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Toxicity of Two Fixed Plant Oils by Using a New Fumigant Method Against *Trogoderma granarium Everts* and *Stegopium paniceum* (L.)

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ABSTRACT

The present research was achieved to study the toxicity of fixed oils of clove buds (*Syzygium aromaticum)* and spearmint leaves(*Mentha spicata*) against larvae and adults of Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and the adults of the drugstore beetle, *Stegobium paniceum* (L.) by using a new fumigant technique. Germination test was conducted in order to assess the effect of tested oils at the highest concentration on wheat seeds after 24 hrs. A GC/MS analysis was performed to record the main chemical constituents in tested oils. Analysis of the toxicity data showed that, clove fixed oil exhibited the strongest fumigant toxicity against all tested insects. *T.granarium* larvae showed a very high resistance against both oils comparing with adults. Germination test showed no adverse effect of the tested oils on wheat seeds germination comparing with control. The GC chromatogram of clove buds fixed oil showed that, the main constituents were eugenol (37.43%) eugenol acetate (11.47%), caryophellene (10.44 %), linoleic acid (9.42%) and caryophellene oxide (8.58%). The GC-MS chromatogram of spearmint fixed oil showed that, the main constituents were carvone (33.9%) and limonene (2.7%).
Keywords: fumigation, *Stegobium paniceum*, *Trogoderma granarium*

INTRODUCTION

Control of stored grain insect populations around the world primarily depends upon applications of organphosphorus, pyrethroides insecticides and the fumigants (i.e. Phosphine and Ecofume). These still the most effective treatments for stored food, feedstuffs and other agricultural commodities protection from insect infestation. Although effective, their repeated use for decades has led to outbreaks of other insect species and sometimes resulted in the development of resistance. It has had undesirable effects on non-target organisms, and fostered environmental and human health concerns (Champ and Dyte 1976; Subramanyam and Hagstrum 1995; White and Leesch 1995). These problems have highlighted the need for the development of selective insect-control alternatives with fumigant action. Essential oils are traditionally used through fumigant or contact action to protect grains against storage pests, a suitable method to preserve products stored in warehouses and on small farms (Shaaya *et al*., 1997). Fumigant toxicity tests conducted with essential oils of plants (mainly belonging to Apiaceae, Lamiaceae, Lauraceae and Myrtaceae) have largely focused on beetle pests such as *Tribolium castaneum, Rhyzopertha dominica*, *Sitophilus oryzae* and *Sitophilus zeamais* (Rajendran and Sriranjini 2008) but little or no attention has been paid towards Khapra beetle and drugstore beetle. This research was achieved to study the toxicity of fixed oils of clove buds and spearmint leaves against larvae and adult of Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) which known as a very resistible insect for most pesticides and plant extracts and the adult of the drugstore beetle, *Stegobium paniceum* (L.) using a new fumigant technique. A GC/MS analysis was performed to record the main chemical constituents in tested oils.

MATERIALS AND METHODS

1-Tested Insects:

For starting a culture of tested insects (F.), adults and larvae of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) reared on wheat seeds and adults of the drugstore beetle, *Stegobium paniceum* (L.) (Coleoptera: Anobiidae) reared on flour wheat in a glass jars (each of approximately 500 ml) and each jar was covered with muslin cloths and fixed with rubber bands.

To have an initial population of *insect* adults homogenous in age, about 500 adults were introduced into jars containing seeds for egg laying and then kept in an incubator at 30±2°c and 65±5 % R.H. After five days, all insects were removed from the media and the jars were kept again at controlled conditions.

2) Tested Plants:

To obtain the fixed oil, 500 gm of clove buds (*Syzygium aromaticum)* (Myrtales : Myrtaceae) and spearmint leaves (*Mentha spicata* var. Viridis) (Lamiales: Lamiaceae) were grounded separately in an electric mill into fine powder. The grounded plant material was soaked in petroleum ether solvent in a large flask for 2 days. The flask was shacked for one hour in a shaker and its content was filtered. The solvent was evaporated at 50°c under reduced pressure using a rotary evaporator as described by Su (1985). The fixed oil in the form of a crude gum was weighted and dissolved by the same solvent to get (w/v) stock solution.Different concentrations were prepared by diluting the stock solution in the solvent.

