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SUSCEPTIBILITY COMPARISON OF BUMBLEBEE AND HONEYBEE TO ACETAMIPRID AND IMIDACLOPRID

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A B S T R A C T

Imidacloprid and acetamiprid are neonicotinoid insecticides introduced in the market for the control of insect pests of agricultural crops, but unfortunately these insecticides are causing harmful effect on non-target organisms including honeybees and bumblebees. Insecticides of this group effect acetylcholine nicotinic receptors. *Apis mellifera*, the European honeybee and *Bombus haemorrhoidalis* are important pollinators for wild and managed crops in Pakistan, but unfortunately their population is declining day by day at alarming rate. This study was design to find out the susceptibility level and insecticidal toxicity comparison on *A. mellifera* and *B. haemorrhoidalis* to acetamiprid and imidacloprid under controlled laboratory conditions by using contact and oral exposure methods. The results of oral method by using sugar solution showed that honeybee is more susceptible as compared to bumblebee on both insecticides. Maximum mortality i.e. 20%, 35%, 66%, in honeybee and 16%, 29%, 45% in bumblebee workers at acetamiprid and at imidacloprid 46%, 67%, 83% in honeybee and 34%, 56%, 72% in bumblebee were observed respectively after the exposure of 3hrs. 6hrs. and 24hrs. Similar susceptibly results were found on pollen and contact method. Overall results demonstrate that these both neonicotinoid insecticides are toxic for bumblebees and honeybees, and honeybee workers are more susceptible as compared to bumblebee workers at company recommended field realistic dose.

Keywords: Neonicotinoid; Acetamiprid; Imidacloprid; Bumblebee; Honeybee; Susceptibility

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INTRODUCTION

Bumblebees, honeybees and solitary bees are the important pollinators of our ecosystem and overall 35% world food crops production depends upon these pollinators (Klein et al., 2007). Both wild and managed bees play a vital for the maintaining of ecosystem (Garibaldi et al., 2011). The honeybee is an important, valuable and useful social insect for human being. In addition to the production of honey, wax, pollen, royal jelly and propolis ect. honeybees are one of the main pollinators for the agricultural food production (Cranston and Gullan, 2005). These bees also play a key role in the gene flow within and between the plant communities (Jaffe et al., 2010).

Beside the importance of bees, their population are decreasing day by day at alarming rate. There are many factors behind this decline, including habitat destruction, predators and parasites, adverse environmental conditions and use of chemicals (Goulson et al., 2015; Sánchez-Bayo and Wyckhuys, 2019). Many wild plants species are also declining due the declining of bee's population in nature (Goulson et al., 2008). More specifically, there is a great concern about the decline of the honey bee (*Apis mellifera*) in several parts of the world (Meixner, 2010). But one of the important factor that play very much role in the population decline in the mind of scientist is use of chemicals (Carreck and Neumann, 2010; Kluser et al., 2010). These chemicals are applied on the crops to control insect pests and diseases and get more production to feed this growing world population (Oerke and Dehne, 2004).

Among these different chemicals including insecticides. Neonicotinoid is a new class of insecticide having systemic in nature properties. This class include thiacloprid, clothianidin, imidacloprid, nitenpyram, acetamiprid and dinotefuran. When these insecticides are applied on crops

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either in form of seed dresser or direct spry, absorbed by the plants and become part of the plants. All these insecticides of neonicotinoid group are neurotoxin and effect on acetylcholine receptors. (Elbert et al., 2008; Matsuda et al., 2001). Among these insecticides imidacloprid which introduced in the market in early 1990s and acetamiprid are extensively used by spraying and as a seed dressing on agriculture crops throughout the world to control insect pests (Elbert et al., 2008). All the insecticides are tested under laboratory and field conditions than approved for commercial use, this risk assessment process confirm that they are harmful or harmless for non-target organisms including these important pollinators. However this assessment process in Europe and USA have significant limitation. They only test the pesticides effect singly (EPPO, 2010), even though when bees visit agricultural field likely to be exposed with combinations of many pesticides (Botías et al., 2017; Tosi et al., 2018). Laboratory analysis showed that multiples residues of these insecticides have been found in honeybees and bumblebees collected nectar and pollen (Dively and Kamel, 2012; Stoner and Eitzer, 2012).

Besides the available literature showing the effects of imidacloprid and acetamiprid on bees including honeybees and bumblebees, little is known about the susceptibility comparison of honeybees and bumblebees against acetamiprid and imidacloprid. We suggest the hypothesis that honeybees are more susceptible as compared to bumblebees to acetamiprid and imidacloprid because honeybee's workers are smaller as compared to bumblebees in size. So the purpose of this study was to find out susceptibility level in honeybees and bumblebees workers at company recommended field realistic dose by contact and oral method.

