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Insecticidal activity of three plants extracts against *Myzus persicae* (Sulzer, 1776) and their phytochemical screening

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ABSTRACT

To reduce the use of synthetic pesticides and their negative effects on the environment, leaves extracts of *Artemisia herba-alba* Asso, *Eucalyptus camaldulensis* Dehnh and *Rosmarinus officinalis* L. were obtained with petroleum ether, ethanol and distilled water as solvents. These extracts were evaluated under laboratory conditions for their insecticidal effect against 3 to 4 days-old *Myzus persicae* individuals (Homoptera: Aphididae) at 1, 2.5, 5, and 10 %. We made observations after 24 hours. Etheric extract of all plants was effective and caused mortalities (100 %, 53 % and 60 % respectively) at the highest concentration. However, ethanolic and aqueous extracts did not show any significant insecticidal effect. The phytochemical screening showed the richness of etheric extract in terpenes. The results obtained suggest that we can make bioinsecticides based on leaves etheric extracts from these plants for use in integrated pest management.

Key words: *Artemisia herba-alba*, botanical insecticides*, Myzus persicae, Eucalyptus camaldulensis, Rosmarinus officinalis*

IZVLEČEK

INSEKTICIDNO DELOVANJE IZVLEČKOV TREH RASTLIN NA LISTNO UŠ *Myzus persicae* (Sulzer, 1776) IN NJIHOVA FITOKEMIČNA ANALIZA

Z namenom zmanjševanja negativnih učinkov sintetičnih pesticidov na okolje so bili narejeni izvlečki listov s petroletrom, etanolom in distilirano vodo iz naslednjih treh rastlin *Artemisia herba-alba* Asso, *Eucalyptus camaldulensis* Dehnh. in *Rosmarinus officinalis* L.. Insekticidni učinki teh izvlečkov so bili ovrednoteni v laboratorijskih razmerah na 3 do 4 dni starih osebkih listnih uši vrste *Myzus persicae* (Sulzer, 1776), Homoptera: Aphididae) v 1, 2.5, 5, in 10 % razredčitvah. Opazovanja so bila opravljena 24 ur po uporabi. Učinkoviti so bili izvlečki s petroletrom vseh rastlin, ki so povzročili smrtnost (100 %, 53 % in 60 %) pri največjih koncentracijah. Etanolni in vodni ekstrakti niso imeli značilnega insekticidnega delovanja. Fitokemična analiza ekstraktov je pokazala veliko vsebnost terpenov v izvlečkih, dobljenih s petroletrom. Rezultati raziskave nakazujejo, da lahko naredimo bioinsekticide iz analiziranih rastlin na osnovi izvlečkov s petroletrom in jih uporabimo v integriranem varstvu rastlin.

Ključne besede: *Artemisia herba-alba*, botanični insekticidi*, Myzus persicae, Eucalyptus camaldulensis, Rosmarinus officinalis*

1 INTRODUCTION

Chemical pest control employs potent chemical pesticides to reduce or eliminate pests and constitutes the major way in crop production throughout the world. In fact, the use of these conventional insecticides have over the years manifested a number of disadvantages, the most

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important of which are the risks involved for human health and for the environment (Ofuya and Okuku, 1994). To reduce the use of synthetic pesticides on fruit and vegetable plantations, phytochemicals and plant extracts have long been a subject of research in an effort to develop alternatives to conventional insecticides but with reduced health and environmental impact (Dancewicz *et al*., 2011). Most plant species that are used in phytomedicine contain ingredients, which inhibit the development of insects, hinder their feeding (antifeedants) or act as repellents and confusants (Laznik *et al*., 2010).

The green peach aphid, *Myzus persicae* (Sulzer, 1776) is found throughout the world. In addition to attacking plants in the field, green peach aphid readily infests vegetables and ornamental plants grown in greenhouses (Capinera, 2011). Its management is generally based upon the use of synthetic insecticides (Ciarla *et al*., 2005). Unfortunately, there is little knowledge in the literature about the effect of a plant extracts such as *Artemisia herba-alba* Asso, *Eucalyptus camaldulensis* Dehnh. and *Rosmarinus officinalis* L. against aphids.

This study aims to determine the toxicity of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* leaves extracts against *Myzus persicae* by using several concentrations.

2 MATERIALS AND METHODS

2.1 Selection of plant species and preparation of leaves extracts

In April 2013, leaves of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* were collected from Batna in the East of Algeria. It is located at 35° 61′ N latitude and 6° 24′ E longitude with an elevation of 1048 meter above sea level.

