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Bioactivity of Indigenous Medicinal Plants against the Two-Spotted Spider Mite, *Tetranychus urticae*

E. Abou-Fakhr Hammad[1], M. Akkary[2], N. Saliba[3], M. Farran[1] & S. Talhouk[4]

[1] Department of Agricultural Sciences, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

[2] Debbane Group, DESCO Holding SAL, Beirut, Lebanon

[3] Department of Chemistry, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon

[4] Department of Landscape Design and Ecosystem Management, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Correspondence: E. Abou-Fakhr Hammad, Department of Agricultural Sciences, Faculty of Agrcultural and Food Sciences, American University of Beirut, Riad El Solh, Beirut 1107-2070, Lebanon. Tel: 961-(1)-350-000 ext. 4493. E-mail: ima27@mail.aub.edu

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Abstract

Forty two methanol extracts and 12 aqueous extracts of 29 indigenous medicinal plant species were tested for their acaricidal bioactivity against the two spotted spider mite, *Tetranychus urticae* adults at the laboratory. Fourteen methanol plant extracts caused significant mortality in mites. This is the first report for the potential effect on survival of mites for 27 tested endemic plant species. Methanol whole plant extracts (WPEs) of *Lotus carmeli*, *Alchemilla diademata*, *Eryngium deserlorum* and aqueous fruit extracts (FrEs) of *Melia azedarach* caused toxic effects against the adult mites in the range of 41-46% mortality. The methanol WPE of *L. carmeli* and the aqueous FrE of *M. azedarach* (1:5) caused the highest mite mortality of 43.55% and 45.55%, respectively and each was used as reference sample for potential acaricidal activity in the methanol and aqueous treatment groups. The latter extract was not significantly different in its effect from aqueous extracts of *M. azedarach* leaf extract (LE) and *Achillea damascena* WPE. Methanol extracts of *Salvia rubifolia* flowers and *Calendula palestina* FrE were found to be more active against the adult mite than their extracts of other plant parts as leaves and flowers, respectively. The former two extracts, flower extracts of *Anthemis scariosa*, *Echinops gaillardoti*, *Nepeta curviflora*, and *Ranunculus cuneatus*, leaves and stems extract of *An. scariosa* and WPEs of *Melissa inodora*, *Ranunuculus myosuroides*, *Origanum libanoticum* and *Ac. damascena* were found to be comparable in their acaricidal activity to that of the whole plant extract of *L. carmeli*. Thus, these bioactive extracts of some local plant species can cause toxicity to adult *T. urticae* mites and consequently could be an alternative control for mite pests.

Keywords: tetranychus, mite, plant extract, botanicals, medicinal, endemic

1. Introduction

The two spotted spider mite *Tetranychus urticae* Koch has been recorded from more than 900 different plant species and its polyphagous nature has been documented worldwide on at least 150 economical crops (Fasulo & Denmark, 2009). Controlling mites by chemicals alone is particularly risky because of the ability of mites to become resistant to a wide range of pesticides especially when exposed to intensive selection pressures by the application of pesticides that encourage the evolution of acaricide resistance in *T. urticae* that consequently lead to outbreaks of *T. urticae* (Funayama, 2015). Resistance of *T. urticae* populations to organophosphates, carbamates, tetradifon, fenbutatin oxide and other acaricides is widely reported (Tsagkarakou et al., 2002; Khajehali et al., 2009). The degree of resistance to many established acaricides has resulted in a demand for new acaricides as plant derived materials with novel modes of action (Khater, 2012; George et al., 2014). Thus, these problems have established the need for alternative pest management control measures that include the search for new classes of pesticides and the reassessment and use of pest control agents of botanical origin.

Many plants develop intrinsically secondary compounds as chemical defenses in their eco-habitat. These plants might be origin of biodegradable pesticides (Farombi, 2003). The world market for pesticides is large; it approximated $54.89 billion in 2016 and projected to reach $70.57 billion by 2021, at a compound annual growth rate of 5.15% from 2016 to 2021 (Anonymous, 2017) with the annual worldwide consumption of pesticides of about two million tons (De et al., 2014). However, there is increase in consumer demand for natural over synthetic pesticides nowadays. The structurally diverse natural compounds of bioactive plant extracts might exhibit against mite and insect pests various behavior responses as repellent, deterrent, antifeedant effects, and might exhibit physiological responses as growth regulator, molting inhibitor, reproduction inhibitor and toxic action. Many indigenous plants are known in the world as medicinal sources for treatment of various human diseases (Farombi, 2003). These plants might be used further locally as insecticides, acaricides, or rodenticides (Mishra et al., 2013).

Al-Alawi (2014) screened 18 plant extracts from the Mediterranean area for acaricidal activity against the mite *T. urticae*. Although all extracts were ineffective against the egg stage and caused less than 30% mortality; Al-Alawi (2014) indicated that the extracts of *Ruta chalepensis* L. and *Astragalus oocephalus* Boiss have the potential to be developed as botanical acaricide for *T. urticae* against both the deutonymph and adult instars. Erdogan et al. (2012) also determined the efficacy of 5 different plant extracts of *Allium sativum* L., *Rhododendron luteum* S., *Helichrysum arenarium* L., *Veratrum album* L., and *Tanacetum parthenium* L. against *T. urticae*. The highest mortality effect of the plant extracts against larvae and adult mite instars occurred at a concentration of 12% while the smallest effect was at 1%.

