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# Biological activity and chemical characteristics of essential oils from the indigenous plant in Palestine

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**ABSTRACT**

New pesticides based on plant extracts and essential oils (EO) are the modern approach to control insect pests and diseases and replacing synthetic pesticides. Therefore, a preliminary screening of nine indigenous plants containing essential oil for antifungal and insecticidal impacts on selected microorganisms and insect pests was evaluated during 2018-19 at Kadoorie Agricultural Research Center (KARC), Palestine Technical University. The biological properties of the plant extracts and EO were tested *in vitro*. Results showed that clove, thyme and eucalyptus oil significantly inhibited the growth of *A. niger, B. cinerea, A. flavus, P. ultimum, P. digitatum* and *F. oxysporum* 24 and 48 hr post-application. $EC_{50}$ and $EC_{90}$ values were significantly the lowest for eucalyptus, thyme then clove. $LC_{50}$ and $LC_{90}$ values against green peach aphid and two-spotted red spider mite were calculated from log-dose probit mortality regression. EO bioassay on aphid showed no significant impact of aphid mortality among all the EO, but a significant effect on the two-spotted red spider mites mortality. Mustard, sage and bitter-cucumber oil applications have high mortality on aphid, while mustard, mint and sesame oil application have high mortality on the two-spotted red spider mite. EO showed no significant impact on plant enzyme activities as in expression values of PPO and POX. The biological activity of the EO investigated on several microorganisms, and insect pests suggested that clove, eucalyptus and thyme showed the highest antifungal activities, while mint, mustard and sesame have the highest insecticidal and acaricidal activities.

**Key Words :** Antifungal, biological activity, chemical characteristics, essential oils, growth inhibition, indigenous plant, insecticide

## INTRODUCTION

The remarkable diversity in the Palestinian flora harbouring more than 3000 plant species for more than 130 families have been recorded in ethnomedicine against many human, animals and plants infectious diseases (Jaradat *et al.*, 2017; Abu-Darwish and Efferth, 2018: Maiti and Singh, 2019). Herbal tea of fresh or dry sage, mint, pandan or thyme leaves has been commonly prescribed for cold, flu, cough, ache, stomachache and antiseptic mouthwash treatment (Abu-Darwish and Efferth, 2018; Minh *et al.*, 2018). The antiseptic rub oil of eucalyptus, sesame or rosemary were used to reduce hoarseness, sore throat, and other cough-related symptoms. Thus, folklore remedies and medicines exhibited a growing interest in these crude plant extracts and their isolates due to their biological activities (Sabbobeh *et al.*, 2016; Jaradat *et al.*, 2017). Indigenous plant extracts and essential oils (EO) role as potential insecticide and fungicide have been described in several studies (Matusinsky *et al.*, 2015; Mossa, 2016). Various herbal plants were identified as pathogen growth inhibitors, anti-inflammatory, antioxidant, and insect repellent. Such compounds were assessed for common insect pests and disease control (Matusinsky *et al.*, 2015). Many studies have been carried out to screen essential oils (EO) from these plants for biological and pesticide activity against phytopathogens and insects on fruits and vegetables (Abdollahi *et al.*, 2011; Shirzad *et al.*, 2011; Boubaker *et al.*, 2016; Roudsari *et al.*, 2017). However, none of the

studies reported the effect of the Palestinian indigenous plant extracts and their essential oils.

## MATERIALS AND METHODS

The present investigation was conducted to screen nine common essential oil of indigenous herbal plants, their biological characteristic as plant resistant elicitors, fungicidal and insecticidal efficacy. The experiments were carried out during 2018-19 at the Kadoorie Agricultural Research Center (KARC) laboratory, Palestine Technical University.

### Essential Oils

Commercial preparations of nine EO processed from medicinal plant leaves or seeds material were purchased from Palsame® essential oils (Jenin-Palestine). According to the manufacturer protocol, plant seeds were used to obtain EO by either hydro-distillation or cold pressing using a Clevenger apparatus. Plant leaves EO were obtained by hydro-distillation using the Clevenger apparatus. The distilled essential oils were stored in a refrigerator at 4°C until the inhibition test was used (Table 1).

### Plant Culture Maintenance

Bean plants were grown in plastic pots in the glasshouse at PTUK and maintained at 25 ± 5° C, 60 ± 5% relative humidity, and 16L:8D photoperiod. Plants were fertilized with irrigation water weekly with Nitrogen: Phosphate: Potassium (NPK) (13:13:13).

