Nigerian Journal of Ecology (2023) 19(1):83-99

ISSN: 1116-753X (Print); E-ISSN: 2955-084X (Online)

Toxicity and Residue of Mexican Mint (*Plectranthus amboinicus* Lour.) Essential Oil as a Bio-insecticide on Cowpea Beetle (*Callosobruchus maculatus* Fabricius)

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(Accepted 23May, 2023)

ABSTRACT

Cowpea (*Vigna unguiculata* L.) (Walp)) is one of the legumes grown worldwide due to its high value in protein. However, the crop faces insects` attack from the field to storage especially *Callosobruchus maculatus* (cowpea grain beetle) which has brought huge economic losses in storage. Insect pests of cowpea had been controlled with various synthetic insecticides but with hazardous effects on human health and the environment. This study assessed plant essential oil which could be used as an alternative to control stored cowpea grains beetles. Therefore, the objectives of this study were: To identify the compounds present in Mexican mint as a bio-insecticide, to determine the residue of the Mexican mint essential oil in treated cowpea grains and to determine the proximate analysis of treated cowpea grains. The study was conducted at Entomology Research Laboratory Department of Crop Protection and Environmental Biology, University of Ibadan using a susceptible cowpea variety Ife Brown cowpea grains. Six treatments: 0.75, 1.25, 1.75 and 2.25 mL/g of Mexican mint essential oil, Phostoxin (0.01g recommended) and without treatment (control) were replicated four times and laid out in a completely randomized design. The essential oil extraction, the essential oil residue in cowpea grains and proximate analysis were determined using standard procedures. Data were collected on mortality of insects corrected with Abbott's formula and analysed using ANOVA at $p<0.05$. Results revealed 26 compounds with two prominent compounds identified; monoterpenes (0.22-6.16%) and terpenes (0.28-4.16%). Toxicity of Mexican mint essential oil concentrations on *C. maculatus* gave a 93.75% mortality rate at 1.25, 1.75 and 2.25 mL/g, respectively which compared favorably with phostoxin (100%), while the control gave highly significant reduction of 12.50%. Compounds found in the cowpea grains residues showed no trace of toxicity which were: alpha. -Pinene (144.98-346.79 mg/L); tau. -Muurolol and Linalool (0.22-0.54mg/L), Di- epi-1,10-cubenol (15.38mg/L) and alpha.-Cadinol (0.35mg/L), respectively from 1.25, 1.75 and 2.25 mL/g. Proximate analysis of treated cowpea grains revealed essential oil at 0.75 mL/g having highest value of 29.75% in crude protein, highest (91.00%) dry matter was obtained from 2.25 mL/g, similar values of 10.75 and 11.14% moisture content was recorded from 0.75 and 1.25mL/g, respectively. Highest similar values ranging from 54.16-54.56% of carbohydrate were obtained from 1.25, 1.75 and 2.25 mL/g, respectively. This study revealed that Mexican mint essential oil at 1.25, 1.75 and 2.25 mL/g contained important insecticidal components, which enhanced high toxicity on *Callosobruchus maculatus* on stored cowpea grains, with no toxic residue of the essential oil in the cowpea grains and no harmful effects on the nutritional components. Therefore, Mexican mint essential oil could give food quality and safety when used as a bio- insecticide to protect cowpea grains in storage.

Keywords: Mexican mint essential oil, Cowpea, *Callosobruchus maculatus,* Toxicity, Cowpea residue and Proximate analysis

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most important food legumes grown in the tropical savannah zones of Africa and other parts of the world (Kebe and Sembene, 2011). The crop is of major importance to the livelihoods of millions of relatively poor people in less developed tropics countries. Cowpea as an agricultural plant stems largely from its use as a short season protein-rich grain crop for human or animal consumption. Its desirability reflects families variously derive food such as; the leaves, immature pods, fresh seeds and dry grains which are eaten, animal feed and cash, together with spill over benefits to their farmlands through its residues. In the African marketplace, harvested cowpea grain provides a cost-effective substitute for the less affordable foods from livestock and fish. Its leaves can be harvested for direct use as needed during times of food scarcity while end of season collection of above-ground biomass after harvest provides valuable feedstock as fodder hay either for direct use or as a transportable commodity for sale or barter (Hollinger and Staatz, 2015). Its grains are highly nutritious and has potential health benefits because of its high protein, high fibre and low glycaemic index, (Aguilera *et al*., 2013; Xu and Chang, 2012).

Regardless of the above, cowpea production is considered too risky an investment by many growers, because of the numerous pest problems associated with it (Singh *et al.*, 2007). Insect damage is the major constraint to cowpea grain production in which insect pests infestation and disease infection had led to huge economic losses. Infestation by several pests from the field (especially cowpea aphids) up to the storage is threatened by various insects (Baidoo *et al.*, 2012) resulting in low yield. The Cowpea beetle (*Callosobruchus maculatus*) is among the major insect pests that can cause economic loss, its population can grow exponentially, leading to significant loss in seed weight, germination viability, and the market value of the crop. (Singh *et al*., 2007; Beck and Blumer 2014). This beetle is considered a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea in the tropics.

Callosobruchus maculatus, damage about 80-100% cowpea grains after harvest, prefers and attacks dried cowpea grains, other beans and peas in storage (Wahedi *et al.*, 2013), in a period of 2-3 months (Kebe and Sembene, 2011). This pest infestation results in substantial quantitative and qualitative losses manifested by seed perforation and reductions in weight, market value and germination ability of seeds. Consumers strong dislike grains that have been damaged by beetle, but can be used as seeds, although, germination percentage may have been reduced.

