

BIO-EFFICACY ASSESSMENT OF SAGE, *SALVIA OFFICINALIS* L. EXTRACTS ON SOME BIOLOGICAL ASPECTS OF SPIDER MITE, *TETRANYCHUS URTICAE* KOCH (ACARI:TETRANYCHIDAE)

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Abstract

The usage of plant extracts for pest control is considered as an alternative control method to synthetic pesticides. The effect of methanol and water extracts obtained from Sage (*Salvia officinalis* L.) plant from the Lamiaceae family on *Tetranychus urticae* Koch was evaluated. The sprayed leaf disk method was used to determine the effect of the plant extracts. The effect of Sage extracts on the eggs and adult females was examined. Four concentrations of the plant extracts 5%, 10%, 15% and 20% were evaluated. Mortality was observed after 1st, 3rd and 5th days from treatment. The highest death rates of *T. urticae* adults were found at 20% concentration as 92.85% and 87.14% for methanol and water Sage extracts, respectively. Both extracts gave poor toxic effect to eggs compared with adult females. Both extracts proved superiority in repellency to spider mite, *T. urticae*. Rate of repellency was decreased gradually by time elapsed after treatment. *T. urticae* females preferred to settle, deposit eggs and feed on the untreated half of the disc and the majority refused to settle on the treated part especially with the high concentrations. Both solvent extracts shortened the longevity and reduced fecundity of adult females of *T. urticae*. As a consequence, Sage extracts are thought to be used as an alternative safe method for mite control.

Keywords: Sage, *Salvia officinalis*, *Tetranychus urticae*, Toxicity, Repellency.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most serious agricultural pests in the world. This mite feeds by puncturing cells and draining the contents causing damage in chlorophyll producing white spots that may become more or less coherent with time Nachman and Zemek (2002). They also produce silk webbing which is clearly visible at high infestation levels. The importance of this mite pest is not only due to direct damage to plants but also decreases in photosynthesis and transpiration Brandenburg and Kennedy (1987). Studies of yield reduction caused by varying population levels of *T. urticae* have demonstrated its potential for damaging crops. Its outbreaks are often a consequence

of repeated and non-selective pesticide applications. The greatest problem with this mite is its ability to rapidly evolve resistance to pesticides Cranham and Helle (1985). In addition, the environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years. Therefore, alternative methods for controlling *T. urticae* are needed. Natural products are an excellent alternative to synthetic pesticides as means to reduce negative impacts to human health and environment. Most of these products are environmentally non persistent and nontoxic to humans so far (with some exceptions) Hjorth et al. (1997). Some of the alternative control methods including plant essential oils, plant preparations and microbial secondary metabolites on the two-spotted spider mite are currently being researched (Calmasur et al. 2006 and Feng and Isman 1995). Plant compounds such as extracts were used as acaricides and, repellents (Venkatachalam and Jebanesan 2001 and Kumral et al. 2010) reported that methanolic extracts of *Daturastramonium* L. (Solanaceae) leaves and seeds exhibited acaricidal, oviposition deterrent activities against *T. urticae*. The Lamiaceae family is recognized for their vital oils, medicinal uses and antimicrobial activity of different species Skaltsa et al. (2003). A review of the chemical breakdown of species in this family has revealed a range of chemical components, predominantly mono and diterpenoids, of which a number possess a range of activities against numerous arthropods (Cole 1992 and Simmonds and Blaney 1992). Sage (*Salvia officinalis*) and Rosemary (*R. officinalis*) belong to the Lamiaceae family are strong aromatic plants, which are predominantly used in conventional medicine, food and medicine industry because of their antioxidant and antimicrobial properties Biljana et al. (2007). Thus, the Objectives of this study were to assess the acaricidal activity of *S. officinalis* against *T. urticae* and to isolate active components.

