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Assessment of the effects of biopesticides of different origins on bumblebee with two exposure scenarios

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Abstract

The present study aimed to determine the damage level of biopesticides with different contents in bumblebees according to two different exposure scenarios: residual contact and spray application. *Bombus terrestris* (L.) (Hymenoptera Apidae) workers were exposed to Nibortem (1.5% *Lecanicillium* (*Verticillium*) *lecanii* strain V1-1), Nostalgist (1.5% *Beauveria bassiana* strain Bb-1), Priority (1.5% *Isaria* (*Paecilomyces*) *fumosoroseus* strain PFs-1), Nimbecidine (0.3 g/L Azadirachtin) and Nematac (*Steinernema feltiae*) at three different doses [1/1, 1/10 and 1/100], with both application methods. In the spray application, the suspension of each biopesticide was sprayed for 20 seconds (0.5 ml) to each box containing 10 workers. In the residual contact application, suspensions were sprayed into empty plastic boxes with the same method and then workers were transferred into these boxes and they remained in the treated boxes for 15 minutes only. The observations were carried out for 14 days in all treatments. Whereas the mean number of dead workers were significantly higher for Nibortem, Nimbecidine, Nostalgist, and 1/1 dose of Nematac in residual contact application, this value was found statistically different for Nibortem, Nimbecidine, Nostalgist, Priority, and 1/1 dose of Nematac in spray application, than control group ($P < 0.05$). The mean number of dead workers in 1/10 and 1/100 doses of Nematac were not significantly different from the control in both application methods. There was no significant difference between both application methods in terms of death values for the control, Nostalgist, 1/1 dose of Nematac.

Key words: entomopathogenic fungi, entomopathogenic nematode, microbial control, non-target organism.

Introduction

Bumblebees are used worldwide for pollinating a variety of economically important vegetable and fruit crops in greenhouses and orchards, as well as many wildflowers (Free, 1993; Proctor *et al*., 1996; Kovach *et al*., 2000; Velthuis and van Doorn, 2006; Dafni *et al*., 2010; Goulson, 2010). About 250 species of this genus were identified in the world and five of them, *Bombus terrestris* (L.), *Bombus lucorum* (L.), *Bombus ignitus* Smith, *Bombus occidentalis* Greene and *Bombus impatiens* Cresson (Hymenoptera Apidae), have been reared for commercial purpose (Williams, 1998, Velthuis and van Doorn, 2006). Approximately three million commercially produced *B. terrestris* colonies in the world and more than 300,000 in Turkey are widely used in the pollination of many greenhouse-grown products, especially tomatoes (95%) (Gosterit and Gurel, 2018). The use of the commercial bumblebees in pollination service provides significant advantages in terms of reducing the workforce, eliminating the need for hormones, limiting the use of chemical pesticides, making products more easily marketable, and food safety. In addition, it positively affects the quality characteristics of the product, while increasing fruit set (Gosterit and Gurel, 2014; Kandemir *et al*., 2016).

It has been reported that the natural populations of bumblebees have decreased remarkably in recent years. These population declines are thought to be due to climate change, the continued reduction of suitable habitats, especially for those adapted to high altitudes, changes in agricultural practices and land use, and the spread of pathogens (Cameron *et al*., 2011; Hoiss *et al*., 2012; Kerr *et al*., 2015; Rasmont *et al*., 2015; Jacobson *et al*., 2018). One of the most important reasons for the decrease in pollinator insect species, including bumblebees, is represented by the use of pesticides in crop management (Vanbergen, 2013; Godfray *et al*., 2015; Potts *et al*., 2016; Wood and Goulson, 2017; Lamsa *et al*., 2018). Bumblebees can be exposed to these pesticides through evaporation of residues from plant surfaces but also by contact with residues on vegetation the contamination of wax layers and soil after pesticides are sprayed or applied as dust particles. In addition, they can assume them by consuming food or water sources containing pesticide residues (Marletto *et al*., 2003; Gradish *et al*., 2018; Demirozer *et al*., 2022a; 2022b). Pollinators are also exposed to different stress conditions due to highly variable environmental factors, malnutrition, parasites, and entomopathogens. They may also be stressed by contact with entomopathogenic nematodes (EPNs) or microorganisms used to control plant pests and diseases when seeking water, food, or nesting material (Erler *et al*., 2022). In this study, it was aimed to determine the effectiveness of biopesticides with different contents on *B. terrestris*, an important pollinator species in agricultural production, according to two different exposure scenarios (residual contact and spray application). Based on this, the lethal effects of *S. feltiae*, a commercial entomopathogenic nematode, and different commercial fungi and plant-derived biopesticides were investigated on *B. terrestris* workers.

