The influence of acetamiprid and deltamethrin on the mortality and behaviour of honeybees (*Apis mellifera carnica* Pollman) in oilseed rape cultivations

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Abstract – The aim of the research was to determine the influence of acetamiprid and deltamethrin on the mortality and behaviour of honeybees of the same variety, coming from the same source and estimated to be in good condition by a professional beekeeper, but in different years and under different weather conditions. Results of research conducted in field isolators in oilseed rape fields in the years 2012–2018 showed no acetamiprid influence either on the mortality or on the behaviour of honeybees. Deltamethrin activity differed from a lack of influence through causing mortality and a weaker condition of honeybee colonies to the destruction of the colony. This demonstrates the relative safety of acetamiprid to honeybees and the high risk of deltamethrin use. The differences in the bee colonies’ reaction to deltamethrin are probably caused by the high acute toxicity of this active ingredient, genetically dependent, variable metabolic capabilities of particular bee colonies, or coexistence of a variety of different stressors—mainly pathogens.

*Apis mellifera* / Acetamiprid / Deltamethrin / Mortality / Behaviour

1. INTRODUCTION

During recent years, many European countries have conducted broad research on the wellbeing, safety and health of honeybees (*Apis mellifera*). It results from an apparent decrease in the pollinators’ population density in many ecosystems and from the lack of a reasonable explanation of the aetiology of the colony collapse disorder (CCD) (Vanengelsdorp et al. 2009). Since 2006, CCD has been observed among honeybees in many places all over the world (EFSA 2013). So far, the cause of this phenomenon has not been identified. Scientists have conducted broad-range studies which indicated a large number of factors (both biotic and abiotic, mainly physicochemical ones) influencing the condition, development and survival rate of bee colonies (Johnson 2015, Neov et al. 2019, Goulson et al. 2015). The Food and Agriculture Organization reports that there were increased amounts of pathogens and parasites found in bees with colony collapse disorder (FAO (Food Agriculture Organization), 2013). The information is supported with scientific research (Cox-Foster et al. 2007). Apart from biotic factors, the widespread use of chemical crop protection products, chiefly insecticides, is most often considered to be the risk factor influencing...
the health of honeybees. Numerous scientific studies show that the physiology, behaviour, immunology, health and survival rate of insects are affected even by minimal contact with insecticides exhibiting different mechanisms of action (Gliński et al. 2011, Johnson 2015, Magesh et al. 2017).

The CCD phenomenon has been widely discussed by scientists all over the world as well as within the European Commission. Many factors are suspected of being the cause of the phenomenon pathogens, weak genetic condition caused by inappropriate breeding, environmental pollution and many others (McMenamin et al., 2016, Neov et al. 2019, Goulson et al. 2015). Still, in public opinion as well as in many scientific environments, chemical plant protection products are indicated as one of the main factors responsible for CCD. The European Food Safety Authority (2013) proposed a “Guidance Document on Risk Assessment of Plant Protection Products on Bees”. Unfortunately, uniform scientific methods have not been accepted yet and so research in different countries is still carried out with the use of different methods (EFSA 2013).

Some of the research carried out so far highlights neonicotinoids, which bind to nicotinic acetylcholine receptors (Tomizawa and Casida 2005), as one of the main causes of CCD. The report suggests that these insecticides disrupt the honeybee nervous system compromising their ability to function normally, even in doses which do not confer mortality (Berenbaum and Johnson 2015; Karahan et al. 2015; Hopwood et al. 2016). Such doses can influence cognitive abilities, navigation, feeding activity and reproduction processes (Belzunces et al. 2012).

Thiamethoxam, clothianidin and imidacloprid are the most dangerous neonicotinoids for bees. Acetamiprid and thiacloprid, which are widely used for plant protection, are recognized to be relatively safe for honeybees at recommended doses (European Commision: https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32018R0113, Regulation (EU) No 485/2013). However, in the opinion of many beekeepers, these as well as many pyrethroids can cause honeybee mortality as a result of acute toxicity or a disturbance in honeybee function and behaviour after the permeation of toxins at sublethal doses.

