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Full Length Research Paper

Acaricidal effects of different plant parts extracts on two-spotted spider mite (*Tetranychus urticae* Koch)

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Extracts of different parts of 12 plant species were evaluated for their potential for acaricidal activity that could lead to the development of new and safe bio-acaricides. The crude methanol extracts of these plants were tested for their acaricidal activity against the two-spotted spider mite *Tetranychus urticae* Koch in a bioassay under controlled conditions. All the extracts exhibited significant adult mite mortality as compared to control. *Lolium perenne* L. (flower, leaf), *Anthemis vulgaris* L. (flower) and *Chenopodium album* L. (flower, leaf) extracts had significantly higher mortality rates than azadirachtin (10 g/L) and the synthetic pesticides tested at 5% concentration in adhesive tape and residual film method. Our results showed that several plant extracts have good potential for acaricidal activity and are worth further investigation.

Key words: *Tetranychus urticae,* plant extract, mortality, acaricidal activity.

INTRODUCTION

Two-spotted spidermite (TSSM), *Tetranychus urticae*, is widely distributed worldwide and is a common pest of many plant species in greenhouses, nurseries, orchards and field crops (Jeppson et al., 1975). The mite causes plant damages by piercing the plant cells and sucking out the contents. The damaged cells appear as yellowish white spots (chlorophyl is destroyed) on the upper surface of leaf. As population increases, the whole leaf eventually turns yellow. Nymphs and adults produce webs and if the population is high the plant can be completely covered with webs. At this point, using miticides is necessary to control the TSSM. But the intensive use of synthetic pesticides in the recent years did not meet the criteria of integrated pest management programs.

There is a growing concern globally, over the continuous use of synthetic chemicals on food crops because of their potential effects on human health and the environment. Mite resistance is another problem as a result of continuous use of synthetic pesticides. For example, there are reports of spider mite resistance to organophosphates, dicofol, hexythiazox, clofentezine and abamectin only a few years after their introduction (Beers et al., 1998; Stumpf and Nauen, 2001, 2002).

There is an increasing interest for natural pesticides which are derived from plants and microorganisms (Isman, 2006; Isman et al., 2007) because they are generally perceived to be safer than the synthetics. These concerns have resulted in a renewed interest in search for alternative control measures. Plant extracts are one of several non-chemical control options that have recently received attention . There are several reports on botanical acaricides. Neem (Sundaram and Sloane, 1995; Martinez-Villar et al., 2005), *Calotropis porcera* (Ait.) (Asclepiadecae), *Nerium oleander* L. (Apocynacae) (Islam et al., 2008), tansy (*Tanacetum vulgare* L.) and wormwood (*Artemisia absinthium* L.) extracts (Chiasson et al., 2001), and garlic oil (Boyd and Alverson, 2000), *Satoreja hortensis* L. (Aslan et al., 2004) were found to be effective against two-spotted spider mite adults.

The objective of this study was to determine the acaricidal effects of different plants and plants' parts extracts on *T. urticae* using adhesive tape and residual film methods.

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Abbreviations: TSSM, Two-spotted spidermite; **SEM,** standard error of mean; **LC_{50},** half maximal lethal concentration.

Table 1. Plants used in contact and residual toxicity bioassay on *T. urticae*.

Family name	Scientific name	Tissue used
Apiaceae	*Conium maculatum* L.	Flowers, leaves
Asteraceae	*Artemisia vulgaris* L.	Leaves
Asteraceae	*Xanthium strumarium* L.	Fruits
Asteraceae	*Xanthium strumarium* L.	Leaves
Asteraceae	*Anthemis vulgaris* L.	Flowers
Asteraceae	*Anthemis vulgaris* L.	Leaves
Canabinaceae	*Humulus lupulus* L.	Flower buds
Chenopodiaceae	*Chenopodium album* L.	Flowers, leaves
Lauraceae	*Laurus nobilis* L.	Leaves
Meliaceae	*Melia azedarach* L.	Fruits
Myrtaceae	*Eucalyptus camaldulensis* Dehn	Flower buds
Myrtaceae	*Eucalyptus camaldulensis* Dehn	Leaves
Solanaceae	*Solanum nigrum* L.	Flowers, leaves
Solanaceae	*Solanum nigrum* L.	Fruits
Styracaceae	*Styrax officinalis* L.	Seeds coats
Styracaceae	*Styrax officinalis* L.	Seed
Poaceae	*Lolium perenne* L.	Flowers, leaves

