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Studies on insecticidal and deterrent effects of olive leaf extracts on *Myzus persicae* and *Phthorimaea operculella*

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Abstract
In the present study, methanol, acetone and petroleum ether extracts of olive leaves were investigated for their insecticidal activity against *Myzus persicae* and deterrent effect on *Phthorimaea operculella* under laboratory conditions. Different concentrations 0.1%, 1% and 10% were implemented in the experiment. The results revealed that acetone and methanol extracts caused 100% mortality of *M. persicae* at 10%. Tests on *P. operculella* generated a very pronounced deterrence of oviposition and larval penetration at 10%. Data indicate that, the acetone extract proved to be most deterrent against larval penetration at 10 % in comparison with other tested extracts and concentrations with 71.7% of deterrent index. Moreover, the highest deterrent effect of oviposition was recorded to acetone extract with 25.9%, 65.2% and 93.3% at 0.1, 1 and 10%, respectively. Therefore, this study provides first report on the insecticidal and deterrent activities of olive leaf extracts against *M. persicae* and *P. operculella*.

Keywords: Olive leaves, extracts, insecticide, *Myzus persicae*, *Phthorimaea operculella*.

1. Introduction
The application of synthetic pesticides has led to several adverse effects such as food, soil, ground water, and air contamination with carcinogenic, teratogenic, and highly and acutely toxic residues [1, 2]. To overcome these problems, it is necessary to seek safe, convenient, environmental and low-cost alternative pest control methods. Among the various alternatives, natural plant products that are non-phytotoxic, biodegradable and ecofriendly are catching the attention of scientists worldwide [3-7]. In fact, plant extracts have several uses in insect control. These products have also been studied for acute toxicity, antifeedant, or repellent, attractant and fumigant effects, as well as inhibiting reproduction of many pest species [8-10].
Many studies were focused on the olive tree (*Olea europaea* L.) and in particular its leaves showing potent biological activities [11, 12]. It is used to enhance the immune system, in heart disease, and as an antimicrobial agent. Experimental animal studies on different total olive leaf extract or their constituents have demonstrated hypoglycemic, hypotensive, antiarrhythmic, antiatherosclerotic, vasodilator, antihepatotoxic, and antinephrotoxic effects [11, 12].
In the present study, different concentrations of methanol, acetone and Petroleum ether extracts of *Olea europaea* leaves were evaluated for their insecticidal and deterrent activities against the peach potato aphids (*Myzus persicae*) and the potato tuber moth (*Phthorimaea operculella*).

2. Materials and Methods
2.1 Plant extraction preparation
Olive, *Olea europaea*, leaves were collected on olive trees (cv. Chemlali) in January 2014 in the region of Chott-Mariem. They were cleaned and dried at 40 °C for one week until crisp. Then, dried leaves were ground and powdered with an electric stainless steel blender.
Powdered olive leaves (100 g) were placed in a 500 ml Erlenmeyer flask with 400 ml of different organic solvents: acetone, methanol or petroleum ether. The preparations were stirred during 24 hours at 25 ± 2 °C. Each extract was filtrated through Whatman n°1 filter paper. Filtrates were let to evaporate at room temperature during 72 h and stored in dark glass bottles at 4 °C until tested. A specific volume of each dry residue was used in the corresponding solvent to make different concentrations (0.1%, 1% and 10%).

2.2 Insect rearing

The insects used in this study were the *Myzus persicae* (Hemiptera: Aphididae) aphids and the potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae).

The peach potato aphids, used in the bioassay, were collected from pepper crop grown in greenhouses of the Regional Center for Research on Horticulture and Organic Agriculture.

The potato tuber moths were reared on potato tubers in the laboratory of entomology in the Regional Center for Research on Horticulture and Organic Agriculture. *P. operculella* colonies were kept in standard conditions at 28 °C and 60% humidity. All bioassays were carried out under the same environmental conditions as the cultures.

