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Research Article

Effect of six ethanolic plant derived products on the pre-imago two-spotted spider mite

Ashrafju Mahla[1,2], Ahmadi Kamal[1*], Purhemati Amin[1,2] and Moshrefi Mandana[1,2]

[1]Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran
[2]Member of Young Researchers Society, Shahid Bahonar University of Kerman, Kerman, Iran

ABSTRACT: **Background:** Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most destructive cosmopolitan pests of many plant families. This pest has shown resistance to many synthetic acaricides applied in the field and glasshouse. Consequently, increased global efforts have been undertaken to develop other methods of pest control such as botanical insecticides. **Methods:** The effects of ethanol (95%) and ethanolic extract of six plant species (*Aloe vera*, *Calendula officinalis*, *Melia azedarach*, *Peganum harmala*, *Syzygium aromaticum* and *Juglans regia*) on *T. urticae* were investigated under laboratory condition. Leaf disks of bean (3.3 cm diameter) were sprayed by ethanol, as negative control, control without spray and plant extracts with 5 $mg.mL^{-1}$ concentration as treatments. Then new hatched larva were placed on the leaves and surveyed daily. Developmental times and mortalities of each treatment were evaluated until adult emergence. **Results:** The susceptibility of each developmental stage fluctuated for each plant extract. Among the applied plant derived chemicals, the greatest total mortality was recorded in *M. azedarach* (87.5%) and *P. harmala* (81.25%) treatments. Moreover, larval developmental time of the spider mite significantly increased in the both above-mention extracts.

KEYWORDS: *Tetranychus urticae*, plant extracts, mortality, acaricidal activity, developmental times

INTRODUCTION

The two-spotted mite, *Tetranychus urticae* (Acari: Tetranychidae), is one of the main pests of Iran and world agriculture which weakens the plants by feeding on cell sap from plant leaves.[1] This mite has a high reproduction rate and short growth period, and its population in low humidity and high temperatures sharply increases.[2] This pest could be controlled by synthetic insecticides or acaricides, but due to resistance, its management has faced difficulties[3] and farmers have to use higher concentrations of pesticides. The use of high pesticide concentrations will also have effects on non-target organisms, natural enemies and on the environment. In addition, due to the low effectiveness of conventional pesticides, farmers spray them frequently which causes a tremendous amount of pesticides that stay on the crop and irreparable consequences will follow. For example, pesticide residues in agricultural products, especially fresh vegetables and fruits will increase cancer risk in consumers and producers of agricultural products.[4]

There is an urgent need to find a safe, effective and natural way to control pests. The use of natural compounds and plant derived chemicals is a promising method for controlling insects and mites.[5] Many of these plant compounds have no toxicity or low toxicity to non-target organisms and mammals and create fewer risks for the environment.[6] These compounds, due to being natural, have far less adverse effects than chemicals and degrade easily in nature and do not have the problems caused by pesticide residues. Accordingly, nowadays the use of plant compounds is highly regarded and there are many scientific records in this field. Dehghani and Ahmadi[7] have investigated the effects of methanol extract of harmal and neem on puparium and nymphal development of greenhouse whitefly and reported significant increases during the nymphal stage. The effect of methanol extract of leaves and fruits of neem was also studied on the *Hyblaea purea*[8] and adverse effects on larval activity were shown.

*Correspondence
Ahmadi Kamal
Department of Plant Protection, Faculty of Agriculture,
Shahid Bahonar University of Kerman, Kerman, Iran
E-mail: kam_ahmadi@yahoo.com
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In this study, the effects of extracts of *Aloe vera* L. (Liliaceae) *Melia azedarach* L. (Asteraceae), *Calendula officinalis* L. (Asteraceae), *Syzygium aromaticum* (L.) (Myrtaceae), *Peganum harmala* L. (Zygophyllaceae) and *Juglans regia* L. (Juglandaceae) on pre-adult stages of *T. urticae* was investigated. These plants were chosen because there are some reports about their toxic and biological effects on other pests.

