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IDENTIFICATION OF CHEMICAL COMPOSITION OF *Eugenia aromatica* AND ITS BIO-EFFICACY AGAINST *Sitophilus oryzae* (L.) AND *Tribolium castaneum* (HERBST)

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ABSTRACT: In this investigation plant oil and powder of *E. aromatica* were evaluated under laboratory conditions to determine their toxicity, fumigant effect and impact on F1 progeny of *S. oryzae* and *T. castaneum* adults. Essential oil and powder of flower buds obtained from *Eugenia aromatica* were purified and analyzed by Gas-Chromatography–Mass Spectrometer (GC-MC). The results obtained showed that the two products of *E. aromatica* had good disorders on the all tested parameters. For toxicity and fumigant effect the oil was the premier compared to powder. In addition, that the two products completely prevented the F1 emergence of the two insects. In respect of the toxicity and fumigation the *T. castaneum* was more tolerant than *S. oryzae* adults. Phytochemical analysis showed that eugenol (89.62%) was the premier component of essential oil, while eugenol (78.66%), caryophyllene (6.30%), -a-Terpinyl acetate (1.35%) were the major components for its powder. These findings suggest application of *E. aromatica* products as suitable tools as a potential source of insecticides, alternative to synthetic insecticides or using these products in integrated pest management program against stored product insects, especially *S. oryzae* or *T. castaneum* adults.

Key words: *E. aromatica*, oil and powder, insecticidal activity, phytochemical, stored product pests, *T. castaneum* and *S. oryzae*.

INTRODUCTION

Many disorders of human health and environment often due to heavy application of chemical synthetic insecticides against the insect attack both in field or storage. The toxicologists and entomologists offered a lot of solutions to those problems. Suggestion of evaluation the plant products (oil, extract and powder) as stored product protectants was the best of them. In respect of, earlier studies were carried out to investigate and identification the components of some plants responsible for the detrimental impacting on biology of some stored product insects. Among the various storage insect pests which damage the stored products, *Sitophilus Spp.* and *Tribolium Spp.* (Lal *et al.*, 2017). One of the most important insects which cause 10-15% weight loss of the stored products is *T. castaneum* Duv. (Albushaba and Al Ameen, 2016). Insect damage may account for 10-40% of loss in stored grains, worldwide (Matthews, 1993) and 50% of some countries (Fornal *et al.*, 2007).

Therefore, biodegradable, nonresidual, equally effective and easily available botanicals may prove to be a better option to control insect pests including storage pests without affecting the quality of grains or seeds. (Lal *et al.*, 2017). Botanical insecticides have broad spectrum activity, they are safe and relatively specific in their mode of action, easy to produce and use. Many studies have demonstrated contact and fumigant toxicity of plant essential oil and their components against several species of stored product insects at different life stages (Papachristos & Stamopoulos, 2003; Pathipati, 2012 and Akinneye *et al.*, 2019).

E. aromatica (clove) is an unopened flower bud belonging to Family Myrtaceae and has numerous medicinal properties, such as anti-oxidant, anti-inflammatory, anti-microbial and antiseptic. The insecticidal activity of (clove) *E. aromatica* was demonstrated to insects of stored product by several authors, (Longe, 2011) evaluated fumigant effect of *E. aromatica* bud and Eucalyptus oil mixed

formulations against *Callosobrochus maculatus* and recorded 100% mortality in all tested parameters. Essential oil of *E. aromatica* was effective as contact biorational against *S. oryzae*, *S. zeamais*, *T. castaneum* and *Callosobrochus maculatus* (Olotuah, 2014). Additionally, ethanolic extract essential oil of *Eugenia aromatica* was effective for the control of *Ephestia caulella* on coca beans since they completely inhibited development of the storage pest from eggs to adult stage at all concentrations, (Akinneye *et al.*, 2019). Therefore, this study was undertaken to investigate contact and fumigant effect of *Eugenia aromatica* oil and powder against two of the most important of stored product pests *S. oryzae* and *T. castaneum*. Further phytochemical analysis was carried out by GC-MC to identify their major components.

MATERIALS AND METHOD

Laboratory experiments were carried out at the Department of Stored Product Pests Research, Plant Protection Research Institute, Sakha Agricultural Research Station Kafr-El-Sheikh, Egypt.

