Acaricidal activities of *Artemisia judaica* L. extracts against *Tetranychus urticae* Koch and its predator *Phytoseiulus persimilis* Athias Henriot (Tetranychidae : Phytoseiidae)

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ABSTRACT

The potential of crude extracts of *Artemisia judaica* L. were evaluated for toxic and repellent effect against adult females and immature stage of *Tetranychus urticae* Koch and its predator *Phytoseiulus persimilis* Athias-Henriot in the laboratory. Ethanolic leaf extraction was more effective as toxic and repellent effect against adult females and immature stage of *T. urticae* , followed by acetone, petroleum ether and aqueous extraction ($P < 0.05$). The results indicated that adults are more susceptible to the leaf extracts than immature. LC_{50} values of *P. persimilis* against ethanolic extract was very low (167.3 gm / ml) as compared to the LC_{50} values of both adult and immature of *T. urticae* which where 0.29 and 2.97 gm / ml, respectively.

Key words: *Artemisia judaica*, Plant extract, Toxicity, Repellent, *Tetranychus urticae*, *Phytoseiulus persimilis*.

INTRODUCTION

Problems associated with the use of synthetic insecticides led researchers to look for natural plant protection compounds such as botanical insecticides and antifeedants. Botanical products are useful tools in many pest management programs because they are effective and specifically target plants natural enemies (Isman, 2006). *T. urticae* control in Egypt has been almost exclusively on pesticides. Unfortunately, spider mites has developed resistance to most available pesticides and the loss of acaricidal efficacy as a result of resistance mite populations in the major problem encountered (Ay, 2005). There is no doubt that widespread indiscriminate pesticide application causing pollution to the health hindered the successful application of such control. So, the intensive use of acaricides in the last few years not acceptable in the modern criteria of integrated pest management (IPM) programs, leading to an increasing interest for alternative pesticides which derived from natural plants (Nauen *et al*., 2001).

Many predaceous Phytoseiid mites are now used as biological control agents in various agricultural ecosystems, and are important predators of phyto phagous mite populations in IPM programs on outdoor and greenhouse crops. *Phytoseiulus persimilis* Athias-Henriot is one of the most important generalist indigenous predator of tetranychid mites and is widely found on various crops and it is considered one of the main predatory mite used in IPM in Egypt. Recently, the extracts of plants have provoked interest as sources of natural products. The genus *Artemisia* L. (Asteraceae) comprises a variable number of species (from 200 to over 400, depending on the authors) found throughout the northern half of the world (Marco and Barbera, 1990). The species *Artemisia judaica* L. known as wormwood grows in wild in Sinai peninsula, Egypt. *A. judaica*, Arabic name “Shih” has enjoyed a reputation among herb experts in Egypt as a medicinal herb (Tackholm, 1974). Isolated compound from this species have been shown anti-malarial, antibacterial, anti-inflammatory (Saban *et al*., 2005), plant growth regulators and anti-tumor activities (El-Massry *et al*., 2002). In another study, essential oils of *Artemisia absinthium* L. was extracted by three methods, such as microwave assisted process, distillation in water, and direct steam distillation, and were then tested for their relative toxicity as contact acaricides to the two-spotted spider mite, *T. urticae* (Chiasson *et al*., 2001), and it was found that all the three extract methods of *A. absinthium* were lethal to the spider mite but in variable degrees.

Some reports also showed that *Artemisia annua* exhibited certain insecticidal bioactivities. Kordali *et al*. (2006) found that the essential oils isolated from the aerial parts of *A. annua* exhibited obvious mortality against *Sitophilus granarius* L. The essential oil of *A. annua* showed very strong fumigant action against *Sitophilus oryzae, Sitophilus zeamais*, *Callosobruchus chinensis* Linnaeus and *Bruchus rufimanus* Boheman . Another research also listed the extract of *A. annua*, which showed highly antifeedant action against *Aphis gossypii*, *T. urticae* (Li *et al*., 2000) and on

T. urticae (Zhang *et al*., 2008). Antifeedant and fungicidal properties of the two major Constituents; piperitone and trans-ethyl cinnamate isolated from the essential oil of *A.judaica* against *Spodoptera littoralis* Boisduval and four plant pathogenic fungi (Abdelgaleil *et al*., 2008).The aim of this work was to evaluate the toxic effects and repellency of different organic solvents extracts of the leaves of *Artemisia judaica* against *T. urticae* and its predator *P.persimilis* under laboratory condition.

