



Insecticidal Efficacy of Geranium Oil Nanoemulsion and Synergism with Sesame Oil and their Acetylcholinesterase Inhibition

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ABSTRACT

Nowadays, many studies have been carried out to develop new eco-friendly alternatives consequently, the essential oils (EOs) have acquired more interest due to their insecticidal activities, low harmfulness, and rapid degradation in the environment. Following this approach, herein we tried to improve the insecticidal activities of *Pelargonium graveolens* (G) through using the geranium oil in nano-emulsion (GN) form and in combination with sesame seed oil (SO). Different concentrations ranged from 0.313 to 10% were prepared from GN and the combination of G with S and tested for their larvicidal and pupicidal activities against *Musca domestica* and *Culex pipiens*, while their adulticidal activities were tested against red flour beetle *Tribolium castaneum*. The LC₅₀ of G alone was 4.29% against house fly larvae. The values of LC₅₀ decreased to 1.50% for GN and 0.32% for the combination of G+S. GO and GN form induced (pupal toxicity) 100% inhibition rate (PIR) in the treated housefly pupae at the concentration of 10%. Meanwhile, GO+SO combination showed 100% PIR at the concentration of 2.5%. Furthermore, the LC₅₀ for GO, GN and GO+SO was 0.22, 0.19 and 0.079 % respectively against larvae of *C. pipiens*, while it was 1.04 % for sesame oil. Regarding the contact and fumigant assays against adult *T. castaneum*, the combination of G and S achieved the best results when compared with GO alone or GN form. All treatments exhibited inhibition in the activity of acetylcholinesterase enzyme in the house fly larvae 24h post application. In addition, all the treatments induced significant increase in the malondialdehyde activity. In conclusion, nanoemulsion tech and mixing GO+SO increased their insecticidal potency against house fly, *C. pipiens* and *T. castaneum*. This is a useful method in the integrated pest management.

Article Information

Received 18 April 2022

Revised 20 February 2023

Accepted 26 March 2023

Available online 19 June 2023

(early access)

Published 13 July 2024

Authors' Contribution

Conceptualization: SMA, A-ASA-B.
Investigation: KMH, AAK.
Methodology: AAK, SMI, KMH.
Validation: AOH, SA-Q, SMA.
Visualization: AAK, SMI, KMH,
AOH. Roles/Writing original draft:
AAK, SMI, KMH. Writing review and
editing: A-ASA-B, SMA.

Key words

Geranium oil, Sesame oil,
Nanoemulsion, Synergism,
Acetylcholinesterase

INTRODUCTION

Insects represent the principal percentage of the world's organisms; they have over one million species and are

still continuing as pests and vectors (Gross, 2006). The housefly *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae) is an important mechanical vector of many diseases for domestic animals and humans (Sasaki *et al.*, 2000; De Jesús *et al.*, 2004; Singh *et al.*, 2009; Sinthusiri and Soonwera, 2014). Also, mosquitoes (Diptera: Culicidae) are considered one of the most dangerous insects throughout the world as they transmit organisms that cause some of the fatal and debilitating diseases to both domestic animals and humans (Service, 2012). Of this disease malaria, West Nile fever, dengue fever, encephalitis, lymphatic filariasis, and yellow fever are able to induce millions of deaths each year (Bhatt *et al.*, 2013; Stanaway *et al.*, 2016; Lee *et al.*, 2017). Because there

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0030-9923/2024/0005-2067 \$ 9.00/0



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are no vaccines for these diseases, prevention strategies are necessary. As well as, stored grain pests are the most damaging insects. Their control is very difficult due to their small size, feeding behavior, and to destroy grain prior to harvest (Raghavendra *et al.*, 2017). *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a major pest of stored products. If no prophylactic measures are taken, infestation of the storage with this pest can prompt losing of stored grains completely within 6 months (Abouelatta *et al.*, 2020; Aboelhadid and Youssef, 2021).

Synthetic insecticides are the main choice to control these insect species, which has resulted in several serious problems, including the development of resistance (Khan *et al.*, 2013), toxic effects to non-specific organisms, and environmental pollution (Shaalán *et al.*, 2005). Consequently, extensive researches have been carried out to develop new eco-friendly alternatives (Carreño *et al.*, 2014). Essential oils (EOs) have acquired considerable interest as insect control agents because of their insecticidal effectiveness with low impacts and rapid degradation in the environment (Chintalchere *et al.*, 2020). These oils comprised mixtures of chemical compounds that cause toxicity in insects through a variety of mechanisms as enzymatic inhibition, membrane, and protein denaturation (Rey *et al.*, 2001; Cavalca *et al.*, 2010).

The main obstacle of field applying the plant EOs as insecticides is their chemical instability in the different environmental conditions, which may lead to rapid evaporation and destruction of active ingredients (Echeverría *et al.*, 2019). In addition, many plant oils have low water solubility which limits their application as insecticides in the field (Aboelhadid *et al.*, 2021). Therefore, several studies have been carried out to develop more effective formulations to avoid the problem associated with their field application as insect control agents (Sharifian *et al.*, 2011; Saranya *et al.*, 2012; Hashem *et al.*, 2018; Massoud *et al.*, 2018). Among the recommended preparations, nanoemulsions may help in solving these problems (Saranya *et al.*, 2012; Hashem *et al.*, 2018; Massoud *et al.*, 2018). Nanoemulsions are more stable and can increase the biological activity by decreasing droplet size (Donsi and Ferreri, 2016). Recently, nanocapsules and nanoemulsions containing cinnamon oil showed high efficacy against engorged female of *Rhipicephalus (Boophilus) microplus* (Dos Santos *et al.*, 2017; Lazcano *et al.*, 2019). *Pelargonium graveolens* L'Her (Geraniaceae) (Geranium) is a South African economic plant that contains essential oil (EO) known commercially as geranium oil (GO) (Bakkali *et al.*, 2008). Egypt is one of the common countries in the production of GO (Abd El-Wahab *et al.*, 2016) which can be extracted from leaves, flowers, and stalks by steam

or hydro distillation (Boukhris *et al.*, 2012). GO is non-toxic, non-irritant, and non-sensitizing with no side effects (Boukhatem *et al.*, 2013). Additionally, GO is known to have antifungal, antibacterial, anti-inflammatory, spasmolytic, and hypoglycemic properties (Lis-Balchin *et al.*, 1997; Maruyama *et al.*, 2006; Lalli *et al.*, 2010; Hassane *et al.*, 2011). Citronellol and geraniol (trans-geraniol) are the two main components found in GO and they are known to have insecticidal activities (Babu and Kaul, 2005; Bouzenna and Krichen, 2013). GO showed a weak toxic effect on *M. domestica* (Pavela, 2008; Saraiva *et al.*, 2020). Also, Norris *et al.* (2015) and Ríos *et al.* (2017) reported moderate toxicity for GO against *Aedes aegypti* and *Anopheles gambiae* only at higher concentrations.