Culturing of insects

Adults of *S. oryzae* and *T. castaneum* were obtained from cultures that were regularly maintained in the laboratory and reared in laboratory-controlled chambers (incubator) at 27 ± 2 °C and $70 \pm 5\%$ relative humidity (R.H). The medium used for the insect culture were wheat grains for *S. oryzae* and crushed wheat for *T. castaneum*. Freshly emerged adults from the cultures were removed and then used for the bioassay in the next experiments.

Eugenia aromatica plant

Essential oil and flower buds of *E. aromatica* used in the current study were purchased from the local market. Clove (*E. aromatica*) flower buds were brought into the laboratory, washed thoroughly with water, after air dried then pulverized into fine powder using electric blinder. The fine powder was kept in airtight plastic container to avoid the absorption of moisture and then used in the next experiments.

Bioassay methods

Contact toxicity (mixing with medium)

Serious concentrations of *E. aromatica* oil were (1, 3, 5, 7 and 10 ml/kg grain and 1, 3, 5, 7 and 10 of powder/100g grain were determined according to preliminary laboratory assessment. The different concentrations of *E. aromatica* oil and powder mentioned above were transferred on plastic containers (11.5 by 6 cm diameter) containing samples of 20g wheat grains, and then mixed with the grains to ensure uniform coating of the grain with the

oil or powder. The jars which treated with oil were exposed to air for 30 minutes to allow the escape of the volatile solvent. Twenty adults of *S. oryzae* and *T. castaneum* (7-14 day old) were chosen randomly and transferred separately to treated samples in the plastic containers. On the other hand, the containers which contain untreated samples were served as control treatment. All treatments were replicated three times. Mortality counts were recorded after 1, 3, 5, 7 and 14 days, all results were corrected with formula by Abbott (1925).

The same experiment continued until the emergence to estimate the mean number of emerged adults, reduction of progeny. The LC50 and slope values were calculated by probity analysis (Finney, 1971).

Fumigation toxicity

a-oil

The fumigant effect of *E. aromatica* oil was determined according to the method described by Prates *et al.* (1998) with minor modification *E. aromatica* oil at rates of 3, 5, 10 and 15 ml/L were tested in 120ml glass jars each of them contains 10 adults of *S. oryzae* and *T. castaneum* (1-7) day old. Whatman No I filter paper were cut to 5cm diameter, impregnated with tested concentrations and attached to the under surface of glass jars screw caps. Another groups of filter papers without oil were used for control. Three replicates were run for each concentration and control. The mortality was counted after 24, 48 and 72 hours.

b- powder

Similarly, *E. aromatica* powder as fumigant was evaluated According to Ofuya *et al.* (2010) with minor modifications. 20 g of wheat grains were infested with ten unsexed adults (7-14 day old) *S. oryzae* and *T. castaneum* separately and then suspended in a piece of muslin cloth, over the powder in glass jars which contain serious of concentrations of 3, 5, 7, 10 and 15% g. The jars were covered with a lid properly screwed up to hold the muslin cloth and wheat grains in space and make the set up airtight. The jars without *E. aromatica* powders serve as control. All treatments plus control were replicated thrice. % mortality was recorded after 1, 2 and 3 days post treatment, and the results were corrected with Abbott's formula.

Phytochemical analysis

Instrument and method set-up

A TSQ triple quadrupole GC-MS/MS instrument coupled with a Thermo Scientific™ TRACETM 1300 GC (Thermo Scientific, Austin, TX, USA) was used. Sample introduction was performed a Thermo Scientific™ AS3000 autosampler, and

chromatographic separation using a Thermo Scientific™ TraceGold TG-5MS 30 m × 0.25 MM I.D. × 0.25 µm film capillary column. Additional details of instrument parameters are displayed below. The column temperature was initially held at 55 °C and then increased by 5 °C/ min to 260 °C with hold 2 min then increased to 300 with 15 °C/min. the injector temperature was kept at 250 °C., helium was used as a carrier gas at a constant flow rate of 1ml/min., the solvent delay was 4 min and diluted samples of 1µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50-650 in full scan mode. The ion source and transfer line were set at 200 °C, and 280 °C respectively. The components were identified by comparison of them.