MATERIALS AND METHODS

Rearing of spider mite, *Tetranychus urticae* Koch

Field samples of bean plant (*Phaseolus vulgaris* L.) leaves infested with spider mite, *T. urticae* were collected and transferred to the laboratory. The culture was initiated by transferring female and male individuals to leaves of mulberry, *Morus alba* L. placed on cotton wool pad, fully saturated with water as a source of moisture and to prevent mite from escaping. Old leaves were changed as necessary. Females were transferred to clean leaves, allowed to oviposit for 24 hr., then removed. Development of these eggs resulted in a cohort of evenly aged mites that were used for all

bioassays. The colony was kept under controlled conditions in the laboratory (28 ± 2 °C and 65 ± 5 % R.H).

Preparation of *Salvia officinalis* extracts

Salvia officinalis leaves were collected during the vegetation period from production areas of El-Arish district. Plant material was dried under shade, powdered by using an electric grinder, and kept in the dark at room temperature in 3 liters glass jars until it was used. The extraction procedure used in the study was described by Gokçe *et al.* (2005) where plant extract was prepared from a representative sample of 100 g of powdered plant material taken into a 2 liters capacity Erlenmeyer flask for each solvent, methanol and water. 300 ml of each solvent were added and shaken for 24 hr. 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. Extract was transferred into a 250 ml evaporating flask and evaporated under a vacuum using a rotary vacuum evaporator at 32°C. The extract solutions were kept in a refrigerator at 4°C until they were used in the bioassay.

Gas chromatography-mass spectrometry analysis (GC/MS)

Volatile compound analysis was performed with a gas chromatography system (Agilent 6890 GC) with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 MS (30 m × 0.32 mm × 0.25 µm film thickness). Helium was used as the carrier gas at a flow about 1.0 ml/min pulsed splitless. The solvent delay was 3 min. and the injection size was 1.0 µl. The mass spectrometric detector was operated in an electron impact ionization mode with an ionizing energy of 70 eV. Scanning from m/z 50 to 500 and the ion source temperature was 230°C. The electron multiplier voltage (EM voltage) was maintained 1250 v above auto tune. The instrument was manually turned using perfluorotributyl amine (PFTBA). Oven temperature program at 45°C (2 min), 150°C (5 min) at a rate of 2°C min⁻¹, then at 150°C (2 min), 280°C (5 min) at a rate of 8°C min⁻¹; split 30:1 during 1.50min, carrier gas He: 1 ml min⁻¹, constant flow; sample volume 1 µl. To identify the parts was based on comparison of their mass spectra with those of Wiley and Nist Tutore Libraries Adams (1995).

Toxicity of *Salvia officinalis* extracts to spider mite, *T. urticae* eggs

Mated mite females were transferred to four leaf discs (15 females each) about (each of 2.4 cm in diameter) which were placed in 4 prepared Petri- dishes on moist cotton to prevent desiccation of leaf. They were allowed to lay eggs on the lower surface of mulberry leaves, for 24 hr. The adult females were removed after 24 hr.

and the number of deposited eggs per disc was counted and recorded about 80 eggs for each concentration (20, 15, 10 and 5%). The disc surface carrying the eggs was gently dipped separately in concentrations from each extract solvents for about 5 seconds. In control test, the leaf discs were dipped in distilled water only. The treated and untreated eggs were kept under constant temperature of $28\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ R.H., In all cases, hatchability percentages and incubation period were assessed.

Toxicity of *Salvia officinalis* extracts to spider mite, *T. urticae* females

The leaf spray method was applied to test the efficacy of the two extracts separately. Each extract was tested with different concentrations (20, 15, 10 and 5 %). $\text{LC}_{50\text{s}}$ from each extract was determined. Total number of 80 individuals (females) as the same age was bio-assayed in 8 replicates of 10 adult females each. Petri-dishes having the same number of females were sprayed with water and used as a control. All discs were placed on moist cotton wool pad in Petri-dishes (9 cm in diameter). Discs were sprayed with tested concentrations using a manual atomizer. The treated females and untreated ones were kept under the same conditions of $28\pm 2^{\circ}\text{C}$ and relative humidity of $65\pm 5\%$ R.H. Mortality percentage was calculated 1, 3, and 5 days after treatment.