We prepared the extracts according to the method presented by N'guessan *et al*. (2009) using various techniques. First, we dried samples at 40 °C into an oven before grinding them with an electric grinder. Crude extracts were obtained by successive extractions with three solvents ordered by increasing polarity. In this order, we used petroleum ether, ethanol and distilled water. For extraction with petroleum ether, we dissolved 80 g of powder obtained from leaves in 240 mL of petroleum ether. A manual agitation was done for 10 min. Then, we filtered the mixture. The filtrate obtained was named etheric filtrate 1. On residual marks, we added 240 mL of petroleum ether; after 10 min of agitation and filtration, we obtained the etheric filtrate 2. The same operation allowed getting the etheric filtrate 3. These three filtrates were grouped together and concentrated on a bath sand. These series of operations resulted in a concentrated solution called etheric extract. After exhaustion by petroleum ether, residual marks were dried. The powder was recovered in 240 mL of ethanol. Homogenization by manual agitation during 10 min allowed getting the ethanolic filtrate 1. The same operation was repeated and gave the ethanolic filtrate 2. The ethanolic filtrates were also grouped and concentrated on a bath sand to give ethanolic extract. To prepare the aqueous extract, we infused dried powder in 800 mL of distilled water during 15 min. After filtration, we obtained aqueous extract. Finally, 4 concentrations were prepared from these extracts: 1, 2.5,5 and 10 %.

2.2 Aphid collecting and rearing

We collected last stage larvae of *Myzus persicae* (Sulzer, 1776) in April 2014 from Biskra in the East of Algeria. It is located at 34° 52′ N latitude and 5° 45′ E longitude with an elevation of 120 meter above sea level. These larvae were found on *Malva sylvestris* L.

A mass rearing of the green peach aphid was started on broad beans (*Vicia faba* L.) in a greenhouse. Each plant was inoculated with apterous adult when emerging in the morning. Aphids were collected after 10 days by brushing them carefully from the leaves.

2.3 Bioassay test

To determine the insecticidal effect of etheric, ethanolic, and aqueous extracts of selected plants, 15 *M. persicae* apterous larvae (3 to 4 days-old)

were placed in a Petri dish containing three leaves of *V. faba* soaked in different concentrations (1, 2.5,5 and 10 %) of these extracts with three replications. The experiment was carried out in the laboratory. The mortality was determined after 24 hours from the beginning of exposure. When no leg or antennal movements were observed, insects were considered dead (Salari *et al*., 2010).

2.4 Phytochemical screening

We characterized the different chemical groups of plants extracts using standard procedures as described by Kayani *et al*. (2007), Benmehdi *et al.* (2012), and Rahim *et al*. (2012).

2.4.1 Test for alkaloids

0.2 g of each extract was warmed with 2 % H_2SO_4 for two minutes. Then, few drops of Dragendorff's reagent (solution of potassium bismuth iodide) were added. Presence of orange red precipitate indicated as positiveness for alkaloids.

2.4.2 Test for terpenoids (Salkowski test)

0.2 g of each extract was mixed in 2 mL of chloroform, and concentrated H_2S0_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.3 Test for saponins

0.2 g of each extract was shaken with 5 ml of distilled water. Then, it heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

2.5 Statistical analysis

The efficiency (E %) of different extracts was calculated according to the Schneider-Orelli formula:

$$E\,\% = [\,(b - k)/(100 - k)\,] \times 100,$$

in which: **b** = percentage of individuals in the treated sample, and **k** = percentage of individuals found dead in the witness sample (Tomescu *et al*., 2009).

A chi-square test was applied to estimate the correlation between mortality and concentrations. Afterwards, data were subjected to the logistic regression model to determine lethal concentrations. We used the statistical program Statistica 8 (StatSoft, Inc., Tulsa, OK) for all analyses.

3 RESULTS AND DISCUSSION

In the conducted experiment, effects of the *A. herba-alba*, *E. camaldulensis* and *R. officinalis* leaves extracts were recorded in the control of the green peach aphid (*Myzus persicae*) after 24 hours. The statistical analyses indicated that only etheric extracts of all plants showed a dependence between the mortality and different concentrations ($P < 0.01$) (Table 1).

Table 1: Mortality of *Myzus persicae* larvae with different types of plants extracts

Plant	Extract	χ^2	*P*
Artemisia herba-alba	Etheric extract	146	0.00
	Ethanolic extract	2.0	0.4
	Aqueous extract	4.0	0.1
Eucalyptus camaldulensis	Etheric extract	75.81	0.00
	Ethanolic extract	4.0	0.1
	Aqueous extract	2	0.4
Rosmarinus officinalis	Etheric extract	91.7	0.00
	Ethanolic extract	1	0.6
	Aqueous extract	1	0.6

We obtained the greatest percentages of mortality of the aphid, 93 % and 100 %, with the two highest concentrations (5 % and 10 %) of *A. herba-alba*. In the case of *E. camaldulensis*, the percentage of mortality was almost 53 % at 10% and 60 % for *R. officinalis* at the same concentration (Figure 1).