MATERIALS AND METHODS

Mites tested

The original population of *T. urticae* was collected from the castor oil plant (*Ricinus communis* L.) in Ismailia Governorate, Egypt. Collected mites were reared continuously on lima bean plants (*Phaseolus vulgaris* L.) under laboratory condition at 27±1°C and 60±5% RH and 16:8 (L : D) photoperiod. The predacious mite *Phytoseiulus persimilis* Athias Henriot was reared in plastic boxes (26 x 15 x 10 cm), a cotton pad was put in the middle of each box, provided with water as a barrier to prevent predatory mite individuals from escaping in addition to a tangle foot strip at the box edges. Highly infested bean leaves with *T. urticae* were provided as food sources to predacious mite in the laboratory.

Plant material

The entire plant was collected from El-Maghara region, Sinai Peninsula, identified and authenticated by a botanist at the department of Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. El-Maghara region situated in the northern part of the central sub region of Sinai Peninsula. It is about 100 km south of Al-Arish city and the Mediterranean coast (latitude 30410 and 30484 N, longitude 331600 and 333630 E).

Preparation for extraction

Leaf samples of *A. judaica* were left to dry under laboratory conditions for two weeks. The dry leaves were powdered using mortar and pestle and screened through an 80-mesh screen. A dried powder (40 gm) was taken and soaked using different organic solvents, Ethanol, Acetone and Petroleum ether (solvent was added at rate of 1mg / 5 ml plant) for 48 h. The extracts were filtered and the filtrates were evaporated till dryness in a rotary evaporator under vacuum. Each crude material obtained was weighted and re-dissolved in the same solvent (1g/10ml solvent), to give 10% (w/v).

Bioassays and treatment

LC_{50} for adult females and immature stages of *T. urticae*

Bioassay was performed in rearing units consisted of four mulberry leaf discs placed on a moist cotton pad in a Petri-dish. Four concentrations, 1.25, 2.5, 5 and 10 gm/ml with four replications per concentration and control were made (solvent only). Effects of the different extracts were tested separately against adult females and immature stage, (20 adult females and immature individuals/ replicate). Mites were transferred to the lower surface of mulberry leaf discs and sprayed with different extracts using a glass atomizer. Mortality was recorded 24, 48 and 72 hrs after treatments. Mortality was corrected using Abott's formula (Abott, 1925) and subjected to probit analysis to estimate LC_{50} with 95% confidence limit according to Finney (1971).

Repellency tests

Mulberry leaves were cut into discs (5 cm in diameter) of symmetrical portion along the midrib obtained per each disc. One half portion of the disc was dipped in tested concentrations and the other half was dipped in solvent as check. The treated discs were left to dry and put on moistened cotton wool in Petri-dishes. Four discs were used as replicates for each concentration. Ten adult females and immature stage were transferred on the midrib of each disc. The mites left to move freely across the two portions of the disc were counted after 24 hr and 48 hr. The number of eggs laid on both sides was recorded after 72 hr. Repellency index were calculated according to Xie and Isman (1992).

Adult females of *P. persimilis* mortality

Five adult females of predacious mite were transferred to each mulberry leaf discs (5 cm in diameter) previously infested with 40 individuals of *T. urticae* and places on moistened cotton wool in Petri-dishes. The leaf discs were sprayed with different concentrations and control ones sprayed with solvent only. Ten replicates for each concentration and control. Mortality of predatory mites was recorded 24, 48 and 72 hr after treatments.

Data analysis

Significant differences among mite groups, solvents and the antifeedant indexes at different treatments were compared using the analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$) for multiple comparisons where significant differences were observed.

