The Effects Of Some Insecticides On Honeybees (*Apis mellifera*)

Rıfat Akça

Ondokuz Mayıs Üniversitesi: Ondokuz Mayıs Üniversitesi

İslam Saruhan (✉ isaruhan@omu.edu.tr)

Ondokuz Mayıs Üniversitesi Ziraat Fakültesi: Ondokuz Mayıs Üniversitesi Ziraat Fakültesi

<https://orcid.org/0000-0003-0229-9627>

Research Article

Keywords: Insecticide, honeybee, neonicotinoid, pyrethroid, toxicology

Posted Date: December 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1152410/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

In the study, the topical application (1 µl/bee), contact (5 ml/pot/10 bees) and residual (5 ml/pot/10 bees) effects of eight insecticides (Methiocarb, Alphacypermethrin, Indoxacarb, Spinosad, Thiacloprid + Deltamethrin, Thiamethoxam, Thiamethoxam + Lambda-Cyhalothrin and Zeta Cypermethrin), which are commonly used in pest control in hazelnut cultivation, were investigated on *Apis mellifera* L. (Hymenoptera:Apidae). The study was conducted with 10 young worker bees in 4 replicates. Knock-down effect and 48-hour mortality were determined in all three methods. The study was conducted under 65-70% humidity and 24 ± 1 °C laboratory conditions. The study findings demonstrated that the impact of the topical application was low in all pesticides, while the other two methods led to 100% mortality after 48 hours at the recommended dose. The highest contact effect was observed with thiamethoxam + lambda-cyhalothrin, thiamethoxam, zeta cypermethrin, methiocarb and indoxacarb, followed by thiacloprid + deltamethrin, Spinosad and alphacypermethrin. Among the insecticides tested for residual effects, thiamethoxam + lambda-cyhalothrin, thiamethoxam, zeta cypermethrin and Spinosad led to over 90% mortality after 5 days.

Introduction

Honey bees (*Apis mellifera* Linnaeus 1758) (Hymenoptera: Apidae) are recognized as an insect with significant agronomic, ecological and scientific functions. Although honey bee products (honey, pollen, royal jelly, propolis and beeswax) are of significant commercial and nutritional value, honeybees are important creatures who play a key role in the pollination of certain plants (Free, 1993; Gallai et al., 2009; Potts et al., 2010; Ye et al. 2014). About 35% of flowering plants need pollinators to reproduce. According to the global 2005 data, pollination is estimated to worth € 153 billion. *A. mellifera* is among the most important pollinators in agriculture. However, there has been a significant decline in pollinator population in recent years in the global agricultural cultivation areas. One of the most important reasons for this is the indiscriminate use of pesticides (Silva et al, 2015). The insecticides that are employed to control pests could have adverse effect on several non-target organisms, including honeybees. Bees could be directly exposed to insecticides due to direct contact with plants in the sprayed fields, weeds, ingestion of the pollen or nectar (Sanchez-Bayo and Goka, 2014; Christen et al. 2017). The direct effect of insecticides on honeybees occurs with the direct contact with the spray droplets when the bees are active in the fields sprayed with insecticides. Another direct effect of insecticides could be observed after the exposure of honeybees when they are active in insecticide-treated orchards, vegetable fields, greenhouses and among the weeds in these areas. Thus, bees could be seriously affected by insecticides, and even the entire colony could be lost.

Honeybees are exposed to various types of insecticides, of which neonicotinoids, pyrethroids and organophosphates are frequently identified in beeswax, pollen and nectar (Christen et al, 2019). In particular, neonicotinoid insecticides are widely employed in the control of several pests that harm the fruits, vegetables and ornamental plants (Muccinelli, 2008). Therefore, the effects and the properties of neonicotinoids suggest that they pose the greatest risk to honey bees (Sanchez-Bayo and Goka, 2014).

Honeybees ingest neonicotinoids on plant parts such as leaves, seeds and pollen. Neonicotinoids act as agonists on the nicotinic Acetylcholine receptor (nAChR) in insect brains, an essential molecular central brain component involved in learning and memory formation (Tison et al, 2019). Besides neonicotinoids, pyrethroids are another insecticide type widely used in plant production (Soderlund and Bloomquist, 1989). Neonicotinoids have high selectivity towards mammals while pyrethroids are not selective (Tomizawa and Casida, 2005). Both insecticide types are highly toxic, particularly for honeybees (Meled et al., 1998; Laurino et al., 2011). Pyrethroids are an important and common type of insecticide. Pyrethroid exposure leads to decreases in queen and sperm count, high mortality in colonies, affects insect memory, a decrease in learning, and a reduction in foraging, flight and sensitivity to stimuli (Liao et al. 2018).

