



HAL
open science

Lethal and sublethal effects of different biopesticides on *Bombus terrestris* (Hymenoptera: Apidae)

Ozan Demirozer, Asiye Uzun, Ayhan Gosterit

► **To cite this version:**

Ozan Demirozer, Asiye Uzun, Ayhan Gosterit. Lethal and sublethal effects of different biopesticides on *Bombus terrestris* (Hymenoptera: Apidae). *Apidologie*, 2022, 53 (2), pp.24. 10.1007/s13592-022-00933-6 . hal-04091304

HAL Id: hal-04091304

<https://hal.science/hal-04091304>

Submitted on 8 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Lethal and sublethal effects of different biopesticides on *Bombus terrestris* (Hymenoptera: Apidae)

Ozan DEMIROZER¹ , Asiye UZUN¹ , and Ayhan GOSTERIT² 

¹ Department of Plant Protection, Faculty of Agriculture, Isparta Applied Science University, 32260 Isparta, Turkey

² Department of Animal Science, Faculty of Agriculture, Isparta Applied Science University, 32260 Isparta, Turkey

Received 14 April 2021 – Revised 29 March 2022 – Accepted 31 March 2022

Abstract – The use of biopesticides against agricultural pests is of great importance for the sustainability of pest management. The goal of the present study was to determine the lethal (acute) and sublethal (first male and young queen emerge time, competition point, total number of workers, males and young queens) effects of four commercial biopesticides [Nibortem (1.5% *Lecanicillium* (*Verticillium*) *lecanii* strain V1-1, Nostalgist (1.5% *Beauveria bassiana* strain Bb-1), Priority (1.5% *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) strain PFs-1) and Nimbecidine (0.3 g/L Azadirachtin)] on *Bombus terrestris*. The impacts of biopesticides were investigated at the individual and colony level on worker bees by two application methods, namely topical and residual contact. While, the worker deaths were significantly higher in each biopesticide used than in the control in the residual contact application, those except Nibortem were found higher than the control in the topical application. The sublethal effect bioassays showed that there were no differences in terms of first male emergence time in the colonies for both topical and residual contact applications. However, the total number of workers was not affected by biopesticides in the residual contact method. The highest number of males was produced in the colonies exposed to Nimbecidine in both application methods. The number of young queens produced in the colonies was not affected by the application methods. In conclusion, Nimbecidine, among the tested biopesticides, caused vitally negative effects on the bumblebee in the residual contact application, in particular.

bumblebee / colony traits / entomopathogenic fungi / side effect / pollinator

1. INTRODUCTION

About 85% of the plants that constitute about 35% of the world's food production are pollinated by insects (Ahmad et al. 2015). In recent years, bumblebees have become an indispensable element for the pollination of greenhouse vegetables, especially for tomatoes (Gradish et al. 2012). Their pollination speed, buzzing behavior and foraging capabilities at low temperature and low light intensity make bumblebees reliable and

more effective pollinators (De Luca et al. 2013; Gosterit and Gurel 2018).

Bombus terrestris L. is the most commercially reared species among bumblebees due to easier breeding and the ability to form colonies with high population size (Velthuis and Van Doorn 2006). It is estimated that the current worldwide commercial bumblebee colonies used as pollinators have reached about three million colonies (about 350,000 colonies used in Turkey in 2020). Although there is a high demand for the use of bumblebees, some factors such as greenhouse features, climatic conditions indoors, and most importantly the intensive use of pesticides may affect the growth and pollination activities of commercial colonies used

Corresponding author: A. Gosterit,
ayhangosterit@isparta.edu.tr

Manuscript editor: Mathieu Lihoreau

in greenhouses (Goulson et al. 2008; Gradish et al. 2010; Potts et al. 2010). Colonies that are affected by these factors have short pollination periods and low performance. Thus, while the cost of pollination increases, the amount and quality of crops decrease causing economic loss for farmers (Gurel et al. 2011; Evans et al. 2017). Bumblebees can be exposed to pesticides directly or indirectly by consuming contaminated nectar and pollen (Thompson 2001; Arce et al. 2017). Worker bee deaths in greenhouses reveal the direct effects of pesticide exposure (Gradish et al. 2010; Wu et al. 2010). On the other hand, abnormalities in foraging behavior and pollen consumption of surviving individuals and the disruption of colony development may also be considered sublethal effects of pesticides (Gradish et al. 2010; Gurel et al. 2011; Mommaerts and Smaghe 2011).

It is rather complicated to determine the risk assessment of pesticides in insects living in colonies, such as honeybees and bumblebees (Blacquiere et al. 2012). However, the worldwide decline in the populations of pollinators, such as bees over the past few decades, has made these assessments necessary and valuable (Potts et al. 2010; Jacobson et al. 2018). It has been recently observed that biopesticides are more frequently used in pest control applications of cultivated plants due to their direct and indirect effects as well as target selectivity. The fungi *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*), *Lecanicillium (Verticillium) lecanii*, *Beauveria bassiana*, and Azadirachtin which constitutes the content of biopesticides that were also included in the present study have lethal effects on important mite and insect pests such as *Tetranychus urticae* Koch. (Acari: Tetranychidae), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) and *Aphis gossypii* Glover, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). For example, as a sublethal effect, they can reduce the fecundity of aphids and whiteflies (Gouli et al. 2009; Gurulingappa et al. 2011; Saranya et al. 2013; Keerio et al. 2020; Abdulle et al. 2021). However, many studies have also been

conducted to reveal the possible side effects of biopesticides on non-target organisms in agricultural production areas (Meikle et al. 2009; Shipp et al. 2012; Smaghe et al. 2013; Barbosa et al. 2015; Karise et al. 2016; Brownbridge and Buitenhuis 2019). Exposure to *B. bassiana* and Azadirachtin has been reported to cause non-lethal effects on *B. impatiens* and *B. terrestris* colonies (Koskor et al. 2009; Barbosa et al. 2015). In relation to that, these biopesticides have been found to lead to a decrease in the number of males and a negative effect on the foraging behavior of bees, resulting in a loss of the body weight in individuals (Mommaerts et al. 2009; Barbosa et al. 2015). However, there are no detailed studies in the literature regarding the lethal and sublethal effects of biopesticides containing *Isaria (Paecilomyces)* spp. and *Lecanicillium (Verticillium)* spp.