2.3 Bioassays

2.3.1 Insecticidal activity assay against *Myzus persicae*

Different concentrations (0.1%, 1% and 10%) of each extract were sprayed directly on pepper leaves attacked by *M. persicae*. Controls received the corresponding organic solvent (Acetone, methanol or petroleum ether). Leaves were placed in Petri dishes measuring 9 cm*1.3 cm coated with filter paper. Plates were maintained in a climatic chamber at 26 ± 2 ºC, relative humidity of 60 ± 10% and photoperiod of 12 h. When no antennal or leg movements were observed, insects were considered dead. The assessment of mortality rate was recorded after 24 hours under binocular microscope as follows:

$M = [(A_i – A_f) / A_i] \times 100$

with M: mortality rate (%), A_i: Initial number of aphids, A_f: Final number of aphids after treatment.

2.3.2 Oviposition deterrence experiment

To study the oviposition deterrence effect of the three extract of *O. europaea* leaves, three concentration tests was carried out: 0.1, 1 and 10%. For this experiment, two new emerged males and females were introduced into plastic box (23×23×23 cm) covered with mesh screen treated by each extract and kept at a temperature of 28±2 °C and a photoperiod of LD 12:12. Eggs in each box were counted three days after starting the experiment. Total number of eggs in each box was used to calculate an oviposition deterrent index (ODI) as follows:

ODI = 100 x (C-T) / (C+T),

Where C represents the total number of eggs in the control box and T the total number of eggs in the treated box.

2.3.3 Larval penetration deterrence experiment

Healthy and uniform potato tubers were soaked in 1 ml of 0.1, 1 and 10% of each extract for 10 min. The organic solvents were eliminated by evaporation at room temperature for 24h. Twelve tubers were introduced into oviposition cages of *P. operculella* for one week. The number of larvae penetrated into each potato tuber was counted and the larval penetration deterrent was determined by a larval penetration deterrent index (LPDI) as follows:

LPDI = 100 x (C-T) / (C+T),

where C represents the total number of larvae in the control tubers and T the total number of larvae in the treated tubers.

2.4 Statistical analysis

Five replications were performed for each test. For statistical comparison among several means, all data were subjected to a one-way analysis of variance (ANOVA) followed by mean comparisons (at $P = 0.05$) and Duncan test (SPSS 20.0).

3. Results

3.1 Insecticidal activity of *O. europaea* extracts against *Myzus persicae*

This experiment was conducted in order to determine the insecticidal activity of olive leaf extracts on *M. persicae* adults. Considerable differences in insect mortality were shown with different extracts at different concentrations.

The mean mortality of *M. persicae* treated with different extracts was shown in Table 1. The minimum mortality among the three extracts was observed at 0.1%. At 1%, the mortality values reached 94.4% for acetone extract. However, statistically, there are no significant differences ($P \leq 0.05$) between olive leaf extracts and between 1% and 10% (Table 1).

Table 1: Mortality rate (Mean ± SE) of *M. persicae* adults treated by foliar application with three concentrations of different extracts of *O. europaea* as compared to controls.

Treatments	Concentrations (%)	Mortality (%) (Mean ± SE)
Acetone extract	0.1	63.8 ± 6.8 [b]
	1	94.4 ± 4.4 [c]
	10	100 ± 0 [c]
Control (acetone)		14.3 ± 11.8 [a]
Methanol extract	0.1	63.2 ± 16.5 [b]
	1	92.5 ± 7.4 [c]
	10	100 ± 0 [c]
Control (methanol)		24.7 ± 12.8 [a]
Petroleum ether extract	0.1	71.6 ± 11.9 [b]
	1	92.3 ± 7.9 [c]
	10	94.6 ± 6.6 [c]
Control (Petroleum ether)		10.5 ± 7.7 [a]

[a] Means followed by different letters in the column indicate significant differences among the treatments.

