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Research Article

Investigations on the Effects of Five Different Plant Extracts on the Two-Spotted Mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae)

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Two-spotted mite, *Tetranychus urticae* Koch (Arac.: Tetranychidae), is an economic pest worldwide including Turkey, causing serious damage to vegetables, flowers, and fruit crops. In recent years, broad-spectrum insecticides/miticides have been used to control this pest in Turkey. Control is difficult mainly due to resistance to conventional pesticides. This study was conducted to determine efficacy of pesticides extracted from five different plants [i.e., *Allium sativum* L. (Amaryllidaceae), *Rhododendron luteum* S. (Ericaceae), *Helichrysum arenarium* L. (Asteraceae), *Veratrum album* L. (Liliaceae), and *Tanacetum parthenium* L. (Asteraceae)] against this mite. Bioassays were tested by two different methods to determine the effects of varying concentrations. Experiments were performed using 3 cm diameter leaf disk from unsprayed bean plants (*Phaseolus vulgaris* L.). In addition, the effects of the extracts on reproduction and oviposition were investigated. The extract yielded high mortality. In the lowest-concentration bioassays, the adult mites laid lower numbers of eggs compared to the untreated control. No ovicidal effect was observed.

1. Introduction

Diseases and insect pests are the major limiting factors in the production of high quality agricultural products. Although conventional pesticides have become an indispensable tool in controlling some pests economically, rapidly, and effectively, extensive use of insecticides may lead to a number of undesirable side effects including the development of insect resistance and resurgence of primary and secondary pests outbreaks. Also they can have adverse effects on nontarget organisms and general environmental contamination [1–4]. The other problems with synthetic insecticides are environmental pollution and insect resistance. According to Nas [5] interest in the application of botanical pesticides for crop protection is on the rise. Many researchers are experimenting and developing alternative plant extracts as pesticides to be used against pest insects.

Plants have the richest source of renewable natural pesticides. Specifically, plant extracts provide a safe and viable alternative to synthetic pesticides and are compatible with the use of beneficial organisms, pest-resistant plants, and to preserving a healthy environment in an effort to decrease reliance on synthetic pesticides. There are many benefits of using botanical pesticides such as reduced environmental degradation, increased safety for farm workers, increased food safety, reduction in pesticide resistance, and improved profitability of production.

As a result, many plant compounds, the majority of which are alkaloids and terpenoids, have now been known to affect insects' behaviour, growth and development, reproduction, and survival [6–9]. Many investigations have recently been performed in relation to effects of plants such as *Chrysanthemum roseum* Web. and Mohr. (Compositae), *Nicotiana tabaccum* L. (Solanaceae), *Derris elliptica* Benth (Fabaceae), neem tree, *Azadirachta indica* A. Juss (Meliaceae), *Melia azaderach* L. (Meliaceae), and *Xanthium strumarium* L. (Solanaceae) on insects [10–13]. The seed kernel extract of neem, known as azadirachtin, has been most

thoroughly tested, and it has been extracted in larger quantities than the other components of neem [14, 15]. High rates of mortality have been found on the two spotted mites fed on the leaves treated with *A. indica* extract. In addition, the same extract significantly reduced the reproductive capacity of mites and the survival of the progeny of treated females greatly diminished in comparison to the control [16].

T. urticae is a very important pest worldwide, causing serious damage to vegetables, flowers, and fruit crops. Many crops must be protected with synthetic acaricides during hot and dry seasons that favor severe outbreaks of *T. urticae*. It is able to transmit many of plants viruses [17].

R. luteum and *V. album* are poisonous plants. It is recorded that the extract of *V. album* has been used as insecticide or rodenticide since the Roman times. Also, today, plants containing toxic alkaloid are used successfully as insecticides and fungicides [18]. In one of the studies evaluating the effectiveness of plant extracts against house flies as indicated, *V. album* inhibited the development of the larvae and the high toxicity [19].

H. arenarium, T. parthenium, and *A. sativum* are important medicinal plants. *H. arenarium*, an infusion of the bright yellow flowers, is used in the treatment of gallbladder disorders and as a diuretic in treating rheumatism and cystitis. It is a component in *zahraa*, an herbal tea used for medicinal purposes in some countries [20]. *A. sativum* and *T. parthenium* have a broad spectrum of biological activity. They have been used for anti-inflammatory, antibacterial, and antifungal activities [21]. It is determined that the extract of *T. vulgare* inhibited the development of *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae). In addition, the same plant extract cultivated showed that it is effective on *T. urticae* [22]. The extract of garlic leaves caused high mortality and reduced reproductive capacity on *T. urticae* [23]. According to the literature, no Works have been published on the acaricidal activity of *H. arenarium*. This study was undertaken in the laboratory at the Central Plant Protection Research Institute in 2009, and the miticidal effect of five plant extracts on *T. urticae* was tested.

