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The effect of different plant extracts on honeybees *Apis mellifera* (Hymenoptera: Apidae) and its parasite, *Varroa destructor* (Acari: Varroidae)

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Abstract

Extracts of 31 cultivated and wild plant species were evaluated for their ability to control *Varroa destructor*, a parasitic mite of honeybees, under laboratory conditions. The bees were fed a solution of 100 mg (dry weight) plant extract diluted in 10 ml sugar water. Numbers of dead mites and bees were recorded daily. Five plant extracts (of *Phoenix dactylifera*, *Zizyphus spina-christi*, *Malva sylvestris*, *Cupressus sempervirens*, and *Rhus coriaria*) were lethal for the mites, whereas the other 26 plant extracts showed no effect. *P. dactylifera and C. sempervirens* were effective against both mites and bees. Thus, the most promising extracts, with minimal insecticidal and maximum acaricidal activities, were from *R. coriaria*, *M. sylvestris* and *Z. spina-christi*.

Keywords: plant extract, honey bees, *Varroa destructor*, natural acaricide.

Introduction

The ecto-parasitic mite *Varroa destructor* endangers worldwide beekeeping industry. *Varroa*, after successfully parasitizing the European honeybee, *Apis mellifera*, has caused severe damage to its new host. *A. mellifera* colonies die from Varroa infestation within few years if the mite population growth is not regulated by beekeepers (RITTER, 1988). Honeybee colonies need chemical treatments to reduce losses caused by this mite. Several synthetic acaricides were used, that include fluvalinate (HENDERSON, 1988; HERBERT *et al.*, 1988a; FERRER-DUFOL *et al.*, 1991), flumethrin (FERRER-DUFOL *et al.*, 1991),

amitraz (HENDERSON, 1988; HERBERT *et al.*, 1988b), clofentezin (YOSHIDA & FUCHS, 1989), apitol (HERBERT *et al.*, 1988b), and coumaphos (DE RUIJTER & VAN DEN EIJNDE, 1986).

Synthetic acaricides have significant drawbacks. Accumulation of residues in bee products as well as potential development of acaricide-resistance in *Varroa destructor* populations are considered as major obstacles (HEMMERLING *et al.*, 1991; LOGLIO, 1993; WALLNER, 1995). These reasons provide considerable incentive to develop new control strategies. Plants may provide an alternative means of *Varroa* control since they constitute a rich source of bioactive chemicals. Some plant extracts tend to have low mammalian toxicity, little environmental effect and wide public acceptance (ISMAN, 2000).

It is well known that many essential oils and their components exhibit acaricidal activity (KIM *et al.* 2004). Before *Varroa destructor* was a worldwide pest, ANGELLOZ (1930) and VECCHI & GIORDANI (1968) tested different components of essential oils for their activity against *Acarapis woodi*. Methyl salicylate and menthol proved to be toxic to the tracheal mite. Researchers testing the effect of natural plant extracts on Varroa mite obtained contradicting results. HOPPE (1990) examined 55 essential oils for Varroa mite toxicity. He found that 24 essential oils caused above 90% mite mortality, whereas only nine extracts caused a below 10% bee mortality. Mite and bee mortality resulting from exposure to marjoram, cinnamon, clove, citronella and lavender oils were investigated by KRAUS (1990). He found that clove oil caused 80% mite mortality with no killing effect on the bees, and marjoram resulted in 100% mite mortality and 20% bee mortality. Lavender, melissa and citral disturbed the bees behavior while wintergreen, fire needle, *Pinus muge*, and neem oils showed no effect (BUNSEN, 1991). Mite mortality was 100% when treated with oregano oil, 87.2% with clove oil, 75.5% with bay oil and 59.4% when treated with tea tree oil (SAMMATARO *et al.*, 1999). Therefore, the objective of this study was to investigate the effectiveness of 31 aromatic plant extracts against *Varroa* mites.

Materials and Methods

Experiments were conducted on *Apis mellifera syriaca* during the summer months of 2006. Sixty sister young honeybee workers were collected from the research hives of the university apiary and were kept in cages (5 x 10 x 10 cm). Each cage was closed at the bottom with a net (meshes 2 x 2 mm) that had an insertion for collecting dropped mites, a cover and two safety glass windows. The edges of the insertion were smeared with a thin layer of Vaseline to prevent the dropped but life mites from escape.