MATERIALS AND METHODS

A. Mite rearing

The initial populations of *T. urticae* were collected from the research greenhouse of Shahid Bahonar University of Kerman, Kerman, Iran [(30°15'15.39"N 57°06'14.38"E, 1772 m (altitude)] and were reared on bean plants. Plexiglas cylinders with 60 cm height and 27 cm diameter were placed and attached on 40 × 40 ceramic tiles to provide a protective cage for pots and mite colonies. Each pot was put in one of the cylinders, and a wide strip (5 cm) of Vaseline was rubbed on the top of the cylinder to prevent escape of mites. After about 2 weeks, colonies were ready for use in the experiments.

B. Extraction

Aloe vera Leaves, *Melia azedarach* fruits, *Syzigium aromaticum* flower buds, *Peganum harmala* seeds, *Calendula officinalis* seeds, and *Juglans regia* leaves were collected from gardens from different regions of Kerman province, except *P. harmala* which was a wild growing plant. The plants were dried in the shade at room temperature and then powdered by an electric steel grinder. The powder (50 g) was poured in a glass Erlenmeyer flask and 100 cm^3 of ethanol (95%) were added. The flask was closed with a Parafilm® layer, and after 15 minutes of shaking, the flasks were placed into the refrigerator for one day. The mixture was filtered by filter paper (Whatman N ° 91 filter paper) and was held in the freezer at –20°C until the experiment. To determine the extract concentration, 100 µl was pippetted and the extract was poured into a container with a specified weight, it was placed at room temperature for some minutes until all the ethanol was evaporated and purified extract remained. After obtaining the weight of pure extract, its concentration was evaluated and the required concentrations were prepared by dilution with ethanol.

C. Experimental protocol

The bean seeds were planted in the pots in the greenhouse with a temperature of 25 ± 5°C and humidity of 65 ± 10 percent. After approximately 20 days the leaves were suitable for testing. The bean leaf discs (3.3 cm diameter (Ø)) were prepared as substrate of experiments. Small Plexiglas containers (4 cm (Ø) and 5.2 cm height) containing a layer with 2 cm of 0.7% agar gel were prepared and each of the leaf disc was placed inside the container on the agar gel. Each of the treatment (with 15 replications) was sprayed with the ethanol extract or ethanol (95%), while the control treatment was not sprayed. After 15 minutes, a new larva (neonate) was put in the center of each leaf disc by a very fine camel hair brush. The lid of each container had a hole covered with mesh to allow ventilation. The dishes were maintained in a growth chamber with temperature of 25 ± 1°C, relative humidity of 60 ± 10% and 16 hour light (artificial light at an intensity of about 4000 lux) and 8 hour dark. The mites were checked daily and any changes including molting and mortality were recorded, and these experiments were continued until adulthood.

D. Data analysis

For statistical comparison of the developmental time, data were subjected to a one-way analysis of variance (ANOVA) followed by a Fisher LSD method (StatPlus version 4.9, 2007). The P values are different and indicated in the results.

RESULTS

Developmental time

The greatest effect on the larval stage was demonstrated in *M. azedarach* extract treatment (Figure 1). The mean larval developmental time of the mite in this extract treatment (1.78 day) was significantly different compare with the control (1.07 days) ($P \leq 0.0001$). Moreover, the extracts of *P. harmala* (1.45) and *A. vera* (1.42) exposure resulted in a significant increase in the larval developmental time. There was no significant difference between *M. azedarach*, *P. harmala*, and *A. vera* ($P \leq 0.02$). Other extracts which were investigated in this experiment showed little difference compared with the control.

During the other pre-imago stages of the mite (Figures 2 and 3), the length of each of life stage was not significantly different between different plant derived chemicals and control treatments.

Statistically, there were no significant differences in the mean total developmental time of the mite in the different treatment (Figure 4).