3-Fumigation bioassay:

To investigate the fumigant toxicity of fixed oils of clove and spearmint against adults and larvae of *T.granarium* and adults of *S. paniceum*, a laboratory experiment was carried out using an investigated technique. To start this experiment, a number of fumigant chambers which are wooden boxes, each of approximately 6000 cm^3 fig (1) were performed. A small electric device with 7 cm^2 disc was fixed in one of the box sides. A glass slab was used to cover the box from the upper side. Each of the electric devices connected to electricity from outside source. Different concentrations (%) were used for each oil. Each concentration was replicated 3 times. 10 individuals of tested larvae and adults were put into separate 150 ml glass jars then the jars were put into the fumigant chambers. 0.5 ml of each concentration was impregnated on to the disc. After evaporation of the solvent, the device was connected to electricity (220v) to help fumigation of oil. The duration of fumigation period used extend up to 24h. A control replicate with solvent only was used for comparing. (%) mortality was then computed at the end of the experiment.

Fig 1. glass jars introduced into the fumigant chamber of approximately 6000 cm^3 with fumigant electric device

5-Seed Germination Test:

Germination test was conducted in order to assess the effect of tested oils at the highest conc. on wheat seeds after 24 hrs. For this assay, 30 seed were randomly picked from each group and placed on moist whatman filter paper No. 1 inside disposable Petri dish of 9.0 cm diameters at the rate of 10 seeds per plate. The treatments were arranged in a completely randomized design with 3 replicates. 7 days later, the percentage germination was calculated thus (Ileke and Oni, 2011; Ojiako *et al*, 2013)

Germination(%)= No.of germinated seeds / No. of seeds planted *100

6) GC/MS Chromatogram:-

The chemical constituents of tested oils were identified by GC/MS (Gas chromatography-mass spectrometry) at central agriculture pesticides laboratory. electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu) coupled to a GCMS QP 5050A mass spectrometer (Shimadzu); fused silica capillary column (30 m x 2.5 mm; 0.25 µm film thickness), coated with DB-1 (J&W); column temperature 100°C (2 min) to 250°C at the rate of 3°C/min; carrier gas, helium at constant pressure of 90Kpa. Acquisition parameters full scan; scan range 40-350 amu.

Identification of the compounds: Compound identification was done by comparing the NIST and WILEY libraries and with the authentic spectra (Adams, 1995). Data of the peaks with those reported in literature, mass spectra of the peaks with literature data. Percentage composition was computed from GC peak areas on BP-I column without applying correction factors.

4) Statistical Analysis:-

Insect mortality data were subject to analysis of variance and differences using ANOVA test (a computer program costate). Mean values were adjusted by Duncan's Multiple Range test Duncan (1951) at 0.05% level of significance with Statistical software version 6.3.0.3.

The mortality (%) was probit analyzed using a computer program named ldp-line according to Finney (1971). From which the toxicity values (Lc_{50} and Lc_{90}) were estimated. Slope values of tested compounds were also estimated and toxicity index.

RESULTS AND DISCUSSION

Fumigant toxicity of clove and spearmint fixed oil against adults and larvae of *T.granarium* and adults of *S. paniceum* after 24 hrs of exposure were recorded in tables (1, 2 & 3).

Analysis of the toxicity data showed that, clove fixed oil exhibited the strongest fumigant toxicity against all tested insects. *T.granarium* larvae showed a very high resistance against both oils comparing with adults.

Table 1. The mortality percentage (mean ± SE) of clove and spearmint oil fumigants against *T.granarium* larvae after 24 hrs

clove		spearmint	
Concentration %	**(%)mortality (mean ± SE)**	**Concentrtion %**	**(%)mortality (mean ± SE)**
900	٨٠±٥.7	٩٠٠	30±5.7
800	63.3±8.7	٨٠٠	16.6±6.6
700	46.6±8.7	٧٠٠	٠±0
600	36.6±3.3	٦٠٠	٠±0
500	33.3±3.3		
400	23.3±3.3		
significance	***	significance	**
LSD 0.05	18.8	LSD 0.05	14.4

Data recorded in table (1) showed that, at the highest conc. *T.granarium* larvae was very resistible to spearmint oil than clove oil as it cause 80% mortality with clove oil while with spearmint oil, mortality was 30%.