### Insect Colony

Inset colony of the green peach aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and two-spotted spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) were reared and maintained in the glasshouse in Kadoorie Agricultural Research Center (KARC)/Palestine Technical University. All stages of the aphid and mite were maintained on the common bean *Phaseolus vulgaris* (L.) (Fabaceae) plants for the laboratory trials following the methodology described by (Stobbs *et al.*, 2015).

### Fungal Isolation and Maintenance

Cultures of five common plant diseases *Pythium ultimum* (trow), *Fusarium oxysporum* (Schlecht. Emend. Snyder & Hansen), *Aspergillus niger* Tiegh., *Botrytis cinerea* Pers., *Aspergillus flavus* Link and *Penicillium digitatum* Sacc. were obtained from the laboratories of the Kadoorie Agricultural Research Center (KARC)/Palestine Technical University. Fungal isolates were sub-cultured as reported by (Gilchrist-Saavedra, 1997) and prepared for assessment of EO activity.

### *In vitro* Antifungal Activity of Plant Essential Oils

### Preliminary Antifungal Screening

Indigenous plant EO's antifungal activities were evaluated against the isolated pathogens using the agar disk-diffusion method, described by Bhalodia and Shukla (2011). A stock solution of 1% of each EO was incorporated into the PDA and poured into Petri dishes, and left to solidify. A single agar disks (0.3 cm in diameter) mycelia of *A. niger*, *B. cinerea*, *A. flavus*, *P. ultimum*, *P. digitatum* and *F. oxysporum* disc were transferred separately to serve as a source of inoculums. Inoculated petri-dishes were sealed with parafilm, then stored at 35±2°C and assessed for pathogen growth at 24, 48 and 72 hrs.

**Table 1.** List of medicinal plants' commercial essential oils used in the study

| S. No. | Scientific name | Common name | Family |
|---|---|---|---|
| 1. | *Rosmarinus officinalis* | Rosemary | Lamiaceae |
| 2. | *Eugenia caryophyllus* | Clove | Myrtaceae |
| 3. | *Mentha spicata* | Mint | Lamiaceae |
| 4. | *Salvia fruticose* | Sage | Lamiaceae |
| 5. | *Citrullus colocynthis* | Bitter cucumber | Cucurbitaceae |
| 6. | *Eucalyptus obliqua* | Eucalyptus | Myrtaceae |
| 7. | *Sesamum indicum* | Sesame | Pedaliaceae |
| 8. | *Thymus vulgaris* | Thyme | Lamiaceae |

**Effective Concentration Bioassay**

Different concentrations of EO of 10, 100, 250 and 500 µL/mL were incorporated into the PDA and poured into Petri dishes and left to solidify as described above. Inoculated petri dishes were incubated for 5-7 days at 35±2°C (Al-Reza *et al.*, 2010). A single agar disks (0.3 cm in diameter), mycelia *P. ultimum*, and *F. oxysporum* disc were transferred separately to serve as a source of inoculums. Plates without EO were used as control treatments. The percent inhibition (PI) of mycelium growth was measured after 24 and 48 hr using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Diameter of control colony (mm) - Diameter of treated colony (mm)}}{\text{Diameter of control colony}} \times 100$$

**Essential Oil Efficacy Bioassays**

As outlined in Table 1, commercial preparations of nine EO were assessed using leaf disk dipping bioassay to determine lethal diagnostic concentrations to green peach aphid and two-spotted red spider mite. Stock solution (10x) of the EO were prepared in water; then, five serial dilutions were prepared.

Leaf disks were cut from leaves of bean plants using a 20 mm diameter cork borer, then dipped for five sec in 20 ml of the different concentrations of each EO tested or the control solution. Treated leaf disks were air-dried before they were transferred on a wetted filter paper in 50 mm Petri-dishes; each lid has 10 small holes for ventilation.

**Aphid Bioassay**

Ten 4$^{th}$ instar GPA per leaf disk were placed on each dish's treated leaves using a fine camel-hair paintbrush. Petri-dishes were kept in a growth chamber at 25 ± 5°C, 60 ± 5% relative humidity, and 16L:8D photoperiod. Numbers of dead and live aphids were recorded after 48 h. Each EO was tested at 5 concentrations and 10 replicates (Samara *et al.*, 2021). Aphid mortality was based on the aphid's inability to withdraw their style or walk in a coordinated manner after being touched gently with a fine camel-hair brush.

**Spider Mite Bioassay**

Ten adult female mites were transferred onto each leaf disc treated with EO using a fine brush as mentioned above; leaf disc was supported on wetted cotton to prevent mites from escaping the treated leaf disc as described by (DeFrancesco *et al.*, 1999). Petri-dishes with each replicate of an EO bioassay were maintained in a growth chamber as described above. Numbers of dead and live mites were counted after 48 h. Each EO was tested at five concentrations and 10 replicates.

