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(Research Article)

Determination of chemical composition and insecticidal potential of leaf essential oil of *Mentha piperita* L.

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Abstract

Pulverised fresh *Mentha piperita* leaves was subjected to hydrodistillation for three hours in a Clevenger-type apparatus. The oil obtained was characterised using Gas Chromatography-Mass Spectrometry (GC-MS) and also evaluated for insecticidal activity on *Callosobruchus maculatus* (bean weevils) and *Sitophilus zeamais* (maize weevils). The oil afforded a yield of 0.78% (w/w). A total of 39 compounds were identified from their mass spectra representing 98.9% of the oil. The classes of compounds identified revealed predominance of oxygenated monoterpenoids (72.4%), hydrogenated monoterpenoids accounted for 6.0%, hydrogenated sesquiterpenoids and oxygenated sesquiterpenoids constituted 12.2 and 4.0% respectively. Other non-terpenic compounds detected in the oil accounted for 4.3% of the total oil. The most abundant compound was menthol (40.4%), other major compounds identified were menthone (12.3%), menthofuran (9.2%), 1, 8-cineole (5.6%) and limonene (5.5%). The percentage mortality of between 68-94.5% and 65-90.2% were recorded for bean weevils and maize weevils respectively after exposure to 10µl essential oil vapour in fumigation chambers for 6, 12, 18 and 24 hours. The insecticidal activity of the oil compared favourably with that of standard insecticide and so can be used to formulate an alternative insecticide from botanical source.

Keywords: Essential oil; Fumigant toxicity; Hydrodistillation; Insecticide; *Mentha piperita*

1. Introduction

Mentha piperita (peppermint) is a perennial aromatic herb which belongs to the Lamiaceae family otherwise known as the mint family. It is widely distributed across Asia, Australia, Europe Africa and North America (Balakrishnan, 2015). It is a natural hybrid of spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatica* L.) (Uribe *et al*., 2016), and is cultivated worldwide for culinary purposes as well as for applications in fragrance, medicine and pharmaceuticals. Peppermint grows well particularly in lands with high water-holding capacity soil (Yingying *et al*., 2016). Generally, mints tolerate a wide range of conditions, and can also be grown in full sun. The plant is widely used in folk medicine for treating both pathogenic and non-pathogenic diseases including digestive disorder, flatulence, morning sickness and anorexia, nausea and vomiting, diarrhea, headache, flu and cold, cough, bronchitis, dental plaque and bad breath, neuralgia and as a remedy for menstrual cramps (Balakrishnan,2015; Brahmi *et al*., 2017; Loolaie *et al*., 2017).

Food security in sub-Saharan Africa generally and particularly in Nigeria has been a cause for concern. As a result, concerted efforts have been made to develop improved food productivity through the use of sustainable agricultural practices (Dahiru *et al*., 2014). However, stored grains are a source of food for many insects and mites which degrade the quality of the product, and post-harvest losses caused by these pests during storage pose a serious problem causing significant economic loss (Ayoub *et al*., 2018).

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Insects are one of the largest, most diverse invertebrates found in all types of environments (Stork, 2018). Some insects, such as bees, butterflies, and ants, have been shown to be beneficial to humans (Warren *et al*., 2021). These insects play an important role in pollinating certain flowering plants, a process that is critical in the agricultural field. However, many insects such as termites have been considered pests by humans because of their damaging effects. Mosquitoes and houseflies transmit certain diseases to humans (Evans & Kasseney, 2019; Shaw & Catteruccia , 2019; Vreysen *et al*., 2021). Other insects such as locusts and weevils sometimes can cause heavy losses in agricultural products (Zhang *et al*., 2019).

Maize weevil (*Sitophilus zeamais* Motsch.) and Cowpea weevils (*Callosobruchus maculatus* Fab.) both have a nearly cosmopolitan distribution, occurring throughout the warmer parts of the world, they are among the most destructive primary insect pests of stored grains. In order to minimize the economic losses caused by these pests, synthetic insecticides such as dichlorodiphenyltrichliroethane (DDT) and benzebnehexachloride (BHC) among others were used. These synthetic insecticides and fumigants widely used to control these pests are harmful to humans and the environment, because they persist for a long time in the environment and in animal tissues (Wojciechowska *et al*.,2016). Other problems are the presence of toxic residues in treated products, toxic hazards to human and other non-target organisms due to their broad spectrum of activity (Kaan *et al*.,2016). Furthermore, many stored grain pests have developed insecticide resistance (Attia *et al*., 2020).