RESULTS

The LC_{50} values at 72 hr exposure to different extracts of *A. judaica* and adult females and immature stage of *T. urticae* and *P. persimilis* are given in Table 1. LC_{50} of *P.persimilis* against ethanolic extract was very low (167.3 gm / ml) as compared to the LC_{50} values of both adult and immature of *T. urticae* which where 0.29 and 2.97 gm/ml,

Table 1. LC values (gm/ml) and probit statistics for tested extracts of *A. judaica* against adult and immature stage of *T. urticae* and its predator *P. persimilis* after 72 hr.

	T. urticae								
	Adult			Immature stage			*P persimilis*		
solvents	Slope	x^2	LC_{50} (95%CL)	Slope	x^2	LC_{50} (95%CL)	Slope	x^2	LC_{50} (95%CL)
Ethanol	289.3	0.2	0.29 [a] 0.01 – 0.56	63.89	0.1	2.97[a] 1.12 – 5.11	10.77	0.33	167.3 [a]122.5 – 265.83
Acetone	13.81	0.9	0.56 [a] 0.15 – 1.21	9.41	1.4	3.61[b] 1.99 – 5.62	23.79	0.27	397.57[b] 189.9 – 453.61
Petroleum ether	18.54	0.1	2.01 [b] 1.3 – 3.79	10.92	0.37	4.57 [c] 2.13 – 8.72	19.1	0.2	2124.1 [c] 1121.63 – 5519.2
Water	23.68	0.35	110.32 [c] 65.43 – 217.27	24.01	0.16	27.13 [d] 18.19 – 59.92	13.53	0.93	2223.9 [d] 1321.5 – 7396.3

respectively. Effectiveness of different leaf extracts on the phytoseiid predatory mite was very low and statistically significant differences were found within all extracts after 72 hr of treatments ($p < 0.05$).

Therefore, sensitivity of *P. persimilis* against different plant extract was higher than *T. urticae* by 1.73, 1.41, 9.46 and 0.05 times for ethanol, acetone, petroleum ether and aqueous extracts, respectively (Table 2). LC_{50} values of adult females of *T. urticae* (0.29, 0.56, 2.01 and 110.32 gm / ml) was lower than immature (2.97, 3.61, 4.57 and 27.13 gm / ml) against plant extracts, respectively. Based on the LC_{50} values it can be arranged in the following descending order of effectiveness: Ethanol, acetone, petroleum ether and aqueous extraction. The data obtained show that the various extracts of *A. judaica* were the most toxic against adult females than immature stages. The LC_{50} measured for adult was not significantly between ethanol and acetone extracts, but there was significant differences with petroleum ether extract and highly significant with aqueous extract (Table 1). On the other hand, The LC_{50} measured for immature stage was significant differences between all extracts.

The data indicated that all extracts of *A. judaica* were more toxic to adult females of *T. urticae* than the females of *P. persimilis*. Thus in a habitat where the predator in associated with phytophagous mites it is necessary to apply the least toxic material to the predator and the most efficient to the prey.

The repellency percentage was highest at 10 gm/ml and 5 gm/ml (100%) after 24hr of treatment for ethanolic leaf extract. After 48 hr of treatment, the repellency percentage was decreased. Ethanolic leaf extract was the highest repellency compared with other extracts especially aqueous extract. In case of treated adults, ethanolic extraction was highest repellency after 24 hr of treatment followed by acetone, petroleum ether and aqueous extraction, respectively. While, after 48 hr of treatment, however, the repellency decreased. The same trend was observed with immature. Thus, at 48 hr mites return to fed on treated leaves indicating that the effective organic compound in the leaf extract are probably volatile. Number of eggs laid by females showed a significant reduction as compared with control. Treated females did not statistically significant between ethanol and acetone extracts, but there was significant differences with petroleum ether extract and highly significant with aqueous extract (Table 3).

