

Determination of the effect of some pesticides on honey bees

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Abstract

Although the bee deaths that started in 2006 have passed for a long time, no solution has been found and even bee deaths have started to increase again in recent years. The end of winter and spring months are periods when bee deaths are seen intensely. When these periods are examined, it can be seen that many factors (disease-harmfulness, hunger, cold, etc.) cause bee deaths. One of these factors is the pesticides used in springtime in the wintering region. In this study, the effects of pesticides, which are commonly used against factors damaging agricultural crops grown in regions where bee deaths is high, on the body motor movements of the bees are investigated. The most commonly used product used for agricultural combat in pesticides used in our study and the label dose (recommended dose) used for this product was fed twice with the label dose and half by oral gavage, after 1, 4 and 24 hours, the bees were checked and some of the body parts (antenna, leg, abdomen and mouth parts) were rated according to motor movements. As a result of the study, pesticides affecting body motor movements of bees are listed as Chlorpyrifos-Ethyl, Imidacloprid, Deltamethrin, Thiacloprid, Acetamiprid, Abamectin and Tau-fluvalinate active substances from high to low. Spirodiclofen, Glyphosate Potassium Salt, and Penconazole active substance chemicals arranged in the same group with control and did not changed their body motor movements.

Keywords: Pesticide, *Apis mellifera*, Honey bee, Bee mortality, Colony losses

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Introduction

Bees continue to exist under pressure in today's world with many factors. The decrease in the amount and variety of flora, the effects of agro-chemicals, the beekeepers' unnecessary applications, the new parasites peculiar to bees, climate change will further widen the size of this problem in the future (Goulson et al., 2015; Mitchell et al., 2017; Williamson and Wright, 2013). In recent years, the decline in the population of honey bees and other pollinizers has also been a source of concern for food safety (Abbo et al., 2016).

Colony losses are found in Turkey (Karahan and Karaca, 2016) and Europe and America (vanEngelsdorp et al., 2011). Severe colony loss in honey bees, called Colony Collapse Disorder (CCD), is widespread across Europe and North America (Stokstad, 2007). Pesticides used against agricultural pests are at the forefront, as are many of the losses in bee populations (Goulson et al., 2015; Krupke et al., 2012; Paradis et al., 2013). In addition, bee deaths constitute environmental risks (Tosi et al., 2017).

Honey bees are important not only because they produce honey and honey products, but also because they offer pollinating services (Yağın, and Turgut, 2016). In addition to pollinating services (Klatt et al., 2013), it also makes a significant contribution to human nutrition with products such as honey, pollen, wax, propolis and bee milk (Ellis et al., 2015; Gray and Peterson, 2017).

Pesticides are also the most important risk factors for honey (Connolly 2013; Baron et al., 2017) and other insect

pollinators (Glenny et al., 2017; Tihelka et al., 2017; Garibaldi et al., 2013), as well as beneficial organisms such as parasitoid bees in the wild (Aydoğdu and Kanev, 2017). For example, only about 5% of the neonicotinoid active ingredient used is consumed by plants (Sur and Stork 2003) and a large part is dispersed in the environment (Goulson, 2014).

Information on how honey bees are affected by pesticides is based on very old ones. It is stated that the Paris Green used against the apple in the USA caused a considerable amount of bee death in 1870 and Carbaryl application as powder in the cotton fields in 1967 destroyed 15% (70,000 bee colonies) of total colony in California (Yıldırım, 2012).

Honey has a different structure than other insects in feeding the baby, and the care of the baby is made by worker bees (Winston, 1991; He et al., 2016). In accordance with the technique, unincorporated chemicals transport nectar and pollen workers through plants and cause collective larvae and adult bee deaths through contact and feeding (Bonmatin et al., 2015; Zhu et al., 2017; Efsa, 2013; Özbek, 2010; Yıldırım, 2012)

It has been determined that the bees in contact with the pesticide become more susceptible to parasites (Goulson et al., 2015) and the life span of the immune system weakens (Woyciechowski and Moroń, 2009). Pesticides affect the queen's bee mating flight negatively and shorten the life span

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of sperm as a result of insufficient sperm intake of sperm (Williams et al., 2015). In the case of workers; this creates a change in the performance of movement, incoherence, orientation and gathering (Desneux et al., 2007; Gill et al., 2012; Schneider et al., 2012; Williamson and Wright, 2013). The beekeeping products in the colonies which are indirectly exposed to beekeeping products are threatening human health (Böhme, et al., 2017).

