Scientific Research and Essays Vol. 6(14), pp. 3041-3044, 18 July, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.535
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

Ovicidal activity of different plant extracts on two-spotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae)

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Accepted July 3, 2011

Methanol extracts of nine plant species were evaluated for their ovicidal activity against the two-spotted spider mite *Tetranychus urticae* Koch in a bioassay under laboratory conditions. These plants and plant parts used in the study were *Xanthium strumarium* L. (fruits and leaves), *Anthemis vulgaris* L. (flowers and leaves), *Humulus lupulus* L. (flower buds), *Chenopodium album* L. (flowers and leaves), *Melia azedarach* L. (fruits), *Eucalyptus camaldulensis* Dehn (flower buds and leaves), *Solanum nigrum* L. (flowers, leaves and fruits), *Styrax officinalis* L. (seed coats, seeds) and *Lolium perenne* L. (flowers and leaves). The greatest mortality was caused by *E. camaldulensis* leaf extract (63.26%), followed by *X. strumarium* fruit (59.64%), *X. strumarium* leaf (57.45%), *S. nigrum* fruit (51.57%), *A. vulgaris* flower (46.80%) and *S. officinalis* seed extract (44.25%). *Lolium perenne* extract (flowers, leaves) caused the least mortality (24.40%). Azadirachtin at 10 g/l concentration was used as a chemical standard and caused 10.09% mortality. Our results show that some of these plant extracts have a potential for ovicidal activity on two-spotted spider mite eggs and are worth further investigation.

Key words: *Tetranychus urticae, Eucalyptus camaldulensis*, plant extract, ovicidal activity.

INTRODUCTİON

Two-spotted spider mite (TSSM), *Tetranychus urticae* (Acari: Tetranychidae), is widely distributed globally and a common pest of many plant species in greenhouses, nurseries, orchards and field crops. Two-spotted spider mites feed by puncturing cells and draining the contents, producing a characteristic yellow speckling of the leaf surface. They also produce silk webbing which is clearly visible at high infestation levels (Jeppson et al., 1975). When the population exceeds economic threshold the use of an acaricide is a necessity to control the TSSM. Plant-based insecticides can be ecologically friendly and safe alternative in managing pests and they can be incorporated in Integrated pest management programs (IPM). There is an increasing interest for natural pesticides which derived from plants and microorganisms due to they are assumed being safer than the synthetic pesticides (Isman, 2006; Isman et al., 2007). These concerns have resulted in a renewed interest in search for alternative control measures. Spider mites have very high reproduction capacity and lay numerous eggs, so that reducing the number of viable eggs is important to keep the mite population below economical injury level. Recent years, ovicidal synthetic and plant based pesticides have been preferred in spider mite management. In many studies, ovicidal activities of plant extracts or active compounds isolated from extracts were reported. Azadirachtin, one of the most active constituents in neem oil, is a good example of this, it acts as a feeding deterrent and limits the growth of insects and mites. Neem extract is also potent repellent, antifeedant, growth regulator and oviposition deterrent affecting more than 200 species of pests (Ascher, 1993). In the course of screening for novel naturally occurring insecticides from plants, the activity of the fruit extract of the Argentinian *Melia azedarach* L. (Meliaceae) and its recently described limonoid meliartenin were investigated by Carpinella et al. (2003). The fruit extract antifeedant activity was tested on a variety of herbivore and granivorous

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insects through choice tests.

16 of 17 species belonging to three orders consume significantly less food when treated with the extract (Carpinella et al., 2003). Mansour et al. (2004) evaluated twenty nine plant extracts and *Capparis spinosa* L., *Cyperus rotundus* L., *Eucalyptus camaldulensis* , *Lupinus pilosus* L., *Punica granatum* L., *Rhus coriaria* L. and *Tamarix aphylla* (L.) H. Karst. extracts caused a significant reduction in the mean number of eggs laid. compared with the control. Yang et al. (2007) tested twenty plant extracts acaricidal activity against *Panonychus citri* (Mc Gregor). Acaricidal effect of leaf and seed extracts of *Datura stramonium* L. were evaluated againts *T. urticae*. Leaf extracts of *D. stramonium* was found much more effective than seed extracts in acute toxicity tests (Kumral et al., 2009). Chiasson et al. (2004) evaluated the acaricidal effect of UDA-245 (based on essential oil extract from *Chenopodium ambrosioides var. ambrosioides*) and its' effects on egg hatch of the two-spotted spider mite (*T. urticae*) and the European red mite (*Panonychus ulmi* Koch). They reported that abamectin , insecticidal soap and neem oil reduced egg hatch of the two-spotted spider mite and the European red mite more significantly than UDA-245. Sarmah et. al. (2009) were tested four aqueous plant extracts of *Acorus calamus* L. , *Xanthium strumarium* L., *Polygonum hydropiper* L. and *Clerodendron infortunatum* (Gaertn) for acaricidal and ovicidal activity under both laboratory and field conditions at 2.5, 5.0 and 10.0% (w/v) concentrations against tea red spider mite, *Oligonychus coffeae* (Nietner).