Statistical analysis

All data subjected statistical analyzed with analysis of variance (ANOVA) using Duncan Multiple Range Test (Duncan, 1955) at 0.05 level of probability for comparison between means of the different treatments.

RESULTS

Laboratory experiments were carried out to evaluate the plant oil and powder of *E. aromatica* against two global insect species of stored grain and

their products *S. oryzae* and *T. castaneum* through mixing with medium method to determination of some criteria.

***E. aromatica* oil**

Effect on mortality

Results obtained in (Table 1) clearly showed that the percent of mortality gradually increased as the concentration and exposure periods increased. For example, the rate of one ml/kg grain achieved 16.7 & 10.0 % correct mortality and 100.0 & 90.0 % after one and 14 days for *S. oryzae* and *T. castaneum*, respectively. While, the rate of 7 ml/kg produced 86.7 & 63.3 and 100 & 93.3 % mortality at one and 3 days post treatment for both insect tested. In addition, whenever the rate increased the exposure periods needed to investigate 100 % mortality decreased. In general *S. oryzae* adults were more susceptible than the *T. castaneum* at the most of periods and concentrations. For pest management, in this study we can use the lower rates to protect the disinfested grain. But with the high infestation the present study suggests use the highest concentration (10 ml/kg) to curative the infested grains. In the case of middle infestation, the concentration of 5 ml/g grain in suitable to produce moderately control to the tested insect species.

Table 1. Mortality percentage of *S. oryzae* and *T. castaneum* adults affected by *E. aromatica* oil at indicated periods

Concen. (ml/kg)	Exposure period (in-days)										% reduction of progeny (F1)	
	1		3		5		7		14			
	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>
1	16.7	10.0	56.7	30.3	60.0	53.3	86.7	80.0	100	90.0	100b	95.5b
3	30.0	23.3	73.3	63.3	93.3	86.7	100	100	-	-	100b	100b
5	50.0	46.7	93.3	83.3	100	100	-	-	-	-	100b	100b
7	86.7	63.3	100	93.3	-	-	-	-	-	-	100b	100b
10	100	100	-	-	-	-	-	-	-	-	100b	100b
control											0.0a	0.0a

S.o: *S. oryzae*

T.c: *T. castaneum*

Toxicity effect

Results shown in Table (2) obviously revealed that the degree of toxicity gradually increased with increasing of exposure period for both insects under test. For example, the LC50 values decreased from 4.4

to 0.97 and from 3.38 to 0.78 ml/kg grain at one and five days post treatment with *T. castaneum* and *S. oryzae*, respectively. Also, results presented that the response of *S. oryzae* to *E. aromatica* oil was higher than that of *T. castaneum* at the all periods of exposure.

Table 2. LC50 values of *E. aromatica* oil against adults of *S. oryzae* and *T. castaneum*

Time in (days)	<i>T. castaneum</i>			<i>S. oryzae</i>		
	LC50	Slope Value	Confidence limits Upper - lower	LC50	Slope value	Confidence limits Upper - lower
1	4.40	2.69	5.5 - 3.52	3.38	2.84	4.22 - 2.70
3	1.83	2.25	2.14 - 1.51	0.82	2.84	1.005 - 0.62
5	0.97	2.71	1.16 - 0.75	0.78	1.32	1.21 - 0.78
7	-	-	-	-	-	-

Effect on progeny

Reduction of, progeny is one of the most criterion to evaluate the ability of a compound to deterrent the development of an insect. Results obtained in Table (1) markedly showed that the all concentrations tested of oil completely prevented the emergence of adults for the two tested insects. Hence the *E. aromatica* oil is considered suitable good alternative to synthetic insecticides to protect the stored grain and their products against the attack of stored product insects.

***E. aromatica* powder**

Similarly, the powder of *E. aromatica* had the same trend of its oil both belong to the % mortality or toxicity as well as the effect on the F1 spring. Results in Tables 1, 2, 3 and 4 clearly showed that the increase of concentration or exposure period had deterrent effect with the all tested aforementioned parameters.

Continually, *S. oryzae* adults were more susceptible than *T. castaneum* at the all tested concentrations along the experiment. The powder against both insects needed higher time or concentration to reach 100% mortality compared to oil.

