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CIRCADIAN CHANGES IN SUSCEPTIBILITY OF YOUNG HONEYBEE WORKERS TO INTOXICATION BY PYRETHROID, CARBAMATE, ORGANOPHOSPHORUS, BENZOYL UREA AND PYRIDINE DERIVATIVE INSECTICIDES

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Abstract: In the years 2009 and 2010, in the apiaries surrounding Tarnobrzeg and Leżajsk, Poland (close to the Carpathian Mountains) research was carried out on diurnal changes in the sensitivity of young honey bee (*Apis mellifera*) workers to insecticides from various chemical groups: pyrethroids (esfenvalerate, cyhalothrin, alpha-cypermethrin, beta-cyfluthrin, deltamethrin), derivatives of pyridine (pyriproxyfen), carbamate (pirimicarb), organophosphate (diazinon), and benzoyl urea derivative (teflubenzuron). The analyses consisted of intoxicating subsequent groups of honey bees in 2-hour intervals, for a period of 24 hours with selected xenobiotics. The results received indicate that the honey bee shows a statistically significant susceptibility to insecticides, changing in the diurnal rhythm.

Key words: honeybee workers, pyrethroid, carbamate, organophosphorus, benzoyl

INTRODUCTION

Biological rhythms are a physiological phenomena occurring in practically all living organisms. The zeitgebers of the rhythms are such factors as temperature (Fuchikawa and Shimizu 2007; Gilbert et al. 2004; Refinetti 1997; Page 1985), moon phase (Mikulecky and Rovensky 2000; Mikulecky and Bounias 1997), access to food (Barrozo et al. 2004), gravitation (Hoban-Higgins et al. 2003) or predation (Kronfeld-Shor and Dayan 2003). However, it is assumed that the most important factor for determining the circadian rhythms in organisms, is daylight (Devlin and Kay 2001; Giebultowicz 1999).

The origin of the occurrence of biological clocks is rather unclear. Most probably, they resulted when primary organisms formed the cyclic mechanisms of escape from ultraviolet radiation. The earth was being bombed by radiation in the period when life was being borne (Pittendright 1993). The confirmation of this theory seems to be the ability of the blue light to strongly modify the operation of biological clocks. Plants, lower and higher animals, and human beings show a reaction to radiation that is blue coloured (Rosato and Kyriacou 2002).

It is similar in workers of honey bees (*Apis mellifera)* where the circadian changes in behaviour are also observed. The amplitude of these changes is different in older workers carrying out flights (Moore and Rankin 1993; Moore et al. 1989; Moore and Rankin 1985) and in young nest carers, cleaning the cells and looking after larvae and the queen (Huang and Otis 1991; Nijland and Hepburn 1985; Spangler 1972). At the same time, it is assumed that the cyclicity in bees is primarily dependent upon colony synchronisation and not upon the cycle of a single individual (Moore et al. 1998; Frisch and Koeniger 1994).

The main objective of this work was to investigate how young honey bee workers, not yet ready to leave the nest, will exhibit circadian rhythm-dependent susceptibility to intoxication by insecticides. The insecticides represent a differing toxicity spectrum.

MATERIALS AND METHODS

Animals

Young imago forms of honey bee workers (less than 48 hours from maturity) of the local race, collected from the cells with bee grubs, were used for the research. At the period of research, they were placed in an incubator Q-Cell model ERC0750 at a temperature of 36±0.5°C and in permanent darkness. The bees originated from the bee-garden, near the localities of Leżajsk (2009) and Tarnobrzeg (2010) not far from the Carpathian Mountains. Individuals from families with an average strength and normal composition were used for the research. In total, 12,960 workers of *A. mellifera* were used for the research. Each test was carried out on 108 individuals (18 individuals in 6 repetitions).

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Plant protection products

The following insecticides were tested:

1. Sumi-Alpha 050 EC – an insecticide from the group of pyrethroids (active substance (a.s.): esfenvalerate – 50 g in 1 liter of the agent). The concentration of the usable liquid: 0.0005%.
2. Karate-Zeon 050 CS an insecticide from the group of pyrethroids (a.s.: lambda-cyhalothrin – 50 g in 1 liter of the agent). The concentration of the usable liquid: 0.0002%.
3. Fastac 100 EC – an insecticide from the group of pyrethroids (a.s.: alpha-cypermethrin – 100 g in 1 liter of the agent). The concentration of the usable liquid: 0.0003%.
4. Bulldock 25 EC – an insecticide from the group of pyrethroids (a.s.: beta-cyfluthrin – 25 g in 1 liter of the agent). The concentration of the usable liquid: 0.0004%.
5. Decis 005 UL – an insecticide from the group of pyrethroids (a.s.: deltamethrin – 5 g in 1 liter of the agent). The concentration of the usable liquid: 0.0003%.
6. Admiral 100 EC – an insecticide from the group of derivatives of pyridine (a.s.: pyriproxyfen – 100 g in 1 liter of the agent). The concentration of the usable liquid: 0.0011%.
7. Pirimor 500 WG – insecticide from the group of carbamate (a.s.: pirimicarb – 500 g in 1 kg of the agent). The concentration of the usable liquid: 0.0050%.
8. Diazol 500 EW – insecticide from the group of organophosphorus (a.s.: diazinon – 500 g in 1 liter of the agent). The concentration of the usable liquid: 0.0006%.
9. Nomolt 150 EC – insecticide from the group of benzoyl urea derivative (a.s.: teflubenzuron – in 1 liter of the agent). The concentration of the usable liquid: 0.0012%.