In the present study, the effects of 8 common insecticides employed in pest control in hazelnut cultivation fields on honeybees were determined in laboratory conditions. The effects of these insecticides on honeybees were determined with three methods (topical application, contact and residual effect). Furthermore, the knock-down effect and 48-hour mortality rate were identified.

Material And Method

Honeybee samples

The honeybees [*Apis mellifera* Linnaeus (Hymenoptera: Apidae)] employed in the study were procured from the Ondokuz Mayıs University, Faculty of Agriculture, Apiculture Department. No chemicals were employed in the hives where honeybees were kept, and all bees were same-age worker bees.

Chemical insecticides

The insecticides employed in the study included Carbamate, Organophosphate, Neonicotinoid, Pyrethroid, and Bacterium types, which are commonly used in hazelnut cultivation. The insecticides employed in the study, recommended doses and doses adopted in the study are presented in Table 1. In the study, the recommended dose and three lower insecticide doses (1/2 - 1/4 - 1/8) were prepared in the experiments.

Table 1
Insecticides used in agricultural application assessed to verify their toxicity against honey bees (*A. mellifera* L.)

Active ingredient (a.i.)	Chemical group	Recommended dose	Concentration used in bioassay (g da ⁻¹ – mL da ⁻¹)
Methiocarb	Carbamate	100 g da ⁻¹	100 – 50 – 25 – 12,5
Alphacypermethrin	Pyrethroid	40 mL da ⁻¹	40 – 20 – 10 – 5
Indoxacarb	Organophosphate	45 mL da ⁻¹	45 – 22,5 – 11,25 – 5,625
Spinosad	Bacterium species	15 mL da ⁻¹	15 – 7,5 – 3,75 – 1,875
Thiacloprid + Deltamethrin	Neonicotinoid+ Pyrethroid	25 mL da ⁻¹	25 – 12,5 – 6,25 – 3,125
Thiamethoxam	Neonicotinoid	20 g da ⁻¹	20 – 10 – 5 – 2,5
Thiamethoxam+Lambda-Cyhalothrin	Neonicotinoid+ Pyrethroid	10 mL da ⁻¹	10 – 5 – 2,5 – 1,25
Zeta Cypermethrin	Pyrethroid	133 mL da ⁻¹	133 – 66,5 – 33,25 – 16,625

Different applications used in the study

In the study, the effects of insecticides on honeybees were determined with three methods: topical application, contact and residual effects. These methods are described below.

Topical application

In the topical application method, 1 µl/bee insecticide diluted with water were applied to the dorsal part of the honeybee thorax with a micropipette. After the application, each honeybee was removed from the hive and 10 bees were transferred into special 1 kg polyethylene containers with lids. Cotton pieces impregnated with 2 ml melted sugar were left in the containers to feed the honeybees. Blotting paper places at the bottom of the containers were moistened with 5 ml distilled water to meet the humidity requirement of the honeybees. The study adopted the recommended doses and three sub-doses of all insecticides listed in Table 1 and was conducted in 4 replicates. No application was conducted in control group honeybees. After the applications, the containers were transferred to the laboratory and checked after 1, 3, 5, 7, 24 and 48 hours and the number of dead honeybees were recorded. Thus, the knock-down effect and 48-hour mortality rate were determined for the topical application.

Contact effect

The contact effect of all recommended and three sub-doses of all the insecticides presented in Table 1 were determined on honeybees. The control containers, where only water was applied, were also included

in the study. The bottom of the special containers was completely covered by blotting paper. 5 mL insecticide solution diluted with water was sprayed thoroughly on the blotting paper. The containers were left to dry for one hour. The study was set up in 4 replicates and 10 worker bees were placed in a container for each replicate. Cotton pieces impregnated with 2 ml sugar water were placed in the container to feed the honeybees. Blotting paper at the bottom of the containers was moistened with 5 ml distilled water to meet the moisture requirement of honeybees. The containers were transferred to the laboratory and dead honeybees were counted to determine the knock-down and contact effects after 48 hours.