Materials and methods

The maximum field recommended doses (MFRD) were used for Nibortem (1.5% *Lecanicillium* (*Verticillium*) *lecanii* strain V1-1; 250 mL/da, 1×10^8 CFU/mL), Nostalgist (1.5% *Beauveria bassiana* strain Bb-1; 250 mL/da, 1×10^8 CFU/mL), Priority (1.5% *Isaria* (*Paecilomyces*) *fumosoroseus* strain PFs-1; 250 mL/da, 1×10^8 CFU/mL), Nimbecidine (0.3 g/L Azadirachtin; 500 mL/100 L water, 16000 IU/mg) (Agrobest Co., Izmir, Turkey). And also, three different doses (1/1, 1/10, 1/100) of Nematac (*Steinernema feltiae*, 150 million infective juveniles (IJs)/100 L water) (Bioglobal Co., Antalya, Turkey) were included in the study. Workers from bumblebee (*B. terrestris*) colonies were reared by a commercial company (Bio Group inc., Antalya, Turkey) and were used to determine the lethal effects of biopesticides.

It is reported that *B. terrestris* workers start foraging activities at the age of 1 week (Gill and Raine, 2014) and forager workers are more likely to be exposed to chemicals under natural or greenhouse conditions. Therefore, we used 8-12 days old workers in the study. For this purpose, old worker pupae collected from different colonies were transferred to empty rearing boxes (20 × 16 × 9 cm) and kept at 27 °C and relative humidity to 50-60%. All emerged workers were transferred to separate boxes and fed in for 7 days (Bulus *et al*., 2020). Biopesticides were applied to these *B. terrestris* workers with 2 different application methods. In the first of these, spray application (SA), suspensions prepared for each biopesticide were sprayed for 20 seconds (0.5 ml) in each rearing box (8 × 12.5 × 9 cm) containing 10 workers. In the second method, residual contact application (RCA), suspensions were sprayed into empty plastic boxes (13 × 5 × 3 cm) at the same pressure and time. After 15 minutes, 10 workers were transferred, for 15 minutes, to these boxes contaminated with biopesticides and then transferred to rearing box (8 × 12.5 × 9 cm) (by modified of USEPA, 2012a; 2012b).

In the experiment, 8 different treatment groups (Nibortem, Nostalgist, Priority, Nimbecidine, three doses of Nematac and control) were constituted and totally of 1600 *B. terrestris* workers (2 application methods × 8 groups × 10 replications × 10 workers for each replication) were used. All workers were immobilized by anaesthesia with CO_2 before treatments. Sterile distilled water was applied to workers from the control groups of both application methods (Mommaerts *et al*., 2009). All workers were fed with sugar syrup ad libitum. The number of dead individuals was recorded every 2 days for two weeks (7 observations during the experiment) after biopesticide applications. Re-isolation was performed from the dead workers to determine whether the cause of death was due to biopesticides. The re-isolation was performed on all workers that died after entomopathogenic fungi (EPF) applications, according to Er *et al*. (2016). Those containing entomopathogenic fungus spores were considered dead due to EPF. The white trap method was used for the re-isolation of EPN from dead individuals (Kaya and Stock, 1997) and the *Bombus* bodies containing IJs were considered dead due to EPN. Data analyses was done in SPSS 20.0 software package. The efficacy of the biopesticides for both methods against *B. terrestris* was analysed by Kaplan Meier survival curves (Omuse *et al*., 2021). The statistical differences between groups for each application were determined by the Log-rank test expressed by χ^2 results and P-values. Data of the dead worker numbers were square root transformed. Tukey's HSD test was run to determine the differences between groups.