Varroa destructor is extremely frail when handled outside the hive away from bees. Each mite was allowed to get in contact with young worker bees (2-3 day old). A total of 40 mites with their host bees (2 days-old) were placed into the cages and incubated at 35°C and 70% relative humidity. In addition, 20 bee workers free of *Varroa* mites were placed in each cage. The

Table 1: List of plant species and parts used for extracts tested to control the parasitic mite, *Varroa destructor*.

No.	Family	Species
1	Anacardiaceae	*Rhus coriaria* L.
2	Apiaceae	*Ammi majus* L.
3	Apiaceae	*Apium graveolens* L.
4	Apiaceae	*Carum carvi* L.
5	Apiaceae	*Coriandrum sativum* L.
6	Apiaceae	*Cuminum cyminum* L.
7	Apiaceae	*Eryngium creticum* Lam.
8	Apiaceae	*Ferula assafoetida* L.
9	Apiaceae	*Foeniculum vulgare* Mill.
10	Apiaceae	*Petroselinum sativum* Hoffman.
11	Apiaceae	*Pimpinella anisum* L.
12	Araceae	*Eminium spiculatum* (Bl.) Kuntze
13	Burseraceae	*Commiphora molmol* Engl.
14	Cupressaceae	*Cupressus sempervirens* L.
15	Cupressaceae	*Tetraclinis articulata* (Vahl.)
16	Euphorbiaceae	*Mercurialis annua*
17	Euphorbiaceae	*Ricinus communis* L.
18	Liliaceae	*Aloe vera* L.
19	Liliaceae	*Asphodelus fistulosus* L.
20	Lythraceae	*Lawsonia inermis* L.
21	Malvaceae	*Althea setosa* L.
22	Malvaceae	*Hibiscus sabdariffa* L.
23	Malvaceae	*Malva sylvestris* L.
24	Myrtaceae	*Eugenia caryophyllus* Thumnb.
25	Palmaeceae	*Phoenix dactylifera* L.
26	Piperaceae	*Piper nigrum* L.
27	Rhamnaceae	*Zizyphus spina christi* (L.) Willd.
28	Rubiaceae	*Rubia tinctorum* L.
29	Salvadoraceae	*Salvadora persica* L.
30	Urticaceae	*Urtica urens* L.
31	Verbenaceae	*Verbena officinalis* L.

experiments were conducted in three replicates for four days using complete randomized design.

Thirty-one cultivated and wild plant species were collected in Jordan and used for obtaining extracts from given plant parts (Table 1). They belong to 17 families and are commonly used in Jordan for nutritional and medicinal purposes. Prof. Dr. Ahmed EL-OQLAH (Yarmouk University, Irbid, Jordan) verified plant classification. Voucher specimens were deposited at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid. Plant material was shade-dried at 25-30°C and 40-50% relative humidity for about three

Table 2: The effect of different plant extracts on Varroa mites (n=40) and worker bees (n=60). * All values in this table are given as mean ± SD. Means followed the same letter are not significantly different (P= 0.05) as determined by the least significant difference test.

Plant species	Dead bees	Killing %	Dead mites	Killing %
Rhus coriaria	0±0.0 c	0	21±3.61 bc*	52.5
Cupressus sempervirens	22±4.36 b	36.7	24±3.61 ab	60
Malva sylvestris	1±1.73 c	1.7	24±5.29 ab	60
Phoenix dactylifera	48±5.29 a	80	26±5.0 a	65
Zizyphus spina-christi	1±1.73 c	1.7	24±6.93 ab	60
Control	1±1.73 c	1.7	2±2.0 c	5

weeks. This material was then grounded in a Wiley grinder with a 2 mm diameter mesh. Fifty grams of the ground material were extracted by cold percolation with 95% ethanol. The ethanolic extract was concentrated under vacuum, weighed and the residue was used in the tests.

The feeding material was prepared by dissolving 1 kg sugar in one liter distilled water. The experimental bees were feed with 10 ml sugar solution mixed with 100 mg plant extract. Bees in the control cages were fed with 10 ml sugar solution only. Numbers of mites dropping on the cage insertions and of dead bees were recorded daily. At the end of the experimental period the whole cages were frozen and the bees were checked under microscope individually for any remaining mites. Data were subjected to analysis of variance (ANOVA), and means were compared using Fisher's least significant differences test at P = 0.05 (STEEL & TORRIE, 1980).