Repellency effect of *Salvia officinalis* extracts against spider mite, *T. urticae* females

To study the repellency effect of Sage extracts against females of spider mite, *T. urticae* at different concentrations (20, 15, 10 and 5 %) of each extract were used. Mulberry leaves were cleaned and cutted into two parts of symmetrical portion along the midrib. One leaf portion of the disc was dipped in tested concentration of each extracts where the other half was dipped in water (control). The treated discs were left to dry and put on top of a wetted filter paper placed inside glass Petri-dishes (10 cm in diameter). Eighty *T. urticae* females were distributed on eight discs and used as replicates of 10 adult females of *T. urticae* each and placed in the middle between the two leaf portions. The number of mites found on each leaf portion was counted after 1, 2, 3 and 4 days from exposure. The repellency percentages were computed.

Statistical analysis

Data were subjected to statistical analysis using one way analysis of variance, ANOVA Duncan(1955).

RESULTS AND DISCUSSION

Chemical composition of *Salvia officinalis* leaves

The bioactive phytochemicals of *S. officinalis* leaves were analyzed by using hydro-distillation and GC–MS. The results revealed 24 compounds representing 99.42% of the contents Table (1). The identification of phytochemical compounds is based on the peak content (1*S*,4*R*,5*R*) 4-Methyl-1-(propan-2-yl)bicyclo[3.1.0] hexan-3-one has peak content 29.90% with RT 15.11 min. followed by 1,7,7-Trimethyl bicyclo [2.2.1] heptan-2-one, (1*S*,4*S*,5*R*)-4-methyl-1-propan-2-yl bicyclo[3.1.0]hexan-3-one and 1,3,3-Trimethyl-2-oxabicyclo [2,2,2] octane have 16.74%, 13.68% and 12.31% with RT 17.20 min, 15.30 min. and 12.20 min., respectively.

Table 1. Compositions and percentages of volatiles from *S. Officinalis* leaves

No.	Compound name	RT/min.	Content%
1	(1 <i>S</i> ,5 <i>S</i>)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	9.21	2.78
2	6,6-Dimethyl-2-methylenebicyclo [3.1.1] heptane	10.93	1.18
3	2,2-Dimethyl-3-methylene-bicyclo [2.2.1] heptane	11.04	3.04
4	7-Methyl-3-methylene-1,6-octadiene	11.66	0.34
5	1,3,3-Trimethyl-2-oxabicyclo [2,2,2] octane	12.20	12.31
6	1-Methyl-4-(1-methylethenyl)-cyclohexene	13.70	0.43
7	1-Isopropyl-2-methylbenzene	14.08	0.58
8	3,7-Dimethylocta-1,6-dien-3-ol	14.50	1.38
9	(1 <i>S</i> ,4 <i>R</i> ,5 <i>R</i>) 4-Methyl-1-(propan-2-yl)bicyclo[3.1.0] hexan-3-one	15.11	29.90
10	(1 <i>S</i> ,4 <i>S</i> ,5 <i>R</i>)-4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-3-one	15.30	13.68
11	(2 <i>S</i> ,5 <i>R</i>)-2-Isopropyl-5-methyl cyclo hexanone	16.21	0.93
12	1,7,7-Trimethylbicyclo [2.2.1] heptan-2-one	17.20	16.74
13	(1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>)-2-Isopropyl-5-methyl cyclohexanol	18.20	1.63
14	Endo-1,7,7-Trimethyl-bicyclo [2.2.1] heptan-2-ol	20.32	1.8
15	2-(4-methyl-1-cyclohex-3-enyl) propan-2-ol	21.24	0.04
16	2-methyl-5-(1-methylethenyl)-2-cyclohexenone	22.20	0.71
17	3,7-dimethylocta-1,6-dien-3-yl acetate	23.10	0.45
18	(trans)-3,7-dimethyl-2,6-octadien-1-ol	23.48	0.21
19	(1 <i>S</i> ,2 <i>R</i> ,4 <i>S</i>)-1,7,7-Trimethyl bicyclo [2.2.1] hept-2-yl acetate	24.15	2.23
20	2-Isopropyl-5-methyl phenol	25.00	1.91
21	2,6,6,9-Tetramethyl-1,4,8-cycloundecatriene	26.18	2.41
22	(2 <i>E</i> ,6 <i>E</i>)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	27.12	0.61
23	5-Isopropyl-2-methyl phenol	28.23	3.32
24	2,3,4,9,10,10a-hexahydrophenanthrene-4a-carboxylic acid	28.80	0.81

