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# Pesticide Impact on Honeybees Declines and Emerging Food Security Crisis

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## Abstract

Bee crisis is threatening worldwide food security. Pesticides are extensively used in the agricultural zone. Unfortunately, these pesticides cause severe toxicity toward pollinators than the target pests such as honeybees. This review summarizes the different studies related to pesticide hazards of bees. This paper reported risks of pesticides neurological and physiological poisoning toward honeybees. Pesticides act as poison and ruin vital functions involved in learning and cognition, behavior and, the body physiological mechanisms. Many laboratory and field research data evaluated the lethal and sub-lethal poisoning on bee foraging dance, learning, and memory abilities of honeybees. Insecticide residues are detected in bee bodies and LD<sub>50</sub> and LC<sub>50</sub> values evaluated. It is also studied that in honeybees systemic insecticide residues and, its metabolite adulterated in their body during foraging activities. Similarly, pesticide-contaminated food stored in a hive consumed continuously by honeybees may cause sub-lethal toxicity effects. Which causes anomalous bee social behavior and ultimately leads to colony collapse disorder. If population of pollinator decline it will disturb the food chain and leads to food crisis. This review emphasized causes of bee decline with the emergence of pesticides in agricultural domains.

**Keywords:** Honeybee (*A. mellifera*), Pesticide poisoning, Pollinator decline, Route of exposure, Lethal effects, Sub-lethal effects

## 1. Introduction

Honeybees are the most important and economically dominant pollinator species for food crop production over the global [1]. It has been studied that 87.5% of flowering and edible plants are pollinated by animals such as honeybees. Honeybees also produce wax, honey, and venom. Economic global estimate concluded that a global economic value of €88bn was associated with bees pollinating crops and products [2]. Afterward, this is revised and estimate and recorded an increase of the economic value of €115bn [3]. This raising trend highlights the dependence upon pollinators in the global food supply. However, researchers noticed the disappearance of bees and their population has been decline [4]. Most important crops, fruits, vegetables, and fodder crops are badly affected by the decline in the honeybee population. Researchers have great concern about the honeybee population decline [5, 6]. In the United States 30–40% disappearance of the honeybee colonies attributed to colony collapse disorder A disease syndrome described as the

sudden and speedy loss of honeybees population [7]. In the United Kingdom, 54% honeybee population lost in the last decades [8]. China has 6 million bee colonies of honeybees. Chinese beekeepers have faced inexplicable colony losses and a decline in the bee population [9]. Various factors such as biotic (parasites and pathogens) and abiotic (climate changes, habitat destruction) are blamed for this bee decline [10]. *Varroa* mites are a serious threat to apiculture globally. *Varroa* mites feed on bee hemolymph and are linked to winter loss of bee colonies. In Germany, it was found that *Varroa* infestation, viral and bacterial infections were associated with winter loss of honeybee colonies [11]. Similarly microsporidium also a worldwide honeybee pathogen [12]. Climatic factors such as increasing temperature, variation in rainfall patterns, and other extreme weather conditions have drastic effects on bees. Changing climatic conditions affect the interaction between pollinators and plants by changing the period of flower blooming [13]. Among various agents, pesticides are the most obvious and significant agent toward the loss of honeybees. They are directly responsible for bee poisoning and bee death [14]. This paper focusing on the (1) pesticide toxicity toward honeybees and route of exposure (2) occurrence of insecticide residues in pollen grains, bee bodies, hive wax, and honey (3) lethal and sub-lethal insecticide toxicity on honeybees. Various laboratory studies described the lethal and sub-lethal pesticide toxicity on honeybee learning, memory, food foraging and, physiological function. While few studies were observed in field studies. This chapter will emphasize the causes of bee decline with the emergence of pesticides in agricultural domains.

## 2. Plants preference for honeybees

Several plant species depend on insect pollinators for seed set honeybees consume pollen and nectar of sunflower, oilseed rape, maize, etc. These plants can possess threatened honeybees if they are treated with systematic pesticides. Bee mortality is observed when bees contact with pesticide treated plants and consume flower pollen and sap plant or nectar e.g. sunflower, *Helianthus annuus* L. [15, 16], oilseed rape, *Brassica napus* L. [17], and *Phacelia tanacetifolia* Benth. [18, 19] which bees consume [20]. It is also observed that pesticide effects on pollinator reproductive success and plant seed set potential. In Canada forest plants were treated with Matacil (amino-carb insecticide) to control spruce budworm. After pesticide application, foraging honeybees reduce, and high mortality was observed. Shortly, afterward as honeybee population reduce a large number of native plant diversity adversely reduce such as lilies flowers showed a reduction in fecundity. Commercial blueberry fields also suffered reduce fruit sets when adjacent lands were sprayed with pesticides.

## 3. Multiple routes of pesticide exposure

An ultimate issue of pesticide poisoning to honeybees is the assessment of the exposure scenario. Honeybees are exposed to insecticides through the following routes.

### 3.1 Exposure via direct contact

Honeybees form direct contact with pesticides. The most common way of pesticide exposure is the aerial spray. They are straightly contaminated while foraging during spray treatment in a field. Treatment also influences by wind drift. Pesticide droplets made direct contact with the honeybee body in the air. In 2003 it was observed that the bee population decline due to direct contact of dust emitted from pneumatic drilling

machine used to plant neonicotinoid coated seeds [21]. Bees flying over sowing fields directly exposed to pesticides at lethal levels especially under humid conditions. In Humid environments pesticides, dust particles stick to the bee abdomen [22]. Herbicides and fungicides were directly applied to the soil before sowing of crops and most insecticides were applied on crops through aerial spraying. Pesticide aerial droplets and dust pesticide particles can make direct contact with flying honeybees and create toxicity. Fine droplets may also carry hundreds of meters from treated site to untreated sites with wind, and cause bee mortality. In addition to food, bees make direct contact with pesticides during water drinking. Pesticide residues from nearby treated fields leach into the water resources or mix with water by drifting from spray applications. Honeybees drink water from trenches, water pools, and rivers, if these water resources are polluted with pesticide, the foraging bees will ingest them.

### **3.2 Exposure via indirect contact or ingestion of pesticide residue**

A systematic characteristic of pesticides provides the translocation of pesticide residues inside all plant tissues, phloem, and nectar. Consequently, honeybees are likely to be vulnerable to the pesticide while feeding on pollen grains and flower nectar [23]. The pesticide used for seed dressing can also possess a noticeable threat to pollinators as they are transported through the plants and contaminate the pollen [17]. In the same way, Early morning when plants produced guttation drops and systematic pesticides appear in such guttation drops in a dose that are lethal to bees [23]. Several studies showed the existence of insecticide residues in seeds, pollen, and nectars. The pesticide used for the treatment of seeds can be transported throughout growing plants and contaminate the nectars and pollens. The presence of imidacloprid residues (3 µg/kg) in pollen grains has been reported in Gaucho seed dresses sunflower [17]. Similarly, in France, a survey study of pesticide residues in pollen loads has been conducted. A survey report was showing the presence of imidacloprid and its metabolites nicotinic acid (49% of 81 analyzed samples), Fipronil, and its metabolites fipronil sulfone and fipronil disulfonil (12% of 81 analyzed samples) [24]. Freshly stored pollen and bee bread are considered to be the principal source of in-hive contamination for adult and larvae bees [25]. In the same way, miticides and fungicide residues are well reported in pollen grain [26]. In Germany, thiacloprid was the extensively abundant pesticide as study results show that it was present in about 33% of the pollen samples at concentration levels up to 199 µg/kg [12]. It is investigated the level of pesticide in the droplets exude by the treated plants [27]. Three different systematic pesticides (imidacloprid, clothianidin, and thiamethoxam) and on the non-systematic pesticide (fipronil) were tested in field and lab trials. Analysis of drops showed that systematic pesticide concentration near to that applied in field sprays. Systematic pesticides found in pollen samples and guttation drops compared to nonsystematic pesticides. In apiaries across Spain, both acaricides and agricultural pesticides were found in beebread. Cypermethrin, deltamethrin, and chlorpyrifos were found at sub-lethal levels [28]. In Slovenia, honeybee colonies present near insecticides treated apple orchards showed insecticide residues in beebread [24]. Pollen samples were collected from different areas of North America. About 5.4% of pollen samples were contaminated with thiacloprid and acetamiprid. Similarly, about 1.9% of bee wax samples were contaminated with thiacloprid [29].