2. Material and Methods

2.1. Plants and Preparation of Extracts. This study covered five plant species; *R. luteum*, *H. arenarium*, *A. sativum*, *V. Album,* and *T. parthenium* were tested as an alternative miticidal. Their leaves and stems were collected when plants were at the flowering stage during the years 2008 and 2009. Only the fruit garlic plant was used for this purpose. Ethanol was used as a solvent to extract the required material from five plants for use as an acaricide. The method of Brauer and Devkota [24] was used in preparation of five plants' ethanolic extract.

The materials were stored in the laboratory to dry up. The dried materials were grounded using a blender, and ethanol was added to the dried powder for 72 hours. This mixture was extracted in 5-6 hours using a Soxhlet machine. The ethanol was removed from the extract in a rotary evaporator (50–60°C). For each plant sample 200 g of dried materials were used to prepare the extract.

2.2. Mites. As a test organism, *T. urticae* was reared on green bean plants, *Phaseolus vulgaris*. The bean plants used in the experiment were grown in a greenhouse.

2.3. Effects of the Extracts of Five Plants on Tetranychus urticae. In all the experiments, first instar larvae and 3-day-old adults were used. Four concentrations and an untreated control were used for all bioassays. Test samples for bioassay were resuspended in distilated water with TritonX.100 at a rate of 0.1 mL/L. Vaseline was used so as to prevent the mites from escaping. Experiments were carried out using (3 cm diameter) leaf discs of green bean leaves. The leaf disks were placed on a moistened filter paper disk and each disk was infested with 10 individuals. Each treatment was replicated 10 times. The concentrations used for mites were 1%, 3%, 6%, and 12% [16].

2.4. Effect on Eggs. Green bean leaf discs were placed into petri dishes on moistened filter paper and females of the same age were put on leaf discs. The eggs were counted after two days. Ten eggs were placed in every petri dish and the other eggs removed. Then the eggs were sprayed with different concentrations of extract (17–20 μL/cm^2) using a small hand-held sprayer. The numbers of hatched larvae were recorded.

2.5. Effect of the Extracts on Larvae and Adults

2.5.1. Leaf-Dipping Method. Green bean leaf discs were treated by dipping them into extract solutions of known concentrations, then left to dry for 30 minutes. The treated leaf discs and individual mites were placed in the petri dishes (9 cm in diameter) that were lined with moistened filter paper. The results were assayed after 1, 3, and 6 days by counting the number of living adults and larvae.

2.5.2. Leaf-Spraying Method. Green bean leaf discs were placed into Petri dishes on moisturized filter paper. Ten adults were placed in every Petri dish. Then eggs were sprayed with different concentrations of extract (17–20 μL/cm^2) using a small hand-held sprayer. The results were assayed after 1, 3, and 6 days by counting the number of living adults.

2.6. Effect on Egg-Laying Capacity. Green bean leaf discs were dipped for 3–5 seconds in prepared concentrations (1, 3, 6, and 12%), then they were dried for 30 minutes and placed in petri dishes with ten adults. After 48 hours of feeding on treated green bean leaves, mites were given untreated green bean leaves. The experiment was repeated 10 times. Daily monitoring was done for fourteen days and the total number of eggs was recorded [25].

The experiments were conducted in a climate chamber at 25-26°C and under long daylight (18 h : 6 h, light : dark). The effect was calculated according to Abbott [26]. The obtained reasults were submitted to a variance analysis and the mean values were compared by Duncan's test ($P = 0.05$) calculated by the program SPSS 13.6). Mortality rate was calculated as; mortality = after treatment the number of died mites/before treatment the number of mites · 100).

Table 1: Effect (mean ± SE) and mortality (%) of extracts obtained from different five plants on *T. urticae*.

