Pol. J. Environ. Stud. Vol. 22, No. 5 (2013), 1457-1463

Original Research

Circadian and Seasonal Changes in Honeybee (*Apis mellifera*) Worker Susceptibility to Diazinon, Teflubenzuron, Pirimicarb, and Indoxacarb

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Received: 17 September 2012
Accepted: 30 April 2013

Abstract

Circadian and seasonal changes in the susceptibility of honeybee (*Apis mellifera*) workers to intoxication by organophosphate, benzoylphenyl urea, carbamate, and oxadiazine insecticides have been studied from 2008 to 2009. Animals were collected in two apiaries located in the surroundings of Mielec and Tarnobrzeg in the Sub-Carpathian Province. The experiments consisted of intoxication by select xenobiotics of the subsequent groups of animals in two-hour intervals for a period of 24 hours and the analysis of their survivability. Experiments were carried out in spring and summer. The results suggest that the susceptibility of bees to different groups of insecticides varies significantly, both in the circadian and seasonal rhythms.

Keywords: honeybee, *Apis mellifera*, biological rhythms, insecticides, pesticides

Introduction

A recent increase in the number of honeybee colonies has been noted in Poland (in the period 2006-12 by nearly 20%). However, this tendency is only artificially maintained by governmental promotion of purchasing bee colonies by beekeepers, thanks to refunds by the National Program to Support Beekeeping in Poland [1]. Unfortunately, research carried out around the world indicates that the honeybee *Apis mellifera,* which pollinates 16% of all wild plant species [2] and which is particularly important from an economic point of view due to direct or indirect participation in the production of about 35% from components of the human diet [3], is increasingly in danger of extinction.

Since 1869 many cases of mass extinction of honeybees around the world have been noted [4]. In many cases, the causes of this phenomenon have not yet been defined, and the episodes of bee colony collapse with unidentified origin which is manifested by a rapid decline in the number of foragers (despite correct egg lying by the queen and the presence of pollen in the hive) have been named colony collapse disorder (CCD). Currently, it has been assumed that CCD is a complex phenomenon composed of many factors, the most important of which are malnutrition of the bees [5], carriage of bee hives [6], viral diseases [7, 8], parasites [9], excessive shortening of telomeres in the wintering generation [3], and a general weakening of the colony that might have been caused by a number of factors [3] and metabolic disorders of bees. However, the effects of pesticides the bees is considered one of the most important causes of CCD [10-13].

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In Europe [14] and in America (e.g. USA [15, 16], although this has not been noted in Latin-American countries yet [17]) the CCD phenomenon occurs more and more commonly, sometimes making a contribution to the extinction of 80-100% of colonies in a given area. Cases of CCD have been also noted in Poland [18]. In 2012 severe intoxications of honeybee colonies on the plantations of rapeseed and fruit crops by pesticides took place in seven provinces (a total of about 1,500 colonies have died). In other provinces cases of poisoning colonies have also been reported [1]. It should be noted here that the detection of those cases, in view of the considerable cost of the analysis, occurs relatively rarely. Studies by Walorczyk et al. that have been performed on found dead bee foragers [19] indicate that the domestic population of *A. mellifera* is largely the object of intoxication by the plant protection products. Moreover, the poisoning by commercial insecticides plays an extremely important role here. Indeed, few of the farmers realize that the death of bees brings significant economic losses not only for the beekeeper, but also significantly reduces the possibilities of optimal pollination of entomophilous crops and is not conducive to maintaining biodiversity.

Since pesticide intoxication seems to be one of the crucial factors that cause the collapse of the bee colonies, the determination of conditions in which this intoxication occurs has great significance. Thus our goal was to check out if the older honeybee *A. mellifera* workers show any circadian and seasonal changes in their susceptibility to insecticides of various mechanisms of action.

