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Effect of some plant extracts in controlling the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract:

Laboratorial experiments are carried out to determine the effects of local plant extracts *Mentha piperita*, *Ricinus communis* and *Punica granatum* (At the rate of 10%) extraction from Water, ethanol and methanol on *Tetranychus urticae* Koch (Acari: Tetranychidae) at 25±5°C; 60±5% RH. The obtained results showed that the treatments of *M. piperita* extraction by methanol (PM), ethanol (PE) and water (PW) have reduction percentage values, 91. 71, 91.13 and 89.14 %, respectively, followed by treatment by *P. granatum* extraction have reduction percentage values, 90.98, 90.02 and 88.02%, respectively, and finally treatment by *R. communis* extraction have reduction percentage values , 88.77, 88.22 and 88.43 %, respectively. Extraction of *M. piperita, R. communis* and *P. granatum* by methanol was more effective than water or ethanol on *T. urticae*. Therefore, it could be concluded that the tested treatments exhibited the potential acaricidal effect ($P \leq 0.05$) on the population numbers of *T. urticae* compared to the control.

Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most common and harmful pests in vegetable production areas. Although more and more farmers consider biological control as a valid option to keep spider mites below economic damage thresholds, control of *T. urticae* is still mainly based on the application of acaricides (Çağatay *et al.*, 2018). *T. urticae* is notorious for its ability to develop acaricide resistance very quickly (Van Leeuwen *et al.*, 2010 and Van Leeuwen and Dermauw, 2016). Its short life cycle, high reproduction and fecundity all contribute to resistance development. Resistance has often been reported to evolve only a few years after the introduction of a new acaricide (Van Leeuwen *et al.*, 2009 and 2010). Another reason for fast resistance development is the polyphagous nature of the species, *T. urticae* is encountered on many crops, resulting in high acaricide exposure. In addition, the evolution to polyphagy might have equipped spider mites with a unique detoxifcation toolkit (Dermauw *et al.*, 2013), although other factors in resistance development might prevail in the broader context of arthropod pests (Dermauw *et al.*, 2018).

As an alternative to synthetic acaricidal agents, essential oils from plants constitute excellent candidates for pest control involving various modes of action that offer less mammalian toxicity and low persistence in the environment. Volatility is one of the most important characteristics of essential oils, enabling their use as fumigating agents for the control of pests under greenhouse conditions (Aslan *et al.*, 2004). Essential oils are complex mixtures comprised of monoterpenes, sesquiterpenes and phenylpropanoids, which are chemical classes of substances known for their acaricidal properties (Araújo *et al.*, 2012; Moraes *et al.*, 2012 and Ribeiro *et al.*, 2016). One of the advantages of using essential oils in the formulation of a plant-based acaricidal agent is the fact that possible resistance in the target pest generally takes a much longer time to develop for a mixture of natural active compounds compared to any single component (Koul *et al.*, 2008).

Mentha piperita is a well-known genus belonging to family *Lamiaceae* shows a reputed medicinal and aromatic value. That genus includes about 30 species that grow in the temperate regions of Eurasia, Australia and South Africa (Dorman *et al.*, 2003).

Pomegranate fruit has been used in China, India, Japan, and the Mediterranean basin as a medicine to cure a variety of diseases. Recently, the traditional usage was corroborated by scientific data indicating that pomegranates are a good source of antimicrobial, anticancer, and antidiabetic compounds (Seeram *et al.*, 2006). Moreover, different pomegranate accessions contain different amounts of bioactive compounds such as punicalagin, punicalin, galagic acid, and ellagic acid (Tzulker *et al.*, 2007). However, the effect of plant-based castor leaves on *T. urticae* has not been studied till date.

The aim of this study is to evaluate the toxic effects and repellence of *M. piperita* and *Punica granatum* and *Ricinus communis* extracts by organic solvents against *T. urticae* under laboratory condition.

Materials and methods

1. Plant extracts:

Leaves of peppermint (*M. piperita*) and castor (*R. communis*) as well as peels of pomegranate (*P. granatum*) against the two-spotted spider mite *T. urticae*. (Table 1)

Table (1): Plant extracts used in the present study

English Name	Scientific Name	Family	Part used	active constituent
Peppermint	*Menth piperita*	Lumiaceae	Leaves	Menthol
Pomegranate	*Punica granatum*	Lythraceae	Peel	Ellagic acid
Castor	*Ricinus communis*	Euphorbiaceae	Leaves	Ricin

2. Extraction procedures:

2.1. Extraction by water:

Peppermint and castor leaves and peels of pomegranate (Table 1) were dried and grinded. 200 gm. powder of each were dissolved in 1000 ml of boiled water, mixed and covered to prevent evaporation of the volatile oils. Incubated for 24h at RT (room temperature). Occasional shaking of mixture was carried out to get maximum extraction, then it was blended for 15 min. in a laboratory blender. The mixture was filtered to eliminate the wastes. The crude extracts were weighed and kept in refrigerator. Series of dilutions were prepared using distilled water to make required concentrations (Berktas and Cam, 2020).

