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Full Length Research Paper

Toxic effect of a selection of medicinal plant products against the parasitic bee mite *Varroa destructor*

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***Varroa destructor* is a dangerous pest directly for beekeeping and indirectly for crops that require insect pollination. Acaricides appeared to be effective against *Varroa* mite but their application within the hives contaminates the wax and honey. The problems associated with the use of acaricides proved considerable incentive to develop new treatment strategies and screening for potential acaricides to minimize these problems. Natural products might provide effective solution to the problem of varroatosis. In the present study, we tested medicinal plant extracts including *Achillea millefolium*, *Artemisia sieberi*, *Mentha longifolia*, *Peganum harmala*, *Satureja sahandica*, *Teucrium polium* and *Thymus kotchyanus*, and *Ferula assa-foetida* resin against *varroa* mite. The mortality was counted after first and second weeks of treatment and total number of dead mites was recorded. All plant extracts and resin showed strong toxicity. However, *F. assa- foetida* resin and *A. sieberi* and *T. kotschyanus* extracts were stronger plant materials in the all tested times and displayed the highest number of mortality. These results demonstrated that tested plant extracts and resin can be suitable alternatives to conventional chemical substances.**

Key words: Honey bee, management, plant extracts, plant resin, *Varroa destructor*.

INTRODUCTION

The varroa mite, *Varroa destructor* Anderson and Truman (Acari: Varroidae), is considered as the most serious pest affecting the honey bee *Apis mellifera* L. It attaches to the body of bee adults, pupae and larvae, weakens them by sucking hemolymph. Varroa also increases the concentration of a series of viruses and then these viruses are also transferred between the bees via the mites. Since the appearance of the mite on *A. mellifera*, honey yield has been reduced, and decreases in both apiary and feral honey bee colonies consequently reduce crop pollination and agricultural yield. Without periodic treatment, most of the honey bee colonies in temperate climates would collapse within a 2 to 3 year period (Fries et al., 1994; De la Rua et al., 2009; Rosenkranz et al., 2010; Nordstrom, 2003). Therefore, varroa research is a challenge for all scientists working in the fields of apiculture, insect pathology and acarology. The control of *varroa* mites is especially difficult as the majority of mites stay in the sealed brood for reproduction and are therefore well protected from different forms of treatment (Hoppe et al., 1989). In the past Varroa mite populations were successfully controlled with the pyrthroid pesticide and fuvalinate (Mavrik) that could be applied in low doses on plywood strips to the brood nests of bee colonies to bring about a high degree of control of varroa mites. Also, 10% of fluvalinate impregnated in plastic strip (10×1 inch) named commercially apistan was used to control varroa mites. In recent years, resistance to this compound has been demonstrated by many authors (Elzen et al., 1999; Macedo, 2002; Thompson et al., 2002; Rodriguez-Vivas et al., 2007; Van et al., 2010). Furthermore, chemicals used to control varroa such as fluvalinate contaminate honey, wax and pollen (Wallner, 1995). In recent years, formic acid was candidate to control varroa mite. Formic acid consumption due to the delayed effect on mortality of mites has been considered. However, application of

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formic acid is limited due to persistence in honey, lack of applicability in areas with temperatures above 32℃ (due to rapid evaporation), damage to the brood (when converted to the larval stage, pupae) is of limited use (Fries et al., 1993; Calderon et al., 2000). The problems associated with the use of acaricides proved considerable incentive to develop new treatment strategies and screening for potential acaricides to minimize these problems. Substances produced from plants can help to solve this problem, since they have the advantage of sustainable supply and are ecologically safe.

Researchers found that plant extracts have acaricidal effects against mite pests. Abdel-Shafy and Zayed (2002) studied the acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae) and indicated that this extract has a highly significant effect in the control of *H. anatolicum excavatum* in economic concentrations of 1.6 and 3.2%. Crude extracts of *Stellera chamaejasme* L. (Thymelaeaceae), a perennial poisonous weed found in meadows and young forest plantations throughout the temperate regions of Asia, demonstrated both contact and systemic toxicity to *Tetranychus viennensis* (Acari: Tetranychidae) in slide dip tests (Shi et al., 2004). Wang et al. (2007) investigated the acaricidal activity of leaf extracts from walnut, *Juglans regia* L., on the mites *Tetranychus cinnabarinus* (Boisduval) and *T. viennensis* Zacher (Acari: Tetranychidae). According to their results, extracts had both contact and systemic toxicity to these mites.

