

Original Research

The Impact of Selected Pesticides on Honey Bees

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Abstract

The presence of honey bee in the environment has a significant impact on global agricultural production. While searching for pollen and nectar, the bee is often exposed to several contaminants such as plant protection products. The aim of this study was to assess the impact of selected pesticides from various groups (fungicides, herbicides, insecticides) on the way the bees harvested food as well as on their behavior, survival rate and the concentration of certain elements in the organisms of bee. The concentration possibly similar to this applied in agriculture and recommended by the manufacturers, were used. The research material was analyzed in terms of the presence of trace elements (manganese, copper, iron, nickel, zinc, lead, cadmium) of different toxicity. The study clearly indicates that selected pesticides used for the purpose of plant protection and plant cultivation may adversely affect bees' behavior and accumulation of trace elements in their body. Among the tested pesticides particular attention should be paid to fungicides that are not commonly tested for their effects on the honey organ. In own studies showed, their effect significantly changed the content of copper and negatively affected the survival of the bees.

Keywords: behavior, trace elements, pesticides, honey bee

Introduction

Intense development of agriculture and animal production has caused exposure to substances with which bees have never before come into contact. The increasing demand for food has forced farmers to use more mineral fertilizers and pesticides to generate higher yields [1]. The

residues of these substances in the form of contaminants are then transferred into grains, vegetables, and fruit [2]. They have also been discovered in herbs such as mint (*Mentha*) or lemon balm (*Melissa officinalis*) [3]. While working on flowers, bees are exposed to direct and indirect contact with pesticides which, depending on the mode of action and the concentration of active substance, can lead to sudden death of pollinating insects or cause death within a couple of hours following exposure [4]. It becomes dangerous when the level of pesticides or their residues in a beehive becomes

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high enough to adversely affect the functioning and development of larvae [5]. This causes the bees to become more susceptible to bacterial infections. This is particularly risky in the case of American foulbrood caused by *Paenibacillus* larvae.

Weakened bees and a disorganized colony become more susceptible to this disease [6]. They also suffer more frequently from nosemosis, also known as bee dysentery, or varroosis [7]. Moreover, the microflora of bee intestines is affected [8]. Some pesticides trigger disorders of the digestive and endocrine systems, leading to distention of the abdomen, as a result of which the bees become slow and apathetic, showing difficulties with active flying [9]. Bees are highly susceptible to environmental changes and pollution, which is strongly reflected in the significant decrease of their survival rate [10].

The search for reasons for this phenomenon has established a new disease entity called colony collapse disorder (CCD). Several studies have indicated the important role of pesticides in the appearance and development of numerous pathological disorders in bee organisms [11]. Although it is still hard to find one cause of CCD, the significant impact of pesticides on its development cannot be questioned. Animals and their produce may also be used in the process of evaluating the level of environmental pollution. Honey bees (*A. mellifera*) serve as a bioindicator of contamination with trace elements of toxic properties (copper, zinc, iron, tin cadmium, lead, arsenic, and aluminum) [12]. Bees constantly penetrate the environment seeking new produce and the raw materials necessary to provide for the functioning of their colony. Even though the aforementioned elements may appear in pesticides commonly used in agriculture, it is impossible to determine unequivocally whether their presence in bees and bee products originates in the environment or results from the contact between bees and pesticides.

Selected manganese (Mn) negatively affects behavior and leads to disturbances in chitin synthesis, while copper (Cu) is a natural component of the hemolymph of bees [13-14]. Lead (Pb) and cadmium (Cd) are very toxic for animals and the environment [15]. Iron (Fe), nickel (Ni), and zinc (Zn) contaminants in the honey bee body can occur because of botanical origin, as well as anthropogenic factors around the colonies [16]. The scientific literature indicates that Pb, Cd, Cu, Mn, Ni, Zn, and Fe are the most important heavy metals, and studies show a tendency for their accumulation in living organisms [17-18]. Pesticides that contain metals as active substances influence the content of these elements in the organism of honey bees.

The aim of this study was to assess the impact of selected pesticides from various groups (fungicides, herbicides, insecticides) on bee behavior, survival rate, and the concentration of selected trace elements in their organisms.

