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Essential oils as ecofriendly fumigants against adults and internal larvae of three stored grain insects

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Abstract
The objective of this research was to evaluate the effectiveness of absolute essential oils (EOs) of Clove buds, *Syzygium aromaticum* (L.); Patchouli, *Pogostemon cablin* and Geranium, *Pelargonium graveolens* as fumigants for killing adults and internal larvae of three stored grain insects, *Sitophilus oryzae* (L.), *Rhizopertha dominica* (F) and *Callosobruchus maculatus* (F). Obtained results declared that the mortality % was increased with increasing of conc. and exposure period. For complete death, *S.oryzae* adults should be fumigated by conc. 10% for 2 days with clove EO, 70% for 2 days with patchouli EO and 50% for 3 days with geranium EO. Fumigation by conc. 40% for 1 day, 50% for 2 days and 25% for 2 days with clove, patchouli and geranium EO respectively were sufficient to cause 100% *Callosobruchus maculatus* adult mortality. For complete death of *Rhizopertha dominica,* adults should be fumigated by conc. 1% for 2 days with clove EO, 3% for 2 days with patchouli EO and 2% for 2 days with geranium EO. The fumigation with LC_{90} for tested EO was able to reduce the F1-progeny of *R.dominica* by 72.7, 70 and 75.7% with clove, patchouli and geranium respectively. While, the three EOs were ineffective against internal larvae of *C.maculatus*. Clove EO were the most effective against internal larvae of *S.oryzae* in which give 53% reduction in F1 progeny compared with geranium which cause 40% reduction while Patchouli EO was ineffective.

Keywords: Essential oils, fumigation, *Sitophilus oryzae, Rhizopertha dominica, Callosobruchus maculatus*

1. Introduction

The rice weevil, *Sitophilus oryzae,* lesser grain borer, *Rhizopertha dominica* and cowpea beetle, *Callosobruchus maculatus* are widespread and destructive insect pests of stored grains. Both adult and larvae are harmful stages for cereals [1] The nonstop and blanket use of chemical fumigants like methyl bromide, phosphine and Ecofume to control stored grains insect pests not only has led to develop resistant strains but also cause accumulation of toxic residues on food grains that became used for human consumption and which has led to many health problems. This awareness has developed an interest in the development of alternate strategies including botanical insecticides which may produce possible alternatives to the presently used chemical agents as they comprised rich source of bioactive compounds and breakdown quickly in the environment, resulting in little risk of residues on food crops [2] In 1996 the EPA established that certain ingredients that pose minimum risk to users no longer require EPA approval to be marketed as insecticides. A number of these ingredients are essential oils which work by disrupting an insect neurotransmitter that is not present in people, pets, or other vertebrates. Essential oils (EOs) are volatile, highly concentrated substances extracted from plant parts have a long history in medical and dietary uses [3] and are "generally recognized as safe" [4] even they show toxic effects against insects of stored product [5] as well as agricultural pests [6, 7]. They may act as fumigants [8], contact insecticides [9, 10], antifeedant [11, 12] or repellents [10, 13]. Essential oils known as any volatile oil(s) which have strong aromatic components and that give distinctive odour, flavor or scent to a plant. These are commonly referred to as volatile plant secondary metabolites [14]. Due to plant-insect interactions, the plants have well developed defense mechanisms against pests and are excellent sources of new insecticidal substances. Due to their low mammalian toxicity [15] they could be used as alternative sources for controlling a number of insect pests including stored product insects [16]. Plant essential oils and their constituents considered potential new fumigants and they may have advantages over conventional fumigants in terms of low mammalian toxicity and low environmental impact.

Past studies have only looked at the effect on insects that were freely exposed to essential oils fumigant but this article included the insect stages developing inside the grains.

The objective of this research was to evaluate the effectiveness of essential oils as fumigants for killing adults and internal larvae of three stored grain insects, *Sitophilus oryzae Rhizopertha dominica* and *Callosobruchus maculates.*

2. Material and Methods

2.1 Tested insects

Adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), lesser grain borer, *Rhyzopertha dominica* (F) (Coleoptera: Bostrichidae) reared on wheat seeds and cowpea beetle, *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae) reared on cowpea under constant condition 30±2°c and 65±5% R.H. at Plant Protection Research Institute, Mansoura branch. The emergence adults were subjected to further bioassay tests.

