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Evaluation of Secondary Effects of some acaricides on *Apis Mellifera Intermissa* (Hymenoptera, Apidae): Acetylcholinesterase and Glutathione S-Transferase Activities

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INTRODUCTION

The honeybee, *Apis mellifera* L., (Hymenoptera, Apidae) is an invaluable beneficial insect in agriculture around the world for its production of honey, and more importantly, for its role in pollination. The parasitic mite *Varroa destructor* (Acari, Varroidae) is a serious world-wide pest of the honeybee *Apis mellifera*. The control of this mite infestation is obtained by the use of several acaricides. Therefore, the aim of this study is to evaluate the secondary effects of the acaricidal treatments on the *Varroa* host, the honeybee, by measuring acetylcholinesterase (AChE) and glutathione S-transferases (GSTs) activities in the larvae, pupae and adult stages of *A. mellifera intermissa*.

MATERIALS AND METHODS

Insect. The experiments were carried out in an apiary of honeybees derived from *A. mellifera intermissa*, placed in Langstroth standard hives in Annaba. Five experimental groups of five colonies of honeybees each (four treated and one control) were used. Acaricides were applied and the bees were collected at larval (day 4); pupal (day 4) stages and adult stages of new (day 0), nurses (day 7) and foragers (day 21), respectively.

Acaricides and treatment. Two plastic strips of Apivar (500 mg of amitraz per strip) were inserted in the brood chamber of each hive. Four strips (3.6 mg of flumethrin per strip) were inserted in the brood chamber of each hive. Strips of Apivar and Bayvarol were left for six weeks and then removed from the treated hives. Apiguard is made with thymol in gelatine manufactured as 50 g gel portions. One tray of Apiguard (12.5 g of thymol per tray) is placed on the top bars of the frames of each brood nest and a second tray at 15 day intervals. ApiLife Var is composed of a porous ceramic carrier impregnated with a mixture of thymol (76%), eucalyptol (16.4%), menthol (3.8%) and camphor (3.8%). The vermiculite tablet is laid on the upper part of the brood combs. After 3 weeks of application, a second tablet is placed in the hive for the same period .

Enzyme assays. The AChE activity was carried out following the method of Ellman *et al.* (1961) using acetylthiocholine as a substrate. Pooled head (each containing 9 heads per series) were homogenized in the following solution containing 38.03 mg ethylene glycol tetraacetic (EGTA), 1ml Triton X-100, 5.845 g NaCl and 80 ml Tris buffer (10Mm, pH 7). After centrifugation (5000g, 5 min), the AChE activity was measured in aliquots (100µl) of resulting supernatants added to 100 µl of 5-5' dithiobis-(2- nitrobenzoic acid) (DNTB) in Tris buffer (0.01 M, pH 8) and 1 ml Tris (0.1 M, pH 8). After 5 min, 100µl acetylthiocholine was added. Measurements were conducted at a wavelength of 412 nm with a run time of 20 minutes.

GST activities were determined with the soluble fraction as enzyme source. GST activities toward 1-chloro-2, 4-dinitrobenzene (CDNB) were measured according to Habig *et al.* (1974). Bees were sampled from control and treated groups and the sting apparatus and venal glands of adults were removed. Each decapitated body was homogenized in sodium; phosphate buffer (0.1 M, pH 6) and centrifuged (14000 g, 30 min). Two hundred microliter of the resulting supernatant was added to 1.2 ml of reaction mixture containing 1 Mm CDNB and 5 Mm reduced glutathione (GST) in the homogenization buffer. Changes in absorbance were recorded at 340 nm. Total protein content was determined according to method of Bradford (1976) using bovine serum albumin as a standard. Enzyme activities were expressed as µmol/min/mg proteins.

Statistics. Results are expressed as mean ± standard deviation (s.d.). All data were subjected to one–way analysis of variance (ANOVA) followed by a *post-hoc* Tukey test. The homogeneity of variances was controlled by the Levene method.

RESULTATS

The specific activity of AChE of *A. mellifera intermissa* was followed during different life stages and data showed that there were no significant differences between colonies treated with acaricides and untreated colonies (Table 1).

Table 1. Effects of some acaricides on the activity of acetylcholinesterase (µmol/minute/mg protein) in three developmental stages of *Apis mellifera intermissa*. Data are expressed as means ± s.d. (n=12-18). In each stage, mean values followed by same letters are not significantly different ($p \geq 0.05$).