## **4. Chronic and acute toxicity**

Chemical pesticide hazards are stated as acute and chronic toxicity. In acute poisoning, the toxic reactions are more violent and occur unexpectedly. Honeybees

Pesticide	Exposure route	Toxicity LD <sub>50</sub>	References
Imidacloprid	Direct contact	18 to 104 ng/bee	[36]
Imidacloprid	Ingestion	4 to 60 ng/bee	[19, 37]
Imidacloprid	oral routes	0.0037 µg/bee	[38]
Imidacloprid	topical application	0.081 µg/bee	[37]
Clothianidin	Oral route	21.8 ng/bee	[34]
Dinotefuran	Oral route	75.0 ng/bee	[34]
Nitenpyran	Oral route	13.8 ng/bee	[34]
Fipronil	Oral route	3.45–3.86 ng/bee	[39]
Thiacloprid	Contact and Oral route	24.2 µg/bee	[40]
Acetamprid	Contact route	7.07 µg/bee	[34]
Oleofin	Oral route	50 µg/bee	[16]
Thiamethoxam	Oral route	0.004 µg/bee	[38]

**Table 1.**

Reported lethal effect toxicity of different pesticides toward honeybee workers.

usually die from contact and ingestion of pesticides [30]. While in case of chronic exposure pesticide absorbed in small and repeated doses, the reaction usually slow but continuous, they may lead to the elimination of the entire colony [31, 32]. Insecticide toxicity generally measured using LD<sub>50</sub> values (Exposure concentration that create death of 50% of the exposed treated population [33]). Pesticide chemical toxicity thresholds for honeybees are generally set as high toxicity (acute LD<sub>50</sub> < 2 µg/honeybee), Moderate toxicity (acute LD<sub>50</sub>: 2–10.99 µg/honeybee), Slightly toxic (acute LD<sub>50</sub>: 11–100 µg/honeybee), Non-toxic (acute LD<sub>50</sub> > 100 µg/honeybee). According to the European regulation on risk valuation of pesticides on honeybees, the toxicity is evaluated by acute LD<sub>50</sub> and LC<sub>50</sub> values. These two parameters measure pesticide poisoning by calculating the number of dead bees that die after 24 hours of treatment. Therefore, the research and knowledge of pesticide toxicity on non-target species is vital to eliminate various agricultural pests without hurting the bees [34, 35]. **Table 1** shows reported LD<sub>50</sub> values of various pesticides toward honeybees.

## 5. Lethal and sub-lethal pesticide toxicity on honeybees

### 5.1 Lethal effects

Pesticide lethal toxicity is the major cause of honeybee decline. Detection of pesticide residues inside the honeybee body is a measure of pesticide poisoning to estimate lethal effects. Pesticides possess high toxicity due to specific mode of action. Neonicotinoids show delay toxicity with sub-lethal effects at low concentrations [11] or they cause the killing of bees if exposed for a long interval of time [41]. Similarly, neonicotinoids and fipronil suppress the immune system and vulnerable them to various infections such as *Nosema* infection [42] and the outbreak of *Varroa* mites. The lethal effects of certain insecticides are enhanced in the presence of fungicides, which act as synergists. These synergistic mixtures inhibit the bee detoxification system. In another study pesticide residues inside honeybee bodies. About 11.2% of honeybee samples were contaminated with imidacloprid, while in about 18.7% samples the dominant metabolite 6-chloronicotinic acid was detected.

Pesticide residue analysis is done to determine bee poisoning [43]. In the UK fungicide and herbicide residues are found in bee bodies. A study carried out by using gas chromatography along with tandem mass spectrometry for the detection of insecticides impurities and its metabolites in honeybees. About more than 200 insecticide and insecticide metabolites were found in tested honeybee body. Clothianidin ranging from 0.5 ng/g- 1.0 ng/g was detected in poisoned honeybees. This indicates the cause of honeybee death, similarly imidacloprid and thiamethoxam residues found in poisoned honeybee [44]. The detected imidacloprid concentration ranging from 0.3 ng/g-240.6 ng/g while thiamethoxam was detected in concentration ranging from 0.5-275 ng/ [45]. Similarly, a second abundant pesticide is a chlorpyrifos in tested honeybee samples. 1.5 ng/g concentration detected in tested honeybee bodies [46]. In Poland during early autumn there was a considerable decline in the honeybee population, it was not having any historical with spray application. An investigation confirmed that these toxic hives were located near cauliflower and brassica fields. The EU allowed seed treatment with sowing. These pesticide residues present in the guttation drops and nectar enter honeybee bodies during foraging activities [28].

## 5.2 Sub-lethal toxicity

Toxic effects of insecticides can be evaluated as sub-lethal toxicity when exposure to the insecticide does not induce death in the experimental population but may disturb biochemical, physiological, and behavioral changes such as by impairing foraging and learning behavior or disrupting another aspect of neurological and physiological functions [43]. Bees exposure at sub-lethal doses also have a negative impact on flower and scent recognition, the spatial orientation of bees, perturbations of the foraging patterns of the honeybee by disturbing navigation memory. In an experimental study, it was demonstrated contact of the honeybees to sub-lethal concentrations of thiamethoxam cause impairment of memory, brain and gut functions which ultimately lead to a shorter lifespan [47]. In laboratory analysis, sub-lethal concentration of deltamethrin disturbed body functions in honeybee at cellular level such as by causing marked dysfunctions in cardiac cells by changing frequency and muscle contraction [43]. The respiratory system is also badly affected after exposure to pesticides. Imidacloprid at sub-lethal exposure created marked changes in the respiratory pattern of bees and also in hypopharyngeal glands growing smaller in size as compared to untreated bees. Similarly, mobility behavior also affects after exposure to low doses of imidacloprid.