2.2 Tested plants essential oils

Absolute essential oils (EO) of Clove buds, *Syzygium* *aromaticum* (L.) (Myrtales: Myrtaceae); Patchouli, *Pogostemon cablin* (Lamiales: Lamiaceae) and Geranium, *Pelargonium graveolens* (Geraniales: Geraniaceae) were obtained from Sakkara essential oils co., Giza, Egypt.

2.3 Fumigation Bioassay against adult insects

To investigate the fumigant toxicity of tested volatile oils against adults of *S.oryzae* (2 weeks old), *R.dominica* (2 weeks old) and *C.maculatus* (1 day old), a laboratory experiment was carried out using an investigated technique. The experiment designed according to [17]**;** a number of fumigant chambers which are wooden boxes, each of approximately 6 L 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 adults were put into separate 150 ml plastic jars covered with fine pores muslin 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 72 h. A control replicate with solvent only was used for comparing. Replicates were investigated after and mortality % of adults was recorded after 24, 48 and 72 h. lethal concentration, LC_{90} was calculated for each oil.

Fig 1: Plastic jars covered with fine small bores muslin introduced into the fumigant chamber of approximately 6 L with fumigant electric device

2.4 Fumigation Bioassay against internal larvae

To investigate the fumigant toxicity of low level LC_{90} conc. of tested EOs against the internal larvae of the three tested insects, the same designed fumigation boxes as illustrated in previous experiment were used. To start this experiment, 50gm of grains were infested separately by 20 adult insects of *C. maculatus, R. dominica and S. oryzae* for 4,7and7 days respectively for egg laying then the adult removed and the replicates were incubated until the larval stage for each insect then the jars were introduced into the fumigant chambers in which LC_{90} conc. of each oil was used. 3 replicates for each insect were used. 0.5 ml of tested 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 periods extend to 72h then the replicates incubated until adult emergency to calculate reduction in F1 generation according to equation by [18] **:** Reduction (%) = $EC - ET / EC \times 100$, where: EC: mean number of emerged adult in control; ET: mean number of emerged adult in treatment.

2.5 Statistical analysis

Insect mortality data were subject to one-way analysis of variance using ANOVA test (a computer program costate). All obtained data were adjusted by Duncan's Multiple Range test [19] 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 [20] from which the toxicity values of tested EOs (LC_{50} and LC_{90}) were estimated. Slope values of tested compounds were also estimated and toxicity index.

3. Results and Discussion

3.1 Fumigation Bioassay against adult insects

Table 1: mortality percentage (mean ± SE) of tested essential oils against *Sitophilus oryzae* after 3 periodic times

Conc.%	Clove			Patchouli			Geranium		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
5	26.6 ± 3.3^a	83.3 ± 3.3^a	100 ± 0^a						
10	36.6 ± 3.3^a	100 ± 0^b	100 ± 0^a	20 ± 5.8^a	56.6 ± 6.6^a	93.3 ± 3.3^a	3.3 ± 3.3^a	13.3 ± 3.3^a	60 ± 5.8^a
15	53.3 ± 3.3^b	100 ± 0^b	100 ± 0^a						
20	70 ± 5.8^c	100 ± 0^b	100 ± 0^a				3.3 ± 3.3^a	20 ± 5.8^a	70 ± 10^a
30	83.3 ± 3.3^d	100 ± 0^b	100 ± 0^a	33.3 ± 3.3^a	86.6 ± 6.6^b	100 ± 0^b	3.3 ± 3.3^a	30 ± 5.8^{ab}	73.3 ± 3.3^{ab}
40	100 ± 0^e	100 ± 0^b	100 ± 0^a	56.6 ± 3.3^b	90 ± 5.8^c	100 ± 0^b	6.6 ± 3.3^a	40 ± 5.8^b	90 ± 10^{bc}
50				63.3 ± 6.6^b	93.3 ± 6.6^c	100 ± 0^b	13.3 ± 3.3^{ab}	56.6 ± 6.6^c	100 ± 0^c
60						100 ± 0^b	23.3 ± 3.3^{bc}	73.3 ± 6.6^d	100 ± 0^c
70				86.6 ± 3.3^c	100 ± 0^c	100 ± 0^b	33.3 ± 3.3^c	86.6 ± 3.3^d	100 ± 0^c
LSD 0.5	11	4.2		14	16.2	4.2	10	16.6	18