MATERIALS AND METHODS

Maintenance of the two-spotted spider mite *T. urticae*

The local strain of *T. urticae* used in this study originated from infested leaves of bean (*Phaseoulus vulgaris* L. Pinto bean) which had not been sprayed with pesticides. The mite stocks were grown under controlled conditions in a controlled climate room at 25°C, 60 ± 5% r.h., with 16 h light (L): 8 h dark (D). Rearing was done on 2 - 3-week-old pinto bean (*P. vulgaris*). Bean plants were grown in 25 x 32 x 8-cm-high pots filled with peatmoss and vermiculite (2:1) and maintained in a growth chamber under controlled conditions (26°C, 60 ± 5% r.h. and 16:8 h (L:D) at Gaziosmanpasa University Research Station in Taşliçiftlik, Tokat. Mites were transferred from aging plants to younger ones by placing old leaves infected with mites near 7- to 10-day-old healthy seedlings. Individual female mites were collected and transferred for bioassay tests using a fine camel's hair brush.

Plants and preparation of crude extracts

In order to study in detail the potential of local plant species as botanical acaricides, different parts (flowers, leaves, fruits and seeds) of 12 plant species (Table 1) were chosen for this study based on results of previous studies (Chiasson et al., 2001, 2004; Italo et al., 2009; Jazzar et al., 2003; Mansor et al., 2004). Different parts of twelve plants were collected during spring and summer of 2004 in Tokat, except *Styrax officinalis* L. obtained from Muğla and *Melia azedarach* L. obtained from Adana. Each plant material was dried under shade and powdered by using electric grinder and kept in a dark condition at room temperature in a 3 L glass jars until used. Plant extracts were prepared from a representative sample of 100 g of each powdered plant material which were taken into a 2 L capacity erlenmeyer flask and 300 ml of methanol was added to it and shaken for 24 h in a horizontal shaker at 120 rpm at room temperature and then the extract was separated using four layers of cheese cloths and transferred into a 250 ml evaporating flask and evaporated under vacuum using a rotary vacuum evaporator (RV 05 Basic 1B, IKA Group) at 32°C. The resulting residue was dissolved in 10% acetone to yield 5 and 50% extract solutions (Gökçe et al., 2006b). The extract solutions were kept in a refrigerator at 4°C until used in the bioassay.

Acaricidal activity

Contact toxicity was assayed by using two different methods (Dent and Walton, 1997).

Adhesive tape method

Each plant extract was sprayed (1 ml) on double sided adhesive tape at 5% concentration and placed in Petri dish and left to dry for 4 to 5 h. Twenty adult female spider mites were placed on each sticky tape in a Petri dish. Ten percent (10%) acetone was used as a control. Petri dishes were incubated in an incubator at 25°C with 70% relative humidity. Mortality was assessed under a binocular microscope at 24 , 48 and 72 h after treatment. Mites were considered dead if movement was imperceptible after repeated gentle probing with a single-hair brush. Acaricidal activities of the extracts were measured in terms of percentage mortality. There were five replicates for each treatment.

Residual film method

Each extract was pipetted into a 90 mm glass Petri dish at 50% concentration (50 µl/dish) and spread with hokey disc and left to dry for 2 to 4 h. Twenty adult mites were placed into each Petri dishes and a tight-fitting lid was placed on the dish. The dish was then sealed with parafilm and incubated as mentioned earlier. Mortality were taken after 24 h. At the end of 24 h incubation, living mites were transferred onto fresh bean leaf and observations of the mite mortality were taken after 48 and 72 h. Four synthetic acaricides (bromopropylate 500 g/L, dicofol 50 g/L, fenpyroximate 50 g/L and spirodiclofen 50 g/L) and commercial azadirachtin (10 g/L) were used as pesticide control and distilled water containing 10% acetone was used as negative control in each bioassays. There

Table 2. Contact effect of methanol extracts from different plants against adult *T.urticae.*