Sesame (*Sesamum indicum*) is an ancient crop cultivated in tropical and sub-tropical regions of the world (Ram *et al.*, 1990). Several literatures documented the synergistic effect of the sesame oil (SO) and also confirmed that sesamin and sesamol were the SO main components. Therefore, several parts of the plant were tested for its insecticidal activities (Kato *et al.*, 1998; Begum *et al.*, 2000). SO showed synergist action when it was used with the botanical insecticides pyrethrin and rotenone against *M. domestica* (Eagleson, 1940). Kranthi (2005) postulated that synergistic effect augments the insecticide efficacy by inhibiting insecticide detoxifying enzymes.

The current investigation aimed to assess the improvement of the insecticidal activities of *P. graveolens* essential oil as nano-emulsion and in combination with SO against 3rd instar larvae and pupae of *M. domestica* and *Culex pipiens* larvae and one economically important adults of *T. castinum*.

MATERIALS AND METHODS

Preparation of geranium (GO) and sesame seed oils (SO)

The oils were purchased from Trust Scientific for Natural Products, Cairo, Egypt. Six concentrations (10, 5, 2.5, 1.25, 0.625 and 0.312%; volume/volume) were prepared for both oils by dissolving in 70 % ethanol. The binary mixtures from GO and SO were prepared at a rate of (1:1) for all concentrations. The GO and SO were analysed using GC-MS and TRACE GC Ultra Gas Chromatographs at the Nawah Scientific Educational Research Center in Egypt (<https://nawah-scientific.com/>) (THERMO Scientific Corp., USA).

Preparation of geranium oil nanoemulsion (GN)

The nanoemulsion was prepared according to Nirmala *et al.* (2020). Briefly, the oil/water macroemulsion was prepared by combination of GO with a surfactant (Tween 80) (one oil to two T80, 1:2V:V) then water to

final volume was added and mixed using a magnetic stirrer (speed of 500 rpm for 10 min) to obtain concentration of 10% of G. An ultrasonicator was used to sonicate the prepared macroemulsion for 10 min (750 W, Branson Probe sonicator-Advanced model, 20 kHz). At 340 nm, a UV-visible spectrophotometer (UV-2600, Shimadzu, Japan) was used to characterise the resulting nanoemulsion.

Rearing of house fly colony

Adult house flies were collected from animal farms in different villages of Beni-Suef province, Egypt. The collected house flies were transported to the Parasitology Lab at Faculty of Veterinary Medicine, Beni-Suef University. The flies were kept at temperature of 28 ± 2 °C and 60–70% relative humidity (RH) in plastic jars (35×15 cm), covered with muslin cloth for several generations. A cotton swab soaked in milk (10% w/v) was introduced as food to the adult flies and also served as a substratum for oviposition. The eggs were transported to another set of jars containing animal feed or cotton swab soaked in milk for hatching and development of larvae. Similarly, pupae were collected and kept in a separate container until they emerged as adults. The bioassays were carried out on larvae and pupae (Jesikha, 2014; Kamel *et al.*, 2019).

Rearing of Culex pipiens

Egg rafts of laboratory reared colony of *C. pipiens* were sieved into large plastic containers with water. The resulting larvae were then placed in enamel trays with 1 liter of dechlorinated water and 0.15 g of Brewer's yeast (lactalbumin) (50:50). Water was replaced every other day and food was added on a daily basis. Adults were kept in 0.51 m^3 aluminum screen cages and fed on 10% sucrose solution on cotton wicks. To blood-feed the insect female, restrained quail was used. A 400 ml plastic container was used to collect the deposited egg rafts. The colony was kept at 26 °C and a 75% RH with a 16 L: 8 D photoperiod. In the bioassays, the third and fourth larval instars were used (Sayed *et al.*, 2018).

Rearing adults of Tribolium castaneum

Adult *T. castaneum* insects were raised in a dark incubator at a temperature of 28°C and RH of 70–80%. The culture for insect rearing was wheat flour mixed with active yeast (10:1, w/w). The adult insect was employed in subsequent applications after a week (Aboelhadid and Youssef, 2021).

Larvicidal and pupicidal bioassays against Musca domestica

The larvicidal and pupicidal bioassays were conducted according to the methods of Busvine (1971) and Palacios

et al. (2009), respectively with few modifications. Briefly, 10 individuals of the 3rd instar larvae/ pupae in each test (5 replicates) were treated with different concentrations (10, 5, 2.5, 1.25, 0.625 and 0.312%) of the investigated materials (G, GN, S and G+S) to evaluate the toxicity and the values of LC_{50} and LC_{90} . For the residual film method, 1 mL of the various concentrations was uniformly applied over filter paper and kept inside a glass Petri dish with a diameter of 9 cm. The treated Petri-dishes were first air-dried for few minutes to allow the solvent to evaporate before being incubated at 28 °C and 75% RH for 24 h. Acetone was used as a negative control, while deltamethrin at 2 ml/L was used in the positive control group. The treated larvae were observed for mortality for 24 h.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100\%$$

In the pupal bioassay, the treated pupae were observed for adult emergence for 6 days. The adult emergence rate was determined according to the method of Kumar *et al.* (2011). Percentage inhibition rate (PIR) was calculated as follows:

$$\%PIR = C_n - T_n / C_n \times 100$$

where C_n is the number of newly emerged houseflies in the control group and T_n is the number of newly emerged houseflies in the treated group.

Larvicidal bioassay against Culex pipiens

The standard method of World Health Organization was used in this bioassay (WHO, 2005). This was done in plastic cups (250 mL). The EOs were dissolved in ethyl alcohol at the tested concentrations 0.078 to 10 % then the working solution prepared by aliquot one mL of these dilutions were added to 99 mL distilled water. Thirty *Culex pipiens* third-instar larvae were placed in the prepared concentrations in the plastic cups (5 replicates for each concentration). In the negative control, larvae were exposed to one mL of the solvent dissolved in the water, however deltamethrin at 3.5 µl/L was used as positive control group. The dead larvae (motionless) were recorded after 24 h, and the average percentage mortality was estimated according to Abbott's formula (Abbott, 1925).

Adulticidal bioassay against T. castaneum

Fumigant toxicity

The fumigant effect of the EO and the nanoemulsion against adults of *T. castaneum* were assessed according to Ko *et al.* (2009). Briefly, filter paper of 6 cm diameter soaked in the prepared dilutions (0.312, 0.625, 1.25, 2.5, 5, and 10%) of the EO and the nanoemulsion forms. This paper was connected to the undersurface of the screw cap of glass jars (170 cm^3). Ten adults of *T. castaneum* were transported to glass jars covered with their screw caps

that were attached with treated filter papers. Filter paper treated with acetone was used as negative control, while deltamethrin (1 ppm) was used in the positive control group (Arthur, 2019). Five replicates were done for each treatment and for each control. After 24 h exposure, the mortality was recorded.

Contact toxicity (Surface film bioassay)

This bioassay was done according to Broussalis *et al.* (1999). The tested compounds were prepared in acetone. To get a series of concentrations ranging from 0.312 to 10 %, one millilitre of each concentration was put to the bottom of a glass Petri dish (9 cm diameter). Before introducing the adults of *T. castaneum*, the Petri dishes were left open for 5 min to allow acetone to evaporate. Ten adults insect were transported to each Petri dish. Control group were treated with acetone, while deltamethrin (1 ppm) (Arthur, 2019) was used in the positive control group. Five replicates were used for each concentration and the control as well. The dead insects were counted after 24 h, and LC₅₀ values were calculated by probit analysis (Finney, 1971).