In view of this, a team of scientists carried out research to extend the current knowledge of risks to honeybee colonies caused by the effect of a pyrethroid (deltamethrin) and a neonicotinoid (acetamiprid) insecticide. These chemical groups were chosen for analysis because they are most frequently used in the crop protection products applied on crops pollinated by honeybees (Ministry of Agriculture and Rural Development: https://www.gov.pl/web/rolnictwo/rejestrodkow-ochrony-roslin). Also, residues of insecticides from the pyrethroid chemical group are most often found in honeybee organisms (Johnson et al. 2010; Mullin et al. 2010; Ch et al., 2014). Both deltamethrin and acetamiprid are recommended for pest protection of rapeseed plants during flowering (Plant Protections Recommendations–Institute of Plant Protection-National Research Institute). They are also recommended for the protection of other crops and orchard plants which bees pollinate (Plant Protections Recommendations–Institute of Plant Protection-National Research Institute). The spraying of pyrethroid compounds in rapeseed protection increased considerably after the withdrawal of neonicotinoid seed dressers (Kathage et al., 2019). The data from the Footprint PPDB, which were prepared at the University of Hertfordshire, also show that deltamethrin is intrinsically a more toxic compound than chlorpyrifos (Sanchez-Bayo and Goka 2014).

The presented research was carried out in semi-field conditions and with the use of normally functioning honeybee colonies, such that observation of short- and long-term effects of insecticide application was possible. The experiments were performed excluding the identification of other possible factors that may influence honeybee mortality or behaviour, because their main aim was to determine the influence of the two widely used insecticides on the mortality and behaviour of honeybee colonies of the same variety, coming from the same source and estimated to be in good condition by a professional beekeeper, but in different years and
weather conditions. Acute poisoning of honeybees is usually reported within 24 h following insecticidal treatment. The experiments were carried out in different years. If the weather conditions in different years were similar during the first 4 days following the treatment, the results of the experiments were treated as repetitions.

2. MATERIAL AND METHODS

The research was carried out according to the modified EPPO method PP 1/170 (4) (https://sitem.herts.ac.uk/aeru/ppdb/en/), first described with modifications by Moores et al. (2012). Research was carried out in the years 2012–2018 at Winna Góra (Wielkopolska region, Poland).

2.1. *Apis mellifera* carnica Pollman

Small colonies of honeybees (*Apis mellifera carnica*) were prepared by a professional beekeeper. Each colony (hive) consisted of five frames of brood and stores and contained approximately 500 bees, including a healthy egg-laying queen and workers. The honeybees had not been treated with any medicinal treatments for at least 4 weeks prior to the experiment. A treatment with Apiwarol® (amitraz) was applied, according to the manufacturer’s recommendations, in August and in the autumn, 7–8 months before the commencement of the experiment to combat any potential effects of varroa mites.

2.2. Chemicals

Two active substances contained in the two commercially available products were used for the experiments. The sprayings were carried out according to the field-recommended application rates. The concentrations of the active substances were calculated assuming that 200 l of water is used per hectare:

Pyrethroids:

- *deltamethrin* (Decis Mega 50 EW with 4.8% of active ingredient): recommended application rate for rapeseed protection in Poland: 0.15 l/ha; concentration of the active substance used in the experiments: 36 ppm,

Neonicotinoids:

- *acetamiprid* (Mospilan 20 SP with 20% of active ingredient): recommended application rate for rapeseed protection in Poland: 0.12 kg/ha; concentration of the active substance used in the experiments: 120 ppm.

The lack of repellent activity of both tested active substances to honeybees was previously reported (Węgorek et al. 2015).

2.3. Field bioassays

All the experiments were conducted at the most appropriate time regarding weather conditions. The weather forecasts were analysed carefully each time to ensure at least 4 days without rain after the treatment and appropriate temperatures (18–30 °C) for the bees to fly outside the hives—also at least for 4 days after the treatment. The aim was to test the insecticidal activity in different temperatures, characteristic for the time of oilseed rape flowering stages. Four days constitute the period for which the weather forecasts are relatively verifiable and the potential effects of negative insecticide influence can easily be observed. This was proved during previous pre-experiments.