3.2 Oviposition deterrent activity of *O. europaea* extracts against *Phthorimaea operculella*

The oviposition deterrent activity for the tested extracts at different concentrations on the *P. operculella* oviposition was reported in Table 2. The three extracts (acetone, methanol and petroleum ether) reduced significantly the number of eggs laid compared to controls (a positive deterrent index). The highest level of deterrent effect was recorded to acetone extract with 25.9%, 65.2% and 93.3% at 0.1, 1 and 10%, respectively. Similarly, methanol extract and petroleum ether extracts had a deterrent effect with 75.3% and 72.4%. Moreover, a positive correlation was observed between the oviposition deterrent and concentrations (Table 2).

In the same way, all olive leaf extracts reduced potato tuber moth larval penetration (Table 2). Data indicate that, the acetone extract proved to be most deterrent against *P. operculella* larvae at 10% in comparison with other tested extracts and concentrations with 71.7%. Furthermore, the number of larvae was reduced for potato tubers treated with methanol extract at 10% with 45.6%. Among the three tested extracts, petroleum ether had the lowest deterrent effect on larval penetration at 10% (Table 2).

Table 2: Deterrent effect of different concentrations of *O. europaea* extracts on the oviposition and larval penetration of *P. operculella*.

Treatments	Concentrations (%)	Oviposition deterrent index (%) (Mean ± SE)	Larval penetration deterrent index (%) (Mean ± SE)
Acetone extract	0.1	25.9 ± 11.6 [ab]	9.4 ± 9.2 [ab]
	1	65.2 ± 19.4 [cd]	38.3 ± 16.2 [bc]
	10	93.3 ± 2.5 [e]	71.7 ± 23.0 [d]
Methanol extract	0.1	8.1 ± 2.0 [a]	23.2 ± 15.8 [abc]
	1	19.5 ± 8.3 [a]	15.4 ± 5.5 [abc]
	10	75.3 ± 3.2 [de]	45.6 ± 20.5 [cd]
Petroleum ether extract	0.1	4.8 ± 3.9 [a]	20.9 ± 11.0 [abc]
	1	43.8 ± 12.4 [bc]	6.6 ± 10.6 [a]
	10	72.4 ± 21.2 [de]	25.3 ± 11.2 [abc]

[a] Means followed by different letters in the column indicate significant differences among the treatments.

4. Discussion

The results revealed insecticidal effects of the three olive leaf extracts at different concentrations. Toxicity of acetone and methanol extracts on *M. persicae* was reached 100% at a concentration of 10%. The insecticidal effect of *O. europaea* was already confirmed for *Euphyllura olivina* [13]. Also findings receive support from the results, that leaves of *O. europaea* produce natural phenolic compounds that are the responsible for the anti-insect effect and could prove useful for pest management [14, 15].

An important deterrence with a particular effect was observed for acetone extract on larval penetration into potato tubers and female oviposition of *P. operculella*. This finding agrees with Ben Hamouda *et al.* [16] that show that olive leaf extract can be used in a preventive control method of the desert locust. Some other different findings were reported by different scientists. Mohammed [17] indicated that olive leaf ethanol extract has a potential repellency against adults and larvae of *Tribolium confusum* with 80.9% and 98.7%, respectively. Barbouche *et al.* [18] have reported the anti-feeding effect of olive-leaf extracts on *Schistocerca gregaria*. It is conceivable that oleuropein plays a fundamental role in this activity, as it is contained in the olive leaves. Studies conducted on the effects of this compound are highly effective against *Dacus olea* females as a strong chemotactile repellent [19, 20].

The results of these investigations suggest that all the olive leaf extracts revealed the insecticidal effect against the peach potato aphids at different concentrations. Moreover, a deterrent activity was observed on larval penetration into potato tubers and female oviposition of *P. operculella.* The highest level was recorded for acetone extract.

As naturally occurring insecticides, these plant-derived materials could be useful as an alternative for synthetic insecticides controlling field populations of *M. persicae* or the potato tuber moth *P. operculella*. However, further studies on isolation of bioactive fraction/constituent may provide futuristic products for field application.

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