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Acaricidal Activity of Medicinal Plants Against the Developmental Stages of the Two Spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

A total of 18 plant extracts from the Mediterranean area were screened for acaricidal activity against the spider mite *Tetranychus urticae* Koch. eggs, deutonymphs and adults using a leaf disc bioassay method. Results showed that all extracts were ineffective against the egg stage and caused less than 30% mortality. Three plant extracts resulted in mortalities exceeding 50% against the deutonymph stage. These were *Ruta chalepensis* L. (65%), *Astragalus oocephalus* Boiss (55%) and *Urtica pilulifera* L. (51%). On the other hand, six plant extracts resulted in mortalities more than 50% to the adult stage. The highest mortality of 65% was achieved by treating adults with the extract of *Phlomis syriaca* Boiss. followed by *Achillea biebersteinii* Afan. (64%), *R. chalepensis* and *Ballota undulate* (Ghassa) (53%), *Alkanna strigosa* Boiss. and Hohenh. and *A. oocephalus* (52%). Further concentration response trials showed that the LC_{50}'s values for the extracts of *R. chalepensis*, *A. oocephalus* and *A. strigosa* were 8.5, 9.9 and 10.8% wt/wt, respectively. These results indicate that the extracts of *R. chalepensis*, *A. oocephalus* have the potential to be developed as botanical acaricides for eco-friendly management of *T. urtica*.

Key words: *Tetranychus urtica*, plant extracts, botanical acaricides, LC_{50}

INTRODUCTION

The two spotted spider mite, *Tetranychus urticae* Koch is considered the most damaging mite species for many vegetables, ornamentals and fruit trees in many parts of the world (Zhang, 2003). Due to its wide host range of over 150 plants (Jeppson *et al*., 1975), its high reproductive capacity and its ability to rapidly develop resistance to pesticides (Cranham and Helle, 1985), *T. urticae* is difficult to control. The predatory mite *Phytoseiulus persimilis* Athias-Henriot has been used commercially for the management of *T. urticae*. However, studies from functional responses of *P. persimilis* to different densities of spider mites showed that the predator might not provide sufficient control for high spider mite populations (Everson, 1979). Moreover, the development of this predator is adversely affected at temperatures exceeding 30°C (Malais and Ravensberg, 1992; Skirvin and Fenlon, 2003). Thus, infestations of *T. urticae* are managed by the application of chemical acaricides (Wilson *et al*., 1999). However, rapid development of pesticide resistance by mite populations (Tsagkarakou *et al*., 1996; Hoy, 2011) as well as public concern over the environmental impact and safety of chemical applications has stimulated research on alternative management tactics.

Plants have been traditionally regarded as a rich source of bioactive chemicals that might play a significant role in pest management. Hence, much effort has been focused on screening plants as potential sources of commercial botanical pesticides. For example, a total of 53 essential oils were

screened for acaricidal activity against *T. urticae* and *P. persimilis* as a fumigant. Caraway seed, citronella, lemon, eucalyptus, pennyroyal, and peppermint oil were found to be highly toxic to both mite species (Choi *et al.*, 2003). Similarly, a 29 plant extract were evaluated for their repellency and toxic effects on the carmine spider mite, *Tetranychus cinnabarinus* (Boisd.). Four of these tested extracts resulted in more than 25% mortality and 12 extracts caused at least 50% mite repellency (Mansour *et al.*, 2004).

Botanical pesticides are generally regarded as more environmentally friendly than synthetic pesticides. They are usually characterized by low mammalian toxicity, reduced impact on non-target organisms and short persistence in the environment (Georges *et al.*, 2008). Thus, the current screened 18 medicinal plants for their acaricidal activity against different life stages of *T. urticae*. The plants under study have long been used for medicinal and/or nutritional purposes in the Mediterranean region (Ali-Shtayeh *et al.*, 2000).

MATERIALS AND METHODS

***T. urticae*:** *T. urticae* was collected from cucumber plants (*Cucumis sativus* L.) grown a plastic house in Jordan Valley. The mites were cultured on bean plants (*Phaseolus vulgaris* L.) grown in 15 cm diameter pots filled with a mixture of 1:1 sand and peat moss. To obtain different life stages of *T. urticae*, bean plants were placed within the infested plants for 24 h. After an extra 24 h, all the adult mites were aspired from the plants. The infested plants were then kept at 24°C±2 and a 16 h photoperiod where *T. urticae* eggs were allowed to develop for the required life stage.