**Plant Enzyme Activity Assessment**

Measuring plant enzyme activities of Peroxidase (POX) and polyphenol oxidase (PPO), treated bean plant was used for protein extraction following the protocol of Scott *et al.* (2017). Bean plants were sprayed with EO, 48 hr post-treatment leaves samples were collected then frozen at -80°C. For protein extraction 0.3 g of the plant were homogenized with 1.25 µL of 0.1M potassium phosphate ($K_3PO_4$) buffer (pH 7, containing 7% (w:v) polyvinyl-pyrrolidone (PVP). Then 100 µL of 10% solution of Triton X-100 was added with mixing vigorously around 10 seconds (sec), centrifuged for 8000 rpm for 15 min (Hettich® MIKRO 200/ 200 R centrifuge, Z652121 SIGMA). Determine POX activity, 10 µL of enzyme extract was added to 2 mL disposable cuvette containing 1 mL of freshly prepared 5 mM guaiacol with 0.02 mM hydrogen peroxide ($H_2O_2$) dissolved in 0.1M $K_3PO_4$ buffer pH 8. For PPO assay, 10 µL of enzyme extract was added to 2 mL disposable cuvette containing 500 µL of fresh prepared 10 mM catechol dissolved in 0.1M $K_3PO_4$ buffer pH 8. Changes in absorbance were measured at 470 nanometers (nm) for 30 sec at room temperature using a spectrophotometer device (Hach Lange DR6000 UV-VIS Spectro-photometer, Germany). Enzyme-specific activity for both enzymes was reported as Absorbance/min/mg of fresh tissue weight (Scott *et al.*, 2017).

**Statistical Analysis**

Concentration-mortality data from all replicates were pooled and subjected to probit

analysis. Aphid and mite mortality was corrected using Abbott's formula for each EO. Then $LC_{50}$ and $LC_{90}$ values and their 95% confidence limits (CL 95%) were calculated. $EC_{50}$, $EC_{90}$, values and their 95% confidence limits (CL 95%) were calculated using SAS software (SAS Institute, Inc. Cary, NC). The percent inhibition and inoculation results were analyzed using ANOVA as a general linear model (PROC GLM) procedure. Duncan's multiple range test was used to compare the means (SAS Institute, 1998).

## RESULTS AND DISCUSSION

Antifungal activities of nine indigenous plant EO's evaluated by agar disc diffusion test against isolated pathogens *A. niger*, *B. cinerea*, *A. flavus*, *P. ultimum*, *P. digitatum* and *F. oxysporum* are shown in Figs 1 to 6. Clove oil showed the highest and an escalation inhibition percentage from 65, 88 and 92%, respectively, 24, 48 and 72 hr against *B. cinerea*. Thyme and Eucalyptus oil inhibition rate increased from 40 to 60% and 40-70%, respectively, post 24 and 48 hr application (Fig. 1). Sage and mustard showed an increased inhibition rate of 13-29, 15-18%, respectively, during the same time. All EO except for clove lose their antifungal activates against *B. cinerea* after 72 hr. A similar pattern was detected against *A. niger* (Fig. 2) and *A. flavus* (Fig. 3). Clove oil inhibition rate increased from 9, 72 to 80% and 11, 85 and 95%, respectively, 24, 48 and 72 hr for both pathogen isolates. Thyme and Eucalyptus oil inhibition rate increased from 33 to 68% and 41-79%, respectively, for *A. niger* isolate (Fig. 2) and from 39 to 80 and 49 to 93% for *A. flavus* isolate post 24 and 48 hr application (Fig. 3). Sage, mustard and rosemary showed relatively significant inhibition potential after 48 hr treatment compared with 24 and 72 hr. Results of the antifungal activities of nine indigenous EO against *P. ultimum* are shown in Fig 4. Sage showed a significantly high inhibition rate (97%) after 24 hr, while clove maintained the same increase pattern 52, 87 and 91% during 24, 48 and 72 hr, respectively. Eucalyptus and thyme showed a significant increase of inhibition rate 44 to 87 and 41 to 77% during 24 to 48 hr, respectively. Results of the antifungal activities of nine indigenous EO against *P. digitatum* are presented in Fig. 5. Clove oil inhibition rate increased from 49, 78 to 90% during 24, 48 and 72 hr, respectively. Simultaneously, eucalyptus and thyme showed a significant increase of the inhibition rate 41 to 77 and 45 to 88% during 24 to 48 hr, respectively. Results of the antifungal activities of nine indigenous EO against *F. oxysporum* are given in Fig. 6. Clove oil inhibition rate increased from 49, 89 to 89% during 24, 48 and 72 hr, respectively. Simultaneously, eucalyptus and thyme showed a significant increase of inhibition rate 40 to 78 and 42 to 75% during 24 to 48 hr, respectively. Sage and mustard showed a relatively significant-high inhibition rate after 24 hr treatment compared with 48 and 72 hr. Thistle and coriander oil showed the lowest antifungal properties for all pathogen tested; all EO tested against pathogen isolates shown significant inhibition potential after 48 hr treatment compared with 24 and 72 hr.

