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Evaluation of Three Plant Essential Oils against Striped Mealybug *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae)

Elhefny, A. A.[1]; Sahar A. Attia[1*]; Rodina A. Hassan[2] and M. S. Salem[1]

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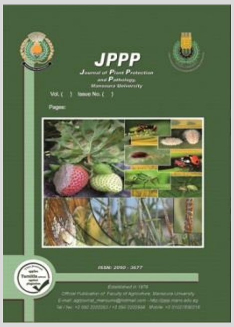
[1] Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.
[2] Economic Entolomgy and Pesticide Department, Faculty of Agriculture, Cairo University, Giza, Egypt.



ABSTRACT

Fruit trees are susceptible to infestations from a variety of harmful pests during developing and growth stages, include striped mealybug, *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae). The current study aims to assess effectiveness of three essential-oils: namely, Thyme, *Thymus vulgaris* L. , Flaxseed, *Linum usitatissimum* L. , and Lemon, *Citrus limon* L. on nymphal and adult populations of *F. virgata* at concentrations (5000, 10000, 15000, and 20000ppm) under laboratory conditions. The oil effects on detoxification enzymes "Acetylcholinesterase AchE., glutathione S-transferase activity and energy reserves (Proteins, carbohydrates, and lipids) at LC_{50}-concentration were also tested. Data obtained indicated that, the highest mortality% of mealybug nymphs and adults was estimated at high concentration (20000) after 24 and 48 hrs. Mortalities were varied according to concentration and type of essential-oil. Lemon-oil was most effective with LC_{50}-values (2309.65 and 50.28 ppm) followed-by thyme-oil (2921.49 and 170.00 ppm) and flaxseed-oil (3999.38 and 528.64 ppm), against nymphs after 24 and 48 hrs., respectively, while they were (542.89 and 39.74), (689.47 and 77.67) and (1621.21 and 341.88 ppm) against adults, respectively. Biochemical analyses showed that, essential-oils caused a significant reduction in activity of glutathione S-transferase and induction in activity of acetylcholine esterase on *F. virgata*. In general, results demonstrated that, high-toxicity was reached by two formulated-oils "Thyme and Lemon" against nymph and adult of *F. virgata*. Therefore, this study suggests use these essential-oils as safe compounds in IPM-strategy of mealybugs.

Keywords: Detoxification enzymes, mealybug *Ferrisia virgata*, acetylcholinesterase AchE, glutathione S-transferase, Energy reserves.

INTRODUCTION

Mealybugs cause damage to several seasonal field crops, vegetables (Black pepper, cashew, cauliflower, cocoa, coffee, cotton, tomato, eggplant, soybean and pineapple), and fruits crops (Citrus, guava, grapes, pomegranate, avocado, banana and mango) by sucking juice leaves, twigs, tender-shoots, branches and fruit (Balikai *et al*., 2011 and Kaydan and Gullan, 2012). Nearly 149 plant species and nearly 246 families of different plants are consumed by mealybugs, of which there have been claimed to be 5000 different species worldwide Ben-Dov, 1994 and 2010 and Afzal, *et al*., 2009). Mealybug *Ferrisia virgata,* Cockerell (Hemiptera: Pseudococcidae) first-recorded with describe in Jamaica, 1893 (Cockerell 1893). Mealybug *F. virgata* one of most commonly occurring-pests in Egypt, and recorded on several hosts. *F. virgata* is called a striped mealybug because; its two dark-dorsal-stripes run lengthwise down the body. Striped mealybug *F. virgata*, considered one of most highly polyphagous mealybugs (Garcia *et al*., 2016) and bad effect on the growth and productivity of plants (Dreistadt, 2001 and Oliveira *et al*. 2014). This mealybug's nymphs and adult females both consume sap of plant-cell from plant various-parts and they excrete honeydew (sugars high in carbohydrates). Honeydew excretion causes sooty-mold growth on fruit foliage (Balikai *et al*., 2011) and disturbs the photosynthesis of plants, finally affecting production. In general, *F. virgata* damages plants both directly and indirectly. Direct damage from feeding causes symptoms including leaf yellowing and defoliation, slowed growth, and in severe cases, death. When opportunistic and saprophytic fungi colonize infected plants, it causes indirect damage (Golan *et al*., 2015).