Plant extracts: Eighteen medicinal plants belonging to fourteen families were used in this study (Table 1). The plants were collected from their natural habitats from different parts in Jordan. When collected, they were in the early flowering stage except for *T. capitatus* that was collected before flowers appear. The selected parts of each plant (Table 1) were left to air dry for 4 to 5 days before grinding using a mortar and pistil to form a powder. Suspensions of 10% wt/wt of each plant powder to sterile distilled water were prepared and then allowed to boil for 10 min. The suspensions were allowed to cool overnight before filtering them through a cheese cloth to separate large particles. The extracts were prepared shortly before application. Tween 80 (Tedia Company, inc. 1000 Tedia way, Fairfield, OH, USA) at a rate of 0.02% was added to the extracts before application to improve adherence to treated plants. Suspensions of 20% wt/wt of the three plant extract: *Ruta chalepensis*, *Astragalus oocephalus* and *Alkanna strigosa* were prepared as above and served as a stock solution for preparing lower concentrations foe further concentration response trials.

Toxicological methods

Eggs: To study the effect of extracts on egg hatching, bean leaf discs 3 cm in diameter were cut from bean leaves and placed in Petri plates with water agar. Fifteen *T. urticae* females were allowed to oviposit on the leaf discs for 24 h. After removal of the females, the leaf discs were immersed for 5 sec in the suspensions of plant extracts, a solution of Propargite (Omite® 57°C) at a rate of 0.1% was used as a positive control and distilled water with 0.02% Tween 80 as a negative control. There were five leaf discs (replicates) for each plant extract and for each control. The leaf discs were allowed to air dry after which, the number of laid eggs for each disc was recorded and the discs were placed in Petri plates with water agar. The plates were incubated at 24°C±2 and a 16 h photoperiod. For egg mortality, numbers of unhatched eggs and newly emerged nymphs were counted 8 days post application.

Table 1: Medicinal plants evaluated against the two spotted spider mite

Scientific name	Family name	Plant part used
Yarrow, *Achillea biebersteinii* Afan.	Compositae	Leaves and stems
Alkanna strigosa Boiss. and Hohenh.	Boraginaceae	Leaves and stems
Anthemis palaestina Reut	Asteraceae	Leaves and stems
Herba- alba wormwood, *Artemisia inculta* Delile.	Compositae	Leaves and stems
Astragalus oocephalus Boiss	Leguminosae	Leaves
Common black horehound, *Ballota undulate* (Ghassa)	Labiatae	Leaves
Spurge, *Euphorbia hierosolymitana* Boiss.	Euphorbiaceae	Stem
Galium longifolium (Sibth and Sm.) Griseb.	Rubiaceae	Leaves
St. John's wort, *Hypericum perforatum* L.	Clusiaceae	Leaves
Garden cress, *Lepidium sativum* L.	Cruciferae	Fruits
Harmal peganum, *Peganum harmala* L.	Zygophyllaceae	Leaves and stems
Phlomis syriaca Boiss.	Lamiaceae	Leaves
Anise, *Pimpinella anisum* L.	Umbelliferae	Fruits
White broom, Juniper bush *Retama raetam* (Forssk.)	Leguminosae	Bark
Rubia tenuifolia d'Urv.	Rubiaceae	Leaves
Fringed rue, *Ruta chalepensis* L.	Rutaceae	Leaves and stems
Wild thyme, *Thymus capitatus* L.	Labiatae	Leaves and stems
Roman nettle, *Urtica pilulifera* L.	Urticaceae	Leaves