Inhibition of acetylcholinesterase (AChE) activity in treated house fly larvae

The treated house fly larvae were suspended in lysis buffer and homogenized with a glass homogenizer on ice. The resulted homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was taken and preserved to be used in the detection of AChE (Chintalchere *et al.*, 2020). The AChE activity in treated larvae was estimated by the modified technique of Ellman *et al.* (1961) and using kits of QuantiChrom™ Acetylcholinesterase Assay Kit (DACE-100) (www.bioassaysys.com). The larvae in the control group were treated with acetone. The AChE activity were detected using UV-spectrometer (at 412 nm). The inhibition of AChE in the treated insects was calculated according to Anderson and Coats (2012): AChE inhibition (%) = 100 - [(As / Ac) x 100], where: As = AChE activity for each concentration; Ac = Negative control.

Lipid peroxidation (Malondialdehyde assay) in treated house flies larvae

The lipid peroxidation (LPO) in the homogenate of house fly larvae was detected by the method of Bar-Or *et al.* (2001) and the reading of the test was done at 535 nm.

Statistical analysis

Three replicates were done for all the treatments and mean ± SE values were calculated. Larval mortality analysis was performed by using ANOVA and subsequent Duncan's multiple range tests (p < 0.05). Probit analysis

was applied to determine the LC₅₀ and LC₉₀ values with their 95% confidence limits (Finney, 1952). All statistical analyses were achieved using SPSS for Windows (version 22.0). The synergistic factor (SF) was calculated by dividing the LC₅₀ value of the individual test insecticide with the corresponding LC₅₀ value of the test insecticide + synergist mixture (Chou, 2006).

$$\text{Synergistic ratio} = \frac{\text{LC50 of insecticide alone}}{\text{LC50 of (insecticide + synergists)}}$$

If the synergistic ratio is < 1 it means antagonistic effect; >1 means synergistic effect; and 1 means no effect.

RESULTS

GC-MAS analysis of GO and SO

The analysis showed that the citronellol (14.44%) and geraniol (11.08%) were the most predominant constituents of the GO with other minor components (Table I), while linoleic acid (37.27 %), oleic acid (26.07 %) and palmitic acid (13.34 %) were the main constituents of the sesame oil (Table II).

Table I. GC-MAS of *Pelargonium graveolens*.

| RT | Compound name | Area% |
|-------|----------------------------------|-------|
| 3.30 | β-Pinene | 0.75 |
| 3.89 | Cis-Linalool oxide | 0.55 |
| 4.32 | Linalool | 7.74 |
| 4.72 | Rose oxide | 0.76 |
| 5.17 | Trans-p-Menthone | 0.33 |
| 5.41 | Isomethane | 4.35 |
| 5.73 | Citronellal | 0.44 |
| 5.85 | Isopulegol | 0.41 |
| 6.63 | Citronellol | 14.44 |
| 6.94 | β-Geraniol | 0.40 |
| 7.31 | Geraniol | 11.08 |
| 7.55 | Citronellyl formate; Formic acid | 7.66 |
| 8.01 | Geranyl formate | 3.91 |
| 8.69 | Geranyl acetal | 0.63 |
| 8.95 | Citronellyl acetate | 1.23 |
| 9.44 | Copaene | 1.05 |
| 9.63 | α-Bourbonene | 3.28 |
| 10.10 | γ-Gurjunene | 0.26 |
| 10.32 | Caryophyllene | 2.55 |
| 10.49 | β-Copaene-4α-ol | 0.36 |
| 10.75 | Aromadendrene | 1.73 |

Table continued on next page.....

| RT | Compound name | Area% |
|-------|-------------------------------|-------|
| 10.88 | ç-Muurolene | 0.96 |
| 10.99 | Humulene | 0.83 |
| 11.13 | Epi-β-Caryophyllene | 0.48 |
| 11.38 | Geranyl propionate | 2.01 |
| 11.56 | Germacrene D | 2.63 |
| 11.65 | Epi-β-Selinene | 0.30 |
| 11.85 | Elemene | 1.60 |
| 11.91 | Epicubebol | 0.53 |
| 12.04 | α-Farnesene | 0.30 |
| 12.19 | Naphthalene | 0.89 |
| 12.39 | σ-Cadinene | 3.27 |
| 12.64 | α-Gurjunene | 0.30 |
| 12.82 | γ-costol | 0.82 |
| 13.08 | Geranyl butyrate | 2.36 |
| 13.48 | Spathulenol | 1.13 |
| 13.60 | phenylethyl tiglate | 2.71 |
| 13.76 | (-)-Globulol | 0.29 |
| 13.91 | Neryl 2-methylbutyrate | 0.41 |
| 14.03 | Caryophyllene oxide | 0.30 |
| 14.34 | 10-epi-γ-eudesmol | 4.93 |
| 14.42 | Cubenol | 0.17 |
| 14.57 | Agarospirol | 0.40 |
| 14.72 | Guaiene | 0.69 |
| 14.89 | Elemol | 1.93 |
| 15.07 | Citronellyl tiglate | 0.79 |
| 15.40 | 2, 6-octadiene, 2, 6-dimethyl | 0.47 |
| 15.75 | Geranyl tiglate | 2.62 |
| 16.02 | Geranyl palmitate | 0.84 |
| 16.66 | Geranyl isobutyrate | 0.37 |
| 17.77 | Citronellol heptanoate | 0.23 |
| 18.36 | Geranyl heptanoate | 0.36 |
| 20.00 | Geranyl caprylate | 0.20 |
| | Total | 100 |

Characterization of GN

The UV-visible spectrophotometer (UV-2600, Shimadzu, Japan) measured the absorbance of GN at 340 nm. The zeta potential (-0.569), droplet size distribution (18.7 d, nm) (analysis by volume) and polydispersity index (PDI) (0.299 d. nm) of nanoemulsions were measured by a zeta sizer apparatus (dynamic light scattering technique) (Nano-ZS90, Malvern, UK). Zeta potential dimensions showed formation of GN (Supplementary Figs. 1, 2 and 3).

Toxicity of GO forms against larvae and pupae of *Musca domestica*

The GO showed no larvicidal effect against the 3rd instar larvae of *M. domestica* at concentrations of ≤ 0.312%. Meanwhile, at 10% concentration the larval mortality was 77.67% with LC₅₀ value (4.29%). However, the GN form revealed better larvicidal activity which is evidenced by the reduced value of the LC₅₀ (1.50%) (Tables III, IV, Fig. 1). Conversely the sesame oil (S) also showed no larvicidal effect against the 3rd instar larvae of *M. domestica*. Interestingly, the combination of GO+S showed significant larvicidal activity at the all tested concentrations with LC₅₀ attained at a concentration of 0.32%. The treated larvae died within 24 h with clear blackening of the cuticle. The synergistic factor was 49.13 (Tables III, IV, Fig. 1).