The present study aimed to determine the lethal and sublethal effects of commercial biopesticides, Nibortem, Nostalgist, Priority, and Nimbecidine on *B. terrestris*. In addition, the effects of different application methods on the impact of biopesticides were also investigated.

2. MATERIALS AND METHODS

2.1. Biopesticides

Four commercial biopesticides [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 fumosorosea* (= *Paecilomyces fumosoroseus*) strain PFs-1; 250 mL/da, 1×10^8 CFU/mL) and Nimbecidine (0.3 g/L Azadirachtin; 500 mL/100 L water, 16,000 IU/mg)] that have been licensed to use against sucking pests in greenhouse vegetable production were obtained from Agrobrest inc. (Izmir, Turkey). The maximum field recommended doses (MFRD) of all biopesticides (250 mL/da for Nibortem, Nostalgist and Priority; 500 mL/100 L water for Nimbecidine) were applied under laboratory conditions.

2.2. Bumblebees

Bombus terrestris L. was used as the bee material for carrying out lethal and sublethal bioassay trials. The bumblebee colonies were obtained from Bio Group inc. (Antalya, Turkey) in two groups according to their developmental stage and maintained in the standard laboratory rearing conditions (27 °C, 50% R.H. and darkness). In lethal effect bioassays, the workers supplied from more than 30 laboratory stock colonies, were used to determine the lethal effects of biopesticides on individuals. A total of 70 colonies including a healthy founder queen, a relatively small brood area, and approximately 30 workers (early stage of colony life) were used to determine the sublethal effect of biopesticides on the colony development (Sublethal effect bioassays). Sugar solution (50° Brix) and fresh thawed pollen were used as food materials.

2.3. Biopesticide application methods

The biopesticides were applied by topical (TA) and residual contact application methods (RCA) to the workers in both experiments. All worker bees were immobilized by CO₂ anesthesia in each application method for all the treatments including controls. In the topical application method, the MFRD of each biopesticides was prepared in water then 50 µL of the suspension of each biopesticide was topically applied to the dorsal part of the thorax of each worker with a micropipette according to the treatment group (Mommaerts et al. 2009). In the residual application method, it was aimed to determine the effects of biopesticide residue. For this purpose, the suspension of each biopesticide was sprayed into empty plastic boxes (13×5×3 cm) in the form of misting at 1 atm pressure for 20 s (0.5 mL). After 15 min, the anesthetized workers were transferred into the biopesticide residue boxes and kept there for 15 min (by modified of USEPA 2012a, b). Thus, the workers that became active immediately after the transfer were allowed to contact the spore suspension during this period. Sterile distilled water was

used in the control treatments of both application methods (Mommaerts et al. 2009). In both experiments, the dead individuals in all biopesticides except Azadirachtin were taken into the PDA (Potato dextrose agar) medium, and a re-isolation process was carried out to determine whether the cause of death was due to the entomopathogenic fungi (Meng et al. 2017).

2.4. Lethal effect bioassays

In this stage of the study, while the direct lethal effect of biopesticide on workers was investigated by the topical application method, their sublethal effect was determined by the residual application method considering that they may come into contact with the biopesticide residues during their foraging activities. Because the *B. terrestris* workers start foraging activities at the age of 1 week (Gill and Raine 2014), the workers aged between 8 and 12 days were used in the lethal effect bioassays. 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. All emerged workers were transferred to separate boxes and fed in for 7 days (Bulus et al. 2020). The trials were set up with these workers (8–12 days old) according to the randomized plot design, with 5 replications with 10 workers each in both RCA and TA methods (totally 500 worker bees). All workers were given sugar syrup ad libitum. The number of dead individuals was recorded daily (24-h interval) for 15 days after biopesticide applications.