Synthetic chemicals' continual and indiscriminate uses for insect pest control (Gbaye *et al.*, 2016) have led to the development of resistant strains of pests, and the accumulation of toxic residues on food crops and animal products consumed by humans (Degri, 2008). According to Longe (2016), cowpea farmers in Africa have depended, over the years, on the use of synthetic insecticides like phostoxin and primiphos methyl (actellic) and lately 2, 2-dichlorovinyl dimethyl phosphate (DDVP, Sniper) in Nigeria on cowpea grains in storage, although very effective for stored products protection, but their use has toxic effects to human health (Fayinminnu *et al.*, 2022) and the environment, if carelessly and indiscriminately used. Altogether these result in health problems such as cancer, birth abnormalities, hormonal imbalance, environmental pollution (Ofuya and Lale, 2001) and food poisoning for both humans and animals (Fayinminnu *et al*., 2022).

Many researchers have reported the insecticidal effects of naturally occurring plant products against a broad range of pests. Some of the techniques explored include the use of garlic, peppermint and chilies (Tiroesele *et al.,* 2015), leaves and seeds powder from mint, tobacco, ginger, moringa, lime and neem among others (Ilesanmi and Gungula, 2013; Longe, 2016). Aromatic plants have both medicinal and aromatic properties and contain a variety of volatile oils which have insecticidal, anti-feedant and repellent effects on insect pests (Tiroesele *et al.,* 2015). An alternative of using oil from the seeds of the neem tree to control insect pests in cowpea storage has been studied (Naniwadekar and Jadhav, 2012).

Mexican mint (*Plectranthus amboinicus*) is a member of the family Lamiaceae or mint family and the paleotropical oil-rich genus. It is among the annual or perennial herbs or sub-shrubs which are often succulents (Wagner and Lawrence, 2016) with economical and medicinal values. *Plectranthus amboinicus* is one the most important highly aromatic medicinal succulent fleshy plants that possesses distinctive smelling leaves with short soft erect hairs and have a tendency for climbing or creeping (Wagner *et al*., 2016). The essential oil obtained from the leaves and stem contains volatile constituents. *Plectranthus amboinicus* essential oil contained a copious quantity of the two major phenolic compounds; carvacrol and thymol, which are pharmaceutically appreciated for various culinary properties (Lukhoba *et al*., 2005). The quality as well as quantity of chemical compounds occurring in the essential oil is directly related to its biological functions. Mexican mint oil is rich in oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Lukhoba *et al*., 2005).

In Nigeria attention on economic issues as storage which enhances shelf life, marketing quality and consumer preferences are neglected in cowpea research. Cowpea grains suffer heavily from insects, both in the field and in storage after harvest and yield reduction can reach as high as 95 percent depending upon the location, year and cultivar (Korletey, 2009). Bio-insecticides are organic formulations recommended for the management of insects that feed on crops and attractive alternatives to synthetic chemical insecticides. Earlier research had revealed that there was no residue for all essential oils used on cowpea grains in storage because they pose little or no threat to the environment or to human health (Isman, 2005).

However, the pests` problem of cowpea is complex and requires diversified efforts. Without a major breakthrough in the control of the more recalcitrant postharvest field pests of this crop, bridging the gap between the present and potential production of cowpea will be a slow and frustrating process. This study therefore, was carried out in order to offer an alternative cowpea preservative technique that is low-cost, accessible and safe. The objectives of this study were to: (i) determine the chemical compounds present in Mexican mint essential oil (ii) evaluate the efficacy of Mexican mint oil as bio-insecticides on stored cowpea grains (iii) determine the residue of Mexican mint oil in treated stored cowpea grains and (iv) determine the proximate analysis of cowpea grains treated with Mexican mint oil.

2.MATERIALS AND METHODS

2.1 Experimental study site

The experiment was carried out at the University of Ibadan, Ibadan, Nigeria, which lies between longitude 7°27.05`N and 3°53.74`E of the Greenwich Meridian (SMUI, 2018). The experiment was conducted under ambient temperature of 29±2°C and relative humidity of 70±5% in the Entomology Research Laboratory, Department of Crop Protection and

Environmental Biology, University of Ibadan.

2.2 Sources of Cowpea Grains, Mexican mint (*Plecthranthus amboinicus*) Leaves and Synthetic Insecticide

Cowpea grains (Ife Brown) were obtained from Institute of Agricultural Research and Training (IAR&T), Moor plantation, Apata, Ibadan, Oyo State, Nigeria. The uninfected grains were sorted to remove perforated/wrinkled ones and were put in clean Kilner jars. Fresh leaves (5.kg) of Mexican mint were sourced from a cultivated plot at Teaching and Research Farm University of Ibadan, Nigeria. Synthetic insecticide Phostoxin was obtained from the Department of Crop Protection and Environmental Biology University of Ibadan, Nigeria.

2.3 Culture of *Callosobruchus maculaus* Fabricius

Twenty four Kilner jars were half filled with clean, sorted and uninfected (250 g/jar) Ife brown cowpea variety. Unsexed adults of *C. maculatus* from infested cowpea grain sourced from the Entomology Unit of Department of Crop Protection and Environmental Biology were introduced into the Kilner jars to initiate oviposition. The rearing jars were covered with 1mm^2 mesh to allow aeration and prevent escape of insects. After seven days of oviposition, beetles were sieved out from the culture and the set up was kept until the emergence of F_1 generations. Newly emerged adults were used for all the laboratory experiment.