Negative impacts on mobility are dose-dependent and change with time [48, 49]. Honeybee foraging and spatial orientation is totally depend upon visual remembering or learning of landmarks. During foraging trips, bees memorized landmarks and used to navigate nectar and pollen sources as well as to communicate accurately to the rest of the colony members about direction of food source and its distance from hive. Exposure to pesticides disturbed the learning of visual landmarks during foraging round trips and the transfer of this information to other bees of the hive. The pyrethroid such as deltamethrin has been studied to change the foraging round trips of worker bees after treated topically with sub-lethal concentrations [50]. Bees exposed to the thiamethoxam treated pollen and nectar under semi-field conditions seem to reduce foraging trips and lost their ways. As a result, the colony becomes weaker and putting it at greater risk of collapse [31]. Similarly, fipronil applied topically at low doses to honeybees reduces their ability to sense sucrose smell by about 40% relative to the capacity of untreated bees [37]. Neonicotinoid pesticides have a toxic effects on the queen bee. They affect queen bee life span and egg-laying capacity. Thus colony size reduced and lead to colony collapse. Pesticides also repel

Pesticide	Sub lethal toxicity	Reference
Deltamethrin	Cardiac dysfunctions in the heart cells	[43]
Imidacloprid	Shorter queen life span and reduced egg laying capacity and colony size, Disrupt development	[52]
Imidacloprid	Mobility and olfactory behavior	[51]
Deltamethrin	Homing trips disturbed	[53]
Thiamethoxam	Colony become weaker, affect foraging behavior, Impair brain and gut functions	[31, 50]
Fipronil	Reduce capacity of bees to detect food sources, olfactory learning and memory	[43, 54]
Diazon	Reduce reproduction and survival of queen, colony maintenance and foraging behavior.	[37]

**Table 2.**  
*Reported sub lethal toxicity of various pesticides toward honeybee.*

the pollinator from treated crops. If pollinators avoid foraging the pesticide contaminated crops and flowers this would adversely influence crop yields dependent upon the pollinator for pollination [51]. **Table 2** showed the sub-lethal toxicity of various pesticides toward honeybees.

## 6. Neurotoxic action of pesticides on bees

Pesticides react at their molecular target site, which may cause to effects impairing behavior and body physiology. Mostly pesticides act as a neurotoxin and disturb normal neurological function. Pesticides showed their lethal and sub-lethal poisoning by acting as an acetylcholinesterase inhibitor, nicotinic acetylcholine receptor agonists, voltage-gated  $\text{Na}^+$  channel agonists, and GABA gated  $\text{Cl}^-$  channel agonist.

### 6.1 GABA gated $\text{Cl}^-$ channel agonist

Fipronil is a phenyl pyrozal insecticide class. When bees are exposed to fipronil reached to target site in brain and binds to the GABA gated  $\text{Cl}^-$  channels, it prevent the  $\text{Cl}^-$  channels from closing and maintained it in open. This state leads to hyperpolarization and the inability to transmit action potential. Phenyl pyrozal is a systemic insecticide and is detected in pollen at 1-4 ppb concentration [53].

### 6.2 Nicotinic acetylcholine receptor agonists

Nicotinic mimics the action of acetylcholine, neurotransmitter, inside the body it react with nicotinic acetylcholine receptor (nAChR) and bind to receptor. After binding it stimulate the repeated generation of action. The new class of pesticides neonicotinoids is synthetic analogs of nicotine with a higher affinity for the nAChR in the bee brain [16]. A study showed that neonicotinoid pesticides such as imidacloprid, clothianidin, and thiamethoxam are highly toxic to bees with acute  $\text{LD}_{50}$  from 0.004 to 0.075  $\mu\text{g}/\text{bees}$  [55]. Imidacloprid metabolites such as 5-hydroxyimidacloprid and olefin have a high affinity for the honeybee nAChR [16]. Another neonicotinoid insecticide thiamethoxam also has affinity for nACh receptors, however, thiamethoxam rapidly degraded in to high affinity clothianidin metabolites. All neonicotinoid pesticides damage the capability of worker bees to forage and go back to the hive [56].

### 6.3 Acetylcholinesterase inhibitor

Organophosphate and methylcarbamate pesticides behave as neurotoxin and inhibit the AChE enzyme activity. AChE enzyme deactivates the acetylcholine neurotransmitter at neuron synapses [57]. Neuro pesticides have wide range of bee poisoning ( $LD_{50} = 0.018\text{--}31.2 \mu\text{g}/\text{bees}$ ) [58]. In United State, about 117 bee poisoning incidents were investigated between 1994 and 2003. The maximum number of these poisoning incidents attributed to dimethoate and bendicarb [59]. Similarly, coumaphos is an organophosphate that is used by beekeepers against varroa mites [60] When beekeepers repeatedly used coumaphos it is concentrated and build up in the colony wax [61]. Poisoning of bee colonies with coumaphos is linked with significant mortality of bee queens and worker bees. It studied that bee larvae raised on food contaminated with 8 mg/L coumaphos were showed more mortality rate than control larvae [62].

### 6.4 Voltage-gated $\text{Na}^+$ channel agonists

A pyrethroid is an extensively used synthetic insecticides. Pyrethroids and DDT are organophosphate. They are neurotoxin and their target site inside brain is voltage-gated  $\text{Na}^+$  channels in the axon of the nerve cell. At their target site pyrethroids delay the closing of  $\text{Na}^+$  channels and prolonged the recovery period after generation of the action potential. The pyrethroid flumethrin and tau-fluvalinate are extensively used to control *varroa* mites, it may be accumulated in bee wax as high as 200 ppm and cause bee death [63].

## 7. Behavior disturbs by pesticide exposure

### 7.1 Habituation

Habituation is a learning behavior that can be explain as steady reduction in the frequency of response to a repeated or useless stimulus. Habituation enable an individuals to tolerant the repeated stimulus and save energy. It is a predominantly vital practice for the honeybees because it permits them to escape both unusable and tedious stimuli. Thus, save time and energy. In honeybee habituation behavior involves the proboscis extension response (PER) stimulated by an antenna touch with a sugar solution. The frequency of antennal contacts with sugar solution calculated to perceive a detention of proboscis extension. Honeybee exposed to neonicotinoid pesticide at sub-lethal concentrations such as imidacloprid enhanced the PER habituation [51]. In another experiment, seven to eight days olds bees were used to elicit antennal receptor stimulations at shorter time intervals [64]. In this research trial approximately 7 and 8 days old bees were used, because in this age bees started their short orientation flight. At start of the exposure (start one hours), imidacloprid upsurges the number of trials required to attain habituation in 7-day-old bees but declines it in 8-day-old bees. As bees become aged or older the habituation behavior become more refine with time. The habituation profile obtained with imidacloprid evolves with time particularly. The development of the habituation learning with time due to the action of imidacloprid metabolites olefin and 5-hydroxyimidacloprid that pause and increase habituation, respectively [63]. These results proposed the presence of two different nicotinic acetylcholine receptors (nAChR), that are differentially expressed as young bees mature.



