International Journal of Agriculture, Environment and Food Sciences

JAEFS e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.4.3 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(4):217-219 (2019)

Effects of some pesticides on *Bombus terrestris* under laboratory conditions

Derya Taplamacioglu[1] **Ismail Karaca**[1,*]

[1]Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, Isparta, Turkey

[*]Corresponding Author: ismailkaraca@isparta.edu.tr

Abstract

Pesticides have not only lethal effect on bees but they can also change their navigational behaviors. The bumblebees exposed to pesticides may not find their food and hive, and even their motor nervous system can acutely be affected. In this study, effects of some pesticides (abamectin, acetamiprid, deltamethrin, imidacloprid) on bumblebees were investigated. The bumblebee colonies were obtained from Koppert Biological Systems, Inc., Turkey. Six doses of each pesticide were tested on motor behaviors of some extremities of the bumblebees and bumblebees were fed on only 50% sucrose as a control group to compare with pesticide applications. Pesticides were applied to bees by feeding and spraying methods. Then, the situations of motor mobility of legs, antennae and proboscis extension of the bumblebees exposed to pesticides were scored. According to the results of the study, the pesticides used in the experiment had an impact on motor nervous system of the bumblebees, and the most effective pesticide was imidacloprid, followed by deltamethrin, acetamiprid, and abamectin, respectively. These results show that imidacloprid, all doses, demage basic motor coordination fundamental to locomotion and foraging and kill at label dose.

Keywords: Bumblebees, Pesticide, Imidacloprid, Proboscis extension reflex

Introduction

Globally, more than 25000 species of the Apoidea (bees) are the key agents of the ecosystem and agricultural pollination in diverse habitat (Donovan, 1980; Michener, 1979; 2007).

Pesticides used against pests in agricultural areas and especially in greenhouses have negative effects on pollinator bees. These negative effects occur as changes in the behaviors of bees, as well as colony losses and the death of bees (Karahan et al., 2015). Besides honey bees, bumble bees are the second most important pollinators for many plants in the natural flora. 239 species were identified and more than 30 countries in the world use these bees as pollinators on 25 different cultivated plants (Goodwin & Steiner, 1997; Williams, 1998; Benton, 2000; Aslan et al., 2017). Because of easy breeding and the large colony populations, *Bombus terrestris* L. (Hymenoptera: Apidae) is one of the main species used in greenhouses for pollination of plants, such as tomatoes, peppers, and melons (Gürel et al., 2001; Gösterit & Gürel, 2005). Populations of bees and other insect pollinators have fallen dramatically in recent years in many nations and there is growing scientific evidence that pesticides had a significant role (Matheson et al., 1996; Allen-Wardell et al., 1998). Currently, many studies were conducted to further understand the toxic effects of pesticides on bees (Kandemir, 2007; Özbek, 2010).

In Turkey, demand for bumblebees has been increased especially in the Mediterranean coastal region for the pollination of plants grown in greenhouses.

Tomato production is widespread in the region and approximately 150000 commercially produced B. terrestris colonies were used in 2012-2013 production season (Gösterit and Gürel, 2010).

Over the past years several laboratory and field tests have been developed to investigate the effect of neonicotinoid insecticides on motor and sensory functions linked to the foraging capacity of bees (Blacquiere et al., 2012).

The aim of this study was to investigate the sublethal effects of the most commonly used pesticides (abamectin, acetamiprid, deltamethrin, imidacloprid) on motor coordination of *B. terrestris*.

Cite this article as:
Taplamacioglu, D., Karaca, I. (2019). Effects of some pesticides on *Bombus terrestris* under laboratory conditions. Int. J. Agric. Environ. Food Sci., 3(4), 217-219
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.3
Received: 04 October 2019 **Accepted:** 09 November 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 217-219
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

Materials and Methods

Stock colonies of *Bombus terrestris* L. (Hymenoptera: Apidae) hives were provided from Koppert/ Turkey (http://www.koppert.com.tr/). Four pesticides used in this study were given in Table 1.

Experiments were carried out under laboratory conditions. Based on the recommended doses by the companies, 6 different concentrations were prepared by half diluting for each step (Table 2). Pesticides were applied to bees by feeding method. Glycose syrup used as control was provided by Koppert company.

Hives including approximately 60-70 bees were opened under red light in the dark. Five bees were taken into the falcon tubes and briefly cooled in the refrigerator at -20 °C until they show first signs of immobility. Immobile bees were taped at their thorax to fixing slots made with syringe. This taping process was performed without preventing the functions of antenna, head, legs and abdominal movements. After bee separation, healthy individuals were controlled for their response by applying water and syrup to their antenna. Then, 5 microliter syrup was mixed with 5 microliter insecticide solution, dropped on the Petri dishes and fed to the bees. Bees are kept under laboratory conditions for 2 hours. At the end of this period, motor movements (antenna, legs, abdomen and the proboscis extension reflex) of bumblebees were controlled and scored. In this scoring system, each body part was scored separately and these scores were totalized for each bee. Evaluation was carried out by giving minimum 0 and maximum 6 points. These points and explanations are given in Table 3.