Column values followed by different letter(s) are significantly different at 0.05 levels

Table 2: The mortality percentage (mean ± SE) of tested essential oils against *Callosobruchus maculatus* after 3 periodic times

Conc.%	Clove			Patchouli			Geranium		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
5				23.3±3.3^{a}	50±5.8^{a}	83.3±6.6^{a}	20±5.8^{a}	60±5.8^{a}	86.6±3.3^{a}
10	23.3±3.3^{a}	26.6±3.3^{a}	56.6±5.8^{a}	36.6±6.6^{ab}	60±10^{ab}	96.6±3.3^{b}	40±5.8^{ab}	80±5.8^{b}	100±0^{b}
15	40±5.8^{b}	66±3.3^{b}	100±0^{b}						100±0^{b}
20	63.3±3.3^{c}	100±0^{c}	100±0^{b}	46.6±3.3$^{\mathbf{bc}}$	76.6±3.3$^{\mathbf{bc}}$	100±0$^{\mathbf{b}}$	53.3±8.8$^{\mathbf{b}}$	93.3±6.6$^{\mathbf{bc}}$	100±0$^{\mathbf{b}}$
25	80±10^{d}	100±0^{c}	100±0^{b}				63.3±8.8$^{\mathbf{bc}}$	100±0$^{\mathbf{c}}$	100±0$^{\mathbf{b}}$
30	93.3±3.3^{de}	100±0^{c}	100±0^{b}	60±5.8$^{\mathbf{c}}$	86.6±3.3$^{\mathbf{cd}}$	100±0$^{\mathbf{b}}$	80±5.8$^{\mathbf{c}}$	100±0$^{\mathbf{c}}$	100±0$^{\mathbf{b}}$
40	100±0^{e}	100±0^{c}	100±0^{b}						
50	100±0^{e}	100±0^{c}	100±0^{b}	83.3±3.3$^{\mathbf{d}}$	100±0$^{\mathbf{d}}$	100±0$^{\mathbf{b}}$			
LSD 0.5	14.8	5.4	6.6	14.8	17.6	10.5	22.5	14.8	4.7

Column values followed by different letter(s) are significantly different at 0.05 levels

Table 3: The mortality percentage (mean ± SE) of tested essential oils against *Rhizopertha dominica* after 3 periodic times

Conc.%	Clove			Patchouli			Geranium		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0.25	30±5.8^{a}	76.6±6.6^{a}	100±0^{a}				20±5.8^{a}	40±5.8^{a}	76.6±6.6^{a}
0.5	46.6±6.6^{ab}	93.3±6.6^{b}	100±0^{a}	26.6±3.3^{a}	53.3±3.3^{a}	86.6±6.6^{a}	33.3±3.3^{a}	56.6±6.6^{b}	93.3±6.6^{b}
1	60±5.8^{bc}	100±0^{b}	100±0^{a}	40±5.8^{ab}	66.6±6.6^{b}	93.3±6.6^{ab}	50±5.8^{b}	86.6±3.3^{c}	100±0^{b}
2	73.3±3.3^{cd}	100±0^{b}	100±0^{a}	53.3±3.3^{bc}	90±5.8^{c}	100±0^{b}	83.3±3.3^{c}	100±0^{c}	100±0^{b}
3	83.3±6.6^{d}	100±0^{b}	100±0^{a}	63.3±6.6^{cd}	100±0^{c}	100±0^{b}	93.3±3.3^{c}	100±0^{c}	100±0^{b}
4				73.3±6.6^{de}	100±0^{c}	100±0^{b}			
5				83.3±3.3^{e}	100±0^{c}	100±0^{b}			
LSD 0.5	18	13.3		15.7	11.8	11.8	14	13.3	13.3

Column values followed by different letter(s) are significantly different at 0.05 levels