## **7.2 Disturb learning and memory**

In social insects honeybee learning and memory is an important behavior for absolute adaptation of the individual to their environment. This behavior enables the honeybees to fulfill the colony requirement. Various pesticides have lethal and sub-lethal toxicity on the honeybee brain and interrupt honeybee learning and memory behavior. A sub-lethal dose of parathion induced an alteration in time of foraging to the early morning. This modification in foraging time can be elucidated by the alteration in the circadian clock [65]. In one study toxic effects of OP methyl-parathion on honeybee foraging, visual and olfactory tasks have been described [66]. Since methyl-parathion is recognized as an acetylcholinesterase inhibitor. The methyl-parathion toxicity stimulated actions has been understood in terms of the advance of cholinergic signaling by inhibiting AChE. Proboscis extension reflex (PER) responses have been studied to evaluate the honeybee learning and memory process. This allows the path to streamlined laboratory procedures and studied the various memory phases. Bees exposed to sub-lethal doses of pyrethroid through tarsal contact may show a weakening of the conditioned responses, which point out that during foraging activities pesticide residual contact impair learning and cognitive process [67]. In another laboratory test, the honeybees were feed on different pyrethroid insecticides contaminated sucrose solution for 11 days. It was concluded that deltamethrin impairs the proboscis extension responses, whereas  $\lambda$ -cyhalothrin, cypermethrin, and  $\tau$ -fluvalinate did not disturb behavior patterns. This demonstrated that lethal toxicity induced by pesticides is more substance-specific than family-specific. Similarly, under a semi-field environment, the presence of deltamethrin prompts a significant decrease in the honeybee foraging trips, which is retreated when the pesticide contact terminates. Results inferred that deltamethrin was considered to be lethal in this study. Similarly, in another study both lethal and sub-lethal doses of imidacloprid elicits a reduction in learning activities, studied by the conditioned PER responses [68]. This effect also depends on the seasonal pattern, as results showed that during the summer season honeybees are more sensitive to imidacloprid, compared to in winter. In the semi-field experiment, the imidacloprid residues in foraging plants generate a considerable decline in the foraging bees. The decline in the foraging bee population is linked with a falling-off of olfactory and learning behavior. T-tube maze is a behavioral approach that is associated with learning by integrating visual and spatial orientation. Honeybees that are exposed to sublethal doses of imidacloprid in a T-tube maze showed a considerable reduction in visual learning capacity and diminished olfactory responses in the PER assay [69]. The toxicity of fipronil on honeybee learning and memory behavior has also been explained by olfactory conditioning of the PER. Sub-chronic exposure of bees to fipronil causes a considerable decrease in the learning process [70]. Results showed that cuticle contact of a sub-lethal dose weakens the olfactory memory whereas a lethal dose administration through thorax changes antennal tactile learning [71].

## **7.3 Olfaction and gustation**

Olfaction and gustation are vital senses in the life of the honeybee colony both at the individual and colony levels. Both responses are trigger and processed at the neural level [69]. They are intricate in odor and sense of taste recognition which is required for the bee to forage and visit flowers, identify foreign bees in the hive, forager workers, social communication for social cohesion of the colony and, recognize allelochemicals and nectar in plants [72, 73]. Pesticides may disturb the honeybee olfactory and gustatory senses by disturbing physiological processes control scent recognition or other neuronal signals [74, 75]. The toxicity of pesticides on gustation

can be estimated by reviewing the gustatory threshold modules, which is defined as the minimum dose of a sugar solution make contact with antenna and able to elicit a PER. Bees topically treated with imidacloprid show an escalation of the gustatory threshold [51]. This effect is upsurges with time. This contrasts with acetamiprid that upturns sensitivity of the antennal stimulation sugar solution by oral route. Thiamethoxam induces no toxicity on sugar sensitivity at the lowest concentration [76] and a reduction in sucrose stimulation detected at higher concentrations [72].

#### **7.4 Navigation and orientation**

Navigation and orientation are important which enable the honeybees to collect the food vital for colony development. It is an integrative component of vision, olfaction, orientation and signal treatment [77, 78]. Navigation involves the food source location communication with other colony members, chiefly when foraging site located at a distance of more than 100 m. The influences of insecticides on the foraging communication dance have been studied in various experiments. Parathion avoids the honeybees to share the correct location of the foraging site by shifting the wrong angle during communication dance. Forager bees showed shorten distances through an intensification of waggle dance rhythm, with no alteration on horizontal and vertical combs. The foraging communication error may be due to a disturbance in neural function [54, 79]. The homing flight is the flight made by the bees from the foraging sites to the hive location. It is a return flight. This flight pattern is used in evaluating the adverse sub-lethal effects of pesticides on honeybees. Deltamethrin hampers return flight or homing activity behavior. Various approaches have been developed for studying honeybee movements and flight in diverse space scales and dimensions [80]. Harmonic radar and radiofrequency identification are two approaches that appear appropriate for investigating navigation and return flight to home. In harmonic radar approach bees are fitted with a transponder to analyze movement in the landscape. This approach allowing researchers to monitor free-flying bees [81]. This approach is utilized to detect specific behaviors of orientation and navigational flight, particularly trip duration related with flight speed that escalate with time [82] and to establish the use of map-like spatial memory in honeybee navigation [83]. Another approach is based on radiofrequency identification (RFID) [84]. Honeybees are tagged with a passive RFID microchip that releases a specific individualized radio signal after activation by a radio frequency. RFID technique widely applied to evaluate the foraging behavior and the circadian foraging rhythm of bees [85]. This technique has been shown that fipronil leads to a decline in the number of individual foraging flights and increased the homing flight time. In the same way contact of imidacloprid and clothianidin cause a lessening of foraging activity.

#### **7.5 Foraging**

Foraging behavior important to maintain the food supply in honeybee colony and so determine honeybee colony's survival and proliferation fortune. Repellent pesticides or anti-feedant usually modify bee foraging behavior, However, prolong exposure to it causes colony starvation and nutritional deficiency. Carbamate and Organophosphate pesticides disturb cholinesterase enzyme i.e. acetylcholinesterase, a vital enzyme control nerve impulse transmission. Organophosphate fenitrothion causes an intense reduction in the foraging bees on flowering. When bees make acute contact with organophosphate, the toxicity effects are more intense [86]. Infield, a reduction in the food at visiting site could simply be elucidated in term of repellent effect. Neonicotinoid insecticides such as imidacloprid show a

moderate toxic action [87]. Toxicity of imidacloprid comprised a reduction in the number foraging trips, less number of active bees at foraging sites, an enhanced in time intervals between visits, irregularities in communication or waggle dance, and disrupt in visual learning and navigatio. This demonstrates that the excessive stimulation of the acetylcholinesterase, inhibition of organophosphate or an agonist action on nAChR, with neonicotinoids [88, 89].

## **7.6 Muscle activity**

Muscle contraction is important in almost all physiological functions and plays an important role in the accomplishment of tasks such as communication dance, flight, digestion of food, and heart and wing thrashing. The pesticides effects on the muscles of honeybees have been studied. In the honeybee research has been carried out on deltamethrin and prochloraz fungicide. In a laboratory study a semi-isolated heart exposed to prochloraz and deltamethrin diluted solutions showed a rapidly reduction in the frequency and the cardiac contractions force [90]. Results indicated that prochloraz seems more cardiotoxic than deltamethrin. Deltamethrin is a neurotoxic substance whereas prochloraz is an inhibitor and an inducer of cytochrome P450 enzymes, which are involved in the detoxication and metabolism of xenobiotics. An association of prochloraz and deltamethrin provokes synergistic effects that entirely and quickly stops heart muscle contractions, thus endorsing the neural basis of the deltamethrinprochloraz lethal and thermogenic synergy. Phenoxyacetic herbicide 2, 4-D also prompts parallel effects. Conversely, triazine herbicides subsidize tranquil neurotransmitter release that results in a boosted frequency and cardiac contractions force [91, 92]. Pyrethroids toxicity correlated with action on voltage-dependent sodium channels. In fact, pyrethroid pesticides and azole fungicides combine together and produce synergistic effects, and disturb normal functioning of target cells such as ATPase and potassium and calcium channels, that play a vital role in muscle and nerve activity [93, 94].