Because the waxes that mealybugs and scale insects produce form a physical barrier that prevents chemical penetration, contact insecticides find it difficult to remove waxy hydrophobic insects like mealybugs and scale insects. (Dreistadt, 2001 and Hollingsworth and Hamnett, 2009). Since synthetic pesticides are hazardous to environments as a responsible of air, water and soil-pollution and kill natural enemies. Nowadays IPM Programs; botanical-extracts and insecticides could be used. Some plant-extracts ecofriendly when use in the mealybug control (Chaudhary, *et al*. 2017), but control more effective with chemicals insecticides. The essential-oils were used long-time to scale-insects control, but achieved limited management against mealybugs (Subramanian *et al* 2021). Botanical oils such as citrus oils (Karamaouna *et al* 2013, EL-badawy, 2015 and Elhefny *et al.*, 2019), thyme-oil *Thymus vulgaris,* (Park, *et al.*, 2017 and Mostafa *et al.*, 2018), some plant natural essential-oils used in management of mealybugs. Current-work aims to study of effect for Egyptian essential-oils (Thyme, Flaxseed and Lemon) on nymph and adult mealybug, *F. virgata.* Furthermore, we evaluated impact of essential-oils on detoxification enzyme activities of the "Acetylcholinesterase,

[*] Corresponding author.
E-mail address: saharali376@yahoo.com
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AchE." and glutathione S-transferases GSTs and energy-resources contained in mealybugs.

MATERIALS AND METHODS

Test insect:

For this study insect culture of the selected insect, striped mealybug, *F. virgata* was obtained from Poinciana trees (*Delonix Poinciana regia*) growing in Orman garden, Giza Governorate. Randomly chosen samples taken from each of four-cardinal direction "East; West; North and South". Leaves were placed in paper-bags before being transported to lab., and kept at temperature of roughly (25°C) and (65°RH). Taxonomy experts from the dept., of Scale-Insects and Mealy-Bugs - Plant Prot. Res. Inst., Agriculture Research Center, Giza - Egypt, recognized insect. Adults and nymph stages of striped mealybug were selected for the insecticidal activity of three essential oils formulations.

Plant materials:

Three plant species, namely thyme, Thyme, (*Thymus vulgaris L.*) (Lamiaceae), Flaxseed, (*Linum usitatissimum L.*) (Linaceae) and Lemon, (*Citrus limon L.*) (Rutaceae), were purchased from the herbal and vegetables market, Cario, Egypt in February 2022. Fresh fruit peels were used for the extraction of lemon oil, while fresh whole plant of thyme and flaxseed seeds were used for essential oils extraction. The samples were identified at the Department of Botany, Faculty of Science, Cairo University, Egypt.

Extraction and preparation of formulated essential oils:

Essential-oils extracted according to (Cavalcanti *et al*., 2004), and whole plants for thyme and flaxseed and the peels of lemon were used in essential oils extraction. Essential oils were extracted from the freshly three plants parts (weight 200gm from each-sample in 400ml distilled-water) by hydrodistillations in a Clevenger-type apparatus (Winzer, Wertheim, Germany), for 4 hrs. at 100 °C. The extracted oils were dried over anhydrous sodium sulfate, and stored in airtight glassware or dark glass tubes at 4 °C until used bioassays tests. Four concentrations of formulated oils from three plants were prepared by the emulsifier, Triton X-100.

Bioassay technique:

The leaf spraying technique was used as a toxicity test for three natural oils (Thyme, Flaxseed, and Lemon) against nymphs and adults of the mealybug insect *F. virgata*. Uninfected Poinciana tree leaves were chosen and put in Petri plates (9cm diameter, 1cm high by 3.5 cm diameter). *F. virgata* individuals were separated from infected Poinciana leaves and placed in a laboratory container, then ten nymphs and ten adults of mealybugs were placed on each leaf in Petri dishes. After that, each dish, leaf, and *F. virgata* individual was sprayed with 1ml for five seconds of concentrations of 5000, 10000, 15000, and 20000 ppm of our different natural essential oils. The dishes were covered and allowed to dry at room temperature. For each treatment, three replicates were carried out. Petri dishes used were placed in lab conditions and checked for mortality after 24 and 48 hrs. Control insects were sprayed with Triton-x100 alone.

Effect of essential oils on detoxification enzymes of mealybug *F. virgata*.

Sample preparation:

Nymphs and adults of mealybug were treated with the lethal concentration (LC_{50}) of tested essential oils after 24 hrs. of exposure. At 4°C, the mealybug was homogenized in 0.1 M phosphate buffer (pH 7.5). After that, the supernatants were collected and kept at -20°C.

Detoxification enzyme assays:

Acetylcholinesterase (AchE) activity assay:

Acetylcholinesterase (AchE) activity was assessed using acetylcholine bromide (AchBr) as substrate, following the method of Simpson *et al*. (1964). The absorbance was read at 515 nm. The enzyme activity was expressed as mg AchBr / min/ g. body wt.