In addition, the toxicity scale of pesticides against beneficial insects was used to evaluate the side effects of the biopesticides on *B. terrestris* individuals (Class 1, non-toxic <25% death; Class 2, weakly toxic 25-50% death; Class 3, moderately toxic 51-75% death; Class 4, highly toxic >75% death) according to the side effect scale of the International Organization for Biological Control (IOBC) (Sterk *et al*., 2002). For this scale, mortality rates calculated from the total death numbers of each treatment on the 14th day were used in both application methods.

Results

Survival probability of workers

The probability of survival of the workers on the observation days when biopesticides are applied with the residual contact method was given in figure 1. The rates of survival for Nibortem, Nimbecidine, Nostalgist, Priority, and 1/1, 1/10, 1/100 doses of Nematac on the 14th day were 38%, 70%, 48%, 86%, and 52%, 74%, 76%, respectively. The survival rates of workers were not significantly different between Nibortem, and Nostalgist while all biopesticides were different from the control on the 14th day after application (χ^2 = 124.313, df = 7, P < 0.001) (figure 1).

The survival probability of workers after the Nibortem, Nimbecidine, Nostalgist, Priority, and 1/1, 1/10, 1/100 doses of Nematac were applied by spraying method were 56%, 40%, 56%, 40%, and 54%, 62%, 70%, respectively. No significant differences were determined in terms of survival between Nibortem, Nostalgist, and Priority (figure 2A), and also, no significant difference in survival probability between Nimbecidine and 1/1 dose of Nematac groups (figure 2B), but these groups were different from control (χ^2 = 103.858, df = 7, P < 0.001) (figure 2).

Lethal effect of biopesticides for spray application

When biopesticides were applied by the spraying method, the mean number of dead workersin Nibortem, Nimbecidine, Nostalgist, Priority, and 1/1 dose of Nematac groups were found to be significantly different from the control group ($p < 0.05$). However, there was no significant difference between 1/10 and 1/100 doses of Nematac and the control ($p > 0.05$) (table 1).

The lethal effects of biopesticides on *B. terrestris* workers after spray application were also evaluated according to the IOBC toxicity scale. The mortality rates for 1/1, 1/10 and 1/100 doses of Nematac, Nimbecidine, Nostalgist, Nibortem and Priority groups were found to be 46%, 38%, 30%, 60%, 54%, 42% and 60%, respectively, for spray application method. According to the IOBC toxicity scale 1/1, 1/10, and 1/100 doses of Nematac and Nibortem were found to be weakly toxic, while other biopesticides were moderately toxic on *B. terrestris* workers.