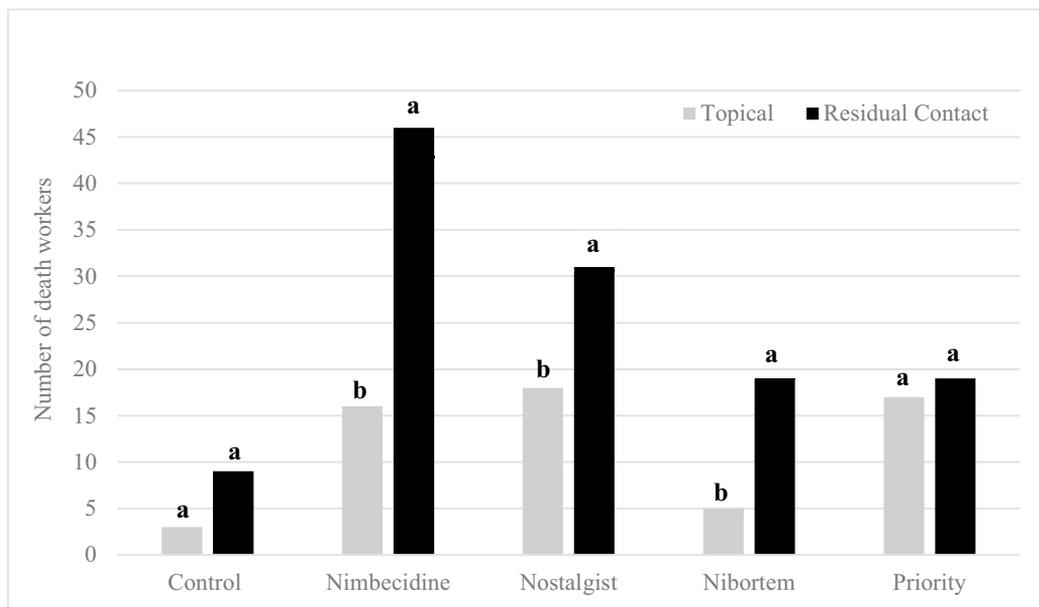
2.5. Sublethal effect bioassays

The sublethal effect bioassays of biopesticides were carried out to determine their impact on some colony developmental traits (first male and young queen emerging time, competition point, total number of workers, males and young queens). A total of 70 colonies (35 for topical and 35 for residual contact application methods; 7 colonies for each treatment) in the early stage of

colony life were used for this purpose. Although it is difficult to achieve a complete homogeneity without harming the normal development of the colony, similar colonies were selected in terms of brood area, and it was ensured that each colony contained a founder queen and about 30 workers. The colonies were observed twice a week under the same conditions until the end of their life cycle. The trials of emergence time of sexuals (males and young queens) and competition point (affecting colony life and also an indicator for the end of the colony life) were counted starting from the biopesticides application day which was considered as time 0. Worker oviposition, oophagy, egg-cup destruction, and two or more open egg cups were accepted as a competition point. During each observation, dead individuals were removed immediately and counted (Gosterit 2016).

2.6. Statistical analysis

All statistical analyses of the data obtained within the scope of the study were performed by the SPSS 20.0 software package. The Fischer’s chi-square test was used in the comparison of the total numbers of deaths obtained at the 15th day for each biopesticide in the topical and residual contact application methods. A square-root transformation was applied to the data obtained in the sublethal effect bioassays and tested for normality before analysis. The difference between the time-dependent survival probability in the topical and residual contact application methods was evaluated by Kaplan–Meier analysis for each time interval (0–5 (log-rank), 5–10 (Breslow (Generalized Wilcoxon)), and 10–15 (Tarone-Ware) days with Bonferroni correction). The descriptive statistics of traits were calculated



P*:	0.1212	0.0002	0.0159	0.0019	0.8351
X²:	3.409	38.200	6.763	10.746	0.174

a, b: dead numbers followed by different letters within each treatment show significant differences between application methods

P*, x²: Fischer's chi-square test

Figure 1. The comparison of the death numbers obtained at the 15th observation day in Topical and Residual contact application methods.

and one-way analyses of variance (ANOVA) was performed. The groups were compared using the Tukey’s multiple comparison test.

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 and Integrated Control (IOBC) (Sterk et al. 2002). For this scale, mortality rates calculated from the total death numbers of each treatment on the 15th day were used in both application methods.

3. RESULTS

3.1. Results of lethal effect bioassays

In the topical application (TA) method, it was seen that the deaths obtained in Nimbecidine ($x^2 = 10.981, P = 0.0016$), Nostalgist ($x^2 = 13.562, P = 0.0003$), and Priority ($x^2 = 12.250, P = 0.0008$) were significantly higher than in the control on the 15th day of observation. Only, Nibortem ($x^2 = 0.5434, P = 0.7149$) was not found different from the control. In the residual contact application (RCA) method, the

number of death in all commercial products was recorded significantly higher (Nimbecidine $x^2 = 55.313, P = 0.0000$; Nostalgist $x^2 = 20.166, P = 0.0000$; Nibortem $x^2 = 4.960, P = 0.0000$; Priority $x^2 = 4.960, P = 0.0000$) than in the control (Table I) at 15 days after application. In addition, when the application methods within each biopesticide treatment were compared, it was found that number of deaths obtained in the RCA for all biopesticides except Priority ($x^2 = 0.173, P = 0.8351$) were significantly higher than in the TA method. Also, the number of deaths obtained was not significantly different from each other in the control treatments in both methods (Figure 1). According to the IOBC’s toxicity scale, the results obtained on the 15th observation day, Nimbecidine (highly toxic) and Nostalgist (moderately toxic) were found toxic to bumblebee workers in the residual contact method. None of the biopesticides was classified as toxic in the topical application method.

The death that occurred in the workers on each observation day is given in Figure 2(a, b). The Kaplan–Meier survival curves plotted for two application methods (TA and RCA) showed that the difference between the number of deaths occurring at each time interval (0–5, 5–10, and 10–15 days) was significant. The survival probability was significantly higher for each biopesticide in the TA than in the RCA method in the time intervals of 0–5 ($x^2 = 22.471, df = 4, P = 0.0001$ for TA, $x^2 = 79.086, df = 4, P = 0.0000$ in RCA) and 5–10 days ($x^2 = 22.469,$

Table I The values obtained from the Fischer’s chi-square test of the cumulative death numbers of biopesticides by comparing with the control groups in topical and residual contact application methods

Treatments	Worker bee numbers***	Topical application			Residual contact application		
		15 th day*	P values**	Fischer’s chi-square	15 th day*	P values**	Fischer’s chi-square
Nimbecidine	50	16	0.0016	10.981	46	0.0000	55.313
Nostalgist	50	18	0.0003	13.562	31	0.0001	20.167
Nibortem	50	5	0.7149	0.543	19	0.0439	4.960
Priority	50	17	0.0008	12.250	19	0.0439	4.960
Control	50	3	-	-	9	-	-