Glutathione S- transferases (GST) activity assay:

The Habig *et al*. (1974) method was used to determine the activity of GST. Chloro-2,4-dinitro- benzene (CDNB) used as substrate and using a molar extinction coefficient of CDNB 9.6/mM/cm, absorbance at 340 nm was measured to calculate the nanomole CDNB/min/g sample. Inhibition percentage of *GST* activity was calculated as follows:

$$\text{GST inhibition \%} = (\text{ODB} - \text{ODT}) / \text{ODB} \times 100$$

Where ODB is the optical density of blank enzyme and ODT is the optical density of treatment.

Effect of essential oils on energy reserves (Proteins, lipids, and carbohydrates):

Estimation of the total protein content:

The Bradford technique (1976) was used to estimate the total proteins by using a standard curve of Bovine serum albumin (10 to 100 µg). The absorbance at 595 nm was measured and the content of protein was estimated as mg/gram body weight.

Estimation of total lipid content:

Total lipid content in mealybug homogenate was estimated according to Knight *et al*. (1972). The developed color was measured at 525 nm and the total lipid was calculated from the standard curve of the oleic acid and palmitic acid mixture (0.5 to 5 mg/ml). The content of lipids was expressed as mg/gram body weight.

Estimation of total carbohydrate content:

The Singh and Sinha (1977) method was used to determine the total carbohydrates using an anthrone reagent. The absorbance was recorded at 620 nm. The carbohydrate content was expressed as mg/gram body weight from the glucose standard curve (50-250 µg).

Statistics:

The mortality rate was calculated as a percentage, and the mortality was adjusted using the Abbott formula (Abbott, 1925). Calculating the LC_{50} values was done using the LdP-line application. The means of trials were compared using Duncan's Multiple Range Test (Duncan, 1955) at $P<0.05$, and data from all experiments were statistically assessed using ANOVA.

RESULTS AND DISCUSSION

Essential-oils effect on *F. virgata*:

Toxicity of formulated essential-oils against F. virgata:

This study was primarily performed to look into the relationship between three essential oils including lemon, thyme and flaxseed and their effectiveness on *F. virgata* nymphs and adults. The insecticidal activities of oils against *F. virgata* nymphs and adults after 24 and 48hrs., are represented in Tables (1,2,3). The results indicated that *C. limon* essential oil significantly achieved highly mortality percentage on nymph and adult with concentrations, (5000, 10000, 15000, and 20000 ppm). After 24 hrs., most potent-rates recorded on nymph and adult by lemon essential-oil

(100±0.00 and 100±0.00%), respectively, at the highest concentration of 20000 ppm, followed by thyme-oil (93.33±3.33 and 96.67±3.33 %) respectively, while, lowest mortality obtained with flaxseed-oil (80.00±5.77 and 86.67±3.33 %), respectively at the same concentration. The mortality of nymph and adult have recorded the same mortality percentages (100±0.00 and100±0.00 %), after 48 hrs. with the three essential oils at the highest concentration of 20000 ppm. The essential oils from lemon and thyme were equally toxic against nymphs and adults of *F. virgata* compared to flaxseed essential oil which was the least. Generally, the percentages of mortality of nymphs and adults for lemon oil were higher than thyme and flaxseed, when exposed for the same time and at similar different concentrations. The insecticidal toxicity of the three essential oils against *F. virgata* adults was more than that against *F. virgata* nymphs (Tables 1,2,3 and 4). All three essential oils exhibited high toxicity rates with time and concentration-dependent (Table 1,2,3 and 4). Between the control and treated variations, there were substantial mortality differences (P<0.05). Our findings indicated that the three essential oils would be useful to us in managing *F. virgata*.

Table 1. Insecticidal effect of lemon (*Citrus limon*) at four concentrations against nymph and adult of striped mealybug *Ferrisia virgata.*

Conc. (ppm)	Mortality (Mean%±SE)			
	24 hour		48 hour	
	Nymph	Adult	Nymph	Adult
5000	70.00±0.00	83.33±3.33	93.33±3.33	96.67±3.33
10000	73.33±6.67	93.33±3.33	96.67±3.33	96.67±3.33
15000	83.33±3.33	90.00±0.00	96.67±3.33	96.67±3.33
20000	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Table 2. Insecticidal effect of Thyme (*Thymus vulgaris*) at four concentrations against nymph and adult of striped mealybug *Ferrisia virgata.*

Conc. (ppm)	Mortality (Mean%±SE)			
	24 hour		48 hour	
	Nymph	Adult	Nymph	Adult
5000	63.33±6.67	60.00±5.77	91.67±0.88	93.33±3.33
10000	70.00±5.77	73.33±6.67	95.00±2.89	95.00±0.00
15000	86.67±6.67	86.67±3.33	96.67±3.33	96.67±3.33
20000	93.33±3.33	96.67±3.33	100.00±0.00	100.00±0.00