2.4 Extraction of Essential Oil from Mexican mint leaves

Hydro- distillation method using Clevenger apparatus was employed as a method of obtaining the essential oil from the leaf sample of 100 g. The sample was carefully placed in a 5 L round bottom flask and water was added until the sample was fully immersed. The essential oil was trapped in 2.0 mL of hexane and then carefully collected using a syringe and placed in pre-weighed sample vial. The weight of extracted oil was recorded and the yield was then stored in the refrigerator at -4^{0}C for further analyses. The extraction process was repeated for the weighed leaf sample for three hours according to the British Pharmacopoeia specification (British Pharmacopoeia, 1980).

2.5 Preparation of Concentrations of Mexican Mint oil

From a portion of the essential oil obtained above, a stock solution was prepared for each concentration in 100 mL standard flax with the methanol using the equation $C_1V_1=C_2V_2$. Four concentrations: 0.75, 1.25, 1.75 and 2.25mL/g were prepared using serial dilution method in methanol as the solvent.

2.6 Identification methods of essential oil from Mexican mint leaves

The analysis was performed using 8860A Gas Chromatograph coupled to 5977C inert mass spectrometer with electron impact source (Agilent Technologies). The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out in CTX-ION Analytics Limited, Ikeja Lagos with the procedures as explained. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. Scanning of possible compounds was from m/z 50 to 550 amu at 2.62s/scan rate and were identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library. All instrumental conditions were as reported in Adegoke and Alo (2013).

2.7 Experimental design

Six treatments were used in this study: 0.75, 1.25, 1.75 and 2.25mL of Mexican mint essential oil, Phostoxin (0.01g) and Control (without treatment). All were replicated four times and laid out in a completely randomised design.

2.8 Toxicity of Mexican mint essential oil from *Plectranthus amboinicus* on mortality of *C. maculatus*

Twenty grams (20g) of Ife Brown cowpea grains was weighed into each of six 1-litre Kilner jar. Concentrations of 0.75, 1.25, 1.75 and 2.25 mL/g of Mexican mint essential oil, respectively was introduced using micro-syringe, 0.01 g of Phostoxin was added to the grains and Control after which each jar received four pairs of unsexed adult *C. maculatus*. Untreated cowpea grains served as the control. Data on mortality were collected at 12h – 24h after infestation, converted to percentage and corrected using Abbott's formula (Abbot, 1925)

P_T = [Po-Pc /100 – Pc] x 100;

Where, PT= corrected mortality (%), PO= observed mortality (%), Pc= control mortality (%)

2.9 Determination and Extraction Procedure of Mexican mint Essential oil residue in stored cowpea grains

The Mexican mint essential oil residue in stored cowpea grains was carried out using the method of UNEP/FAO/IAEA (1986). Instruments and reagents used were; Ultrasonic Bath: CLEAN 120-HD (China), Rotary Evaporator: BUCHI Rotavapor R-215 (Switzerland) and Analytical Balance: ADAM AAA250LE Weighing Balance (UK). Acetone: GC Ultratrace Scharlau (Spain), n-Hexane: 96% GC Ultratrace Scharlau (Spain), Silica Gel: Loba Chemie (India) and Anhydrous Sodium Sulphate: Merck (US). A 5g of properly homogenized samples were weighed into beakers and mixed with 10ml n-Hexane: Acetone (1:1). The beakers were then placed into an ultrasonic bath and sonicated for 20 mins. The mixture was allowed to settle and solvent layer was decanted and concentrated down to 2 mL using a rotary evaporator (UNEP/FAO/IAEA (1986)).

2.10 Cleanup procedure of Mexican mint Essential oil residue in stored cowpea grains

Granular silica gel (Mesh Size 60-200A) was activated by heating at 130^0 C for 16hrs and stored in a desiccator. A glass column was packed with 5g of silica gel and 1g of Anhydrous Na_2SO_4 was added. A 20 mL n-Hexane was added to the column and eluted into a beaker; the sample extract was added to the top of the column quantitatively. Another 10 mL of n-Hexane was added to the column and eluted to waste. Before the column head dried out 10mL (1+1) Dichloromethane + Hexane was added and the eluent was collected. The eluent was then concentrated to 2mL using a rotary evaporator and analyzed.

Proximate Analyses of the Mexican mint Essential Oil on treated cowpea grains

Using the method of AOAC (1998), proximate analyses of treated cowpea grain samples for % crude protein, ash, ether fat, crude fibre, dry matter, moisture content and carbohydrate were carried out at the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT) at p<0.05 probability.

RESULTS

Identification of Compounds from Mexican mint essential oil using

Twenty-six (26) compounds were identified in Mexican mint essential oil as shown in Table 1 and Figure1. The compounds were divided into different compound classes as follows: Monterpenoids; 3-carene, Linalool, Camphene. Linalool had the highest percentage composition of 1.49%, while the lowest percentage composition was recorded in camphene with 0.21%.