## **7.7 Thermoregulation**

Thermoregulation is a phenomenon in honeybees to regulate their body temperature. Thermoregulation comprises thermogenesis by unremitting flight muscles contraction, fanning and beating wings, and evaporation of water at the individual as well as colony level. The thermoregulation allows the bees to fly at temperatures ranging from 11–46°C and heat their swarms and their broods. It is also plays a vital role in the exchange of information during foraging, waggle dnce, social communication and speedy nectar processing [95, 96]. Insecticides disturbed the normal thermoregulation process. Pyrethroid pesticides and azole fungicides (imidazoles and triazoles) usually act synergistically to prompt lethal toxicity. Azole fungicides act as both inhibitors and inducers of cytochromes P-450 enzymes involved in the metabolism of xenobiotics [97]. This initially suggested the action of azole fungicides on pyrethroid metabolism. Pyrethroids display a negative temperature coefficient below 28–30°C and induce higher toxicity at low temperatures. Honeybees were exposed to sub-lethal doses of deltamethrin to evaluate the toxicity. It was concluded that sub-lethal concentrations of deltamethrin triggers extreme hypothermia conditions for about 4 h [98]. Similar organophosphate also created thermogenesis process impairment by inhibiting acetylcholinesterase. These finding suggested association of a cholinergic pathway in the negative control of thermogenesis. Deltamethrin exposure induces hypothermia and leads to disruption of homing flight during foraging. The results of these study showed that condition of hypothermia created after impairment of muscle contraction

rhythms and disturb nerve impulse process. Hence, honeybees would be capable of coordinated flight muscle contraction but would not be able to perform the neural program to attain shivering. Hypothermia become more severe in winter and spring when temperature already low and necessitating efficient thermogenesis [99].

## **7.8 Effects on reproduction**

Reproduction is an important natural phenomenon that guarantee the existence and proliferation of the colony population. Indeed, a loss of reproductive brood consider as more detrimental for the bee colony than the loss of older bees (foragers). In hive, bees showed eusocial perform division of tasks to maintain the colony. Worker bees accomplish numerous chores throughout their lifespan. A few studies have revealed the adverse effects on larval development after exposure to pesticides [100]. It is evaluated a delay in hatching and development of honeybee when fed with imidacloprid contaminated food.

## **8. Minimizing honeybee exposure to pesticides**

The threats to pollinators are significant and complex. Managing all threats in an integrated way will be an immense and fundamentally necessary task. Transforming the current chemical-intensive agriculture system into an ecological farming system will have much positive impact on the environment, pollinators, and human food security. However, these methods are often neglected as potentially effective tools for protecting the bee population. A recent study in Sweden clearly showed how strawberry crops benefited from organic farming. Organic strawberries received more pollinators and achieved higher pollination success than conventionally grown strawberries, and this difference was evident quickly after the conversion from conventional to organic farming. The authors concluded that organic agriculture benefited crop pollination in terms of both the quantity and quality of the yield [99]. Diversified farming systems, like those under organic or ecological production methods, bring out many benefits in addition to increased pollination services; they enhance the control of weeds, diseases, and insect pests. However, these systems have received significantly less public funding for research as a means of improved management, compared to conventional farming systems. This lack of support is remarkable, given that ecological and organic farming systems can produce approximately the same amount of food and profits as conventional farming while generating far less environmental and social harm [101]. More public and private funding are needed for research and development on ecological farming practices that enhanced ecological services, alongside food production and environmental protection, while at the same time helping social and economic development.

## **9. Food security crises due to honeybee decline**

Scientists accepted that a world without honeybees would have a critical situation of food shortages and possibly leads to famine. There is much debate over whether Albert Einstein once said, that “if bees disappeared off the face of the Earth, humans would have only four years left to live. If all bees completely vanished, it would not cause humans to go extinct”. Among all the edible crops that fulfill about 90% of food requirements throughout the world, among them 70% are pollinated by honeybees and other pollinators. Pollinators also improve the quality and shelf-life of crops and, also increase the genetic variability. It is estimated that the economic value of

insect pollinating crops is about 761 € per ton [102]. Further, the majority of calories of human bodies also fulfill from insect-pollinated plants. The decline of the pollinator population cause decrease of a substantial amount of high economic values crops and fruits. These crops are a key element of the majority of calories and vitamins. In terms of nutrients in the human diet, they account for more than 90% of vitamin C, 100% of Lycopene and almost 100% of the antioxidants  $\beta$ -cryptoxanthin and  $\beta$ -tocopherol, the majority of the lipids (74%), vitamin A (>70%) and related carotenoids (98%), calcium (58%) and fluoride (62%), and a large portion of folic acid (55%). In total, pollinator-mediated crops account for about 40% of the global nutrient supply for humans [103]. Pollinators are essential to fulfill all nutrient requirements as they are responsible for essential crop pollination. If all the insect pollinators kill it would lead to a drastic decrease in fruit setting and growth of edible crops, which would affect all the population that is depending on it. It would also disturb the herbivores population that depends upon plants, which in turn disturb carnivores. In simple whole food chain will be disturb. Honeybees are under great threat due to the combined effects of global warming, intensive agriculture practices, habitat loss and, insecticide uses. FAO's Director-General said that the absence of honeybees and other pollinators will lead to wiping out coffee, orange, apple, peaches, tomatoes, and other cereal crops that rely upon pollination. Countries need to move toward more pollinator-friendly practices. It is estimated that only in North America 30% of consuming food is produced from bee pollinating vegetation. The value of bee pollination is estimated at \$16 billion only in the USA. In the last few years particularly between 2007 to 2016 honeybee population size has been dramatically reduced, dropping by 89 percent. In the United States, 40 percent of honeybee colonies were lost in 2018 alone. In the USA annual income of fruits, seed, and nuts plants, corn crops decrease due to the decline or disappearance of 90% pollinator decline. In the USA extensive use of insecticides has been considered as a prominent factor for colony disappearance, bee population decline, honey production, and wax yield and cause losses of about \$283 million per year. In Africa, the economic value of insect-pollinating crops is \$11.6 million per year. In Pakistan yield of some fruit reduces up to 33.4% due to pollinator decline [104]. In the Himalayan region of Pakistan, the population of pollinators declines due to farmers' and institutions' unawareness about pollination benefits.

Globally, pollination has an estimated market value of up to \$577 billion annually which represents about 10 percent of the global crop market [105]. Without these biotic pollinating services, decreases in crop production could both surge prices for consumers and lead to producers a loss of nearly \$2 billion annually. It argues a future with compromised pollination due to the absence of pollinating insects points to a dominant urge for hand pollination or any other innovative technology. But this involved labor cost in terms of hand pollination or investment by innovative technology. The labor costs involved in hand pollination are potentially significant, estimated at \$90 billion per year in the United States alone.

## **10. Conclusion and future recommendations**

Pesticide exposure can induce more or less harmful effects on neural functions and cause disturbance of maintenance behavior and physiological functions. The mechanisms by which pesticides produce their lethal and sub-lethal effects are not limited to the exclusive interaction between the pesticide active substance and the target molecular. The effects of pesticides encompass several molecular targets sites of diverse affinities at various exposure and contact levels. Exposure time was also studied as a significant factor in insecticide and pesticide toxicity. The toxicity of

pesticides is correlated to circadian rhythms, the exposure duration, the developmental stage of the bees exposed to pesticides, and the seasonal stress. The route and the mode of exposure (acute, sub-chronic, or chronic) play a predominantly decisive role in pesticide toxicity. Metabolic processes modulate the intrinsic toxicity and resultant metabolites may cause high or low toxicity than that of the parent pesticide. Researches on synergistic effects of pesticides should take a more dominant place in honeybee toxicology in the future. The practices of a large number of pesticides at a single site, synergistic effects by pesticide combinations will need to be prioritized. This could be based on the spatio-temporal presence of pesticide active substances. A promising approach to avoiding side effects of pesticides on pollinators has been derived by improving the mode of action of pesticides in targeted pests and honeybees. Mode of action can be improved by selecting the active substances that act selectively on pests [106].