Mortality

The neem extract showed the highest mortality rate with 87.5% during the developmental time, and nonetheless, there was little difference in the mortality at different

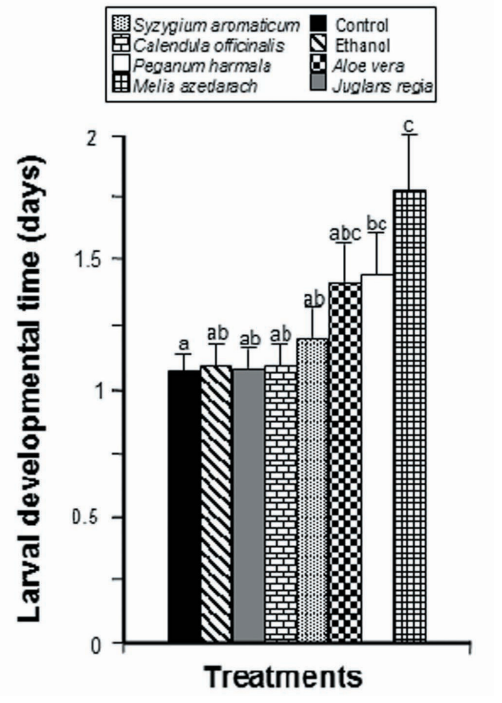

Figure 1. The average larval developmental time in *Tetranychus urticae* in control, ethanol treatment and plant extract treatments (different lowercase letters indicate significant differences between different treatments and the same letters indicate no significant differences).

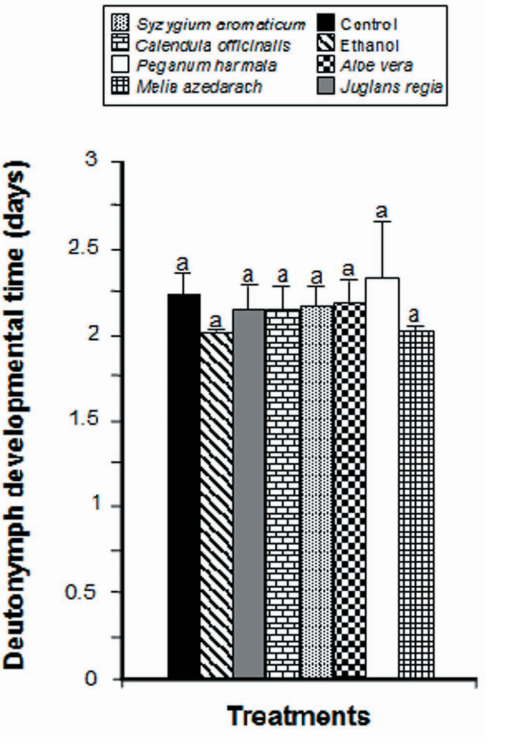

Figure 3. The average deutonymph developmental time in *Tetranychus urticae* in control, ethanol treatment and plant extracts.

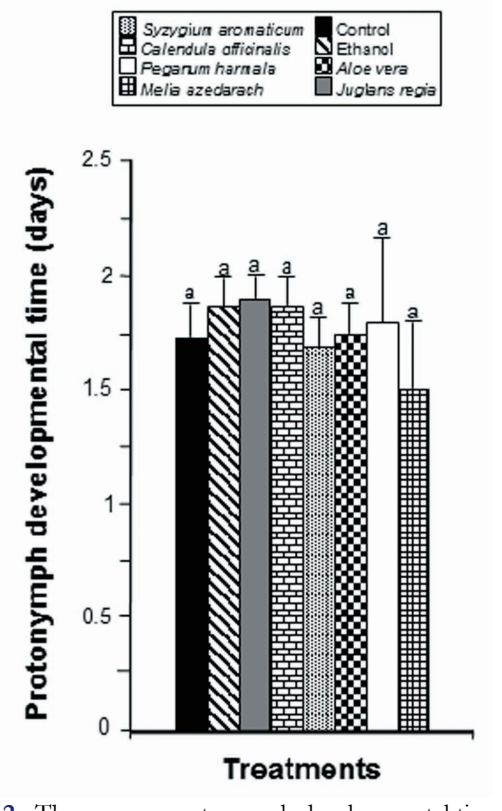

Figure 2. The average protonymph developmental time in *Tetranychus urticae* in control, ethanol treatment and plant extracts.