Menthane monoterpenoids; beta-Phellandrene, Terpinen-4-ol. Terpinen-4-ol was observed to have the highest percentage composition (0.31%), while the lowest percentage composition (0.17%) was recorded in beta- Phellandrene. Sesquiteterpenoids; beta-Bourbonene, alpha-Muurolene, Cubenene, gamma.-Elemene, Epicubenol, tau-Cadinol, alpha-Cadinol. The highest percentage composition was recorded in alpha-Cadinol with 3.52%, while beta-Bourbonene with 0.16% was the lowest. Other compounds identified were; alpha. Pinene (Terpenes) 0.28%, (+)-4 Carene 6.16%, o-Cymene 4.41%, trans-beta-Ocimene 0.29%, gamma-Terpinene 5.50%, alpha-Cubebene 0.14%, Copaene 4.56% (tricyclic sesquiterpene), Germacrene D 0.76%, Carophyllene 2.64%, Humulene (monocyclic sesquiterpenes) 4.69%, p-Cymene 0.15%, Ledol 0.31%, and beta-Vatirenene 0.20%.

Table 1: Identification of compounds from Mexican mint (*P. amboinicus*) essential oil using GC-MS

/N	Compound	Composition%	Compound Class
1	alpha-Pinene	0.28	Terpene
2	beta-Phellandrene	0.17	Menthane monoterpenoids
3	3-Carene	0.22	Monoterpenoids
4	(+)-4-Carene	6.16	Bicyclic monoterpene
5	o-Cymene	4.41	Alkylbenzene related to a monoterpene4
6	trans-beta.-Ocimene	0.29	Acyclic monoterpenoids
7	gamma-Terpinene	5.50	Monoterpene
8	Fenchone	0.20	Bicyclic monoterpenoids
9	Linalool	1.49	Monoterpenoid
10	Terpinen-4-ol	0.31	Menthane monoterpenoids
11	alpha-Cubebene	0.14	Sesquiterpenes
12	Copaene	4.56	Tricyclic sesquiterpene
13	beta-Bourbonene	0.16	Sesquiterpenoids
14	Germacrene D	0.76	Sesquiterpenes
15	Caryophyllene	2.64	Bicyclic sesquiterpene
16	Humulene	4.69	Monocyclic sesquiterpene
17	alpha-Muurolene	3.31	Sesquiterpenoids
18	Cubenene	0.23	Sesquiterpenoids
19	p-Cymene	0.15	Alkylbenzene related to a Monoterpene
20	gamma-Elemene	0.22	Sesquiterpenoids
21	Ledol	0.31	Aromadendrane sesquiterpenoids
22	Epicubenol	0.30	Sesquiterpenoids
23	tau-Cadinol	3.14	Sesquiterpenoids
24	alpha-Cadinol	3.52	Sesquiterpenoids
25	beta-Vatirenene	0.20	Eremophilane
26	Camphene	0.21	Monoterpenoids

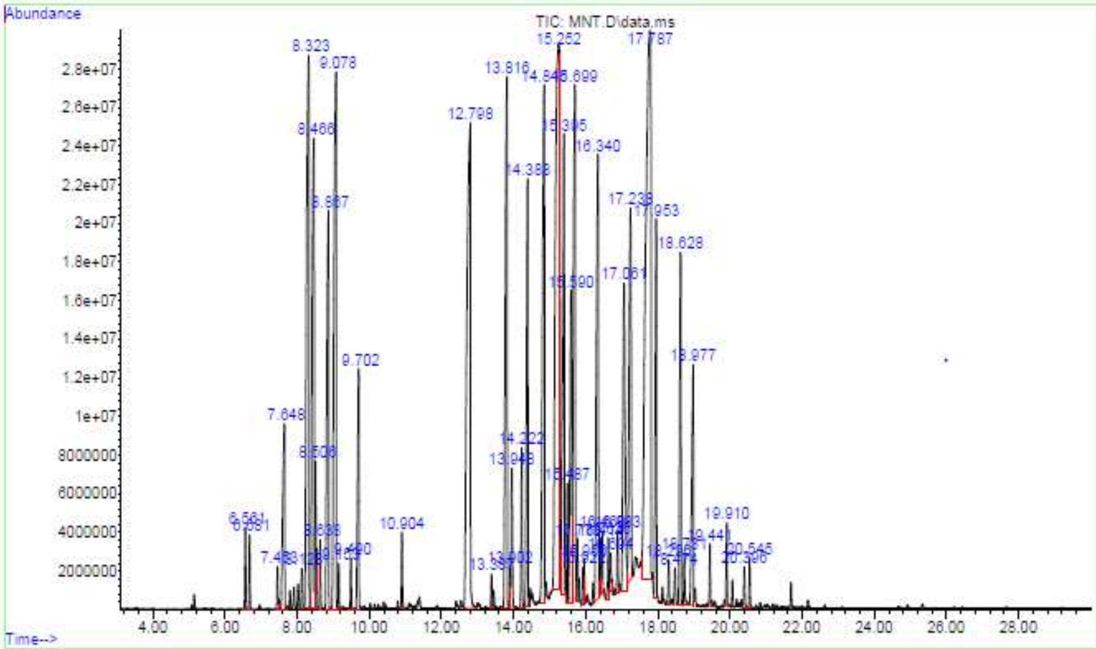

Figure 1: Identification of compounds from Mexican mint (*P. amboinicus*) essential oil using GC-MS

Toxicity of Mexican mint oil on *Callosobrochus maculatus*

Results revealed significant differences ($p<0.05$) amongst the Mexican mint essential oil treatments as toxic effects on insects` mortality at 12hrs after application on stored cowpea grains (Table 2). Synthetic phostoxin treatment had the highest mortality rate (100%) with no significance difference ($p>0.05$) from concentrations of Mexican mint essential oil except 0.75 mL. However, the control (untreated) treatment recorded the lowest mortality rate (12.50%) with significant difference ($p<0.05$) from other treatments. Toxicity at 24 hours exposure on mortality rate revealed significant effects with application of Mexican mint essential oil on stored cowpea grains (Table 2). Result showed no significant differences ($p>0.05$) between phostoxin and Mexican mint essential oil treatments. However, control (untreated) recorded the lowest value (37.50%) mortality rate which was significantly different ($p<0.05$) from others.