Table 3. Insecticidal effect of Flaxseed (*Linum usitatissimum*) at four concentrations against nymph and adult of striped mealybug *Ferrisia virgata.*

Conc. (ppm)	Mortality (Mean%±SE)			
	24 hour		48 hour	
	Nymph	Adult	Nymph	Adult
5000	63.33±3.33	73.33±3.33	91.00±0.58	91.67±0.88
10000	73.33±6.67	90.00±0.00	93.00±3.33	93.30±2.89
15000	76.67±3.33	83.33±3.33	95.00±2.89	97.67±1.45
20000	80.00±5.77	86.67±3.33	100.00±0.00	100.00±0.00

SE= Standard error

The results in Table (4), revealed that LC_{50} values of nymphs and adults were (2309.65 and 542.89), (2921.49 and 689.47) and (3999.38 and 1621.21) ppm, for lemon, thyme, and flaxseed after 24 hrs., respectively and (39.74 and 50.28); (170.00 and 77.67) and (528.64 and 341.88) ppm after 48 hrs., respectively. While LC_{90} values were (17104.00 and 12786.79); (19458.59 and 21319.86) and (47150.65 and 27991.39) ppm, for lemon, thyme, and flaxseed after 24 hrs., respectively, and (5190and 6902.04); (6890.80and 6306.12) and (6496.74 and 6559.21) ppm after 48 hrs., respectively. Data obtained in Table (4), clarified that, lemon-oil showed mostly potent- effects on nymph and adult followed by thyme oil, while flaxseed oil was the lowest potent. Toxicity-assays clarified, activities of nymphicidal and adulticidal in three essential-oils: lemon followed-by thyme then flaxseed. The LC_{50} values of essential-oils ranged 39.74 to 3999.38ppm on nymphs and 50.28 to 1621.21ppm on adults depending-on toxicity of essential-oils, treatment-time, and mealybug-life stages. This indicates that the three essential oils' LC_{50} values differ against *F. virgata* based on the formulation's toxicity and mealybug-life stages. Additionally, LC_{50} for each essential-oil show a clear distinction between nymphs and adults.

Table 4. Toxicological values (LC_{50} and LC_{90} ppm) of three different essential oils on striped mealybug *Ferrisia virgata.*

Essential oil	24hrs.							
	Nymph				Adult			
	LC_{50}	LC_{90}	X^2	Slope	LC_{50}	LC_{90}	X^2	Slope
Lemon	2309.65	17104.00	1.474	0.077	542.89	12786.79	1.011	0.060
Thyme	2921.49	19458.59	0.446	1.866	689.47	21319.86	0.110	2.107
Flaxseed	3999.38	47150.65	1.061	0.058	1621.21	27991.39	1.036	0.209
	48hrs.							
	Nymph				Adult			
Lemon	50.28	5190.95	0.4358	0.6579	39.74	6902.04	0.6036	0.7968
Thyme	170.00	6890.80	0.1283	0.6057	77.67	6306.12	0.2149	0.6108
Flaxseed	528.64	6496.74	0.8245	1.002	341.88	6559.21	1.3265	1.1733

Several studies investigated the insecticidal effects of citrus species' essential oils against mealybugs and scale insects, such as the results of Elhefny *et al* (2019) indicated that *F. virgata* and *Milviscutulus mangiferae* nymphs and adults were highly poisonous to the two formulated navel orange (*Citrus sinensis*) oils. They recommended using special navel orange oil as a safe, potential natural alternative to treat *F. virgata and M. mangiferae*. Additionally, El-Badawy (2015) revealed that all citrus oils tested, particularly those from Baladi and Navel orange, had significant effects insecticidal and repellant on mealybug nymph and adult of *Icerya seychellarum*, (Westwood) (Hemiptera: Monophlebidae). These results are supported by Karamaouna *et al*. (2013), who demonstrated that the citrus peel essential oils of lemon (*C. limon*) and navel orange (*C. sinensis*) were the most toxic of all the tested essential oils against third-instar nymphs and female adults of the vine mealybug, *Planococcus ficus* (Signoret). Tacoli *et al* (2018) concluded similar results of the insecticidal activity of citrus essential oil against some vineyard mealybugs including *P. ficus*. Pure limonene the main component of the citrus *spp*., exhibited insecticidal activity against nymphs and adults of some mealybug species such as *Planococcus citri* (Risso), *Nipaecoccus nipae* (Maskell) and *Pseudococcus longispinus* (Hollingsworth, 2005 and Munuru,2021).