Table 3. Mortality percentage of *E. aromatica* powder against *S. oryzae* and *T. castaneum* adults at indicated periods

Concen. (%)	Exposure period (in-days)										% reduction of progeny (F1)	
	1		3		5		7		14			
	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>	<i>S. o</i>	<i>T. c</i>
1	13.3	6.7	40.0	26.7	53.3	56.7	76.7	63.3	83.3	80.0	100b	92.0b
3	20.0	13.3	63.3	60.0	80.0	70.0	86.7	73.3	93.3	80.0	100b	100b
5	26.7	20.0	86.7	76.7	93.3	83.3	100	90.0	100	100	100b	100b
7	56.7	43.3	93.3	86.7	100	96.7	-	100	-	-	100b	100b
10	76.7	53.3	100	93.3	-	100	-	-	-	-	100b	100b
15	100	93.3	-	100	-	-	-	-	-	-	-	-
control											0.0 a	0.0 a

S.o: *S. oryzae*

T.c: *T. castaneum*

Table 4. LC50 values of *E. aromatica* powder against adults of *S. oryzae* and *T. castaneum* at indicated periods

Time in (days)	<i>T. castaneum</i>			<i>S. oryzae</i>		
	LC50	Slope value	Confidence limits Upper - lower	LC50	Slope value	Confidence limits Upper - lower
1	8.26	2.24	10.32 - 6.61	5.40	2.39	6.75 - 4.32
3	2.10	2.07	2.46 - 1.74	1.52	2.15	2.07 - 0.67
5	0.95	1.59	1.19 - 0.76	0.97	2.07	1.22 - 0.70
7	0.72	1.55	0.90 - 0.58	0.36	1.53	0.45 - 0.29
14	0.14	1.03	0.42 - 0.002	-	-	-

For example, the rate of 7ml/kg oils achieved 100% mortality for *S. oryzae* and *T. castaneum* after 3 days post treatment, while % powder of *E. aromatica* powder produced the same results for each insect at 7 days exposure period, respectively Table 1 & 3. With regard to that the concentration of 1% powder (10000 PPM) nearly equal 10 times more than the concentration of oil, 1ml/kg (1000 PPM). In addition, the effect of powder on F1 progeny had the same trend of oil in preventing the emergency of adults. Therefore, the oil was the strongest and the faster against the two tested insects.

Fumigant effect on mortality

a-oil

Results in (Table 5) showed that no one of the concentration used achieved 100% mortality through the three days of exposure except *S. oryzae* with 10 and 15 ml/l after three and two days, respectively. Also, results revealed that *T. castaneum* adults were more tolerant than *S. oryzae*. The percent of mortality of both insects increased with the increasing of concentration and the exposure period.

Table 5. Fumigant toxicity of *E. aromatica* oil and powder on adults of *S. oryzae* and *T. castaneum*

Concentration (ml/l)	Exposure period (in-days)					
	Oil					
	1		2		3	
	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>S. oryzae</i>	<i>T. castaneum</i>
3.0	10.0	0.0	26.7	6.7	43.3	13.3
5.0	20.0	10.0	63.3	36.7	80.0	43.3
7.0	43.3	20.0	70.0	43.3	83.3	63.3
10.0	76.7	30.0	93.3	50.0	100.0	80.0
15.0	93.3	53.3	100.0	76.7	-	86.7

Concentration % (w/w)	Powder					
	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>S. oryzae</i>	<i>T. castaneum</i>
3.0	6.7	0.0	23.3	0.0	36.7	6.7
5.0	10.0	3.3	43.3	23.3	63.3	40.0
7.0	20.0	10.0	60.0	30.0	76.7	60.0
10.0	66.7	20.0	80.0	50.0	100.0	73.3
15.0	93.3	56.7	100.0	73.3	-	86.7

b-powder

Results of *E. aromatica* powder (Table 5) had the same trend of its oil where the mortality gradually increased with the increasing of concentration and period of exposure. % mortality of *T. castaneum* was weak with the rates of 3, 5 and 7% compared to that of *S. oryzae*. In general, the influence of *E. aromatica* oil had the strongest effect on the two tested insects if compared with that of powder. For example, the rate of 7ml/l of oil performed % mortality ranged from 20-83.3% with the both insects. While the rate of 7% of powder resulted % mean mortality between 10-76.7%

with take account that rate of 7% powder nearly equal 10 times of 7 ml/l of oil.