2.2. Extraction by the organic solvent:

2.2.1. Methanol:

Peppermint and castor leaves and peels of pomegranate (Table, 1) were dried and grinded. 200 gm. powder of each were dissolved in methanol (MeoH) (1 gm: 7 ml methanol: 3ml Water) for 24/hrs., and then blended for 15 min. The mixtures were set aside for 3 days at RT. with shaking each day and then filtrated. The mixtures were transferred to round bottles in a rotary evaporator adjusted at 60°C until dryness. The mixtures were evaporated. The crude extracts were weighed and kept in refrigerator. Series of dilutions were prepared using distilled water to make required concentrations (Ahmed *et al* ., 2021 and Pramila *et al.*, 2012).

2.2.2. Ethanol

Peppermint and castor leaves and peels of pomegranate (Table, 1) were dried and grinded. 200 gm. powder of each were dissolved in ethanol (1 gm: 7 ml ethanol: 3ml Water) for 24/h, and then blended for 15 min. The mixtures were set aside for 3 days at RT. with shaking each day and then filtrated. The mixtures were evaporated. The crude extracts were weighed and kept in refrigerator. Series of dilutions were prepared using distilled water to make required concentrations (Vasavada and Inampudi, 2020).

3. Rearing technique of mites:

A pure culture of *T. urticae* was reared at (25±5°C) in the Pharmaceutics laboratory, Pharmaceutics Department, Faculty of Pharmacy, The British University in Egypt. *T. urticae* was hosted on the lower surface of mulberry leaves (*Morus nigra* L.) which placed on filter paper in Petri-dishes (20 cm in diameter) padded with moist cotton. The cotton pads were moistened daily to avoid disc dryness, and to prevent mite escape. The damaged infested leaves were placed on new ones to maintain mites feeding which assure its proliferation and transformation. Females were collected, then each female was cultured in Petri-dishes provided with surplus of *T. urticae* to get small colonies which used for further tests.

4. Effect of extracts on different developmental stages of *Tetranychus urticae*:

To test the susceptibility of *T. urticae* to different groups of extracts, mulberry leaves were placed in Petri dishes (15 cm in diameter) padded with moist cotton which represented five replicates for each treatment. To study the effect of the extracts on *T. urticae*, five gravid females for 24 h. At the end of this time the spider mites were removed, and the number of eggs was adjusted to 50 by destroying, removing or adding eggs using a fine brush. All discs except controls were treated with extracts by spraying. The treated discs were kept at RT. The number of adult females and the deposited eggs were counted daily.

5. Treatments:

5.1. 10% peppermint extract water (PW) on 55 *T. urticae*; **5.2.** 10% peppermint extract ethenol (PE) on 55 *T. urticae*; **5.3.** 10% peppermint extract methanol (PM) on 55 *T. urticae*; **5.4.** 10% pomegranate extract water (PeW) on 55 *T. urticae*; **5.5.** 10% pomegranate extract ethanol (PeE) on 55 *T. urticae*; **5.6.** 10% pomegranate extract methanol (PeM) on 55 *T. urticae*; **5.7.** 10% castor extract water (CW) on 55 *T. urticae*; **5.8.** 10% pomegranate extract ethanol (CE) on 55 *T. urticae*; **5.9.** 10% pomegranate extract methanol (CM) on 55 *T. urticae* and 5.10. Control without any plant extract.

The reduction percentages of *T. urticae* average number were calculated according to the equation of Henderson and Tilton (1955).

$$\text{Reduction} = 1 - \frac{\text{Treatment after x control before}}{\text{Teaiment before x control after}} \times 100$$

6. Statistical analysis:

One-way analysis of variance (ANOVA) and mean comparison using Fisher's least significant difference (LSD) were conducted for the number of spider mite, using the software packages SPSS 16.0.0 (USA) for windows. Significance level was $P \leq 0.05$.

Results and discussion

The extract of peppermint (Menthol), pomegranate (Ellagic Acid) and castor (Ricin) were used to evaluate toxic effect on *T. urticae* population. Figure (1) shows the relation between time (Days) and the mean average numbers of *T. urticae* (Individual) for the previously mentioned 9 treatments and the control. Also, Table (1) shows the results of the average number of *T. urticae* population and their reduction percentage for all treatments. The experimental results showed that the spider mite population and their reduction percentage showed no significant difference between different extract treatments (LSD; *P* 0.895). However, there was significant difference of the spider mite population in the control treatment. (LSD; $P < 0.004$).