Each year, the experiments were carried out in oilseed rape cultivations (variety Poznaniak, non-hybrid oilseed rape) between the BBCH (phenological development stages of plants—Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) stages 64 and 67—for 9 days. Sown seed was pretreated with fungicide seed dressing T 75 DS/WS containing 750 g of thiuram, with no further insecticide or fungicide application after sowing. Three large isolator cages, built of wooden frames covered with mesh, each 1.5 × 5 × 1.8 m, were placed within the rape field before the start of spring vegetation. The size of the isolators was
adequate for the experimental conditions (the observation period time, the number of bees in each colony—500, the number of flowering plants necessary for the working colony) as discussed and approved by a professional beekeeper. One isolator constituted a control version, whilst plants in the other ones were treated with the recommended doses of deltamethrin or acetamiprid.

In each isolator, 1 m² was cleared of plants in order to site a small hive. Each hive was placed on a wooden construction approximately 80 cm high. Hives were placed in isolators 1 day prior to spraying for colony acclimatization. Spraying was carried out in the evening, after bee flight. The hives were removed from the isolators just before spraying and placed at about 10–15 m from the isolators to secure the bees from the possibility of having contact with the insecticides. The hives were placed back inside the isolators after spraying. For each of the following 9 days, assessments were made on the health and behaviour of the bees. Elements of behaviour indicating good or ill health were observed: normal, quick or slow movements inside the hive, gathering nectar and pollen or resting outside the hive, etc. Numbers of bees outside a hive in relation to the starting population. Dead bees were counted in defined areas: the soil surface was searched around the interior edges of each cage as well as the area cleared of plants around the hives. Dead bees found on the wooden construction on which the hive was placed were also counted. Dead bees were removed each morning. Numbers of dead bees in the tables (see results section) are always the sum of dead bees found outside the hives from the beginning of the experiment. The behaviour of bees at the entrance of the hive was also observed: flying inside or outside the hive (or lack thereof), creating groups at the entrance. These observations were conducted at midday on each day of assessment.

When the oilseed rape flowers started fading and the bees remained on the mesh, the hives were removed from the isolators and transferred to a professional beekeeper, who conducted further observations for the next 2 months. After this time, the beekeeper assessed the final condition of the bee colonies, percentage of live bees inside the hive and the presence of brood and a queen. The percentage of live bees was always assessed considering the natural mortality and development of bee colonies (new bees from eggs laid by the queen), and the initial assessment before the experiments.

3. RESULTS

In some cases, the percentage of bees inside and outside the hive, in spite of a small number of dead bees found outside the hive, was not 100%. This stemmed from the fact that it was impossible to find all dead bees among rape plants inside the isolators. It was taken into account by a beekeeper in the end of the experiment.

Results presented in Table I show that the percentage of bees inside and outside the hives did not always constitute 100%. It results from the fact that dead bees are either thrown away from the hives by other bees or do not come back to the hives and fall down among plants within the isolator. In the last case, they cannot always be found and collected.

In 2012 (Table I a; 2), there were ideal weather conditions for honeybees to collect nectar and pollen during the first 4 days after the insecticide treatment (19–20 °C, sunny). On the 5th day, cold temperatures and rain prevented the bees from leaving the hives. On the 7th day, the weather was favourable again. Towards the end of the experiment, on the 9th day, the unfavourable weather and reduction of flowers induced the insects to stay in the hives or rest on the mesh. During the experiments, no differences in honeybee mortality or behaviour were observed among the treatment groups (control, acetamiprid, deltamethrin). The final assessment made by the beekeeper revealed a 20% reduction in the percentage of live bees and slightly weakened condition of the colony in the deltamethrin treatments. The number of bees and the colony condition were comparable in the control and acetamiprid groups.