Material and Methods

Laboratory tests were carried out from June to mid-August. The studied material consisted of honey bee workers (*A. mellifera*) of the Carniola race obtained from one bee colony to each repeat [19]. Ten-day-old worker bees were distributed into seven dietary treatments. They were transported to the laboratory where the container was left for 30 min (at about 26°C). The bees were then rendered unconscious by exposure to ammonium nitrate. After about 30 seconds, the insects became unconscious for about 2-3 min, when they were moved to experimental cages at 160 individuals per cage. The cages were supplied with lids, each with two holes with 5 cm³ food dispensers containing test or control solutions. Bees were placed in identical cages of wood and glass with dimensions of 50 × 150 × 150 mm. Inside each cage were placed frames with wax foundations (size approximately 120 × 120 mm) [20]. The cages were put in an incubator where constant temperature and humidity (the first 24 hours of experience T 35°C, subsequently 27°C, H 75%) were maintained [21]. Throughout the study, bees were fed with 2 mol/dm³ sugar syrup. The first 24 h were dedicated to adjusting to the new environmental conditions, feeding, and then – after removal of dead individuals – starting the actual experiment.

Each dietary treatment was divided into six replicates. Pesticides were selected in the manner allowing for double representation of each group (two kinds of insecticides, two herbicides, and two fungicides). The concentration of each pesticide was in accordance with the recommendation of the manufacturer. Each dose of pesticide was dissolved in 100 cm³ of sugar syrup (2 mol/dm³). Experimental groups were:

- Group K: control, pesticide-free sugar syrup.
- Group A: Miedzian 50WP (fungicide 1) with active substance of copper oxychloride- authorized for trade and distribution until 2019, dose 0.35 g/100 cm³ of syrup.
- Group B: thiram granuflo 80WG (fungicide 2) main active ingredient thiram, authorized for use until 2020, dose 0.60 g/100 cm³ of syrup.
- Group C: Fastac 100EC (insecticide) active substance α-cypermetrin, authorized for distribution until 2020, dose 0.04 cm³/100 cm³ of syrup.
- Group D: Actara 25WG (insecticide 2) active substance thiamethoxam, authorized for trade until 2021, dose 0.04 g/100 cm³ of syrup.
- Group E: Basagran 480SL (herbicide 1) active substance bentazone, authorized for distribution until 2020, dose 1.2 cm³/100 cm³ of syrup.
- Group F: Metafol 700SC (herbicide 2) active substance metamitron, authorized for production until 2016, dose 0.60 cm³/100 cm³ of syrup.

Every day the solutions in food dispensers were replaced with new ones in order to minimize the risk of chemical changes in the tested substances. Dead individuals were collected and stored in a freezer (-20°C). After completion of the experiment the samples

were defrosted and triturated using a ceramic crucible. Afterward the samples were transferred quantitatively to a petri dish and placed in a dryer laboratory for 8 h at 45°C. In order to obtain precisely dried and homogenized samples, dried material was triturated and placed on RADWAG WPX 50S moisture balances, where it was dried to a constant weight at the same temperature as in the incubator. Each sample was weighed at 1 g (to the nearest 0.10 mg) and samples of biological material were weighed in Teflon dishes using a RADWAG WAS 220/X analytical balance. The samples were covered with 5 cm³ of spectrally pure 69% nitric acid (TRACEPUR EMD Millipore Corporation). These sample preparations were mineralized in ANTON PAAR MULTIWAVE 3000 microwave digestion for 30 minutes. The whole process lasted two hours.

After mineralization the obtained mineralisates were centrifuged and the content was moved into new Falcon tubes. The resulting mineralisates were analyzed quantitatively using flame atomic absorption spectrophotometry (AAS). Elements to which bees might be exposed in the natural environment and elements that appeared in the chemical structure of active substances of the pesticides were indicated. The indicated trace elements included Cu, Mn, Fe, Ni, Cd, Pb, and Zn. Moreover, behavioral observation was carried out during the study. The results obtained in experimental groups were related to a control group. The following behavioral factors were taken into account: aggression, walking, grooming, reversal, immobility, and attempts to reconstruct comb foundations. If no irregularities were noticed during observation it was marked with '-' in the table, whereas any behavioral changes or dysfunctions were marked with a '+' (small), '++' (medium), or '+++' (big). An assessment was made based on frequency of given behavior. Observations were carried out in 15-min periods for each group by two observers. The results obtained were analyzed statistically using Statistica for Windows v. 10.0. The mean, standard deviation, and relevance of differences between given groups were established. Statistical analysis was made based on Duncan test ($P \leq 0.05$).