Park, *et al* (2017) evaluated five different essential oils against *Pochazia shantungensis* adults and nymphs. The results indicate that *T. vulgaris* oil recorded the highest insecticidal toxicity and this may be attributed to the major two compounds carvacrol and thymol. Also, Mostafa *et al*. (2018) examined the toxicity of eleven plant essential oils on adult female *Phenacoccus solenopsis* (Tinsley) cotton mealybugs (Hemiptera: Pseudococcidae) and they found that *T. vulgaris*, *Mentha longifolia*, and *Cyperus articulates* were the three most toxic essential oils after 24 and 72 hrs. of treatment. The findings of Erdemir and Erler (2017) revealed that all of the tested essential oils, anise (*Pimpinella anisum*), rosemary (*Rosmarinus officinalis*), peppermint (*Mentha piperita*), Turkish oregano (*Origanum onites*), and thyme (*Thymus vulgaris*) had a repellent activity against female adults of *Planococcus citri* Risso. The two oils of *O. onites* and *T. vulgar* achieved the highest repellency percentage with the longest period (96 hrs.). Additionally, the insecticidal activity against several species of mealybugs was also demonstrated by different aromatic essential oils. (Cloyd *et al*., 2009; Karamaouna *et al*., 2013, Peschiutta *et al*., 2017, Erdemir and Erler, 2017, Mostafa *et al*., 2018, Prabowo and Damaiyani, 2019, Mwanauta *et al*., 2021, Saad *et al*., 2021 and Brahmi *et al*., 2022) have been reported.

Effects of essential oils on detoxifying enzymes:

Effects of essential oils on AchE., activity:

Results of the effects of the LC_{50} concentration of the three essential oils on acetylcholinesterase (AchE) enzyme activity of *F. virgata* after 24 hrs. exposure are represented in Table (5). Acetylcholine esterase showed a significant increase at the LC_{50} concentration of *C. limon* (1.83±0.04 *mg AchBr/ min/ g. body wt.*) compared to the control (0.740±0.02 *mg AchBr/ min/ g. body wt.*), and there is low increase recorded with essential oil of *L. usitatissimum* (0.864±0.017 *mg AchBr/ min/ g. body wt.*), while essential oil of *T. vulgaris* caused a decrease in Acetylcholine esterase activity (0.585±0.02 *mg AchBr/ min/ g. body wt.*), compared with the control. According to these results, *C. limon* and *L. usitatissimum* oils caused significant AchE induction (stimulation) and the oil stimulation of enzyme activity based on their AchE activities values. The oil of *C. limon* was a more potent enzyme stimulator than *L. usitatissimum*. In addition, the oil of *T. vulgaris* recorded enzyme activity lower than the control this meaning that it caused an inhibitory effect against AchE. Acetylcholine esterase activity increased in the *F. virgata* treated with both the essential oils of *C. limon* and *L. usitatissimum* in our study, while *T. vulgaris,* caused a decrease in AchE activity.

Effects of essential oils on GST activity:

Table (5), shows GST activity after 24-hour exposure to the LC_{50} concentration of the chosen essential oils. In comparison to the control, the results showed considerable inhibition of the enzymatic activity. The oil of *T. vulgaris* was the most potent enzyme inhibitor (69.16±3.04 *nanomole CDNB/ min/ g* followed by the *C. limon* oil which caused inhibition of enzyme activity (100.73 ± 9.22 *nanomole CDNB/ min/ g*), while the oil of *L. usitatissimum* recorded the weakest enzyme inhibition (136.67±2.03 *nanomole CDNB/ min/ g*) based on their GST activities values compared to control (144.47±0.87 *nanomole DNB/ min/ g*) after the same exposure period. The *C. limon* oil induced an inhibitory effect higher than *L. usitatissimum* oil, while the highest inhibitory effect was achieved with *T. vulgaris*. These findings were confirmed by the inhibition percentage values (I %) of *T. vulgaris* (I=52.19 %), *C. limon* (I=30.38 %), and *L. usitatissimum* (I= 1.14 %) Table (6).

Table 5. Specific activities of acetylcholinesterase and gluthatione S-transferases in mealybug, *F. virgata* after 24 h of exposure to the lethal concentration LC_{50} of *C. limon, T. vulgaris* and *L. usitatissium* essential oils.

Treatment	Acetylcholinesterase mg AchBr/min/g (means ±SE)	Gluthatione S-transferases nanomole CDNB/min/g (means ±SE)
Control	0.704±0.20[c]	144.47±0.868[a]
C. limon	1.837±0.05[a]	100.73±9.220[b]
T. vulgaris	0.582±0.04[d]	69.16±3.034[c]
L. usitatissimum	0.844±0.02[b]	136.67±2.027[a]
F value	254.06	39.50
P	0.0000	0.0002

Table 6. Inhibition values of Glutathione S-transferases (GST) activity of mealybug, *F. virgata* by *C. limon, T. vulgaris and L. usitatissium* essential oils.