* Occurred cumulative death numbers until the 15th day

** P values obtained Fischer’s chi-square test, P values shown in bold in the same column are different

*** Fifty individuals were used for each treatments in both application methods (Topical and residual contact application)

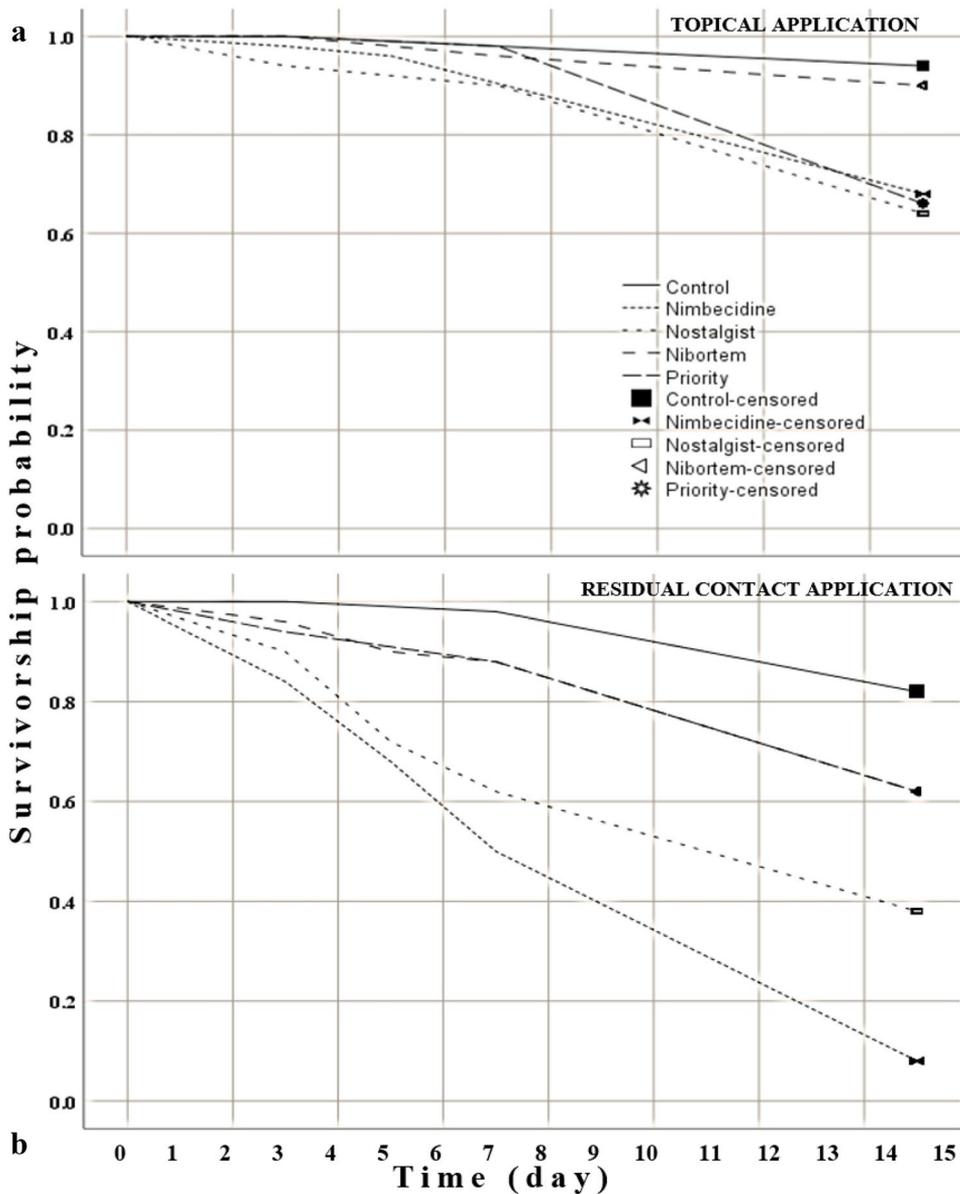


Figure 2. (a) Kaplan–Meier survival curve for all biopesticides in topical application method. (b) Kaplan–Meier survival curve for all biopesticides in residual contact application method.

$df=4, P=0.0001$ for TA, $\chi^2=77.471, df=4, P=0.0000$ in RCA). It was found that the survival probability differed according to the application methods and biopesticides in the time interval of 10–15 days ($\chi^2=22.470, df=4, P=0.0001$ and $\chi^2=78.486, df=4, P=0.0000$

for TA and RCA, respectively). At the end of the study, the highest survival probability was observed for Nibortem in the TA method, and for Nibortem and Priority in the RCA method. On the last observation day (15th day), the highest death was determined for Nostalgist in the

TA method, for Nimbecidine in the RCA method (Figure 2a, b).

It was observed that the side effects caused by the biopesticides on *B. terrestris* workers could be classified into the different groups based on the IOBC classification according to the different application methods used in this study. Priority, a myco-pesticide, was weakly toxic in both application methods with the mortality of 34–38%, whereas Nibortem was classified as non-toxic with 10% mortality when it was applied topically. It was weakly toxic in the residual contact method with the mortality of 38%. On the other hand, Nostalgist was weakly toxic in the topical application method with 36% mortality, while moderately toxic in the residual contact application method with the mortality of 62%. Nimbecidine, a plant-derived biopesticide, was found to be weakly toxic with 32% mortality in the topical application method and highly toxic with remarkably high 92% mortality in the residual contact application method.