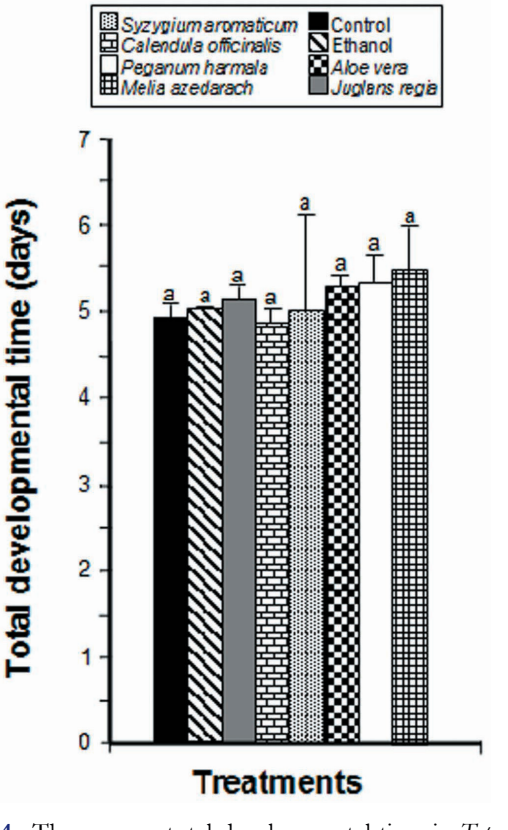

Figure 4. The average total developmental time in *Tetranychus urticae* in control, ethanol treatment and plant extracts.

Table 1: The average mortality in *Tetranychus urticae* in control, ethanol treatment and plant extracts

Treatment	Mortality during of nymphal development (%)			
	Larva	Protonymph	Deutonymph	Total
Control	0	0	13.13	13.13
Ethanol (95%)	26.67	27.27	25	60
Aloe vera	14.28	0	8.33	21.43
Juglans regia	13.33	16.67	30	50
Peganum harmala	25	50	50	81.25
Calendula officinalis	20	25	22.22	53.33
Melia azedarach	43.75	55.55	50	87.5
Syzygium aromaticum	11.76	13.33	7.69	29.41

pre-imago stages (Table 1). The mortality of larval stage in *P. harmala* extract treatment was 25% but it was increased to 81.25% until adult emergence. Among the different plant extract, the lowest mortality rate was recorded in *A. vera* treatment (21.43%).

DISCUSSION

As neem and harmal extracts showed maximum mortality in immature mites as well as significant increases in the larval period, plant-based acaricides are proposed as alternatives for managing two-spotted spider mite. Previous studies indicated that fruit of *M. azedarach* contains gedumin, nimbin, nimbolide and azadirachtin that have effects on insects and mites.[1]

The increase in larval developmental time might be the result of antifeedant plant compounds, and then mortality might be the result of starving. Therefore, this method can indirectly assess antifeedant effects of plant compounds on *T. urticae*. In another study by Nathan and Sehoon,[8] a methanolic leaf extract *M. azedarach* had adverse effect on larval activity of *Hyblaea purea* and reduced the rate of larval development. Dehghani and Ahmadi[7] stated that the greenhouse whitefly nymphal stages were significantly increased due to treatment by harmal and neem extracts. Another study[9] showed that the extract of harmal is effective on insect biology.

Generally, while mites are in ideal conditions, the growth rate in immature mites is high although they are very sensitive and vulnerable. If a low-concentration acaricide destroys immature populations, the whole population growth rate will decrease highly and the pest damages will decrease as well.

CONCLUSION

Herbal extracts of *M. azedarach* and *P. harmala* increased pre-adult developmental time in two-spotted spider mite and also impacted on the population dynamics so that population growth reduces. As a result, these herbal extracts can be considered as an alternative to conventional synthetic pesticides. But it is important to note that to use these plant compounds and formulate them, more scientific studies should be conducted at the biochemical and technical levels.

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