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Review

Effects of Plant Protection Products on Biochemical Markers in Honey Bees

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Abstract: Plant protection products (PPPs) are pesticides that protect crops and ornamental plants. PPPs include primarily insecticides, herbicides, and fungicides. Bees' contact with PPPs can cause immediate death or, in sublethal dose, may affect their physiology and/or behavior. Understanding the effect of PPPs' sublethal doses is especially important. Contact with a sublethal dose of PPPs generally allows the bee to return to the hive, which may expose the whole colony to the harmful substance. Biochemical changes may affect colony condition, health, and performance. Most of the research on the biochemical effects of PPP in honey bees focuses on insecticides and among them neonicotinoids (especially imidacloprid). The vast majority of research is carried out on *Apis mellifera* workers. A small part of the research has been conducted on drones and queens. Pesticides, including fungicides and herbicides, may alter antioxidant defense, detoxification, gene expression, and immune response of the bee. They affect the drones' semen quality and metabolic rate of the queen. In this review, the biochemical effect of PPP products in the honey bee was examined, with a focus on the effect on cytochrome P450 monooxygenases, glutathione transferases, and carboxylesterases, which take part in toxin metabolism or the detoxification process. PPPs effects on the activity of glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), proteases, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and phenoloxidase (PO) are also presented.

Keywords: detoxification; pesticide; fungicide; insecticide; herbicide; P450; glutathione transferases; esterases

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1. Introduction

Plant protection products (PPPs) are pesticides that protect crops and ornamental plants. PPPs include the following primarily: insecticides (for insect control), herbicides (for unwanted-plant control), and fungicides (for fungi control). The use of these compounds increases yield, which is of great importance in food production [1]. The Nobel Prize received by Paul Muller for the invention of the insecticidal properties of dichlorodifenylotrichloroetan (DDT) emphasizes the importance of pesticides for humans [2]. However, non-selective action and bioaccumulation potential are one of the main disadvantages of pesticides [3]. Many of the once widely used pesticides have been withdrawn from the market because of their toxicity to humans and other homeothermic species [4].

More attention is paid to the toxicity of PPPs to honey bees, which as a pollinator has a positive effect on increasing yield and maintaining biodiversity. Bees' contact with PPPs can cause immediate death or, at sublethal doses, may affect their behavior and/or physiology. Understanding the effects of PPPs sublethal doses is especially important. Contact with a sublethal dose of PPPs generally allows the bee to return to the hive, which may expose the whole colony to the harmful substance [5]. Since field studies with colonies of bees are much more complicated and expensive than laboratory experiments, the majority of research is carried out in the laboratory using honey bee workers [6].

So far it has been shown that exposure of honey bees to sublethal doses of PPPs (mainly insecticides) affects motor activity, orientation in the field, feeding, development, learning ability, and memory; weakens the immune and reproductive system; and triggers the antioxidant and detoxification mechanisms [7–12]. The major enzyme families which take part in the toxin metabolism or detoxification process are the cytochrome P450 monooxygenases (P450), glutathione transferases (GST), and carboxylesterases (COEs). These parameters are most often analyzed in studies on the biochemical effects of PPPs in honey bees [13].

P450 are involved in the metabolism of a large spectrum of xenobiotics. The genes encoding P450 are one of the largest gene superfamilies [6]. The P450 are responsible for *Drosophila melanogaster* resistance to DDT (CYP6G1) and for *Anopheles gambiae* ability to quickly metabolize pyrethroid insecticides (CYP6M2). In addition, P450 are involved in honey bee's detoxification of tau-fluvalinate and coumaphos (CYP9Q1, CYP9Q2, and CYP9Q3) [14–16]. GST can catalyze the metabolism of xenobiotics by conjugation of the reduced glutathione [6]. COEs include, among others, acetylcholinesterase (AChE) and esterases. AChE catalyzes the degradation of the neurotransmitter acetylcholine (ACh). Some pesticides act as AChR agonists and cannot be degraded by the enzyme AChE. As a result, nerve signal transmission is disturbed. Many other COEs are important in the metabolism of xenobiotics [17].

Other analyzed enzymes inclue glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G6PD), proteases, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and phenoloxidase (PO) [10,11,18–20]. GPX, CAT, SOD, and GP6D are involved in the antioxidant protective capacity against free radicals. Proteases participate in the digestion of protein chains. AST and ALT take part in amino-acid metabolism and ALP catalyzes the dephosphorylation of various phosphate esters. PO controls melanization of pathogenes. The action of PO is regulated by the activation of zymogen prophenoloxidase (proPO) [21]. Most studies on biochemical effects of the PPPs in honey bees' concern insecticides, especially neonicotinoids.