Table 2. The mortality percentage (mean ± SE) of clove and spearmint oil fumigants against *T.granarium* adults after 24 hrs

clove		spearmint	
Concentration %	**(%)mortality (mean ± SE)**	**Concentration %**	**(%)mortality (mean ± SE)**
15	100±0	١٥	80±5.7
10	83.3±6.6	10	66.6±3.3
5	63.3±3.3	5	40±5.7
3	43.3±6.6	3	23.3±3.3
1	20±5.7	1	6.6±3.3
significance	***	significance	***
LSD 0.05	16.3	LSD 0.05	14

Data recorded in table (2) showed that, at all tested conc. clove oil was more toxic than spearmint oil with *T.granarium* adults. Mortality ranged from (20-100%) with clove oil and (6.6-80%) with spearmint oil.

Table 3. The mortality percentage (mean ± SE) of clove and spearmint oil fumigants against *S.paniceum* adults after 24 hrs

clove		spearmint	
Concentraion %	**(%)mortality (mean ± SE)**	**Concentration %**	**(%)mortality (mean ± SE)**
10	100±0	10	100±0
5	86.6±8.7	7.5	83.3±3.3
3	56.6±8.7	5	63.3±3.3
1	36.6±3.3	3	43.3±6.6
0.5	23.3±6.6	1	26.6±6.6
significance	***	significance	***
LSD 0.05	20.5	LSD 0.05	14.8

According to data recorded in table (3), high Significance mortality of *S.paniceum* adults was recorded with both tested oils ($p<0.05$). The highest conc. of both tested oils has the same toxic effect against adults as it causes 100% mortality. Mortality ranged from (23.3-100%) with clove oil and (26.6-100%) with spearmint oil

Essential oils recently have received much attention due to their multi-functions as antimicrobial, antifungal, antitumor and insecticidal agents (de Souza *et al*. 2005).Essential oils and especially their main compounds monoterpenoids, offer promising alternatives to classical fumigants (Peterson and Ems-Wilson 2003, Aslan *et al*. 2004). Essential oils are volatile and can act like fumigants offering the prospect for use in stored-product protection (Lee *et al*. 2001), contact insecticides (Tapondjou *et al*. 2002), antifeedent or repellent effects (Kim *et al*.2003, Park *et al*. 2003, García *et al*. 2005) and may also affect some biological parameters such as growth rate, life span and reproduction (Tunç *et al*. 2000, Kathuria and Kaushik 2005, Rahmat *et al*. 2006). During recent years, some plants have been receiving global attention and their secondary metabolites have been formulated as botanical pesticides for plant protection since they do not leave residues toxic to the environment, have lower toxicity to mammals and medicinal properties for human uses (Duke,1985) .The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Coats *et al*., 1991; Konstantopoulou *et al*., 1992; Regnault and Hamraoui, 1995; Ahn *et al*., 1998) due to their high volatility, they have fumigant action and gaseous action might be of importance for stored-product insects.

Many previous researchers tested the fumigant toxicity of plant oils against stored grain insects according to the traditional method described by Prates *et al*., (1998) in which, insect adults were put into separate glass jars. Tested oil at different conc. was applied to 5 cm diameter filter paper. The filter papers were attached to the underside surface of the screw caps of the glass jars. The jars were first covered with nylon mesh. The caps were then attached. However, in this research, the toxicity of plant oils was tested using a new fumigant Technique. This Technique was used in a previous research by Sameeh, *et al*., (2016) in which fumigant toxicity of fixed and volatile oils of clove, cinnamon and moringa were tested against adults of *S.oryzae* and *T.castaneum* and the results showed that, this method was promising and give a good mortality % against both tested insects after 24 hrs from exposure. Results of this research and earlier studies indicated that, some plant extracts and essential oils might be useful for managing coleopterous insects in enclosed spaces such as storage bins, glasshouses, or buildings because of their fumigant action.