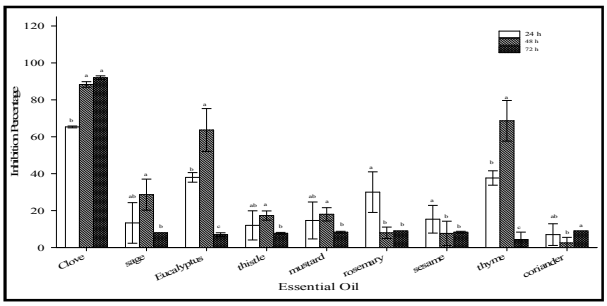


Fig. 1. Antifungal activity of nine EOs against the radial growth and spore germination of Botrytis cinerea, based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth after 24,48, and 72 hr [Means followed by the same letter are not significantly different (Duncan's multiple range test, P > 0.05)].

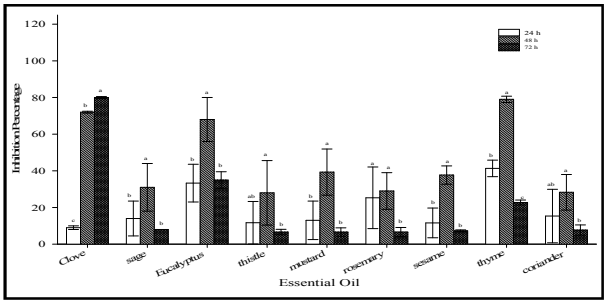


Fig. 2. Antifungal activity of nine EOs against the radial growth and spore germination of Aspergillus niger based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth after 24,48, and 72 hr [Means followed by the same letter are not significantly different (Duncan's multiple range test, P > 0.05)].

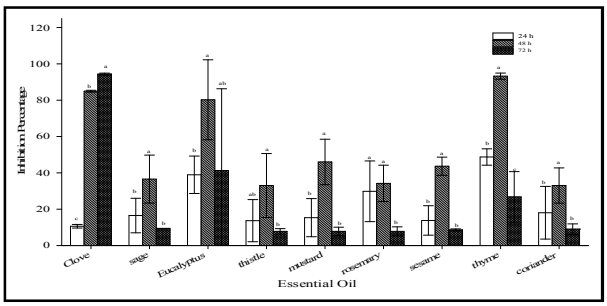


Fig. 3. Antifungal activity of nine EOs against the radial growth and spore germination of Aspergillus flavus, based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth after 24,48, and 72 hr [Means followed by the same letter are not significantly different (Duncan's multiple range test, P > 0.05)].

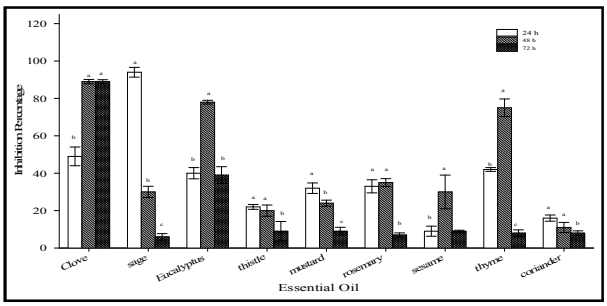


Fig. 4. Antifungal activity of nine EOs against the radial growth and spore germination of Pythium ultimum based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth after 24,48, and 72 hr [Means followed by the same letter are not significantly different (Duncan's multiple range test, P > 0.05)].

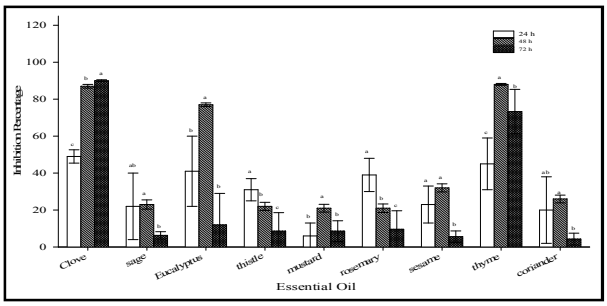


Fig. 5. Antifungal activity of nine EOs against the radial growth and spore germination of Penicillium digitatum based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth after 24,48, and 72 hr[Means followed by the same letter are not significantly different (Duncan's multiple range test, P > 0.05)].