Control: Number of spider mites increased, and this increase continued till reached its peak (about 228 individual/Petri Dish) on day 15. After that, the population of spider mite started to decrease sharply due to the damage of leaves caused by the spider mite population, the population extinction on day 19 (Figure 1 and Table 2).

In all treatments the average of spider mite populations (Eggs, adults and total of spider mite; Figure 1) declined immediately after spraying the plant extracts.

- Peppermint 10% water (PW): Number of spider mites in this treatment decreased gradually compared to the control directly after spraying and reached zero on the 7th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 91.13% (Table 2).

Peppermint 10% ethanol (PE): Similarly, number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 7th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 89.14% (Table 2).

Peppermint 10% methanol (PM): Likewise, number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 6th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 91.71% (Table 2).

- Also, Pomegranate 10% water (PeW): Number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 9th day. (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 88.02% (Table 2).

Pomegranate 10% ethanol (PeE): Similarly, number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 9th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 90.02% (Table 2).

Pomegranate 10% methanol (PeM): The same number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 9th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 90.98% (Table 2).

- Finally, Castor 10% water (CW): Number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 9th day (Figure 1). The obtained reduction percentage of spider mite

population under this treatment was 88.43% (Table 2). **Castor 10% ethanol (CE):** Number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 8th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 88.22% (Table 2).

Castor 10% methanol (CM): Number of spider mites decreased gradually compared to the control directly after spraying and reached zero on the 10th day (Figure 1). The obtained reduction percentage of spider mite population under this treatment was 88.77% (Table 2).