Materials and Methods

The main material of this work is the Anatolian bee species (*Apis mellifera anatoliaaca*) which is the most common bee in our country (Sirali, 2017) and pesticides used in regions where bee deaths were intensively observed. The usage and quantities of these pesticides widely used in agricultural production areas are given in Table 1 (Karahan and Karaca, 2016).

Table 1 lists the licensed products and doses used for the pesticides used intensively in the area where the bees died. These pesticides are used intensively, regardless of the license group in regions where agricultural production. It is also thought that the target is better struggled by using over the label dose against the harmful factors. These chemicals are used extensively when the bees are spread (food collection) or during the flowering period.

In the study, the most commonly used product of agricultural use of pesticides is fed the label dose (recommended dose) twice the label dose and half of the label by oral (in 2M sugar syrup) and some of the body parts of the bees (legs, abdomen and mouth parts) were evaluated by looking at the motor movements. After 1, 4 and 24 hours after feeding, the bees were checked and scored. In this point; "1" for each organ, if all the organs are healthy and two "2" points if all antennae, mouthpieces, legs and abdomen are moving slowly and irregularly (Duell, 2012). If all of the

organs of the bees followed according to this scoring system were working, "8", if not, they got "0".

In order to obtain the bees used in the study, 2M of sugar water was placed in a petri dish in front of the hives 10 meters. Worker bees that came to the petri dish were caught randomly through small plastic boxes and brought to the laboratory. Afterwards, these bees were kept frozen for about 3-4 minutes in the freezer of the refrigerator (Hranitz et al., 2010). The immobilized bees were removed from the boxes and attached to the previously prepared injector reservoirs between the head and thorax. After the bees have been soaked, they have been chosen to react with their ankles by touching their antennae with the aid of an ear garbage impregnated with water and syrup (sugary syrup), and the responding healthy bees have been selected for use in the study (Abramson et al., 2004). All of these selected bees were fed with 2 moles of prepared syrup until saturated and kept at normal room temperature for 24 hours (Duell, 2012). At the end of this training, a second selection was made to compensate for mistakes caused by practice, leaving the healthy ones in groups of 5.

The solution containing pesticide in 2M sugar syrup was fed to the bees with 10 µl of micropipette in each experimental group. The control group was not given pesticide, but only 2M sugar syrup was given.

Five trials were established for each pesticide. In each trial 5 bees were used per dose. A total of 1000 bees for 10 different pesticides in Table 1 were used in this study.

In this study, it was determined how body motor movements of bees exposed to pesticides change.

As a result of this process it was revealed that reactions of bees given different dose. In statistical analysis, one-way variance analysis was used with the help of SPSS (ver.17) program and benefitted from Tukey test ($P < 0.05$).

Table 1. Type and amount of pesticides used in the study

	Pesticides* (Active Ingredient)	Registered Crops	Product Type	Rate (Dose)	Formulation Type
1	Chlorpyrifos-Ethyl	Corn	Insecticide	180 ml/100 L water	EC (Emulsion concentrate)
2	Deltamethrin	Corn / Apple	Insecticide	50 ml/100 L water	EC (Emulsion concentrate)
3	Imidacloprid	Apple	Insecticide	20 ml/100 L water	SC (Suspension concentrate)
4	Thiacloprid	Apple	Insecticide	40 ml/100 L water	OD (Oil based suspension concentrate)
5	Acetamiprid	Apple	Insecticide	20 g/100 L water	SP (Water soluble powder)
6	Abamectin	Citrus	Acaricide	25 ml/100 L water	EC (Emulsion concentrate)
7	Tau-Fluvalinate	Apple	Insecticide	30 ml/100 L water	EW (Emulsion, Oil in water)
8	Penconazole	Grape	Fungicide	25 ml/100 L water	EC (Emulsion concentrate)
9	Spirodiclofen	Citrus	Acaricide	20 ml/100 L water	SC (Suspension concentrate)
10	Glyphosate Potassium Salt	Citrus	Herbicide	600 ml/100 L water	SL (Water soluble concentrate)

* Pesticides used in areas where bee deaths occur



Results and Discussion

The doses were consumed to bees as stated in the material method section and the scores and statistical groups were given in Table 2. According to the body motor movements, Table 3 shows the percentage of decrease in the body motor movements of the bees, which are given the label dose depending on the control group. These scores obtained by bees according to their body movements indicate the health status of bees. When bees are released, the bees whose body motion score below 5 cannot fly but the bees who receive more than 5 points can fly. The bees, whose score is above 6, fly comfortably, and the bees, which score between 5 and 6, have difficulty flying.