The objective of this study was to determine the ovicidal effects of different plants and plant parts extracts on eggs of *T. urticae* to examine the possibility of use these extracts in the control of this pest.

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 Pinto bean (*Phaseoulus vulgaris* L.) 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 two or three week old pinto bean. 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 Agriculture Research Station, Tokat. Mites were transferred from aging plants to younger one 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.

Preparation of plants and of crude extracts

The plant species and their part used in the study are presented in Table 1. The plants were collected from Tokat, except *Styrax officinalis* L. obtained from Muğla and *M. azedarach* L. obtained from Adana provinces. Each plant material was dried under shade and powdered by using electric grinder and kept in a dark condition at room temperature in the 3 L glass jars until used. The extraction procedure used in the study was described by Gökçe et al. (2005). Plant extracts were prepared from a representative sample of 100 g of each powdered plant material 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. The plant suspension was sieved through four layers of cheese cloths to separate plant parts from the supension and it was 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% (w/w) acetone/water to yield 10% (w/w) extract solutions. The extract solutions were kept in a refrigerator at 4°C until used in the bioassay. Azadirachtin (Neemazal T/S) was used as chemical standart (3 ml/1 L dose). Water containing 10% aceton was used as control.

Ovicidal activity

For the assessment of ovicidal properties of the extracts, 10 adult females of two- spotted spider mite were introduced on detached bean leaf for oviposition and kept overnight in the petri dish. The leaves were padded with water soaked cotton. After 24 h the introduced mites were removed with the help of camel's hair brush. The eggs laid on bean leaves were counted under microscope as pre-treatment count. Bean leaves containing 20 eggs were sprayed with 0.5 ml of 10% each plant extract by using glass atomizer. There were five replication for each treatment, for the last trial we had four replication. Trials were repeated three times. The control eggs were also segregated as the aforementioned manner and treated with distilled water containing 10% aceton. The viability of eggs was determined for both experimental and control batches of eggs for a period of 10 days after oviposition. Those eggs that did not hatch after this period were regarded as non-viable.

Statistical analysis

Data were corrected for mortality in the control using Abbott's formula (Abbott, 1925) and then normalized using arcsine transformation (Zar, 1999). Transformed data were analysed using analysis of variance (ANOVA). SPSS 10.00 program used for analysis. Treatment means were compared by Tukey HSD test at $p = 0.05$. Means (±SEM) of untransformed data are reported.

RESULTS

Ovicidal activity

Ovicidal effects of all plant exracts were significantly higher than the control except perennial *ryegrass L. perenne* (flower, leaf) extract at 10% concentration (Table 2). The greatest egg mortality was observed in river red gum *E. camaldulensis* leaf extract (63.26%), this was followed by common cockleburr *X. strumarium* fruits (59.64%), *X. strumarium* leaves (57.45%), black nightshade *S. nigrum* fruits (51.57%), wild chamomile *A. vulgaris* flowers (46.80%) and storax *S. officinalis* seed (44.25%), *E. camaldulensis* flower buds (43.46%), common hop *H. lupulus* flower buds (43.15%) and *S.*

Table 1. Plants used in ovicidal activity bioassay on *Tetranychus urticae*.

Family name	Scientific name	Common name	Tissue used
Asteraceae	*Xanthium strumarium* L.	Common cockleburr	Fruits
Asteraceae	*Xanthium strumarium* L.	Common cockleburr	Leaves
Asteraceae	*Anthemis vulgaris* L.	Wild chamomile	Flowers
Asteraceae	*Anthemis vulgaris* L.	Wild chamomile	Leaves
Canabinaceae	*Humulus lupulus* L.	Common hop	Flower buds
Chenopodiaceae	*Chenopodium album* L.	White goosefoot	Flowers, leaves
Meliaceae	*Melia azedarach* L.	Bakain, chinaberyy	Fruits
Myrtaceae	*Eucalyptus camaldulensis* Dehn	River red gum	Flower buds
Myrtaceae	*Eucalyptus camaldulensis* Dehn	River red gum	Leaves
Solanaceae	*Solanum nigrum* L.	Black nightshade	Flowers, leaves
Solanaceae	*Solanum nigrum* L.	Black nightshade	Fruits
Styracaceae	*Styrax officinalis* L.	Storax, snowbell	Seeds coats
Styracaceae	*Styrax officinalis* L.	Storax, snowbell	Seed
Poaceae	*Lolium perenne* L.	Perennial ryegrass	Flowers, leaves

Table 2. Ovicidial effect of methanol extracts from different plant parts against *Tetranychus urticae* eggs.

