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Toxicity of Thiacloprid and Fenvalerate on the black bean aphid, *Aphis fabae*, and biosafety against its parasitoid, *Lysiphlebus fabarum*

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ABSTRACT

Considering the undeniable need to use synthetic pesticides in agriculture, there are new steps to find new pesticides and new ways to manage them to act safer to human and the environment. In this study, the toxicity of thiacloprid and fenvalerate were tested under field conditions at recommended field rates on the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), and also evaluated biosafety against its parasitoid, *Lysiphlebus fabarum*. This aphid is one of the most polyphagous sap-sucking pests that make primary and secondary damages to more than 200 leguminous host plants. While *L. fabarum* plays an important role in the control of *A. fabae* (34-79% parasitism in different conditions), insecticides must be used in special conditions to reduce their adverse effects. The Black bean aphids were sprayed with the insecticides or water after adaptation on the *Vicia faba* leaves. Thiacloprid and fenvalerate caused 89.21±3.6% and 81.14±4.2% mortality against aphids, respectively. The insecticides were also applied to the immature stages of parasitoid which were exposed to the materials by briefly dipping mummified aphids into insecticide emulsion or water (control). 34.65±1.91%, 11.64±1.38% and 38.79±1.84% of adults emerged after exposure to thiacloprid, fenvalerate and control, respectively. Results demonstrate that both insecticides have significant mortality effects on *A. fabae*, while thiacloprid does not significantly reduce the adult emergence of *L. fabarum*. Based on these results, thiacloprid could be used and classified as a slightly dangerous insecticide to *L. fabarum* in immature stages, but mortal to *A. fabae*.

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INTRODUCTION

Aphids are a major group of pests that have high reproductive rate. Aphids cause both direct and indirect damages to their host plants including plant pathogenic viruses. The Black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is one of the most polyphagous aphid species that attacks more than 200 Leguminous plant species and can damage all plant parts (Barnea *et al*., 2005). This pest can stop growth in broad bean, *Vicia faba* L. and flowering might be stopped (Nuessly *et al*., 2004). Apterous *A. fabae* can over-winter in areas with temperate climates that make it possible to continue their lives without sexual phase or holocycle (Aghajanzadeh *et al*., 1997).

Lysiphlebus fabarum (Marshall) (Hymenoptera: Braconidae: Aphidiinae) is a specialized parasitoid of *A. fabae* on both crops and weeds and is biquitous in many agro-ecosystems (Stary, 1986; Hildebrand *et al*.,1997; Völkl and Stechmann, 1998; Raymond *et al*., 2000, Nuessly *et al*., 2004). Members of the subfamily Aphidiinae complete pre-imaginal stages inside the body of the aphid (Völkl and Stechmann, 1998; Carver, 1989; Stary, 1999). Aphidiinae subfamily members, including this parasitoid, pass their pre-adult stages with in the body of aphids (Carver 1989).

Parasitoids have often been shown to be more sensitive to synthetic insecticides than their hosts. In order to integrate the use of biological control with pesticide applications, synthetic pesticides should be selected for minimal impact on biological control agents (Sabahi *et al*., 2010). Thiacloprid and fenvalerate are two effective insecticides on aphids of legume crops. Thiacloprid is an insecticide active against various chewing and sucking pests (Elbert *et al*., 2001). Fenvalerate was used widely against

different pests such as aphids (Syrett and Penman, 1980).

Standardized methods involving both laboratory and field tests have been developed to test the safety of pesticides to beneficial organisms in accordance with IOBC guidelines. The aim of this study was to determine the toxicity of thiacloprid and fenvalerate on *A. fabae* and its parasitoid *L. fabarum* under laboratory condition.

MATERIALS AND METHODS

Insect collecting and rearing

Primary colonies of the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), were collected from Alhagi (*Alhagi maurorum*) in the experimental teaching garden of Bahonar University of Kerman, Kerman, Iran in May 2012. The aphids were deployed and adapted on broad bean leaves which were put on 7% Agar gel inside plastic Petri dishes. The colonies were reared under the temperature condition of 25±2 °C, 60±5% relative humidity and16:8 (Light: Dark) photoperiod. Then, the population was purified. The parasitoids of *A. fabae*, *Lysiphlebus fabarum* (Hymenoptera: Braconidae: Aphidiinae), were not reared in the laboratory. Mummified aphids necessary to perform the tests were collected directly from the experimental teaching garden of Bahonar University of Kerman.