Fumigant toxicity of oil and powder

When highlighting on the obtained results in Table (6), it is shown that the effect of the both powder and oil had the same trend where the action of them increases with the increasing of exposure period. Continually the impact of oil was the stronger according the concentration units used (ml/land % w/w). In addition, the response of *S. oryzae* to both materials was continuously higher than *T. castaneum*.

Table 6. LC50 values of fumigant toxicity of *E. aromatica* oil and powder against adults of *T. castaneum* and *S. oryzae*

Time in (days)	<i>T. castaneum</i>			<i>S. oryzae</i>		
	LC50 ml/l	Slope Value	C. L U- Lower	LC50 ml/l	Slope value	C. L U- Lower
1	7.16	4.19	6.66-11.47	14.2	3.01	12.46-16.92
2	4.42	3.86	4.00-8.52	8.52	2.71	6.33-13.23
3	3.31	3.82	2.65-4.14	5.93	3.23	5.40-6.47
powder						
1	8.58	4.88	6.86-10.23	14.61	4.02	13.15-16.87
2	5.40	3.50	4.93-5.86	9.76	3.40	8.94-10.80
3	3.95	3.45	3.47-4.36	6.56	3.50	6.03-7.12

Phytochemical analysis

Chemical analysis by GC-MS of *E. aromatica* was carried out and the results summarized in tables (7, 8) concluded that, *E. aromatica* contains nine and five components for essential oil and powder respectively. Essential oil mainly contains high Eugenol, (89.62%), 14-a-h-pregna (2.72%) and 17-pentatriacontene (2.25%) as well as minor components were 14-a-h-pregna (0.73) HAhnfett (0.55%), tetrapentacontane,

1, 54 dibromo. On the other hand the principle components of *E. aromatica* powder were phenol (Eugenol 78.66%), caryophyllene (6.30%), phenol, 2-methoxy – 4 -92- propenyl) -,acetate (11.62%) and trans -13- octadecenoic acid, methyl ester (1.06). Therefore, our results of chemical analysis, indicated that phenolic compound (Eugenol) was the major component of *E. aromatica* over (89.62 %) in oil and (78.66 %) in powder.

Table 7. Major components of *E. aromatica* essential oil

R.T	Area %	Name of compounds	Molecular formula	Molecular weight
15.02	89.62	Eugenol	C10 H12 O2	164
18.14	0,63	Docosane	C22 H46	380
24.03	2.72	14 - a - H – Pregna	C21 H36	288
24.17	2.60	Tetrapenta contane, 1,54- dibromo	C54 H108 BR2	914
26.51	2.25	17 – Pentatriacontene	C35 H70	490
26.92	0.73	14 - a - H – Pregna	C21 H36	288
27.89	0.55	HAhnfett	N/A	0.0
27.59	0.47	HAHNFETT	N/A	0.0
27.89	0.55	Tetrapenta contane, 1,54- dibromo	C54 H108 Br2	914

Table 8. Names of chemical components present in *E. aromatica* powder

R.T	Area %	Name of compounds	Molecular formula	Molecular weight
13.03	1.35	- a - Terpinyl acetate	C12 H20 O2	196
13.56	6.30	Caryophyllen	C15 H24	204
14.38	1.00	Humulene	C15 H24	204
14.82	78.66	Eugenol	C10 H12 O2	164
18.16	11.62	Phenol- 2- methoxy-4- (2- propenyl)-acetate	C12H14O3	206
24.14	1.06	Tran-13-Octadecenoic acid, methyl ester.	C19H36O2	296