Results

Results showed that 24 extracts had a positive killing action on the mites, as compared to the control. Five extracts caused a high mortality (= 50%) and they were from *P. dactylifera*, *Z. spina-christi*, *M. sylvestris*, *R. coriaria*, and *C. sempervirens*. No acaricidal activity was observed with extracts from *P. anisum*, *A. graveolens*, *E. spiculatum*, *C. molmol*, *T. articulata*, *R. communis*, *L. inermis*, *A. setosa* and *E. caryophyllus*. The other extracts showed a very low acaricidal activity.

A poisoning effect on bee workers was observed with 26 extracts, and 13 extracts caused over 10% bee mortality. *R. coraria*, *F. vulgare*, *A. graveolens*, *T. articulate*, and *A. setosa* were totally not poisonous to the bee workers, while *F. assafoetida*, *E. creticum*, *P. anisum*, *C. sativum*, *E. spiculatum*, *C. molmol*, *C. sempervirens*, *A. vera*, *P. dactylifera*, *P. nigrum*, *R. tinctorum*, and *S. persica* extracts killed significantly more than 10% of the bees as compared to the control. The plant extracts from *C. molmol* and *V. officinalis* were totally ineffective against the mites, but killed all bees.

The most promising extracts, which have minimal insecticidal and maximum acaricidal activities (Fig. 1), were from *R. coraria*, *M. sylvestris* and *Z. spina-christi*.

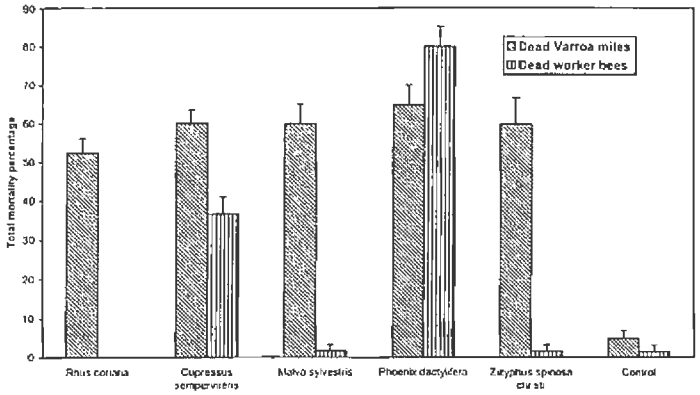

Figure 1: Total mortality percentage of *Varroa destructor* mites (n=40) and worker bees (n=60) after treatment with the most effective plant extracts.

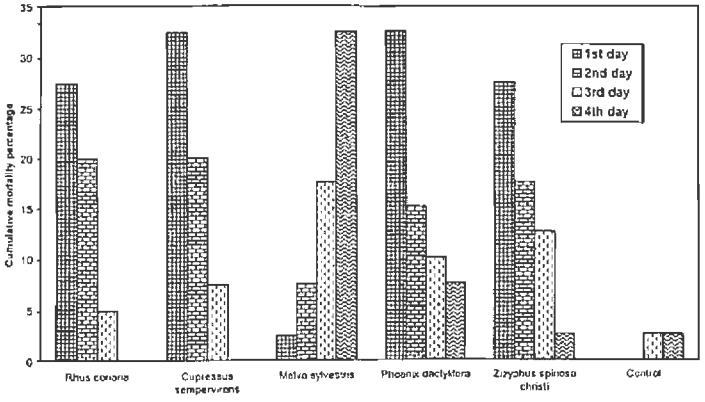

Figure 2: Daily mortality percentage of *Varroa destructor* mites (n=40) in four days interval after treatment with the most effective plant extracts

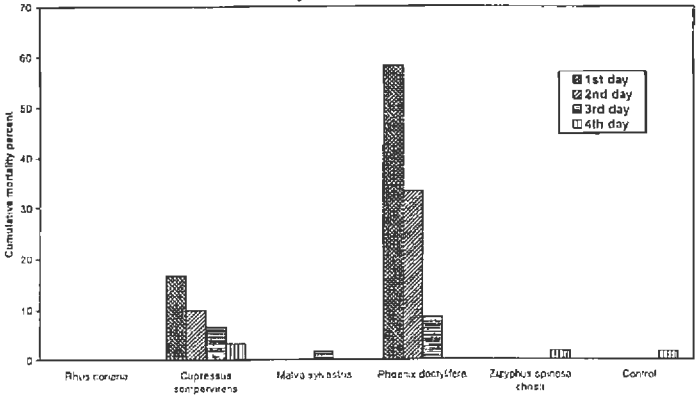

Figure 3: Daily mortality percentage of bees (n=60) in four days interval after treatment with most effective plants extracts

For the five plants species listed in Table 2, their extract killed most mites at the beginning of the experiment, that is, especially in the first day after application, except for the extract from *M. sylvestris* where mortality increased over time (Fig. 2). The daily mortality percentages of bee workers are illustrated in Fig. 3. This mortality caused by the extracts appeared only upon the use of *P. dactylifera* and *C. sempervirens*.