Preserving the natural resources of endemic plants is one means for developing sustainable agro-ecosystems. It has been well established that certain plant-derived extracts and phytochemicals may provide potential alternatives to be used as pest control agents (Calmasur et al., 2006). The main objective of this study is to screen 29 medicinal plant species native to Lebanon for the bioactivity of their extracts against the adult *T. urticae* which has been reported to have resistance to pesticides in Lebanon; knowing that methanol extracts of these plant species, except *Calendula palestina* Pers. (Site 1) flowers and fruit extracts, were studied previously for their insecticidal effects against the cotton whitefly *Bemisia tabaci* (Gennadius) within a bioprospect context (Abou Fakhr et al., 2014), for utilizing local plant species as an alternative in pest management.

2. Materials and Methods

2.1 Plant Extract Preparation

Plant extraction was performed according to a methodology set for testing bioactivity of plant extracts at the Center of Initiative for Biodiversity Studies in Arid Regions-IBSAR (currently The Nature Conservation Center) located at the premises of the American University of Beirut (AUB), Lebanon.

2.2 Plant Material

Three hundred and eighteen plant genera indigenous to Lebanon with local medicinal and/or agricultural uses were investigated at IBSAR center. The initial plant list of 399 species was based on different bioactivity categories depending on the plant use by local people including pesticidal activity. Out of the latter list, 109 plants with acaricidal/insecticidal use have been recognized; this indicates that 27.3% of total plants revised for all above categories have pesticidal bioactivity, same as in previous study with *B. tabcai* (Abou Fakhr et al., 2014). In this study, the selected tested plant species were 30 plant species collected from different locations or sites (Tables 1 and 2). Plants in 11 botanical families were collected in Spring and Summer 2002 (between March and August) during which plants were collected for their fruits, flowers, leaves, or stems.

2.3 Extraction of Plant Material

Extraction of collected plant parts was executed at the Department of Chemistry, Faculty of Arts and Sciences, AUB. Harvested plant material was washed with distilled water and was dried at the laboratory at 25-32 ºC with enough aeration for 2 weeks. Plants were separated physically into leaves, fruits, flowers and stems or used as a whole plant (stems, leaves and flowers combined), in case of low availability of the plant species. Then plant parts were ground into fine particles (0.3 mm in diam.) by using a grinder (SM 100 Cutting Mill, Brinkman, Germany) at a speed of 1600 rpm at 60 HZ.

The grinded plant material was soaked in methanol for 16 h and placed in a shaker-incubator at room temperature; as extraction of plant material at higher temperatures would denature or would change the chemical composition of the plant potential pesticidal bioactivity. The methanol solvent was used to extract most of the semi-polar and polar constituents. This MeOH extraction was performed using the standard plant material/solvent concentration of 1:10 (w:v), this concentration was selected to avoid phytotoxicity by the

unknown bioactivity of the tested plant extracts against the two-spotted spider mite. The extract was filtered by vacuum pressure (Buchi V-500, Switzerland) and filtrate of each raw extract was used in the bioassays. Forty two methanol extracts of 28 plant species were tested for their acaricidal bioactivity in this study (Table1).

Similar to the above procedure, 13 aqueous extracts of different plant parts of 8 plant species (Table 2) were prepared at a concentration of 1:5 (w:v); only *Melia azedarach* L. was tested against another concentration of (1:2) for leaf and fruit extracts (Table 2). These extracts were used in one bioassay as a preliminary test for a few medicinal plant species.

Table 1. List of 42 methanol extracts of 28 indigenous plant species tested for their acaricidal bioactivity against *Tetranychus urticae* adults