Residual effect

To determine the residual effect, only the recommended doses of the five insecticides that exhibited the highest contact effect were tested on the honeybees. The study was planned with five selected insecticides, a control group and in 4 replicates. Thus, the adequate number of containers were sprayed with 5 ml insecticide solution on the same day, and these containers were stored in the laboratory. Certain containers were tested on the 1st day, while others were tested on the 2nd, 3rd, 4th and 5th days of the trial. On different days, 10 honeybees were placed in the containers that were sprayed the same day, and the dead honeybees were counted after 1, 3, 5, 7, 24 and 48 hours. Thus, the persistence of the selected insecticides were determined up to the 5th day.

Statistical analysis

Mortality rate was calculated by dividing the dead honeybee count by the initial honeybee count. The mortality data were corrected with Abbott's Formula (Abbott 1925). Serial-time bioassay mortality data were examined with probit analysis, and SPSS software was used to calculate 50% lethal time (LT_{50}), 90% lethal time (LT_{90}) (in hours), 50% lethal dose (LD_{50}) and 90% lethal dose (LD_{90}) (95% fiducial limits) (SPSS Inc., Version 21 Chicago, Illinois, USA).

Results

The effects of the tested insecticides on honeybees were investigated with three methods. The results obtained with these methods are presented in different sections below.

Table 2
The results of the topical application of certain insecticides to honeybees

Pesticides	Dose	n	Time after application (h)							
			1	3	5	7	24	48	LT ₅₀	LT ₉₀
Thiamethoxam (g da ⁻¹)	20	40	22,5	62,5	80	82,5	100	100	2,75	7,24
	10	40	17,5	47,5	60	60	60	62,5	12,0	115,55
	5	40	12,5	35	42,5	42,5	45	50	40,81	147,89
	2,5	40	10	37,5	37,5	37,5	42,5	45	51,32	171,51
LD ₅₀								4,92		
LD ₉₀								15,47		
Thiamethoxam+ Lambda- Cyhalothrin (mL da ⁻¹)	10	40	45	52,5	55	55	65,5	70	2,32	105,54
	5	40	37,5	42,5	47,5	47,5	52,5	67,5	7,92	108,08
	2,5	40	17,5	25	27,5	30	35	42,5	60,38	167,65
	1,25	40	2,5	10	12,5	20	25	32,5	66,84	133,76
LD ₅₀								3,90		
LD ₉₀								15,52		
Spinosad (mL da ⁻¹)	15	40	37,5	47,5	65	65	72,5	75	2,46	76,47
	7,5	40	37,5	50	62,5	62,5	72,5	75	2,53	76,39
	3,75	40	5	7,5	7,5	15	25	40	55,05	103,97
	1,875	40	0	0	2,5	5	12,5	40	53,16	83,47
LD ₅₀								4,41		
LD ₉₀								20,54		
Zeta Cypermethrin (mL da ⁻¹)	133	40	65	82,5	87,5	87,5	90	90	0,09	35,79
	66,5	40	3	32,5	35	40	42,5	50	41,58	121,85
	33,25	40	15	15	17,5	17,5	22,5	42,5	60,96	133,79
	16,625	40	5	5	7,5	7,5	22,5	37,5	56,95	102,38
LD ₅₀								49,26		
LD ₉₀								145,49		
Alphacypermethrin	40	40	60	65	70	70	75	80	0,18	85,69

Pesticides ($\mu\text{L da}^{-1}$)	Dose	n	Time after application (h)							
			1	3	5	7	24	48	LT ₅₀	LT ₉₀
	20	40	27,5	27,5	27,5	27,5	30	42,5	74,6	223,09
	10	40	10	12,5	15	20	22,5	32,5	77,3	165,26
	5	40	0	0	0	0	2,5	30	56,0	76,04
LD ₅₀	20,97									
LD ₉₀	53,19									
Methiocarb (g da ⁻¹)	100	40	22,5	45	65	65	72,5	85	4,9	51,24
	50	40	2,5	15	35	35	37,5	50	43,2	106,93
	25	40	2,5	7,5	12,5	12,5	17,5	27,5	78,2	148,12
	12,5	40	0	5	10	10	12,5	20	94,6	169,93
LD ₅₀	51,44									
LD ₉₀	110,61									
Thiacloprid + Deltamethrin (mL da ⁻¹)	25	40	5	5	5	5	27,5	50	45,9	79,99
	12,5	40	0	0	0	0	32,5	45	45,7	71,31
	6,25	40	2,5	2,5	2,5	2,5	15	30	62,4	102,11
	3,125	40	0	0	0	2,5	20	30	56,9	88,84
LD ₅₀	23,06									
LD ₉₀	72,87									
Indoxacarb (mL da ⁻¹)	45	40	0	0	2,5	5	12,5	40	53,16	83,47
	22,5	40	0	0	0	5	25	40	49,90	79,59
	11,25	40	0	0	0	2,5	22,5	30	56,40	88,67
	5,625	40	0	0	0	5	20	25	62,42	99,91
LD ₅₀	63,98									
LD ₉₀	193,64									
Control	-	40	0	0	0	2,5	2,5	5	-	-