Adults and 2nd nymphal stage: The toxicity of the plant extracts to *T. urticae* was studied under laboratory conditions using a leaf-dip bioassay. A leaf cage was prepared from two 9 cm Petri plates by adhering the bottom of the upper plate to the cover of the lower plate. A 4 mm hole was made trough the two plates in which the treated leaves were inserted after treatment. A 2 cm opening covered with fine muslin was cut in the cover of the upper plate. The bottom of the lower plate was filled with water to prevent the wilting of the bean leaflet. A 9 cm filter paper with few water droplets was placed in the bottom of the upper plate to provide additional humidity. For leaf treatment, a twenty 2nd nymphal stage or adults of *T. urticae* were transferred to a bean leaf. To prevent the mites from escape, a sticky substance in the form of a ring (about 3 cm in diameter) was made on the upper side of each leaf using a plastic cylinder (Stumpf and Nauen, 2001). The leaves were immersed in the plant extracts and the positive and negative controls as above. After drying, they were inserted through the holes in the leaf cages. There were 5 replicates (leaf cages) for each plant extract or control. The leaf cages were incubated at 24°C±2 and a 16 h photoperiod and *T. urticae* mortality was recorded on the 2nd and 5th days post application. *T. urticae* were considered dead if they did not move after probing with a fine hair brush.

Concentration response trials: Based on the results obtained from the trials above, three plant extracts were selected for concentration response tests. These plant extracts were: *R. chalepensis*, *A. oocephalus* and *A. strigosa*. Concentrations of 2.5, 5, 10 and 20% were prepared from each plant extract and bio-assayed against adults of *T. urticae* as above.

Statistical analyses: Data were corrected for control mortality using Abbott (1925) formula before analysis. Percent of unhatched eggs and percent mortality of 2nd nymphal stage and adults of *T. urticae* were arcsine square-root transformed before subjecting to one-way ANOVA (PROC GLM, SAS, 2002). Means were separated using the Student-Newman-Keuls test (SNK) multiple range test. To calculate the overall effect, mortalities of 2nd nymphal stage and adults were averaged for

each plant extract. Probit analysis was used to estimate the lethal concentration 50 (LC_{50}) and the lethal time 50 (LT_{50}) for the plant extracts (PROC PROBIT, SAS, 2002). LC_{50}'s and LT_{50}'s are considered significantly different if their 95% confidence intervals did not overlap. The type I error rate (%) was set at 0.05 level for all tests. Mortality data were back-transformed to their original scales for presentation in tables.

RESULTS

Toxicity to eggs: Statistical analysis showed that the tested plant extracts significantly affected egg hatching of *T. urticae* ($F_{18,79}$ = 13.8, p<0.01). Percentage of unhatched eggs treated with the medicinal plants ranged between 1-29% (Table 2). The highest percentage of unhatched eggs was achieved by the acaricide Omite treatment which was significantly higher than all the tested plant extracts (Table 2). The highest percentage of unhatched eggs among the extracts resulted form the treatment with *A. palaestina* followed by *B. undulate* but with no significant reduction in egg hatching compared to most of the other plant extracts (Table 2).

Toxicity to adults and 2nd nymphal stage: Analysis of mortality data two days post treatment with the different plant extracts or the acaricide significantly affected the survival of the 2nd nymphal stage ($F_{18,76}$ = 11.7, p<0.01), the adults ($F_{18,76}$ = 20.4, p<0.01) and the overall effect ($F_{18,171}$ = 17.4, p<0.01). Further mean separation showed that the highest mortality of 2nd nymphal stage resulted from treatment with Omite which was significantly higher than all the tested plant extracts (Table 3). Three plant extracts resulted in mortalities exceeding 50%. These were *R. chalepensis* (65%), *A. oocephalus* (55%) and *U. pilulifera* (51%). Extracts of *A. biebersteinii*, *A. palaestina*, *P. syriaca* and *A. strigosa* resulted in mortalities ranging between 45-50% (Table 3).

Table 2: Percentage of unhatched eggs (±SE) of *T. urticae* treated with 18 plant extracts

Plant	Percentage of unhatched eggs
Omite	70±3.9^{a}
Anthemis palaestina	32±4.4^{b}
Ballota undulata	27±4.2^{c}
Rubia tenuifolia	22±6.0^{bc}
Pimpinella anisum	21±3.4^{bcd}
Urtica pilulifera	20±5.9^{bcd}
Thymus capitatus	20±3.8^{bcd}
Peganum harmala	19±2.9^{bcd}
Retama raetam	18±3.3^{bcd}
Euphorbia hierosolymitana	18±2.4^{bcd}
Ruta chalepensis	14±2.7^{cd}
Artemisia inculta	12±4.6^{cd}
Phlomis syriaca	11±3.7^{cd}
Astragalus oocephalus	11±4.8^{cd}
Hypericum perforatum	11±3.2^{cd}
Alkanna strigosa	8±3.1^{cd}
Galium longifolium	7±2.9^{cd}
Achillea biebersteinii	6±2.5^{d}
Lepidium sativum	4±2.1^{d}