Plant Family	Plant species	Plant part/s[a]	Plant collection location[b]
Apiaceae	*Eryngium deserlorum* Zohary	Whole plant	Zahle
Asteraceae	*Achillea damascena* L. (Loc. 1 and Loc. 2)	Whole plant	Rassem El Hadath
		Whole plant	Hasroun
	Anthemis hebronica Boiss. and Kotschy	Whole plant	Marjeyoun
	Anthemis scariosa Boiss.	Flowers	Marjeyoun
		Leaves and stems	
	Calendula palestina Pers. (Loc.1)	Leaves and stems	Ibel El Saki (site1)
		Flowers	
		Fruits	
	Calendula palestina Pers. (Loc. 2)	Leaves and stems	Ibel El Saki (site 2)
		Flowers	
		Fruits	
	Centaurea ainetensis Boiss.	Flowers	Aineta
	Centaurea erengoides Lam.	Flowers	Jroud Hasroun
	Cirsium sp. Boiss.	Whole plant	Bcharree
	Echinops gaillardoti Boiss.	Flowers	Marjeyoun
	Hieracium sp. L.	Whole plant	Kneisseh
	Onopordum cynarocephalum Boiss.	Flowers	Sannine
	Serratula pusilla Dittr. (Loc. 1 and Loc. 2)	Flowers	Mahmeit Baalabeck
		Flowers	Aineta
	Taraxacum sp. Poir.	Leaves and stems	Nabee El Safa
		Flowers	Bcharree
Boraginaceae	*Heliotropium rotundifolium* Boiss.	Leaves	Zahle
		Flowers	Sannine
Dioscoreaceae	*Tamus orientalis* L.	Leaves and stems	Ibel El Saki
Fabaceae	*Lotus carmeli* Boiss.	Whole plant	Chouf region
Lamiaceae	*Melissa inodora* Bornm.	Whole plant	Nahr El Kaleb
	Nepeta curviflora Boiss.	Flowers	Deir El Kamar
		Leaves	
		Stems	
	Origanum libanoticum Boiss.	Whole plant	Nabee El Aassal
	Phlomis damascena Born.	Whole plant	Mahmeit Baalabeck
	Phlomis syriaca Boiss.	Whole plant	Mikne
	Salvia rubifolia Boiss.	Leaves	Barouk mountains
		Flowers	
Ranunculaceae	*Ranunculus cuneatus* Hook.	Flowers	Yanta
	Ranunculus myosuroides Boiss. and Kotschy	Whole plant	Hasroun
Rosaceae	*Alchemilla diademata* Rothm.	Whole plant	Aineta
Scrophulariaceae	*Verbascum blancheanum* Boiss.	Leaves and stems	Rass Baalabeck
	Verbascum leptostychum DC.	Leaves	Aineta
		Flowers	Nabee El Safa
Urticaeceae	*Urtica fragilis* Thiebaut	Leaves and stems	Baskinta

Note. [a]Whole plant includes leaves, flowers, and stems of the same plant ground together; [b]Loc. = Location.

Table 2. List of 13 aqueous extracts of 8 indigenous plant species tested for their acaricidal bioactivity against *Tetranychus urticae* adults

Plant Family	Plant species	Plant part/s[a]	Plant collection location[b]
Asteraceae	*Achillea damascena* L.	Whole plant	Hasroun
	Calendula officinalis L.	Flowers	Ibel El Saki
	Serratula pusilla Dittr.	Whole plant	Aineta
Lamiaceae	*Phlomis damascena* Born.	Leaves	Mahmeit Baalabeck
Ranunculaceae	*Ranunculus cuneatus* Hook.	Flowers	Yanta
		Leaves	
Scrophulariacea	*Verbascum blancheanum* Boiss.	Whole plant	Rass Baalabeck
		Leaves	
Urticaeceae	*Urtica fragilis* Thiebaut	Leaves and stems	Baskinta
Meliaceae	*Melia azedarach* L.	Leaves (1:5)	AUB campus
		Fruits (1:5)	
		Leaves (1:2)	
		Fruits (1:2)	

Note. [a]Whole plant includes leaves, flowers, and stems of the same plant ground together; [b]AUB = American University of Beirut.

2.4 Screening Bioassays with Plant Extracts against T. urticae

All experiments were performed at the Entomology Laboratory, Faculty of Agricultural and Food Sciences, AUB at 25±2 °C, 60±10% RH and 16:8 (L:D) photoperiod. Treatments included 55 extracts of 30 plant species with 1 negative control: Methanol (10%) or distilled water in case of aqueous extracts. Each treatment was replicated 3 times on one date of the experiment. The experiment was replicated at 3 different dates during the study. Due to the availability of consistent conditions during the experiments with adult mites; about 15 treatments were performed simultaneously on different days of the experiment allowing about 45 Petri dishes to be assessed at one date.

2.4.1 Mite Colony

T. urticae colony was raised in a glasshouse compartment under controlled conditions of 28±5 °C, 80±10% R.H. and a photoperiod of 14:10 (L:D). The colony, originally from a field population (previously exposed to pesticides) was reared on cucumber plants of the variety Perla (Edena Seeds, USA) in a mite proof cage (140 × 85 × 130 cm) covered completely with mesh (270 × 770 µm). Two true leaf seedlings were grown to provide a source of healthy plants to the mite colony and bioassays. Irrigation and fertilization with Floral® (20-20-20+ microelements; Cifo S.p.A., Bologna, Italy) was applied about 2 times a week, as needed.

2.4.2 Experimental Setup with Adult mites

Non-infested soft cucumber leaves of 4 d old were detached from the tip of plants to be used in the bioassays. Each leaf was placed in a glass dish (12.5cm diam. × 5 cm depth); the petiole of the leaf was inserted in a narrow necked small vial filled with water to prolong the rigidity of the leaf throughout the experiment.

Five ml of each methanol extract was rotovaped (Centrivap console, Labconco, USA), after which 5 ml of distilled water was added and mixed with a vortex (Vortex V1 plus, Boeco, Germany) to homogenize the solution before spraying the mites on the leaf surface. Leaves and mites were sprayed topically by methanol or aqueous extract; each leaf received an average of 2.5 ml of the extract or the control (10% Methanol or distilled water) using 10 ml glass sprayers.