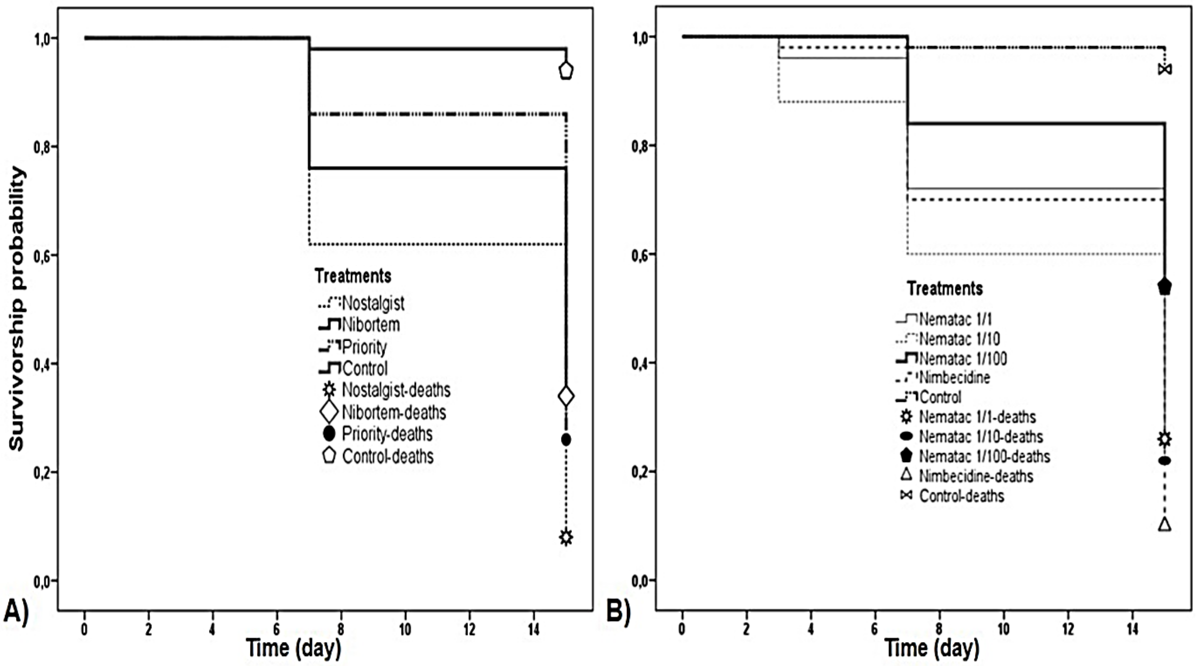

Figure 1. Kaplan-Meier survival curve for (**A**) fungal biopesticides, (**B**) plant-derived and nematode biopesticides in the residual contact application method. Deaths were censored data to calculate survival probability in Kaplan Meier analysis.