With the extensive use of pesticides, the conservation of foraging honeybees is challenging. Even if a single worker of bee brings pesticide-contaminated pollen it can harm large numbers of honeybees. Systematic insecticides are a great threat to bees. Systemic insecticide disrupts the foraging behavior by impairing cholinergic signaling. Pesticide exposure also disturbs long and short-term memory. Insecticide treatments are not recommended in plantations and crops during flowering. Based on these findings it is recommended to apply methods of integrated pest management (IPM) of crops should be used, avoiding the use of chemical products. This report shows scientific evidence about the harmful effects of pesticides on honeybees and proving that pesticides play important role in bee decline. As consequence policymakers should ban the bee-harming pesticides. Through national action plans to support and promote agricultural practices such as crop rotation and organic farming that benefits the pollinator population. For this purpose promote research on ecological and organic farming practices that move away from reliance on chemical pest control.

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## References

- [1] Velthuis HH, A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*, 2006; 37 (4): p. 421 - 451.
- [2] Rockstrom J, Steffen W. A safe operating space for humanity. *Nature*, 2009; 461 (7263): 472 - 475.
- [3] Gallai N, Salles JM, Settele J, Vaissia BE. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological economics*. 2009; 68 (3): 810 - 821.
- [4] Biesmeijer JC, Roberts SPM, Reemer M, Ohlemuller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas, CD, Settele J, Kunin W E. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 2006; 313 (5785): 351 - 354.
- [5] Stokstad E. The case of the empty hives. *Science*, 2007; 316 (5827):970 - 972.
- [6] Aizen M A, Harder, LD. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current biology*, 2009; 19(11): p. 915-918.
- [7] Lebuhn G, Droege S, Connor EF, Gemmill HB, Potts SG, Minckley RL, Griswold T, Jean R, Kula E, Roubik DW, Cane J, Wright K W, Frankie G Parker F. Detecting insect pollinator declines on regional and global scales. *Conservation biology*, 2013; 27(1): p. 113-120.
- [8] Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: trends, impacts and drivers. *Trends in ecology and evolution*, 2010; 25(6): 345 - 353.
- [9] Kluser S, Peter N, Chauzat MP, Pascal P, Ron W, Mwangi T. Global honey bee colony disorders and other threats to insect pollinators. 2010; 16: 1 - 16.
- [10] Decourtye A, Devillers J, Aupinel P, Brun F, Bagnis C, Fourrier J, Gauthier M. Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. *Ecotoxicology*, 2011; 20(2): 429 - 437.
- [11] Genersch E, Von der O W, Kaatz H, Schroeder A, Otten C, Bachler R, Berg S, Ritter W, Mohlen W, Gisder S, Meixner M, Liebig G and Rosenkranz P. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie*, 2010; 41(3): 332 - 352.
- [12] Higes M, Martin R, Meana A. *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *Journal of invertebrate pathology*. 2006; 92(2): 93 - 95.
- [13] Memmott J, Craze P G, Waser N M, Price M V. Global warming and the disruption of plant-pollinator interactions. *Ecology letters*, 2007; 10(8): 710 - 717.
- [14] Rabea E I, Nasr, HM Badawy ME. Toxic effect and biochemical study of chlorfluazuron, oxymatrine, and spinosad on honey bees (*Apis mellifera*). *Archives of environmental contamination and toxicology*, 2010; 58(3): 722 - 732.
- [15] Nauen RU, Ebbinghaus-Kintscher, Schmuck, R. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Management Science: formerly Pesticide Science*, 2001; 57(7): 577 - 586.
- [16] Bonmatin J M, Moineau I, Charvet R, Fleche C, Colin M E, Bengsch E R. A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in

pollens. *Analytical chemistry*, 2003; **75**(9): 2027 - 2033.

[17] Bonmatin J M, Giorio C, Girolami V, Goulson D, Kreuzweiser D P, Krupke C. Environmental fate and exposure; neonicotinoids and fipronil. *Environmental science and pollution research*, 2015; **22**(1): 35 - 67.

[18] Decourtye A, Lacassie E, Pham-Delegue M H. Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. *Pest Management Science*, 2003; **59** (3): 269 - 278.

[19] Wallner K. Sprayed and seed dressed pesticides in pollen, nectar and honey of oilseed rape. *Julius-Kühn-Archiv*, 2009; (423): 152 - 153.

[20] Rortais A, Arnold G, Halm M P, Touffet B F. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie*, 2005; **36** (1): 71 - 83.

[21] Greatti M, Barbattini R, Stravisi A, Sabatini A G, Rossi S. Presence of the ai imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. *Bulletin of insectology*, 2006. **59**(2): p. 99.

[22] Tapparo A, Marton D, Giorio C. Assessment of the environmental exposure of honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environmental science and technology*, 2012; **46**(5): 2592 -2599.

[23] Skerl M I S, Bolta S V, Cesnik H B, Gregorc A. Residues of pesticides in honeybee (*Apis mellifera carnica*) bee bread and in pollen loads from treated apple orchards. *Bulletin of environmental contamination and toxicology*, 2009; **83**(3): 374 - 377.

[24] Chauzat M P, Faucon J P, Martel A C, Lachaize J, Cougoule N, Aubert M A

A survey of pesticide residues in pollen loads collected by honey bees in France. *Journal of economic entomology*, 2006; **99**(2): 253 - 262.

[25] Krupke C H, Hunt G J, Eitzer B D, Andino G, Given K. Multiple routes of pesticide exposure for honeybees living near agricultural fields. *PLoS One*, 2012; **7**(1): e29268.

[26] Bernal J, Garrido BE, Nozal MJD, Gonzalez PAG, Hernandez MR, Diego JC, Jimenez JJ, Bernal JL, Higes M. Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. *Journal of economic entomology*, 2010; **103**(6): 1964 - 1971.

[27] Girolami V, Mazzon L, Squartini A. Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *Journal of economic entomology*, 2009; **102** (5): 1808 - 1815.

[28] Orantes B F J, Pajuelo A, Megias M, Torres F P C. Pesticide residues in beeswax and beebread samples collected from honey bee colonies (*Apis mellifera* L.) in Spain. Possible implications for bee losses. *Journal of apicultural research*, 2010; **49**(3): 243 - 250.

[29] Mullin C A, Frazier M, Frazier J L, Ashcraft S, Simonds R and Pettis JS. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *Plos One*, 2010; **5**(3): e9754.

[30] Henry M, Beguin M, Fabrice R, Oriane R, Jean O, Pierrick A, Jean A, Sylvie T, Axel D A. Response to comment on “A common pesticide decreases foraging success and survival in honeybees”. *science*, 2012; **337**(6101): 1453 - 1453.

[31] Chakrabarti P, Rana S, Sarkar S, Smith B, Basu P. Pesticide-induced oxidative stress in laboratory and field



populations of native honeybees along intensive agricultural landscapes in two Eastern Indian states. *Apidologie*, 2015; **46**(1): 107 - 129.

[32] Zaluski R, Kadri S M, Alonso D P, Ribolla P E, Oliveira O R. Fipronil promotes motor and behavioral changes in honey bees (*Apis mellifera*) and affects the development of colonies exposed to sublethal doses. *Environmental toxicology and chemistry*, 2015; **34**(5): 1062 - 1069.

[33] Iwasa T, Motoyama N, Ambrose JT, Roe RM. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee. *Crop protection*, 2004; **23**(5): 371 - 378.