Adult female mites recognized by their elliptical body and non-tapering caudal end and 12 pairs of dorsal setae (Fasulo & Denmark, 2009) were collected from the rearing colony and introduced into each glass dish and placed over the leaf before treatment; each dish was sealed with parafilm after treatment. Each treatment was replicated 9 times. Each replicate comprised 10 adult female mites per cucumber leaf. Numbers of adult mites dead or alive in each dish were recorded at 48 h after treatment; this selected timing was the best based on the healthy status of the leaves. Assessment of mite mortality corresponded to failure of mite response to probing with a thin needle: with no movements of legs, proboscis, or abdomen.

2.4.3 Statistical Analysis

The experiments were laid out in a completely randomized block design with two factors: treatment and date of experiment. Each treatment was replicated 9 times on 3 different dates. Data was analyzed by the statistical package MSTAT (Anonymous, 1991). The data were pooled and analyzed as a one-way ANOVA with treatment as the main factor, as there was no significant interaction among the 2 factors. Thus, there were 42 treatments with methanol extracts and 1 negative control (Methanol 10%) with 9 replicates per treatment. On the other hand, there were 13 treatments with aqueous extracts and 1 negative control (distilled water) with 9 replicates per treatment. The % mortality of adult mites per leaf were used in the data analysis, after ensuring their normal distribution by transforming the data using Arcsin Sqrt. (% x), with *x* being the adjusted % mortality, to stabilize the variances. The adjusted % mortality is the mortality data corrected according to the control mortality using Abbott's (1925) formula as the mites mortality in the control of the experiments was of an average of 5%. All means were separated by LSD test, if significant F values were obtained.

3. Results

This study is the first report for the potential effect/s on survival of *T. urticae* for the tested indigenous medicinal plant species (Tables 1 and 2), except for *Phlomis syriaca* Boiss. and *M. azedarach* that were studied previously against *T. urticae* (Al-Alawi, 2014; Ashrafju et al., 2014, respectively). Results of our current study have shown efficacy in decreasing number of live adult mites in some treatments with plant extracts of these medicinal plants, under laboratory conditions.

3.1 Effect of Organic Plant Extracts

In our bioassays with methanol extracts, there were significant differences in % mortality of the adult mites among treatments. The 42 methanol extract treatments were divided into seven groups for discussion simplification of the results. The first major treatment groups included: 13 whole plant extracts (WPEs), 15 flower extracts (FEs), 7 leaf and stem extracts (LSEs) and 4 leaf extracts (LEs). The 5th treatment group including the 3 remaining extracts, 2 fruit extracts and one stem extract, are discussed in a comparative manner related to different plant parts of the same plant species collected from one location; the latter group included 13 extracts of *Sa. rubifolia*, *An. scariosa*, *N. curviflora* and *Ca. palestina* (plant parts collected from 2 locations). The 6thtreatment group dealt with effect of plant source (collection location/site) on bioactivity of the extracts. This group included 10 extracts related to *Ac. damascena*, *Ca. palestina* and *Se. pusilla*, each sp. collected from 2 locations/sites (Table 1). The 7thtreatment group dealt with effect of similar plant parts of different species within one plant genus on bioactivity of the extracts; this group included 4 extracts of *P. damascena*, *P. syriaca*, *Ce. erengoides* and *Ce. ainetensis*.

For the whole plant extracts (WPEs), there was significant difference in % mite mortality among treatments. The WPE of *L. carmeli* showed the highest mortality of 43.55% among all treatments and hence it is used as the reference sample for high acaricidal activity in methanol extracts treatment groups. Whole plant extracts of *Al. diademata*, *Er. deserlorum*, *Melis. inodora*, *R. myosuroides*, *Or. libanoticum*, and *Ac. damascena* caused 42.96, 41.23, 36.92, 35.09, 31.80 and 31.48% mortality, respectively and were not significantly different in their effect from that of *L. carmeli* (Table 3). However, the other 6 WPEs were significantly lower in their effect from *L. carmeli* with *P. damascena* causing the lowest mite mortality of 18.41%.

For the flower extracts (FEs), there was significant difference in % mortality of adult mites among treatments with 15 FEs of different plant species (including two plant species, *Se. pusilla* and *Ca. palestina*, collected from 2 locations, Table 1). Flower extracts of *Sa. rubifolia*, *An. scariosa*, *Ec. gaillardoti*, *N. curviflora* and *R. cuneatus* caused high mortality of 38.48, 33.57, 32.83, 31.41 and 29.95%, respectively and were not significantly different in their effect from that of the WPE of *L. carmeli* (Table 3). However, the other 10 FEs were significantly lower in their effect from *L. carmeli* with *Ca. palestina* causing the lowest mite mortality of 15.63%.

For the leaf and stem extracts (LSEs), there was significant difference in % mortality of adult mites among treatments with 7 LSEs of different plant species. Only LSE of *An. scariosa* was not significantly different in its effect from *L. carmeli* causing a mortality of 34.33%. On the contrary, the other 6 LSEs were significantly lower in their effect from *L. carmeli* with *V. blancheanum* causing the lowest mite mortality of 17.75% (Table 3). However, the latter LSEs were not significantly different in their effect from that of *An. scariosa*.