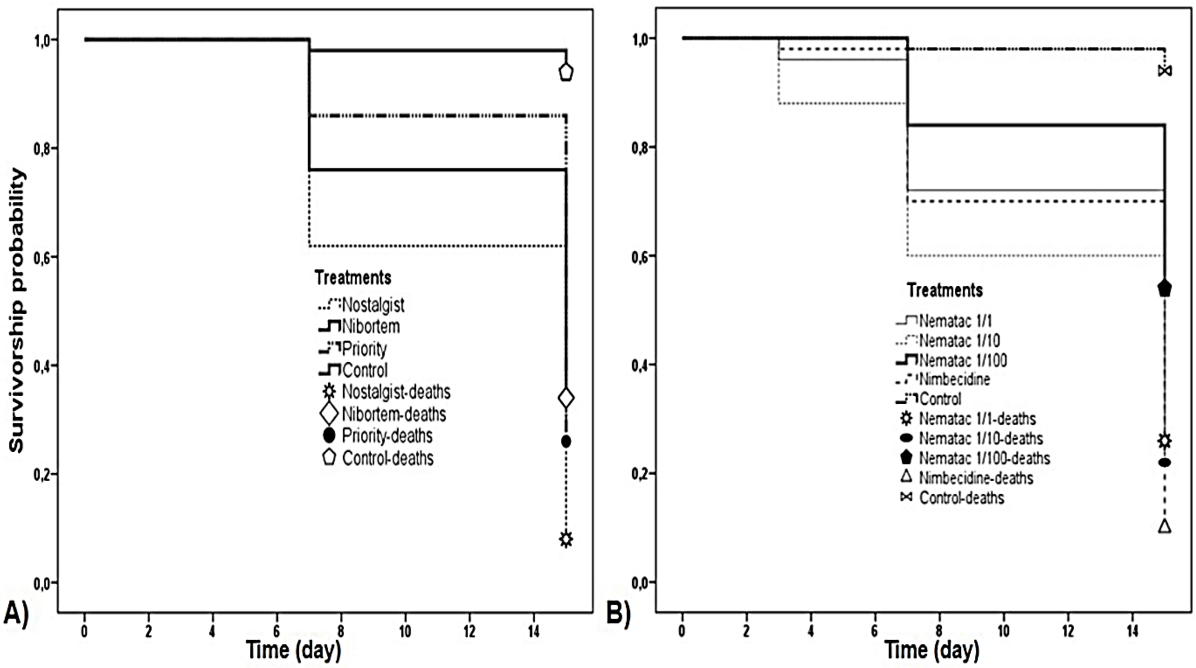

Figure 2. Kaplan-Meier survival curve for (**A**) fungal biopesticides, (**B**) plant-derived and nematode biopesticides in the spray application method. Deaths were censored data to calculate survival probability in Kaplan Meier analysis.

Lethal effect of biopesticides for residual contact applications

The mean number of dead workers in Nibortem, Nostalgist, and 1/1 dose of Nematac groups were significantly higher than in the control group ($p < 0.05$). However, Nimbecidine, Priority, 1/10 and 1/100 doses of Nematac groups were not found significantly different from the control group in terms of the mean number of dead workers for residual contact applications (table 1). The mortality rates calculated for each treatment on the 14th day was used to evaluate the lethal effects of the biopesticides on workers. The mortality rates on the 14th observation day after residual contact applications for 1/1, 1/10 and 1/100 doses of Nematac, Nimbecidine, Nostalgist,

Table 1. The mean number (± SE) of dead workers in treatment groups for the residual contact and spray applications.

Treatments	Application methods	
	Residual contact	Spray
1/1 dose of Nematac	2.152 ± 0.204 a	0.701 ± 0.047 a
1/10 dose of Nematac	1.540 ± 0.238 ab	0.573 ± 0.147 ab
1/100 dose of Nematac	1.512 ± 0.187 ab	0.516 ± 0.128 ab
Nimbecidine	1.665 ± 0.237 ab	0.831 ± 0.078 a
Nostalgist	2.251 ± 0.179 a	0.774 ± 0.075 a
Nibortem	2.460 ± 0.190 a	0.656 ± 0.098 a
Priority	0.892 ± 0.388 b	0.843 ± 0.124 a
Control (Distilled water)	0.565 ± 0.346 b	0.122 ± 0.075 b

Means followed by different letter in the same column are different (P < 0.05).

Nibortem, and Priority were 48%, 26%, 24%, 30%, 52%, 62%, and 14%. Priority, 1/10 and 1/100 doses of Nematac were found non-toxic, whereas 1/1 dose of Nematac and Nimbecidine were weakly toxic, Nostalgist and Nibortem were moderately toxic to bumblebee workers in the residual contact application.

Comparison of the effect of application methods on pesticide toxicity

It was determined that there was no significant difference in terms of death values for the control ($\chi^2 = 23.958$, df = 1, P = 0.089), Nostalgist ($\chi^2 = 42.320$, df =1, P = 0.121), 1/1 dose of Nematac ($\chi^2 = 46.142$, df = 1, P = 0.133) between the residual contact method and spray application on *B. terrestris*. However, significant difference between application methods was determined for other treatments. The number of dead workers obtained in the spray application method for 1/10 ($\chi^2 = 28.663$, df = 1, P < 0.001) and 1/100 ($\chi^2 = 34.962$, df = 1, P < 0.001) doses of Nematac, Nimbecidine ($\chi^2 = 14.286$, df = 1, P < 0.001) and Priority ($\chi^2 = 5.426$, df = 1, P = 0.002) were significantly higher than the residual contact method. However, the number of dead workers that occurred in Nibortem ($\chi^2 = 22.191$, df = 1, P < 0.001) with the residual contact method were significantly higher than that occurred with the spray application (figure 3).