Treatment	Inhibition (%) (Mean I %±SE)
Control	00.00±0.00
C. limon	30.378±1.107
T. vulgaris	52.188±1.871
L. usitatissimum	1.140±6.715

After 24 hrs. of exposure at the lethal concentration of LC_{50}, acetylcholine esterase activity was induced in the *F. virgata* treated with both the essential oils of *C. limon* and *L. usitatissimum* in our study, while *T. vulgaris,* inhibited AchE activity. Several essential oils from aromatic plants have exhibited inhibition or induction against AchE of mealybugs (Brahmi, *et al* 2022 and Arokiyaraj *et al* 2022), stored products insects (Abdelgaleil *et al*. 2009; Abdelgaleil *et al*. 2016, Oboh, *et al*. 2017 and Kim *et al*. 2013) other insect species (Miyazawa *et al*. 1997 and Kumrungsee *et al.2022*). The toxicity of the essential oils may be related to an increase in acetylcholinesterase activity because of the experiments' observed hyperactivity and knocked-down effect. Most neurotoxic pesticides target the enzyme acetylcholinesterase (Liao *et al*. 2017). Because impaired neuromuscular functions result in neural excitement and knocked-down effects, the essential oil's insecticidal properties may be related to the inhibition or activation of acetylcholinesterase. Acetylcholine, a crucial neurotransmitter involved in the transmission of nerve impulses, is broken down by the enzyme acetylcholinesterase. Accumulation of acetylcholine at neuromuscular junctions due to acetylcholinesterase may result in neural stimulation, hyperactivity, paralysis, and finally insect mortality (Rajashekar *et al*. 2014). The essential oils' main active ingredients may be responsible for the acetylcholinesterase inhibitory action that was found (Oboh *et al*. 2014). Numerous studies have shown that essential oils and monoterpenes interfere with the activity of the AchE enzyme in insects, which is a key factor in the neurotoxic modes of action of insects (Yeom *et al*. 2013). Previous investigation has revealed that limonene the main compound of citrus species is an inhibitor of acetylcholinesterase activity (Abdelgaleil *et al*. 2009).

In the current study, treatment of *F. virgata* with the investigated essential oils reduced the activity of GST compared to the control. The essential oils of *T. vulgaris*, *C. limon* and *L. usitatissimum* at the lethal concentration of LC_{50} substantially inhibited the detoxification enzyme activity of GST in mealybug, *F. virgata* after 24 hrs. exposure. The GST more susceptible to essential-oils, than “AchE” based-on inhibition percentage (I%) results, indicating that GST may be the primary target for the essential oils. Most organisms include a significant category of detoxification enzymes called glutathione S-transferases. They contribute to the protection of cells from oxidative stress and chemical toxins by the excretion of electrophilic and lipophilic chemicals (Hayes and Pulford 1995 and Enayati *et al*. 2005). Several investigations have shown that when insects are exposed to pathogens, plant allelochemicals, and chemical pesticides, GST is activated (Qin *et al*. 2011 and Acheuk *et al*. 2018). This agrees with our findings, which showed that *F. virgata* exposed to the tested essential oils had high GST activity.

AchE and GST are the main enzymes that researchers are focusing on to learn more about the biochemical alterations in insects among the many detoxification enzymes (Koodalingam *et al*., 2011 and Shojaei *et al*., 2017). The results of Dolma *et al*. (2021), which showed that *Tagetes minuta* oil inhibited the AchE and GST activities against *Plutella xylostella*, confirmed the current findings. Similarly, *Artemisia maritima* essential oil suppressed the GST activity against *Callosobruchus chinensis* L. and *Callosobruchus maculatus* (Chauhan *et al*., 2022). In general, botanicals have strong insecticidal effects by controlling the neuro-endocrine system and metabolism of the previously mentioned target insects (Parthiban *et al*., 2020 and Ramachandran *et al*., 2022). This study indicates that the essential oils exhibited stimulation to acetylcholinesterase (AchE) and inhibition to Glutathione S-transferase (GST) and oils from *C. limon* and *T. vulgaris* could be effective in the biocontrol of nymphs and adults of *F. virgata* and this might be related to how it affects GST and acetylcholinesterase activity. In general, the insect activates its immunological or defense systems, such as increasing detoxifying enzymes, which will demand a lot of energy or cost, regardless of the sort of external element influencing it (Abd El-Kareem, *et al* 2022). In most cases, insects metabolize xenobiotics using detoxification enzymes (Li, *et al* 2007, Ramsey, *et al* 2010 and Russell, *et al* 2011). However, chemical and botanical insecticides can stimulate enzymes, which are crucial for developing pest resistance (Yu, *et al* 1993 and Bouayad, *et al* 2013). The essential oils have a variety of mechanisms against insect pests, including inhibiting enzymes such as AchE, GST, and cytochrome P450 monooxygenase, as well as growth and neurotoxicity (Park and Tak, 2016).