Materials and Methods

Research Material

Animals: Adult honeybee foragers (more than 19 days of age) were used in the experiment. During each experiment groups of 18 workers were collected directly from the entrances to the hive and then intoxicated. The honeybees came from two apiaries: one near Podleszany (2008) and another in the surroundings of Tarnobrzeg (2009) in the Sub-Carpathian Province. In total, 12,960 workers of *A. mellifera* were used for the research. Both control and pesticide intoxication experiments were carried out in six trials.

Insecticides

The following insecticides were used for the experiment:

Diazol 500 EW. Producer: Makhteshim-Agan Industries. Insecticide formulation belonging to organophosphorus compound (active substance: diazinon – 500 g in a 1 litre of the agent) – the concentration of the usable liquid 0.0006%

Nomolt 150 EC. Producer: BASF Agro B.V. Insecticide formulation belonging to benzoylphenyl urea compound (active substance: teflubenzuron – 150 g in a 1 litre of the agent) – the concentration of the usable liquid is 0.0012%

Pirimor 500 WG. Producer: Syngenta Limited. Insecticide formulation belonging to carbamate compound (active substance: pirimicarb – 500 g in a 1 litre of the agent) – the concentration of the usable liquid is 0.005%

Steward 30 WG. Producer: Du Pont de Nemours. Insecticide formulation belonging to oxadiazines compound (active substance: indoxacarb – 30 g in a 1 kilogram of the agent) – the concentration of the usable liquid is 0.0093%.

All insecticides that were used were water-soluble formulations.

The Experiment

Experiments on the insecticides' impact on the survivability of the honeybee were conducted in two similar repetitions that took place in 2008 and 2009. They were carried out on the turn of April and May (spring measurement) and in July (summer measurement). During the experiment, bee workers were placed in a modified incubator (type: Q-Cell model ERC0750) in stable thermal conditions (ambient temperature 27±0.5°C), with the use of the natural L/D cycle of diffuse sun-light, and with unlimited access of the animals to food (sugar syrup), and water.

Methods 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 surface of an insect's thorax near the paraoesophageal ring. Water has been used in the control group.

The Duration of the Experiment

Assumed duration of the measuring cycle, i.e. time from placing animals into an incubator to survivability measurement, was 72 hours.

Data Analysis

The results were analyzed using Statistica for Windows software, version 10 with the use of multivariate ANOVA (Tukey's test).

Results

The results are summarized on Tables 1-5. The results shows that the time of day in which contact of the animal with insecticides occurs determines their toxic effect. The study of the seasonal changes of susceptibility to different insecticide groups showed that, in most cases, the bees intoxicated in the spring were characterized by higher survival rate than those treated by the insecticide in summer, whereby, in many cases, most clear dependencies were in groups of animals intoxicated in morning hours. In the spring season (Table 1) this usually coincided with maximum sensitivity 2-4 hours later than in summer (Table 2).

Table 1. The effect of insecticide intoxication with diazinon, teflubenzuron, pirimicarb, and indoxacarb on survivability of honeybee workers (*A. mellifera*) in spring.

Active substance		Time of application											
		02:00	04:00	06:00	08:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00	00:00
Control	survi. (%)	100.0	99.1	99.1	98.1	93.5	89.8	92.6	97.2	99.1	99.1	100.0	100.0
	std. err.	0.00	0.17	0.17	0.33	0.17	0.17	0.42	0.34	0.17	0.17	0.00	0.00
Diazinon	survi. (%)	100.0	94.4	100.0	88.9	89.8	38.9	50.0	44.4	72.2	77.8	94.4	100.0
	std. err.	0.00	0.45	0.00	0.45	0.65	0.45	0.52	0.45	0.68	0.58	0.52	0.00
Teflubenzuron	survi. (%)	100.0	100.0	93.5	94.4	67.6	28.7	33.3	62.0	77.8	81.5	100.0	100.0
	std. err.	0.00	0.00	0.40	0.45	0.60	0.65	0.45	0.40	0.26	0.33	0.00	0.00
Pirimicarb	survi. (%)	90.7	100.0	99.1	100.0	51.9	27.8	49.1	57.4	87.0	91.7	88.9	91.7
	std. err.	0.21	0.00	0.17	0.00	0.49	0.45	0.65	1.98	0.49	0.34	0.52	0.43
Indoxacarb	survi. (%)	93.5	92.6	89.8	88.9	83.3	40.7	46.3	42.6	70.4	83.3	93.5	97.2
	std. err.	0.75	0.42	0.60	0.45	0.58	0.76	0.88	0.67	1.05	1.00	0.48	0.50