Discussion and conclusion

The growing demand for the reduction of chemical inputs in agriculture and increasing resistance to insecticides have given great impetus to the development of alternative forms of insect-pest control. Entomopathogenic fungi normally infect through the outer cuticle of insects and do not need to be eaten by the insect to initiate disease (Sevim *et al*., 2015). They can reduce non-target insect populations by infecting them directly (Roy and Pell, 2000). The entomopathogenic nematodes, which are other biopesticides, *Steinernema* (Rhabditida Steinerne-

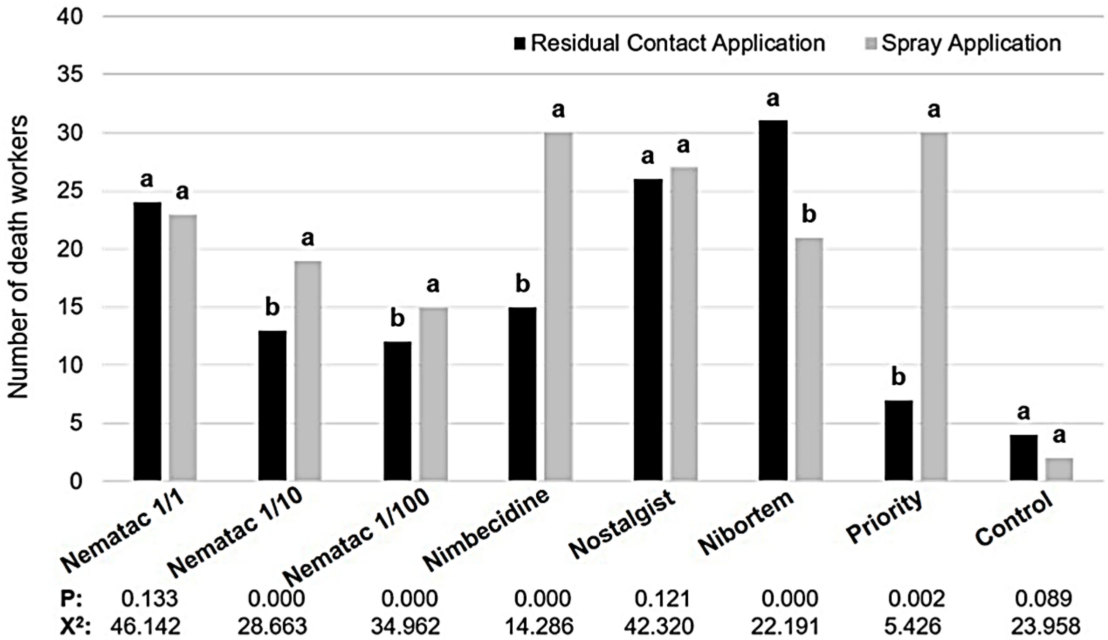

Figure 3. The comparison of the dead numbers obtained on the 14th observation day with the two application methods (RCA and SA). Dead numbers followed by different letters within each treatment show significant differences between application methods (Fischer's χ^2 test).