Treatment		Leaf-dipping method				Leaf-spraying method	
		Larvae		Adult		Adult	
	Concentration (%)	Mortality (%)	Effect (%)	Mortality (%)	Effect (%)	Mortality (%)	Effect (%)
H. arenarium	1	46	31.59 ± 4.00^{c}	37	25.32 ± 4.10^{c}	52	39.76 ± 5.18^{c}
	3	53	41.59 ± 5.47^{bc}	47	37.22 ± 6.77^{bc}	66	59.88 ± 4.65^{b}
	6	58	46.09 ± 2.53^{b}	51	42.36 ± 5.61^{b}	76	71.82 ± 1.76^{ab}
	12	71	62.72 ± 2.28^{a}	64	56.85 ± 5.63^{a}	85	82.38 ± 1.92^{a}
A. sativum	1	46	31.30 ± 5.01^{b}	29	16.43 ± 2.43^{b}	66	59.76 ± 4.45^{b}
	3	50	37.80 ± 5.96^{b}	34	27.59 ± 5.17^{ab}	69	65.45 ± 5.16^{ab}
	6	56	43.37 ± 5.95^{b}	45	34.35 ± 6.76^{a}	77	72.79 ± 4.38^{a}
	12	68	58.35 ± 6.31^{a}	49	39.49 ± 5.07^{a}	78	73.92 ± 3.16^{a}
V. album	1	50	35.83 ± 4.33^{c}	51	29.07 ± 4.71^{c}	47	33.57 ± 4.12^{c}
	3	65	54.93 ± 5.22^{b}	61	42.41 ± 6.33^{b}	59	49.72 ± 3.39^{b}
	6	75	65.37 ± 3.15^{ab}	78	51.57 ± 5.37^{a}	70	62.58 ± 2.98^{b}
	12	77	70.55 ± 2.44^{a}	79	79.02 ± 3.76^{a}	81	75.77 ± 3.81^{a}
T. parthenium	1	49	38.22 ± 5.83^{c}	64	54.49 ± 4.34^{c}	47	33.61 ± 4.14^{c}
	3	64	54.06 ± 3.14^{b}	77	69.58 ± 1.52^{b}	60	49.75 ± 3.41^{b}
	6	75	67.89 ± 2.56^{a}	85	82.41 ± 1.94^{a}	71	62.62 ± 2.96^{b}
	12	82	76.54 ± 3.51^{a}	88	83.47 ± 1.95^{a}	81	75.68 ± 3.77^{a}
R. luteum	1	44	31.87 ± 3.31^{b}	27	31.66 ± 4.50^{b}	37	25.81 ± 2.94^{c}
	3	48	35.38 ± 4.05^{b}	44	34.02 ± 3.62^{b}	42	43.23 ± 3.40^{b}
	6	74	66.14 ± 4.50^{a}	53	44.35 ± 4.43^{b}	58	50.31 ± 3.28^{ab}
	12	81	75.62 ± 3.03^{a}	67	63.66 ± 2.44^{a}	68	61.97 ± 3.75^{a}
Control		22	0	15	0	15	0

Within columns, means ± SE followed by the same letter are not significantly different (DUNCAN's multiple F-test $P < 0.05$).

3. Results and Discussion

3.1. Effect on Eggs. All of the eggs treated were found to have hatched. It is determined that the ethanolic extracts of *R. luteum, H. arenarium, A. sativum*, *V. album*, and *T. parthenium* did not have an ovicidal effect. The hatched larvae continued to develop as it was in the control.

3.2. Effect of the Extracts on Larvae

3.2.1. Leaf-Dipping Methods. From Table 1, it can be observed that ethanol extracts of five plants had a significant mortality and the highest effect on *T. urticae* larvae. In all of the plant extracts, the highest effect occurred at a concentration of 12% while the smallest effect was at 1%. The increased concentration led to increased larval mortality. Statistical analysis showed $P < 0.05$ importance between the treatments. The extract of *T. parthenium* showed the highest effect on the *T. urticae* larvae. The smallest effect was at the extract of *A. Sativum*.

3.3. Effect of the Extracts on Adult

3.3.1. Leaf-Dipping Methods. As shown in Table 1, for the adults placed on leaf discs treated with different plant of extracts, the highest effect was determined at a concentration of 12% the extract of *T. parthenium*. Among the plant extracts, the extract of *T. parthenium* indicated the highest mortality. On the other hand, the smallest mortality was found at the extract of *A. sativum.* The increased concentration led to increased adult mortality.

3.3.2. Leaf Spraying Method. For the larvae placed on leaf discs treated with different plant of extracts at concentration of %12, mortality at the extract of *H. arenarium, A. sativum, V. album*, *T. parthenium*, and *R. luteum* was 85, 78, 81, 81, and 68%, respectively. In all of the extracts the highest effect was determined at a concentration of 12% while the smallest effect was at 1% (Table 1).

In both methods, similar results were obtained and there was not a significant difference on the mortality when leaf-dipping method was compared with direct spraying on the plant.

3.4. Effect on Egg-Laying Capacity. The numbers of eggs laid by mites feeding on extract-treated bean leaves were found to be statistically significant ($P < 0.05$) for all extracts with the maximum number of eggs obtained from the control. The lowest number of eggs was found at the 12% concentration of the extract of *R. luteum,* and the number of eggs laid was reduced significantly by increasing concentration (Table 2).