Discussion

Despite the efforts of many researchers, the question of the possible causes of CCD has not yet been fully resolved. It is believed that one of the important causes of mass disappearance of bee colonies is, among others, the indiscriminate use of insecticides in agricultural practice. This investigation is focused on only one of the aspects of the effect of these substances on the body of the insect that, therefore, could provide a solution to the existing state of the current threat to the species *Apis mellifera* L. Namely, we were trying to determine circadian and annual changes in the susceptibility of worker honeybees to insecticides.

The lowest susceptibility to insecticides and thus their highest survivability was noted in the spring. Also, a time of day when the insecticide was used had an influence on the survivability of the honeybee workers. These results suggest, proving our reports and those of other investigators [20-22] that cyclic changes in various physiological parameters and the activity rhythm of the honeybees also are reflected in their resistance to insecticides.

The presence of cyclic changes in the activity of honeybees has been observed for about 100 years [23]. With the time passing, it has been proven that the largest impact on the functioning of biological clocks of these animals comes from age [24] and weight [25] of the individuals, the functions they fulfil in the nest [26], synchronization with other individuals in the colony [27], and the time of gathering nectar from plants [23].

As indicated in the studies on the genesis of daily bee rhythms, they are more similar to the ones observed in vertebrates than in other invertebrates. Despite the honeybee brain contain only four times more neurons than in Drosophila melanogaster [28], honeybees were able to develop social behavior together with the development of communication language [29], and they also have learned to adjust their daily and seasonal behavior to the rhythm of plant nectaring [30]. On the other hand, the honeybee individuals have weakly developed immune and detoxifying systems. The amount of monooxygenase coding genes P450 or glutation-S-transferase of this species is nearly 50% lower than in other representatives of the insects class [28]. Therefore, many functions fulfilled by these systems in other species were compensated in bees by means of various forms of social behavior [31, 32].

The insecticides used in this study, although the mode of their action is significantly different [33-35], at the same time showed increased toxicity in relation to workers of honey bee, in both the diurnal and the seasonal rhythms. Hence the assumption is possible that the effectiveness of detoxification mechanisms in honeybee organisms is mainly determined by endogenous biological clocks. This is confirmed by our previous research on circadian changes in the sensitivity of older honeybee workers to pyrethroids (unpublished information), in which the results obtained were similar to those presented in this paper. However, in analogous tests carried out on young workers still before the cleansing flight, daily changes in the susceptibility were not so evident [20]. This proves that the daily changes in sensitivity on toxic agents are age-dependent and, therefore, in older bee foragers (which are characterized by a marked circadian rhythm of activity) it shows up more evidently than in younger bees. [23].

In Poland, where over 500 species of bees live, nearly half of them are on the red list of animals threatened by extinction. The CCD problem requires further detailed study. Among others, important studies are presented in this publication. In addition to the scientific analysis of this phenomenon, it is also necessary to take steps to reduce the impact of individual risk factors, for example environmental contamination by insecticides. Otherwise, in a very short time, the honeybee species also will find its way to this list

Table 2. The list of statistically significant differences in the survivability of honeybees (*A. mellifera*) under the influence of diazinon, teflubenzuron, pirimicarb, and indoxacarb during the spring, dependent on the hour of intoxication. [1]P<0.05, [2]P<0.01, [3]P<0.001.