2. Effects of Insecticides Used in Plant Protection on Biochemical Markers in Honey Bees

2.1. Effects of Neonicotinoids on Biochemical Markers in Honey Bees

2.1.1. Introduction

Neonicotinoids' toxicity to bees and association with Colony Collapse Disorder (CCD) has been the subject of public debate and many studies over the past twenty years [9,22]. Neonicotinoids are neuro-active insecticides. They are acetylcholine agonists at its receptors on postsynaptic membranes in insect nerve cells. By binding to the receptors instead of acetylcholine, it is not degraded by the enzyme acetylcholinesterase, which causes excitation and disturbance in the conduction of nerve impulses [23]. The neonicotinoid family includes, among others, acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. Cyano-substituted neonicotinoids (acetamiprid, thiacloprid) have a lower LD_{50} value than nitro-substituted and are considered to be much less toxic than nitro-substituted compounds (imidacloprid, clothianidin, and thiamethoxam) [10]. Neonicotinoids are generally less toxic to mammals, birds, and fish which is the main reason for their popularity and high use. Neonicotinoid acts systemically, which means that plants transport this insecticide to all of their parts [6].

2.1.2. Imidacloprid

Effects of Imidacloprid on Detoxification Enzymes in Honey Bees

Apis Mellifera Workers

The activity of carboxylesterases (COEs) was altered after imidacloprid oral administration at the dose of 4.3 ng/bee [24] and concentration of 7 ppb [25]. Depending on the dose and exposure duration, imidacloprid caused an alteration of acetylcholinesterase (AChE) activity [24,26] (Table 1). The concentration of 5 and 200 ppb in syrup decreased the

activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) [10,11,27]. Depending on the dose and type of sample used for analysis, imidacloprid caused the upregulation or downregulation of glutathione-S-transferase (GST) (Table 2) and cytochrome P450 activity [28–30] (Table 3). In addition to the dose, the effect of imidacloprid in bees is also influenced by the ambient temperature and the age of the bees [10,11,27,31]. The toxic effect seems to be particularly dangerous for young honey bees [11].

Apis Mellifera Queens

The concentration of 7 ppb increased the activity of GST and GPX and altered the activity of COEs [10,25]. This pesticide reduced the activity of AST, ALT, and ALP (orally, the concentration of 5 and 200 ppb) [11]. Moreover, imidacloprid in the concentration of 2.5 ppb reduced the standard metabolic rate of queen bee [32].

Apis Mellifera Drones

Treating drones with imidacloprid (orally, the concentration of 5 and 200 ppb) reduced the activity of AST, ALT, and ALP in drones' hemolymph [11]. In addition, imidacloprid administered orally at the concentration of 5 and 200 ppb reduced sperm motility and viability, while no effect on sperm concentration was observed [33].

Apis Cerana Workers

In *Apis cerana,* imidacloprid did not affect activity of GST [24,34]. This pesticide decreased the activity of cytochrome P450 [34] and upregulated COEs and AChE activities [24] (Tables 1–3).

Effects of Imidacloprid on Antioxidants and Immune-Related Biochemical Mechanisms in Honey Bees

Apis Mellifera Workers

Imidacloprid did not affect glutathione content (orally, at the concentration of 0.7 or 2 ppb) [35]. This insecticide caused a decrease in the level of two carotenoids (α-carotene and α-cryptoxanthin) and α-tocopherol (orally, in the concentration of 0.06, 0.2, 0.6, 2 ppb) [36]. The concentration of 5 and 200 ppb in syrup induced downregulation of ferric ion reducing antioxidant power (FRAP) [10,11,27]. The dose of 4.3 ng/bee decreased prophenoloxidase (proPO) activity [24]. Moreover, imidacloprid at the concentration of 0.7, 2, 5, and 200 ppb increased membrane lipid peroxidation and altered the activity of catalase (CAT) [10,35]. The concentration of 5 and 200 ppb in syrup dysregulated the activity of proteases [10,11,27]. Honey bees fed with imidacloprid showed a lower concentration of thiol group of proteins and did not affect pyridine nucleotides content (orally, at the concentration of 0.7 or 2 ppb) [35]. Imidacloprid reduced hemocyte density, encapsulation response, and antimicrobial activity at the concentration of 1 and 10 ppb [37]. The concentration of 5 and 200 ppb in syrup decreased the average protein content and upregulated global DNA methylation [10,11,27].