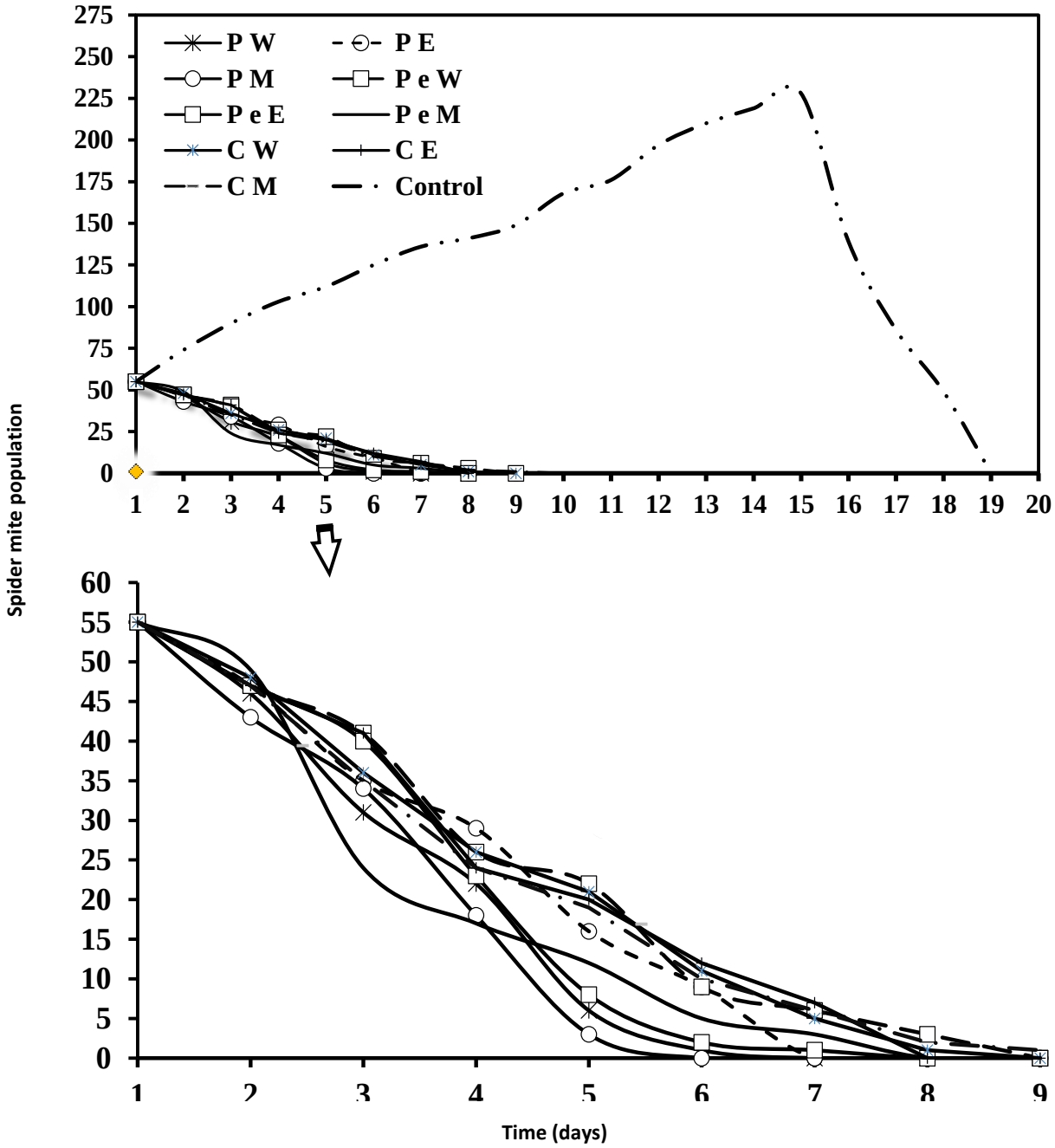

Figure (1) : The average numbers of *Tetranychus urticae* reduction by peppermint, pomegranate and castor extractions by methanol, ethanol and water as well as the control treatment.

Table (2): The average numbers of *T. urticae* and their corresponding reduction percentage (%) by peppermint, pomegranate and castor extraction by methanol, ethanol and water as well as the control treatment.

Treatments	Average ± SE	Max.	Reduction %
Peppermint 10% water (PW)	**10.73±4.83 $_{ab}$**	**55.00**	**91.13 %$_{A}$**
Peppermint 10% ethanol (PE)	**12.73±4.98 $_{ab}$**	**55.00**	**89.14 % $_{A}$**
Peppermint 10% methanol (PM)	**10.20±4.78 $_{a}$**	**55.00**	**91.71 %$_{A}$**
Pomegranate 10% water (PeW)	**13.93±5.02 $_{ab}$**	**55.00**	**88.02 % $_{A}$**
Pomegranate 10% ethanol (PeE)	**11.73±5.06 $_{ab}$**	**55.00**	**90.02 % $_{A}$**
Pomegranate 10% methanol (PeM)	**11.00±4.71 $_{ab}$**	**55.00**	**90.98 % $_{A}$**
Castor 10% water (CW)	**13.53±4.95 $_{ab}$**	**55.00**	**88.43 % $_{A}$**
Castor 10% ethanol (CE)	**13.73±5.00 $_{ab}$**	**55.00**	**88.22% $_{A}$**
Castor 10% methanol (CM)	**13.27±4.83 $_{ab}$**	**55.00**	**88.77% $_{A}$**
Control	**145.53±13.85 $_{c}$**	**228.00**	**-**

Means followed by different subscript letters within columns are significantly different from each other (P < 0.05) LSD test.

The previous results show that all treatments have close reduction percentage values. The menthol (Methanol) treatment has the highest reduction percentage value (91.71%). while, ellagic acid *(*Water) treatment has the lowest reduction percentage value (88. 02%). The curve of menthol treatments (Methanol, ethanol and water) reached zero on (6^{th} day, 7^{th} day and 7^{th} day, respectively) .The curve of ellagic acid treatment (Methanol, ethanol and water) reached zero on (8^{th} day, 8^{th} day and 9^{th} day, respectively). The curve of ricin (Methanol, ethanol and water) reached zero on (10^{th} day, 8^{th} day and 9^{th} day, respectively; Figure 1).

There are several studies in different countries to assess the effect and potential of plant extraction for controlling the pest without the use of pesticides without economic damage to the crop (e.g. Kotb, 2003; ElMougy and Alhabeb, 2009; Hussein *et al.*, 2013 and Abdallah *et al.,* 2015). Successful biocontrol can be obtained in many cases (e.g., Brødsgaard and Enkegaard, 1997; Messelink *et al.,* 2005 and 2006). Our result is closely and agree with Gorski and Piatek *(*2008); El-Zemity *et al*. (2009) and Abdallah *et al*. (2015) who recorded that the peppermint oil was the best extract to control of *T. urticae* with 92.70% redaction. The three essential oils that extracted by (Methanol, ethanol and water) have high efficiency in eliminating *T. urticae*. Menthol extraction by methanol better than extraction by water or ethanol in reduction percentage. Also, ellagic acid extraction by methanol better than extraction by ethanol or water in reduction percentage. Finally, the Ricin extraction by methanol better than extraction by ethanol or water in reduction percentage.

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