DISCUSSION

Laboratory experiments were carried out to evaluate the insecticidal toxicity of oil and powder of *E. aromatica* plant against two global species of stored grain insect pests, *S. oryzae* and *T. castaneum* through studying toxicity, fumigant effect, offspring (F1 progeny). Results obviously showed that, oil and powder of *E. aromatica* have significant effect on all studied parameters with the two tested species. The contact toxicity of *E. aromatica* to adults of *S. oryzae* and *T. castaneum* depends on concentration, exposure period and insect species for example, the rate of 1ml/kg grain gave 16.7 & 10.0% mortality and 100 & 90% after one and 14 days for *S. oryzae* and *T. castaneum* respectively, while the rate of 7 ml/kg grain produced 86.7 & 63.3 and 100 & 93.3% mortality at one and 3 days post treatment for both tested insect, the effect of *E. aromatica* powder has the same trend. This report confirmed with **Park et al. (2003)** they concluded that, response of *C. chinensis* and *S. oryzae* exposed to direct contact of essential oil of *Chamaecyparis obtuse* varied according to insect species and application dose. Additionally, the contact toxicity of *E. aromatica* to adults of *S. oryzae* and *T. castaneum* depends on concentrations and exposure period, (**Akinneye et al 2019**). Results confirmed that, *S. oryzae* continually more susceptible than *T. castaneum* along the experiments as reported by **Olotuah (2014)**. The differences in the response by the different insect pest species could be attributed to the morphological and behavioral characters of each specie (**Tanpondju et al., 2002**), and might be due to their feeding habit also (**Olotuah, 2014**). The results markedly showed that all studied concentrations completely prevented F1 progeny for two tested insects, therefore *E. aromatica* oil is considered good alternative to synthetic insecticides to protect stored grain against the attack of stored product pests. In this regard, the oil extract of *E. aromatica* is used to control stored product Coleopteran and Lepidoptera because of their high efficacy on all developmental stages of insect (**Olotuah, 2014**), the present results were also supported by the finding of **Akinneye and Ogungbite (2016)** in which some botanical oils were found to prevent the hatching of the eggs as well as adult emergence. The efficacy of this botanical oil could be as a result of inability of the insect to feed through the oil coat which may in return leads to starvation. On the other hand, botanical oils may also have disrupted the normal respiratory activity of the insects and this may lead to asphyxiation and sudden death (**Akinneye and Ogungbite, 2016**). Additionally, secondary metabolites which are present in plant oils could be responsible for the inability of the adult insect to emerge as reported by **Mordue-Luntz and Nisbit (2000)** and **Yang et al. (2006)** that secondary metabolites in botanicals are found to disrupt growth and reduce larvae survival as well as disrupt life cycle of insect species. Concerning to *E. aromatica* powder

in the present study was also found to be completely preventing adult emergence at all concentrations used. These results agreed with **Akinneye et al. (2019)**, who reported that powder of *E. aromatica* was found to be completely inhibit eggs hatching and emergence of adult of *E. cautella* with all concentration used, and this effect may be due to that the *E. aromatica* powder inhibit gaseous exchange **Akinneye (2003)**. The action of the *E. aromatica* powder may be attributed to their storage odour in accordance with **Lale and Abdulrahman (1990)**, that mortality of storage insects may be associated with the pungent odour produced by *E. aromatica* powder. Chemical analysis of *E. aromatica* was carried out to identify its principle components. The toxicity of essential oils tested against stored product was attributed to the chemical components of the oils (**Koul et al., 2008 and Batish et al., 2008**). The chemical composition of plant oils or powder was considered the subject of many studies by several authors (**Zrira et al., 2003; Savan and Kucubay, 2013; Singh et al., 2008 and Akinneye et al., 2019**). Some bioactive compounds such as the terpenoids, monoterpenes, and other compounds have been reported to be present in the (clove) *E. aromatica* essential oil (**Bensky, 2004**). In addition, **Katunku et al. (2014)** reported that saponin that is found in *E. aromatica* affect the respiratory system of insects.