Results

The highest intake of fodder was observed in Control Group K: 250.10 mm³ per one bee in 24 hours. This group presented the lowest daily mortality of bees at the average level of 1.92 individuals, which represents 1.20% of all bees in a given cage. The highest daily mortality – 68.91% – was noticed in Group D, where the insecticide Actara 25WG was supplied. Daily fodder intake in this group was the lowest at 0.15 mm³ per individual (Table 1). In Group B, fed with the addition of the fungicide Thiram Granuflo 80WG, and in Group E, fed with the herbicide Basagran 480SL, similar syrup intakes of 16.78 and 16.16 mm³ per insect were observed. Daily mortality rate in these groups (3.23% and 2.00%, respectively)

Table 1. Daily intake of syrup and the mortality rate

Group	Syrup intake [mm ³] /bee/24h	Mortality rate bees/24h	Time of experiment [h]
K	250.10*	1.92*	168
A	4.75	35.15	108
B	16.78	5.17	168
C	2.25	32.48	120
D	0.15**/**	110.25**/**	36
E	16.16 **	3.21 **	168
F	13.87	13.00	168

* differences between groups assessed highly significant on a level of $P \leq 0.05$.

** differences between groups assessed highly significant on a level of $P \leq 0.01$.

showed another resemblance (Table 1). In Group F the volume of syrup intake was slightly lower (13.87 mm³), whereas the mortality rate was three times as big and amounted to 13 individuals, representing 8.13% of all bees in this cage. Very low syrup income was observed in Groups A (fungicide Miedzian 50W) and C (insecticide Fastac 100EC) on the level of 4.75 and 2.25 mm³/bee, respectively. Remarkably, both groups showed very similar mortality rates.

The fewest behavioral changes were observed in the Control Group (Table 2). The bees were calm and did not show increased aggression during human interference in the cage. They made an effort to reconstruct the comb foundation, which is fairly natural. Generally, no behavioral irregularities were observed in relation to usual course of behavior in the colony from which the bees originated. It is therefore relevant to use this group as reference material for other test groups. The most significant changes were observed in the behavior of bees in Group D, fed with the additive of insecticide Actara 25WG. Their behavior was typical for bees exposed to neonicotinoid pesticide. The second insecticide (Fastac 100EC) provided in Group C did not affect the insects' behavior.

Table 2. Evaluation of honey bee behavior

Behavior	Group						
	K	A	B	C	D	E	F
Aggression	-	++	+	-	+++	+++	++
Walking	-	++	++	-	+++	+++	+
Grooming	+	+	+	+	+++	+	+++
Reversal	-	+	-	+	++	-	+
Still	-	-	-	-	++	-	+
Rebuild of comb	++	-	- ¹	-	-	+	+

¹bees cut the comb foundation

Table 3. The concentration of trace elements

Group	The average contents ($\pm SD$) of chemical elements [$\text{mg}\cdot\text{kg}^{-1}$]						
	Manganese	Copper	Iron	Nickel	Zinc	Lead	Cadmium
K	58.80 (± 2.21) ^{ab}	27.70 (± 1.65) ^b	201.94 (± 7.53) ^b	0.68 (± 0.15) ^c	118.21 (± 4.42) ^a	4.59 (± 0.72)	0.2 (± 0.08)
A	49.68 (± 5.39) ^{cd}	2102.90 (± 203.13) ^a	276.19 (± 29.62) ^a	3.18 (± 0.97) ^a	119.31 (± 1.63) ^a	4.78 (± 0.43)	0.15 (± 0.08)
B	47.71 (± 2.45) ^d	32.24 (± 3.13)	188.03 (± 8.12) ^{bc}	2.10 (± 0.09) ^b	114.62 (± 2.45) ^{bc}	5.36 (± 0.32)	0.16 (± 0.10)
C	51.25 (± 2.33) ^{cd}	30.44 (± 0.43)	171.26 (± 2.68) ^c	1.66 (± 1.17) ^c	119.71 (± 1.34) ^a	4.55 (± 0.31)	0.16 (± 0.08)
D	60.17 (± 1.35) ^a	28.49 (± 0.40)	205.48 (± 14.39) ^b	3.33 (± 1.09) ^a	117.03 (± 1.25) ^{ab}	5.36 (± 0.58)	0.22 (± 0.07)
E	46.73 (± 0.4) ^d	26.28 (± 0.73)	173.24 (± 16.96) ^c	1.58 (± 0.35) ^{bc}	112.26 (± 1.09) ^c	4.83 (± 1.2)	0.20 (± 0.15)
F	54.30 (± 7.3) ^c	28.31 (± 0.49)	206.07 (± 15.18) ^b	2.44 (± 0.94) ^{ab}	118.52 (± 1.823) ^a	5.05 (± 0.52)	0.18 (± 0.06)