Table 2: Toxicity of Mexican mint Essential oil on stored cowpea grains weevil (*C. maculatus)* adults at 12hours and 24hours after application

Concentrations (mL/g)	Mortality % at 12 Hr	Mortality at 24 Hr
0.75	56.25b	75.00b
1.25	70.00a	87.50ab
1.75	93.75a	93.75ab
2.25	100.00a	93.75ab
Phostoxin (0.01g/20g)	100.00a	100.00a
Control (Untreated)	12.50c	37.50c

Means followed by the same letter (s) within a column are not significantly different at ($p<0.05$) from each other using Duncan Multiple Range Test

Determination of Mexican mint essential oil residue in stored cowpea grains at 1.25, 1.75 and 2.25 mL/g concentrations using GC-MS

Twelve different compounds class were detected in the stored cowpea residue analysis in various concentrations of Mexican mint essential oil as shown in Table 3 and Figures 2, 3 and 4**.** Results from 1.25 mL/g concentration showed highest value (144.98mg/L) which was recorded from alpha-Pinene, followed by (+)-4-Carene having the value of (76.87mg/L), while the lowest concentration (0.22mg/L) was from tau-Muurolol. The highest retention time (RT) (17.209min) was observed in alpha-Cadinol, while the lowest RT (6.675min) was recorded from alpha-Pinene (Table 3 and Figure 2).

The same trend of twelve different compounds class were observed in the stored cowpea residue at 1.75 mL/g concentration of Mexican essential mint oil. Highest concentration value (346.79mg/L) was recorded from alpha-Pinene, followed by gamma -Terpinene with the value of (90.45mg/L), the lowest concentration value (0.54mg/L) was recorded from Linalool. The highest retention time RT (17.226min) was observed in alpha-Cadinol, while the lowest RT (6.681min) was recorded from alpha-Pinene (Table 3 and Figure 3).

Results presented on Table 3 and Figure 4 revealed twelve different compounds class in the stored cowpea residue analysis for 2.25 mL/g concentration of Mexican essential mint oil. Highest concentration value (15.38mg/L) was recorded from Di-epi-1,10-cubenol, followed by gamma - Terpinene having the value of (10.30mg/L) the lowest concentration value (0.35mg/L) was recorded from alpha-Cadinol. However, (+)-4-Carene was not detected in the cowpea residue analysis. The highest retention time RT (17.255min) was observed in alpha-Cadinol, while the lowest RT (6.680min) was recorded from alpha-Pinene.

Table 3: Determinations of Mexican mint Essential Oil Residues in Stored Cowpea Grains

		1.25mL Concentration		1.75 mL Concentration			2.25 mL Concentration		
Serial No.	Compounds	Retention Time (RT)	Concentrations mL/g	Compounds	Retention Time (RT)	Concentrations mL/g	Compounds	Retention Time (RT)	Concentrations mL/g
1	Alpha-Pinene	6.675	144.98	Alpha-Pinene	6.681	346.79	Alpha-Pinene	6.680	8.85
2	(+)-4-Carene	8.111	74.87	(+)-4-Carene	8.231	29.10	(+)-4-Carene	0.000	ND
3	Gamma-Terpinene	9.050	8.94	Gamma-Terpinene	8.975	90.45	Gamma-Terpinene	9.095	10.30
4	Linalool	9.862	0.54	Linalool	9.742	0.54	Linalool	9.564	0.51
5	Copaene	13.856	0.59	Copaene	13.747	3.18	Copaene	13.747	2.34
6	1H-cycloprop[e]	14.245	4.67	1H-cycloprop[e]	14.222	4.69	1H-cycloprop[e]	14.251	1.55
7	Caryophyllene	14.325	2.39	Caryophyllene	14.337	5.49	Caryophyllene	14.342	2.50
8	Humulene	14.766	1.40	Humulene	14.772	2.58	Humulene	14.771	1.85
9	Ledol	17.072	3.71	Ledol	16.671	3.71	Ledol	16.688	3.64
10	di-epi-1,10-cubenol	17.198	15.85	di-epi-1,10-cubenol	16.883	13.04	di-epi-1,10-cubenol	16.894	15.38
11	Tau-muurolol	17.009	0.22	Tau-muurolol	17.009	0.72	Tau-muurolol	17.020	0.44
12	Alpha-cadinol	17.209	0.81	Alpha-cadinol	17.226	0.77	Alpha-cadinol	17.255	0.35