Topical application

The topical application of the recommended dose of Thiamethoxam led to 100% mortality rates in honeybees on the 48th hour. The mortality rates obtained with the other insecticides were as follows: Zeta Cypermethrin (90%), Methiocarb (85%), Alphacypermethrin (80%), Spinosad (75%), Thiamethoxam + Lambda-Cyhalothrin (70%), Thiacloprid + Deltamethrin (50%) and Indoxacarb (40%). The topical application method exhibited a relatively low effect on honeybees. The analysis of the LT_{90} values for the insecticides used in the topical application revealed that the most effective insecticide was Thiamethoxam (7.24 days), followed by Zeta Cypermethrin (35.79 days), Methiocarb (51.24 days), Spinosad (76.47 days), Thiacloprid + Deltamethrin (79.99 days), Indoxacarb (83.), Alphacypermethrin (85.69 days) and Thiamethoxam + Lambda-Cyhalothrin (105.54 days) (Table 2).

Table 3
The contact effect of certain insecticides on honeybees

Pesticides	Dose	n	Time after application (h)							
			1	3	5	7	24	48	LT ₅₀	LT ₉₀
Thiamethoxam (g da ⁻¹)	20	40	30	87,5	100	100	100	100	1,67	3,16
	10	40	07,5	47,5	77,5	82,5	100	100	3,71	7,05
	5	40	2,5	20	22,5	30	100	100	9,04	15,75
	2,5	40	2,5	10	22,5	35	100	100	8,35	13,53
Thiamethoxam+ Lambda-Cyhalothrin (mL da ⁻¹)	10	40	37,5	55	77	82,5	100	100	2,32	8,10
	5	40	15	47,5	67,5	70	97,5	100	4,21	13,63
	2,5	40	7,5	25	42,5	47,5	85	85	14,29	42,95
	1,25	40	2,5	15	32,5	37,5	72,5	82,5	20,06	47,87
Spinosad (mL da ⁻¹)	15	40	0	15	55	70	100	100	5,35	8,22
	7,5	40	0	0	20	37,5	100	100	7,47	10,32
	3,75	40	0	2,5	10	12,5	100	100	11,10	16,32
	1,875	40	0	0	5	7,5	67,5	95	22,98	36,86
Zeta Cypermethrin (mL da ⁻¹)	133	40	25	42,5	75	77,5	100	100	4,12	7,40
	66,5	40	12,5	30	57,5	60	92,5	100	6,91	19,49
	33,25	40	0	15	35	45	67,5	100	14,69	31,89
	16,625	40	0	5	20	35	67,5	100	16,89	31,50
Alphacypermethrin (mL da ⁻¹)	40	40	52,5	55	70	77,5	100	100	1,186	10,96
	20	40	37,5	40	57,5	62,5	92,5	100	4,33	20,72
	10	40	32,5	35	42,5	55	90	100	6,95	23,35
	5	40	7,5	7,5	15	30	77,5	100	15,37	28,76
Methiocarb (g da ⁻¹)	100	40	70	100	100	100	100	100	0,20	2,00
	50	40	37,5	100	100	100	100	100	1,26	2,62
	25	40	25	100	100	100	100	100	1,56	2,81
	12,5	40	15	100	100	100	100	100	1,71	2,44
Thiacloprid + Deltamethrin	25	40	2,5	7,5	22,5	27,5	85	100	13,88	25,34
	12,5	40	2,5	7,5	17,5	20	87,5	97,5	15,95	29,09

Pesticides (mL da ⁻¹)	Dose	n	Time after application (h)							
			1	3	5	7	24	48	LT ₅₀	LT ₉₀
Indoxacarb (mL da ⁻¹)	6,25	40	2,5	2,5	7,5	10	40	70	34,53	60,29
	3,125	40	2,5	2,5	5	7,5	32,5	67,5	37,02	62,63
	45	40	0	7,5	42,5	70	100	100	5,72	8,26
	22,5	40	0	2,5	22,5	60	100	100	6,48	8,83
	11,25	40	0	5	25	47,5	100	100	6,97	10,11
	5,63	40	0	2,5	27,5	40	85	85	19,10	41,43
Control	-	40	0	0	0	2,5	2,5	2,5		