Discussion

A majority of plant extracts used in our tests had no real effect against *Varroa* mites. These extracts probably did not contain acaricidal compounds, or any other deleterious chemicals for the parasitic mites. However, the method of extraction was perhaps not appropriate, which may have affected pesticide concentration and properties. Several factors have been identified as possible source of variation for the chemical composition and toxicity of extracts, such as phenological age of the plant (JACKSON & HAY, 1994), humidity of the harvested material (CHIALVA *et al.*, 1983; TATEO & RIVA, 1991), plant parts chosen for extraction (CHIALVA *et al.*, 1983; JACKSON & HAY, 1994) and the method of extraction (PEREZ-SOUTO *et al.*, 1992). Pesticides made from plant extracts are notorious for variable toxicity to the target species even when they are made from the same plant species (CHIASSON *et al.*, 2001).

Some of our plant extracts showed a lethal activity against honeybee workers and the bee parasitic mites (Tab. 2), and *C. molmol* and *V. officinalis* even showed no acaricidal effect, but had a high insecticidal effect. Such variations of effectiveness on mites versus bees could be related to the concentration of the compounds involved, the length of treatment, the delivery method and environmental conditions (COX *et al.*, 1989; CALDERONE & SPIVAK, 1995; CHIASSON *et al.*, 2001; KIM *et al.*, 2004). In the present study, two symptoms of poisoning appeared. Most of dropped mites had a knockdown-type death, while the other dropped mites were still alive without any movement. These two types of mites poisoning were reported by FURUNO *et al.* (1994); IGNATOWICS (1981) and KWON & AHN (2002). Several plant compounds possess significant acaricidal activity against the parasitic tracheal mite, *A. woodi*. Menthol and eucalyptus oil contain citronellal and cause significant mortality of *A. woodi* and *V. jacobsoni* (CALDERONE *et al.*, 1991).

Generally, the seven plant extracts which were most effective against *Varroa* mites knocked them down in the first day after exposure to the extract, and had a gradually decreasing effect afterwards, except for *M. annua* and *M. sylvestris*. This clear difference in the toxicity of the extracts against the parasitic mite may be due to variation in the chemical composition and toxicity of the extracts (JACKSON & HAY, 1994; RASIKARI *et al.*, 2005). Other involved factor could include the repellent character of these extracts. KRAUS *et al.* (1994) reported that bee brood infestation with *V. jacobsoni* was significantly diminished when brood wax comb contained 0.1% marjoram oil.

The toxicity of extracts from plant origin to honey bees has been reported in

several studies on the control of the bee parasitic mite *Varroa destructor* (HOPPE, 1990). Honeybee mortality from exposure to marjoram, cinnamon, clove, citronella, lavender oils and *Melissa* were reported by KRAUS (1990) and BUNSEN (1991). LINDBERG *et al.* (2000) mentioned that honeybees appear somewhat susceptible to different plant extracts. The insecticidal effect of plant extracts was also reported against other insect pest species. *Thymus serpyllum* and *Origanum majorama* found to be toxic to bean weevil *Acanthoscelides obtectus* (Bruchidae) (REGNAULT-ROGER & HAMRAOUI, 1993). Contact, fumigant and antifeedant effects of extracts of *Syzygium aromaticum* and *Illicium verum* against the red flour beetle *Tiobolium castaneum* and maize weevil *Sitophilus zeamais* were also proved (HO *et al.*, 1995, 1997; HUANG & HO 1998; HUANG *et al.*, 1998).

In conclusion, the extracts of *Z. spina-christi*, *M. sylvestris* and *R. coriaria* have a good potential to be used in a sustainable management approach of *Varroa destructor* populations, by keeping the pest below a damage threshold, and thereby maximizing the health of *A. mellifera* colonies. Investigations on the characterization of the dose response relationships between plant extracts and bee/mite toxicity in the field and contamination of bee hive products will be performed in the near future.

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