Apis Mellifera Queens

The concentration of 7 ppb increased the activity of CAT and superoxide dismutase (SOD) [10,25]. Antioxidant defense after imidacloprid treatment was not changed in 1 day old queens but highly upregulated in 2 year old queens (orally, the concentration of 5 and 200 ppb) [10]. Moreover, imidacloprid at the dose of 0.02 ppb decreased sperm viability in queens by 50% [29].

Apis Mellifera Drones

Treating drones with imidacloprid (orally, the concentration of 5 and 200 ppb) altered the activity of proteases and increased global DNA methylation [11].

Apis Cerana Workers

In *Apis cerana,* imidacloprid had little effect on phenoloxidase (PO) activity and did not affect the activities of GST [24,34] (Tables 1–3).

Effects of Imidacloprid on Immunity, Detoxification, and Antioxidation-Related Genes Expression in Honey Bees

Biochemical markers are confirmed in gene expression tests. Christen et al. [37] exposed honey bee workers to imidacloprid which significantly altered (mainly downregulated) gene expression. Imidacloprid affected the expression of immunity, detoxification, and antioxidation-related genes [24,28,31,34,37–41]. Imidacloprid dysregulated the expression of three immune-related genes (hymenoptaecin (AmHym), basket (AmBask), and lysozyme (AmLyso2)); antioxidant-related gene (vitellogenin (AmVit2) and the poly-U binding factor (AmPuf68)) [28]; and the detoxification-related gene (CYP9Q3 and CYT P450) [28,39]. Feeding larvae with imidacloprid result in increased immune-related gene expression in adult bees [38]. Gregorc et al. [40] tested the effects of imidacloprid on the expression of antioxidant-related genes. Imidacloprid at the dose of 20 ppb induced upregulation of Cat, TrxR1, SelK, and MsrB expression and the downregulation of Sod2. The dose of 5 ppb caused downregulation of Cat, MsrA, and TrxR1 expression. Gene expression increased with the dose of 2.5 ppb, which was intensified in bees kept in the cold treatment (20 °C) [31]. The dose of 0.02 ppb decreased the expression of P450 genes and antioxidant-related, immunity-related, and development-related genes [29].

2.1.3. Other Neonicotinoids

Effects of Other Neonicotinoids on Detoxification Enzymes in Honey Bees

Apis Mellifera Workers

Thiamethoxam at a concentration of 300 ppb decreased ALP activity [20]. This pesticide at the dose of 51.16, 5.12, and 2.56 ng/bee caused alteration in the activity of COEs, GST, and ALP [42]. Depending on the dose and type of exposure, clothianidin altered AChE and GST activity [20,24,30,42–44] (Table 1). In the study of Shi et al. [45], thiacloprid (at the dose of 120 ng/bee) affected 115 metabolites associated, among others, with detoxification. Exposition on acetamiprid (3660 ppb) altered P450 and GST activity (Tables 2 and 3) [46].

Apis Mellifera Queens

Clothianidin at the concentration of 0.1 ppb increased the activity of GPX and GST in drones' semen, while it decreased semen volume and sperm concentration [18,19]. Thiamethoxam administration to queens' larvae (4.28 and 8.56 ng) decreased the amount of sperm in spermatheca in adult queens [47].

Apis Mellifera Drones

Acetamiprid (the concentration of 10 ppb) reduced sperm concentration [48].

Effects of Other Neonicotinoids on Antioxidants and Immune-Related Biochemical Mechanisms in Honey Bees

Apis Mellifera Workers

Thiamethoxam at a concentration of 300 ppb increased polyphenol oxidase (PPO) activity [20]. In addition, thiamethoxam increased alfa-carotene and alfa-tocopherol levels (orally, at the concentration of 0.12, 0.4, 1.2, and 4 ppb) and alfa-cryptoxanthin (4 ppb) [36]. This pesticide at the dose of 51.16, 5.12, and 2.56 ng/bee caused alterations in the activity of CAT [42]. Clothianidin at the dose of 20.0, 10.0, 5.0, and 2.0 ng/bee altered the NF-κB signaling [49]. In the study of Shi et al. [45], thiacloprid (at the dose of 120 ng/bee) altered 115 metabolites associated, among others, with oxidative stress in honey bee workers. Exposition on acetamiprid (3660 ppb) altered CAT activity [46]. The concentration of 40 ppb caused a decrease in the total protein content of bee heads [50]. Thiamethoxam at the concentration of 1200 and 2000 ppb reduced hemocyte numbers [51,52] and, at the dose of 200 and 2000 ppb, reduced hemocyte density, encapsulation response, and antimicrobial activity [52]. Clothianidin affected these parameters at 100 ppb [52]. Chronic exposure to clothianidin (5 and 50 ppb) lowered the lipids and glycogen content of bees [53]. Nitenpyram (the concentration of 3000 ppb) caused significant alterations in gut microbiotas, which is responsible for homeostasis and immunity [54].