3.2. Results of sublethal effect bioassays

The results of the first male and young queen emergence time and competition point are shown in Table II. No significant differences were found between biopesticide treatment groups in terms of the first male emergence time in any application methods. However, the first young queen emergence time in colonies was affected significantly by the biopesticide exposure in both topical ($F = 4.58$; $P = 0.0080$) and residual contact ($F = 5.08$; $P = 0.0060$) application methods. Nimbecidine exposure, in particular, caused the start of young queen production at an earlier period of colony life when compared to the other biopesticides. In addition, the application methods did not cause any difference in the effects of biopesticides on the first young queen emergence time.

As explained in detail in Sect. 2, one of the most important traits that affect the colony life of *B. terrestris* is the competition point. This critical stage is considered an indicator for the end of the colony life cycle. The results showed

that there were significant differences between the biopesticide treatment groups in terms of the competition point in the topical application. Nimbecidine significantly differed from the other biopesticides tested and caused a late competition point. However, in the residual contact application method, the effect of the biopesticides on the competition point was not significant. The results regarding the total number of workers, males and young queens produced in colonies are given in Table III. The differences between the biopesticide treatment groups in terms of the total number of workers ($F = 2.76$; $P = 0.0460$) were significant in the topical application method, but not significant in the residual contact method. In the topical application method, while the highest number of workers was observed in the colonies treated with Nimbecidine, the lowest number of workers was in the Nostalgist treated colonies. The effect of the biopesticides on the total number of males produced in the colonies was not significant in TA. But, there were significant differences between the applied biopesticides in terms of the total number of males produced in the colonies only in the residual contact method. The average total number of males was significantly higher in the colonies treated with Nimbecidine (120.0 ± 21.40) than in the control (50.83 ± 9.44) and Nibortem (45.00 ± 7.75) treatment groups ($F = 4.52$; $P = 0.0070$). However, in the residual contact method, the number of males in Nibortem treated colonies, despite being the lowest value, was not significantly different than in the colonies treated with Nostalgist or Priority.

In this study, we determined that the number of young queens produced in the colonies was not affected by the application method of the biopesticide. However, the results obtained in the topical application method showed more variation than those in the residual contact method. The number of young queens produced in the colonies treated with Nimbecidine was significantly higher than in all other biopesticide groups in the residual contact method ($F = 7.85$; $P = 0.0010$), but differed only from the Nibortem and Control treatment groups in the topical application method ($F = 4.58$; $P = 0.0080$).

Table II Some colony traits of *Bombus terrestris* exposed to biopesticides by different application methods

Treatments	First male emerging time (day)				First gyne emerging time (day)				Competition point (day)			
	Residual Contact		Topical		Residual Contact		Topical		Residual Contact		Topical	
	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	n
Control	30.00±3.17	6	35.83±2.75	0.1950 1.93	40.67±3.33	3	45.67±2.33	0.2860 1.51	9.71±1.41 A	7	8.29±1.66	0.5240 0.43
Nibortem	31.75±3.97	7	28.71±4.04	0.6350 0.24	41.00±3.21	5	39.00±5.13	0.7910 0.08	10.00±2.09 AB	7	10.29±2.16	0.9260 0.01
Nostalgiist	23.83±5.11	7	28.43±4.29	0.5020 0.48	32.71±0.97	5	31.80±2.01	0.6620 0.20	9.14±1.50 A	7	8.27±0.92	0.6350 0.24
Priority	29.89±3.69	6	26.50±4.10	0.5540 0.37	30.67±3.30	6	34.00±2.29	0.4270 0.69	6.00±1.51 A	7	10.29±1.19	0.0460 4.96
Nimbecidine	24.00±2.73	5	20.00±2.81	0.3430 0.99	27.71±2.11	5	25.20±2.06	0.4300 0.68	17.00±2.20 B	7	14.14±1.18	0.2760 1.30
P values	0.4720		0.1320		0.0080	0.0060		0.0030		0.057		
F values	0.91		1.95		4.58	5.08		5.16		2.58		

A, B Means followed by different letter in the same column are significantly different from each other

Table III Effects of biopesticides on the total number of individuals produced in *B. terrestris* colonies in topical and residual contact applications

Treatments	Total number of workers						Total number of males						Total number of queens					
	Topical		Residual contact		P		Topical		Residual contact		P		Topical		Residual contact		P	
	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	F	F	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	F	F	n	$\bar{x} \pm S.E.$	n	$\bar{x} \pm S.E.$	F	F
Control	7	160.00 ± 22.40	7	143.60 ± 17.20	0.5720	0.5720	6	70.80 ± 14.30	6	50.83 ± 9.44	0.2710	0.2710	3	13.00 ± 3.21	3	11.00 ± 1.53	3	0.6040
		AB																
Nibortem	7	176.90 ± 12.70	7	152.00 ± 15.00	0.2290	0.2290	4	68.80 ± 14.80	6	45.00 ± 7.75	0.1560	0.1560	3	23.33 ± 1.67	5	25.20 ± 5.34	5	0.8050
		AB			0.34						1.36							0.32
Nostalgist	7	119.10 ± 21.70	7	157.30 ± 10.40	0.1390	0.1390	6	61.70 ± 15.80	7	71.40 ± 10.90	0.6140	0.6140	7	30.71 ± 4.56	5	27.00 ± 7.84	5	0.6710
		A			1.60						2.45							0.07
Priority	7	151.40 ± 12.90	7	162.40 ± 10.30	0.5180	0.5180	7	67.60 ± 14.00	6	97.80 ± 20.10	0.2320	0.2320	6	45.70 ± 11.90	6	33.50 ± 8.41	6	0.4230
		AB			2.51						0.27			AB				0.19
Nimbecidine	7	191.29 ± 7.66	7	168.6 ± 16.4	0.2330	0.2330	7	77.1 ± 16.3	5	120.00 ± 21.4	0.1350	0.1350	7	97.3 ± 23.4	5	108.0 ± 25.3	5	0.7660
		B			1.57						2.64			B				0.09
P values		0.0460		0.7650				0.9640		0.0070				0.0080		0.0010		
F values		2.76		0.46				0.14		4.52				4.58		7.85		