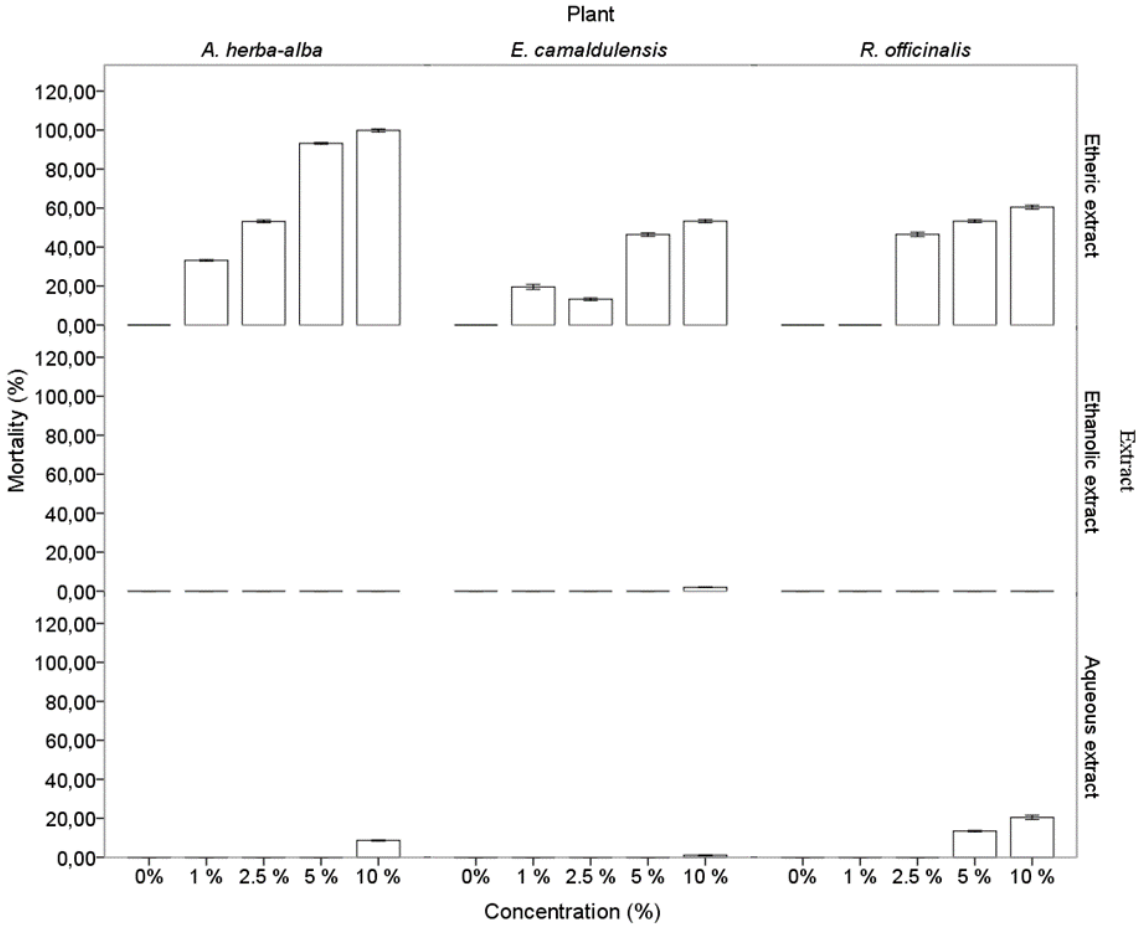

Figure 1: Average mortality (% ± standard error) of *Myzus persicae* larvae with leaves extracts from *A. herba-alba, E. camaldulensis and R. officinalis* with several concentrations after 24 hours of exposure

However, ethanolic extract of all plants did not show any dependence between mortality and concentrations (Table 1). The mortality of *M. persicae* obtained was 0% for all plants at a concentration of 5%, and almost 9% at 10 % when *E. camaldulensis* was used (Figure 1). Also, no dependence between the mortality and concentrations concerning aqueous extract of all plants ($P > 0.05$) (Table 1) was established. Nearly 8 % was obtained with *A. herba-alba* at 10 % and 13 % with *R. officinalis* at the same concentration (Figure 1).

From the Logit analyses, the LC_{50} (Lethal Concentration) was 2.07 % for *A. herba-alba,* 8.35 % for *E. camaldulensis* and 6.6 % in the case of *R. officinalis*. These data proved that *A. herba-alba* is more toxic than the other two plants etheric extracts (Table 2).