[34] Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, Pham-Delegue MH. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Archives of environmental contamination and toxicology*, 2005; **48**(2): 242 - 250.

[35] Desneux N, Decourtye A, Delpuech, JM. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology* 2007; **52**: 81 - 106.

[36] Nauen R, Denholm I. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Archives of insect biochemistry and physiology*: Published in Collaboration with the Entomological Society of America, 2005; **58**(4): 200 - 215.

[37] El Hassani A K, Dupuis JP, Gauthier M, Armengaud C. Glutamatergic and GABAergic effects of fipronil on olfactory learning and memory in the honeybee. *Invertebrate neuroscience*, 2009; **9**(2): 91.

[38] Schmidt H W, Brasse D, Kunast C, Muhlen W, Vonder OW, Tornier I, Wallner K. Introduction of indices for

the evaluation of tent tests and field tests with honeybees. *Bulletin of insectology*, 2003; **56**: 111 - 118.

[39] Laurino D, Porporato M, Patetta A, Manino A. *Toxicity of neonicotinoid insecticides to honeybees: laboratory tests*. *Bulletin insectology*, 2011; **64**(1): 107 - 13.

[40] Elbert C, Erdelen C, Kuehnhold J, Nauen R, Schmidt H W, Hattori Y. Thiacloprid, a novel neonicotinoid insecticide for foliar application. in *The BCPC Conference: Pests and diseases, Volume 1. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13 - 16 November 2000*. British Crop Protection Council.

[41] Rondeau G, Bayo S F, Tennekes H A, Decourtye A, Romero R, Desneux, N. Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites. *Scientific reports*, 2014; **4**(1): 1 - 8.

[42] Pettis J S, Johnson J, Dively G. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften*, 2012; **99**(2): 153 - 158.

[43] Chauzat M P, Martel A C, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Caroentier P, Faucon J P. An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. *Environmental toxicology and chemistry*, 2011; **30**(1): 103 - 111.

[44] Kiljanek T, Alicja N, Stanisław S, Marta G, Milena B, Andrzej P. Multi-residue method for the determination of pesticides and pesticide metabolites in honeybees by liquid and gas chromatography coupled with tandem mass spectrometry—Honeybee poisoning incidents. *Journal of chromatography*, 2016; **1435**: 100 - 114.

[45] Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S,

- Porrini C. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bulletin of insectology*, 2003; **56**: 63 - 68.
- [46] Porrini C, Sabatini A G, Girotti S, Fini F, Monaco L, Celli G, Bortolotti L, Ghini, S. The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators. *Bulletin of insectology*, 2003; **56**(1): 147 - 152.
- [47] Oliveira RA, Roat TC, Carvalho SM, Malaspina O. Side-effects of thiamethoxam on the brain and midgut of the africanized honeybee *Apis mellifera* (Hymenoptera: Apidae). *Environmental toxicology*, 2014; **29**(10): 1122 - 1133.
- [48] Suchail S, Guez D, Belzunces LP. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environmental Toxicology and Chemistry*, 2001; **20**(11): 2482 - 2486.
- [49] Lambin M. Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. *Archives of insect biochemistry and physiology*: Published in Collaboration with the Entomological Society of America, 2001; **48**(3): 129 - 134.
- [50] Van dame R, Meled M, Colin MA. Alteration of the homing-flight in the honey bee *Apis mellifera* L. Exposed to sublethal dose of deltamethrin. *Environmental toxicology and chemistry*, 1995; **14**(5): 855 - 860.
- [51] Easton AH, Goulson D. The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations. *PLoS One*, 2013; **8**(1): e54819.
- [52] Whitehorn PR, Connor S, Wackers FL, Goulson D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, 2012; **336**(6079): 351 - 352.
- [53] Levot GW, An insecticidal refuge trap to control adult small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae) in honeybee colonies. *Journal of apicultural research*, 2008; **47**(3): 222 - 228.
- [54] Esch, HE, Zhang SW, Srinivasan MV, Tautz J. Honeybee dances communicate distances measured by optic flow. *Nature*, 2001; **411**(6837): 581 - 583.
- [55] Cresswell JE. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honeybees. *Ecotoxicology*, 2011; **20**(1): 149 - 157.
- [56] Tomizawa M, Casida JE, Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annual review of pharmacology and toxicology*. 2005; **45**: 247 -268.
- [57] Casida J E, Durkin K A. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annual review of entomology*, 2013; **58**: 99 - 117.
- [58] Hardstone MC, Scott JG. Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest management science*, 2010; **66**(11): 1171 - 1180.
- [59] Barnett E A, Charlton AJ, Fletcher MR. Incidents of bee poisoning with pesticides in the United Kingdom, 1994 - 2003. *Pest management science*, 2007; **63**(11): 1051 - 1057.
- [60] Johnson R M, Ellis M D, Mullin C A, Frazier M. Pesticides and honeybee toxicity—USA. *Apidologie*, 2010; **41**(3): 312 - 331.
- [61] Chauzat M P, Faucon JP. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. *Pest Management Science: formerly Pesticide science*, 2007; **63**(11): 1100 - 1106.

- [62] Haarmann T, Spivak M, Weaver D, Weaver B, Glenn T. 2002. Effects of fluvalinate and coumaphos on queen honey bees (Hymenoptera: Apidae) in two commercial queen rearing operations. *Journal of economic entomology*, 2002; **95**(1): 28 - 35.
- [63] Guez D, Belzunces L, Maleszka R. Effects of imidacloprid metabolites on habituation in honeybees suggest the existence of two subtypes of nicotinic receptors differentially expressed during adult development. *Pharmacology biochemistry and behavior*, 2003; **75**(1): 217 - 222.
- [64] Guez D, Suchail S, Gauthier M, Maleszka R, Belzunces L P. Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiology of learning and memory*, 2001; **76**(2): 183 - 191.
- [65] Moore D. Honey bee circadian clocks: behavioral control from individual workers to whole-colony rhythms. *Journal of insect physiology*, 2001. **47** (8): 843 - 857.
- [66] Guez D, Zhu H, Zhang S W, Srinivasan M V. Enhanced cholinergic transmission promotes recall in honeybees. *Journal of insect physiology*, 2010; **56**(9): 1341 - 1348.
- [67] Decourtye A, Devillers J, Cluzeau S, Charreton M, Pham-Delegue M H. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pesticide biochemistry and physiology*, 2004; **78**(2): 83 - 92.
- [68] Han P, Niu C Y, Lei C L, Cui J J, Desneux N. Use of an innovative T-tube maze assay and the proboscis extension response assay to assess sublethal effects of GM products and pesticides on learning capacity of the honeybee *Apis mellifera* L. *Ecotoxicology*, 2010; **19**(8):1612 - 1619.
- [69] Goulson D, Chapman J W, Hughes W O. Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *Journal of insect behavior*, 2001; **14**(5): 669 - 678.
- [70] Aliouane Y, Adessalam K, El Hassani A K, Gray V, Armengaud C, Lambin M, Gauthier M. Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. *Environmental Toxicology and Chemistry: An international Journal*, 2009; **28**(1):113 - 122.
- [71] Bernadou A, Demares F, Couret-Fauvel T, Sandoz J C, Gauthier M. Effect of fipronil on side-specific antennal tactile learning in the honeybee. *Journal of insect physiology*, 2009; **55**(12): 1099 - 1106.
- [72] Reinhard J, Srinivasan M V, Zhang S. Scent-triggered navigation in honeybees. *Nature*, 2004; **427** (6973) 411 - 411.
- [73] Gawleta N, Zimmermann Y, Eltz T. Repellent foraging scent recognition across bee families. *Apidologie*, 2005; **36**(3): 325 - 330.
- [74] Couvillon M J. Alarm pheromones do not mediate rapid shifts in honeybee guard acceptance threshold. *Journal of chemical ecology*, 2010; **36**(12): 1306-1308.
- [75] Kather R, Drijfhout F P, Martin S J. Task group differences in cuticular lipids in the honeybee *Apis mellifera*. *Journal of chemical ecology*, 2011; **37**(2): 205 - 212.
- [76] El Hassani A K, Dacher M, Gary V, Lambin M, Gauthier M, Armengaud C. Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). *Archives of environmental contamination and toxicology*, 2008; **54**(4): 653 - 661.
- [77] Barron A B, Zhu H, Robinson G E, Srinivasan M V. Influence of flight time and flight environment on distance