As a general trend, all treatments investigated were found repellence to adult females and immature of *T. urticae* than the control experiment at all the inspected times. Comparison among treatments revealed no significant differences between extracts. Minimal repellent effect was observed for aqueous extracts as it expressed significantly lower repellency than the other treatments.

DISCUSSION

Herbal therapies are natural products, environmental friendly and cheap. The need for alternative, non chemical,

Table 2. LC_{50} ratio of plant extract against *T. urticae* and its predator *P. persimilis*.

Mites	Ethanol		Acetone		Petroleum ether		Water	
	LC_{50}	Ratio	LC_{50}	Ratio	LC_{50}	Ratio	LC_{50}	Ratio
T. urticae	0.29	1.00	0.56	1.00	2.01	1.00	110.32	1.00
P. persimilis	167.3	1.73	397.57	1.41	2124.1	9.46	2223.9	0.05

Table 3. Repellent effect of *A. judaica* solvent extracts on adult and immature stages of *T. urticae*

Solvents	Conc. gm / ml	*T. urticae*				Average no. of eggs/ female after 48 hr	
		Adult		Immature			
		24	48	24	48	T	C
Ethanol	1.25	92.1	88.3	96.1	94	1.21 [a]	5.19
	2.5	95.9	90.2	96.1	94		
	5	100	90.2	100	96		
	10	100	92.1	100	97.9		
Acetone	1.25	73.2	73.5	83.5	80.2	1.9 [b]	7.11
	2.5	76.7	73.2	85.2	81.8		
	5	78.2	76.8	86.9	83.5		
	10	81.5	78.2	88.7	85.2		
Petroleum ether	1.25	67.8	65	85.2	83.5	1.5 [b]	6.9
	2.5	69.2	66.4	88.7	85.2		
	5	70.7	69.3	90.5	86.9		
	10	73.7	70.7	92.3	88.7		
Water	1.25	48.8	47.1	70.9	68.1	3.41 [c]	9.13
	2.5	50.4	48.8	73.9	70.9		
	5	51.5	49.3	75.9	72.4		
	10	53.8	50.4	76.9	73.9		

T = Treatment C = Control

control strategies in crop protection systems has increased in the last decade due to development of resistant strains of mites. *A. judaica* is an important traditional medicine plant, and it is well known for its antimalarial activity, attributed to the presence of artemisinin. *A. judaica* contains artemisinin, artemisinic acid, methyl wormwood, artemisinic alcohol, and the volatile oil, mainly including eucalyptol, artemisia ketone, camphor, caryophyllene, oxidation caryophyllene, and so on. Volatile oil has antibacterial, antiviral, anti-parasitic, the regulation of immune function, antipyretic and anti-tumor and other roles (Wei *et al*., 2004).

The current results reported the acaricidal bioactivities of *A. judaica* against adult females and immature stage of *T. urticae* and its predator *P. persimilis*. The leaf extracts of *A. judaica* had shown stronger biological activity, and there were significant differences ($P < 0.05$). These results provided basis for further screening and separation of the acaricidal activity components from *A. judaica*, and brought important reference value for the mite pests control new pesticides.

The data obtained show that the various extracts of *A. judaica* were the most toxic against adult females than immature stages except the aqueous extraction. Although, the aqueous extract of *Artemisia* was used herein, the obtained results were in line with the finding of Abd-Elshafy *et al*. (2007) who found that diethyl ether, ethyl acetate and ethanol extracts of *Artemisia* manifested the highest toxicity against larvae of *Hylomma dromedarii*. Also, Hassanein *et al*. (2004) recorded that hexane, chloroform, ethyl acetate and ethanol extracts of *Artemisia* were toxic to the fourth instar larvae of *Spodoptera littoralis* . Moreover, Soliman *et al*. (2005) found that successive extracts of *Artemisia* with petroleum ether, chloroform, ethyl acetate and ethanol were toxic for the two-spotted spider mite *T. urticae*, also, Saber (2004) found that *A. monosperma*, have repellency, mortality and oviposition deterrent effects against female of *T. urtica*. Abdel-Shafy *et al*. (2009) found that Hexane, ethanol extracts and diethyl ether extracts of *A. herba-alba* and ethyl acetate extract of *A. monosperma* were highest effect against the third instar larvae of *Chrysomyia albiceps*. Shekari *et al*. (2008) found that the methanolic leaf extract of *A. annua* L. was effective against 3rd instars larvae and adult of elm leaf beetle *Xanthogaleruca luteola* Mull., with LC_{50} 48% and 43.77% for adult at 24 and 48 hr, respectively. Tripathi *et al*. (2000) showed that adults of *Tribolium castaneum* were more susceptible to cineole which had been extracted from *A. annua*. Ahmed *et al*. (2009) found that the cold *Artemisia* aqueous extracts (Soaking) is more effective than the hot water (Boiling), and mentioned that this could be due to boiling has a negative effect on the active component of the herb. The