Contact effect

The contact effect application led to 100% mortality with all insecticides in honeybees at the recommended dose after 48 hours. All employed insecticides exhibited a high contact effect on the honeybees. The contact effect LD₉₀ values were as follows: Methiocarb (2 days), Thiamethoxam (3.16 days), Zeta Cypermethrin (7.40 days), Thiamethoxam + Lambda-Cyhalothrin (8.10 days), Spinosad (8.22 days), Indoxacarb (8.26 days), Alphacypermethrin (10.96 days), and Thiacloprid + Deltamethrin (25.34 days) (Table 3).

Table 4
The residual effect of certain insecticides on honeybees

Pesticides	Days	n	Time after application (h)							
			1	3	5	7	24	48	LT ₅₀	LT ₉₀
Thiamethoxam (g da-1)	1	40	20	52,5	70	85	100	100	3,31	7,51
	2	40	12,5	37,5	47,5	65	100	100	5,16	10,62
	3	40	10	42,5	47,5	50	92,5	100	7,57	20,69
	4	40	5	30	47,5	52,5	80	100	10,18	26,91
	5	40	10	30	40	40	70	90	15,49	43,67
Thiamethoxam+ Lambda-Cyhalothrin (mL da-1)	1	40	15	27,5	37,5	60	97,5	100	7,10	16,47
	2	40	7,5	22,5	27,5	45	80	100	12,35	27,59
	3	40	5	17,5	30	40	72,5	100	14,19	30,65
	4	40	2,5	17,5	30	40	67,5	97,5	16,15	35,61
	5	40	0	5	20	30	60	97,5	19,73	37,18
Spinosad (mL da-1)	1	40	25	10	25	55	100	100	7,49	15,08
	2	40	0	2,5	20	50	90	100	11,88	21,64
	3	40	0	2,5	15	35	75	100	15,96	28,48
	4	40	0	7,5	15	30	70	97,5	18,35	34,23
	5	40	0	0	10	12,5	47,5	92,5	26,10	43,72
Zeta Cypermethrin (mL da-1)	1	40	35	40	47,5	77,5	95	100	4,19	17,62
	2	40	27,5	35	37,5	62,5	82,5	100	7,91	26,88
	3	40	12,5	27,5	32,5	37,5	70	100	13,57	32,82
	4	40	5	15	22,5	30	50	100	19,23	38,77
	5	40	2,5	10	17,5	22,5	42,5	75	30,31	60,21
Alphacypermethrin	1	40	5	62,5	82,5	85	97,5	100	2,90	9,95
	2	40	32,5	37,5	50	67,5	80	100	5,91	27,85
	3	40	2	32,5	37,5	50	77,5	97,5	12,32	32,08
	4	40	1,5	32,5	35	37,5	62,5	95	16,43	40,57
	5	40	15	30	40	40	475	75	23,07	70,49
Control	1	40	0	0	0	0	0	0		

2	40	0	0	0	0	0	0
3	40	0	0	0	0	0	0
4	40	0	0	0	2,5	2,5	2,5
5	40	0	0	0	2,5	2,5	2,5

Residual effect

To determine the residual effect, five insecticides that were effective in topical application and contact effect tests were selected and recommended doses were applied in the trial. On the 5th day of the residual effect trial, the most effective insecticide was Thiamethoxam + Lambda-Cyhalothrin (97.5%), followed by Spinosad (92.5%), Thiamethoxam (90%), Zeta Cypermethrin (75%) and Alphacypermethrin (75%). The residual effect LT_{90} values were as follows on the 5th day: Thiamethoxam + Lambda-Cyhalothrin (37.18 days), Thiamethoxam (43.67 days), Spinosad (43.72), Zeta Cypermethrin (60.21) and Alphacypermethrin (70.49 days) (Table 4).