For the leaf extracts (LEs), there was significant difference in % mortality of adult mites among 4 LEs of different plant species and *L. carmeli*. Leaf extracts of *N. curviflora*, *He. rotundifolium*, *V. leptostychum*, and *Sa. rubifolia* caused significantly low acaricidal effect in the range of 15.26%-25.20% mite mortality in comparison to WPE of *L. carmeli* (Table 3).

In comparing extracts of different plant parts of one plant sp. collected from same location, there was significant difference in % mite mortality (Table 3) among 13 treatments with extracts of different parts of each of the following plant species: *Sa. rubifolia*, *An. scariosa*, *N. curviflora* and *Ca. plaestina*. There was a significant difference in mite mortality between the FE and LE of *Sa. rubifloia*, but there was no significant difference in % mite mortality between the LSE and the FE of *An. scariosa*. Similarly, for the 3 extracts of *N. curviflora*, there was no significant difference in % mite mortality among extracts of different plant parts: leaves, stems and flowers. On the other hand, the fruit extract (FrE) and FE of *Ca. palestina* from one location (labeled as location 2, Table 1) was significantly different in % mite mortality from that of its LSE. On the contrary, there was no significant difference among the FrE, LSE and FE of *Ca. palestina* collected from another location (labeled as location 1, Table 1); all 3 extracts were not comparable in their effect to that of *L. carmeli* WPE (Table 3). However, the FEs of *Sa. rubifolia*, *An. scariosa* and *N. curviflora* and LSE of *An. scariosa* and FrE of *Ca. palestina* seem to be comparable in their acaricidal effects to WPE of *L. carmeli*.

In comparing extracts of similar plant parts of a plant species collected from different locations/sites, there was no significant difference in % mite mortality among 10 treatments with extracts of similar plant parts of three plant species; each species collected from 2 locations/sites. The WPEs of *Ac. damascena*, FEs of *Se. pusilla*, FEs, LSEs and FrEs of *Ca. palestina* collected from different locations were not significantly different in their effect against the mites (Table 3).

In comparing treatments with extracts of same plant part of four species within 2 plant genera: *Centaurea* and *Phlomis*, there was no significant difference in % mite mortality among these extracts. Difference in bioactivity effect of species within plant genus is not reflected in this study for the tested plant extracts of *Centaurea* sp. and *Phlomis* sp. Furthermore, FEs of *Ce. erengoides* and *Ce. ainetensis* and WPEs of *P. syriaca* and *P. damascena* were significantly lower in % mite mortality from that of WPE of *L. carmeli* and caused low mite mortality of 18.41%-28.26% (Table 3).

3.2 Effect of Aqueous Plant Extracts

In our bioassays with 13 aqueous plant extracts, there were significant differences in % mite mortality among treatments (Table 4). *M. azedarach* FrE (1:5) had shown the highest mite mortality of 45.55% and hence it is used in comparison with other aqueous extracts as reference sample for high acaricidal activity. The latter extract was not significantly different in its effect from *M. azedarach* LE (1:5) and *Ac. damascena* WPE causing mite mortality of 35.55 and 26.6%, respectively. However, the other 10 extracts were significantly lower in their effect from the reference *M. azedarach* FrE (1:5).

Table 3. Mortality of *T. urticae* adult mites caused by methanol extracts of different plant species under laboratory conditions

Treatment[a]	**Plant part/s**[b]	**% mortality**[c]
Lotus carmeli	Whole plant	43.55±8.40 a
Alchemilla diademata	Whole plant	42.96±10.73 ab
Eryngium deserlorum	Whole plant	41.23±8.65 abc
Salvia rubifolia	Flowers	38.48±3.45 a-d
Melissa inodora	Whole plant	36.92±4.49 a-e
Ranunculus myosuroides	Whole plant	35.09±7.11 a-f
Anthemis scariosa	Leaves and stems	34.33±9.14 a-f
Anthemis scariosa	Flowers	33.57±8.42 a-g
Echinops gaillardoti	Flowers	32.83±1.68 a-h
Origanum libanoticum	Whole plant	31.80±6.35 a-i
Achillea damascena (Loc.1)	Whole plant	31.48±9.23 a-i
Nepeta curviflora	Flowers	31.41±7.85 a-i
Calendula palestina (Loc. 2)	Fruits	30.52±5.24 a-j
Ranunculus cuneatus	Flowers	29.95±6.81a-k
Serratula pusilla (Loc. 2)	Flowers	28.81±7.85 b-l
Nepeta curviflora	Stem	28.46±6.97 b-l
Tamus orientalis	Leaves and stems	28.32±5.19 c-l
Centaurea erengoides	Flowers	28.26±4.67 c-l
Verbascum leptostychum	Flowers	27.77±8.33 c-l
Achillea damascena (Loc. 2)	Whole plant	26.72±6.45 c-l
Serratula pusilla (Loc. 1)	Flowers	26.58±7.12 d-l
Taraxacum sp.	Leaves and stems	26.29±6.53 d-l
Anthemis hebronica	Whole plant	25.77±5.80 d-l
Nepeta curviflora	Leaves	25.20±5.46 d-l
Centaurea ainetensis	Flowers	24.97±4.61 d-l
Heliotropium rotundifolium	Leaves	24.75±6.50 d-l
Calendula palestina (Loc. 1)	Fruits	23.49±4.59 e-l
Urtica fragilis	Leaves and stems	23.23±7.60 e-l
Phlomis syriaca	Whole plant	22.64±4.55 e-l
Calendula palestina (Loc. 1)	Leaves and stems	21.79±2.90 f-l
Heliotropium rotundifolium	Flowers	20.54±7.54 f-l
Calendula palestina (Loc. 2)	Flowers	19.59±5.53 g-l
Hieracium sp.	Whole plant	18.96±5.57 h-l
Cirsium sp.	Whole plant	18.48±3.71 h-l
Phlomis damascena	Whole plant	18.41±2.95 h-l
Taraxacum sp.	Flowers	18.18±5.97 I-l
Calendula palestina (Loc. 2)	Leaves and stems	18.05±7.54 f
Verbascum blancheanum	Leaves and stems	17.75±7.54 f
Onopordum cynarocephalum	Flowers	16.75±4.06 j-l
Calendula palestina (Loc. 1)	Flowers	15.63±3.17 k-l
Verbascum leptostychum	Leaves	15.43±2.17 k-l
Salvia rubifolia	Leaves	15.26±4.65 l