Apis Mellifera Queens

Clothianidin at the concentration of 0.1 ppb increased the activity of SOD, CAT, cell redox potential, ATP content, lactate dehydrogenase activity, and malonialaldehyd levels in drones' semen while decreasing protein content [18,19]. The exposition of young queens to thiacloprid or clothianidin resulted in a reduction in the total hemocyte number and the proportion of active and differentiated hemocytes. Moreover, thiacloprid (200 or 2000 ppb) weakened the antimicrobial activity of the hemolymph and melanization response [55].

Table 1. Effect of plant protection products on acetylcholinesterase (AChE) activity.

	Pesticide	Dose (ng/bee)/Concentration (ppb)	Exposure Type and Duration	Effect on Honey Bee Physiology	Sample for Enzyme Activity Analysis	References
Neonicotinoids	Imidacloprid(I)	0.1, 1, and 10 ppb	Orally, 10 and 20 days	↔	Head	[26]
		4.3 ng/bee	Orally, 2, 24, and 48 h	↓ after 48 h	Tissue homogenate	[24]
		1 ng/bee	Orally, 2, 24, and 48 h	↓ after 2, 24 h ↑ after 48 h in *Apis cerana*	Tissue homogenate	[24]
	Thiamethoxam(I)	300 ppb	Orally, acute	↑	Head	[20]
		0.00001, 0.001, and 1.44 ppb	Larvae feeding, 6 days	↑	Head	[43]
		51.16, 5.12, and 2.56 ng/bee	Topically, acute	↔	Head	[42]
	Clothianidin(I)	1 ng/bee	Orally, 2, 24 and 48 h	↔	Tissue homogenate	[24]
		0.3 ng/bee	Orally, 2, 24 and 48 h	↓ in *Apis cerana*	Tissue homogenate	[24]
	Acetamiprid(I)	3660 pbb	Orally, 1, 5, and 10 days	↔ in *Apis cerana*	Head	[46]
Pyrethroids	Lamda-cyhalothrin(I)	26,400,000 ppb	Orally, acute	↑	Head	[20]
		62,200 ppb	Spraying	↓	Head and thorax	[30]
	Beta-cyhalothrin(I)	10,400,000 ppb	Orally, acute	↓	Head	[20]
Oxamyl(I)		68,000 ppb	Spraying	↔	Head and thorax	[30]
Acephate(I)		168 ppb	Orally, 6, 12, 24, 48, 72 h	↓ after 48, 72 h	Head and thorax	[44]
		88,300 ppb	Spraying	↓	Head and thorax	[30]
Sulfoxaflor(I)		58,500 ppb	Spraying	↑	Head and thorax	[30]
Abamectin(I)		33 ppb	Orally, acute	↓	Head	[20]
Propiconazole(F)		24,000 and 60,000 ppb	Orally, 1, 5, and 10 days	↔ in *Apis cerana*	Head	[46]
Difenoconazole(F)		0.1, 1, and 10 ppb	Orally, 10 and 20 days	↔	Head	[26]
Tetraconazole(F)		512,500 ppb	Spraying	↔	Head and thorax	[30]
Glyphosate(H)		0.1, 1, and 10 ppb	Orally, 10 and 20 days	↑ after 20 days (0.1 ppb)	Head	[26]
		1,217,500 ppb	Spraying	↔	Head and thorax	[30]

↓, decreased activity; ↑, increased activity; ↔, no effect; I—insecticide; F—fungicide; H—herbicide; data relates to *Apis mellifera* workers unless otherwise stated.

Table 2. Effect of plant protection products on glutathione S-transerases (GST) activity.