Treatment	24 h mortality (%)
Lolium perenne (Flowers, leaves)	93.50 ± 2.48[a]
Anthemis vulgaris (Flowers)	92.34 ± 1.73[a]
Chenopodium album (Flowers, leaves)	91.15 ± 1.72[a]
Xanthium strumarium (Fruits)	85.88 ± 1.47[ab]
Conium maculatum..(Flowers, leaves)	81.11 ± 4.22[abc]
Xanthium strumarium (Leaves)	79.85 ± 0.83[abc]
Solanum nigrum (Flowers, leaves)	79.36 ± 4.65[abc]
Anthemis vulgaris (Leaves)	76.63 ± 2.08[abc]
Melia azedarach (Fruits)	76.45 ± 2.92[abc]
Artemisia vulgaris (Leaves)	75.12 ± 2.36[abc]
Styrax officinalis (Seeds coats)	73.25 ± 4.32[abc]
Laurus nobilis (Leaves)	69.72 ± 0.84[abcd]
Humulus lupulus (Flower buds)	67.84 ± 2.52[abcd]
Eucalyptus camaldulensis (Leaves)	55.57 ± 2.12[abcd]
Solanum nigrum (Fruits)	53.29 ± 0.58[abcd]
Eucalyptus camaldulensis (Flower buds)	47.15 ± 0.93[abcd]
Styrax officinalis (Seed)	31.28 ± 1.50[abcd]
Azadirachtin 10 g/l	16.28 ± 3.07[cd]
Bromopropylate 500g/l	19.07 ± 0.54[bcd]
Dicofol 50 g/l	23.21 ± 0.32[bcd]
Fenpyroximate 50 g/l	22.18 ± 1.74[bcd]
Spirodiclofen	22.41 ± 2.38[bcd]
Control	7.81 ± 0.06[d]

Values are expressed as mean ± SEM.
*The mean difference is significant at the 0.05 level (Tukey test).

were five replicates for each treatment.

Dose-mortality tests

Lolium perenne, Anthemis vulgaris (flower), *Chenopodium album* (leaf and flower), and *M. azedarach* L (fruit) extracts were further tested in dose-response bioassay based on their significant acaricidal effects on *T. urticae* adults in the initial mortality experiment. Residual film method was used as described in Dent and Walton (1997). Different concentrations: 0.05, 0.1, 0.5, 1, 5, 10 and 50% (w/w), were prepared from the stock solution. Fifty microliter (50 µl) of each concentration for every plant species was pipeted into a 90 mm glass Petri dish and spread with a hokey disc and left to dry for 2 to 4 h. In the control, 50 µl aceton water mixtures were applied to each dish. Each treatment was repeated three times. Twenty adult mites were placed into each Petri dishes and a tight-fitting lid was placed on the dish. The mites were incubated at 25°C with 70% relative humidity and mortalities were recorded for 7 days at 24 h intervals. Whole trial was repeated at three different days.

Statistical analysis

Data were corrected for mortality in the control using Abbott's Formula (Abbott, 1925) and then normalized using arcsine transfromation (Zar, 1999). Transformed data were analysed using analysis of variance (ANOVA). SPSS 10.00 program was used for analysis (SPSS, 2000). Treatment means were compared by Tukey HSD test at p = 0.05. Means (±standard error of mean) of untransformed data are reported. Dose-mortality data was analyzed using POLO-PC (LeOra Software, 1994) probit and logit analysis software according to Finney (1971). All calculations of half maximal lethal concentration (LC_{50}) values, lethal concentartion ratios and the tests of the equality and parallelism of slopes were carried out with POLO-PC program.

RESULTS

Acaricidal activity of plant extracts on adult mites

Crude extracts of different parts of 12 plant species were tested to evaluate their toxic effect at 24, 48 and 72 h against adult TSSM using two different methods (adhesive tape and residual film methods) and the obtained results have been summarized in Tables 2 and 3. All the extracts exhibited significant adult mite mortality as compared to control.

In adhesive tape method, *L. perenne* (flower, leaf), *A.vulgaris* (flower) and *C. album* (flower and leaf) extracts had significantly higher mortality rates than azadirachtin and the synthetic pesticides tested at 5.0% concentration

Table 3. Residual effect of methanol extracts from different plants against adult *T. urticae*.