These environmental concerns and demand for food safety have necessitated the need for alternative insecticide from botanical source in integrated pest management programmes. In the last decades, plant essential oils have been reported to be a potential alternative for many applications including herbicide uses (Lins *et al*., 2019; Isman, 2020). More particularly, essential oils also have repellent, oviposition, larvicidal and insecticidal properties to replace synthetic insecticides (Huang *et al*., 2020; Isman,2020; Shaw & Catteruccia, 2019; Mapossa *et al*., 2021; Mwingira *et al*., 2020). Increasing interest in essential oils as an alternative to synthetic insecticides can be attributed to their characteristics. They have the advantage over conventional fumigants in terms of local availability, high volatility, temperature and Ultra Violet (UV) light degradation sensitivity and low mammalian toxicity (Campolo *et al*., 2018). Furthermore, essential oils are less persistent in the environment and are therefore considered eco-friendly, they are also cheaper and have medicinal properties for humans (Mossa, 2016). It is on the basis of all these that this research is aimed at characterizing the essential oil of *Mentha piperita* and evaluating the insecticidal potential of the oil on beans and maize weevils.

2. Material and Methods

2.1. Plant Material

Fresh leaves of *Mentha piperita* was harvested from the garden in Tudun Wada area of Kaduna, Kaduna State. The sample was identified at the Herbarium of Kaduna State University, where voucher specimen was deposited and voucher number KASU/BSH/162 was issued. A 500g of pulverized sample was subjected to hydrodistillation for three hours in a Clevenger-type apparatus according to British Pharmacopoeia Specification. The resulting oil was collected, preserved in a sealed sample bottle and stored under refrigeration prior to analysis.

2.2. Gas-Chromatography-Mass Spectrometry (GC-MS) Analysis

A Hewlett-Packed HP 5890A GC, interfaced with a VG analytical 70-250s double focusing mass spectrometer was used, with helium as the carrier gas at 1.2mL/min. The mass spectrometry operating conditions were: ionization voltage 70eV, ion source 230^{0}C. The GC was fitted with a 25m x 0.25mm fused silica capillary column coated with Cp-Sil 5, with a film thickness 0.15μm. The mass spectrometry data was acquired and processed by online desktop with computer equipped with disc memory. The identification of individual compounds was performed by comparing their retention indices determined with respect to homologous series of n-alkanes, and by comparison with the mass spectra fragmentation pattern with those found in the MS databases (Adams, 2007; Joulang and Konig, 1998).

2.3. Insecticidal Activity

Mentha piperita leaf essential oil was screened for insecticidal activity based on the procedure of Ilboudo *et al*. (2010) with slight modification. Maize weevils and cowpea weevils were reared on whole maize and beans (10:1w/w) respectively. The insects were cultured in a dark growth chamber at a temperature of 27 ± 1ºC, relative humidity 65 ± 5% with a 12:12 h light: dark cycle.

The insecticidal activity of the essential oil against the adult *C. maculatus* (bean weevils) and *S. zeamais* (maize weevils) was determined by fumigant bioassay according to the procedure of Liang *et al*. (2016) with slight modification, using the closed container method. The experimental concentration of essential oils on the insect were determined by preliminary tests. A group of 10 insects were put into the bottom of a 50 ml plastic container. Paper discs were treated with 10µl essential oils. The discs were attached to the inside top of the container and the container was closed. All the treated insects were held in the same rearing conditions. Acetone was used as control and dichlorvos was used as a positive control. The control sets received no essential oil. Each experiment and controls were replicated five times. The number of dead insects was counted. Mortality was determined after 6, 12, 18, and 24h from the start of exposure. An insect was considered dead if it did not move when observed outside of the container and when lightly probed.

2.4. Statistical analysis

Data are presented as mean ± standard error. Duncan and Tukey tests were used to determine statistical significance; ANOVA was used to determine whether results obtained for insecticidal activity assays were statistically different. Statistical significance was set at $P < 0.05$.