ND-Not Detectable

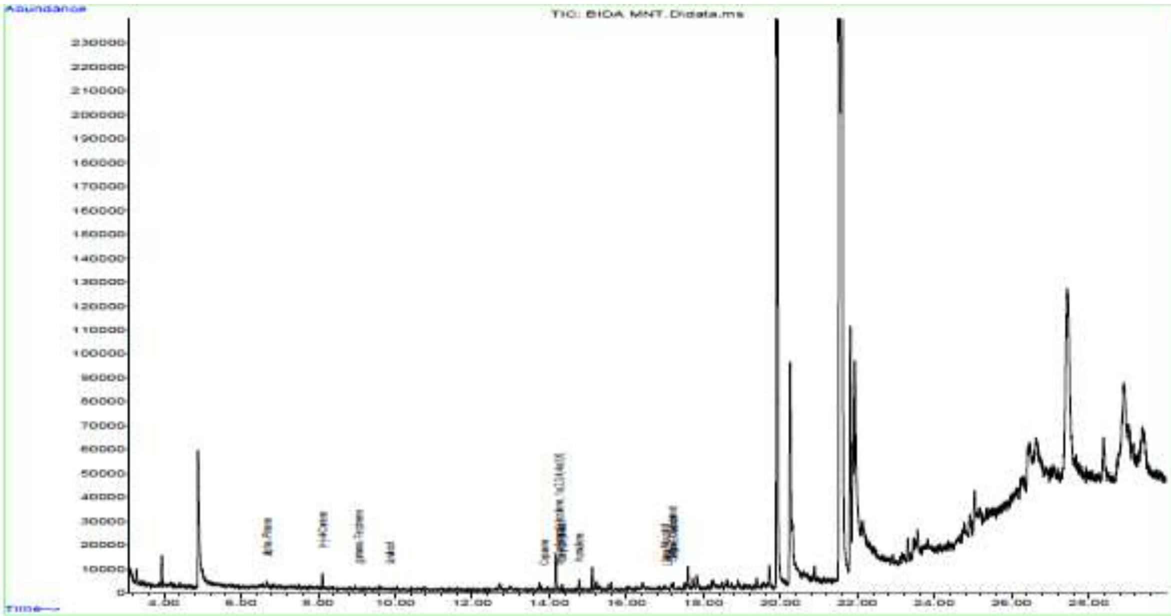

Fig. 2: Determination of Mexican mint essential oil residue in stored cowpea grains at 1.25mL/g concentration using GC-MS

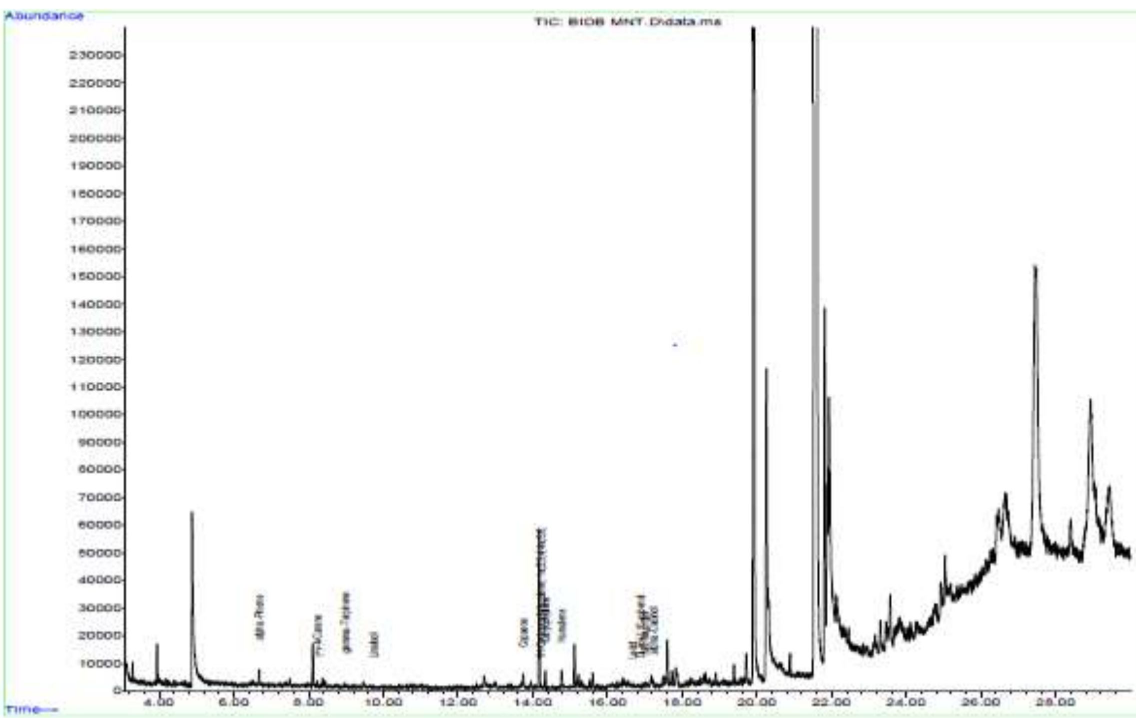

Fig. 3: Determination of Mexican mint essential oil residue in stored cowpea grains at 1.75mL/g concentration using GC-MS