Ethanolic extracts were made from different plants and their effects were tested on two-spotted mite for the first time

Table 2: Effect of extracts from obteined different five plants on egg laying capacity of *T. urticae*.

Concentrations (%)	Treatment				
	H. arenarium	*A. sativum*	*V. album*	*T. parthenium*	*R. luteum*
	Number of eggs (mean ± SE)				
Control	162.5 ± 11.80^{c}	162.5 ± 11.80^{c}	162.5 ± 11.80^{c}	162.5 ± 11.80	162.5 ± 11.80^{c}
1	145.5 ± 5.91^{c}	184.0 ± 12.10^{b}	152.6 ± 10.50^{c}	158.0 ± 12.1^{b}	152.6 ± 10.50^{c}
3	94.5 ± 6.0^{b}	154.3 ± 10.3^{b}	137.6 ± 13.43^{c}	153.3 ± 10.3^{b}	137.6 ± 13.40^{c}
6	81.8 ± 6.40^{b}	115.2 ± 9.13^{a}	108.9 ± 19.9^{b}	136.2 ± 9.12^{b}	88.9 ± 19.92^{b}
12	62.5 ± 6.33^{ab}	98.2 ± 8.60^{a}	96.4 ± 2.52^{b}	87.2 ± 8.60^{a}	18.4 ± 2.50^{a}

Within columns, means ± SE followed by the same letter are not significantly different (DUNCAN's multiple F-test $P < 0.05$).

in the world. It was observed that some extracts showed a high rate of mortality and reduced fecundity on *T. urticae.*

There were no references in the literature of other studies using four plant extracts ethanolic extract on *T. urticae* except that *A. sativum*. However, other plant extracts have been investigated and the findings for *T. urticae* are similar to those of our study. Neem seed kernel extracts and its formulation are reported to influence mortality, repellency, and fecundity of mites [27–29]. It was found out that the two commercial preparations of neem seed extracts (Margosan-0 and Neem Azal S, Neem Azal T/S) were effective on *T. urticae* [16, 30]. Several herbal extracts of *Achillea millefolium* L. (Asteraceae), *Taraxacum officinale*s F. H. (Asteraceae), *Matricaria chamomilla* L. (Asteraceae), and *Salvia officinalis* L. (Lamiaceae) demonstrated strong inhibition of the feeding activity of mites [31, 32]. It was determined that the extracts of yew showed a high mortality, decrease in female fecundity and shortened longevity [33, 34]. Shi et al. [35] revealed that the extract of *Bassia scoparia* (L.) A. J. Scott. (Chenopadiaceae) showed contact and systemic effects, and it caused high rates of mortality in all the three species (*T. urticae, T. cinnabarinus*, and *T. viennensis*). Pure azadirachtin reduced the reproductive capacity and feeding of *T. urticae* [36]. Crude foliar extracts of 67 species from six subfamilies of Australian Lamiaceae showed both contact and systemic toxicity to these mites [37]. The extracts of wild tomato leaf showed strong repellency effect on *T. urticae* [38]. The acaricidal activities of plant extracts on *T. urticae* were tested. The mortalities were high in extracts *Albizia coreana* Twig., *Pyracantha angustifolia* F. (Rosaceae), and *Ligustrum japonicum* Thunb. (Oleaceae) within 48 h treatment [39]. Attia et al. [23] revealed that the extract of garlic led to a rise in female mortality and a reduction in fecundity with the increasing of concentration. Essential oils of *Artemisia absinthium* L. (Asteraceae) and *Tanacetum vulgare* L. (Asteraceae) were extracted by three methods, a microwave-assisted process (MAP), distillation in water (DW), and direct steam distillation (DSD), and tested for their toxicity as contact acaricides to *T. urticae.* DSD and DW extracts of *T. vulgare* were more toxic (75.6 and 60.4% mite mortality, resp., at 4% concentration) to *T. urtica* than to the MAP extract (16.7% mite mortality at 4% concentration) [22]. The ethanol extracts *of Croton rhamnifolius* H.B.K. (Euphorbiaceae) *C. sellowi, C. jacobinensis, and C. micans* had a high mortality on *T. urticae,* whereas *C. sellowi* extract showed the highest effect [40]. Garlic extract showed a mortality at 48–57% on *T. urticae* [41]. Wang et al. [42] revealed that the crude extract of walnut leaf had some contact and systemic effect on *T. cinnabarinus* and *T. viennensis*.

It was found out that the extract of *V. album* and *T. parthenium* had a high rate mortality and reduced fecundity for *T. urticae.* Ethanolic extracts of *V. album* and *T. parthenium* can be useful to control *T. urticae* populations on vegetable plants grown through Integrated Pest Management (IPM) and organic systems of agriculture.

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