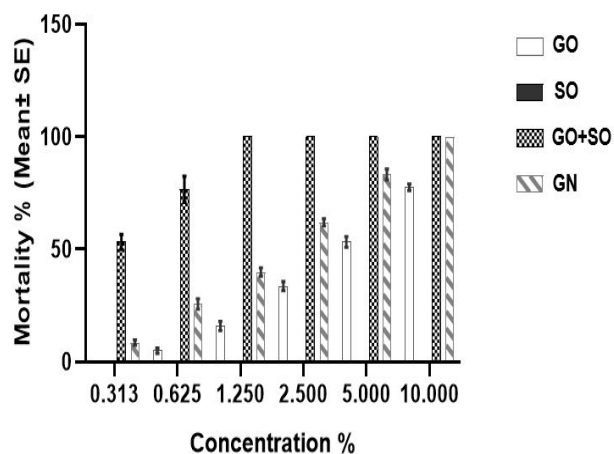


Fig. 1. Mortality percentages of geranium (GO and GN) and the synergistic action with sesame oil (SO) against *Musca domestica* larvae.

Regarding the pupal toxicity, GO showed a percentage inhibition rate (PIR) ranging from 6.67% to 100% at different concentrations after six days of application with LC₅₀ at concentration of 1.48%. Meanwhile, the GN induced PIR ranged from 3.3 to 100% with LC₅₀ achieved at concentration of 1.98%. The 100% of PIR for both GO and GN achieved only at the concentration of 10%. In contrary, SO showed no any pupicidal activity at the all-tested concentrations. The combination of GO+SO showed significant pupicidal activity at the all tested concentrations with LC₅₀ reached at a concentration of 0.50% and 100% PIR was achieved at a concentration of 2.5% concentration. The synergism factor was 2.96 (Tables V, VI).

Table II. The phytochemical composition of sesame oil (SO) by GC-MS.

| Peak | R.t* | Name | Area % | Molecular weight | Molecular formula | MF** |
|------|-------|--|--------|------------------|-------------------|------|
| 1 | 17.63 | Phenol, 2, 4-bis(1, 1-Dimethylethyl)- | 0.40 | 206 | C14H22O | 932 |
| 2 | 22.24 | Methyl tetradecanoate | 0.27 | 242 | C15H30O2 | 933 |
| 3 | 26.39 | Hexadecanoic acid, methyl ester (palmitic acid) | 13.34 | 270 | C17H34O2 | 925 |
| 4 | 29.61 | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester (Linoleic acid) | 37.27 | 294 | C19H34O2 | 905 |
| 5 | 29.73 | 9-Octadecenoic acid (Z)-, methylester (oleic acid) | 26.07 | 296 | C19H36O2 | 939 |
| 6 | 30.12 | Methyl stearate | 10.22 | 298 | C19H38O2 | 929 |
| 7 | 30.35 | 9,12-Octadecadienoic acid (Z, Z)- | 1.26 | 280 | C18H32O2 | 898 |
| 8 | 30.45 | Oleic Acid | 1.78 | 282 | C18H34O2 | 930 |
| 9 | 30.61 | Linoleic acid ethyl ester | 0.36 | 308 | C20H36O2 | 912 |
| 10 | 30.71 | Ethyl oleate | 0.32 | 310 | C20H38O2 | 908 |
| 11 | 32.93 | 11-Eicosenoic acid, methyl ester | 0.47 | 324 | C21H40O2 | 913 |
| 12 | 33.40 | Eicosanoic acid, methyl ester | 2.14 | 326 | C21H42O2 | 884 |
| 13 | 36.52 | Docosanoic acid, methyl ester | 0.30 | 354 | C23H46O2 | 880 |
| 14 | 38.27 | 9,12,15-Octadecatrienoic acid, 2, 2-dimethyl-1, 3-dioxolan-4- Ylmethyl ester, (Z, Z, Z)- | 0.82 | 392 | C24H40O4 | 797 |
| 15 | 38.33 | Oleic acid, (2, 2-dimethyl-1, 3-dioxolan-4-yl)methyl ester | 0.95 | 396 | C24H44O4 | 892 |
| 16 | 38.94 | 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 4.03 | 356 | C21H40O4 | 883 |

Table III. Mortality percentages (Mean±SEM) of *P. graveolens* (GO and GN) and the synergistic action with sesame oil (SO) against *Musca domestica* larvae.

| Concentration (%) | GO | GN | SO | GO+SO |
|---|--------------------------|--------------------------|--------------------------|--------------------------|
| 0.312 | 0.00±0.00 ^g | 8.67±1.20 ^f | 0.00±0.00 ^b | 53.33±3.33 ^c |
| 0.625 | 5.00±1.15 ^f | 25.77±2.33 ^c | 0.00±0.00 ^b | 76.67±5.77 ^b |
| 1.25 | 16.00±2.08 ^e | 40.00±1.76 ^d | 0.00±0.00 ^b | 100.00±0.00 ^a |
| 2.50 | 33.67±2.03 ^d | 62.00±1.53 ^c | 0.00±0.00 ^b | 100.00±0.00 ^a |
| 5.00 | 53.33±2.40 ^c | 83.33±2.40 ^b | 0.00±0.00 ^b | 100.00±0.00 ^a |
| 10.00 | 77.67±1.45 ^b | 100.00±0.00 ^a | 0.00±0.00 ^b | 100.00±0.00 ^a |
| Deltamethrin (2ml/L) (control positive) | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Acetone (negative control) | 0.00±0.00 ^g | 0.00±0.00 ^g | 0.00±0.00 ^b | 0.00±0.00 ^d |

Means within a column followed by the same letter are not significantly different (Duncan's multiple range test: $P > 0.05$).

Table IV. Larvicidal activity of *P. graveolens* (GO and GN) and the synergistic action with sesame oil against *Musca domestica* larvae.

| Treatment | LC ₅₀ (%) | 95% CL | | LC ₉₀ | 95% CL | | X ² (df = 4) | P* | Synergism factor |
|--|----------------------|--------|------|------------------|--------|-------|-------------------------|-------|------------------|
| | | LCL | UCL | | LCL | UCL | | | |
| <i>P. graveolens</i> oil (GO) | 4.29 | 3.71 | 5.07 | 14.08 | 11.02 | 19.42 | 1.74 | 0.784 | - |
| <i>P. graveolens</i> nanoemulsion (GN) | 1.50 | 1.31 | 1.72 | 6.27 | 5.09 | 8.15 | 8.26 | 0.082 | - |
| Sesame oil (SO) | - | - | - | - | - | - | - | - | - |
| GO+SO | 0.32 | 0.26 | 0.36 | 0.77 | 0.66 | 0.95 | 6.92 | 0.140 | 13.41 |

LCL, lower confidential limit; UCL, upper confidential limit; X², Chi-square; df, degree of freedom; LC₅₀ and LC₉₀ were lethal concentration at which 50% and 90% population dies, respectively. * $p > 0.05$ is non-significant

Table V. Percentage inhibition rate (PIR) (Mean±SEM) of *P. graveolens* (GO and GN) and the synergistic action with SO against housefly pupae.