3. Results and Discussion

3.1. Percentage Oil yield

Extraction of the oil afforded a yield of 0.78% (w/w). This is in agreement with the yield of 0.75%(w/w) reported by Souza *et al*. (2022).

3.2. Chemical Composition of *Mentha Piperita* Leaf Essential Oil

The chemical composition of leaf essential oil of *M. piperita* is shown in Table 1. In the Table, a total of 39 compounds were identified representing 98.9% of the oil. The oil was predominated by oxygenated monoterpenoids accounting for 72.4 % of the oil. Menthol (40.4 %) was the major oxygenated monoterpenoid and the most abundant. Other principal oxygenated monoterpenoids include menthone (12.3%), menthofuran (9.2%) and 1, 8-cineole (5.6%). The predominant hydrocarbon monoterpenoid was limonene (5.5%). Hydrogenated sesquiterpenoids were found in appreciable and minute quantities, those detected in appreciable amounts include γ-elemene (2.3%), β-caryophyllene (2.8%) and Germacrene D (2.3%). Oxygenated sesquiterpenoids were detected in minute quantities, among them were epicubenol (1.3%) and α-cadinol (0.9%). Other non-terpenic compounds detected accounted for 4.3% of the oil.

The chemical composition of essential oils including mint species may vary considerably with different organs of the plant, plant maturity, genetic factors and evolution, environmental and agronomical factors such as climate, soil, geographical regions, harvesting season, and processing conditions (Riachi and De Maria, 2015; Chen and Zhong, 2015; Loolale *et al*., 2017). However, the compositional pattern of the oil compares favourably with those reported in the literature (Sahib *et al*., 2013; Stanescu *et al*., 2014; Balakrishnan, 2015; Aishwarya, 2015).

Table 1 Chemical Composition of Leaf Essential Oil of *M. piperita*

S/N	Compound[a]	RI[b]	RI[c]	Composition (%)	Mass Spectral Data[d]
1	α-Thujene	931	928	Tr	136 93 77 65
2	α-Pinene	936	936	0.2	136 121 93 77
3	Octen-3-ol	974	978	0.2	99 85 72 57
4	*p*-cymene	1022	1024	Tr	134 124 119 91
5	Limonene	1027	1031	5.5	136 121 93 68
6	1,8-cineole	1028	1033	5.6	154 139 81 71
7	γ-terpinene	1057	1062	0.3	136 121 93 77
8	α-terpineol	1188	1189	0.2	136 121 93 59
9	*Trans*-limonene oxide	1136	1138	0.8	137 108 94 79
10	Menthone	1156	1137	12.3	154 139 112 69
11	Isopulegol	1158	1146	0.2	154 138 109 94

12	Menthol	1165	1163	40.4	138 123 95 71
13	Menthofuran	1176	1165	9.2	150 108 79 39
14	Carvone	1261	1242	1.1	150 108 82 54
15	Menthyl acetate	1295	1281	1.6	138 95 81 43
16	Neoisomenthol	1166	1166	0.8	156 138 81 71
17	*Trans*-carveyl acetate	1335	1337	2.2	152 119 109 84
18	Decanoic acid	1374	1376	0.3	172 129 73 60
19	γ-Elemene	1554	1555	2.3	204 161 121 95
20	Aromadendrene	1438	1439	0.2	204 161 105 91
21	β-caryophyllene	1417	1418	2.8	204 161 133 93
22	β-farnesene	1456	1458	0.2	204 161 93 69
23	Cis-Muurola-4,5-diene	1462	1464	0.4	204 161 94 81
24	Germacrene D	1479	1480	2.3	204 161 105 91
25	α-Humulene	1452	1456	0.2	204 147 121 93
26	Bicyclogermacrene	1492	1494	1.3	204 161 121 95
27	γ-muurolene	1475	1477	0.8	204 161 119 105
28	*Trans*-calamenene	1522	1528	1.1	202 159 144 129
29	Spathulenol	1575	1578	0.2	220 205 159 91
30	Epicubenol	1628	1626	1.3	204 189 161 119
31	Caryophyllene oxide	1582	1581	0.6	220 109 79 43
32	Carotol	1595	1595	0.3	204 179 161 69
33	α-cadinol	1655	1653	0.9	220 189 91 41
34	Aromadendrene oxide	1703	1702	0.3	220 189 91 41
35	δ-Cadinene	1523	1524	0.3	204 161 119 69
36	Pulegone	1240	1234	1.1	155 152 109 81
37	Piperitone	1248	1341	0.6	152 137 110 82
38	Valencene	1490	1492	0.3	204 161 105 91
39	Viridiflorol	1596	1591	0.4	204 161 109 43
	Total (%)			98.9	