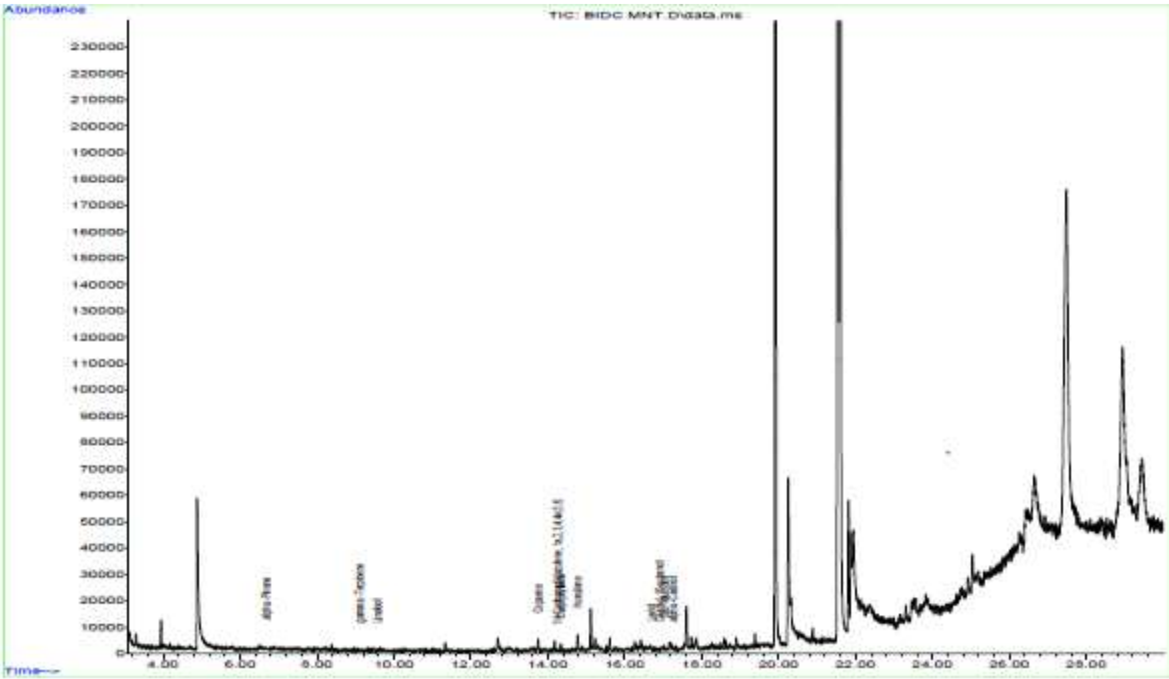

Fig. 4: Determination of Mexican mint essential oil residue in stored cowpea grains at 2.25 mL/g concentration using GC-MS

Toxic Effects of Mexican mint Essential Oil on Nutritional Compositions (Proximate Analysis) of Cowpea Grains

Results of Crude Protein revealed significant differences with the highest value (29.75%) from 0.75 mL/g Mexican mint essential oil, while 2.25 mL/g and control treatments recorded the lowest values of 22.05 and 22.75%, respectively (Table 4). Cowpea grains from all Mexican mint Essential Oil in different concentrations, phostoxin and control treatments revealed no significant effects on Ether, Crude Fibre and Ash (Table 4). Cowpea grains treated with 2.25mL/g Mexican mint essential oil had a significant highest value of 91.00% Dry matter content, while grains treated with 1.25mL/g recorded the lowest value of 88.86% with no significant differences ($p>0.05$) from Mexican mint essential oil of 0.75mL/g (89.25%) and 1.75 mL/g (89.60%), respectively.

3.4Moisture content revealed the maximum value of 11.14% from cowpea grains treated with 1.25 m3 L/g with no statistical differences ($p>0.05$) from Mexican mint essential oil of 0.75 mL/g (10.75%) and 1.75 mL/g (10.40%), respectively. However, the lowest value 9.00% was from 2.25mL/g treated cowpea grains (Table 4). Mexican mint essential oil at 1.25mL/g concentration gave the highest value 54.56% of Carbohydrate with no significant differences ($p>0.05$) from those treated with 2.25 mL/g (54.25%) and 1.75mL/g (54.16%), respectively. The minimum value 44.50% however, was obtained form 0.75mL/g concentration (Table 4).

Table 4: Results of Mexican mint Essential Oil on Proximate Analysis of Cowpea Grains

Treatments mL/g	%Crude Protein	%Ether (Fat)	%Crude Fiber	%Ash	% Dry Matter	%Moisture Content	%Carbohydrate
0.75	29.75a	5.50a	4.50a	5.00a	89.25c	10.75a	44.50d
1.25	20.30d	4.90a	4.60a	4.50a	88.86c	11.14a	54.56a
1.75	21.00d	5.10a	4.90a	4.44a	89.60bc	10.40ab	54.16a
2.25	22.05c	5.10a	4.80a	4.80a	91.00a	9.00c	54.25a
Phostoxin(0.01/20g)	25.91b	4.80a	4.10a	4.30a	90.10b	9.90b	51.32c
Control (Untreated	22.75c	5.30a	4.40a	4.90a	90.19b	9.81b	52.84b

Means followed by the same letter (s) within a column are not significantly different at ($p<0.05$) from each other using Duncan Multiple Range Test

DISCUSSION

Phytochemical screening of *Plecthranthus amboinicus* essential oil revealed that its primary compounds were monoterpenes, alpha pinene, gamma terpinene and cubenol which is in accordance with Bett *et al*. (2016); Zhang *et al*. (2006). Monoterpenes have been described to possess strong insecticidal toxicity against various insect species especially those attacking stored products (Ajayi *et al*., 2014; Bett *et al*., 2016). The interest in and utilisation of botanical insecticides, particularly essential oils have become increasingly relevant in the control of insect pests in storage (Regnault-Roger *et al*., 2014). In this study, the essential oil of *P. amboinicus* was observed to have a better performance in toxicity resulting to highly significant value (almost 100%) on *Callosobruchus maculatus* at a shorter period. However, insecticidal activities varied with essential oil concentrations and exposure times.