	Pesticide	Dose (ng/bee)/Concentration (pbb)	Exposure Type and Duration	Effect on Honey Bee Physiology	Sample for Enzyme Activity Analysis	References
Neonicotinoids	Imidacloprid(I)	0.1, 1, and 10 ppb	Orally, 10 and 20 days	↓ µg/L in abdomen after 10 days (1 ppb) and 20 days	Head, midgut, or abdomen	[26]
		968 ppb	Orally, 1 h, 2 h, 4 h, 8 h, 12 h, 16 h, and 20 h.	↔ in *Apis cerana*	Abdomen	[34]
		4.3 ng/bee	Orally, 2, 24, and 48 h	↑ after 48 h	Tissue homogenate	[24]
		1 ng/bee	Orally, 2, 24, and 48 h	↔ in *Apis cerana*	Tissue homogenate	[24]
		7 ppb	Orally, 8 days	↑ in queen	Midgut or head	[25]
	Thiamethoxam(I)	300 ppb	Orally, acute	↑	Abdomen	[20]
		0.00001, 0.001, and 1.44 ppb	Larvae feeding, 6 days	↔	Head	[43]
		51.16, 5.12, and 2.56 ng/bee	Topically, acute	↔	Midgut	[42]
	Clothianidin(I)	11,000 ppb	Orally, 3 weeks	↑	Midgut	[44]
		1 ng/bee	Orally, 2, 24, and 48 h	↔	Tissue homogenate	[24]
		0.3 ng/bee	Orally, 2, 24, and 48 h	↑ *in Apis cerana* after 24 and 48 h	Tissue homogenate	[24]
	Acetamiprid(I)	3660 ppb	Orally, 1, 5, and 10 days	↓ after 10 days *in Apis cerana*	Midgut	[46]
Pyrethroids	Lamda-cyhalothrin(I)	26,400 ppb	Orally, acute	↑	Abdomen	[20]
		7300 ppb	Orally, 3 weeks	↑	Midgut	[44]
		62,200 ppb	Spraying	↓	Head and thorax	[30]
	Beta-cyhalothrin(I)	10,400 ppb	Orally, acute	↓	Abdomen	[20]
	Abamectin(I)	33,000 ppb	Orally, acute	↑	Abdomen	[20]
	Oxamyl(I)	179 ppb	Orally, 3 weeks	↑	Midgut	[44]
		68,000	Spraying	↔	Head and thorax	[30]
	Sulfoxaflor(I)	58,500 ppb	Spraying	↔	Head and thorax	[30]
	Chlorpyrifos(I)	1860 ppb	Orally, 3 weeks	↔	Midgut	[44]
	Acephate(I)	168 ppb	Orally, 6, 12, 24, 48, and 72 h; 3 weeks	↓ after 48 and 72 h ↔ after 3 weeks	Midgut	[44]
	Tetraconazole(F)	84 ppb	Orally, 3 weeks	↔	Midgut	[44]
		512,500 ppb	Spraying	↔	Head and thorax	[30]
	Difenoconazole(F)	0.1, 1 and 10 ppb	Orally, 10 and 20 days	↓ in midgut after 10 day (1 ppb)	Head, midgut, or abdomen	[26]
	Propiconazole(F)	24,000 ppb	Orally, 1, 5, and 10 days	↔ in *Apis cerana*	Midgut	[46]
	Glyphosate(H)	0.1, 1 and 10 ppb	Orally, 10 and 20 days	↓ in head and abdomen after 10 days and in midgut after 20 days (1 ppb)	Head, midgut, or abdomen	[26]
		35,000 ppb	Orally, 3 weeks	↔	Midgut	[44]
		1,217,500 ppb	Spraying	↔	Head and thorax	[30]

↓, decreased activity; ↑, increased activity; ↔, no effect; I—insecticide; F—fungicide; H—herbicide; data relates to *Apis mellifera* workers unless otherwise stated.

Table 3. Effect of plant protection products on cytochrome P450 activity.