Note. Values = Mean ± Std. Error; [a] Loc. = Location; [b] Whole plant includes leaves, flowers, and stems of the same plant ground together; [c] Means followed by the same letter within a column are not significantly different (LSD test; $P > 0.05$).

Table 4. Mortality of *T. urticae* adult mites caused by aqueous extracts of different plant species under laboratory conditions

Treatment	Plant part/s[a]	% mortality[b]
Melia azedarach (1:5)	Fruits	45.55±5.3 a
Melia azedarach (1:5)	Leaves	35.55±6.48 ab
Achillea damascena	Whole plant	26.66±8.82 abc
Melia azedarach (1:2)	Leaves	18.88±6.55 bc
Verbascum blancheanum	Leaves and stems	18.88±6.55 bc
Phlomis damascena	Whole plant	15.55±5.55 bc
Calendula officinalis	Flowers	13.33±3.72 bc
Melia azedarach (1:2)	Fruits	12.22±3.64 bc
Serratula pusilla	Flowers	11.11±3.09 bc
Urtica fragilis	Leaves and stems	11.11±3.89 c
Verbascum blancheanum	Whole plant	8.88±3.89 c
Ranunculus cuneatus	Leaves	6.66±2.88 c
Ranunculus cuneatus	Flowers	6.66±2.88 c

Note. Values = Mean ± Std. Error; [a] Whole plant includes leaves, flowers, and stems of the same plant ground together; [b] Means followed by the same letter within a column are not significantly different (LSD test; $P > 0.05$).

4. Discussion

In general, the bioactivity effect of the plant extracts against arthropods as mites might be related to the extracted plant part, the plant species, the source (*i.e.* collection location/site) of the plant material and the mite instar. A few studies have reported various effects on mites by a number of other plant species belonging to the same genera of the medicinal plant species that were tested in this study. Our treatments with methanol or aqueous extracts of some medicinal plants applied topically over adult mites infesting cucumber leaves caused a relative high toxic effect against mites depending on the extracted plant part/s and biochemical characteristics of the plant species. In comparing the bioactivity of methanol extracts of the four major plant parts, 53.84% of the WPEs, 33.33% of the FEs and 14.28% of the LSEs were having acaricidal activity above 29.95%. Similarly, the fruit extract of *Ca. Palestina* has comparable acaricidal effect to the aforementioned extracts. However, all tested LEs, methanol and aqueous LSEs of *U. fragilis* caused lower mortality of mites less than 26% (Table 3). Similarly, Al-Alawi (2014) found that the extract of *Urtica pilulifera* L. did not influence young adult female mites of *T. urticae*, but it caused high deutonymph mortality of 51%.

In our study, methanol WPEs of *L. carmeli*, *Al. diademata*, *R. myosuroides* and *Ac. damascena* were found to have potential acaricidal effect under laboratory conditions. Both *L. carmeli* and *Al. diademata* WPEs were previously reported to have a repellent effect against adult *B. tabaci*, but WPEs of *R. myosuroides* and *Ac. damascena* caused both repellent and toxic effects against adult and 2nd nymphal instars of *B. tabaci*, respectively (Abou Fakhr Hammad et al., 2014). Thus, these WPEs seem to have acaricidal toxic effect in addition to their latter determined insecticidal effects. However, WPEs of *Phlomis* sp. were also found to have repellent effect against *B. tabaci*, but caused low acaricidal activity in our study (Table 3). The acaricidal effect of our former plant extracts (Table 3) seems to be similar to that of other synthetic chemical acaricides as Hexythiazox that caused 17-30.83% *T. urticae* mortality in treated plots compared to other acaricides as Fenpyroximate, Spiromecifen and Pyridaben that caused 82-95% mite mortality; the lower effect of Hexythiazox was also reported under laboratory conditions (Ali et al., 2015).