In the present study, GC-MS analysis of *E. aromatica* essential oil mainly contain, Eugenol, (89.62%), Ducosane (0.63%), 14-a-h-pregna (2.72%) 17-pentatria contend (2.25%) HAhnfett (0.55%), tetrapentacotane, 1, 54 dibromo. On the other hand, the principle components of *E. aromatica* powder were phenol (Eugenol 78.66%), caryophyllene (6.30%), a- Terpinyl acetate (1.35%), Humulene (1.00%), phenol, 2- methoxy – 4 -92- propenyl) - ,acetate (11.62%) and trans -13- octadecenoic acid, methyl ester (1.06). Therefore, our results of chemical analysis, indicated that phenolic compound (Eugenol) was the major component of *E. aromatica* over 89.62 and this findings are in agreement with **Alma (2007)** who concluded that the main components of essential oil of Turkey clove buds *E. aromatica* were Eugenol 87%, Eugenol–acetate 8%, B-caryophellene (3.56%). Chemical constituents of essential oil of *E. aromatica* were determined by GC-MS, Eugenol, caryophellene, humolene, 2- methoxy- 3- (2- propenyl), 2- methoxy- 4- (2-propenyl) and 2- methoxy -5- (1-propenyl) represent a main component, **Akinneye et al. (2019)**. In addition, the insecticidal potency of *E. aromatica* was suggested to be caused by Eugenol, caryophellene, humolene and other chemical compounds which have been proven to be insecticidal. This finding also agrees with the reports of **Liu et al. (2010)**, **Kim et al. (2003)** that caryophellene and its derivatives are widely distributed among plant oils and reportedly possess acaricidal, insecticidal and repellent properties. Plant essential oils have potential as products for control of

stored product pests because some of them are selective and have little or no harmful effects on –non – target organisms (Isman, 1999). *E. aromatica* is known to have sharp smell and mainly contain Eugenol over (87.62) and caryophellene (6.30%) and the action of *E. aromatica* on these beetles could be as a result of stomach poisoning through feeding on whole or fragmented grain. In general, the complexity of the chemical composition of most of the volatile oils gives them low specificity (Bakkali *et al.*, 2008), because biological activity is not assigned to a single mechanism of action, since the wide variety of chemical groups allows multiple targets in the cell (Burt, 2004).

The present study presented that oil or powder of *E. aromatica* had markedly fumigant effect on the tested insects. The potential use of essential oils as fumigants to control stored grain pests has been the subject of many studies (Shaaya *et al.*, 1991 & 1997; Bouda *et al.*, 2001 and Lee *et al.*, 2004). Its well-known however that ,the toxicity of essential oils to stored product insects is influenced by the chemical composition of the oil which in turn depends on several factors and experimental conditions such as, climatic conditions harvesting time, method of extractions, nutritional status, plant part used and analytical conditions (Savan and Kucukbay, 2013). The insecticidal mode of action of the plant oils may be largely attributed to fumigant action; they may be toxic by penetrating the insect body via the respiratory system. The volatile oil of many plants have fumigant and gaseous action due to high volatility, there for considered importance for control of stored product pests. Similar results reported by Kim *et al.* (2003) concluded that response of *S. oryzae* and *C. chinensis* to essential oil of cinnamon, garlic, and mustard oil using fumigation method, varied with insect species and time of exposure. Monoterpenoids are typically volatile and rather lipophilic compounds which can rapidly penetrate into insects and interfere with their physiological functions (Lee and Peterson, 2002). Regardless Ryan and Byrne (1988) suggested that the toxic effect of plants products may be attributed to a reversible competitive inhibition of acetylcholinesterase by occupation of the hydrophobic site of the enzyme active center. Additionally, Enan (2004) concluded that response of insects to essential oils and its constituents could be attributed to compound structure–activity relationship and physiological structure induced cellular changes resulting in poisoning of insects by blocking octopamin receptors.

CONCLUSION

The results obtained from this study suggest that both oil and powder of *E. aromatica* was effective for the controlling *S. oryzae* and *T. castaneum* since they able to prevent adult emergence with all concentrations used. On the other hand, the effect of

E. aromatica depends on the time of exposure, concentrations, and insect species. Continually, *S. oryzae* was more sensitive followed by *T. castaneum*. Furthermore, phytochemical analysis of *E. aromatica* reveals the major components responsible for insecticidal action, such as, eugenol, caryophellene, humulen, -a-Terpinyl acetate. According to the finding mentioned above it can be safely concluded that *E. aromatica* either powder or oil are potential bio-pesticide and have no risk in handling, in contrast to synthetic chemical which have many complications. In addition, the effects of crude volatile oils are not particularly dangerous to consumer since they are commonly used in many pharmaceutical preparations (Bauer *et al.*, 1990).

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