The acaricidal activity of crude extracts and fractions from stems and leaves of *Petiveria alliacea* (Phytolaccaceae) was reported against larvae and adults of the cattle mite, Rhipicephalus (Boophilus) microplus (Acari: ixodidae), by Rosado-Aguilar et al. (2010).

The aim of the present investigation is to search for effective plant extracts to control *V. destructorb* for their advantages of low cost and low health and environmental hazards for both consumers and house bee keepers. In the present study, the toxicity of the extracts obtained from eight medicinal plants including *Achillea millefolium* L. (Asteraceae), *Artemisia sieberi* (Asteraceae), *Mentha longifolia* L. (Lamiaceae), *Peganum harmala* L. (Zygophylaceae), *Satureja sahandica* (Lamiaceae), *Teucrium polium* (Lamiaceae) and *Thymus kotchyanus* (Lamiaceae) and *Ferula assa-foetida* (Apiaceae) resin were investigated against *V. destructor*.

MATERIALS AND METHODS

Plant material and extraction

The aerial parts of *A. millefolium* (37°56′ 01.0′′ N, 48°11′ 00.9′′ E, altitude=alt= 1636 m), *A. sieberi* (37°56′ 01.0′′ N, 48°11′ 00.9′′ E, alt= 1636 m), *M. longifolia* (37°56′ 01.0′′ N, 48°11′ 00.9′′ E, alt= 1636 m), *P. harmala* (37°56′ 01.0′′ N, 48°11′ 00.9′′ E, alt= 1636 m), *S. sahandica* (37°56′ 4.35′′ N, 48°00′ 20.34′′ E, alt= 2046 m), *T. polium* (37°56′ 01.0′′ N, 48°11′ 00.9′′ E, alt= 1636 m) and *T. kotchyanus* (37°56′ 4.35′′ N, 48°00′ 20.34′′ E, alt= 2300 m) were collected at the beginning stage in Ardabil province, Iran. This material was air dried in the shade at room temperature (26 to 28℃). The specimens were ground with electrical grinder. Ten grams from each plant material with 100 cc water were placed in autoclave and kept on shaker for two h and finally, filtration was carried out by standard filter paper (Whatman N° 1). Achieved extracts were dried by rotary evaporator. The resin of *F. assa-foetida* was purchased from Fars province, Iran.

Bioassay

The 54 hives were selected from Pars Abad, Ardabil, Iran and determination of the percentage of pollution performed with sugar test. Experiments were performed according to study of Shimanuki and Knox (2000) with nine treatments and three replications in a randomized complete blocks design. Counting of the number of dead mites in each hive was considered as a measure of acaricidal effects. A hundred bees from each hive were selected and placed in the glass jars containing powdered sugar. Mites were separated via shaking of glass jars and contact the infected bees with powdered sugar.

The number of separated mites in the container were counted and expressed as infection percentage for each hive. After sugar test, 27 hives were selected from 54 treated hives and were arranged based on infection percentage in three replicates with 2 to 4, 4 to 6 and 6 to 8%. On the other hand, 9 hives were placed in one block (Table 3). Then, 1.5 g from each extract and resin was decanted in 100 cc water and used for each hive as spray method. Medicinal plant materials along with apistan (as a positive control) randomly utilized into the blocks. A newspaper was spread in front of and under the comb of the each hive. The number of dead mites was counted after one and two weeks after treatment. Results were analyzed by analysis of variance (ANOVA) and the mean of mortality was compared by LSD (least significant difference, $\alpha = 0.05$) by SAS software.

RESULTS

The extracts and resin of herbs showed strong toxicity on varroa mite for first and second weeks and the total of two weeks and the analysis of variance showed significant difference for this toxicity (Table 1). The mean comparison of mortality for tested plant extracts and resin against varroa mites is shown in Table 2. Comparison of the mean at first week showed that the greatest mean for killed mites was related to *F. assa- foetida* resin and positive control (apistan) and minimum mean achieved with *P. harmala* extract. Comparison of the mean at second week showed that highest means were for apistan and *F. assa- foetida* resin. In this case, the lowest means were for *M. longifolia* and *P. harmala* extracts. For total number of dead mites in two weeks, the highest means obtained with apistan and *F. assa- foetida* resin, too and the lowest mean was related to *P. harmala* extract (Table 2).

Results showed that *F. assa-foetida* resin, *A. sieberi* and *T. kotschyanus* extracts were respectively the strongest plant materials against varroa mite in the all tested times and displayed the highest mean mortality.