| Concentration (%) | G | GN | SO | GO+SO |
|---|--------------------------|--------------------------|--------------------------|--------------------------|
| 0.312 | 6.67±3.33 ^f | 3.33±3.33 ^f | 0.00±0.00 ^b | 30.00±5.77 ^d |
| 0.625 | 20.00±5.77 ^c | 11.67±4.41 ^c | 0.00±0.00 ^b | 60.00±5.77 ^c |
| 1.25 | 43.33±3.33 ^d | 30.00±5.77 ^d | 0.00±0.00 ^b | 83.33±3.33 ^b |
| 2.50 | 66.67±3.33 ^c | 60.00±5.77 ^c | 0.00±0.00 ^b | 100.00±0.00 ^a |
| 5.00 | 86.67±3.33 ^b | 83.33±3.33 ^b | 0.00±0.00 ^b | 100.00±0.00 ^a |
| 10.000 | 100.00±0.00 ^a | 100.00±0.00 ^a | 0.00±0.00 ^b | 100.00±0.00 ^a |
| Deltamethrin (2ml/L) (control positive) | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Acetone (negative control) | 0.00±0.00 ^f | 0.00±0.00 ^f | 0.00±0.00 ^b | 0.00±0.00 ^c |

Means within a column followed by the same letter are not significantly different (Duncan's multiple range test: $P > 0.05$).

Table VI. Pupicidal activity of *P. graveolens* (GO and GN) and the synergistic action with SO against *Musca domestica* pupae.

| Treatment | LC ₅₀ (%) | 95% CL | | LC ₉₀ | 95% CL | | X ² (df = 4) | P* | Synergism factor |
|--|----------------------|--------|------|------------------|--------|------|-------------------------|------|------------------|
| | | LCL | UCL | | LCL | UCL | | | |
| <i>P. graveolens</i> oil (GO) | 1.48 | 1.31 | 1.68 | 5.29 | 4.39 | 6.66 | 4.05 | 0.39 | - |
| <i>P. graveolens</i> nanoemulsion (GN) | 1.98 | 1.76 | 2.23 | 6.32 | 5.29 | 7.87 | 6.95 | 0.12 | - |
| Sesame oil (SO) | - | - | - | - | - | - | - | - | - |
| GO+SO | 0.50 | 0.44 | 0.57 | 1.38 | 1.18 | 1.70 | 4.38 | 0.36 | 2.96 |

For abbreviations and statistical details, see [Table IV](#).

Table VII. Mortality percentages of *P. graveolens* (Mean± SE) (GO and GN) and the synergistic action with SO against *Culex pipiens* larvae.

| Concentration (%) | GO | GN | Sesame oil (S) | GO+SO |
|---|--------------------------|---------------------------|--------------------------|--------------------------|
| 0.078 | 8.67±1.3 ^e | 11.67± 4.41 ^d | 0.00±10.00 ^c | 50.00±5.77 ^c |
| 0.156 | 33.33±3.33 ^d | 40.00± 5.77 ^c | 1.67±1.67 ^c | 86.67±3.33 ^b |
| 0.312 | 70.00±5.77 ^c | 70.00± 5.77 ^b | 10.00±2.89 ^c | 100.00±0.00 ^a |
| 0.625 | 90.00±5.77 ^b | 100.00± 0.00 ^a | 23.33±1.67 ^d | 100.00±0.00 ^a |
| 1.25 | 100.00±0.00 ^a | 100.00± 0.00 ^a | 50.00±5.77 ^c | 100.00±0.00 ^a |
| 2.50 | 100.00±0.00 ^a | 100.00± 0.00 ^a | 88.33±1.67 ^b | 100.00±0.00 ^a |
| 5.00 | 100.00±0.00 ^a | 100.00± 0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| 10.000 | 100.00±0.00 ^a | 100.00± 0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Deltamethrin (3.5µl/L) (control positive) | 100.00±0.00 ^a | 100.00± 0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Ethyl alcohol70% (negative control) | 1.67±1.67 ^c | 1.67±1.67 ^c | 1.67±1.67 ^c | 1.67±1.67 ^d |

Means within a column followed by the same letter are not significantly different (Duncan's multiple range test: $P > 0.05$).

Larvicidal activity of GO forms against *Culex pipiens*

GO caused 100% mortality in *Culex pipiens* larvae at concentration of 1.25 % and LC₅₀ was achieved at concentration of 0.22 %. While, GN showed 100% larval mortality at concentration of 0.625 % with LC₅₀ was reached at 0.19 % ([Tables VII, VIII, Fig. 2](#)). SO showed

larval toxicity at of 5.00 and 10.00 % with LC₅₀ attained at 1.04 %. Furthermore, the combination of G+S induced significant larvicidal activity at the all tested concentrations with LC₅₀ reached at concentration of 0.079 %. The synergistic factor was 2.78 ([Tables III and V, Fig. 2](#)).

Table VIII. Larvicidal activity of *P. graveolens* (GO and GN) and the synergistic action with sesame oil against *Culex pipiens*.

| Treatment | LC ₅₀ (%) | 95% CL | | LC ₉₀ | 95% CL | | X ² (df = 6) | P* | Synergism factor |
|--|----------------------|--------|-------|------------------|--------|-------|-------------------------|-------|------------------|
| | | LCL | UCL | | LCL | UCL | | | |
| <i>P. graveolens</i> oil (GO) | 0.22 | 0.19 | 0.24 | 0.56 | 0.48 | 0.67 | 1.81 | 0.936 | - |
| <i>P. graveolens</i> Nanoemulsion (GN) | 0.19 | 0.17 | 0.21 | 0.44 | 0.38 | 0.53 | 8.101 | 0.231 | - |
| Sesame oil (SO) | 1.04 | 0.93 | 1.16 | 2.87 | 2.46 | 3.47 | 9.81 | 0.133 | - |
| GO+SO | 0.079 | 0.068 | 0.089 | 0.162 | 0.142 | 0.196 | 1.22 | 0.976 | 2.78 |

For abbreviations and statistical details, see Table IV.

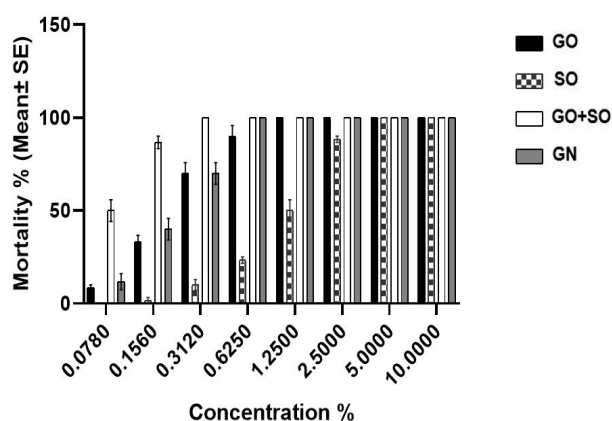


Fig. 2. Mortality percentages of geranium (GO and GN) and the synergistic action with SO against *Culex pipiens* larvae.