different extracts possess a fairly good mite repellency against adult females and immature stage of *T. urticae* after 24 and 48 hr of treatment, this might due to the main component in *A. judaica* as eugenol, carnation oil, -pinene and geraniol. The effect of *A. judaica* extract on *T. urticae* indicates that the extract affect either feeding behavior acting like a deterrent or biochemical processes including digestion and metabolism. However, after 2 days the chemical may evaporate from the leaf or loose its potency and this may explain the loss of deterrency. Shekari *et al*. (2008) showed that the methanolic extract of *A. annua* has antifeedant effect on *X. luteola*. Abdelgaleil *et al*. (2008) tested that the two essential oils of *Artemisia judaica* namely piperitone and trans-ethyl cinnamate as antifeedant activity against the third instar larvae of *Spodoptera littoralis* (Boisd). They found that trans-ethyl cinnamate was more toxic than piperitone. The same two essential oils when tested for antifungal activity against four plant pathogenic fungi, the isolated compounds exhibited a moderate to high activity. The need for new natural pesticides with different mode of action of the entire plant demonstrated here may encourage further studies on their using as biodegradable and mammalian and environmentally safe mites control agents.

REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *Journal Economic Entomology*, **18**: 265 - 267.

Abdelgaleil, S. A. M., Abbassy, A. M., Belal, A. H. and Abdel Rasoul, M. A. A. 2008. Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L. *Bioresource Technology*, **99**: 5947 – 5950.

Abdel-Shafy, S., Soliman, M. and Habeeb, S. M. 2007. Invitro acaricidal effect of some crude extracts and essential oils of wild plants against certain tick species. *Journal of Acarologia*, **22**: 33 - 42.

Abdel-Shafy, S., El-Khateeb, R. M., Soliman, M. M. M. and Abdel-Aziz, M. M. 2009. The efficacy of some wild medicinal plant extracts on the survival and development of third instar larvae of Chrysomyia albiceps (Wied) (Diptera: Calliphoridae). *Trop Anim Health Prod.*, **41**:1741 – 1753.

Ahmed, W. M., Habeeb, S. M., El Moghazy, M. F. and Hanafi, E. M. 2009. Observation on Pediculosis in Buffalo-Cows with emphasis on its impact on ovarian activity and control by herbal remedies. *World Applied Sciences Journal*, **6** (8): 1128 - 1138

Ay, R. 2005. determination of susceptibility and resistance of some greenhouse populations of *Tetranychus urticae* Koch to chlorpyrifos (Dursban 4) by the Petri dish-Potter tower method. *Journal of Pest Science*, **78**: 139 - 143.

Chiasson, H., Bélanger, A., Bostanian, N., Vincent, C. and Poliquin A. 2001. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction. *Journal of Economic Entomology*, **94**: 167 - 171.

El-Massry, K. F., El-Ghorab, A. H. and Farouk, A. 2002. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L.. *Food Chemistry,* **79**: 331 – 336.

Finney. D. J. 1971. Probit Analysis 3rd ed., *Cambridge Univ. Press, London*., 383 **PP**.