Table 1. Result of analysis of variance.

S.O.V.	df	MS		
		For first week	Second week	Two weeks
Replication	2	72574.70**	10633.44**	138698.81**
Treatment	8	48138.78**	18508.5**	123356.12**
Error	16	1613.70	788.57	3905.65
C.V. %		15.68	24.63	16.88

**, significant at P≤1%.

Table 2. Comparison mean of the mortality of *Varroa destructor* exposed to different medicinal plant extracts and resin and apistan, as a one of the conventional chemical compound, with LSD test at p = 0.05.

Experimental material	Mean first week	Mean second week	Total two weeks
T. kotschyanus extract	293	133.67	426.67
A. millefolium extract	239.00	110.00	349.00
A. sieberi extract	345.33	155.67	501.00
T. polium extract	180.33	81.33	261.67
M. longifolia extract	168.67	25.67	194.33
P. harmala extract	30.33	14.33	44.67
S. sahandica extract	211.67	56.00	267.67
F. assa- foetida resin	418.33	208.00	626.33
apistan	417.67	241.33	659.00
LSD	69.53	48.60	108.17

Table 3. Data of the mortality of *Varroa destructor* exposed to different medicinal plant extracts, resin and apistan in three blocks (2 to 4, 4 to 6 and 6 to 8%).

Replication	Treatment	First week	Second week	Total two weeks
Block 1 (2-4%)	*T. kotschyanus*	192	96	288
	S. sahandica	118	45	163
	M. longifolia	86	14	100
	P. harmala	11	0	11
	F. assa- foetida	278	134	412
	A. sieberi	235	113	348
	A. millefolium	157	79	236
	apistan	273	185	458
	T. polium	114	31	145
Block 2 (4-6%)	*T. kotschyanus*	298	123	421
	S. sahandica	215	67	282
	M. longifolia	171	14	185
	P. harmala	33	29	62
	F. assa- foetida	435	203	638
	A. sieberi	367	175	542
	A. millefolium	245	136	381
	apistan	406	227	633
	T. polium	203	96	299
Block 2 (6-8%)	*T. kotschyanus*	389	182	571
	S. sahandica	302	56	358

Table 3. Contd.

M. longifolia	249	49	298
P. harmala	47	14	61
F. assa- foetida	542	287	829
A. sieberi	434	179	613
A. millefolium	315	115	430
apistan	574	312	886
T. polium	224	117	341

DISCUSSION

The effect of many plant products such as essential oils and extracts as acaricides in protecting varroa mite infestation has been studied, and it has been shown that this mite is susceptible to the some plant derived materials. Most of these extracts cause the mite to release from the bees, but do not cause mite mortality, and are therefore of limited value. For example, Smirnov et al. (1984) used a plant acaricidal preparation (KAS-81) with sugar syrup for the control of varroa during the whole season without harming the bees. Calderone et al. (1997) used plant extracts for the control of the varroa mites and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of *A. mellifera*. They found that mite prevalence values increased 28.3% in the control colonies by the following May, but decreased 22.4% in colonies receiving thymol and citronella. Whittington et al. (2000) and Dimetry et al. (2005) found that Neem oil spray killed 90 to 94% of varroa mites but thymol oil spray reduced varroa mites to 79% in honey bee colonies. Abd El-Wahab and Ebada (2006) studied the toxicity of *Citrus aurantium*L. (sour Orange), *Cymbopagon flexuosus* (lemon grass) and Citronella volatile oils against varroa mite and found that the mean percentage of varroa infestation on the worker brood and adult workers of honey bee reduced to 100% after the fourth week of treatment with *Citrus aurantium* L. (sour Orange), *Cymbopagon flexuosus* (lemon grass) and after the third week of the treatment with Citronella oils. Romeh (2009) showed that sycamore leaves (*Ficus sycamorus*) extract is effective in the control of varroa mite.

In all cases, control of varroa mite using naturally plant products are more recommended than other chemical acaricides to keep the social life of honeybee away from any harmful effect (Dimetry et al., 2005). These results demonstrated that tested plant extracts and resin can be suitable alternatives to conventional chemical materials such as apistan and apivar. Although, this knowledge should be taken into account to control mite based on natural products, further studies are need to identify some other factors that may be involved in a successful control of Varroosis. Further studies will be needed to evaluate the possible side effects of the active plant extracts on honey bee and the taste of honey.

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