a, b, c, d - differences between the elements assessed highly significant on a level of $P \leq 0.05$.

All behavioral factors were similar to the Control Group and reflected the low toxicity of this substance to bees. Although general behavioral changes were similar in Groups A, E, and F, different levels of intensity were observed. Increased aggression and mobility was noticed among bees in Group E, whereas in the other two groups they were on a low or middle level. The least significant changes were observed in Group B, fed with the addition of fungicide, where the behavior was similar to Control Group. Trace elements determined in their organisms are presented in Table 3. The largest fluctuation in concentration among all tested elements was observed in the concentration of copper – particularly in the case of Miedzian 50WP, as in that additive copper was one of the components of active substance (CuOCl).

There is a clear connection between the presence of copper in provided fungicide and the increased level of copper in the organisms of bees. Said product caused mortality among bees at the rate of 17.47% of overall daily mortality, which means that a significant number of bees fed Miedzian 50WP would have been able to return to the hive. No statistically significant differences in the presence of copper were observed in other groups in reference to Control (Table 3). Cd appeared to be the least estimating element, as its amount was not detected at a representative level and in the sample containing Basagran 480SL the amount of cadmium was below the limit of quantification. Mn, another analyzed element, exhibited more variability than Cu. The level of Mn in bees from groups A, B, C, and E showed significant differences in reference to other groups. Bees in Groups A and B were fed syrup with the addition of fungicide, bees from Group C were fed insecticide Fastac 100 EC, and bees from Group E were fed herbicide Basagran 480 SL. The level of Mn in given sample material was getting lower in comparison to Control, except for the bees fed the addition of Atacara 25 WG, where the level of said element increased by 2.33%.

The concentration of Fe and Cu in tested bees presented the highest standard deviation among analyzed elements. The presence of iron in bees fed with the addition of various pesticides presented significant fluctuations. The

impact of Miedzian 50 WP (Group A) stood out among all tested products. The concentration of iron in bees from the above-mentioned group increased more than in any other group and in reference to Control it increased by 36.77%. Whereas in the case of bees from Groups C and E, fed with the addition of Fastac 100 EC and Basagran 480 SL, a significant decrease of iron concentration was observed. In all sample groups, where the bees were provided with the addition of pesticides, the amount of Ni was bigger than in Control. The highest level of said element was observed in group D, where it was greater by 2.65 $\text{mg}\cdot\text{kg}^{-1}$ d.m in comparison to Control. A similar result was obtained in Group A, where the difference reached 2.5 $\text{mg}\cdot\text{kg}^{-1}$ d.m. These results are statistically significant and have an impact on the research outcome.

Indicated concentrations of Zn did not show any significant dispersion of values in reference to mean value. Considerable fluctuation of its content in sample material was marked between the group fed sugar syrup with addition of Actara 25 WG and the one fed Thiram Granuflo 80 WG fungicide and the group supplied with Basagran 480 SL (Table 3). The variation of Zn in bees from subsequent groups was distributed irregularly. Therefore, it may be stated that no set tendency in variation among certain researched groups exists.

Another two elements, i.e., Cd and Pb, did not represent statistically significant fluctuations within tested groups. Supplied products did not affect the distribution of said elements in the organisms of experimental bees. It may therefore be presumed that the contents of Cd and Pb in sample material indicates their presence in the habitat of the bees. It should therefore be explicitly stated that the fungicide Miedzian WP 50, which led to average levels of mortality, had the most significant impact on the content of tested elements in sample material.

