



Studies on Acaricidal Bioactivities of *Artemisia judaica* L. extracts against *Eutetranychus orientalis* (Klein), (Acari : Tetranychidae)

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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 *Eutetranychus orientalis* (Klein), in the laboratory.

Ethanol leaf extraction was more effective as toxic and repellent effect against adult females and immature stage of *Eutetranychus orientalis* (Klein), 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.

Key words: *E. orientalis*, *A. judaica*, Toxicity, Repellent.

INTRODUCTION

Spider mites (Acari: Tetranychidae) are the most important phytophagous mite pests of agricultural crops worldwide, whose population outbreaks can cause serious damage and yield losses. Among them, the citrus brown mite, *Eutetranychus orientalis* (Klein), is especially significant as the most polyphagous species in field and greenhouses in the Middle East, Africa, and Asia (Sewify & Mabrouk 1991). It usually attacks citrus and is a persistent pest in Egypt, especially in Upper Egypt, preferring lemon and orange. *E. orientalis* is a serious pest of a wide variety of agricultural, ornamental, and medicinal plants (Rasmy 1978).



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).

E. orientalis is primarily controlled by acaricides in Egypt. As long-term reliance of spider mite control on chemical acaricides has caused resistance and public concerns of residues in agroproducts (Iskander *et al.* 1993), it was necessary to search for alternatives to conventional pesticides such as biopesticides to control spider mites (Nauen *et al.*, 2001).

Recently, the extracts of plants have provoked interest as sources of natural products.

Artemisia judaica L. known as wormwood grows in wild in Sinai peninsula, Egypt (Tackholm, 1974). Several isolated compound from this species have been shown plant growth regulators and cyto-toxicity (anti-tumor) activities (El-Massry *et al.*, 2002, Saban *et al.*, 2005). Essential oils of *Artemisia* were tested for their relative toxicity as contact acaricides to the two-spotted spider mite, *T. urticae* (Chiasson *et al.* 2001), and it was found lethal to the spider mite.

Extracts of *A. judaica* were tested against *Spodoptera littoralis* as Antifeedant and fungicidal (Abdelgaleil *et al.* 2008).

Some reports also showed that the other species; *Artemisia annua* L. was exhibited certain insecticidal and fungal bioactivities. Kordali *et al.* (2006) found that the essential oils separated 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 *S. oryzae*, *S. zeamais*, *Callosobruchus chinensis* and *Bruchus rufimanus*. 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 *Tetranychus cinnabarinus* Biosduval (Zhang *et al.*, 2008).

The aim of this work was to evaluate the toxic effects and repellency of different organic solvents extracts having different polarity of the leaves of *Artemisia judaica* against *Eutetranychus orientalis* under laboratory condition.

MATERIALS AND METHODS

Mites tested

The pure cultural of the citrus brown mite, *Eutetranychus orientalis* was collected from the castor oil plant (*Ricinus communis* L.) and reared on castor leaves as host plant under laboratory condition at $27\pm 1^{\circ}\text{C}$ and $60\pm 5\%$ RH and 16:8 (L : D) photoperiod.

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.

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 5 ml/1mg 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 suitable solvent (1g/10ml solvent), to give 10% (w/v).



The effect on adult females and immature stages of *Eutetranychus orientalis* Bioassay was performed in rearing units consisted of four castor 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. 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 castor leaf discs and sprayed with different extracts. Mortality was recorded 24, 48 and 72 hrs after treatments. Data from laboratory bioassays (cumulative mortality data for immature and adult females) were 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

Castor 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).

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



Direct toxicity

The LC₅₀ values with a 95% confidence limit and regression slope at 72 hr exposure to different extracts of *A. judaica* and adult females and immature stage of *Eutetranychus orientalis* are given in Table 1.

LC₅₀ values of adult females of *Eutetranychus orientalis* (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 LC₅₀ 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 LC₅₀ 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).

Repellency effect

The repellency percentage of adult females and immature of *Eutetranychus orientalis* and number of deposited eggs per female are shown in Table 3. 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). Mukind and Syce (2007) found that the extract of *Artemisia afra* was non toxic in mice and rats when administrated orally in doses up to 2000 and 1000 mg/kg, respectively.

As a general trend, all treatments investigated were found repellence to adult females and immature of *Eutetranychus orientalis* 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.

Table (1): LC values (gm/ml) and probit statistics for tested extracts of *A. judaica* against adult and immature stage of *Eutetranychus orientalis* after 72 hr.

Solvents	<i>Eutetranychus orientalis</i>					
	Adult			Immature stage		
	Slope	χ^2	LC ₅₀ (95%CL)	Slope	χ^2	LC ₅₀ (95%CL)
P. ether	17.51	0.1	4.01 ^b (1.3 – 3.79)	10.92	0.37	4.57 ^c (2.13 – 8.72)
Acetone	13.81	0.9	0.49 ^a (0.15 – 1.11)	9.41	1.4	3.61 ^b (1.39 – 5.32)
Ethanol	269.7	0.4	0.29 ^a (0.01 – 0.56)	63.89	0.1	2.97 ^a (1.12 – 5.41)
Water	21.69	0.31	111.32 ^c (61.43 – 215.27)	24.01	0.16	27.13 ^d (16.19 – 54.92)



Table (3): Repellent effect of *A. judaica* solvent extracts on adult and immature stages of *Eutetranychus orientalis*

Solvents	Conc. gm / ml	<i>Eutetranychus orientalis</i>				Average no. of eggs/female after 48 hr	
		Adult		Immature		Treatment	Control
		24	48	24	48		
P. ether	1.25	68.1	65	85.2	84.5	1.5 ^b	6.6
	2.5	69.2	66.4	88.7	85.2		
	5	70.7	69.3	90.5	81.9		
	10	75.9	77.7	92.3	82.7		
Acetone	1.25	73.2	73.5	83.5	81.2	1.8 ^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	87.2		
Ethanol	1.25	92.1	88.3	96.1	94	1.11 ^a	4.91
	2.5	96.9	90.2	96.1	91		
	5	100	90.2	100	93		
	10	100	92.1	100	97.9		
Water	1.25	48.8	47.1	70.9	68.1	4.41 ^c	11.13
	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		

DISCUSSION

The current results reported the acaricidal bioactivities of *A. judaica* against adult females and immature stage of *Eutetranychus orientalis*. 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.

Herbal therapies are natural products, environmental friendly and cheap. The need for alternative, nonchemical, 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).

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*

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*.

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 *A.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.

Tripathi et al. (2000) showed that adults of *Tribolium castaneum* were more susceptible to cineole which had been extracted from *Artemisia annua*. Ahmed et al (2009) found that the cold *Artemisia* aqueous extracts



(Soaking) is more effective than the hot extract (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 *Eutetranychus orientalis* 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 *Eutetranychus orientalis* 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.

Saber (2004) found that *Artemisia 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 *Artemisia 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 *Artemisia annua* L. was effective against 3rd instars larvae and adult of elm leaf beetle *Xanthogaleruca luteola* Mull., with LC₅₀ 48% and 43.77% for adult at 24 and 48 hr, respectively.

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.



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