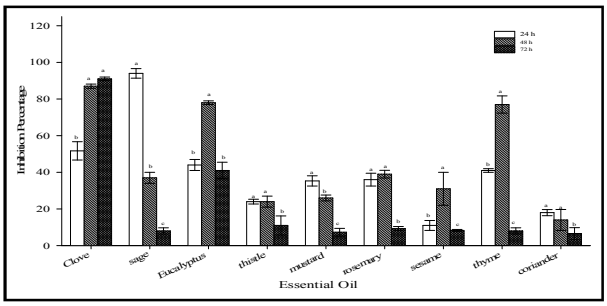


Fig. 6. Antifungal activity of nine EOs against the radial growth and spore germination of Fusarium oxysporum based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth after 24,48, and 72 hr [Means followed by the same letter are not significantly different (Duncan's multiple range test, P > 0.05)].

*P. ultimum* and *F. oxysporum* mycelium sensitivity against nine EO was calculated by the concentration producing 50 and 90% fungal growth inhibition ($EC_{50}$ and $EC_{90}$ values) are given for each EO in Table 2. Eucalyptus oil has the significant lowest $EC_{50}$ (357 µL/mL) against *P. ultimum*, followed by thyme (400 µL/mL) then clove ($2.3E^3$ µL/mL). As for *F. oxysporum*, significant low $EC_{50}$ was found for clove (432.1 µL/mL), Eucalyptus 729.9 (µL/mL) and Thyme 861 (µL/mL).

Lethal concentration values ($LC_{50}$ and $LC_{90}$ values) against GPA, calculated from log-dose probit mortality regression including $LC_{50}$ and $LC_{90}$ with corresponding 95% confidence limits, degree of freedom, regression equations, Pearson Chi-square results are given for the nine EO in Table 3. GPA mortalities from these leaf disk bioassays for the control treatments were always below 10% after 48 h. GPA $LC_{50}$ values after 48 hr did not differ significantly among all the EO ($\alpha$= 0.05). The highest mortalities recorded were for mustard, sage and bitter-cucumber (997, 891, 1624 (µL/mL), respectively.

Lethal concentration values ($LC_{50}$ and $LC_{90}$ values) against spider mite, calculated from log-dose probit mortality regression including $LC_{50}$ and $LC_{90}$ with corresponding 95% confidence limits, degree of freedom, regression equations, Pearson Chi-square results are given for the nine EO in Table 3. Spider mite mortalities from these leaf disk bioassays for the control treatments were always below 10% after 48 h. spider mite $LC_{50}$ values after 48 hr differed significantly among

**Table 2.** $EC_{50}$ and $EC_{90}$ values (with corresponding 95% confidence limits) for *Pythium ultimum* and Fusarium oxysporum fungal growth after 48 h exposure to four different EO using agar disk-diffusion. The percent inhibition of radial growth measured the percent inhibition (PI) of mycelium growth results are presented as $EC_{50}$ and $EC_{90}$ with corresponding 95% confidence limits (CL), Pearson Chi-square results, degree of freedom (df) and regression equations