Discussion of Results

An assessment of pollution of the environment has long been one of the world's priority issues. Sources of contamination and the explanation of its origins are

constantly sought after in order to foresee its long-term effects. Various methods and simulations are applied for said purposes. It is very common to use plants and animals in the process of bio-indication. Honey bees also play an important role here, as they have the ability to indicate climate change or the degree of environmental pollution [22]. There are not only field studies with bees taken directly from the environment, but also laboratory research where external factors that adversely affect the insects are easier to reproduce. In the latter, it is common to use dispenser cages in order to control food intake and mortality.

It has been confirmed that the best results are obtained when applying 20 ml syringes as food dispensers [23]. In the current research, however, 5 ml syringes were used in order to avoid overproduction of pesticide waste. Similar dispensers were used by Ptaszynska et al. [24] in research on the impact of ethanol on nosema spore-formers, and Pareja et al. [25] in research on the impact of pesticides on bees. Among tested pesticides the highest mortality was observed in the group fed with Actara 25 WG, which caused 100% mortality. Similar results were obtained by Roman et al. [26] during research on the toxicity of various substances against bees.

The same observations were obtained by Laurino et al. [27], who researched the impact of neonicotinoid pesticides on bees of a different genotype. Our own studies show the impact of fungicides on the mortality rate, but in scientific literature these pesticides are without such an effect. Mayer and Lunden [28] tested the fungicides triforine, triflumizole, and DuPont 6573, and the acaricide hexythiazox for honey bee toxicity and their effects on bee foraging. There was no increased mortality in bees in contact with these pesticides. Similar results have been obtained for Ladurner et al. [29] of the impact of fungicides on bees (*Osmia lignaria*). They noticed reduced female activity and increased mortality.

The study enabled recognition of changes in the behavior of tested insects. It was particularly noticeable in Group D, where bees were fed syrup supplemented with one of the neonicotinoids. Similar behavioral disorder caused by exposure to pesticides has been presented by Thompson [30], whereas Barbieri et al. [31] confirm the influence of neonicotinoids on insects from different systematic groups such as ants. Brandt et al. [32] indicated the severe impact of several pesticides on the immune system of bees. The present study showed the significant impact of pesticides on content of metals in bee organisms. This was particularly high in the case of Cu in Group A, which clearly resulted from the presence of this element in the provided pesticide. Similarly, an increase of Fe content was observed, and Ni, during his research on seasonal variations of four trace elements (Cd, Pb, Cu, and Se) also pointed to Cu, whose concentration in bees was the highest even though its level amounted to 22.6 mg·kg⁻¹ d.m., barely exceeding the level obtained Group K during the present study [33]. This puzzling trend has been observed in the accumulation of Cd.

In bees from all groups, except for Group D, the Cd content was lower than in Control (0.200 mg·kg⁻¹ d.m.). However, these differences were statistically insignificant. The lower accumulation of Cd to be obtained in conventional studies is observed in the case of beeswax (0.01-0.1 mg/kg), and in the case of propolis (0.006-3.8 mg/kg) and pollen (0.05-2.3 mg / kg). This suggests a certain accumulation of this in the bee body [34]. Hladum et al. [35] indicated that worker bees accumulated all tested elements (Cd, Cu, Pb, and Se) in amounts greater than those accumulated by the queen (very little amounts), whereas the concentrations of said elements were highest in the bodies of deceased worker bees.

Conclusions

The deterioration of health and general condition of honey bee colonies may be caused by pesticides commonly used in agriculture. This study clearly indicates that selected pesticides used for the purpose of plant protection and plant cultivation may adversely affect bee behavior and the accumulation of trace elements in their bodies. Among the tested pesticides, particular attention should be paid to fungicides that are not commonly tested for their effects on the honey organ. Our own studies showed that their effect significantly changed the content of Cu and negatively affected the survival of bees. The negative impact of insecticides on bees and their behavior have been confirmed. The herbicides and insecticides had their greatest impact on behavior. All selected pesticides changed natural behavior, mortality rates, and accumulation compared to Control.

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