This finding are in harmony with that of Mahfuz (2007) investigated the fumigant toxicity of five essential oils, cardamom, cinnamon, clove, eucalyptus an neem oils against the cowpea weevil, *C. maculatus* adults. The efficacy in respect of the toxicity followed in the order: clove > cinnamon > cardamom > neem > eucalyptus after 24 h after treatment, and clove > cinnamon > cardamom > eucalyptus > neem after 48 h after treatments. Ahmed (2010) tested the fumigant activity of 7 essential oils against the *C. maculatus* and *S. oryzae* and found that (%) mortality increased with increasing concentration of different oils and exposure period. Both species were found to be highly susceptible to cinnamon. *C. maculatus* was more sensitive than *S. oryzae* to the essential oils of clove and eucalyptus. Abo-El-Saad, *et al*., (2011) tested the fumigant toxicity of clove buds fixed oil against *Tribolium casteneum*,fumigation assay has also exhibited strong fumigant activity toward the adults of *T. castaneum*. at 100 µL oil/L air. This result was in agreement with that of Eliopoulos, *et al*., (2015) tested the fumigant activity of essential oil vapors distilled from *O. basilicum* L. and spearmint, *M. spicata* L. against *E. kuehniella* and *P. interpunctella*. Both oils were highly effective against adult moths, given that notable mortality (>80%) was recorded after exposure to low doses such as 2.5 µl/liter. Toxicity data indicated that larvae and pupae were the most tolerant stages in all cases. Valdeany, *et al*., (2016) evaluated the fumigant activity of essential oils of *O. basilicum* L., *C. aurantium* L., *M. spicata* L. and *C. pulegiodorus* Baill on adult *R. dominica* in stored maize. The essential oil of *O. basilicum* exhibited strong fumigant toxicity. The *C. aurantium* essential oil required higher concentrations than *O. basilicum*, *M. spicata* and *C. pulegiodorus* to kill insects.

2) Data Analysis

The probit statistics, estimate of LC_{50}, LC_{90} and the slope of regression lines of fixed oils of clove and spearmint against adults and larvae of *T.granarium* and adults of *S. paniceum* after 24 hrs from exposure is represented in Table (4) and fig. (2, 3&4)

Based on Lc_{50} values, it was found that clove oil was more toxic against all tested insects than spearmint oil. When probit regression lines of fixed oil against tested insects were calculated, they showed a linear relationship between mortality percentage and concentration.

Table 4. toxicity values of tested oils on tested insects

oil	*T.granarium* larvae			*T. granarium* adults			*S.paniceum* adults		
	Lc_{50}	Lc_{90}	Slope	Lc_{50}	Lc_{90}	Slope	Lc_{50}	Lc_{90}	Slope
Clove	655.4	1348.8	4±0.84	3.2	16.4	1.8±0.36	1.6	9.9	1.6±0.32
spearmint	-	-	-	6.4	23.8	2.3± 0.46	2.8	16	1.7±0.38

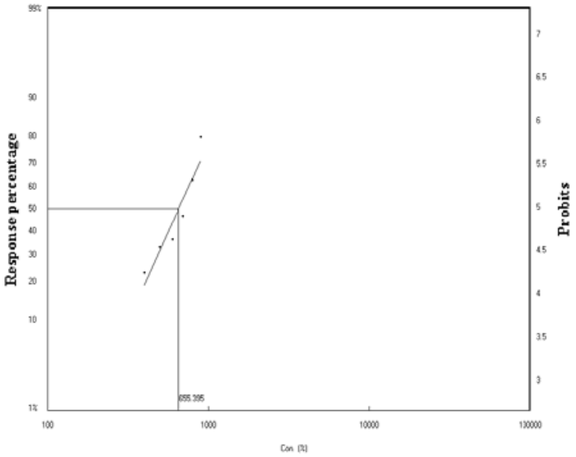

Fig. 2. Toxicity lines of clove fixed oil against *T.granarium* larvae

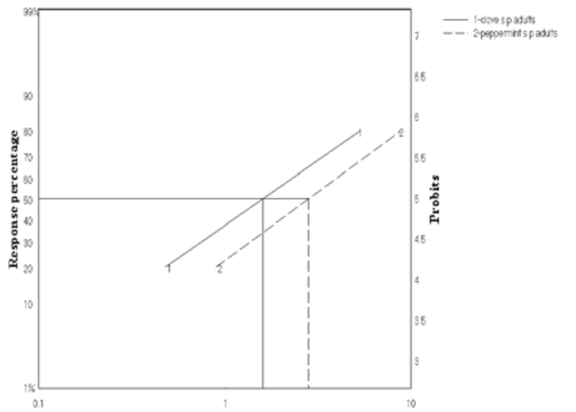

Fig. 3. Toxicity lines of clove and spearmint fixed oil against *S.paniceum* adults

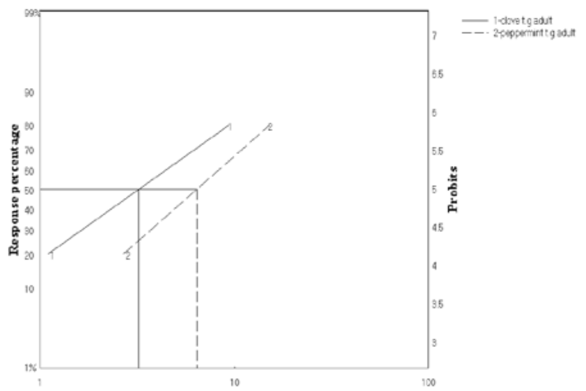

Fig. 4. Toxicity lines of clove and spearmint fixed oil against *T.granarium* adults