RT: Retention Time

Toxicity of *Salvia officinalis* against eggs and adult females of spider mite, *T. urticae*.

The results showed that the ovicidal action of the experimented natural extract against eggs of *T. urticae* varied considerably Table (2). Both extracts were effective on eggs and caused unhatchability. 20 % concentration caused 35 % and 25 % for methanol and water extracts while, 5 % concentration was the lowest one that caused 22.5 % and 18.75% for the same previous order, respectively. In addition to increasing the incubation period to 5.32 days compared with control of 3.47 days. These results are agreement with Hussein *et al.* (2006) who stated that ethyl acetate extract of the plant *Capparis aegyptia* leaves and fruits were the most potent extracts tested against *T. urticae* egg Barakat *et al.* (1984) tested some plant extracts against adults and eggs of *T. urticae*. Eggs were less affected than adults, but extracts of *Piper nigrum* and *Datura stramonium* showed considerable ovicidal properties. Mortality percentages of females increased with increasing the concentrations of both solvents extracts. Methanol extract was more effective extract against females that caused 92.85% mortality 5 days after treatment at 20% concentration compared with water extract 87.14% mortality at the same concentration. However, each of 10% and 15% concentrations caused variable mortality percentages ranged from 70 % to 84.29%. The lowest concentration caused 57.14 % and 65.71% mortality for water and methanol extracts, respectively. These results agree with Saber and Isman (2006) who assessed the efficacy of rosemary, *Rosmarinus officinalis* L., essential oil against the two-spotted spider mite. Laboratory bioassay results indicated that pure rosemary oil caused complete mortality of spider mite at concentrations that aren't phytotoxic to the host plant.

Table 2. Effect of the Sage extracts against eggs and adult females of *T. urticae*

Concentrations A. I. %	Solvents	Eggs		Mortality % females		
		Unhatchability %	Incubation period (days)	1 day	3 days	5 days
5 %	Methanol	22.5 ^c	4.09±0.04	14.28	27.4	65.71 ^e
	Water	18.75 ^d	3.52±0.02	10.00	18.57	57.14 ^f
10 %	Methanol	27.5 ^b	4.58±0.05	22.85	51.42	78.57 ^c
	Water	21.25 ^c	3.95±0.12	17.14	42.85	70.00 ^d
15 %	Methanol	33.75 ^a	5.03±0.03	34.85	64.28	84.29 ^b
	Water	22.5 ^c	4.24±0.06	28.57	52.85	71.42 ^d
20 %	Methanol	35.00 ^a	5.32±0.04	44.28	77.14	92.85 ^a
	Water	25.00 ^b	4.66±0.01	31.42	72.85	87.14 ^{ab}
Control		0	3.47±0.02	0	2.85	4.28

Means in the columns followed by the same letter are not significantly different at 5% level (Duncan's multiple range tests). ± Standard Error

Mwandila *et al.* (2013) evaluated the effect of Syringa (*Melia azedarach*) fruit and seed extracts (SSE) on red spider mite (*Tetranychus* spp.) eggs, nymphs and adults which it was high toxic and raised the mortality percentage to 90% two days after treatment.

Repellency effect of *Salvia officinalis* extracts on *T. urticae* females.