MATERIALS AND METHODS

The study was conducted to observe the susceptibility level of bumblebee *Bombus haemorrhoidalis* and honeybee *Apis mellifera, to* two different neonicotinoids insecticides i.e. acetamiprid and acetamiprid in the Department of Entomology, University of Poonch Rawalakot, Azad Jammu and Kashmir, Pakistan.

Insect collection

Workers of *A. mellifera* were collected from the honeybee colony with the help of 50 ml falcon tubes. Only forager bees were collected loaded with pollen. Workers of bumblebee were collected from the filed with the help of arial net from different localities of Rawalakot. These collected specimens were transported form field to laboratory in 50ml falcon tubes having cotton socked with sugar solution for their survival.

Laboratory conditions

Optimum lab conditions were maintain for successful experimentation, temperature and humidity (25±2^{o}C and 60-80% relative humidity) were maintain with the help of fan heater and humidifier. All the equipments used during the experiments were clean with the help of cotton piece soaked with 70% ethyl alcohol.

Insecticides

Technical grade two insecticides acetamiprid and imidacloprid were used with recommended field dose 125µl/100 ml and 250µl/100 ml respectively. The reason behind the selection of these two insecticides from the whole group of neonicotinoid was the most commonly used insecticides in Pakistan

Bioassays

Susceptibility test of bees to these two insecticides acetamiprid and imidacloprid were performed by using contact and ingestion methods. For experiment, small plastic boxes of size (10.5×14.5×6.5 cm^3) were used. The toxic effects of these insecticides were assessed by ingestion and contact methods. The first part of experiment was ingestion method; in this method field recommended dose (125ul/100 ml for Acetamiprid and 250ul/100 ml for Imidacloprid) was mixed with 50% sugar solution and used as a nectar substitute. These insecticides mixed sugar solution was filled in Petri dishes with socked foam. In second ingestion method pollen grains were grind and mixed with these contaminated sugar solution and used for bees feeding. In the second part of experiment contact method was used to test toxicity of these two insecticides through contact contamination. In this method filter paper was cut in size of box and dip in field recommended dose than spread in bottom of each box. Both bumblebees and honeybees collected workers were released in each box separately with non-treated sugar solution and pollen. Twenty bees were released in each box with four replications.

Data analysis

Percentage mortality of honeybees and bumblebees workers were recorded after 3 hours, 6 hours, and 24 hours of treatment. The bumblebees and honeybees workers were observed with dissecting forceps as dead bees could not give response.

RESULTS AND DISCUSSION

Susceptibility comparison of two neonicotinoid insecticides acetamiprid and imidacloprid were tested against *Apis mellifera* and *Bombus haemorrhoidalis* workers. The results revealed that when field realistic dose of these two insecticides were

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mixed in sugar solution and used as a substitute of nectar, maximum mortality was observed in honeybees as compared to bumblebees when treated with acetamiprid. Results showed that honeybees are more susceptible to acetamiprid as compared to bumblebee on all three observed time duration (3hrs, 6hrs and 24hrs). Similar trend was observed when bumblebees and honeybees were exposed at imidacloprid. Results showed that 34%, 56% and 72% mortality were observed in bumblebees and 46%, 67% and 83% mortality observed in honeybees when observed after 3hrs, 6hrs, and 24hrs respectively. Overall results showed that imidacloprid is more toxic as compared to acetamiprid on both bees, and honeybees are more susceptible to imidacloprid as compared to bumblebees (Figure 1).

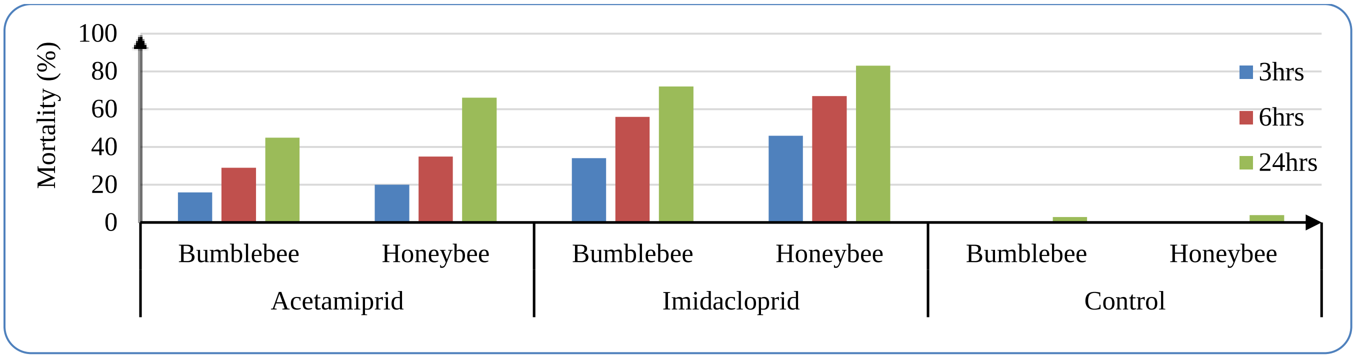

Figure 1. Effect of acetamiprid and imidacloprid at recommended field dose on honeybee and bumblebee using sugar solution as an ingestion method.