The average body movement scores obtained after 1, 4 and 24 hours after consumption of the pesticides used in the study are given in Table 2. Among the pesticides used, Chlorpyrifos-Ethyl is the pesticide that affects the body movements of bees the most. The control bees were included in 3 groups and statistically different group in the follow-up period. Among the pesticides used, Imidacloprid, Deltamethrin, Thiacloprid all of the bees were included in the different statistic group according to the control group.

Acetamiprid and Abamectin active pesticides did not affect the bees at the end of 1 and 4 hours but at the end of 24 hours they were statistically in different groups with control bees affecting the body movement scores of the bees. Spirodiclofen, Glyphosate Potassium Salt, Penconazole active substance pesticides and control group were similar to each other and statistically, they were in the same group after 1, 4 and 24 hours. After the recommended dose of Chlorpyrifos-Ethyl was administered, 96.00% of the bees died at the end of the first hour and all at the end of 4 hours (Table 3).

After applying the label dose of pesticide used in Table 3, the average score of bees was calculated and the percentage of decrease in body movements was given depending on the control group. Table 3 shows that there was no change in the body movements of the control group bees in the experiment with Chlorpyrifos-ethyl at the end of 1 hour, while 96.00% decrease in the body movements of the bees fed with Chlorpyrifos-ethyl label dose was observed. After 4 and 24 hours after the application of Chlorpyrifos-ethyl, the body movements of bees have stopped completely (100%) that is, bees have died. The decrease in body motor movements after the application of other pesticides was given in Table 3.

Table 2. Average points given to body motor movements of pesticide applied bees (Average \pm SE)

Pesticides	Doses	1 Hour	4 Hour	24 Hour
Chlorpyrifos-Ethyl	Control (0 ml/100 L)	8.00 \pm 0.00 a	7.88 \pm 0.08 a	7.28 \pm 0.18 a*
	3. Dose (90 ml/100 L)	0.76 \pm 0.11 b	0.00 \pm 0.00 b	0.00 \pm 0.00 b
	2. Dose (180 ml/100 L)**	0.32 \pm 0.04 c	0.00 \pm 0.00 b	0.00 \pm 0.00 b
	1. Dose (360 ml/100 L)	0.00 \pm 0.00 d	0.00 \pm 0.00 b	0.00 \pm 0.00 b
Imidacloprid	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.48 \pm 0.12 a
	3. Dose (10 ml/100 L)	3.68 \pm 0.33 b	0.20 \pm 0.08 b	0.56 \pm 0.24 b
	2. Dose (20 ml/100 L)	1.92 \pm 0.41 c	0.08 \pm 0.10 c	0.00 \pm 0.00 c
	1. Dose (40 ml/100 L)	1.16 \pm 0.26 c	0.00 \pm 0.00 c	0.00 \pm 0.00 c
Deltamethrin	Control (0 ml/100 L)	7.96 \pm 0.04 a	7.92 \pm 0.08 a	7.12 \pm 0.12 a
	3. Dose (25 ml/100 L)	3.84 \pm 0.20 b	1.72 \pm 0.21 b	1.12 \pm 0.16 b
	2. Dose (50 ml/100 L)	2.44 \pm 0.22 c	0.64 \pm 0.19 c	0.12 \pm 0.08 c
	1. Dose (100 ml/100 L)	1.40 \pm 0.14 d	0.00 \pm 0.00 d	0.00 \pm 0.00 c
Thiacloprid	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.48 \pm 0.10 a
	3. Dose (20 ml/100 L)	4.96 \pm 0.24 b	1.20 \pm 0.12 b	1.16 \pm 0.14 b
	2. Dose (40 ml/100 L)	3.20 \pm 0.20 c	0.36 \pm 0.04 c	0.28 \pm 0.08 c
	1. Dose (80 ml/100 L)	2.16 \pm 0.22 d	0.00 \pm 0.00 d	0.00 \pm 0.00 c
Acetamiprid	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.12 \pm 0.23 a
	3. Dose (10 g/100 L)	7.36 \pm 0.07 a	7.04 \pm 0.11 b	4.48 \pm 0.34 b
	2. Dose (20 g/100 L)	6.68 \pm 0.24 b	6.28 \pm 0.28 c	2.92 \pm 0.31 c
	1. Dose (40 g/100 L)	5.04 \pm 0.20 c	4.52 \pm 0.18 d	1.76 \pm 0.14 d
Abamectin	Control (0 ml/100 L)	8.00 \pm 0.00 a	7.96 \pm 0.04 a	7.48 \pm 0.08 a
	3. Dose (12.5 ml/100 L)	7.88 \pm 0.08 a	7.24 \pm 0.20 a	7.36 \pm 0.27 a
	2. Dose (25 ml/100 L)	6.96 \pm 0.16 b	5.08 \pm 0.23 b	5.32 \pm 0.19 b
	1. Dose (50 ml/100 L)	4.56 \pm 0.20 c	2.56 \pm 0.31 c	1.92 \pm 0.16 c
Tau-fluvalinate	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.52 \pm 0.10 a
	3. Dose (15 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.36 \pm 0.26 a
	2. Dose (30 ml/100 L)	8.00 \pm 0.00 a	7.88 \pm 0.08 a	7.44 \pm 0.07 a
	1. Dose (60 ml/100 L)	8.00 \pm 0.00 a	7.48 \pm 0.08 b	6.56 \pm 0.13 b
Penconazole	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.64 \pm 0.17 a
	3. Dose (12.5 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.40 \pm 0.27 a
	2. Dose (25 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.48 \pm 0.17 a
	1. Dose (50 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.48 \pm 0.16 a
Spirodiclofen	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.48 \pm 0.17 a
	3. Dose (10 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.20 \pm 0.26 a
	2. Dose (20 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.32 \pm 0.21 a
	1. Dose (40 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.44 \pm 0.19 a
Glyphosate Potassium Salt	Control (0 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.56 \pm 0.16 a
	3. Dose (300 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.24 \pm 0.24 a
	2. Dose (600 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.44 \pm 0.29 a
	1. Dose (1200 ml/100 L)	8.00 \pm 0.00 a	8.00 \pm 0.00 a	7.60 \pm 0.07 a