On the other hand, *L. carmeli* belongs to the Family Fabaceae/Leguminosae which frequently contains alkaloids; phytochemical analysis of *Lotus* spp. also included coumarins, flavonoids and tannins with potential antimicrobial activity (Girardi et al., 2014). Similarly, *Alchemilla* species (Rosaceae) were found to have tannins and catechins, phytochemical profile of various *Ranunculus* species (Ranunculaceae) included different secondary metabolite groups as triterpene saponins, alkaloids, cyanogenic glucosides that might be related to antioxidant and antibacterial activities (Wink, 2015). It is important to note that the 3 main chemical classes in plant species that were known for insecticidal, fungicidal and other biological activities were terpenoids, phenylpropanoids and alkaloids as in *Heliotropium* sp. (Boraginaceae), *Tamus* sp. (Dioscoreaceae), *Verbascum* sp. (Scrophulariaceae), *Urtica* sp. (Urticaceaea), Lamiaceae (as *Phlomis* & *Origanum* spp.), Apiaceae and

Achillea species (Asteraceae) which are also known to have polyacetylenes or polyenes, phenolic acids, coumarins, and sterols which are usually related to their medical effects (Wink, 2015).

Flower extracts of *Sa. rubifolia*, *An. scariosa*, *Ec. gaillardoti*, *N. curviflora* and *R. cuneatus* were also found to have potential toxic acaricidal effect. However, only *Ec. gaillardoti* FE caused significant decrease in number of whitefly live nymphs on treated plants which indicates that this extract seems to have both acaricidal and insecticidal effects, but FEs of *Se. pusilla* and *On. cynarocephalum* which were found to have an insecticidal effect against the whitefly (Abou Fakhr et al., 2014) were not found to have a significant acaricidal effect in our current study (Table 3).

Only *An. scariosa* LSE seems to have potential toxic acaricidal effect among the 7 tested LSEs. The former extract seems to have both acaricidal and insecticidal effects against *B. tabaci*, but LSEs of *Ca. palestina*, *T. orientalis* and *V. blancheanum* and LEs of *N. curviflora*, *He. rotundifolium*, *V. leptostychum*, and *Sa. rubifolia* which were found to have significant insecticidal effects against *B. tabaci* (Abou Fakhr et al., 2014) were found to have low acaricidal effect in our current study.

In comparing bioactivity of extracts of different plant parts of one plant sp. collected from same location in terms of acaricidal activity against *T. urticae* (our current study) and insecticidal effect against *B. tabaci* (Abou Fakhr Hammad et al., 2014), *Salvia rubifloia* FE, LE and FrE, LSE of *Ca. palestina* have acaricidal and insecticidal effects, respectively in each plant species; whereas, LSE of *An. scariosa* seem to have both acaricidal and insecticidal effects, but its FE has only acaricidal effect which is similar to that of *N. curviflora* FE. Thus, the secondary metabolites vary depending on part of the plant studied as flower, leaf, stem or fruit. Furthermore, methanol LEs and FrEs of *M. azedarach* tree were found to be repellent to whitefly adults, while the fruit extracts have caused a significant detrimental effect against nymphs of *B. tabcai* (Jazzar & Abou-Fakhr Hammad, 2003) and *Bemisia argentifolii* (Bellows & Perring) (Abou-Fakhr Hammad & McAuslane, 2006). Furthermore, the secondary plant metabolites may vary due to several factors besides the plant part studied; as the degree of plant maturity at the collection period of the sample and other factors as geography of the collection location/site.

In our study, there was no location effect on the bioactivity of the plant part extract of one species collected from different locations/sites against the mite *T. urticae*. However, the WPE of *Ac. damascena* (collected from labeled location 1, Table 1) and fruit extract of *Ca. palestina* (collected from labeled location 2) seems to have potential acaricidal effects. Similarly, there was no location effect on the extracts bioactivity of the plant sp. *Ca. palestina*, *Se. pusilla* and *Ac. damascena* against the insect pest *B. tabaci* (Abou Fakhr Hammad et al., 2014). However, WPE of *Ac. damascena* (collected from labeled location1) have both acaricidal and insecticidal effects, but the FrE of *Ca. palestina* (collected from labeled location 2) have only acaricidal effect. This "no location" effect could be attributed to the fact that the two collection locations/sites for particular species are geographically not widely distant from each other. For example, plant samples of *Ca. palestina* LSEs were collected from 2 sites in one location, Ibel El Saki; plant samples of *Se. pusilla* FEs were collected from 2 locations in Baalbek District; plant samples of *Ac. damascena* WPEs were collected from two locations in close districts (Table 1). However, certain studies have confirmed that some plant species have distinct chemotypes or populations that are often separated geographically (Pascual-Villalobos & Ballesta-Acosta, 2003). Thus, the location or site factor is associated to a phenomenon which relates the variability in production of a secondary plant compound to strong biogeographic dependence; for example, it was reported that crude extracts of *M. azedarach* from Paraguay were devoid of any anti-molting activity whereas this activity was found in the Brazilian *M. azedarach* (Cabral et al., 1996).