Means within columns with different letters are significantly different at 0.05 level using Student-Newman-Keuls test (SNK) multiple range test

Table 3: Percentage mortality (±SE) of *T. urticae* developmental stages treated with 18 plants extracts two days post application

	Life stage		
Plant	2nd nymphal stage	Adult	Overall
Omite	81±4.1	84±2.5^{a}	83±2.4^{a}
Ruta chalepensis	66±5.1^{b}	53±4.3^{bc}	60±3.7^{b}
Astragalus oocephalus	55±2.5^{bc}	52±6.9^{bc}	54±3.5^{bc}
Urtica pilulifera	51±1.2^{bc}	31±2.9^{def}	42±3.5^{cd}
Achillea biebersteinii	50±2.5^{bc}	64±2.5^{b}	58±3.0^{bc}
Anthemis palaestina	48±3.5^{bc}	18±5.3^{f}	34±5.7^{de}
Phlomis syriaca	48±4.3^{bc}	65±1.2^{b}	57±3.8^{bc}
Alkanna strigosa	46±4.6^{bc}	52±6.1^{bc}	50±3.8^{bcd}
Artemisia inculta	44±6.4^{c}	24±6.9^{ef}	35±5.4^{de}
Pimpinella anisum	43±6.1^{c}	50±5.1^{bc}	47±4.0^{bcd}
Ballota undulata	42±4.3^{c}	53±2.9^{bc}	48±3.2^{bcd}
Peganum harmala	41±8.5^{c}	47±4.2^{cd}	45±4.6^{bcd}
Galium longifolium	39±2.9^{cd}	44±2.5^{cd}	42±2.1^{cd}
Hypericum perforatum	38±2.2^{cd}	45±2.5^{cd}	42±2.1^{cd}
Retama raetam	33±6.1^{cde}	5±5.4^{g}	19±5.9^{e}
Euphorbia hierosolymitana	33±6.1^{cde}	38±5.6^{cde}	36±4.0^{d}
Thymus capitatus	20±6.6^{ef}	20±2.0^{f}	20±3.3^{e}
Lepidium sativum	18±1.6^{ef}	21±3.3^{f}	20±1.9^{e}
Rubia tenuifolia	8±5.2^{f}	32±2.7^{def}	21±5.0^{e}

Means within columns with different letters are significantly different at 0.05 level using Student-Newman-Keuls test (SNK) multiple range test

Adults of *T. urticae* treated with the tested plant extracts suffered significantly lower mortalities compared to those treated with Omite (Table 3). Among the tested plant extracts, the highest adult mortality of 65% was achieved by treating adults with the extract of *P. syriaca* followed by *A. biebersteinii* (64%), *R. chalepensis* and *B. undulate* (53%), *A. strigosa* and *A. oocephalus* (52%) (Table 3). When the overall effect of each plant extract was determined, results showed that the highest overall effect was for Omite which was significantly different than the plant extracts followed by *R. chalepensis*, *A. biebersteinii*, *P. syriaca* and *A. oocephalus*. The overall effect for Omite and the plant extracts were 83, 60, 58, 57 and 54%, respectively.

Mortality data five days post treatment showed a significant effect on the 2nd nymphal stage ($F_{18,76} = 10.7$, $p<0.01$), the adults ($F_{18,76} = 24.5$, $p<0.01$) and the overall effect ($F_{18,171} = 16.6$, $p<0.01$) upon treatment with the tested plant extracts and Omite. For the 2nd nymphal stage, the highest significant mortality (93%) was achieved by the Omite treatment. The extracts of *R. chalepensis*, *A. oocephalus*, *A. strigosa*, *U. pilulifera* and *A. inculta* resulted in more than 50% mortality (Table 4). Moreover, the extracts of *A. biebersteinii* and *P. syriaca* resulted in 50% mortality. These mortalities were significantly higher than the mortalities resulted from the treatment with *T. capitatus*, *L. sativum* and *R. tenuifolia* extracts (Table 4).