Susceptibility results of bumblebees and honeybees to these two neonicotinoid insecticides when mixed in pollen showed that maximum mortality was observed in honeybees as compared to bumblebees feed on pollen treated with acetamiprid. Results revealed that 4%, 11% and 36% mortality was observed in bumblebee and 7%, 16% and 43% mortality observed in honeybee after 3hrs, 6hrs and 24hrs exposure at acetamiprid. The results showed that honeybees are more susceptible to acetamiprid as compared to bumblebees. Susceptible result of imidacloprid also showed the same trend i.e. maximum mortality was observed in honeybees as compared to bumblebees. Data showed that 9%, 21% and 38% mortality observed in bumblebee and 14%, 36% and 49% observed in honeybees. Overall results showed that imidacloprid is more toxic as compared to acetamiprid when mixed in pollen and honeybees are more susceptible to these two insecticides as compared to bumblebees (Figure 2).

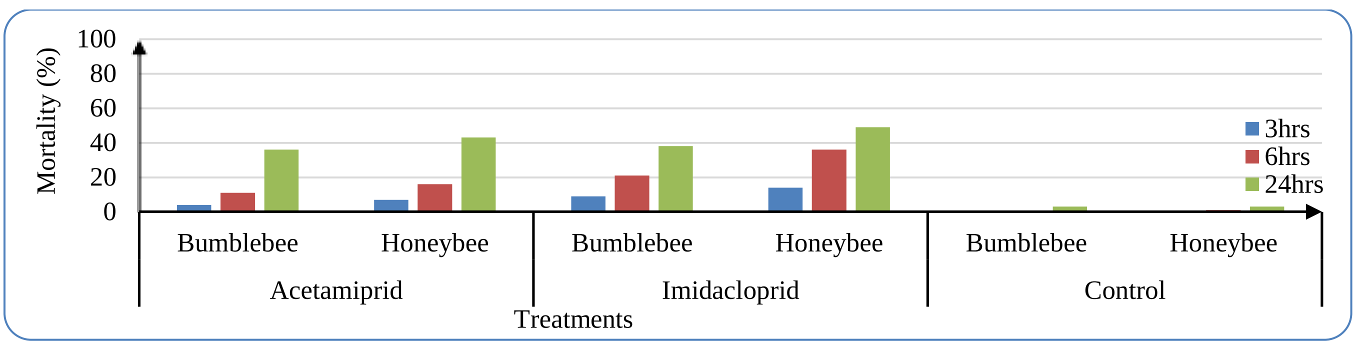

Figure 2. Effect of acetamiprid and imidacloprid at recommended field dose on honeybee and bumblebee using pollen as an ingestion method.

Toxicity comparison results of acetamiprid and imidacloprid by using contact method on honeybees and bumblebees showed that imidacloprid is more toxic as compared to acetamiprid on both tested bees. Susceptibility index showed that honeybees are more susceptible to acetamiprid than bumblebees when treated with wit filter paper using contact method. Similar results were observed when these bees were exposed on imidacloprid. Data revealed that 21%, 44% and 65% mortality observed in bumblebees and 34%, 54%, 73% observed in honeybees after the exposure of 3hrs, 6hrs and 24hrs respectively (Figure 3).

Overall results of this study concluded that these both tested neonicotinoid insecticides i.e. acetamiprid and imidacloprid are toxic for bumblebees and honeybees. Susceptibility level showed that honeybees *Apis mellifera* workers are more susceptible as compared to bumblebees *Bombus haemorrhoidalis* workers to acetamiprid and imidacloprid.