In 2013, the weather conditions were favourable until the 8th day after the treatment. No signs of poisoning were observed in acetamiprids. In the deltamethrin-treated group, although higher temperatures were present compared with the previous year, which usually weakens pyrethroid activity, on the 7th day after
Table 1. Observations of honeybee mortality and behaviour in the experimental conditions in the years 2012–2018

| Number of days before treatments | Number of days after treatments |
|----------------------------------|--------------------------------|
| Weather                          | 19°C, partial cloudiness       |
| Active substance                 | D                              |
| Mortality [number of individuals]| 16                             |
| Behaviour at the hive entrance   | flying into and outside         |
| Percent of bees inside the hive  | normal                         |
| Behaviour of bees outside the hive| collecting nectar and pollen    |
| Percent of bees outside the hive | 15                             |
| Behaviour of bees outside the hive| absence                         |

| Number of days after treatments |
|----------------------------------|
| Weather                          | 20°C, sunny                    |
| Active substance                 | D                              |
| Mortality [number of individuals]| 16                             |
| Behaviour at the hive entrance   | flying into and outside         |
| Percent of bees inside the hive  | normal                         |
| Behaviour of bees outside the hive| collecting nectar and pollen    |
| Percent of bees outside the hive | 15                             |
| Behaviour of bees outside the hive| absence                        |

| Number of days after treatments |
|----------------------------------|
| Weather                          | 20°C, sunny                    |
| Active substance                 | D                              |
| Mortality [number of individuals]| 16                             |
| Behaviour at the hive entrance   | flying into and outside         |
| Percent of bees inside the hive  | normal                         |
| Behaviour of bees outside the hive| collecting nectar and pollen    |
| Percent of bees outside the hive | 15                             |
| Behaviour of bees outside the hive| absence                        |

| Number of days after treatments |
|----------------------------------|
| Weather                          | 13°C, rain                     |
| Active substance                 | D                              |
| Mortality [number of individuals]| 18                             |
| Behaviour at the hive entrance   | absence                        |

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treatment, bees in the hives were observed to be nervous and affected, with unnatural, quick movements. The situation was similar on the 9th day. On that day, in spite of the total cloudiness and low temperature which made bees in the control and acetamiprid treatments stay mainly in the hives, in deltamethrin treatments, 15% of bees were outside, whilst inside the hives, there were 80% of bees. Since the beginning of the experiment, 55 dead individuals were found. When considering individuals that were lost among the plants and not found, it points to a decrease in the number of individuals within the family. The final assessment 2 months after the end of the experiment showed 50% decrease in the number of individuals and the weakened condition of the colony in the deltamethrin treatment group.

In 2014, there were high temperatures throughout the entire experiment, which does not favour pyrethroid action. The weather conditions for bee flight were ideal all the time. No signs of poisoning were observed. It was also confirmed by the final assessment—all colonies were in good condition.

In 2015, there were favourable temperature conditions for pyrethroid activity and good conditions for bees to collect nectar and pollen for the first 6 days after the treatment (only weak rain towards the end of the 6th day). Later, the temperature was higher—up to 30 °C on the last day. In spite of the ideal temperature for pyrethroid action during the first day of the experiment, no signs of poisoning were observed in the deltamethrin group nor in the acetamiprid and control groups. This was confirmed by the final assessment made by a beekeeper.

In 2016, for the first 4 days, there were good weather conditions for bee flight but not for pyrethroid activity. On the 5th day, the temperature increased but later suddenly decreased which prevented bees from flying. No signs of poisoning were observed in any experimental version, which was confirmed by the final assessment.

In 2017, for the first 6 days, there were ideal conditions for bee flight but not for pyrethroid activity. Towards the end of the experiment, rain forced the insects to stay in the hives. No signs of poisoning were observed in the control and acetamiprid groups. In the deltamethrin group,
unnatural, quick movements of bees inside the hive were observed on the 3rd, 5th, 7th and 9th day after the treatment. Although not many dead individuals were found on the 5th day, higher mortality was observed as 10% of the bees were outside and 80% inside the hive. Other dead individuals were probably lost among the plants. On the 9th day, 112 (since the beginning of the experiment) dead individuals were found and only 70% of the colony—all bees inside the hive. The final assessment revealed the total decline of the colony.