A, B Means followed by different letter in the same column are significantly different from each other

4. DISCUSSION

In recent years, a considerable number of scientific studies are available proving the negative effects of pesticides on human and environmental health (Ries et al. 2003). Therefore, bio-origin plant protection products used in the controlling of harmful arthropods are attracting more attention and their use has been increasing (Pineda et al. 2008; Shahid et al. 2012; Maina et al. 2018). As with any pesticide, clarifying the effects of biopesticides on target and non-target organisms is vital for the sustainability of agricultural production. Nimbecidine derived from neem tree shows insecticidal effects due to tetranortriterpenoids it contained (Zhong et al. 2017). It has been reported that Nimbecidine poses potential risks to non-target arthropods, especially bees, and has strong biological properties as an anti-feedant, repellent, sterilant, and insect growth regulator (Morgan 2009). According to our results, among the biopesticides tested, Nimbecidine stood out with its lethal effect (92%) in the RCA method. The application methods of biopesticides can be decisive on their effect levels on bumblebees. Barbosa et al. (2015) determined that the exposure of *B. terrestris* to Nimbecidine in the concentration of between 3.2 and 320 mg per liter sugar water inhibited their reproduction and also caused 32 to 100% mortality in the colony. Previous studies have also indicated that Nimbecidine concentrations in different ranges (low/high) shorten the ovarian length. Therefore, it stops the ovulation and thus the production of males. Consequently, antifeedant effects of Nimbecidine may lead to a low food intake and thus adversely affect bees' ovarian development (Mordue and Nisbet 2000; Melathopoulos et al. 2000; Trumm and Dorn 2000). In another study, when Nimbecidine has been applied to honeybees by the leaf disk method, it has caused 60% death in adults and 42% death in larvae (Xavier et al. 2015). Similarly, the mortality rate with Nimbecidine application was 92% for the contact method and 32% for the topical application method. The product named Nostalgist containing *B.*

bassiana strain, Bb-1 caused 36% death in the workers when it was applied topically, and 62% death in the residual application. Different results have been reported about the effects of different isolates of *B. bassiana* on bumblebee species. Karise et al. (2016) reported that Botanigard caused a high mortality on *B. terrestris* workers, consistent with our results. On the contrary, Shipp et al. (2012) reported that *B. bassiana* did not cause a significant mortality on *B. impatiens*. Mommaerts et al.'s (2009) results showed that the *B. bassiana* killed 92% of *B. terrestris* workers in the topical application. In addition, they found that when the *B. terrestris* colonies were exposed to *B. bassiana* with pollen and sugar syrup, it negatively affected the production of males in the colonies. In general, *B. terrestris* colonies produced a large number of workers in the early stage of colony life. To produce the next generations, young queens and males are expected to be produced at the end of the life cycle. However, in our study, Nostalgist was determined not to have an adverse effect in the total number of males in both application methods. Besides, it had no effect on the first male emergence and the competition point but shortened the first young queen emergence time in the topical application. On the other hand, it has been reported that no growth parameters of *B. impatiens* were adversely affected in the exposure to the same product by the topical application method (Ramanaidu and Cutler 2013). The application of Nibortem (*Lecanicillium (Verticillium) lecanii* strain V1-1) resulted in the lethality of 10% in the topical application and 38% in the residual contact application, but did not cause any negative effect on the colony development of the bees. On the other hand, Priority (*Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) strain PFs-1), another bioinsecticide tested, was weakly toxic in both application methods and its effects on the colony life varied. It caused a decrease in the total number of workers when applied topically and an increase in the total number of young queens and males in the residual contact application. In general, bumblebees by nature produce a

large number of queens and males toward the end of the colony life.

In conclusion, the assessment of the lethal and sublethal effects of these plant protection products at the individual and colony level on bumblebees is vital for the food chain cycle in nature. It is also crucial for the future and sustainability of agricultural activities. Further studies is recommended to be conducted in the conditions of greenhouse and open field production areas to contribute to the elimination of many doubts on biopesticides by considering the results obtained in the present study.

ACKNOWLEDGEMENTS

The author thanks Assoc. Prof. Tufan NAYIR for his assistance with the statistical analysis of the study (MD Consultant, World Health Organization, Country Office, Turkey).

AUTHOR CONTRIBUTION

Study conception and experimental design were performed OD and AG; data collection were performed by AU, data analysis were performed by OD and AU. The manuscript was written by OD and AG.

FUNDING

This study was supported by The Scientific and Technological Research Council of Turkey (Project number 119O442).

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this article.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Conflict of interest The authors declare no competing interests.

Ethics approval and consent to participate No ethical approval is required.

Consent for publication Not applicable.