- communication by dancing honey bees. *Insectes sociaux*, 2005; **52**(4): 402 - 407.
- [78] Menzel R, De Marco R J, Greggers U. Spatial memory, navigation and dance behaviour in *Apis mellifera*. *Journal of comparative physiology*, 2006; **192**(9): 889 - 903.
- [79] Dacke M, Srinivasan M V. Honeybee navigation: distance estimation in the third dimension. *Journal of Experimental Biology*, 2007; **210** (5): 845 - 853.
- [80] Reynolds D, Riley J. Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. *Computers and electronics in agriculture*, 2002; **35**(2): 271 - 307.
- [81] Riley J R, Greggers U, Smith A D, Reynolds D R, Menzel R. The flight paths of honeybees recruited by the waggle dance. *Nature*, 2005; **435** (7039): 205 - 207.
- [82] Capaldi E A, Smith A D, Osborne J L, Fahrbach S E, Farris S M, Reynolds D R, Edwards A S, Martin A, Robinson G E, Poppy G M, Riley J R. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature*, 2000; **403**(6769): 537 - 540.
- [83] Menzel R, Greggers U, Smith A, Berger S, Brandt R, Brunke S, Bundrock G, Hulse S, Plumpe T, Schaupp F, Schuttler E, Stach S, Stindt J, Stollhoff N, Watzl S. *Honey bees navigate according to a map-like spatial memory*. *Proceedings of the National Academy of Sciences*, 2005; **102**(8): 3040 - 3045.
- [84] Streit S, Bock F, Pirk CWW, Tautz J. *Automatic life-long monitoring of individual insect behaviour now possible*. *Zoology*, 2003; **106**(3): 169 - 171.
- [85] Ohashi K, Souza D D, Thomson J. *An automated system for tracking and identifying individual nectar foragers at multiple feeders*. *Behavioral Ecology and Sociobiology*, 2010; **64**(5): 891 - 897.
- [86] Guez D, Zhang S.W, Srinivasan M.V. *Methyl parathion modifies foraging behaviour in honeybees (Apis mellifera)*. *Ecotoxicology*, 2005; **4**(4): 431 - 437.
- [87] Colin M E, Bonmatin J M, Moineau I, Gaimon C, Brun S, Vermandere JPA *method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides*. *Archives of environmental contamination and toxicology*, 2004; **47**(3): 387 - 395.
- [88] Ramirez R, Chaufaux J, Pham-Delegue MH. *Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee Apis mellifera, a comparative approach*. *Apidologie*, 2005; **36**(4): 601 - 611.
- [89] Yang E C, Chuang Y C, Chen Y L, Chang L H. *Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae)*. *Journal of economic entomology*, 2008; **101**(6): 1743 - 1748.
- [90] Papaefthimiou C G. *Theophilidis, The cardiotoxic action of the pyrethroid insecticide deltamethrin, the azole fungicide prochloraz, and their synergy on the semi-isolated heart of the bee Apis mellifera macedonica*. *Pesticide biochemistry and physiology*, 2001; **69**(2): 77 - 91.
- [91] Papaefthimiou C, Theophilidis G. *Triazines facilitate neurotransmitter release of synaptic terminals located in hearts of frog (Rana ridibunda) and honeybee (Apis mellifera) and in the ventral nerve cord of a beetle (Tenebrio molitor)*. *Comparative biochemistry and physiology Part C: Toxicology & Pharmacology*, 2003; **135**(3): 315 - 330.
- [92] Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, Weiner ML. *Mechanisms of*

pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology*, 2002; **171**(1): 3 - 59.

[93] Calore EE, Cavaliere MJ, Puga FR, Calore NMP, Rosa A R, Weg R, Dias S S, Santos R P. Histologic peripheral nerve changes in rats induced by deltamethrin. *Ecotoxicology and environmental safety*, 2000; **47**(1): 82 - 86.

[94] Neal AP, Yuan Y, Atchison WD. Allethrin differentially modulates voltage-gated calcium channel subtypes in rat PC12 cells. *Toxicological sciences*, 2010; **116**(2): 604 - 613.

[95] Stabentheiner A, Vollmann J, Kovac H, Crailsheim K. Oxygen consumption and body temperature of active and resting honeybees. *Journal of insect physiology*, 2003; **49**(9): 881 - 889.

[96] Kleinhenz, M., Bujok, B., Fuchs, S., Tautz, J. Hot bees in empty broodnest cells: heating from within. *Journal of experimental biology*, 2003; **206**(23): 4217 - 4231.

[97] Babin M, Casado S, Chana A, Herradon, B., Segner, H., Tarazona, J.V., Navas, J.M. Cytochrome P4501A induction caused by the imidazole derivative Prochloraz in a rainbow trout cell line. *Toxicology in vitro*, 2005; **19**(7): 899 - 902.

[98] Vandame R, Belzunces LP. Joint actions of deltamethrin and azole fungicides on honey bee thermo-regulation. *Neuroscience letters*, 1998; **251**(1): 57 - 60.

[99] Andersson GK, Rundlof M, Smith HG. Organic farming improves pollination success in strawberries. *PloS one*, 2012; **7**(2):e31599.

[100] Tasei JN. Effects of insect growth regulators on honey bees and non-Apis bees. A review. *Apidologie*, 2001; **32**: 527 - 545.

[101] Davis AS, Hill JD, Chase CA, Johanns AM, Liebman M. Increasing cropping system diversity balances productivity, profitability and environmental health. *PloS One*, 2012. **7**(10): p. e47149.

[102] Chagnon M. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental science and pollution research*, 2015; **22**(1): 119 - 134.

[103] Eilers EJ, Claire K, Sarah SG, Andrea KG Alexandra MK. Contribution of pollinator-mediated crops to nutrients in the human food supply. *PLoS One*, 2011; **6**(6): e21363.

[104] Aslam M, Razzaq M, Hussain S Akhter W. Effect of insect pollinators on fruit setting on Mango (*Mangifera indica* L.). *Journal of Science Research*, 2004; **15**: 53 - 58.

[105] Chaplin-Kramer R, Dombeck E, Gerber J, Knuth K A, Mueller ND, Mueller M, Ziv G, Klein AM. Global malnutrition overlaps with pollinator-dependent micronutrient production. *Proceedings of the Royal Society B: Biological Sciences*, 2014; **281**(1794): 20141799.

[106] Courjaret R, Grolleau F, Lapied B. Two distinct calcium-sensitive and-insensitive PKC up-and down-regulate an  $\alpha$ -bungarotoxin-resistant nAChR1 in insect neurosecretory cells (DUM neurons). *European journal of neuroscience*, 2003; **17**(10): 2023 - 2034.