Effect of essential oils on the energy reserves

The results of the total protein, lipid, and carbohydrate content of *F. virgata* treated by LC_{50} concentration of *T. vulgaris, C. limon,* and *L. usitatissimum* essential oils after 24 hrs. exposure are shown in Table (7).

The amount of the total protein (33.14±2.46, 47.50±0.50 and 23.89±1.84 mg/ g. body wt.) of *F. virgata* treated with LC_{50} concentration of *T. vulagris, C. limon*, and *L. usitatissimum* essential oils respectively, increased after 24 hrs. exposure, comparison with the control (13.50±0.12 mg/ g. body wt.). The data show that the LC_{50} concentration of *T. vulgaris*, *C. limon*, and *L. usitatissimum* essential oils significantly increased total proteins.

The three essential oils tested reduced the lipid value content, except with *C. limon* oil (38.61±0.49 mg/ g. body wt.) which was increased. The lipid content of *T. vulagris* and *L. usitatissimum* essential oils reduced the amount of lipid to (9.16±0.50 and 17.79±2.30 mg/ g. body wt.), respectively, compared with the control (34.40±0.22 mg/ g. body wt.). Compared to the control, this reduction was more pronounced for *T. vulgaris* than *L. usitatissimum*. The essential oils' impact on lipid metabolism and the usage of lipid reserves for energy production as a result of induced stress may both contribute to the reduction of lipid levels in *F. virgata* treated with them.

The amount of carbohydrates in *F. virgata* was significantly decreased by the essential oils examined in this study. The carbohydrates content of *F. virgata* decreased by all essential oils, the reduction affected by *C. limon* (12.29±0.11 mg/ g. body wt.), *T. vulgaris* (11.27±0.13 mg/ g. body wt.) and *L. usitatissimum* (9.55±0.32 mg/g. body wt.) essential oils was significant compared to the control group (19.08±3.06). More sugars may be metabolized to meet energy needs when under stress. This might be the reason the treated insects decreased their carbohydrate levels.

Table 7. Effect of LC_{50} concentration of *Citrus limon, Thymus vulgaris* and *Linum usitatissimum* essential oils on energy reserves (Mean%±SE) of *Ferrisia virgata.*

Treatment	Energy reserves (Mean%±SE)		
	Proteins	Lipids	Carbohydrates
Control	13.50±0.12	38.61±0.49	19.08±3.06
C. limon	47.50±0.50	34.40±0.22	12.29±0.11
T. vulgaris	33.14±2.46	9.16±0.50	11.27±0.13
L. usitatissimum	23.89±1.84	17.79±2.30	9.55±0.32

SE= Standard error

The essential oils had an impact on energy resources. All essential oils raised the protein content, whereas *T. vulgaris* and *L. usitatissimum* essential oils had the opposite effect and lowered the carbohydrate content. According to Abd El-Kareem *et al*. (2022), insects can often convert carbohydrates into lipids, which may account for the somewhat lower reduction in lipid content caused by *C. limon* essential oil compared to other essential oils. In this study, *F. virgata* treated with the three essential oils had less lipid and carbohydrate content. This was likely because the pest needed more of these energy reserves to produce esterase enzymes. These findings corroborated those of Yazdani *et al*. (2014) and Moutassem *et al*. (2021), who found that insect pests treated with Thymus essential oils had lower carbohydrates and lipids levels. The enzymatic activities of an organism were significantly impacted by exposure to lethal and sublethal doses, reflecting the biochemical disruptions (Kiran and Prakash,2015). The measurement of total protein and other macromolecules, such as lipids and carbohydrates, is vital in physiological research. The LC_{50} concentration values had a significant effect on the protein, carbohydrates, and lipid content. In comparison to the control, the treatment of *F. virgata* with LC_{50} values of all examined essential oils considerably increased their total protein content. One or more factors, such as a decrease in protein synthesis or an increase in protein breakdown to detoxify the active ingredients found in plant extracts or essential oils, were attributed to the changes in protein composition in insects (Vijayaraghavan *et al*. 2010). Reducing an insect's intake of proteins, carbohydrates, and lipids is one of the most effective strategies for controlling insect pests since these nutrients are essential in the metabolic processes that control an insect's development, metamorphosis, reproduction, and diapause. (Nestel, *et al* 2003 and Senthil-Nathan, 2013).