* Within same columns, the values (\pm standard error) means sharing a letter are not significantly different from each other (Tukey's at $P < 0.05$).

** 2. Dose is label dose of all pesticides used (recommended dose).

**Table 3.** Decrease rate of body movements of label-administered bees (%)

Decrease in body motor movements (%)				
Doses	1 Hour	4 Hour	24 Hour	
1 Chlorpyrifos-Ethyl	96.00	100.00	100.00	
2 Imidacloprid	76.00	99.00	100.00	
3 Deltamethrin	69.50	92.00	98.50	
4 Thiocloprid	60.00	95.50	96.50	
5 Acetamiprid	16.50	21.50	63.50	
6 Abamectin	13.00	36.50	33.50	
7 Tau-fluvalinate	0.00	1.50	7.00	
8 Spirodiclofen	0.00	0.00	8.50	
9 Glyphosate Potassium Salt	0.00	0.00	7.00	
10 Penconazole	0.00	0.00	6.50	

Conclusion

As seen in the findings of the work, some pesticides have restricted the body motor movements of bees or caused the death of bees. Some of the pesticides used did not affect the body movements of the bees. These pesticides may not have affected the body function of the bees, but may have affected some sense organs (such as direction, vision, hearing, smell, taste). This can end their lives because the bees lose their properties such as smell, taste, direction or sight, cannot find their husk, or mix their husk. The most important result of this study is that some pesticides do not directly kill the bees and they are transported to the hives by worker bees and they pass on products like honey, pollen. These pesticide-depleted foods are consumed by human beings as well as consumed by other bees in the hive, creating a major problem for human health.

The bees exposed to different pesticide mixtures in the field conditions are lost on the field and between the field and the colony (Zhu et al., 2017). Therefore, as the strength of the colon weakens, the movements of the bees are diminished and their duties are delayed, the abdomen swells, the wings and legs become paralyzed and die (Yıldırım, 2012). The study showed similarity with the studies of' Williamson et al. 2014; Oliver et al.,2015; Karahan et al., 2015; Zhu et al., 2017; Bovi et al., 2018 ', and it was observed that some pesticides used were found to be extremely harmful in honey bees and cause paralysis and deaths afterwards.

In order to reduce pesticide damages and protect bees, the spraying should not be done during the flowering periods, and if the pesticide has to be expelled, it should be applied in the evening hours when there are no bees. In addition, the beekeeping site should be located far away from areas where intensive spraying is made, if it cannot be transported during the application, it should be kept closed and covered in order to protect it from pesticides (Yıldırım, 2012).

As a result, the awareness of the harm that all pesticides have on the bees and the environment has not yet been understood. The substances used to make benefits can damage the environment and cause the bees to die in climate changes. New works should be done to protect the lives of the bees and the surrounding area. Protection of bees and the environment is more important and necessary for people than anything else.

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