Active substance	Time of application											
	02:00	04:00	06:00	08:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00	00:00
Control	10:00[1], 12:00[3], 14:00[2]	12:00[3], 14:00[1]	12:00[3], 14:00[1]	12:00[3]	02:00[1], 22:00[1], 00:00[1]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 16:00[2], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[2], 04:00[1], 06:00[1], 18:00[1], 20:00[1], 22:00[2], 00:00[2]	12:00[2]	12:00[3], 14:00[1]	12:00[3], 14:00[1]	10:00[1], 12:00[3], 14:00[2]	10:00[1], 12:00[3], 14:00[2]
Diazinon	12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 12:00[3], 14:00[3], 16:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 12:00[3], 14:00[3], 16:00[3], 22:00[3], 00:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]
Teflubenzuron	10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[2]	10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[2]	10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[1]	10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[2]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 12:00[3], 14:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 12:00[3], 14:00[3], 18:00[1], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[1], 08:00[2], 12:00[3], 14:00[3], 16:00[1], 22:00[3], 00:00[3]	02:00[2], 04:00[2], 12:00[3], 14:00[3], 16:00[3], 22:00[2], 00:00[2]	10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[2]	10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[2]
Pirimicarb	10:00[3], 12:00[3], 14:00[3], 16:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 12:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 14:00[2], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 12:00[2], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 12:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]	10:00[3], 12:00[3], 14:00[3], 16:00[3]
Indoxacarb	12:00[3], 14:00[3], 16:00[3], 18:00[2]	12:00[3], 14:00[3], 16:00[3], 18:00[2]	12:00[3], 14:00[3], 16:00[3], 18:00[1]	12:00[3], 14:00[3], 16:00[3]	12:00[3], 14:00[3], 16:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 18:00[2], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 08:00[3], 10:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[2], 04:00[2], 06:00[1], 12:00[3], 14:00[2], 16:00[3], 22:00[2], 00:00[3]	12:00[3], 14:00[3], 16:00[3]	12:00[3], 14:00[3], 16:00[3], 18:00[2]	12:00[3], 14:00[3], 16:00[3], 18:00[3]

Table 3. The effect of insecticide intoxication with diazinon, teflubenzuron, pirimicarb, and indoxacarb on survivability of honeybee workers (*A. mellifera*) in summer.

Active substance		Time of application											
		02:00	04:00	06:00	08:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00	00:00
Control	survi. (%)	100.0	98.1	99.1	99.1	95.4	93.5	93.5	98.1	99.1	100.0	94.4	99.1
	std. err.	0.00	0.33	0.17	0.17	0.17	0.17	0.40	0.21	0.17	0.00	0.26	0.17
Diazinon	survi. (%)	88.9	94.4	44.4	83.3	77.8	33.3	100.0	100.0	100.0	100.0	94.4	88.9
	std. err.	0.26	0.45	0.52	0.71	0.26	0.68	0.00	0.00	0.00	0.00	0.26	0.52
Teflubenzuron	survi. (%)	94.4	83.3	77.8	50.0	43.5	76.9	53.7	88.0	75.0	91.7	92.6	87.0
	std. err.	0.45	0.68	0.77	0.77	0.75	0.75	0.33	0.40	0.50	0.67	0.33	0.33
Pirimicarb	survi. (%)	92.6	90.7	71.3	32.4	31.5	43.5	61.1	58.3	55.6	88.0	81.5	91.7
	std. err.	0.21	0.61	0.48	0.40	0.33	0.31	0.86	0.81	0.86	0.17	0.49	0.34
Indoxacarb	survi. (%)	91.7	91.7	50.9	59.3	67.6	42.6	98.1	96.3	92.6	99.1	95.4	91.7
	std. err.	0.34	0.50	0.91	1.12	0.60	0.88	0.21	0.33	0.42	0.17	0.17	0.62

Table 4. The list of statistically significant differences in the survivability of honeybees (*A. mellifera*) under the influence of diazinon, teflubenzuron, pirimicarb, and indoxacarb during the summer, dependent on the hour of intoxication. [1]P<0.05, [2]P<0.01, [3]P<0.001.