Treatments / Stages (days)	Control	Amitraz	Flumethrin	Thymol	Thymol blended with essential oils
Larvae (4)	1.64 ± 0.61 A	1.18 ± 0.55 A	1.23 ± 0.56 A	1.70 ± 0.51 A	2.06 ± 1.01 A
Pupae (4)	21.02 ± 2.46 A	20.02 ± 2.84 A	19.55 ± 2.80 A	20.83 ± 2.30 A	20.50 ± 3.22 A
Adult (0)	34.68 ± 6.82 A	30.90 ± 5.49 A	31.97 ± 5.26 A	34.07 ± 6.42 A	30.60 ± 4.17 A
Adult (7)	20.40 ± 2.89 A	18.16 ± 2.58 A	18.07 ± 2.23 A	21.80 ± 1.49 A	19.08 ± 2.33 A
Adult (21)	18.30 ± 0.93 A	17.22 ± 3.13 A	16.32 ± 2.77 A	16.64 ± 2.17 A	18.00 ± 1.61 A

The specific activities of GST in the larvae were significantly higher with amitraz and flumethrin (Table 2). In the pupae stage, the highest activity was found with amitraz followed by flumethrin. In the groups treated with thymol and thymol blended with essential oils, the activities were not significantly different. For control group, the activity value was lower than the other treatments.

Table 2. Effects of some acaricides on the activity of glutathione S-transferases (µmol/minute/mg protein) in the larvae and pupae stages of *Apis mellifera intermissa*. Data are expressed as means ± s.d. (n=12-18). In each stage, mean values followed by different letters are significantly different ($p < 0.05$).

Treatments / Stages (days)	Control	Amitraz	Flumethrin	Thymol	Thymol blended with esential oils
Larvae (4)	21.91 ± 6.23 A	73.26 ± 8.39 C	63.62 ± 5.27 C	38.36 ± 4.69 B	46.77 ± 7.87 B
Pupae (4)	24.69 ± 2.69 A	94.45 ± 3.09 D	73.45 ± 6.66 C	38.14 ± 3.13 B	35.64 ± 1.57 B
Adult (0)	14.58 ± 3.15 A	40.64 ± 5.57 C	23.79 ± 3.35 B	16.04 ± 3.56 A	14.96 ± 2.77 A
Adult (7)	6.38 ± 3.23 A	23.48 ± 3.14 C	14.87 ± 3.66 B	8.05 ± 2.87 A	10.60 ± 3.84 AB
Adult (21)	16.12 ± 5.13 A	23.53 ± 6.81 A	22.98 ± 3.65 A	16.19 ± 5.25 A	18.20 ± 2.62 A

The specific activity in newly emerged workers showed a significant increase in the groups treated with the synthetic acaricides for amitraz and flumethrin. The negative effect remained in workers of 7 days old. But later on, at 21 days in the adult life the specific activity was almost identical in all experimental groups (Figure 1).

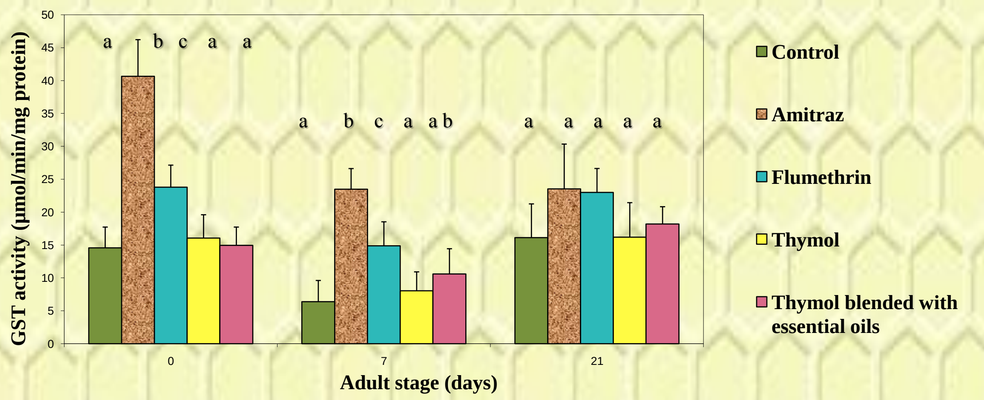

Figure 1. Effects of some acaricides on the activity of glutathione S-transferase (µmol/minute/mg protein) in the adults of *Apis mellifera intermissa*. Data are expressed as means ± s.d. (n =12-18). In each date, different letters above bars indicate significant differences at $p < 0.05$.

CONCLUSION

The acaricides used to control *V. destructor* did not affect the activity of AChE but induce the increase of GST indicating that bees are exposed to toxic stress when acaricides, especially synthetic ones, are used in hives. Therefore, the two thymol formulations can be recommended as treatment and synthetic varroacides as flumethrin and amitraz should be minimized. Also, efforts are necessary to optimise the diversity and availability of mite control strategies and to use acaricides under supervision by the authorities in order to preserve bee health.