Hassanein, A. A., Abou-Yousef, M. H., Soliman, M. M. and Shaaban, M. N. 2004. The biological effects of certain plant extractions against cotton leafworm. *The second international conference on the Role of Biochemistry in Environment and Agriculture*, 404 - 414 **PP**.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, **51**: 45 – 66.

Kordali, S., Aslan, I., Calmasur, O. and Cakir, A. 2006. Toxicity of essential oils isolated from three Artemisia species and some of their major components to granary weevil, *Sitophilus granaries* (L.) (Coleoptera: Curculionidae). *Industrial Crops and Production*, **23**: 162 - 170.

Li, Y. S., Tang, S. Z., Zou, H. Y., Wang, L. X., Yang, Y. Z., Li, W. Y., Na, X. Y. and Er, Z. 2000. Insecticidal activity of extracts from *Artemisia annua*. *Pesticide*, **39**: 25 - 26.

Marco, J. A. and Barbera, O. 1990. Natural products from the genus *Artemisia* L. **In:** Atta-ur-Rahman (Ed.), Studies in natural products chemistry, vol. 7 Elsiever Science Publishers BV, Amsterdam, 201 - 264 **PP.**

Nauen, R., Stumpf, N., Elbert, A., Zebitz, C. P. W. and Kraus, W. 2001. Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* (Acari : Tetranychus). *Pest Management Science*, **57**: 233 - 261.

Saban, K., Recep, K., Ahmet, M., Ahmet, C. A. A., Ali, Y., 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *Artemisia dracunculus*, *Artemisia santonicum*, and Artemisia spicigera essential oils. *Journal of Agricultural Food Chemistry*, **53**: 9452 – 9458.

Saber, S. A. 2004. Influence of Artemisia monosperma Del. Extracts on repellency, oviposition deterrence and

biological aspects of the two-spotted spider mite *Tetranycus urticae* Koch. Egypt. *Journal of Biological Pest Control*, **14**: 345 - 348.

Shekari, M., Sendi, J. J., Etebari, K., Zibaee, A. and Shadparvar, A. 2008. Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). *Pesticide Biochemistry and Physiology,* **91**: 66 – 74.

Soliman M., Saber, S. A. and Amer, S. A. A. 2005. Toxocological evaluation of the desert plant Artemisia monosperma Delile extracts and their isolates on the two-spotted spider mite, Tetranychus urticae Koch. *Egypt Journal of Biology and Pest Control*, **15**: 113 - 117.

Tackholm, V. 1974. Student Flora of Egypt, 2nd ed. Cairo University Press, Cooperative printing Co., Beirrut, Lebanon, 581 **PP.**

Tripathi, A. K. V. , Prajapati, A. K. Aggarwal-Khanuja, S. P. S. and Kumar, S. 2000. Repellency and toxicity of oil from Artemisia annua to certain stored product beetles. *Journal of Economic Entomology,* **93**: 43 - 47.

Tunon, H., Thorsell W., Mikiver, A. and Malander, I. 2006. Arthropod repellency, especially tick (*Ixodes ricinus*), extracted by extract from *Artemisia abrotanum* and essential oil from flowers of *Dianthus caryophyllum*. *Fitoterapia*, **77**: 257 - 261.

Wei, X. G., Dong, Y., Cui, Q. X. and Zhang, G. L. 2004. GC/MS analysis of chemical constituents of volatile oil in uncultivated *Artemisia annua* L. in Dezhou. *Journal of Shandong University of TCM,* **28**: 140 - 143.

Xie, Y. S. , Isman, M. B. 1992. Antifeeding and repellent effect of Meliaceous plants to some insect pest. *Journal of South China. Agri. Univ.*, **4**: 1 - 7

Zhang, Y. Q., Ding, W., Zhao, Z. M., WU, J. and Fan, Y. H. 2008. Studies on Acaricidal Bioactivities of *Artemisia annua* L. Extracts Against *Tetranychus cinnabarinus* Bois. (Acari: Tetranychidae). *Agricultural Sciences in China*, **7**(5): 577 - 584.

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