Discussion

The present study aimed to determine the effects of certain common insecticides in different chemical groups on honeybees with three methods. In the study, certain insecticide application methods were determined by considering based on real-life scenarios where the honeybees could be exposed to insecticides. Thus, the insecticides were applied with three methods (topical application, contact and residual effects). The effects of the chemicals on honeybees vary based on the region of application (Barron et al., 2007; Badawy et al. 2015). Furthermore, the effects of insecticides in different chemical groups and even insecticides in the same chemical group on honeybees could differ (Wang et al. 2020). The effects of pesticides on honeybees vary based on the metabolite and formulations that include a mixture of two different chemicals (Belzunces et al. 2012).

Topical application: This method was adopted to determine the effect of insecticide applications on honeybees during the active hours. Differences were determined between the effects of insecticides in different chemical groups on honeybees. In the topical application, the most effective insecticide was Thiamethoxam, a Neonicotinoid chemical group insecticide, and it was followed by the Zeta Cypermethrin in the Pyrethroid chemical group. Furthermore, Indoxacarb in the Organophosphate chemical group exhibited the lowest effect on honeybees with the topical application method. In a previous study, Badawy et al. (2015) reported that dinotefuran, a neonicotinoid, was highly effective on honeybees ($LD_{50} = 0.0006$ µg/bee in topical application and $LC_{50} = 1.29$ mg/L in oral feeding), and in similar applications, it was determined that acetamiprid (Neonicotinoid) and pymetrozine (Pyridine azomethine) were relatively less toxic (mortality <25%). In another study, Iwasa et al., (2004) applied certain neonicotinoid insecticides topically on the thorax of the honeybees. The findings demonstrated that the most effective was Clothianidin ($LD_{50} = 0.0218$ mg/bee) and the least effective was Thiacloprid ($LD_{50} = 14.6$ mg/bee). These

studies demonstrated that the effects of neonicotinoid insecticides on honeybees were significantly different. In a study on the topical effects of insecticides on *Aphis mellifera*, the toxicity of the insecticides was ordered as permethrin, azinphosmethyl, Carbaryl and methyl parathion (Danka et al. 1986). The other study examined the acute toxicity of eight agricultural insecticides such as Lambda-cyhalathrine, Furathiocarb, Carbaryl, Carbosulfan, Benfurocarb and Methiocarb. Research results also revealed that Carbaryl contained the fastest insecticide toxic effects on bees (Akca et al., 2009a). Based on these findings, the employment of an active substance with a lower effect on honeybees or an organophosphate group insecticide should be preferred, and neonicotinoid group insecticides should be used in pest control applications conducted during the active hours of the honeybees. Efficacy of three insecticides including Carbaryl, Carbosulfan and Methiocarb were evaluated in laboratory bioassays and lethal concentrations were determined against honeybee adults. The highest toxicity based on LC₅₀ values was observed in carbosulfan (1.3 mg ai L⁻¹). The LC₅₀'s (mg ai L⁻¹) for methiocarb and carbaryl in topical application method were 65.9 and 71.7, respectively (Akca et al., 2009b). The toxicity of bifenthrin and deltamethrin on workers of *Apis mellifera ligustica* was also evaluated, and the results from the study showed that the median lethal effects of bifenthrin and deltamethrin were 16.7 and 62.8 mg/L, respectively (Dai et al., 2010).

Contact effect: The contact effects of the insecticides were higher on the honeybees when compared to the topical application. This could be due to the differences between the pesticides when passing through the insect cuticula (Zawora et al., 2019). 100% mortality was observed only with the application of the recommended dose of Thiamethoxam on the 48th hour after topical application, while the contact effect led to 100% mortality with all insecticides. In a previous study, the toxicity of different insecticides on the 8th hour after contact on honeybees was ranked as follows: malathion, cypermethrin, demon-s-methyl, fenvalerate and deltamethrin, and all insecticides were found to be effective on honey bees (Sharma and Abrol, 2005). In a study where the contact toxicity of neonicotinoid insecticides was investigated on honeybees after 48 hours, it was reported that the LC₅₀ values were 3.35 ng/bee for clothianidin, 90.09 ng/bee for imidacloprid, and 4.27 ng/bee for thiamethoxam. In the study, the toxicity of imidacloprid in honeybees was lower than other pesticides (Laurino et al. 2013).

Residual effect: It was conducted to determine the residual effect of 5 insecticides that exhibited the highest topical and contact effects. The neonicotinoid group insecticides exhibited a residual effect of over 90% at the end of the 5th day. All insecticides employed to determine the residual effect were effective on honeybees even on the 5th day. This demonstrated that the insecticides could have an effect on honeybees even days after the application. Bee mortality generally started to decrease after 3 and 4 days. Kim et al. (2008) reported that Fenproprathrin, a common pyrethroid insecticide, led to 72.5% mortality even 5 days after the application. The residual effect studies conducted with several insecticide groups and honeybees reported 100% mortality on the 5th day (Ahn et al. 2008).