CONCLUSION AND RECOMMENDATIONS

The goal of the current study is to use phytochemicals, which are safer, more environmentally friendly, and more effective than chemical pesticides, to control mealybugs. In our investigation, the essential oils *T. vulgaris* and *C. limon*, were found to be most effective against *F. virgata* with all treatments. Essential oils changed the activity level of acetylcholinesterase and glutathione S-transferase (GST) through increased (induced) acetylcholinesterase activity and dramatically decreased (reduced) glutathione S-transferase. *C. limon* essential oils, had an impact on the neurological system of *F. virgata*, as shown by the considerable induction of AchE., activity. The essential oils decreased carbohydrate and lipid contents while, total protein content was increased. Therefore, essential oils have an impact on mealybug macromolecule activity as well as its detoxifying enzymes. In fact, the probability of pest resistance is low due to the numerous mechanisms of action of essential oils, which include the activation and inhibition of acetylcholinesterase, inhibition of glutathione-S-transferase activity, and reduction in energy reserves.

It was concluded that the used essential oils may be as an alternative to the conventional mealybug control agents because it was determined that they were toxic to *F. virgata* and had irreversible effects on important metabolic processes. Further research should be done on the side effects of these essential oils on mealybugs and other beneficial non-target insects, as well as on the pesticidal effects of their constituent parts on other insect pests and the implications on human health. In the context of integrated pest management programmes, these findings on neurotoxicity and toxicity bioassays offer new information for formulating efficient essential oil-based insecticides to treat *F. virgata*.

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تقييم ثلاث زيوت نباتية على حشرة البق الدقيقى *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae)

أحمد عبد المنعم الحفنى[١] ، سحر على عطية[١] ، ردينه احمد حسن[٢] و سالم محمد سالم[١]

[١] معهد بحوث وقاية النباتات - مزكز البحوث الزراعية - الدقى - الجيزة - مصر
[٢] قسم الحشرات الاقتصادية - شعبة المبيدات - كلية الزراعة - جامعة القاهرة - الجيزة

الملخص

تتعرض أشجار الفاكهة للأصابة بأنواع مختلفة من الأفات الضارة أثناء مراحل نموها وتطورها بما فى ذلك حشرة البق الدقيقى *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae) . تمت الدراسة الحالية لتقيم الكفاءة الأبادية لثلاثة زيوت نباتية (زيت الزعتر، وبذور الكتان وقشر الليمون) على تعداد حوريات وبالغات البق الدقيقى *F. virgata* بأربع تركيزات (٥٠٠٠، ١٠٠٠٠، ١٥٠٠٠ و ٢٠٠٠٠ جزء/المليون) تحت الظروف المعملية ، وتأثير التركيزات النصف مميتة لتلك الزيوت على انزيم مزيل السموم (الأستيل كولين استيريز) وعلى نشاط جلوتاثيون استرين وايضا على احتياطات الطاقة (البروتين والكربوهيدرات والدهون) . وقد اشارت النتائج أن أعلى نسبة موت للحوريات والبالغات سجلت مع أعلى تركيز (٢٠٠٠٠ جزء/المليون) بعد ٢٤ و٤٨ ساعة ، وأختلفت نسبة الموت حسب نوع الزيت وتركيزه . وأظهرت النتائج أن زيت الليمون أعلى كفاءة ثم زيت الزعتر وزيت بذور الكتان على التوالى . قيم التركيزات ٥٠٪ لزيوت (قشر الليمون والزعتر وبذور الكتان) كانت (٣٠٩,٦٥ و ٣٩,٧٤) ، (٢٩٢١,٤٩ و ٧٧,٦٧) و (٣٩٩٩,٣٨ و ٣٤١,٨٨ جزء/المليون) بينما كانت قيم التركيز ٩٠٪ (٥٤٢,٨٩و ٥٠,٢٨) ، (٦٨٩,٤٧و ١٧٠,٠٠) و (١٦٢١,٢١و ٥٢٨,٦٤ جزء/المليون) ضد الحوريات والبالغات بعد ٢٤و٤٨ساعة على التوالى . وقد أشار التحليل البيوكيميائى ان الزيوت تسببت فى انخفاض معنوى لنشاط الجلوتاثيون والأستيل كولين . وكان زيت الزعتر والليمون اعلى سمية ضد الحوريات والبالغات للحشرة المختبرة . لذلك يمكن استخدام هذه الزيوت كمركبات آمنة فى مكافحة حشرة البق الدقيقى *F. virgata* في التجارب الحقلية و النصف حقلية.