matidae) and *Heterorhabditis* (Rhabditida Heterorhabditidae) are widely used as biological agents against a large number of pests and are considered to be promising alternative to replace pesticides (Labaude and Griffin, 2018). In addition, azadirachtin, which was extracted from seeds of the *A. indica*, is one of the most well-known plant-based biopesticides preferred for pest control (Boeke *et al.*, 2004). Despite these benefits, biopesticides may have potential risks to beneficial insects such as bees (Morgan, 2009; Barbosa *et al.*, 2015). Health and environmental concerns, along with the many negative effects of pesticides, have promoted safe and harmless pest control strategies (Kilani-Morakchi *et al.*, 2021). Currently, there are studies related to the effects of biopesticides (Mommaerts and Smagghe, 2011; Barbosa *et al.*, 2015; Karise *et al.*, 2016; Demirozer *et al.*, 2022b) on *B. terrestris* with different application methods. However, studies regarding the effects of nematodes are limited (Dutka *et al.*, 2015). In the present study, the lethal effects of biopesticides with different contents on *B. terrestris* according to two different application methods were investigated. Some microbial control agents, including *B. bassiana*, have been reported to be safe for bumblebees when exposed topically or orally (Sterk *et al.*, 2002). However, Karise *et al.* (2016) stated that the survival of *B. terrestris* was significantly reduced with BotaniGard (which contains *B. bassiana* GHA strain) application. It has been reported that *B. bassiana* GHA SF86-21 has strong pathogenicity against *B. terrestris* and increased dose-related mortality rates (Hokkanen *et al.*, 2003; Karise *et al.*, 2018). Mommaerts *et al.* (2007) detected the worker mortality was up to 90% on the *B. terrestris* after exposure to dermal contact and orally via the drinking of the sugar water of Botanigard (Maximum Field Recommended Concentration, MFRC). In another study, when the MFRC (2.5×10^{10} CFU L^{-1}) of Botanigard was applied to *B. terrestris* workers with the topical application method, mean worker mortality was 92 ± 3% in the eleventh weeks after application (Mommaerts *et al.*, 2009). In the present study, Nostalgist, containing *B. bassiana* strain Bb-1, caused 52% and 54% death on the 14th day for residual contact and spray application methods, respectively. According to the previous study (Demirozer *et al.*, 2022a), when the same commercial preparation was applied to *B. terrestris* by the residual contact method and topically, the mortality rates were 62% and 36% on the 15th day after the application. Demirozer *et al.* (2022a) also found that when other biopesticides with the same content of Nibortem, Priority, and Nimbecidine were used against *B. terrestris* by the topically and residual contact method, the mortality rates were ranged from 10% and 38% for Nibortem, 34% and 38% for Priority, and 32% and 92% for Nimbecidine on the 15th day. In the current study, on the 14th day after application, the mortality rates were ranged from 42% and 62% for Nibortem, 60% and 14% for Priority, and 60% and 30% for Nimbecidine in the spray and residual contact method, respectively. While the mortality rates for the residual contact method were found to be higher than the topical application in previous studies, the mortality rates for the spray application method in the current study were higher than the residual contact method for 1/10 and 1/100 doses of Nematac, Nimbecidine and Priority. On the contrary, the number of dead workers in Nibortem in the residual contact method were significantly higher than the spray application method. In a different study, Dutka *et al.* (2015) investigated the effectiveness of the entomopathogenic nematode product containing the mixture of commercially available *Heterorhabditis* spp. and *Steinernema* spp. against *B. terrestris*. When exposed to soil-applied nematodes at the recommended dose (50 nematodes per cm^2 soil), the mortality rate in *B. terrestris* individuals was found to be over 80% within 96 hours. Although a mixture of *Heterorhabtidis* spp. and *Steinernema* spp. was used in the aforementioned study, the effect of *S. feltiae*, when used alone, was revealed in our study. According to our results, when *S. feltiae* was applied at the MFRD, the mortality rates in *B. terrestris* workers were 46% in the spray method and 48% in the residual contact method, on the 14th day after application.

In accordance with the IOBC classification, Demirozer *et al.* (2022b) determined that when *B. terrestris* workers are exposed to Nimbecidine, Priority, Nostalgist, and Nibortem with sugar water, the toxicity classes were highly toxic (100%), weakly toxic (44%), non-toxic (8%), and non-toxic (14%), respectively at 15th observation day. All biopesticides are classified as non-toxic (6%-16%) when exposed to the same biopesticides with pollen. In the present study, on the 14th day after application, for the spray method, toxicity classes were weakly toxic for 1/1, 1/10, and 1/100 doses of Nematac and Nibortem, while other biopesticides were moderately toxic on *B. terrestris* workers.

Consequently, mortality rates that may occur when bumblebees used as pollinators are exposed to biopesticides during (spray method) or after spraying (residual contact method) have been obtained. According to our results, biopesticides may have weakly (Nematac and Nibortem) or moderately toxic (Nimbecidine, Nostalgist and Priority) effects on *B. terrestris* workers. These data are thought to be important for the safe use of these biopesticides, which are alternatives to chemical pesticides.

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