In 2018, the weather conditions were excellent for bees to collect food. As for pyrethroid action, they were ideal for the first 2 days. Nevertheless, no signs of poisoning were observed under any condition, except for deltamethrin on the 7th day inside the hive. However, the bees quickly reverted to their natural behaviour. The final assessment revealed that all bee colonies were in a good condition.

In all experimental years (Table II; Figs. 1 and 2), despite different weather conditions, no signs of poisoning were observed in the control or acetamiprid groups. In the case of deltamethrin, irrespective of favourable or unfavourable weather conditions for pyrethroids, no signs of poisoning were revealed in 2014, 2015, 2016 and 2018. In 2012 and 2013, the colonies were weaker and a reduction in the numbers of individuals was observed, even though the conditions were not very favourable for pyrethroid action. However, the colonies were able to metabolize the insecticide and survive, which proves the presence of queens and brood. In 2017, despite the weather conditions being unfavourable for pyrethroids, a total decline in the colony was noted.

Similar weather conditions for the first 4 days following treatment were recorded in 2012 and 2015. Whilst in 2012, a 20% decrease in the number of bees in the deltamethrin version was recorded in the final assessment; no decrease was observed in 2015. For acetamiprid, no differences within these years were noted. Similarly, weather conditions at the beginning of the experiments in 2013 and 2018 were comparable. In spite of this, in 2013, there was a considerable weakening of a bee family in the case of deltamethrin, whilst no such signs were observed in 2018. Also, no differences were observed within these years for acetamiprid. Similar weather conditions during the first days of the experiments did not influence the deltamethrin toxicity in the year 2016 and 2017. In 2016, no signs of poisoning were observed, but in 2017, the total decline of a family was reported.

The results demonstrate the safety of acetamiprid treatment at the recommended dose

| Year | Percent of alive bees | The presence of a queen | The presence of brood | General condition of a family |
|------|-----------------------|------------------------|----------------------|-------------------------------|
|      | D A C                 | D A C                  | D A C                | D A C                         |
| 2012 | 80% 100% 100%        | Yes Yes Yes            | Yes Yes Yes          | Slightly weakened Good Good   |
| 2013 | 50% 100% 100%        | Yes Yes Yes            | Fewer Yes Yes Yes    | Good Good                     |
| 2014 | 100% 100% 100%       | Yes Yes Yes            | Yes Yes Yes          | Good Good                     |
| 2015 | 100% 100% 100%       | Yes Yes Yes            | Yes Yes Yes          | Good Good                     |
| 2016 | 100% 100% 100%       | Yes Yes Yes            | Yes Yes Yes          | Good Good                     |
| 2017 | 10% 100% 100%        | No Yes Yes Yes         | Very few Yes Yes Yes | Decline Good Good             |
| 2018 | 100% 100% 100%       | Yes Yes Yes            | Yes Yes Yes          | Good Good                     |
Figure 1. Percent of alive bees.

Legend:
- **good**
- **slightly weakened**
- **weakened**
- **decline**

| Active substance / Year | Deltamethrin | Acetamiprid | Control |
|-------------------------|--------------|-------------|---------|
| 2012                    |              |             |         |
| 2013                    |              |             |         |
| 2014                    |              |             |         |
| 2015                    |              |             |         |
| 2016                    |              |             |         |
| 2017                    |              |             |         |
| 2018                    |              |             |         |

Figure 2. Final condition of bee colonies.
for honeybees. In the case of deltamethrin, the results show that although its use may be dangerous for bees, the insects are equipped in detoxification mechanisms enabling metabolism of the toxin. The possible negative influence of deltamethrin on bee colonies seemed to be due to the general condition of a bee colony and different factors, identification of which is the aim of further research.