KEY: a-compounds listed in order of elution from DB-5 column; b-retention indices in relation to n-alkane series; c-retention indices from literature; tr-trace, d-mass spectral data, base peak in bold.

3.3. Insecticidal Activity

The result of the percentage mortality of *Callosobruchus maculatus* (bean weevils) and *Sitophilus zeamais* (maize weevils) after exposure to 10µl vapour of essential oils from leaves of *M.piperita* in fumigation chambers for 6, 12, 18 and 24 hours is presented in Table 2.

At the end of six hours of exposure, the oil obtained from the leaves of *M. piperita* caused 60 and 56% mortality of *C. maculatus* and *S. zeamais* respectively. The % mortality increased to 66 and 62% respectively after 12 hours' exposure time. After 18 hours' exposure time, a mortality of 84 and 74% were recorded, and at the end of 24 hours' exposure time, 90 and 88% mortality was obtained respectively. No mortality was observed in the negative control. The standard insecticide (Dichlorvos) reported % mortalities of 66, 74, 82 and 92% after exposure times of 6, 12, 18 and 24 hours respectively. The % mortality increased with exposure time for both insect pests.

Table 2 Insecticidal Activity of *Mentha piperita* Essential Oil on *Callosobruchus maculatus and Sitophilus zeamais*

Exposure Time(h)	**% Mortality**		
	Beans weevils (*Callosobruchus maculatus*)	Maize weevils (*Sitophilus zeamais*)	Dichlorvos
6	60±7.07	56±5.48	66±5.48
12	66±5.48	62±4.47	74±5.48
18	84±5.48	74±5.48	82±4.47
24	90±8.37	88±8.37	92±4.47

Values are % mean ± std dev of 5 replications

Mentha piperita oil was generally more active on beans weevils (*Callosobruchus maculatus*) than maize weevils (*Sitophilus zeamais*). The insecticidal activity of the oil compared favourably with that of the standard insecticide.

The grains were treated directly with a standardized quantity of oil, and a determined number of insects of the same age group were directly placed on the grains in order to be as close as possible to realistic application. Consequently, the observed mortality was a result of contact with the treated grain, attempts at nutrition and a fumigation effect.

The insecticidal components of a great number of essential oils are mostly mono- and sesquiterpenoids. Monoterpenoids have strong toxicity towards insects due to high volatility, and lipophilic properties can penetrate into insects rapidly and interfere in physiological function.

According to several literature surveys, the main active ingredients of *M. piperita* essential oil are pulegone, carvone, menthone, menthol and to a lesser extent dihydrocarvone (Saeidi and Mirfakhraie, 2017). The high toxicity effects of *M piperita* essential oil against cowpea and maize weevils can be attributed to the presence of high concentrations of components such as caryophyllene, menthol, menthone and other constituents in minor quantities. The toxicity of essential oils on stored products insect pests had been linked to the separate and synergistic actions of the constituents of the oils (Pang *et al*., 2020).

4. Conclusion

Enhancing food storage life, while guaranteeing its quality and safety, is a focal interest of farmers, agro-food industry and government agencies. Essential oils have been extensively evaluated for insecticidal activity, and they have been considered as promising insect control agents. This study showed that essential oil of *M. piperita* and its constituent compounds have potential for development into natural insecticides/fumigants for the control of insects in stored grains.

Recommendation

Storage life of any food item refers to the time duration between which it will stay unaltered, retain sensorial properties, physical, biochemical, microbiological, and functional attributes. Synthetic insecticides have been used to prolong the shelf life of food items effectively. However, they are associated with deleterious side effects. Further research is therefore needed to evaluate the mechanisms underlying the insecticidal activity of the essential oil in order to develop environmentally safe, inexpensive, readily available and agronomically viable insecticides from plant origin.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest.

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