	Pesticide	Dose (ng/bee)/Concentration (pbb)	Exposure Type and Duration	Effect on Honey bee Physiology	Sample for Enzyme Activity Analisis	References
Neonicotinoids	Imidacloprid(I)	968 ppb	Orally, 1 h, 2 h, 4 h, 8 h, 12 h, 16 h, and 20 h.	↑ after 4 h ↓ after 8–20 h in *Apis cerana*	Midgut	[34]
		39.5 ng/bee	Orally, acute	↑ 24 and 72 h after application	Bee homogenate	[28]
		27.7 ng/bee	Topically, acute	↑ 72 h after aplication	Bee homogenate	[28]
		4300 ppb	Spraying	↑	Head and thorax	[30]
		0.02 ppb	Topically, acute	↓ in queens ↓ in workers	Abdomen	[29]
	Clothianidin(I)	14.7 ng/bee	Orally, acute	↑ 24 and 72 h after application	Bee homogenate	[28]
		0.63 ng/bee	Topically, acute	↑ 72 h after aplication	Bee homogenate	[28]
	Acetamiprid(I)	3660 ppb	Orally, 1, 5, and 10 days	↑ after 5 days in *Apis cerana*	Midgut	[46]
	Carbaryl(I)	205 ng/bee	Orally, acute	↑ 24 and 72 h after application	Bee homogenate	[28]
		112 ng/bee	Topically, acute	↑ 72 h after aplication	Bee homogenate	[28]
	Acephate(I)	168 ppb	Orally, 6, 12, 24, 48, and 72 h; 3 weeks	↓ after 48 ↔ after 3 weeks	Midgut	[44]
	Propiconazole(F)	2400 ppb	Orally, 1, 5, and 10 days	↔ in *Apis cerana*	Midgut	[46]

↓, decreased activity; ↑, increased activity; ↔, no effect; I—insecticide; F—fungicide; data relates to *Apis mellifera* workers unless otherwise stated.

2.2. Effects of Other Insecticides on Biochemical Markers in Honey Bees

2.2.1. Introduction

In addition to neonicotinoids, a frequently used group of insecticides are pyrethroids. Pyrethroids are derivatives of chrysantemic acid, similar to the naturally occurring pyrethrins produced by the flowers of Chrysanthemum L. and Tanacetum L. [56]. Dried flowers of *Tanacetum cinerariifolium* secrete natural pyrethrins with insecticidal properties [57,58]. Pyrethroids, which are more persistent agents than naturally occurring pyrethrin, cause the prolongation of the opening of sodium channels in the nerve cells of insects, which in turn results in disturbances in the conduction of nerve impulses. It results in excitation, exhaustion, and subsequent paralysis and death of insects and mites [58]. Studies of the biochemical effect in honey bees involved deltamethrin, lambda-cyhalothrin, and beta-cyhalothrin [30,44,48,59].

Another group of PPPs are carbamate insecticides. They blocked the activity of cholinesterases and disturb the functioning of the nervous system. In turn, they result in uncontrolled movement, paralysis, and eventually death [60]. Studies of the biochemical effect in honey bees involved carbaryl and oxamyl [28,30,48].

The organophosphate insecticides form a large group of PPPs. They disturb the action of acetylcholine. A compound that originates from the metabolism of organophosphate binds the acetylcholinesterase. Thus, the deactivation of acetylcholine in the synaptic cleft is not possible, which in turn results in overexcitation followed by paralysis and death. Studies of the biochemical effect in honey bees involved chlorpyriphos and acephate [30,44,61,62].

Sulfoxaflor (sulfoximine insecticide) and abamectin (avermectin insecticide) are pesticides further involved in studies of the biochemical effect in honey bees [20,30]. Similar to the neonicotinoids, sulfoxaflor is a nicotinic acetylcholine receptor agonist. Sulfoxaflor

binding causes uncontrolled nerve impulses resulting in muscle tremors followed by paralysis and death [30]. Abamectin is the product of *Streptomyces avermitilis* fermentation. It binds to the glutamate-gated chloride channels that are found in insect neurons and muscle cells. It results in paralysis and death [63].

2.2.2. Effects of Other Insecticides on Detoxification Enzymes in Honey Bees

Apis Mellifera Workers

Lambda-cyhalothrin and acephate altered GST and AChE activity [30,44] (Tables 1 and 2). Chlorpyrifos decreased esterase activity [44]. Oxamyl in spray did not affect esterase, GST, and AChE activity [30] (Tables 1 and 2). Sulfoxaflor administered in spray increased AChE activity, while no effect on esterase and GST activity was observed [30] (Tables 1 and 2). Abamectin (the concentration of 33 ppb) increased ALP activity [20].

Apis Mellifera Drones

Deltamethrin (orally, 0.53 ng/bee) affected the drones by reducing sperm concentration [48].