Similarly, adults of *T. urticae* treated with Omite suffered the highest significant mortality (89%) compared with adults treated by the tested plant extracts (Table 4). Followed by the Omite treatment, the extracts of *A. biebersteinii*, *P. syriaca* and *A. oocephalus* resulted in more than 60% mortality while the extracts of *P. harmala*, *B. undulate*, *A. strigosa*, *P. anisum*, *R. chalepensis* and *G. longifolium* resulted in more than 50% mortality (Table 4). On the other hand, the extracts of

Table 4: Percentage mortality (±SE) of *T. urticae* developmental stages treated with 18 plants extracts five days post application

	Life stage		
Plant	2nd nymphal stage	Adult	Overall
Omite	93±0.0^{a}	89±1.2^{a}	91±0.7^{a}
Ruta chalepensis	66±5.1^{b}	53±4.3^{bc}	59±3.7^{b}
Astragalus oocephalus	56±2.5^{bc}	65±4.1^{b}	60±2.8^{b}
Alkanna strigosa	54±3.7^{bc}	55±6.0^{bc}	54±3.3^{bc}
Urtica pilulifera	51±1.2^{bc}	34±4.4^{ef}	42±3.4^{bcde}
Artemisia inculta	51±6.2^{bc}	29±4.6^{ef}	40±5.1^{cde}
Achillea biebersteinii	50±2.5^{bc}	67±2.2^{b}	58±3.4^{b}
Phlomis syriaca	50±3.7^{bc}	66±1.0^{b}	58±3.4^{b}
Anthemis palaestina	48±3.5^{bcd}	22±5.9^{f}	35±5.3^{def}
Peganum harmala	48±8.6^{bcd}	58±4.5^{bc}	51±5.0^{bcd}
Ballota undulata	45±4.6^{bcd}	58±2.7^{bc}	51±3.3^{bcd}
Pimpinella anisum	44±6.0^{bcd}	54±2.5^{bc}	49±3.6^{bcd}
Retama raetam	42±5.6^{cd}	7±5.7^{g}	24±6.8^{f}
Euphorbia hierosolymitana	41±7.5^{cd}	45±6.4^{cd}	43±4.7^{bcde}
Galium longifolium	39±2.9^{cd}	51±1.9^{bc}	45±2.7^{bcde}
Hypericum perforatum	39±2.2^{cd}	50±3.0^{c}	44±2.8^{bcde}
Thymus capitatus	26±8.6^{e}	24±1.2^{f}	25±4.1^{f}
Rubia tenuifolia	20±7.2^{e}	42±3.5^{cde}	31±5.4^{ef}
Lepidium sativum	18±1.6^{f}	23±4.0^{f}	20±2.3^{f}

Means within columns with different letters are significantly different at 0.05 level using Student-Newman-Keuls test (SNK) multiple range test

Table 5: Lethal time 50 (LT_{50}) and lethal concentration 50 (LC_{50}) of *T. urticae* developmental stages treated with plant extracts that resulted in more than 50% mortality

	Life stage					
	2nd nymphal		Stage		Adult	
Treatment	LT_{50}	C.I. (95%)	LT_{50}	C.I. (95%)	LC_{50}	C.I. (95%)
Omite	1.61	0.75-2.46	1.62	0.60-2.54	NA	NA
Ruta chalepensis	2.75	1.50-4.20	3.54	2.42-5.41	8.5	10.23-12.15
Astragalus oocephalus	3.51	2.40-5.33	3.06	2.15-4.19	9.9	11.64-13.70
Alkanna strigosa	3.80	2.79-5.54	3.56	2.39-5.64	10.8	12.14-13.72

LT_{50}'s and LC_{50}'s values with overlapping confidence intervals are considered not significant at 0.05 level

R. raetam, *T. capitatus* and *L. sativum* were significantly the lowest among the tested plant extracts (Table 3). For the overall effect, the acaricide Omite significantly outperformed the tested plant extracts resulting in 91% mortality (Table 4). The extracts of *A. oocephalus*, *R. chalepensis*, *A. biebersteinii*, *P. syriaca* were the highest among the tested plant extracts in their overall effect. Theses extracts resulted in 60, 59, 58 and 58% mortality, respectively (Table 4).