Treatment	24 h mortality (%)
Chenopodium album (Flowers, leaves)	96.99 ± 0.61[a]
Conium maculatum..(Flowers, leaves)	95.18 ± 0.97[ab]
Anthemis vulgaris (Flowers)	92.37 ± 1.45[abc]
Lolium perenne (Flowers, leaves)	91.43 ± 0.86[abc]
Anthemis vulgaris (Leaves)	82.33 ± 1.60[abcd]
Melia azedarach (Fruits)	74.57 ± 0.39[abcd]
Solanum nigrum (Flowers, leaves)	69.88 ± 0.41[abcd]
Solanum nigrum (Fruits)	68.78 ± 0.17[abcd]
Xanthium strumarium (Fruits)	68.24 ± 0.94[abcd]
Styrax officinalis (Seed)	68.17 ± 0.24[abcd]
Laurus nobilis (Leaves)	66.11 ± 1.22[abcde]
Styrax officinalis (Seeds coats)	64.11 ± 0.82[abcde]
Eucalyptus camaldulensis (Leaves)	62.61 ± 0.07[abcde]
Humulus lupulus (Flower buds)	56.37 ± 0.99[bcde]
Artemisia vulgaris (Leaves)	54.13 ± 0.81[cde]
Xanthium strumarium (Leaves)	52.48 ± 0.88[cde]
Eucalyptus camaldulensis (Flower buds)	51.91 ± 1.27[cde]
Azadirachtin 10 g/l	45.66 ± 1.29[de]
Bromopropylate 500g/l	47.80 ± 1.61[de]
Dicofol 50 g/l	44.65 ± 1.06[def]
Fenpyroximate 50 g/l	18.96 ± 1.99[ef]
Spirodiclofen	53.93 ± 1.14[cde]
Control	5.38 ± 0.45[f]

Values are expressed as mean ± SEM.
*The mean difference is significant at the 0.05 level (Tukey test).

($p < 0.05$). The adult mite mortality rate was varied among plant extracts but the differences among them are not statisticaly significant ($p > 0.05$) (Table 2). The extract of *L. perene* (flower and leaf) revealed 93.5% mortality after 24 h treatment. Lowest mortality rate (31.28%) was obtained with *S. officinalis* (seed) extract at 5% concentration (Table 2).

In residual film bioassay, the highest mortality rate was obtained with *C. album* (flower, leaf) extract (96.99%) followed by *C. maculatum* (flower and leaf) (95.18%), *A.vulgaris* (flower) (92.37%) and *L. perenne* (flower and leaf) (91.43%). Least mortality rate was seen in *Eucalyptus camaldulensis* (Flower buds) with 51.91% mortality. All the plant extracts had higher mortality rates than the acaricides and azadirachtin used in the trials.

In both bioassays (residual film and adhesive tape method), toxicity of the extracts to the twospotted spider mite range from 31 to 96% after 24 h incubation and all the tested plant extracts resulted in significantly higher mortality than the control ($p < 0.05$). The least acaricidal action (31.28%) was noticed after 24 h at 5% concentration of *S. officinalis* (seed) extract in adehsive tape method. Besides, *S. officinalis* showed 68.17% mortality in the residual film method. In both bioassays, *C. album*, *L. perenne* and *A. vulgaris* showed high mortality rates when compared with control, acaricides (bromopropylate 500 g/L, dicofol 50 g/L, fenpyroximate 50 g/L and spirodiclofen) and azadirachtin 10 g/L.

Dose-mortality

Slopes of the dosage-mortality relationship for the two-spotted spider mite treated with *L. perenne*, *A. vulgaris*, *S. officinalis* (seed), *S. officinalis* (seed coat) and *C. album* extracts were 0.65, 1.64, 2.08, 1.93 and 2.79, respectively (Table 4).

Significant variation in response of the two-spotted spider mite to the tested plant extracts were observed and *C. album* extract was significantly different from other tested extracts, except *S. officinalis* (seed coat) ($p < 0.05$). The 50 values (fiducial limits 95%) were 1.73 (0.23 to 5.30) for *L. perenne*, 5.24 (1.82 to 7.63) for *S. officinalis* (seed), 3.88 (0.60 to 6.06) for *S. officinalis* (seed coat), 3.06 (1.10 to 5.02) for *A. vulgaris* and 10.09 (7.35 to 13.06) for *C. album* (w/w). Lethal concentration ratios were 0.33, 0.45, 0.56 and 0.17 for *S. officinalis* (seed coat), *S. officinalis* (seed), *A. vulgaris* and *C.*

Table 4. LC_{50} values and fiducial limits of different plant extracts for two spotted spider mite.