2.2.3. Effects of Other Insecticides on Antioxidants and Immune-Related Biochemical Mechanisms in Honey Bees

Apis Mellifera Workers

Chlorpyriphos increased lipid peroxidation in tissue homogenate treating with 1 µg [62]. Feeding bees with syrup with the addition of chlorpyriphos (15, 30, 60, 125, 250, 350, and 500 ng/bee) did not affect NF-κB signaling [61]. Abamectin (the concentration of 33 ppb) increased PO activity [20].

2.2.4. Effects of Other Insecticides on Immunity, Detoxification, and Antioxidation-Related Gene Expression in Honey Bees

Deltamethrin (orally, 0.53 ng/bee) induced the cyp9q2 expression (detoxification-related gene cytochrome P450) [59]. Carbaryl topically administrated on workers upregulated immune-related gene AmHym (112 ng/bee), while orally administration (205 ng/bee) did not affect the tested genes [28].

3. Biochemical Effects of Fungicides and Herbicides in Honey Bees

3.1. Introduction

Fungicides and herbicides are considered much less toxic to bees than insecticides. For this reason, only a few studies on the biochemical effects in honey bees involved these agents. The biochemical effect in honey bees was studied on triazole fungicides (propiconazole, tetraconazole, and difenoconazole), strobilurin fungicide (picoxystrobin), and the herbicide glyphosate.

3.2. Fungicides and Herbicides Used in Plant Protection

After 10 days of the exposition, fungicide difenoconazole (orally, 1 ppb) altered the activity of GST and decreased the activity of G6PD in *Apis mellifera*. The fungicide tetraconazole in spray (512,500 ppb) and orally (84 ppb) did not affect esterase, GST, and AChE activity [30,44]. The herbicide glyphosate (the concentration of 0.1 ppb) increased AChE activity after 20 days (Table 1) and GST after 10 days while the dose of 1 ppb altered GST activity after 10 and 20 days (Table 2) and decreased glucose-6-phosphate dehydrogenase after 10 days [26]. Glyphosate in spray (1,217,500 ppb) did not affect esterase, GST, and AChE activity [30] (Tables 1 and 2). The fungicide picoxystrobin (orally, 18 ppb) did not affect the hemocyte number in *Apis mellifera* [51]. The fungicide propiconazole (orally, 2400 and 6000 ppb) had no significant effect on GST and AChE activity but changed P450 activity in *Apis cerana* [46].

4. Biochemical Effects of Plant Protection Products Mixtures in Honey Bees

4.1. Introduction

Bees, especially near crops, have contact with many PPPs at the same time. Various PPPs can react with each other and change their properties. The best-known example of an interaction between pesticides is the synergic effect of ergosterol-biosynthesis-inhibiting (EBI) fungicides and pyrethroid insecticides. Prochloraz, an active ingredient in EBI fungicides, increases the toxic effect of pyrethroids [64]. Studies on PPPs mixtures on honey bees' physiology contribute to enhancing knowledge about the PPPs effects. However, there is still little research on this topic.

4.2. Mixtures of Plant Protection Products

In *Apis mellifera*, the mixture of acephate and oxamyl had less impact on esterase and GST activity than a single pesticide application. On the other hand, the effect of acephate in the composition with clothianidin, tetraconazole, glyphosate, lambda-cyhalothrin, or chlorpyriphos did not differ significantly from treatment with a single pesticide [44]. A mixture of imidacloprid with clothianidin or tetraconazole increased esterase activity. The effect of these mixtures was stronger than a single pesticide treatment. Imidacloprid in combination with lambda-cyhalothrin, oxamyl, sulfoxaflon, or glyphosate did not affect AChE, esterase, and GST in a different manner than a single pesticide [30]. Lambda-cyhalothrin and abamectin reduced the toxicity of thiamethoxam. The mixture of these pesticides had less impact on PPO, AChE, and GST activity than a single pesticide treatment. In addition, a combination of thiamethoxam and beta-cyhalothrin had less effect on ALP activity than single pesticide administration [20]. In *Apis cerana* mixture of propiconazole and acetamiprid caused an increase (after 1 day) or a decrease (after 10 days) in P450 activity [46].

5. Potential Effects of Pesticides on Honey Bee Biology

5.1. Introduction

PPPs may alter detoxification, antioxidant, and immune-related biochemical mechanisms in honey bees, which impact the functioning of their entire organism. Many studies confirmed that pesticides caused an increase or decrease in enzyme activity and changed the content of some crucial substances (e.g., ATP, proteins, and glutathione) [10,11,20,24–30,37,41,42,46,47,52,61,62]. However, the reference values were not estimated [65]. There are many limitations in the interpretation and comparison of the results of biochemical studies on honey bees. Workers differed in age in an individual study (physiological parameters and sensitivity to pesticide change with the bee's age [65,66]). In addition, various materials are collected for analysis (e.g., hemolymph, head, and intestines) while the content and/or activity of indicators may vary in different tissues and organs [28–30]. Pesticides used in studies had a wide range of concentrations or doses; they were applied in different manners and for various exposure times, which is also an important issue in interpreting the results.