Toxicity activities of GO forms against *T. castaneum*

Contact toxicity

In contact toxicity assay, the mortality percentage of *T. castaneum* was increased with increasing the exposure time. After 24 h of exposure, the concentration 10% achieved 100% mortality for each oil and their combination. The LC₅₀ was attained at concentrations of 1.74%, 0.711%, 0.97% and 0.302% for GO, S, GN and GO+S, respectively. The synergistic factor was SF=5.26 (Tables IX, X).

Fumigant toxicity

At 10% concentration the GO and GN showed 80.00% and 94.67% mortality, respectively after 24h of exposure with LC₅₀ value at 4.65% and 3.5%, respectively (Tables XI, XII, and Fig. 4). Oppositely, sesame oil showed no effect on *T. castaneum*. Furthermore, the combination of GO+S at 5 and 10% showed 90 and 100% mortality, respectively, with an LC₅₀ reached at a concentration of 1.97%. The synergistic factor was 2.36 (Tables XI, XII; Fig. 4).

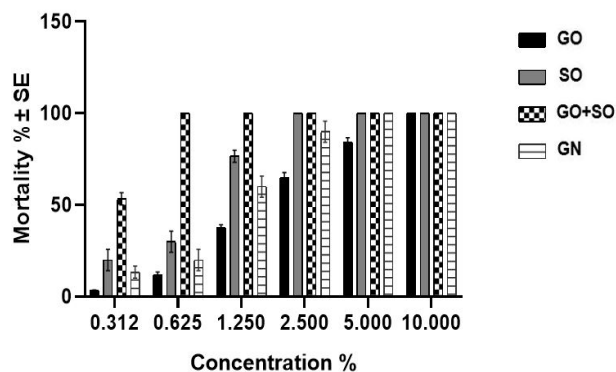


Fig. 3. Mortality percentages of geranium (GO and GN) and the synergistic action with sesame oil against *T. castaneum* in contact toxicity assay after 24 h of exposure

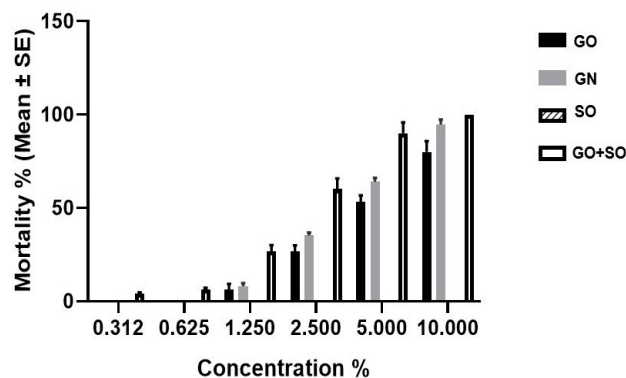


Fig. 4. Mortality percentages of geranium (GO and GN) and the synergistic action with sesame oil against *T. castaneum* in fumigant toxicity assay.

AchE inhibition and MDA activity in house fly larvae

As shown in Figure 5A, all treatments induced reduction in the activity of AchE enzyme of the house fly larvae after 24 h with non-significant difference between these treatments. Meanwhile, all treatments exhibited significant increase in the MDA production when compared with that of the control untreated larvae (Fig. 5B).

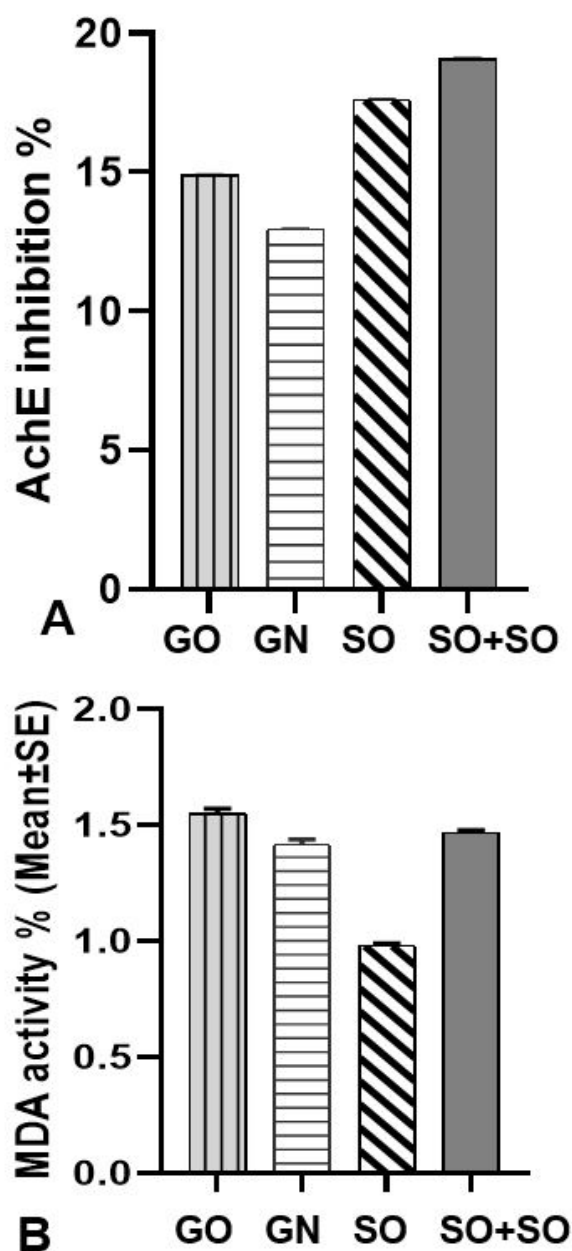


Fig. 5. Acetyl-cholinesterase inhibition % (A) activity of malondialdehyde (MDA) (B) in *Musca domestica* larvae treated with with geranium oil (GO and GN), SO and the combination of geranium and sesame.

DISCUSSION

Traditional insecticides are commonly used to control various insect species; however, many of them do not have the expected efficacy, due to the development of insecticidal resistance (Scott *et al.*, 2000). In addition, some insecticides are known to pose severe threats to human

health and the environment. Therefore, it is necessary to investigate natural products such as essential oils as alternative insecticides (Koul *et al.*, 2008). Following this prospective, the present work was aimed to investigate the insecticidal activities of the geranium essential oil and to evaluate the improvement in its activity when it was in nano-emulsion form and when it was combined with sesame oil.

Generally, the larval stage of insect causes most of the damage, thus determining the larvicidal activity of essential oils is critical (Ebadollah, 2012). The GO showed moderate activity against 3rd instar larvae of *Musca domestica* with LC₅₀ 4.29%. Similarly, Pavela (2008) reported moderate toxicity for geranium oil against the adults of *M. domestica*. In addition, Saraiva *et al.* (2020) found no larvicidal activity for the GO at concentrations from 2.5% to 20% against *M. domestica*. Meanwhile, the GN showed 100% larval mortality at concentration of 10% with LC₅₀ achieved at a concentration of 1.50%. Our findings are in agreement with Boito *et al.* (2018) as they reported higher insecticidal effect for the cinnamon nanoemulsion with respect to the cinnamon oil against *M. domestica* and *Haematobia irritans* flies. Also, SO did not show larvicidal activity against *M. domestica*. Similarly, Mesbah *et al.* (2006) reported a weak toxic effect for the SO on the cotton leaf-worm *Spodoptera littoralis*. In addition, Soe *et al.* (2019) found no insecticidal activity for the SO against the pulse beetle *Callosobruchus maculatus*.