| EO | *Pythium ultimum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NO* | Regression equations[1] | $X^2$(df) | Slope±SE | $EC_{50}$** (µL/mL) | (95%CL)[2] | $EC_{90}$** (µL/mL) | (95%CL) |
| Clove | 25 | y=0.1516x+0.6201 | 172.96(6) | 5.35±0.32 | $2.3E^3$ abc | $1.7E^3$-$3.2E^3$ | 5.40E+04 | $2.8E^4$-$1.3E^5$ |
| Sage | 25 | y=1.0406x-3.7843 | 4.83(6) | 3.48±0.29 | $6.5E^{15}$ c | $40E^8$-$1.5E^{124}$ | 2.50E+24 | $1.4E^{14}$-$4.0E^{203}$ |
| Mustard | 25 | y=0.2554x+1.124 | 22.74(6) | 5.43±0.52 | $5.3E^5$ c | $6.0E^4$-$9.1E^6$ | 1.90E+08 | $4.1E^6$-$2.1E^{12}$ |
| Rosemary | 25 | y=0.2482x+0.4165 | 54.42(6) | 4.63±0.35 | $4.7E^4$ bc | $1.6E^4$-$2.9E^5$ | 7.70E+06 | $9.2E^5$-$2.9E^8$ |
| Eucalyptus | 25 | y=0.0459x+1.7238 | 572.75(6) | 13.46±0.57 | 357.3 a | 339.4-376.1 | 932.6 | 855.7-1029 |
| Sesame | 25 | y=0.2443x+0.2691 | 4.33(6) | 3.56±0.30 | $3.4E^{15}$ c | $1.9E^8$-$2.7E^{225}$ | 1.30E+25 | 1.74E+14 |
| Thyme | 25 | y=0.0516x+1.6727 | 546.82(6) | 12.19±0.52 | 400.2 ab | 378.7-422.9 | 1178 | 1066-1323 |
| Coriander | 25 | y=0.2617x-0.088 | 58.53(6) | 4.28±0.31 | 41170 bc | $1.5E^4$-$2.3E^5$ | 9.60E+06 | $1.2E^6$-$3.4E^8$ |
| Thistle | 25 | y=0.158x+1.1527 | 80.18(6) | 6.12±0.46 | 9911 bc | 5461-24664 | 2.70E+05 | $8.3E^4$-$1.7E^6$ |
| EO | *Fusarium oxysporum* | | | | | | | |
| | NO* | Regression equations[1] | $X^2$(df) | Slope±SE | $EC_{50}$** (µL/mL) | (95%CL)[2] | $EC_{90}$** (µL/mL) | (95%CL) |
| Clove | 25 | y=0.1306x+1.5249 | 530.31(6) | 12.05±0.52 | 432.1 a | 408.5-457.3 | 1307 | 1176-1477 |
| Sage | 25 | y=0.0529x+1.683 | 173.72(6) | 7.18±0.42 | 7507 b | $5.6E^3$-$1.1E^4$ | 1.30E+05 | $6.9E^4$-$3.2E^5$ |
| Mustard | 25 | y=0.1495x+0.7656 | 212.94(6) | 5.59±0.29 | 2864 b | 2152-4112 | 6.50E+04 | $3.5E^4$-$1.5E^5$ |
| Rosemary | 25 | y=1.0435x-5.1854 | 7.85(6) | 3.31±0.27 | $2.30E^{12}$ b | $2.3E^8$-$8.24E^{34}$ | 3.62E+20 | $1.57E^{13}$-$1.63E^{62}$ |
| Eucalyptus | 25 | y=0.0811x+1.4041 | 484.66(6) | 8.54±0.38 | 729.9 a | 679.5-786.93 | 3977 | 3350-4869 |
| Sesame | 25 | y=0.9965x-4.0193 | 5.84(6) | 3.38±0.28 | $8.27E^{13}$ ab | $7.5E^8$-$1.2E^{62}$ | 9.31E+22 | $7.5E^{13}$-$8.8E^{109}$ |
| Thyme | 25 | y=0.1025x+1.0907 | 298.62(6) | 6.93±0.36 | 861.46 b | 764.4-989.2 | 7345 | 5409-10746 |
| Coriander | 25 | y=0.1766x+0.7229 | 96.85(6) | 5.34±0.37 | 7968 b | 4.7E3-$1.7E^4$ | 320048 | $1.0E^5$-$1.7E^6$ |
| Thistle | 25 | y=1.1255x-4.2885 | 4.17(6) | 3.43±0.29 | $9.36E^{15}$ b | 2.80E+09 | 1.56E+26 | 4.65E+14 |

* Number of samples used in the bioassay; ** $EC_{50}$ and $EC_{90}$ values in (µL/mL); *** $EC_{50}$ values in (µg/L) having different letters are significantly different (95% CL did not overlap). [1]Regression equations estimated by Probit regression; [2]Confidence limits 95% for $EC_{50}$ and $EC_{90}$ in (µL/mL).

**Table 3.** $LC_{50}$ and $LC_{90}$ values (with corresponding 95% confidence limits) for *Myzus persicae* and *Tetranychus urticae* mortality after 48 h exposure to four different concentrations of EO using leaf disc bioassay. Control mortality was always below 10%. The results are presented as $LC_{50}$ and $LC_{90}$ with corresponding 95% confidence limits (CL), Pearson Chi-square results, degree of freedom (df) and regression equations