5.2. Effects on Detoxification and Antioxidation

Changes in the activity of detoxification enzymes may result in the accumulation of harmful substances in the honey bee's body. Lower activities of the ALP, ALT, and AST enzymes in bees may impair, among others, the ATP synthesis, Krebs cycle, oxidative phosphorylation, and beta-oxidation. Disorders in the antioxidant system may cause toxic effects through the production of peroxides and free radicals, causing oxidative damage to all cell components. The damage to proteins, lipids, and DNA is particularly severe for the cell.

5.3. Effects on Immunity

Changes in hemocyte numbers may contribute to weakened phagocytosis and encapsulation response. Phagocytosis allows the engulfing of pathogens and infected cells, which reduced the spread of infectious agents in organisms. During encapsulation, the

bound hemocytes isolate pathogens and neutralize them by anoxia, toxic reactive oxygen species, or starvation [67]. Alteration of the transcription factor NF-κB can cause disturbance in inflammatory responses [68]. Changes in the activity of proteases may result in the poor extracellular or intracellular digestion of proteins, disruption in processes of zymogen activation, the reduced release of hormones and proteins, and hindered translocation across membranes. It may weaken the immunity and resistance to varroosis and nosemosis [69,70]. An especially important function of proteases (more precisely serine proteases) is the activation of the proPO cascade, which is responsible for melanization and sclerotization of the cuticle and participates in immunological processes [71,72].

5.4. Effects on Behavior

Changes in AChE activity after pesticide exposure can indicate malfunction of the nervous system. AChE hydrolyzes Ach, which is a major neurotransmitter associated with learning in the insect brain [73]. Changes in AChE activity may affect the learning ability and memory [73–75]. Disorders can impact, among others, navigation skills and olfactory learning which are necessary for searching the food and returning to the hive [76,77]. Moreover, alteration in AChE activity may affect grooming behavior, motor function, and cause abdominal spasms [78].

5.5. Effects on Individual Development

Serine proteases participate in regulatory cascade pathways. Changes in protease activity may affect individual development [79]. Royal jelly produced in the hypopharyngeal glands of nurse bees contains trace amounts of ACh, which are necessary for the proper larval development [80]. Changes in the level of AChE may result in developmental impairments [80,81].

5.6. Effects on Colony Strength

All of the mentioned changes at the molecular level may cause dysregulation in the functioning of the bee's organism, which may manifest itself in behavioral changes, disease resistance, development, production of brood, and finally the weakening of the colony condition and performance. Although most of the studies involved honey bee workers, PPPs are not indifferent to queens and drones [10,18,19,25,29,32,33,47,48,55]. PPPs altered the activity of detoxification, antioxidant, and immune-related enzymes and reduced sperm concentration and viability, which altogether can result in reduced quality and quantity of the bee brood, thereby weakening the bee colony. A strong colony with a sufficient number of individuals is able to provide the right amount of food and take care of the microclimate and hygiene in the hive.

6. Conclusions

The activity of individual enzymes after treating bees with PPPs differs depending on the dose, method of administration, duration of exposure, and type of the sample for analysis. PPPs may disturb the honey bees' physiology and their effects can vary even if they belong to the same chemical group. Most studies on the biochemical effects of PPPs on honey bees focus on insecticides, especially neonicotinoids, and among them imidacloprid. Cyano-substituted neonicotinoids (acetamiprid and thiacloprid), pyrethroids, and other insecticides are less common subjects of research on the honey bees' biochemical markers. However, results showed that these insecticides affects honey bee biology. The biochemical effects of fungicides and herbicide (glyphosate) in honey bees were the subject of several studies. Although they are considered less toxic, fungicides and herbicides may affect honey bees. The vast majority of research is carried out on *Apis mellifera*. It is worth emphasizing that results indicated that different physiological mechanisms occurred in *Apis mellifera* and *Apis cerana*. Therefore, findings on *Apis mellifera* physiology should not always be applied to *Apis cerana*. A small part of the research involves drones and queens.

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