Data in Table (3) showed that four levels of concentrations (5, 10, 15 and 20%) for methanol and water extracts were tested for repulsion percentages of *T. urticae*. The repellency effect reached an average of 81.25% at 20% concentration of methanol extract one day after application and that considered the most effective one. The rate of repellency effects were decreased gradually by time elapsed after treatment, which was pronounced with all the tested extracts ranged between 81.25%-16.25% and 73.75% – 12.50% one day and four days after treatment for methanol and water extract, respectively. Each of 10% and 15% concentrations were succeeded in repulsion *T. urticae* individuals, where the repellency reached 50%. At 5% concentration, the repellency effect dropped from 33.75% to 3.75% and from 38.75% to 6.25% for methanol and water extracts four days after application, respectively. These findings indicated that when concentration increased the repellency percent also increased. The female mites may control eating when food is abundant. However, under food shortage the mites feed in other areas to guarantee their survival. These results are in agreement with Saber (2004) who tested the effect of petroleum ether, chloroform, ethyl acetate and ethanol extracts of sand wormwood (*Artemisia monosperma*) against *T. urticae* females. The majority of mite females preferred settle and feeding on untreated discs, while few individuals fed on the treated portions.

Table 3. Repellency effect of Sage extracts against *T. urticae*

Concentrations A. I. %	Solvents	Repellency% / days after treatment			
		1	2	3	4
20	Methanol	81.25 ^a	65.00	36.25	16.25 ^a
	Water	73.75 ^b	60.00	40.00	12.50 ^b
15	Methanol	71.25 ^b	60.00	30.00	11.25 ^b
	Water	62.5 ^c	50.00	33.75	8.75 ^c
10	Methanol	56.25 ^d	45.00	23.75	6.25 ^c
	Water	50 ^d	38.75	21.25	7.50 ^c
5	Methanol	33.75 ^e	25.00	16.25	3.75 ^d
	Water	38.75 ^e	27.50	18.75	6.25 ^c

Means in columns followed by the same letter are not significantly different at 5% level (Duncan's multiple range tests)

The petroleum ether extract was the most effective in repellency effect 99.76%. Dhroug, *et al.* (2000) indicated that Bitter apple seed hexane extract proved superiority in repellency effect against spider mites followed by camphor / olive oil mixture. They added that the crude American aloe juice and crude banana leaves juice gave inefficiency effect for mite.

Latent effect of LC₅₀ of *Salvia officinalis* extractson longevity and fecundity of *T. urticae* females.

Data in Table (4) showed that all solvents extracts shortened the longevity and reduced fecundity of *T. urticae*. Pre-oviposition period lasted 2.13 and 1.98 days for methanol and water extract as compared with 1.82 days for untreated one, respectively. On the other hand, oviposition period was 13.75 and 16.07 days for the same solvents while it was 2.44 and 2.51 days for post-oviposition while it was 2.9 days for untreated one, respectively. So, longevity averaged 18.32 and 20.56 days for methanol and water extract and 23.93 days for control, respectively. The total number of deposited eggs for methanol and water extract were (31.39 and 38.92 eggs) while it was (65.31 eggs) for untreated females. These results similar to those of Kawka and Tomczyk (2002)who evaluated both extracts made of fresh and dry leaves of *S. officinalis* that reduced the total number of eggs produced by *T. urticae* females by about 35-45%. The female longevity was reduced by about 25%. Activity of females feeding on ivy leaves was strongly affected by Sage extracts.