Tested plant extract (tissue used)	LC_{50}	Fiducial limits for LC_{50}	Slope ± SE	Lethal dose ratios
Lolium perenne (Flower)	1.73	0.23-5.30	0.65±0.10	-
Anthemis vulgaris (Flowers)	3.06	1.10-5.02	1.64±0.37	0.56
Styrax officinalis (Seed)	3.88	0.60-6.06	2.08±0.60	0.45
Styrax officinalis(Seed coat)	5.24	1.82-7.63	1.93±0.56	0.33
Chenopodium album (flower and leaves)	10.09	7.35-13.06	2.79±0.49	0.17

SE, Standard error.

album, respectively.

DISCUSSION

Application of plants or their extracts to control microorganisms has been used for hundreds of years by practitioners of traditional medicine. For the past decades, the acaricidal and insecticidal properties of the plant extracts have been widely used against phytophagous pests. For example, methanolic fruit extracts of 24 hot pepper accessions and ethanolic extracts of *Datura stramonium* leaves and seeds exhibited acaricidal, ovicidal and repellent activities against two-spotted spider mite, *Tetranychus urticae* (Koch) (Antonious et al., 2006; Kumral et al., 2009). In our findings, *X. strumarium* (fruits) extracts had more mortality (85.88% in adhesive tape method and 68.24% in residual film method) than *X. strumarium* (leaves) (79.85% in adhesive tape method and 52.48% in residual film method). In addition, caraway seed, citronella java, lemon eucalyptus, pennyroyal and peppermint oils gave 90% mortality against adult *T. urticae*, whereas 82 and 81% mortality was observed with sage and spearmint oils, respectively (Choi et al., 2004), while chloroform leaf extracts of *L. hirsutum* f. *glabratum* accessions (PI-251304, PI-134417, PI-134418, and PI-126449) exhibited greatest antibiotic activity on two-spotted spider mites. On the other hand, the hexane extracts of the same accessions showed greatest repellency (Antonious and Snyder, 2006). Our results demonstrated that the best plant extracts, which resulted in more than 82% mortality, were *L. perene, Anthemis vulgaris, C. album* and *C. maculatum* (flower and leaf extract) in both adhesive tape and residual film bioassays (Tables 2 and 3). These results are in agreement with previous studies showing the insecticidal activitiy of leaf and flower extracts of these four plant species to various developmental stage of different phytophagous insect species (Çalmasur et al., 2006, Sharma et al., 2006, Gökçe et al., 2006a, 2006b; Tayoub et al., 2006). *M. azedarach* (fruits) extract had 76.45 and 74.57% mortality in adhesive tape method and residual film method, respectively. Azadirachtin (10 g/L) had higher mortality in residual film method (45.66%) than adhesive tape method (16.28 %). There are many studies on insecticidial and acaricidial activities of neem products (Mansor et al., 1997, Italo et al., 2009, Jazzar et al., 2003). The results of the present study showed similarity with the results of previous studies which revealed that *M. azedarach* extracts had insecticidial and acaricidial activities on plant pests.

There were nearly ten fold difference in residual toxicities of *L. perenne* and *C. album* extracts to the mite. This difference could be as a result of different secondary metabolite contents of the extracts, for example, terpenoid, phenolic and alkaloid compounds. Among the tested extracts, *C. album* had the most steper slope as *L. perenne* had the shallower slope. These indicate that the two-spotted spider mite did not homogenously respond to the tested plant extract. Similar variations were observed with plant extracts tested on other arthropods (Gökçe et al., 2006a).

The biological activity of plant extracts is due to the various compounds present in the extracts. These compounds may independently or jointly contribute to cause acaricidal and ovicidal action against *T. urticae*. Results of this study suggest the possibility of developing suitable natural acaricide from *C. album, L. perenne* and *A. vulgaris* extracts. Further study is needed to identify the active compounds of these plant extracts responsible for their acaricidal activities under controlled and field conditions.

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