Furthermore, difference in bioactivity effect of species within plant genus is not reflected in this study for the tested methanol plant extracts. The FEs of *Ce. erengoides* and *Ce. ainetensis* and WPEs of *P. syriaca* and *P. damascena*, caused low acaricidal effect against the mite *T. urticae.* Similarly, this bioactivity effect was not reflected for the plant extracts of the genera: *Centaurea* and *Phlomis* against *B. tabaci* (Abou Fakhr Hammad et al., 2014), but these extracts caused high insecticidal effects against the whitefly.

For the treatments with our aqueous extracts against the mite *T. urticae*, FrE and LE of *M. azedarach* (1:5) caused the highest mite mortality of 45.55% and 35.55%, respectively (Table 4). However, Ashrafju et al. (2014) found that *M. azedarach* extract concentrations of 4 and 5 mg/ml caused a reduction of more than 50% in the egg laying of *T. urticae* mites and a significant increase in their pre-mature period. These latter extracts proved to be more efficacious as the volume of water used was larger than that of the FrE and LE of *M. azedarach* (1:2), leading to extracting more of the bioactive material; this is similar to Neem, a related meliaceous tree, which is known to be of very low solubility in water which urges the need for a larger amount of water to be used for

extracting the bioactive constituents (Anonymous, 1992). Furthermore, all extracts in our study were tested as raw extracts without the addition of any surfactant; the aqueous WPE of *P. damascena* caused only 15.55% mite mortality whereas, Al-Alawi (2014) found that aqueous LEs of *P. syriaca* with Tween-80 caused mortality of 65%, followed by *Achillea biebersteinii* Afan. (64%) against the adult *T. urticae*.

In comparing the bioactivity of our plant extracts in different solvents, the aqueous and methanol WPEs (collected from labeled locations 2 and 1) of *Ac. damascena* caused comparable mite mortality of 26.66, 26.72 and 31.48%, respectively. Similarly, the aqueous and methanol LSE of *V. blancheanum* caused a comparable mite mortality of 18.88% and 17.75%, respectively. However, other aqueous extracts had caused in general a lower mite mortality of 6.66 till 15.55% (Table 4) compared to their corresponding methanol extracts (Table 3). Furthermore, *M. azedarach* methanol extracts were found to be more active against *B. tabaci* than extracts with other solvents as acetone, ether and water (Abou-Fakhr Hammad et al., 2000). On the other hand, neem methanol extracts were reported to be 50 times more concentrated than water extracts that were effective as pesticides (Anonymous, 1992). Govindachari et al. (1999) also found that water extracts of neem seeds contain all the principal triterpenoids but much less than subsequent extraction with methanol.

In general, raw plant products are not highly water-soluble for extraction of the active ingredients which makes them difficult for application, but these products are convenient for farmers, as they do not involve any costly solvents. Although, extraction with water is the most effective for bulk application of plant extracts, one limitation is the level of stability of secondary compounds in the plant extracts. Furmanowa et al. (2002) found that aqueous extracts of *Taxus cuspidata* Siebold & Zucc. stems and leaves have strong antifeedant activity against mites, but hot water extraction of this plant lowered the reproductive rate of *T. urticae* from 70.24 in the control to 6.70%. They also indicated that aqueous extracts of *Taxus baccata* var. *elegantissima* had a significant effect on mortality, development and fecundity of *T. urticae*. Thus, the extraction method may affect the constituents of the extract and consequently its bioactive properties.

Therefore, for transferring laboratory results to the field application, besides the continuous search needed for suitable organic solvents to extract pesticidal secondary plant compounds, other factors are also needed. Plant extracts might also have low residual stability on plants especially that it is known that pesticides of botanical origin are susceptible to UV degradation. For example, Azadirachtin (Azamax® 12 g/L) was efficient against *T. urticae*, with a mortality rate similar to that of abamectin, but with less persistence level of 7 days versus 21 days for abamectin (Bernardi et al., 2013). Thus, increasing the persistence level of the botanical insecticidal extract on the plant is needed to increase the pesticide effect and to enhance the application of botanical products. The activity of neem based formulations on mites as solvents and additives in neem seed kernel extracts were reported to influence the mortality, repellency, and fecundity of mites; the two commercial preparations of neem seed kernels Margosan-O and Neem azal-S with 3000 ppm and 3500 ppm azadirachtin, respectively were effective in decreasing the population of *T. urticae* (Dimetry et al., 1993).

Finally, we conclude that 14 extracts out of 42 methanol extracts and 3 extracts out of 13 aqueous extracts of endemic medicinal plants were found to have a potential high bioactivity against *T. urticae* adults. The effect of these bioactive extracts would be enhanced by selecting other organic solvents for extraction or by increasing the concentration of the active ingredient of these extracts, pending there will be no phytotoxicity to the tested plants. Furthermore, the most bioactive extracts as *L. carmeli* (WPE) and *M. azedarach* fruit and leaf extracts need to be further investigated against the immature instars of the mite, with more concentrated aqueous or other organic solvent extracts and/or in combination with other additives that would enhance the bioactivity against different stages of the mites at the laboratory and under field conditions.

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