Conditions for the tests

The research on how the insecticides with different spectrums of insecticidal activity affect the survivability of honey bees, was carried out in two similar repetitions which took place in July 2009 and 2010. For the whole measuring period, intoxicated insects were placed in preset thermal conditions of 36±0.5°C and in permanent darkness. Throughout the whole period, they had unlimited access to food and water.

Method of intoxication

Contact apitoxicity was determined by the individual dosing method. An applicator was used to place a drop of the preparation (volume of 4 µl) on the ventral part of an insect's thorax near the paraoesophageal ring. Water was used in the control group. The unabsorbed part of xenobiotic was removed with a paper tissue after 20 seconds.

The time of research conducted

The assumed period for conducting each measurement cycle was 72 hours.

The development and presentation of results

The results were developed with the use of Statistica for Windows software, version 7.0 with the use of multivariate analysis of ANOVA type (Tukey's test). Microsoft Excel 2010 software was used for the graphic presentation of the results.

RESULTS

During the research, it was proved that the control individuals showed higher survivability than bees from groups treated with the remaining preparations (Table 1). It was never the case that the research starting time in the control group had any statistically significant impact on the survivability of bees from this group (Table 2).

In the case of insects treated with plant protection, the susceptibility of bees to the activity of biocides in the circadian rhythm proved to be variable; depending on the time of intoxication and on the preparation applied (Table 1).

There was a significant quantity of statistically material differences in the survivability of animals, depending upon the time of intoxication and the preparation applied. These differences were given in table 2 exclusively in the quantitative form.

Table 1. Survivability of young honey bee *(A. mellifera)* workers intoxicated with agents in which the agents have various mechanisms of action

Active substances	Survivability/ Standard error	Time of a day											
		02.00	04.00	06.00	08.00	10.00	12.00	14.00	16.00	18.00	20.00	22.00	24.00
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	survi. [%]	100.0	92.6	100.0	88.9	90.7	100.0	88.9	100.0	100.0	91.7	100.0	100.0
	std. err.	0.0	0.7	0.0	0.7	0.9	0.0	0.7	0.0	0.0	1.1	0.0	0.0
Esfenvalerate	survi. [%]	65.7	60.2	44.4	34.3	11.1	16.7	54.6	60.2	66.7	77.8	65.7	75.9
	std. err.	1.0	0.6	1.3	0.5	0.6	0.8	0.8	0.5	0.6	1.1	0.3	0.3
Lambda-cyhalothrin	survi. [%]	71.3	49.1	59.3	40.7	28.7	94.4	82.4	90.7	58.3	71.3	27.8	73.2
	std. err.	0.2	1.5	1.1	0.7	0.9	0.7	0.8	1.0	1.2	0.9	1.0	0.5
Alpha-cypermethrin	survi. [%]	65.7	60.2	44.4	34.3	11.1	16.7	54.6	60.2	66.7	77.8	65.7	75.9
	std. err.	0.9	0.8	0.6	0.5	0.3	0.5	0.6	1.3	0.4	1.4	1.3	0.8

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Beta-cyfluthrin	survi. [%]	5.6	37.1	52.8	49.1	25.9	5.6	0.0	15.7	0.0	10.2	0.0	0.0
	std. err.	0.5	1.0	1.2	0.9	0.8	0.3	0.0	0.7	0.0	0.5	0.0	0.0
Deltamethrin	survi. [%]	78.7	77.8	53.7	42.6	77.8	5.6	0.0	5.6	0.0	0.0	71.3	78.7
	std. err.	1.1	1.3	1.0	0.2	0.6	0.4	0.0	0.4	0.0	0.0	0.9	0.3
Pyriproxyfen	survi. [%]	49.1	37.9	5.6	48.2	20.4	4.6	0.0	0.0	26.8	0.0	0.0	55.6
	std. err.	1.3	0.6	0.4	0.3	0.4	0.4	0.0	0.0	1.1	0.0	0.0	1.2
Pirimicarb	survi. [%]	92.6	66.7	30.6	6.5	27.8	71.3	5.6	60.2	93.5	61.1	94.4	83.3
	std. err.	0.7	0.9	0.7	0.5	0.7	1.5	0.5	1.1	0.3	0.9	0.5	0.7
Diazinon	survi. [%]	70.4	59.3	42.6	32.4	38.9	70.4	77.8	50.0	81.5	50.0	67.6	66.7
	std. err.	1.0	1.2	0.9	0.8	0.9	0.6	1.3	1.0	0.9	0.7	0.8	0.8
Teflubenzuron	survi. [%]	53.7	46.3	29.6	31.5	40.7	0.0	15.7	18.5	10.2	31.5	28.7	54.6
	std.err.	0.9	0.7	0.6	0.8	1.0	0.0	0.7	0.7	0.5	0.5	0.7	0.6