Table 2: Toxicity of etheric extracts on *M. persicae* larvae (LC_{50}'s [%] calculated by logistic regression)

Plant extract	B	Wald χ^2	*P*	Odds Ratio	LC_{50} (%)
A. herba-alba etheric extract	0.8	67.5	< 0.01	2.2	2.1 %
E. camaldulensis etheric extract	0.3	69.0	< 0.01	1.3	8.3 %
R. officinalis etheric extract	0.22	48.0	< 0.01	1.2	6.6 %

Soliman (2007) reported that *Artemisia herba-alba* oil gave a high toxicity with LC_{50} 0.023 % and caused 90.44 % reduction in the population on *Aphis gossypii* (Glover , 1877). Abdel-Shafy *et al*. (2009) evaluated the crude extracts with different solvents of *A. herba-alba* against the third instar larvae of *Chrysomyia albiceps* (Wiedemann, 1819). Results showed that all extracts had toxic effects on larvae. The settling of *M. persicae* on host plant leaves was strongly deterred by wormwood (*Artemisia absinthium* L.) (Dancewicz, 2008). *Artemisia seiberi* Besser oil extract was the most toxic to woolly apple aphid (*Eriosoma lanigerum* [Hausmann, 1802]) in terms of concentration and time responses compared with other tested oil extracts (Ateyyat *et al.*, 2012). Işık and Görür (2009) demonstrated that between 7 essential oils used against cabbage aphid (*Brevicoryne brassicae* [Linnaeus, 1758]), rosemary oil can be considered as an important aphidicide to control aphid population. It can be used also as an acaricide against the two-spotted spider mite (*Tetranychus urticae* Koch, 1836), causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants (Miresmailli, 2006). According to Rojht *et al*. (2012), the mortality rate of the *Acanthoscelides obtectus* (Say, 1831) adults after 7 days using ethanol extract of *R. officinalis* reached 91.2 % between the concentrations of 50 % and 100 %. The use of rosemary essential oils could significantly affect pest insects as well. In fact, the longevity, fecundity, and fertility of the cowpea weevils (*Callosobruchus maculatus* [Fabricius, 1775]), (Coleoptera, Bruchinae) were negatively affected by these oils (Douiri *et al*., 2014). Furthermore, 81.7 % adult mortality at 1 % concentration on sycamore lace bug (*Corythucha ciliata* [Say, 1832]) (Rojht *et al*., 2009). The highest mortality percentage against *Hyalopterus pruni* (Geoffroy, 1762) adults was achieved with extract of *E. camaldulensis* leaves, reached 92.6 % at 10 % after 48 days of treatment (Haji Younis, 2013). Elbanna (2006) recorded that 20 ml of the eucalyptus seed extract at concentration 1000 ppm caused 80 and 100 % mortality in larvae of *Culex pipiens* (Linnaeus, 1758) within 14 hours.

Phytochemical screening showed the presence of terpenoids in the etheric extract of all plants used in this study (Table 3).

Table 3: Phytochemical screening of *A. herba-alba*, *E. camaldulensis* and *R. officinalis* using different solvents

Plant species	Alkaloids			Terpenoids			Saponins		
	PE	E	W	PE	E	W	PE	E	W
A. herba-alba	-	-	-	+	+	+	-	-	+
E. camaldulensis	-	-	-	+	+	+	-	-	+
R. officinalis	-	-	+	+	+	+	-	-	+

(+) : positive, (-) : negative

Many works showed the richness in terpenoids of *A. herba-alba,* like monoterpene hydrocarbons (Behtari, 2012), oxygenated monoterpenes (Hudaib and Aburjai, 2006) and sesquiterpenes (Laid, 2008; Paolini, 2010). Qualitative and quantitative analyses of *A. herba-alba* and *Artemisia monosperma* Delile essential oils showed that both oils were mainly characterized by high concentration of total terpene compounds (75.281 and 78.69 % respectively) and low concentration of sesquiterpenes (24.357 and 21.023 % respectively) (Soliman, 2007). Miresmailli *et al*. (2006) and Verma (2012) pointed out the presence of monoterpenes in rosemary whereas Zhang *et al*. (2014) reported its richness in diterpenoid and triterpenoid glycosides. Terpenoids are abundant also in the foliage of *E. camaldulensis*, providing the characteristic smell as well as being valuable economically and influencing ecological interactions (Leicach, 2010; Külheim *et al*., 2015). Moreover, in *E. camaldulensis* oil, monoterpenes were prevalent while sesquiterpene hydrocarbons and oxygenated sesquiterpenes were less represented (Mediouni Ben Jemâa, 2012).

4 CONCLUSIONS

A. herba-alba, *E. camaldulensis* and *R. officinalis* leaves etheric extract were efficacious against *Myzus persicae*. The results obtained suggest that we can make bioinsecticides based on extracts from these plants for use in integrated pest management, which are a good alternative to conventional synthetic insecticides. This study is a first step and its purpose was to compare the effect of several plants leaves crude extracts against aphids. More studies will necessary to test the activity of each identified compounds against aphid species and other pests.

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