Insecticides

Thiacloprid as commercial formulation, Calypso® 480 SC (Bayer CropScience, Monheim, Germany) and fenvalerate EC 20 (formulated in Moshkfam Pesticide Producing Company) were applied in these tests. These pesticides were tested at a single rate of application, corresponding to their minimum label-recommended rate, which is generally used by farmers against aphids in field condition. Concentration of thiacloprid and fenvalerate were 0.144 gram and 0.1 mL of active ingredient per liter of water.

Contact toxicity of pesticides on aphid

Ten aphids of 4-5-days old were reared on broad bean leaves which were put on 0.7% Agar gel inside plastic Petri dishes as a unit. The aphids were placed into a growth chamber for 10 minutes to get adapted. Tests were conducted with 10 replications for each treatment and 10 aphids in each replication. Then, the aphids of each unit were sprayed using thiacloprid, fenvalerate and tap water (control), separately for five seconds. Finally, the lids of containers were closed with meshes and were placed inside growth chamber under the condition of 25±2°C, 60±5% relative humidity and 16:8 (Light: Dark) photoperiod. Mortality of insects was recorded after 24 hrs. Percentage mortality was calculated using the corrected formula of Abbott's for natural mortality in untreated controls (Abbotts, 1925).

Dipping bioassay on *L. fabarum*

A. maurorum plants in the field were checked daily for mummification by the parasitoid. After the aphids' shapes were changed, branches of *A. maurorum* with numerous newly mummified aphids were cut from the plants in the field that each branch had different numbers of mummified aphids. Then, the number of mummified aphids for each replication was not fixed. Each replication was dipped in thiacloprid, fenvalerate and tap water (control), separately for five seconds and then left under laboratory hood for drying of extra water. Finally, each branch was put in a Plexiglas cylinder (8 cm height and 4 cm diameter) which had ventilated lid and were kept in field condition in May. Adult emergence was determined after 7 days by counting dead parasitoids inside each cylinder.

Statistical analysis

The toxicity data of thiacloprid and fenvalerate on *A. fabae* was calculated using the corrected formula of Abbott's (1925). All experimental data were subjected to a one-way ANOVA to determine differences between samples, using the statistical software Statplus 2007. Means were separated using the Tukey's HSD test.

RESULTS AND DISCUSSION

Mortality percentage of *A. fabae* due to application of thiacloprid and fenvalerate after 24 hrs resulted 89.21±3.6% and 81.14±4.2%, respectively (Figure 1). This showed that both insecticides were strongly lethal to 4-5-days old black bean aphid. The results are comparable to those of Rouhani *et al*. (2013) who observed toxicity of four insecticides such as flonicamid, imidacloprid, thiacloprid and

thiamethoxam on the pomegranate aphid, *Aphis punicae*. Also Wang *et al*. (2008) reported about mortality of *Myzus persicae* (Sulzer) treated with several insecticides including fenvalerate.