| EO | *Myzus persicae* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NO* | Regression equations[1] | $X^2$(df) | Slope±SE | $LC_{50}$** (µL/mL) | (95%CL)[2] | $LC_{90}$** (µL/mL) | (95%CL) |
| Sage | 30 | y = 0.1777x - 0.2 | 5.01(5) | 4.08±2.15 | 997.20 a | 0.22-3668 | 41011 | 8431-6.26E[12] |
| Mustard | 30 | y = 0.0492x + 2.0642 | 2.56(5) | 14.50±8.92 | 891.92 a | - | 2497 | - |
| Eucalyptus | 30 | y = 0.0968x + 1.5992 | 10.42(5) | 8.35±2.69 | 2192 a | 648.22-6088 | 16580 | 5996-399585 |
| Sesame | 30 | y = 0.0944x + 1.7999 | 11.07(5) | 8.97±2.85 | 3156 a | 889.89-7649 | 22729 | 9022-319467 |
| Thyme | 30 | y = 0.1344x + 0.8502 | 6.43(5) | 5.89±2.39 | 1857 a | 290.83-9113 | 30857 | 7163-4.3E[8] |
| Coriander | 30 | y = 0.259x - 1.0439 | 2.86(5) | 3.38±2.03 | 4144 a | - | 932122 | - |
| Mint | 30 | y = 0.2005x - 0.182 | 4.49(5) | 4.13±1.98 | 2676 a | 90.40-1.0E[5] | 177340 | 18576-1.14E[28] |
| Bitter-cucumber | 30 | y = 0.1232x + 0.9921 | 7.24(5) | 6.30±2.45 | 1624 a | 285.23-5695 | 21381 | 5970-1.6E[7] |
| EO | *Tetranychus urticae* | | | | | | | |
| | NO* | Regression equations[1] | $X^2$(df) | Slope±SE | $LC_{50}$** (µL/mL) | (95%CL)[2] | $LC_{90}$** (µL/mL) | (95%CL) |
| Sage | 30 | y = 0.1275x - 0.8045 | 1.95(5) | 2.83±2.66 | 30.92 ab | - | 444.91 | - |
| Mustard | 30 | y = 0.2485x - 4.8296 | 0.57(5) | 0.35±2.60 | 0.44 a | - | 79.45 | - |
| Eucalyptus | 30 | y = 0.2011x - 2.11 | 2.41(5) | 1.82±1.73 | 32.34 ab | - | 2169 | - |
| Sesame | 30 | y = 0.1988x - 2.3289 | 2.55(5) | 1.52±1.71 | 17.79 ab | - | 1139 | - |
| Thyme | 30 | y = 0.1017x + 0.6688 | 3.95(5) | 5.95±3.21 | 315.35 b | 4.07E[-9] -2503 | 2644 | 749.95- 1.28E[61] |
| Coriander | 30 | y = 0.3601x - 3.7537 | 0.97(5) | 1.83±1.65 | 535.40 b | - | 9.9E[5] | |
| Mint | 30 | y = 0.2577x - 4.5651 | 1.21(5) | 0.07±1.77 | 1.19 a | - | 260.17 | - |
| Bitter-cucumber | 30 | y = 0.0903x + 0.1707 | 5.12(5) | 4.82±2.61 | 62.57 ab | 0.20-243.14 | 413.65 | 119.045-1.6E[6] |

*Number of samples used in the bioassay; **$LC_{50}$ and $LC_{90}$ values in (µL/mL); ***$LC_{50}$ values in (µg/L) having different letters are significantly different (95% CL did not overlap). [1]Regression equations estimated by Probit regression; Confidence limits (95%) for $LC_{50}$ and $LC_{90}$ in (µL/mL).

all the EO (α= 0.05). The highest mortalities recorded were for mustard, mint and sesame (0.44, 1.19 and 17.8 µL/mL), respectively.

Results of bean plants enzyme activities measured by POX and PPO level post EO treatments are presented in Fig 7. EO showed no significant impact on PPO level than the control (Fig 7A). Similar findings were recorded on the POX level, with no significant expression of POX value of treated bean plants with the EO than the control (Fig 7B).

Nine essential oil extracts from indigenous Palestinian plants have been screened for their fungicidal and insecticidal properties. The biological activity of the EO has been investigated on several microorganisms and insect pests. Clove, eucalyptus and thyme showed the highest antifungal activities against *A. niger*, *B. cinerea*, *A. flavus*, *P. ultimum*, *P. digitatum* and *F. oxysporum.*

Ethnobotanical essential oils and plant extract have been reported to their antimicrobial, antifungal properties in several studies. Clove oils have been reported for their antibacterial, antiviral, antifungal characteristic and insecticidal capacity (Chaieb *et al.*, 2007; Pinto *et al.*, 2009; Estrada-Cano *et al.*, 2017; Nisar *et al.*, 2018). EO's basic mode of action is still not clear; it could be due to their chemical nature and compositions; phenolic, monoterpenes, terpenoid, and terpenes affect the cell membrane and inhibit cell wall synthesis. EO was reported to increase cell membrane permeability, triggering cellular leakage and producing alpha-toxins.