Table 2. Shortened, tabular presentation of statistically significant differences in the survivability of honey bee workers *(A. mellifera)*, depending upon the time of intoxication

Active substances	$p < 0.001$	$p < 0.01$	$p < 0.05$
The control	0	0	0
Esfenvalerate	11	2	3
Lambda-cyhalothrin	29	0	5
Alpha-cypermethrin	25	2	6
Beta-cyfluthrin	30	2	0
Deltamethrin	43	0	0
Pyriproxyfen	31	9	0
Pirimicarb	39	5	4
Diazinon	13	10	2
Teflubenzuron	23	7	3

DISCUSSION

About 35% of our diet is from honey bee *(A. mellifera)* pollinated plants. Yet, for some time the honey bee has also become a species threatened by extinction. Colony Collapse Disorder (CCD) had already been noted in 1869. It is a complex phenomenon in which a reduced number of collectors in the colony takes place (Underwood and van Engelsdorp 2007). There are many reasons suggested for CCD, such as: viral diseases (Bonning 2009; Bacandritsos *et al*. 2010), parasites (Paxton 2010), pesticides (Desneux *et al*. 2007; Johnson *et al*. 2010), a great shortening of telomeres in the wintering generation (Stindl and Stindl 2010) or secondarily, general weakening of the colony resistance caused by disease (Johnson *et al*. 2009) and the reduction in the metabolic competence of insects (Naug 2009). The first observations of cases similar to CCD, under names such as "Fall-Dwindle-Disease" or "Spring Dwindle", occurred in the territory of the United States (USA). Today this happens to be a global phenomenon, with only a rare exception (Vandame and Palacio 2010).

A significant part of the currently applied insecticides are highly lipophilic preparations. This means that in a beehive full of bee's wax, even individuals which had no contact with the environment outside the nest may be continuously exposed to the activity of biocides (Johnson *et al*. 2010). Biocides are one of the components suspected of contributing to the occurrence of CCD. Biological rhythms, may determine to a significant degree, the toxicity of biocides (Jamali *et al*. 1998; Pszczolkowski and Dobrowolski 1999). In our research, we decided to verify whether the biological rhythms which may determine biocide toxicity, act on young honey bee workers.

The intoxication of young honey bee workers was carried out with substances belonging to pyrethroids, benzoyl urea derivative, carbamate and organophorus compounds. These are preparations having variable mechanisms of action (van Eck 1979; Fukuto 1990; Soderlund *et al*. 2002). The results obtained indicate that the diurnal time in which the intoxication takes place determines the toxicity of the insecticides. In the case of deltamethrin, alpha-cypermethrin, teflubenzuron and pirimicarb, it was close to the middle of the day that *A. mellifera* individuals turned out to be most susceptible to the activity of biocides (Table 1). In the case of substances like pyriproxyfen, beta-cyfluthrin, esfenvalerate, lambda-cyhalothrin and diazinon in the dose we applied, the time of highest susceptibility was not so unambiguous. Between 2008 and 2009, we conducted research on circadian and seasonal susceptibility of bee collectors to the same insecticides. In the case of practically all compounds, we were able to prove the highest susceptibility of older workers to the activity of biocides occurs around the middle of the day. Such information could confirm that the natural circadian cyclicity of lighting may determine circadian changes of insects' susceptibility to insecticides. On the other hand, in the research on older bees, we used natural sunlight which could have a crucial impact on the results received.

Our work has demonstrated that *A. mellifera* is characterized by a statistically significant susceptibility to insecticides, and this susceptibility is related to its daily rhythm. These results support the results of other studies on the spectrum of action of chemical pesticides, depending on the time of intoxication (Eesa and Cutkomp 1995; Pszczolkowski and Dobrowolski 1999). The results from the work of *A. mellifera*, show that natural periods of activity and rest of honey bees must be taken into account. These periods must be noted when developing programs of agricultural treatments with chemical preparations meant to be used close to nectaring plants.

CONCLUSIONS

In the present work, the functioning of the circadian clock in young honey bee workers, not exposed to cyclic changes of light phases during a 24-hour period, was studied. We found that preparations containing deltamethrin, alpha-cypermethrin, teflubenzuron and pirimicarb when

applied at mid-day were most toxic to young honey bees workers. But, in the case of pyriproxyfen, beta-cyfluthrin, esfenvalerate, lambda-cyhalothrin and diazinon, the obtained results were inconclusive with no clear relationship between the time of the day and susceptibility to insecticide intoxication. Nevertheless, the studied insecticides, even when applied at significantly lower rates than those recommended to control pests on crops, were characterized by high toxicity to the studied young honey bee workers.

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