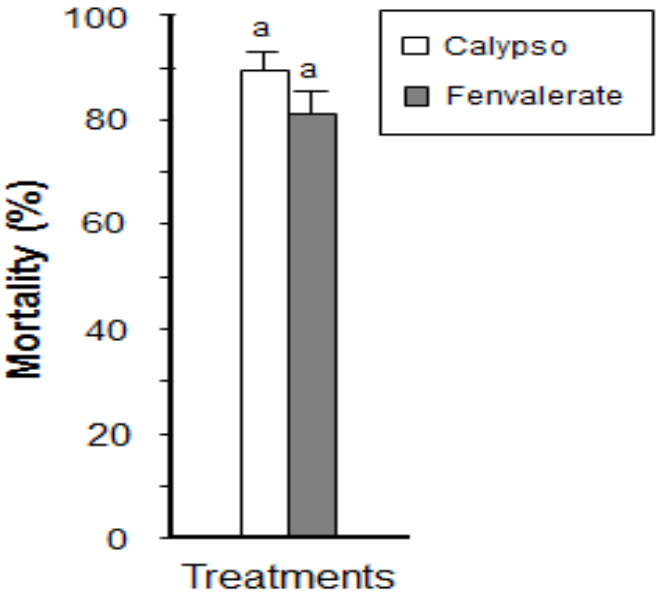

Figure 1. Mortality percentage of *A. fabae* , 24 hours after exposure with thiacloprid and fenvalerate. Different small letters indicate significant differences between different treatments ($P \leq 0.05$) (one-way ANOVA)

Results of the percentage of the adult emergence after exposure of mummified aphids to thiacloprid, fenvalerate and control indicated 34.65±1.91%, 11.64±1.38% and 38.79±1.84%, respectively (Figure 2). These results showed significant ($P<0.05$) differences between control and fenvalerate treatments which states that this insecticide is harmful to *L. fabarum*, while there were no significant difference between control and thiacloprid treatments. According to studies of Matin *et al*. (2005) adult parasitoid emergence rate ranges from 34.38% to 79.17% due to different temperature and relative humidity. While the aphids were mummified in low relative humidity and a moderate temperature (27-30°C) for this study, middle range percent of parasitism in control (38.75±4.98%) could be justified. Vogt and Ternes (2005) suggested that thiacloprid did not affect protected stages of *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) within the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), even when mummies were directly sprayed, likely due to protection afforded by the host integument. These results are comparable to Sabahi *et al*. (2010) that studied the effects of three insecticides to *L. fabarum* and *A. fabae*. Therefore, when biological control fails to maintain aphids below threshold such that a pesticide application becomes necessary, a portion of the parasitoid population in the mummy stage may experience a functional refuge.

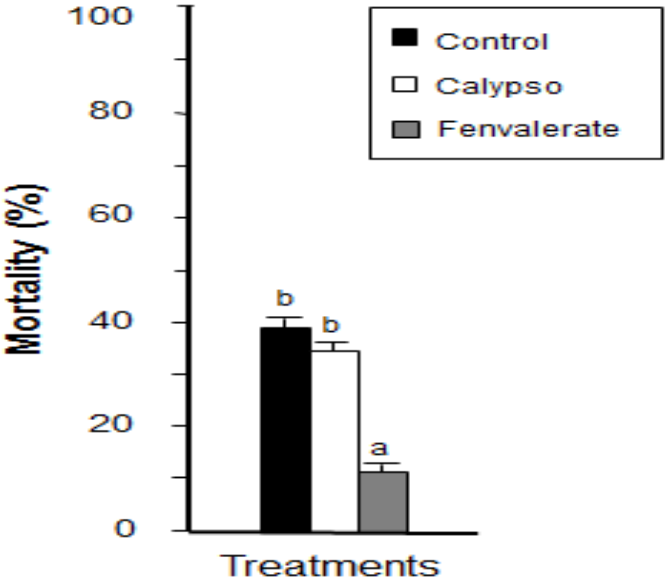

Figure 2. Percentage of adult emergence of *Lysiphlebus fabarum* after exposure of the mummified aphids to thiacloprid, fenvalerate and water. Different small letters indicate significant differences between different treatments ($P \leq 0.05$) (one-way ANOVA)

Our observations revealed that thiacloprid and fenvalerate are highly lethal to *A. fabae*, while thiacloprid is slightly lethal to mummified *A. fabae*. Then, thiacloprid could be recommended for spraying against *A. fabae* while mummified aphids are present. This can guide us through IPM for this polyphagous sap-sucking insect. The use of pesticides may cause undesired effects on non-target beneficial organisms and lead to secondary pest outbreaks. To avoid this, the harmful effects of insecticides on natural enemies of the target pest should be minimized, either through careful timing of applications or the use of materials with selective activity (Sabahi *et al*., 2010). Future area of focus would be to investigate other insecticides against black bean aphid and other aphids to obtain a better control of this important group of pests.

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