Interestingly, combination of GO and SO showed strong larvicidal activities against *M. domestica* with LC₅₀ reached at concentration of 0.32% that attributed to synergistic action between SO and GO as evidenced by the synergist factor of 49.13. Several authors found that the combination of EOs induced great improvement in the potency of their insecticidal effects due to the synergetic effect between the different oils constituents like terpenes and phenylpropanoids (Gallardo *et al.*, 2012; Tong and Bloomquist, 2013; Faraone *et al.*, 2015; Gallardo *et al.*, 2015). Similarly, Mesbah *et al.* (2006) realized that the combination of clove and SOs greatly improved its larvicidal effect when compared with their use alone against the 4th larval instar of *Spodoptera littoralis*. Also, Soe *et al.* (2019) observed synergistic effect for SO after mixing with clove oil against the pulse beetle, *Callosobruchus maculatus*.

The pupicidal effect of the tested EOs was also important for the control of the housefly population (Chintalchere *et al.*, 2020). In the present study, GO and GN induced 100% inhibition of pupal emergence at concentration of 10% which was similar to those reported by Pavela (2008), Boito *et al.* (2018) and Saraiva *et al.* (2020). Meanwhile, SO showed no pupicidal activity at

Table IX. Contact toxicity of *P. graveolens* (Mean±SE) (GO and GN) and the synergistic action with SO against adults of *Tribolium castaneum* after 24h.

| Concentration (%) | G | GN | SO | GO+SO |
|---|--------------------------|--------------------------|--------------------------|--------------------------|
| 0.312 | 3.33±0.33 ^f | 13.33±3.33 ^c | 20.00±5.77 ^c | 53.33±3.33 ^b |
| 0.625 | 11.67±1.76 ^c | 20.00±5.77 ^c | 30.00±5.77 ^c | 100.00±0.00 ^a |
| 1.25 | 37.70±1.45 ^d | 60.00±5.77 ^b | 76.66±3.33 ^b | 100.00±0.00 ^a |
| 2.50 | 65.00±2.65 ^c | 90.00±5.77 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| 5.00 | 84.33±2.33 ^b | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| 10.000 | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Deltamethrin (1 ppm) (control positive) | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Acetone (negative control) | 16.67±3.33 ^c | 16.67±3.33 ^c | 16.67±3.33 ^d | 16.67±3.33 ^c |

Means within a column followed by the same letter are not significantly different (Duncan's multiple range test: $P > 0.05$).

Table X. Adulticidal activity of *P. graveolens* (GO and GN) and the synergistic action with SO against *T. castaneum* in contact toxicity assay after 24h.

| Treatment | LC ₅₀ (%) | 95% CL | | LC ₉₀ | 95% CL | | X ² (df = 4) | P* | Synergism factor |
|--|----------------------|--------|------|------------------|--------|------|-------------------------|-------|------------------|
| | | LCL | UCL | | LCL | UCL | | | |
| <i>P. graveolens</i> oil (GO) | 1.74 | 1.54 | 1.95 | 5.43 | 4.57 | 6.71 | 4.47 | 0.346 | - |
| <i>P. graveolens</i> nanoemulsion (GN) | 0.97 | 0.73 | 1.27 | 2.59 | 1.86 | 4.64 | 11.72 | 0.02 | - |
| Sesame oil (SO) | 0.711 | 0.496 | 0.99 | 1.75 | 1.21 | 3.88 | 17.04 | 0.002 | - |
| GO+SO | 0.302 | 0.27 | 0.33 | 0.46 | 0.42 | 0.56 | 1.50 | 0.83 | 5.76 |

For abbreviations and statistical details, see [Table IV](#).

Table XI. Fumigant toxicity of *P. graveolens* (Mean ± SE) (GO and GN) and the synergistic action with SO against adults of *Tribolium castaneum* after 24h.

| Concentration (%) | GO | GN | SO | GO+SO |
|---|--------------------------|---------------------------|--------------------------|--------------------------|
| 0.312 | 0.00±0.00 ^c | 0.00±0.00 ^f | 0.00±0.00 | 4.21± 0.53 ^d |
| 0.625 | 0.00±0.00 ^c | 0.00±0.00 ^f | 0.00±0.00 | 6.33± 0.88 ^d |
| 1.25 | 6.00±3.33 ^d | 8.00±1.73 ^c | 0.00±0.00 | 26.67± 3.33 ^c |
| 2.50 | 26.6±3.33 ^c | 35.33±1.45 ^d | 0.00±0.00 | 60.00± 5.77 ^b |
| 5.00 | 53.33±3.33 ^b | 64.00±2.08 ^c | 0.00±0.00 | 90.00± 5.77 ^a |
| 10.000 | 80.00±5.77 ^a | 94.67±2.60 ^b | 0.00±0.00 | 100.00±0.00 ^a |
| Deltamethrin (1 ppm) (control positive) | 100.00±0.00 ^a | 100.00± 0.00 ^a | 100.00±0.00 ^a | 100.00±0.00 ^a |
| Acetone control negative | 0.00±0.00 ^c | 0.00±0.00 ^f | 0.00±0.00 | 0.00±0.00 ^d |

Means within a column followed by the same letter are not significantly different (Duncan's multiple range test: $P > 0.05$).

Table XII. Adulticidal activity of *P. graveolens* (GO and GN) and the synergistic action with SO against *T. castaneum* in fumigant toxicity assay.

| Treatment | LC ₅₀ (%) | 95% CL | | LC ₉₀ | 95% CL | | X ² (df = 3) | P* | Synergism factor |
|--|----------------------|--------|------|------------------|--------|-------|-------------------------|-------|------------------|
| | | LCL | UCL | | LCL | UCL | | | |
| <i>P. graveolens</i> oil (GO) | 4.65 | 4.13 | 5.29 | 13.88 | 11.27 | 18.29 | 1.717 | 0.633 | - |
| <i>P. graveolens</i> Nanoemulsion (GN) | 3.50 | 3.16 | 3.89 | 8.64 | 7.39 | 10.52 | 3.187 | 0.527 | - |
| Sesame oil (SO) | - | - | - | - | - | - | - | - | - |
| GO+SO | 1.97 | 1.77 | 2.19 | 4.91 | 4.23 | 5.91 | 1.783 | 0.619 | 2.36 |

For abbreviations and statistical details, see [Table IV](#).

the all tested concentrations which in agreement with the finding of Mesbah *et al.* (2006) and Soe *et al.* (2019). Furthermore, the combination of GO+SO induced strong pupicidal effect and reduced the LC₅₀ concentration to 0.50% with SF of 2.96. Several previous studies reported similar synergistic effect for sesame oil when mixed with other EOs (Mesbah *et al.*, 2006; Karso and Al-Mallah, 2015; Soe *et al.*, 2019).