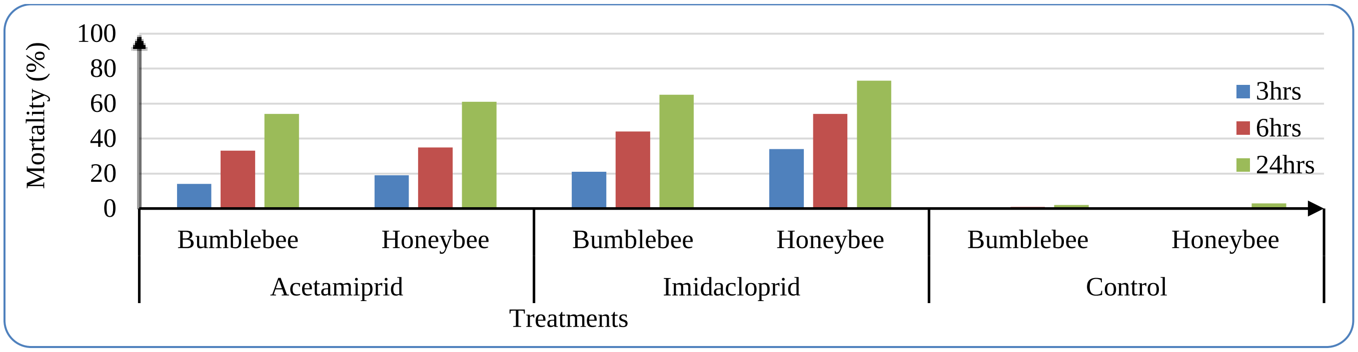

Figure 3. Effect of acetamiprid and imidacloprid at recommended field dose on honeybee and bumblebee using wet filter paper as a contact method.

Despite the importance of honeybees and bumblebees in pollination service (Kleczkowski et al., 2017; Stanley et al., 2015), this study is to test the susceptibility level of honeybees and bumblebees to acetamiprid and imidacloprid. These bees are the vial pollinators of our managed and wild corps and fruits trees but unfortunately their population is decreasing at alarming rate (Whitehorn et al., 2012). Our results demonstrate that honeybee's workers are more susceptible as compared to bumblebee's workers when tested on acetamiprid at label dose by using sugar solution in oral method. This work also indicates that acetamiprid is less toxic on both bees as compared to imidacloprid. Previous study conducted on bumblebees in both bioassay method i.e. oral and contact by using imidacloprid showed that bumblebees are sensitive to these compound (Nauen et al., 2001). According to EPA (Environmental Protection Agency) imidacloprid is also categorized as a highly toxic on both honeybees and bumblebees (USEPA, 2014).

It might be due to its neurotoxin effects, previously study conducted on bees and neonicotinoid showed that even when bees are treated at very low dose of these insecticides, their flight activity disturbed (Sánchez-Bayo et al., 2016). Similar finding were found when susceptibility of these both bees were tested by using pollen in oral method. Results showed that honeybee's workers are more susceptible as compared to bumblebee's workers on label dose and also acetamiprid is less toxic as compared to imidacloprid. Our results also confirmed the previous finding in which different neonicotinoid insecticides were tested on sensitivity of honeybee, and results indicate that imidacloprid is most toxic from all others (Imran et al., 2018). Difference of susceptibility level between honeybee and bumblebee might be due to their body size and amount of fat bodies. Another study conducted on the olfactory behavior of honeybees show that imidacloprid impaired more behavior as compared to acetamiprid in neonicotinoid group of insecticides (Imran et al., 2019). The possible elucidation for these conflicting finding is due to differences between in the structure of these compounds (Kayser et al., 2004; Marletto et al., 2003). Study conducted on sensitivity of bumblebees showed that bumblebees are more sensitive to imidacloprid as compared to others neonicotinoid insecticides (Heard et al., 2017).

Results of sensitivity by contact method are also same as mention above i.e. bees are less sensitive to acetamiprid as compared to imidacloprid. Study conducted under laboratory and field (Cresswell et al., 2012; Rundlöf et al., 2015) on both bumblebees and honeybees to test the neonicotinoid insecticides, and finding showed that there are differences in the toxicity of neonicotinoid over time (Heard et al., 2017). Insecticides of this group are the agonists and binds the insect nicotinic acetylcholine receptors (Déglise et al., 2002) and these chemicals activates these receptor to produce a biological response (Moffat et al., 2016). Due to such type of effects of neonicotinoid insecticides on non-target organisms especially on important pollinators including honeybees, these are partially banned in Europe (Erickson, 2013), but still commonly used in other parts of the world. The direct effects of these neonicotinoid insecticides on honeybee homing and flight ability therefore need further study.

CONCLUSION

Overall finding of this study clearly revealed that honeybees are more susceptible to acetamiprid and imidacloprid as compared to bumblebees and these both insecticides are toxic for bees. This is the basic research and still research is needed on molecular basis to find out this susceptibility mechanism.

AUTHORS' CONTRIBUTIONS

All authors contribute equally for collection, conducting experiment, data collection and analyses and in write-up of this manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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