3-B) Seed Germination Test:-

Germination test showed that none of the tested oils at the highest conc. had any adverse effect on seeds germination after 24 hrs from exposure comparing with control. It has been reported that plant products generally do not affect the viability of seeds treated with them (Oparaeke and Dike, 1996; Akinkurolere *et al*., 2006). The present findings are almost in agreement with those of Islam (2001); Khaire *et al*., (1992), Gupta *et al*., (1988), where they reported that seeds treated with plant materials did not adversely affect the seed germination.

4) GC/MS Chromatogram Analysis:

According to previous paper, The GC chromatogram of clove buds fixed oil showed that, the main constituents were eugenol (37.43%) eugenol acetate (11.47%), caryophellene (10.44 %), linoleic acid (9.42%) and caryophellene oxide (8.58%). The GC-MS chromatogram of spearmint fixed oil showed that, the main constituents were carvone (33.9%) and limonene (2.7%).

The results in the line with Alma *et al*., (2007) analyzed the essential oils of clove buds by GC and GC-MS. The results showed that, the mainly contained about 87.00% eugenol, 8.01% eugenyl acetate and 3.56% β-Caryophyllene. Nazrul *et al*., (2010) recorded that the main components of clove were eugenol (74.3%), eucalyptol (5.8%), caryophellene (3.85%) and α cadinol (2.43%). Mejdi *et al*., (2015) identified chemical constituents in spearmint oil using GC/MS. The main components were carvone (40.8%) and limonene (20.8%). Jasim *et al*., (2007) reported that, the essential oil from *M. spicata* contains carvone (73.29 %), d-limonene (7.59 %) and dihydrocarvone (3.83 %) as major constituents out of the 21 components. Kaddem *et al*., (2009) revealed that GC-MS analysis of M. viridis was rich in carvone (50.47%), 1,8-cineole (9.14%), and limonene (4.87%). lamiri, *et al*., (2001) demonstrated that the insecticidal activity of an essential oil could be attributed either to the major compounds present in the oil or to the synergistic and/or antagonistic effects of all compounds of the oil. Jyotika and Thakur (2015) tested the fumigant toxicity of eight pure monoterpenes: citronellol, geraniol, linalool, eugenol, thymol, cinnamaldehyde, p-cymene and α-pinene against four stored product pests, *C. analis, S.oryzae, S. paniceum* and *T. casteneum* and found that, Eugenol and thymol showed potent fumigant toxicity causing high mortality for all the insect species. Among the four insect species tested, *C. analis* and *S.paniceum* were the most susceptible, having highest mortality of the species tested for each Compound.

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سمية اثنين من الزيوت الثابته باستخدام طريقة تبخير جديدة ضد حشرتى خنفساء الصعيد (الخبرة) وخنفساء العقاقير

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اجرى هذا البحث لاختبار سمية زيتى القرنفل والنعناع باستخدام طريقة حديثة للتبخير ضد حشرتى خنفساء الصعيد (الخبرة) وخنفساء العقاقير كما اجريت اختبارات تاثير الزيتين على انبات حبوب القمح وايضا تم تحليل المكونات الكيميائية لكلا الزيتين. وقد اثبتت النتائج مدى فعالية طريقة التبخير الجديدة ضد الحشرتين محل الدراسة حيث ان زيت القرنفل اثبت فعالية اكثر من زيت النعناع ضد الحشرتين المختبرتين. كما اظهرت يرقات خنفساء الصعيد مقاومة عالية جدا ضد الزيوت المستخدمة مقارنة بالحشرة البالغة. اثبتت النتائج انه لا يوجد اى تاثير سلبى على انبات الحبوب المعرضة لاعلى تركيز بعد ٢٤ ساعة من التعرض. اثبت تحليل GCMS ان المواد الاساسية فى زيت القرنفل هى eugenol (37.43%) eugenol acetate (11.47%), caryophellene (10.44 %), linoleic acid (9.42%) and caryophellene oxide (8.58%). والمواد الاساسية فى زيت النعناع هى carvone (33.9%) and limonene (2.7%).