The larvicidal activity of GO against *Culex pipiens* reached to 100% mortality at concentration of 1.25 % with LC₅₀ achieved at concentration of 0.22 %. Our findings are similar to those of Norris *et al.* (2015) as they recorded moderate toxicity for the GO against *Aedes aegypti* and *Anopheles gambiae*. Also, Ríos *et al.* (2017) reported larvicidal effect of 80% for the GO against *Aedes aegypti* larvae especially at higher concentrations (130mg/L). However, the lower concentration of GN (0.625 %) induced 100% larval mortality with LC₅₀ achieved at concentration of 0.19 %. Several literatures demonstrated the strong insecticidal activity for the nano-emulsified preparations of EOs (Sugumar *et al.*, 2014; Ramar *et al.*, 2017; Sundararajan *et al.*, 2018). Also, Jesser *et al.* (2020) reported significant larvicidal effect for *Geranium maculatum L. nanoemulsion* form against *Culex pipiens* and decreasing the LC₅₀ from 80.97 ppm for EO to 48.27 ppm. Interestingly, combination of GO+SO led to decrease in concentration of the LC₅₀ to 0.079 % with SF of 2.78. Our results are constant with work of Salama *et al.* (2002) as they confirmed a synergistic action for the SO against a Baygon-resistant strain of *C. pipiens*. Also, Jan (2001) emphasized the synergistic and antioxidant effects of SO and attributed these effects to presence of sesamin, sesamol, and sesamol. In addition, Karso and Al-Mallah (2015) reported synergistic effect for SO when mixed with acetamiprid against the larvae of *Trogoderma granarium*.

Regarding the adulticidal activity against *Tribolium castaneum*; G was found to exert high contact toxicity than the fumigant effect. In contact assay, 100% mortality was achieved at concentration of 5% for GN after 24 h of exposure, with LC₅₀ was reached at concentration of 0.97%. While in fumigant assay, 10 % GO showed 80.00% mortality with LC₅₀ was achieved at concentration of 4.65%. The variation in the results of the two techniques could be attributed to the high volatility of EOs while in the contact assay the insects were in direct contact with the tested substance (Kabera *et al.*, 2011). Similar variation was also previously reported by Odeyemi *et al.* (2008) and Kabera *et al.* (2011). In contrary, Abouelatta *et al.* (2020) noticed no toxic effect for *P. graveolens* EO on *T. castaneum* in the contact assay, while it showed a fumigant effect. This contradiction might be attributed to the variation in components of the used essential oil. GN induced no

significant mortality at the lower concentration while at 10% concentration; mortality of 100% and 94.6 was achieved in both contact and fumigant assays, respectively similar to those reported by Boito *et al.* (2018). SO has no effect against *T. castaneum* adult in fumigant assay but it induced 100 % mortality in the direct contact assay after 24h of exposure. The mixture of GO and SO exerted 100% adulticidal activity against *T. castaneum* either by contact or fumigant assays with LC₅₀ was reached at concentration of 1.97% and SF of 5.76 in agreement with the results of Mesbah *et al.* (2006), Karso and Al-Mallah (2015) and Soe *et al.* (2019). Several studies reported the synergistic action for the sesame oil to various insecticides through the obstruction of the detoxification process (Gowda, 1996; Visetson *et al.*, 2003; Vastrad *et al.*, 2004).

The insecticidal action of *P. graveolens* EO is due to the fact that it contains large quantity of monoterpenes, mainly citronellol and trans-geraniol (Babu and Kaul, 2005; Bouzenna and Krichen, 2013). The insecticidal effect of the constituents found in essential oils is caused by the oil penetration into the insect's tissues and altering the insect's main physiological functions (Babu and Kaul, 2005). According to Pavela (2005), the effectiveness of essential oils may vary based on the method of application and the ability of each insect species for detoxification. Although the mode of action of EOs is unknown, it was found to have acute, sub-acute, and sub-lethal effects (Hummelbrunner and Isman, 2001).

Our results demonstrated that the predominant constituents in SO are linoleic acid (37.27 %), oleic acid (26.07 %) and palmitic acid (13.34 %). Similar findings were reported by Rounizi *et al.* (2021). The action of SO on insects may refer to that the oil disrupting gas exchange (respiration) and destruct the cell membrane function or structure of the tick. So, SO toxic action is more physical than chemical and is short-lived (Cranshaw and Baxendale, 2013).

In the present study, to improve the efficacy of GO; two forms were prepared; GN formulation and a binary mixture with SO. Anjali *et al.* (2012) proposed that the GN preparation as a new promising way to improve the properties and effectiveness of botanical insecticides for commercial use. Our results revealed that the lower concentrations of GN induced no significant mortality while the higher concentrations caused 100% mortality. Another approach to increase the efficiency of GO is the combination with the SO for its synergistic action. This combination increased the percentage of mortality even at the lower concentrations when compared to their use alone against insects.

In the current study, all treatments caused neurotoxicity as evidenced by inhibiting the AchE enzyme

of the *M. domestica* larvae after 24h of treatment. Many secondary metabolites of aromatic plants such as EOs and monoterpenes were known to be able to inhibit the AchE activity of insects (Senthil-Nathan *et al.*, 2007). Our findings go parallel with Chintalchere *et al.* (2020) as they reported inhibition in AchE activity of *M. domestica* larvae treated with bay EO. Similarly, Anderson and Coats (2012) and Rajashekar *et al.* (2014) found inhibition in AchE of *M. domestica* treated with carvacrol and coumaran, extracted from *Lantana camara*.

All treatments induced an increase in the MDA production in *M. domestica* larvae which reflect the role of oxidative stress in the insecticidal effects of GO. These results are in accordance with Rahimi *et al.* (2018) as they observed increase in the level of MDA in *Helicoverpa armigera* larvae treated with *P. persicaria* agglutinin. Rahimi *et al.* (2018) postulated that the plant-derived compounds were able to enhance high levels of lipid peroxidation, that induces cytotoxicity in insect midgut epithelial cells.

It is worth mentioning that the search for synergy among the essential oil constituents is a promising approach for increasing the insecticidal activity of natural compounds. Synergistic mixtures enable a defined level of effect to be achieved at lower dose of constituents than that of using them alone. Thus, in the present study, mixing the GO with the SO could be an effective strategy to improve insecticidal activity and decrease insect control costs due to the lower price of sesame than geranium oil. This synergy may be attributed to the different mode of action of both oils. The geranium effects on the nervous system of insect, meanwhile, sesame oil acts through physical and mechanical effect on the insect.

CONCLUSIONS

Mixing the GO with SO increased the insecticidal activity with higher percentage of mortality even at lower concentrations when compared with the use of each one alone. The SO is of low price; thus, its use in combination with GO (expensive) decreasing its dose and saving money. This form of natural products is safe for environment, animals and human consequently it could be utilized in the integrated pest management.

ACKNOWLEDGMENTS

The authors appreciate the help of veterinarians and technicians for conducting this work.

Funding

This work was supported by Researcher supporting

Project (RSP-2023/3), King Saud University.

IRB approval

This work was approved by the Institutional Review Board of Beni-Suef University.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

All the study data are in this article.

Consent to Publish

Not applicable.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20220418100455>

Statement of conflict of interest

The authors have declared no conflict of interest.

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