major component in the *P. nigrum* essential oil, and it can be responsible for the contact and fumigating activities against *C. maculates* and *S. oryzae*. The *P. nigrum* essential oil has toxic effects on the studied insect pests. The essential oil is more toxic against *C. maculates* at a lower exposure dose and a smaller duration. The ease of handling and accessibility to farmers, and the toxicity against stored-grain insects make this insecticide product a potential bio-insecticides in Africa.

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