Table 1. List of the four different pesticides tested, their active ingredients (AI) and commercial names, formulation types, and dosages

Active ingredient	Common name	Formulation	Dosage
Abamectin	Agrimec	EC	0.25 ml / l
Acetamiprid	Mostar	SP	0.30 ml / l
Deltamethrin	Decis	EC	0.10 ml / l
Imidacloprid	Confidor	SC	0.10 ml / l

Table 2. Doses of four pesticides used in the study for 100 ml suspension

AGRIMEG Abamectin	MOSTAR Acetamiprid	DECIS Deltamethrin	CONFIDOR Imidacloprid
Control	Control	Control	Control
0.781 µl	0.937 µl	0.312 µl	0.312 µl
1.652 µl	1.875 µl	0.625 µl	0.625 µl
3.125 µl	3.75 µl	1.25 µl	1.25 µl
6.25 µl	7.5 µl	2.5 µl	2.5 µl
12.5 µl	15 µl	5 µl	5 µl
25 µl	30 µl	10 µl	10 µl

Table 3. The motor movement scores of *Bombus terrestris* body parts

Scores	Explanation
0 point	Bees couldn't move any of their body parts
1 point	Bees move their proboscis, antenna, legs or abdomen slowly
2 point	Bees move their body parts normally

Experiments were replicated 5 times and 30 bees were used for each pesticide. Totally 120 bees were used for this experiment. Experiments were conducted in a climate chamber with 25°C temperature, 60% relative humidity and 16:8 h (light: dark) photoperiod.

Statistical analyses was conducted using the statistical program IBM SPSS (Ver. 20) SPSS Inc., Chicago, Illinois, ABD. Tukey's HSD test was used to detect significant differences among the doses and pesticides.

Results and Discussion

Effects of Abamectin, Acetamiprid, Deltamethrin and Imidacloprid on motor movements of *Bombus terrestris* are given in Table 4.

Abamectin didn't affect the motor movements of *B. terrestris* and all doses were statistically similar. Effects of Acetamiprid and Deltamethrin on the motor movements of *B. terrestris* individuals were intermediate level. The most efficient pesticide was Imidacloprid.

The results of the study in Table 4, showed that Imidacloprid has the maximum negative effect on motor movements of bumble bees and lowest score was recorded with Abamectin treatment followed by Acetamiprid and Deltamethrin. The control, where there was no insecticide spray, had the highest score according to motor movements.

Recent research suggested that widespread agricultural use of imidacloprid and other pesticides may cause honeybee colony collapse disorder. For this reason, several countries have restricted the use of imidacloprid and other neonicotinoids (Woodcock et al., 2016). Likewise, worries are raising concern about the impact of neonicotinoids on wild bumble bee populations (Laycock et al., 2012). Cresswell et al. (2013) reported that imidacloprid reduced feeding and locomotory activity in bumblebees. In our study, imidacloprid showed most adverse effects against *B. terrestris*. Recommended dose (40 µl/100 ml) of imidacloprid used for the greenhouse pests was significantly different from lower doses and control group. Bees exposed to this recommended dose showed no mobility. As a result, it can be mentioned that this pesticide should not be applied during pollination in order to reduce severe effects on *B. terrestris*.

Conclusion

Tests, like the behavioral observations we report here, would be a rapid means of assessing the impact of longer-term exposure to pesticides on bee motor functions and could be used as a reliable bioassay for sublethal effects on pollinators. However, further research is required to establish imidacloprid's impact in greenhouse populations.

Table 4. Comparison of the motor movement scores of *Bombus terrestris* subjected to four insecticides

Doses	Abamectin	Acetamiprid	Deltamethrin	Imidacloprid
Control	6.00±0.00 a*	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
1	6.00±0.00 a	6.00±0.00 a	5.00±0.57 ab	2.66±0.33 b
2	6.00±0.00 a	6.00±0.00 a	4.66±0.33 ab	2.33±0.88 b
3	5.66±0.33 a	5.33±0.33 ab	4.00±0.00 b	1.33±0.33 bc
4	5.66±0.33 a	5.33±0.33 ab	4.00±0.00 b	0.66±0.33 bc
5	5.00±0.57 a	5.00±0.10 ab	2.00±0.57 c	0.66±0.33 bc
6	5.33±0.66 a	2.66±1.66 b	1.66±0.33 c	0.00±0.33 c

*Means in each row shown by the same letter (s) are not significantly different at (P< 0.05%) according to Tukey Multiple Range Test.

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