Obtained results in tables 1, 2 and 3 declared that the mortality % was increased with increasing of conc. and exposure period. Adult's mortality % of *S. oryzae* fumigated with different conc. of clove, patchouli and geranium essential oil for 3 periodic times 24, 48 and 72 h were recorded in Table 1. For complete death, *S. oryzae* adults should be fumigated by conc. 10% for 2 days with clove EO, 70% for 2 days with patchouli EO and 50% for 3 days with geranium EO. Clove EO was the most effective fumigant followed by patchouli then geranium EO. High conc. of the 3 tested EO was significant toxic compared with low conc. Fumigant toxicity of different conc. of clove, patchouli and geranium essential oil for 3 periodic times 24, 48 and 72 h against *C.maculatus* adults were tabulated in Table 2. Results showed that, fumigation by conc. 40% for 1 day, 50% for 2 days and 25% for 2 days with clove, patchouli and geranium EO respectively were sufficient to cause 100% adult mortality. High conc. of the 3 tested EO was significant toxic compared with low conc. Adult mortality % of *R. dominica* exposed to different conc. of clove, patchouli and geranium essential oil fumigant for 3 periodic times 24, 48 and 72 h was recorded in Table 3. According to observed results, for complete death adults should be fumigated by conc. 1% for 2 days with clove EO, 3% for 2 days with patchouli EO and 2% for 2 days with geranium EO. High conc. of the 3 tested EO was significant toxic compared with low conc. According to obtained results, clove, patchouli and geranium essential oils could provide a save ecofriendly fumigant for stored grain insects if exposed for sufficient time. Previous researchers examined the fumigant toxicity of plant essential oils against stored grain insects according to the traditional method described by [21] using filter paper while during this research; the toxicity of plant oils was tested employing a new fumigant technique. This method was designed and tested in a previous research by [17] during 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 provides an honest death rate against both tested insects after 24 h from exposure also using the same technique [22] reported that clove and spearmint fixed oil exhibited strong fumigant toxicity against *S.paniceum* which cause 100% mortality at 10 % conc. after 24 h from exposure. Based on fumigation box technique utilized in the recent paper, [23] found that tested oils of patchouli, geranium, clove and lemon grass at high concentrations were highly significant toxic (p<0.05) with Stegobium paniceum and *Gibbium psylloides* adults. Applied combination between LC_{50} of geranium and patchouli essential oil with LC_{50} of ozone gas was additative and promising alternative against both tested insects in which give 100% mortality comparing with mortality % of applied LC_{50} of essential oil or ozone alone. Clove essential oil was effective fumigant against the three tested insects. The present results corroborate the findings of previous studies by [24] using the filter paper technique found that the essential oils of clove, Cinnamon, and the eugenol compound are the most promising to control *C. maculatus*, via fumigation. Also [25] using filter paper technique reported that, the toxicity of Clove and Dill oils against adults of *S. oryzae*, *R. dominica* and *T. castaneum* was much higher in the fumigant bioassay tests than in the contact method. Clove oil was more effective than dill oil against the three insect species. Geranium is an economic plant in Egypt that can resist insect infections. Geranium essential oil was effective fumigant against tested insects. A results obtained by [26] Was in harmony in recent results in which found that, based on filter paper technique the fumigant activity of geranium absolute oil was effective against *T. castaneum* and *R. dominica* than crude oil. The presence of volatile compounds having strong odour would have blocked the tracheal respiration of the insects leading to their death. Similar observation was made by [27] pointed out that the amount of fumigant absorbed depends on whether the insect's initial contact with the fumigant resulted in supplication or stimulation of the tracheal opening. The mode of action of oils was partially attributed to interference in normal respiration, resulting in suffocation [28]. Moreover, the ability of the insect to exclude vapour from its cuticle and prevent dehydration of body fluid plays a vital role in susceptibility or tolerance to fumigants of various life stages of insects particularly beetles and weevils infesting stored products [29]. The presence of volatile compounds is responsible for strong odour that could block the tracheal respiration of the insects leading to their death [30].