In conclusion, it was determined in the study that insecticides in various chemical groups were effective on honeybees. The two methods employed in the experiments revealed different effects on honeybees. The topical application method exhibited lower effects when compared to the contact method. It was

determined that the residual effects of certain insecticides were significant in both methods even after 5 days. Thus, it was concluded that the most effective was the neonicotinoid group insecticides on honeybees, followed by other chemical insecticide groups.

Declarations

Acknowledgements

This work was supported by Ondokuz Mayıs University Scientific Research Projects Coordination Unit. Project Number: PYO.ZRT.1904.16.012.

Funding

No funding

Availability of data and materials

All data are available at the end of the article and the materials used in this work are of high quality and grade.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Authors' contributions

RA and IS designed the study, supervised the work, and wrote the manuscript with input from all author. RA and IS carried out the experiments. IS analyzed the data. All authors read and approved the final manuscript.

Competing interests

The author declare that they have no competing interests

References

1. Ahn, K. S., Oh, M. G., Ahn, H. G., Yoon, C., Kim G. H. (2008) Evaluation of toxicity of pesticides against honeybee (*Apis mellifera*) and bumblebee (*Bombus terrestris*). Korean J. Pestic. Sci. 12:382-390
2. Akca, I., Tuncer, C., Guler, A., Saruhan, I. (2009a) Residual toxicity of 8 different insecticides on honey bee (*Apis mellifera* Hymenoptera: Apidae). Journal of Animal and Veterinary advances, 8(3), 436-440

3. Akca, I., Tuncer, C., Guler, A., Saruhan, I. (2009b) Determination of lethal concentration of some insecticides to honey bee *Apis mellifera* (Apidae, Hymenoptera) with laboratory bioassays. *Journal of Animal and Veterinary Advances*. 8(11), 2380-2382
4. Badawy, M. E., Nasr, H. M. and Rabea, E. I. (2015) Toxicity and biochemical changes in the honey bee *Apis mellifera* exposed to four insecticides under laboratory conditions. *Apidologie*, 46(2), 177-193. DOI: 10.1007/s13592-014-0315-0
5. Barron, AB., Maleszka, J., Vander Meer, RK., Robinson, GE., Maleszka, R., (2007) Comparing injection, feeding and topical application methods for treatment of honeybees with octopamine. *Journal of Insect Physiology* (53) 187–194. DOI: 10.1016/j.jinsphys.2006.11.009
6. Belzunces, L. P., Tchamitchian, S., & Brunet, J. L. (2012) Neural effects of insecticides in the honey bee. *Apidologie*, 43(3), 348-370. DOI: 10.1007/s13592-012-0134-0
7. Christen, V., Bachofer, S., Fent, K. (2017) Binary mixtures of neonicotinoids show different transcriptional changes than single neonicotinoids in honeybees (*Apis mellifera*). *Environmental Pollution*, 220, pp.1264-1270. DOI: 10.1016/j.envpol.2016.10.105
8. Christen, V., Joho, Y., Vogel, M., Fent, K. (2019) Transcriptional and physiological effects of the pyrethroid deltamethrin and the organophosphate dimethoate in the brain of honey bees (*Apis mellifera*). *Environmental Pollution*, 244, pp.247-256. DOI: 10.1016/j.envpol.2018.10.030
9. Dai, P. L., Wang, Q., Sun, J. H., Liu, F., Wang, X., Wu, Y. Y., Zhou, T. (2010) Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee *Apis mellifera ligustica*. *Environmental Toxicology and Chemistry: An International Journal*, 29(3), 644-649. DOI: 10.1002/etc.67
10. Danka, R. G., Rinderer, T. E., Hellmich, R. L., Collins, A. M. (1986) Comparative toxicities of four topically applied insecticides to Africanized and European honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 79(1), 18-21
11. Free, J.B. (1993) *Insect Pollination of Crops*. 2nd Enlarged Edition, Academic Press, London, 684
12. Gallai, N., Salles, J. M., Settele, J., & Vaissière, B. E. (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological economics*, 68(3), 810-821. DOI: 10.1016/j.ecolecon.2008.06.014
13. Iwasa, T., Motoyama, N., Ambrose, J. T., & Roe, R. M. (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop protection*, 23(5), 371-378. DOI:10.1016/j.cropro.2003.08.018
14. Kim, B. S., Park, Y. K., Lee, Y. H., Joeng, M. H., You, A. S., Yang, Y. J., Ahn, Y. J. (2008) Honeybee acute and residual toxicity of pesticides registered for strawberry. *The Korean Journal of Pesticide Science*, 12(3), 229-235
15. Laurino, D., Manino, A., Patetta, A., Porporato, M. (2013) Toxicity of neonicotinoid insecticides on different honey bee genotypes. *Bulletin of Insectology*. 66(1), 119-126
16. Laurino, D., Porporato, M., Patetta, A., Manino, A. (2011) Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. *Bulletin of Insectology* 64 (1): 107-113