Estimation of the lethal time 50 (LT_{50}) for the acaricide and the plant extracts that resulted in more than 50% mortality for both the adults and 2nd nymphal stage showed that there were no significant differences between the Omite treatment and the extracts of *R. chalepensis* and *A. oocephalus* (Table 5). However, the estimated LT_{50} for the extract of *A. strigosa* was significantly lower than that of the Omite treatment for the 2nd nymphal stage but not the adult (Table 5).

On the other hand, the lowest estimated LC_{50} was for *R. chalepensis* (8.5%) followed by *A. oocephalus* (9.9%) and then *A. strigosa* (10.8%). No significant differences in LC_{50}'s were found among these plant extracts.

DISCUSSION

Using chemicals of botanical origin for pest suppression is an old practice that dates back ancient civilizations in China, India, Egypt and Greece (Isman, 2006). By the advent of synthetic chemical pesticides, the role of botanicals as well as other biological and cultural means of pest control tactics was drastically relegated by the new comers. The currently recognized hazards of chemical pesticides on humans and the environment generated great interest in eco-friendly pest control means such as botanicals. Few studies screened plant extracts for acaricidal activity against spider mites. Two out of 29 screened ethanol plant extracts resulted in more than 25% mortality against the carmine mite *T. cinnabarinus* (Mansour *et al.*, 2004). More work was focused on screening plant essential oils against spider mites (Choi *et al.*, 2003; Sertkaya *et al.*, 2010; Khani and Asghari, 2012). Therefore, this study was initiated to screen 18 plant extract for their acaricidal activity. The acaricidal activity of the tested plant extracts was clearly demonstrated as 4 and 7 plant extracts resulted in more than 50% mortality for nymphs and adults of *T. urticae*, respectively after 2 days of application. Moreover, the two plants extracts *R. chalepensis* and *A. oocephalus* caused more than 50% mortality for nymphs and adults of *T. urticae* after 2 and 5 days of applications indicating strong acaricidal activity. Previous acaricidal activity for those two plant extracts was not reported although, a strong insecticidal activity against the sweet potato whitefly, *Bemisia tabaci* was found for *R. chalepensis* water based extract (Al-Mazraawi and Ateyyat, 2009).

Some of the tested plant extracts belong to the same family (Table 1). However, plant extracts from the same family showed different effects on *T. urticae*. For example, *A. oocephalus* and *R. raetam* belong to the family Leguminosae but, the former caused 54% overall mortality to *T. urticae* while the later caused 19% after 2 days. of application. Similarly, the extract from *A. biebersteinii* caused 58% overall mortality while the extract from *A. inculata* resulted in 35% overall mortality although both of them belong to the compositae family.

All the tested plant extracts were ineffective against the egg stage as the percentage of unhatched eggs was less than 30%. Eggs of many mites and insect pests are generally regarded less susceptible to adverse effects such as chemicals or unfavorable weather conditions. Similar finding were reported when 20 plant extracts were screened against the sweet potato whitefly. Few of the tested extracts showed high activity against the adult and nymphal stages of the whitefly while none of them was effective against the egg stage (Al-Mazraawi and Ateyyat, 2009).

With the vast increase in public awareness towards the harmful effects of chemical insecticides on human health and the environment, plant extracts are expected to become increasingly important tools in pest management particularly in the developing world where ancient medicine based on plant extracts is largely practiced. The majority of the tested plants in the current study have been traditionally used as either food flavourings or in ancient medicine (Ali-Shtayeh et al., 2000). Therefore, extracts from these plants might be perceived as more safe for human health. Furthermore, chemicals based on plant extracts are expected to be more readily acceptable by growers which facilitate there incorporation in pest management programs.

In conclusion, out of 18 plant extracts screened for acaricidal activity against *T. urticae*, the extracts of *R. chalepensis* and *A. oocephalus* showed the highest activity. These findings open

avenues for more research in isolation and identification of the active secondary metabolites that might be responsible for that activity in an attempt to develop botanical acaricides for the management of *T. urticae*.

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