Several previous studies have demonstrated the distinct susceptibility of stored-product beetle species such as *Callosobruchus maculatus* to essential oils. Callosobruchus species were found to be more susceptible to essential oils or their components than other insect species (Tripathi *et al*., 2003; Lee *et al*., 2004). Chaubey (2017) reported contact toxicity of gamma terpinene against both *Tribolium castaneum* and *Sitophilus oryzae*. Therefore, the insecticidal activity of essential oil from *P. amboinicus* may be related to these components. Also, this essential oil could be used as a postharvest botanical insecticide since it contains monoterpenoids and sequiterpenoids similar to conventional insecticides. In this study *P. amboinicus* essential oil showed effectiveness as an alternative control method for *C. maculatus* because it displayed insecticidal activity with 100% adult mortality after treatment for 24 hours by contact toxicity. The results indicated that essential oil of *P. amboinicus* could be a potential toxic agent against *C. maculatus* and reduce the environmental risk associated with the use of synthetic insecticides.

Results obtained in the analysis of *P. amboinicus* essential oil in cowpea grains residue showed biodegradability of the bio-insecticide with no trace in the treated cowpea grains. This is in agreement with Fayinminnu *et al*. (2013) who reported that botanicals are biodegradable and less harmful to humans. Although reports have shown hepatotoxic properties of some monoterpenes and sesquiterpenes (plant secondary metabolites) (Seif, 2016). The major components of essential oils found in the cowpea grains residue in this study were used in folk medicines, pharmaceutical industries and cosmetics (Seif, 2016). Most of the terpenes identified in cowpea grains residue had been demonstrated to easily enter the human body through ingestion, penetration through the skin, or inhalation and thereby present in measurable levels in the blood without any known metabolism and toxic effects in human liver (Bartikova *et al*., 2014).

The proximate composition of cowpea samples in this study showed that crude protein value was higher than the values reported by Ilesanmi and Gungula (2010; 2016), which might be due to the effective control of the essential oil on cowpea grains against *Callosobruchus maculatus* and agreement with the work of Gayan *et al*. (2006). However, cowpea protein was similar to the value reported by Fayinminnu and Adesiyan (2013), Onwulari and Obu (2002) gave an average crude cowpea protein value of 20.50 and 31.70% for some commercially cowpea grains. High moisture content (9.00-11.14%) obtained in this study is in agreement with Ilesanmi and Gungula (2010; 2016) and similar to the study of Fayinminnu and Adesiyan (2013). This could be as a result of differences in the relative humidity of the experimental environment during the treated cowpea grains storage period. The treated cowpea samples however, gave the most acceptable safe moisture level (10-12%) for grain storage as reported by Anderson and Alcock (2013). Also, this level is less than the critical (14%) moisture value for cereals, which might be owing to the removal of water, with absorption of *P. amboinicus* essential oils into the cowpea grains. The oil might have formed moisture barrier for the cowpea grains during storage even when the relative humidity was high.

The obtained values of ether (fat), crude fiber and ash in this study were similar and not statistically differed, respectively but were in agreement with the research of Adekola and Oluleye (2007) and Fayinminnu and Adesiyan (2013). Higher values of dry matter content attained is an agreement to the earlier observation of Fayinminnu and Adesiyan (2013) which was a revealing of comparatively more resilience (durability) in storage as a result of low moisture. The high levels of carbohydrate contents were within some of treated cowpea in storage with neem-moringa seed oils from the work of Ilesanmi and Gungula (2016) and Fayinminnu and Adesiyan (2013). This result might be due to the cowpea carbohydrate contents been bulky within the endosperm of the grains and also the levels of *P. amboinicus* essential oil preserving them against infestation whereby the carbohydrate contents were not affected (Ilesanmi and Gungula, 2016; Ojimelukwe *et al*.,1999).

5. CONCLUSION AND RECOMMENDATION

This study revealed that *Plecthranthus. amboinicus* essential oil contained monoterpenes, alpha pinene, gamma terpinene and cubenol that possessed strong insecticidal toxicity against *Callosobruchus maculatus*. Concentrations of *P. amboinicus* essential oil at 0.75, 1.25, 1.75 and 2.25 mL/g, respectively gave 100% mortality on *Callosobruchus maculatus* within 12-24 hrs after application being similarly effective as the synthetic insecticide Phostoxin. There was no trace of *P. amboinicus* essential oil residue in treated cowpea grains, which showed biodegradability of the oil. Also, there was no hazardous effects on the nutritive components of treated cowpea grains.

It is therefore recommended that Mexican mint essential oil concentrations at 1.25, 1.75 and 2.25 mL/g should be used as a bio-insecticide on cowpea grains in storage due to 100% mortality rate on *Callosobruchus maculatus. Plecthranthus. amboinicus* (Mexican mint) is accessible, readily available, inexpensive, safe to handler, ecofriendly and with no toxic residue in cowpea grains. Hence a good natural cowpea storage bio-insecticide product for farmers to have quality nutrition and food safety.

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