Table 4. Latent effect of LC_{50s}different Sage extracts on *T. urticae* female biology

Extracts	Pre-oviposition period	Oviposition period	Post-oviposition period	Longevity days	Fecundity
Methanol	2.13±0.36	13.75±1.14	2.44±0.54	18.32±2.04 ^b	31.39±3.12 ^c
Water	1.98±0.12	16.07±2.07	2.51±0.26	20.56±2.35 ^b	38.92±3.95 ^b
Control	1.82±0.09	19.21±2.11	2.90±0.18	23.93±3.18 ^a	65.31±4.68 ^a

Means in columns followed by the same letter are not significantly different at 5 % level (Duncan's multiple range tests). ± Standard Error

Generally, it was found that Sage plant methanol extract showed an obvious effect on *T. urticae* eggs and adults under laboratory conditions. Synthetic pesticides, which are widely used, are known for causing adverse effects on human beings, the environment, and other creatures. It is considered that some components involved in the Sage extracts show contact effect as well as different effects such as preventing feeding of *T. urticae*. This agrees with Badawy *et al.* (2010) who reported that monoterpenes such as 1,8-cineole, limonene and carvone have a strong fumigant activity against *T. urticae*. Hori and Komatsu (1997) also found that the 1,8-cineole

and camphor components of Sage and Rosemary had repellency effect on *T. urticae* adults and nymphs. Pesticidal efficiency was proved against *T. urticae* that decreased the oviposition period and the number of deposited eggs Bakr, (2013). Ebadollahi *et al.* (2014) studied the effect of essential oils from fennel, *Foeniculum vulgare* Mill and lavender, *Lavandula angustifolia* Miller against adult females of *T. urticae* and chemical composition of these oils was analyzed by GC-MS, Anethole, limonene, α -fenchone, linalool, 1,8-cineole and 1- borneol that affected on *T. urticae* with contact and fumigant toxicity. However, it is necessary to perform experiments of Sage plant extract under field conditions and compare them with the laboratory treatments. In addition, it is believed that performing different types of research by determining the effects of this plant extract on natural enemies would be helpful.

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تقييم الكفاءة الحيوية لمستخلص المرمرية *Salvia officinalis* L. على بعض
الظواهر الحيوية للحلم العنكبوتى *Tetranychus urticae* Koch
(Acari:Tetranychidae)

داليا عبدالرحمن أحمد واكد

معهد بحوث وقاية النباتات - مركز البحوث الزراعية -الدقى-الجيزة-مصر

- تعتبر المستخلصات النباتية أحد البدائل الآمنة بيئياً فى مكافحة بعض الآفات الثاقبة الماصة ويعتبر العنكبوت الأحمر العادى أحد هذه الآفات الهامه الذى يصيب معظم المحاصيل الحقلية والبستانية حيث تم استخدام مستخلص نبات المرمرية بإستخدام كلاً من الميثانول والماء كان له أثر فعال ضد إناث الحلم العنكبوتى وطور البيضة حيث أستخدم أربعة تركيزات مختلفه ٢٠، ١٥، ١٠، و ٥ % من مستخلص المرمرية وتم حساب نسبة الموت بعد ١، ٣، و ٥ يوم من المعاملة.
- أوضحت النتائج أن نسبة الموت تزداد بزيادة التركيز حيث بلغت ٩٢,٨٥ % و ٨٧,١٤ % عند تركيز ٢٠% للمذيب الميثانولى والمائى على التوالى.
- كان لهذا المستخلص تأثير إيدى لطور البيضة حيث سبب نسبة عدم فقس للبيض المعامل مع زيادة مدة حضائه مما أدى إلى طول مدة دورة الحياة بالمقارنة بالكنترول.
- كان لمستخلص نبات المرمرية تأثير طارد ضد الإناث البالغة من الحلم العنكبوتى حيث بلغت نسبة الطرد ٨١.٢٥ % لمستخلص الميثانول بينما كانت ٧٣.٧٥ % للمستخلص المائى وذلك عند تركيز ٢٠ % وإنخفضت هذه النسب بإنخفاض التركيزات والأيام بعد المعاملة.
- تأثر بيولوجى الحلم تأثيراً معنوياً خاصة الإناث المعاملة بالتركيز النصف قاتل من مستخلص المرمرية حيث قلل فترة حياة الإناث البالغة مما أدى إلى نقص عدد البيض للأنثى بالمقارنة بالكنترول.