17. Liao, C. H., He, X. J., Wang, Z. L., Barron, A. B., Zhang, B., Zeng, Z. J., Wu, X. B. (2018) Short-term exposure to lambda-cyhalothrin negatively affects the survival and memory-related characteristics of worker bees *Apis mellifera*. Archives of environmental contamination and toxicology, 75(1), 59-65. DOI: 10.1007/s00244-018-0514-1
18. Meled, M., Trasyvoulou, A., Belzunces, LP. (1998) Seasonal Variations In Susceptibility of *Apis mellifera* to Thesnergistic Action Of Prochloraz And Deltamethrin. Environmental Toxicology and Chemistry, Vol. 17, No. 12, pp. 2517–2520. <https://doi.org/10.1002/etc.5620171220>
19. Muccinelli M. (2008) Prontuario degli agrofarmaci.- Edagricole, Bologna, Italy
20. Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E. (2010) Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345-353. DOI: 10.1016/j.tree.2010.01.007
21. Sanchez-Bayo, F., Goka, K. (2014) Pesticide residues and bees–a risk assessment. PloS one, 9(4), e94482. <https://doi.org/10.1371/journal.pone.0094482>
22. Sharma, D., Abrol, D. P. (2005) Contact toxicity of some insecticides to honeybee *Apis mellifera* (L) and *Apis cerana* (F.). Journal of Asia-Pacific Entomology, 8(1), 113-115. [https://doi.org/10.1016/S1226-8615\(08\)60079-5](https://doi.org/10.1016/S1226-8615(08)60079-5)
23. Silva, PD., Oliveira, FAS., Pedroza, HP., Adelha, ICN., Melo, MM. (2015) Benito Soto-Blanco Pesticide exposure of honeybees (*Apis mellifera*) pollinating melon crops Idalécio. Apidologie 46:703–715. DOI:10.1007/s13592-015-0360-3
24. Soderlund, D.M., Bloomquist, J.R. (1989) Neurotoxic actions of pyrethroidinsecticides. Annu. Rev. Entomol. 34, 77–96. DOI: 10.1146/annurev.en.34.010189.000453
25. Tison, L., Hahn, M. L., Holtz, S., Rößner, A., Greggers, U., Bischoff, G., et al. (2016) Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. Environ. Sci. Technol. 50, 7218–7227: <https://doi.org/10.1021/acs.est.6b02658>
26. Tomizawa, M., Casida, JE. (2005) Neonicotinoid Insecticide Toxicology: Mechanisms Of Selective Action. Annu. Rev. Pharmacol. Toxicol. 45:247–68. DOI:10.1146/ANNUREV.PHARMTOX.45.120403.095930
27. Wang, F., Yang, J. F., Wang, M. Y., Jia, C. Y., Shi, X. X., Hao, G. F. and Yang, G. F. (2020) Graph attention convolutional neural network model for chemical poisoning of honey bees' prediction. Science Bulletin, 65(14), 1184-1191. DOI: 10.1016/j.scib.2020.04.006
28. Ye, Z. M., Dai, W. K., Jin, X. F., Gituru, R. W., Wang, Q. F., & Yang, C. F. (2014) Competition and facilitation among plants for pollination: can pollinator abundance shift the plant–plant interactions?. Plant Ecology, 215(1), 3-13. DOI 10.1007/s11258-013-0274-y
29. Zaworra, M., Koehler, H., Schneider, J., Lagojda, A., Nauen, R. (2018) Pharmacokinetics of three neonicotinoid insecticides upon contact exposure in the western honey bee, *Apis mellifera*. Chemical research in toxicology, 32(1), 35-37. <https://doi.org/10.1021/acs.chemrestox.8b00315>