REFERENCES

- Abdulle YA, Nazir T, Sayed S, Mahmoud SF, Majeed MZ, Aslam HMU, Iqbal Z, Nisar MS, Keerio AU, Ali H, Qiu D (2021) Sub-lethal effects of *Lecanicillium lecanii* (Zimmermann)-derived partially purified protein and its potential implication in cotton (*Gossypium hirsutum* L.) defense against *Bemisia tabaci* Gennadius (Aleyrodidae: Hemiptera). *Agriculture* 11: 778. <https://doi.org/10.3390/agriculture11080778>
- Ahmad M, Bodlah I, Mehmood K, Sheikh UAA, Aziz MA (2015) Pollination and foraging potential of European bumblebee, *Bombus terrestris* (Hymenoptera: Apidae) on tomato crop under greenhouse system. *Pakistan J Zool* 47: 1279–1285.
- Arce AN, David TI, Randall EL, Rodrigues AR, Colgan TJ, Wurm Y, Gill RJ (2017) Impact of controlled neonicotinoid exposure on bumblebees in a realistic field setting. *J App Ecol* 54: 1199–1208. <https://doi.org/10.1111/1365-2664.12792>
- Barbosa WF, De Meyer L, Guedes RNC, Smagghe G (2015) Lethal and sublethal effects of azadirachtin on the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). *Exotoxicology* 24: 130–142. <https://doi.org/10.1007/s10646-014-1365-9>
- Blacquiere T, Smagghe G, Van Gestel CAM, Mommaerts V (2012) Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Eco-toxicology* 21: 973–992. <https://doi.org/10.1007/s10646-012-0863-x>
- Brownbridge M, Buitenhuis R (2019) Integration of microbial biopesticides in greenhouse floriculture: the Canadian experience. *J Invertebr Pathol* 165: 4–12. <https://doi.org/10.1016/j.jip.2017.11.013>
- Bulus IY, Uzun A, Demirözler O, Gosterit A (2020) Effects of acetamiprid on brood development in bumblebees *Bombus terrestris*. *J. Fac Agric* 15: 91–99.
- De Luca PA, Bussiere LF, Souto-Vilaros D, Goulson D, Mason AC, Vallejo-Marin M (2013) Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. *Oecologia* 172: 805–816. <https://doi.org/10.1007/s00442-012-2535-1>
- Evans LJ, Smith KE, Raine NE (2017) Fast learning in free-foraging bumble bees is negatively correlated with lifetime resource collection. *Sci Rep* 7: 496 (1–10). <https://doi.org/10.1038/s41598-017-00389-0>
- Gill RJ, Raine NE (2014) Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Funct Ecol* 28: 1459–1471. <https://doi.org/10.1111/1365-2435.12292>

- Gosterit A (2016) Adverse effects of inbreeding on colony foundation success in bumblebees, *Bombus terrestris* (Hymenoptera: Apidae). *Appl Entomol Zool* 51: 521–526. <https://doi.org/10.1007/s13355-016-0427-2>
- Gosterit A, Gurel F (2018) The role of commercially produced bumblebees in good agricultural practices. *Sci Papers Ser D Anim Sci* 1: 201–204.
- Gouli VV, Gouli SY, Skinner M, Shternshis M (2009) Effect of the entomopathogenic fungi on mortality and injury level of western flower thrips *Frankliniella occidentalis*. *Arch. Phytopathol* 42: 118–123. <https://doi.org/10.1080/03235400600951829>
- Goulson D, Lye GC, Darvill B (2008) Decline and conservation of bumble bees. *Annu Rev Entomol* 53: 191–208. <https://doi.org/10.1146/annurev.ento.53.103106.093454>
- Gradish AE, Scott Dupree CD, Shipp L, Harris CR, Ferguson G (2010) Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest Manag Sci* 66(2): 142–146. <https://doi.org/10.1002/ps.1846>
- Gradish AE, Scott-Dupree CD, Frewin AJ, Cutler GC (2012) Lethal and sublethal effects of some insecticides recommended for wild blueberry on the pollinator *Bombus impatiens*. *Can Entomol* 144: 478–486. <https://doi.org/10.4039/tce.2012.40>
- Gurel F, Gosterit A, Argun Karshi B (2011) The influences of greenhouse conditions on pollination performance of *Bombus terrestris* L. colonies. *Derim* 28: 47–55.
- Gurulingappa P, McGee PA, Sword G (2011) Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. *Crop Protection* 30: 349–353.
- Jacobson MM, Tucker EM, Mathiasson ME, Rehan SM (2018) Decline of bumble bees in northeastern North America, with special focus on *Bombus terricola*. *Bio Conserv* 217: 437–445. <https://doi.org/10.1016/j.biocon.2017.11.026>
- Karise R, Muljar R, Smaghe G, Kaart T, Kuusik A, Dreyersdorff G, Williams IH, Mand M (2016) Sublethal effects of kaolin and the biopesticides Prestop-Mix and BotaniGard on metabolic rate, water loss and longevity in bumble bees (*Bombus terrestris*). *J Pest Sci* 89: 171–178. <https://doi.org/10.1007/s10340-015-0649-z>
- Keerio AU, Nazir T, Anwar T, Majeed MZ, Abdulle YA, Jatoi GH, Gadhi MA, Qiu D (2020) Sub-lethal effects of partially purified protein extracted from *Beauveria bassiana* (Balsamo) and its presumptive role in tomato (*Lycopersicon esculentum* L.) defense against whitefly (*Bemisia tabaci* Genn.). *Insects* 11(9): 574(1–13). <https://doi.org/10.3390/insects11090574>
- Koskor E, Muljar R, Drenkhan K, Karise R, Bender A, Viik E, Luik A, Mänd M (2009) The chronic effect of the botanical insecticide Neem EC on the pollen forage of the bumble bee *Bombus terrestris* L. *Agron Res* 7: 341–346.
- Maina UM, Galadima IB, Gambo FM, Zakaria D (2018) A review on the use of entomopathogenic fungi in the management of insect pests of field crops. *J Entomol Zool Stud* 6: 27–32.
- Meikle WG, Mercadier G, Annas F, Holst N (2009) Effects of multiple applications of a *Beauveria* based biopesticide on *Varroa destructor* (Acari: Varroidae) densities in honey bee (Hymenoptera: Apidae) colonies. *J Apic Res* 48: 220–222. <https://doi.org/10.3896/IBRA.1.48.3.13>
- Melathopoulos AP, Winston ML, Whittington R, Higo H, Le Doux M (2000) Field evaluation of neem and canola oil for the selective control of the honey bee (Hymenoptera: Apidae) mite parasites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae). *J Econ Entomol* 93: 559–567. <https://doi.org/10.1603/0022-0493-93.3.559>
- Meng X, Hu J, Ouyang G (2017) The isolation and identification of pathogenic fungi from *Tessaratoma papillosa* Drury (Hemiptera: Tessaratomidae). *Peerj* 5: 1–14. <https://doi.org/10.7717/peerj.3888>
- Mommaerts V, Sterk G, Hoffmann L, Smaghe G (2009) A laboratory evaluation to determine the compatibility of microbiological control agents with the pollinator *Bombus terrestris*. *Pest Manag Sci* 65: 949–955. <https://doi.org/10.1002/ps.1778>
- Mommaerts V, Smaghe G (2011) Entomovectoring in plant protection. *Arthropod-Plant Inte* 5: 81–95. <https://doi.org/10.1007/s11829-011-9123-x>
- Mordue AJ, Nisbet AJ (2000) Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *An Soc Entomol Bras* 29: 615–632.
- Morgan ED (2009) Azadirachtin, a scientific gold mine. *Bioorgan Med Chem* 17: 4096–4105. <https://doi.org/10.1016/j.bmc.2008.11.081>
- Pineda A, Marcos-Garcia MA (2008) Use of selected flowering plants in greenhouses to enhance aphidophagous hoverfly populations (Diptera: Syrphidae). *Ann Soc Entomol Fr* 44: 487–492.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impact and drivers. *Trends Ecol Evol* 25: 345–353. <https://doi.org/10.1016/j.tree.2010.01.007>
- Ramanaidu K, Cutler GC (2013) Different toxic and hormetic responses of *Bombus impatiens* to *Beauveria bassiana*, *Bacillus subtilis* and spirotetramat. *Pest Manag Sci* 69: 949–954. <https://doi.org/10.1002/ps.3456>
- Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Fay MP, Feuer EJ (2003) Edwards BK (eds). SEER Cancer Statistics Review, 1975–2000, National Cancer Institute. Bethesda, MD, <https://seer.cancer.gov/csr/19752000/2003>. Accessed 01 June 2021
- Saranya S, Ramaraju K, Jeyarani S (2013) Pathogenicity of entomopathogenic fungi to two spotted spider

- mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Biopestic Int* 9: 127–131.
- Shahid AA, Rao AQ, Bakhsh A, Husnain T (2012) Entomopathogenic fungi as biological controllers: new insight into their virulence and pathogenicity. *Arch Biol Sci* 64: 21–42. <https://doi.org/10.2298/ABS1201021S>
- Shipp L, Kapongo JP, Park HH, Kevan P (2012) Effect of bee-vectored *Beauveria bassiana* on greenhouse beneficials under greenhouse cage conditions. *Biol Control* 63: 135–142. <https://doi.org/10.1016/j.biocontrol.2012.07.008>
- Smaghe G, De Meyer L, Meeus I, Mommaerts V (2013) Safety and acquisition potential of *Metarhizium anisopliae* in entomovectoring with bumble bees, *Bombus terrestris*. *J Econ Entomol* 106: 277–282. <https://doi.org/10.1603/EC12332>
- Sterk G, Heuts F, Merck N, Bock J (2002) Sensitivity of non-target arthropods and beneficial fungal species to chemical and biological plant products: results of laboratory and semi-field trials. 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, USA, pp. 306–313.
- Thompson HM (2001) Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* 32: 305–321. <https://doi.org/10.1051/apido:2001131>
- Trumm P, Dorn A (2000) Effects of azadirachtin on the regulation of midgut peristalsis by the stomatogastric nervous system in *Locusta migratoria*. *Phytoparasitica* 28: 7–26. <https://doi.org/10.1007/BF02994020>
- USEPA (2012a) Ecological effects test guidelines: OCSPP 850.3020: honey bee acute contact toxicity test. United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0016>. Accessed on 02 December 2021
- USEPA (2012b) Ecological effects test guidelines: OCSPP 850.3030: honey bee toxicity of residues on foliage. United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. <https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0154-0017>. Accessed 02 December 2021
- Wu J, Li JL, Peng WJ, Hu FL (2010) Sensitivities of three bumblebee species to four pesticides applied commonly in greenhouses in China. *Insect Sci* 17: 67–72. <https://doi.org/10.1111/j.17447917.2009.01286.x>
- Velthuis HHW, Van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* 37: 421–451. <https://doi.org/10.1051/apido:2006019>
- Xavier VM, Message D, Picanco MC, Chediak M, Santana Junior PA, Ramos RS, Martins JC (2015) Acute toxicity and sublethal effects of botanical insecticides to honey bees. *J Insect Sci* 15: 1–6. <https://doi.org/10.1093/jisesa/iev110>
- Zhong B, Chaojun L, Weiyan Q (2017) Effectiveness of the botanical insecticide azadirachtin against *Tirathaba rufivena* (Lepidoptera: Pyralidae). *Fla Entomol* 100: 215–218. <https://doi.org/10.1653/024.100.0215>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.