Treatment	Ovicidial effect (%) mean ± SEM
Eucalyptus camaldulensis (leaves)	63.26±1.77 a
Xanthium strumarium (fruits)	59.64±1.83 a
Xanthium strumarium (Leaves)	57.45±0.67 a
Solanum nigrum (fruits)	51.57±1.84 ab
Anthemis vulgaris (flowers)	46.80±1.07 ab
Styrax officinalis (seed)	44.25±2.35 ab
Eucalyptus camaldulensis (flower buds)	43.46±1.26 ab
Humulus lupulus (flower buds)	43.15±1.47 ab
Solanum nigrum (flowers, leaves)	42.24±1.14 ab
Chenopodium album (flowers, leaves)	36.62±0.88 ab
Melia azedarach (fruits)	36.07±0.92 ab
Styrax officinalis (seeds coats)	35.39±0.78 ab
Anthemis vulgaris (leaves)	25.38±1.13 ab
Lolium perenne (flowers, leaves)	24.40±0.77 abc
Azadirachtin 10 g/l	10.09±0.51 bc
Control	0.00±0.00 c

nigrum (flowers and leaves) (42.24%). The least mortality was recorded with *L. perenne* (flowers, leaves) 24.40% mortality. Positive control Azadirachtin 10 g/l (3 ml/l) caused 10.09% mortality. Our results show that several plant extracts have a potential to use in control of two-spotted spider mite and are worth to further investigation.

DISCUSSION

For the past decades the acaricidal and insecticidal properties of the plant extracts have been widely tested against phytophagous pests for the past two decades. The results of present study showed that leaf and flower bud extracts of *E. camaldulensis* exhibited 63.26 and 43.46% mortality respectively on eggs of two-spotted spider mite at 10% extract concentration. Similarly Tunç et al. (2000) reported that essential oil of *E. camaldulensis* caused 45 and 12% mortality respectively on eggs of *E. kuehniella* and *T. confusum* at 196.9 µl/l air dose. In our findings, *X. strumarium* fruit and leaf extract caused 59.64 and 57.45% mortality respectively. Sarmah et al. (2009) reported 87.09% egg mortality at 10.0% (w/v) concentration of aqueous plant extracts of *X. strumarium*. Ethanolic extracts of *D. stramonium* leaves and seeds exhibited acaricidal, oviposition deterrent activities against two-spotted spider mite, *T. urticae* (Kumral et al., 2009). Leaf extract of *E. camaldulensis* exhibited the greatest eggs mortality with 63.26% at 10% extract concentration. It was followed by *X. strumarium*

fruit and leaf extracts with 59.64 and 57.45% eggs mortality respectively. The mortality rates of *T. urticae* eggs treated with *E. camaldulensis* (leaf), *S. nigrum* (fruit), *A. vulgaris* (flowers), *X. strumarium* (fruit),and *Styraxs officinalis* (seed) extracts were higher than that of other plant part extracts. These differences may be related to the difference in the chemical content of each plant species (Isman et al., 2007; Tayoub et al., 2006). Additionally, we also observed difference between different parts of the same plant species and this difference could be attributed the chemical contents of the leaf, flower, seed and seed coats extracts of these plants. For example, Kumral et al. (2009) reported that toxic effects of *Datura stramonium* seed extracts was lower than that of leaf extracts.

Chiasson et al. (2004) evaluated six concentrations of UDA-245 (based on essential oil extract from *Chenopodium ambrosioides var. ambrosioides*) at 0.5% (vol:vol), 0.7% (ai) of neem oil, 1% (ai) of insecticidal soap, 0.006% (ai) of abamectin, and a control (water) against two-spotted spider mite (*T. urticae*) and the European red mite (*Panonychus ulmi* Koch). Egg hatching rate for the two-spotted spider mite was significantly reduced after 5 and again after 9 days of application. The values were 8.2 and 8.8% egg hatch abamectin and 1.8 and 2.2% egg hatch neem oil respectively. These were significantly more effective than insecticidal soap (59.5 and 64.6% egg hatch, respectively) and UDA-245 (83.6 and 86.1% egg hatch, respectively). Egg hatching rate for the European red mite was not significantly reduced by UDA-245 (85.7%) compared with the control treatment (83.4%) 13 days after application. Abamectin was as effective as insecticidal soap and neem oil with 11.0, 14.2 and 29.6% egg hatch respectively, and all three were significantly more effective than UDA-245 and the control treatment (Chiasson et al., 2004). In the present study, egg hatch of two-spotted spider mite was reduced by white goosefoot *C. album* (63.38% egg hatch) and bakain *M. azedarach* (63.93% egg hatch) more than that caused by azadirachtin (89.91% egg hatch). In that study, neem product was reported to be more effective than our result. This could be related to difference between the dose used in our study and the dose used in that of Chiasson et al. (2004). Among the plant extracts used in this study, river red gum *E. camaldulensis* leaf and flower bud, common cockleburr *X. strumarium* fruit and leaf, black nightshade *S. nigrum* fruit, and wild chamomille *A. vulgaris* flower extract significantly reduced the egg hatch of two-spotted spider mite as compared to control. Besides the direct acaricidal effects of plant extracts on larvae, nypmh and adults of two-spotted spider mite, their ovicidal effects were also significant.

These results are very promising and encourage to further studies for isolation and characterization of the ovicidal compounds and their further evaluation for crop protection strategies.

ACKNOWLEDGMENT

This work was supported by a grant from the Scientific Research Council of Gaziosmanpasa University.

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