Clove was used repeatedly for it to have many uses in medicine, including antiviral, antimicrobial, and antifungal properties (Estrada-Cano *et al.*, 2017). Clove's main chemical constituents are carvacrol, thymol, eugenol and cinnamaldehyde, all of which are anti-inflammatory, cytotoxic, insect repellent and anesthetic (Chaieb *et al.*, 2007). Clove EO was strongly inhibitory against fungal mycelial growth such as *Candida*, *Aspergillus* and dermatophyte clinical strains (Pinto *et al.*, 2009). Eucalyptus oil was tested against *Fusarium* spp., *S. sclerotiorum*, *A. flavus*, *A. tubingensis*, *B. cinerea* (Davari and Ezazi, 2017), *Phytophthora cactorum*, and *Cryponectria parasitica* (Lee *et al.*, 2008). Similar results were reported for thyme, sage and sesame against *P. expansum* and *B. cinerea* (Baratta *et al.*, 1998; Fraternale *et al.*, 2005; Park, 2011; Sabbobeh *et al.*, 2016; Grzegorczyk-Karolak *et al.*, 2019). Phillips *et al.* (2012) observed that citrus EO reduced spore germination of pathogens *P. chrysogeum*, *A. niger* and *A. alternata*. Thyme and mint oil have a strong aromatic odour; they have antiseptic, antioxidant, antibacterial and antifungal properties (Park, 2011); they have been used for centuries herbalist for medical, food preservation and pest control.

Aphidicidal and acaricidal effects of several essential oils were reported (Momen *et al.*, 2001; Kordali *et al.*, 2007; Niroumand *et al.*, 2016; Roy *et al.*, 2018). Monoterpene is one of the important essential oil components. Monoterpenes were reported to compose variable toxic insecticidal and acaricidal action against several insect species, such as limonene, terpinen, cineole, menthone, carvacrol, myrcene and α-pinene. Some compounds have a mode of action similar to synthetic insecticides, with a high mortality rate and quick knockdown effect. Others are insect repellents, where toxic volatiles enter

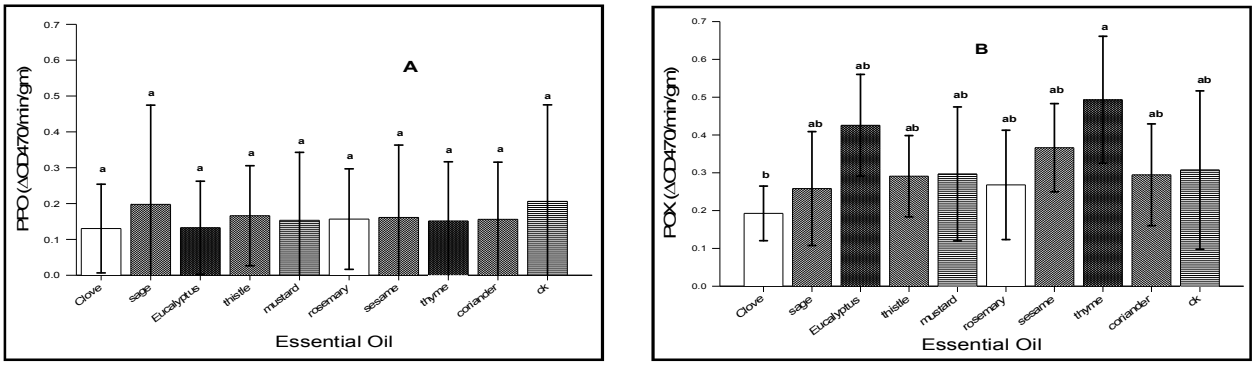


Fig. 7. Impact of nine EO on plant Enzyme activities measured by PPO (A) and POX (B) activities post 48 h of treatment. Enzyme activities were reported by ? in absorbance (optical density/min/g fresh tissue weight) [Bar with different lowercase letters indicated a significant difference (ANOVA PROC GLM, Duncan's multiple grouping test, P= 0.05)].

the insect body through their spiracle into the tracheal system (Sfara *et al.*, 2009). In crops like mustard and sesame (Roy *et al.*, 2018) and rosemary (Momen *et al.*, 2001) were recorded as toxic adulticide, ovicidal, repellent and anti-oviposition against spider mites. Rosemary was strongly toxic against Colorado potato beetle (Kordali *et al.*, 2007).

## CONCLUSION

Indigenous Palestinian EO could control insects and diseases to alternate conventional synthetic pesticides, especially in organic farms and small farms. The current study indicated that EO could be used in insect pest and plant disease control. However, future research on these indigenous plants must identify essential oil's active constituents, their mode of action, and potential phytotoxic effect on target plants based on their application rates.

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