Active substance	Time of application											
	02:00	04:00	06:00	08:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00	00:00
Control	12:00[1], 14:00[1]					02:00[1], 20:00[1]	02:00[1], 20:00[1]			12:00[1], 14:00[1]		
Diazinon	06:00[3], 12:00[3]	06:00[3], 10:00[3], 12:00[3]	02:00[3], 04:00[3], 08:00[3], 10:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	06:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]	04:00[3], 06:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3]	02:00[3], 04:00[3], 08:00[3], 10:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 10:00[3], 12:00[3]	06:00[3], 12:00[3]
Teflubenzuron	06:00[2], 08:00[3], 10:00[3], 12:00[2], 14:00[3], 18:00[3],	08:00[3], 10:00[3], 14:00[3]	02:00[2], 08:00[3], 10:00[3], 14:00[3], 22:00[1]	02:00[3], 04:00[3], 06:00[3], 12:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 12:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[2], 08:00[3], 10:00[3], 14:00[3], 20:00[1], 22:00[1]	02:00[3], 04:00[3], 06:00[3], 12:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	08:00[3], 10:00[3], 14:00[3]	02:00[3], 08:00[3], 10:00[3], 14:00[3], 20:00[2], 22:00[2]	08:00[3], 10:00[3], 12:00[1], 14:00[3], 18:00[2]	06:00[1], 08:00[3], 10:00[3], 12:00[1], 14:00[3], 18:00[2]	08:00[3], 10:00[3], 14:00[3]
Pirimicarb	06:00[2], 08:00[3], 10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3]	06:00[1], 08:00[3], 10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3]	02:00[2], 04:00[1], 08:00[3], 10:00[3], 12:00[3], 00:00[1]	02:00[3], 04:00[3], 06:00[3], 14:00[3], 16:00[3], 18:00[2], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 06:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 08:00[3], 10:00[3], 20:00[3], 22:00[1], 00:00[3]	02:00[3], 04:00[3], 08:00[3], 10:00[3], 20:00[3], 22:00[2], 00:00[3]	02:00[3], 04:00[3], 08:00[2], 10:00[3], 20:00[3], 22:00[3], 00:00[3]	08:00[3], 10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3]	08:00[3], 10:00[3], 12:00[3], 14:00[1], 16:00[2], 18:00[3]	06:00[1], 08:00[3], 10:00[3], 12:00[3], 14:00[3], 16:00[3], 18:00[3]
Indoxacarb	06:00[3], 08:00[3], 10:00[2], 12:00[3]	06:00[3], 08:00[3], 10:00[2], 12:00[3]	02:00[3], 04:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	02:00[2], 04:00[2], 12:00[2], 14:00[3], 16:00[3], 18:00[2], 20:00[3], 22:00[3], 00:00[3]	02:00[3], 04:00[3], 10:00[2], 14:00[3], 16:00[3], 18:00[3], 20:00[3], 22:00[3], 00:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[2], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]	06:00[3], 08:00[3], 10:00[3], 12:00[3]

Table 5. The list of statistically significant seasonal differences in the survivability of honeybees (*A. mellifera*) dependent on the influence of diazinon, teflubenzuron, pirimicarb, and indoxacarb. [1]P<0.05, [2]P<0.01, [3]P<0.001.

Active substance	time of application
Control	-
Diazinon	06:00[3], 14:00[3], 16:00[3], 18:00[3], 20:00[3]
Teflubenzuron	04:00[2], 06:00[1], 08:00[3], 10:00[3], 12:00[3], 14:00[3], 16:00[3]
Pirimicarb	06:00[3], 08:00[3], 10:00[1], 18:00[3]
Indoxacarb	06:00[3], 08:00[3], 14:00[3], 16:00[3], 18:00[2]

and we will have to cope with either the Chinese model of manual pollination of plants and synthetic honey or to use other species of bee-like insects, compensating for lower honey productivity than in *A. mellifera* with a higher level of aggression.

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