3.2 Data analysis

The probit statistics, estimate of LC_{50}, LC_{90} values and the slope of regression lines of tested volatile oils against adults of *S.oryzae* (2 weeks old), *R.dominica* (2 weeks old) and *C.maculatus* (1 day old), after 24h from exposure was represented in Tables (4,5,6) and Fig. (2,3,4). from probit analysis, it was found that clove EO cause the highest toxicity with R. dominica and S. oryzae while geranium EO cause the highest toxicity with *C. maculatus*. When probit regression lines of EOs against tested insects were calculated, they showed a linear relationship between mortality percentage and concentration.

Table 4: Relative potency values of EOs on C.maculatus

Line name	LC_{50}	index	RP	SLOP	LC_{90}	Lower limit	Upper limit
geranium	14.52	100	1	1.87	70.26	42.9	211
clove	16.15	89.9	1.11	3.93	34.16	26.7	60
patchouli	16.7	86.9	1.15	1.45	128.5	66.2	609

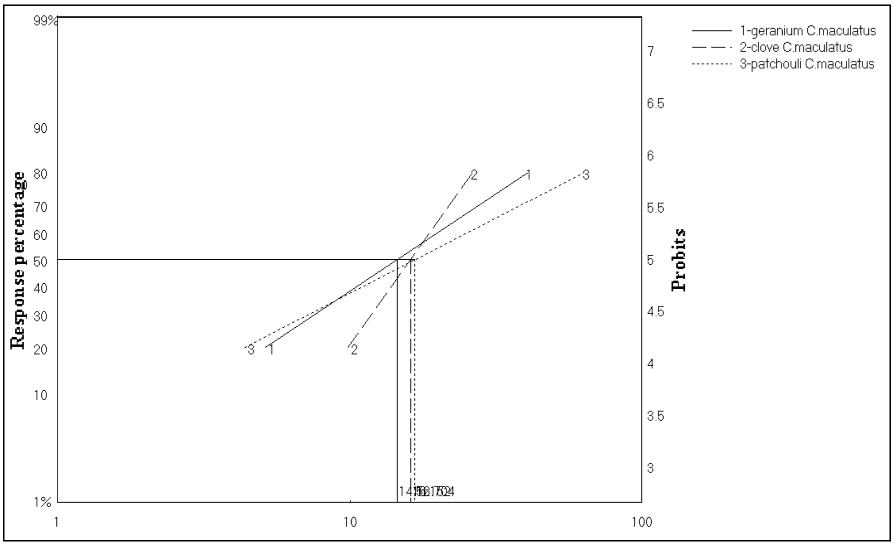

Fig 2: Toxicity lines of EOs on C.maculatus

Table 5: Relative potency values of EOs on R.dominca

Line name	LC_{50}	index	RP	SLOP	LC_{90}	Lower limit	Upper limit
clove	0.62	100	1	1.3	5.8	3	24.5
geranium	0.8	77.5	1.3	1.9	3.6	2.2	11.2
patchouli	1.5	40.8	2.4	1.4	12	6.8	38

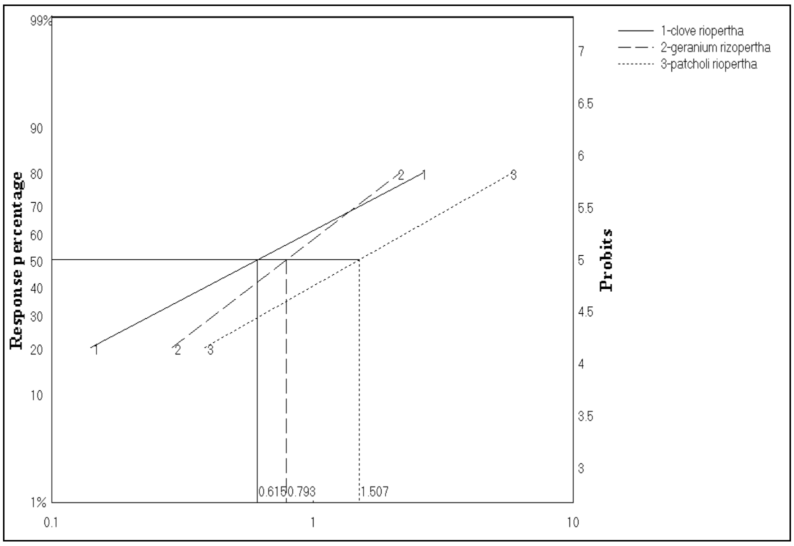

Fig 3: Toxicity lines of EOs on R.dominica

Table 6: Relative potency values of EOs on S.oryzae

Line name	LC_{50}	index	RP	SLOP	LC_{90}	Lower limit	Upper limit
clove	12	100	1	2	49.4	32.3	124.3
patchouli	32.2	37.2	2.7	2.1	128.8	85.4	305.5
geranium	40.8	29.3	3.4	3.4	95.8	75.8	148