4. DISCUSSION

In the present study, the reaction of honeybees to acetamiprid treatments was always the same, just as in the control version, irrespective of weather conditions. No signs of poisoning (increase in mortality or changes in behaviour) were recorded in all years of the research. Also, the development of the colonies was similar in these two versions. On the other hand, honeybees’ reaction to deltamethrin was different depending on the year of the experiments. In some years, an increased mortality or even a family collapse was observed. In others, honeybee colonies survived the treatments and developed properly. The differences in honeybee reactions to deltamethrin were not related to different weather conditions within the years. Such results suggest that acetamiprid, at the recommended doses, is safe for honeybees, and deltamethrin treatment is dangerous for honeybee colonies. The fact that bees can survive treatment with deltamethrin demonstrates the existence of mechanisms that can metabolize the toxin in the absence of other factors weakening the health and wellbeing of a bee colony.

Research on acetamiprid effect on honeybees showed that this substance did not have any influence on bees (Węgordek et al. 2015). Iwasa et al. (2004) pointed to a very high toxicity of nitrosubstituted compounds (imidacloprid, clothianidin, thiametoxam, dinotefuran and nitenpyram) to honeybees when compared with cyano-substituted neonicotinoids (acetamiprid and thiacloprid). The authors also demonstrated the role of detoxification enzymes blocked with piperonyl butoxide in the mechanism of honeybee resistance to acetamiprid and thiacloprid. A significant difference between imidacloprid and acetamiprid influence on honeybees was confirmed by other authors (Johnson R.M. 2015, Iwsa et al. 2004, Zaworra et al. 2018).

Research presented in this study on the influence of field doses of insecticide active substances on honeybees in semi-field (field isolators) conditions showed high differentiation of the toxicity of pyrethroids. Some of the presented results pointed to the risk of deltamethrin application for honeybees, whilst others do not reveal any risk. It has already been demonstrated that deltamethrin and imidacloprid affected foraging activity of honeybees, whilst deltamethrin additionally influenced a reduction in bee learning capacities (Decourtye et al., 2004a, b, Ramirez-Romero et al. 2005; Telangre et al. 2018). The authors observed flight disorientation in honeybees contaminated with sublethal doses of deltamethrin, without detecting any deltamethrin residues 3 h after bee exposure. The influence of some pyrethroid active ingredients (deltamethrin, bifenthrin and fluvalinate) on neuronal excitability of honeybee brain neurons has also been reported.
An extensive literature review of toxicity data of various insecticides’ influence on honeybees and other insects showed that honeybees were moderately or highly sensitive to deltamethrin in comparison with other insects (Zhou et al. 2011, Zhang et al., 2020, Belzunces et al. 2012).

Other authors consider the possibilities of reducing the number or quantity of chemicals ingested by honeybees (Berenbaum and Johnson 2015). Despite the relatively few detoxification genes, honeybees have other mechanisms, e.g. the forager discrimination system, dilution by pollen mixing or colony food processing by microbial fermentation (Hardstone and Scott 2010). This may be the explanation for the fact of a great difference between deltamethrin contact acute toxicity—LD$_{50}$ 0.0015 (μg bee$^{-1}$)—and oral acute toxicity—LD$_{50}$ 0.07 (μg bee$^{-1}$) for honeybees (Zhang et al., 2020 2014; Berenbaum and Johnson 2015), which means high risk for this species and our field data, in which bee colonies are able to overcome the toxin and survive—sometimes without any negative effects.

The question of the necessity of a holistic approach towards risk assessment for honeybees has been undertaken by The European Food Safety Authority (EFSA). The EFSA Guidance Document firmly emphasizes the need for the inclusion of other multiple stressors such as chemical mixtures and biological agents including bee pests (i.e. Aethina tumida, Vespa velutina) or beekeeping management practices when working out the risk approach strategy, as they can modify the insecticide influence (Sharma and Abrol 2005).

So far, the factors affecting different reactions of bee colonies to insecticide treatments have not been identified. It still remains a challenge for scientists and necessitates further research.

CONTRIBUTIONS

DD, GM, JZ, PW and PS conceived the research and designed experiments; DD and PS performed the experiments and analysis; DD, GM, JZ and PW interpreted the data and wrote the paper.

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