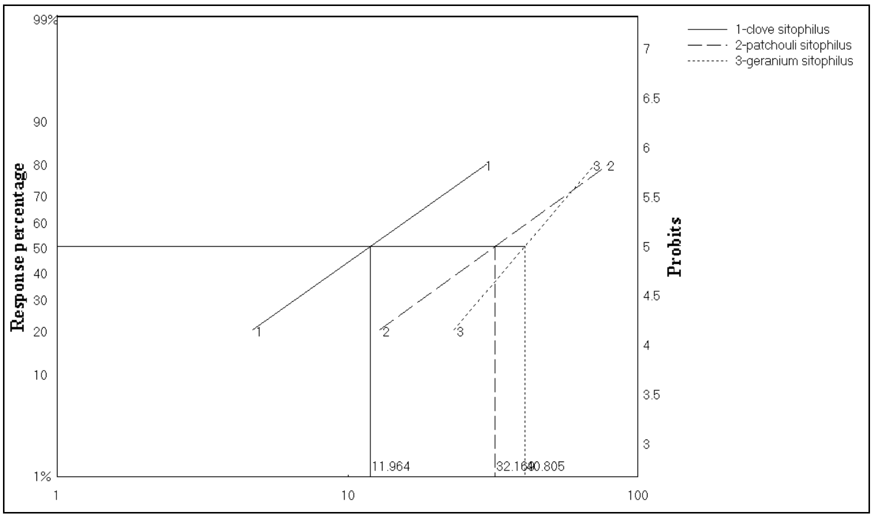

Fig 4: Toxicity lines of EOs on S.oryzae

3.3 Fumigation Bioassay against internal larvae

Table 7: Mean reduction % in F1 progeny after fumigated internal larvae of tested insects by lower limit LC_{90} essential oils for 72 h.

Essential oil	Lower limit LC_{90}	S. oryza	Lower limit LC_{90}	C. maculatus	Lower limit LC_{90}	R. dominica
clove	32.3%	53%	42.9	27.3%	3%	72.7%
patchouli	85.4%	0	26.7	0	6.8%	70%
Geranium	75.8%	40.5%	66.2	0	2.2%	75.7%

The mean reduction % in F1 progeny after fumigated internal larvae of all tested insects by lower limit LC_{90} essential oils for 72 h. of clove, patchouli and geranium were recorded in Table 7. According to obtained data, the fumigation with LC_{90} for tested EO was able to reduce the F1-progeny of *R. dominica* by 72.7, 70 and 75.7% with clove, patchouli and geranium respectively. While, the three EO were ineffective against internal larvae of *C. maculatus*. Clove EO were the most effective against internal larvae of *S. oryzae* in which give 53% reduction in F1 progeny compared with geranium which cause 40% reduction while Patchouli EO was ineffective.

No previous data was recorded about studying the fumigant toxicity of plant essential oils on insect stages developing inside the grains as past studies have only looked at the effect on insects stages that were freely exposed to essential oils fumigant like a study by [22] who recorded that *T.granarium* larvae showed a very high resistance against fixed oils fumigant of clove buds (*Syzygium aromaticum*) and spearmint leaves comparing with adults. Also [31] reported that, larvae of *T. castaneum* was less susceptible to the fumigant action of two synthetic volatile compounds, namely Propionic acid and Benzaldehyde and two essential oils namely, Ocimum basilicum and Mentha piperita and their different possible combinations compared with the adults of Callosobruchus maculatus and Tribolium castaneum.

4. Conclusion

Clove, patchouli and geranium essential oils could provide a save ecofriendly fumigant for stored grain insects if exposed for sufficient time. Essential oil fumigant is not efficient against internal stages of stored grain insects.

5. References

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