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Exploring the Adverse Impact of Pesticides in Honey Bees and Their Virulence

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Abstract: Honey bees (*Apis mellifera* L.) significantly enhance seed production, fruit set, and yield through pollination. However, the world is facing a major challenge of honey bee loss due to the excessive use of pesticides facilitated by technological advancements. Pesticides are harmful to pollinators and can cause lethal and sublethal effects through dermal contact, ingestion, or inhalation of contaminated air. Pesticide exposure in agricultural fields has led to honey bee toxicity, which affects their neurophysiological and immune systems and, consequently, their life cycle. This toxicity also leads to contamination of bee products, which can negatively impact human health, affecting the nervous and digestive systems, among others. While there is limited knowledge about the impact of pesticides on wild honey bees, it is crucial to understand this to guide agricultural practices and legislation aimed at safeguarding these important pollinators. It is, therefore, essential to take major steps to control pesticide toxicity in honey bees and prevent further harm.

Keywords: pesticides; honey bee; toxicity; neurophysiological.

1. Introduction

In the past few years, the use of pesticides has increased rapidly in agriculture to control pests and other insects. The bee (*Apis mellifera*), a globally distributed organism, is cultivated by farmers and beekeepers to produce goods with high commercial value, including honey, wax, propolis, royal jelly, pollen, and bee venom. Germination is the second reason beekeepers retain bees; honeybees are horticulture's most effective and widely used pollinating agent . As the world population grows, so does the demand for food and agricultural products, which results in the overuse of pesticides to develop high yields. To protect the plant from pests, the formulation of different pesticides is used [1]. They can be used to treat soil and seeds or directly sprayed on the flowering plant, based on the type of pesticide and the suggested technique. The various methods and persistence of applied pesticides in the field play a major role in honey bees' exposure to the pesticides[2]. Honey bees are exposed to pesticides used to defend agricultural crops against harmful species such as insects, mites, plant diseases, and weeds at various phases of their lives [3]. Close contact with the toxin while eating the pesticide-treated plant is one of the major possible scenarios. Other common exposure pathways include pesticide-contaminated plant bits that attach to bees or are transported to the hive by the air, water, physical intimacy, etc. [4]. Application of pesticides to untreated agricultural crops near the treated field and pesticide residues in pollen by seed treatment [1]. Pesticide uptake in plants is mainly through three pathways: direct application on plants, i.e., absorption and translocation through stem, leaves, and fruits, sorption through the soil, and then biological degradation through soil microorganisms and mainly through ground and percolated water by root uptake [5,6]. Depending on the application method and physiochemical characteristics, toxins(pesticides) distributed residue in different plant components, bees can be exposed to which plant pollinators like bees can be exposed [7]. This exposure occurs through dermal contact, oral intake, and inhalation [8]. Bee colonies can get contaminated due to the various routes where honeybees might be exposed to pesticides [9]. Workers bees foraging in the farms facilitate oral ingestion of agrochemicals [10]. In dermal (cuticular), they come into direct exposure to agrochemical toxins while forging around a hive, searching for food, etc. [11]. These classes of pesticides are lethal in very small quantities or concentrations. The primary route of cuticular pesticide exposure is thought to be through the body wall, specifically the chitinous cuticle of the honeybee thorax [12]. There are several classes of pesticides that are commonly stable in the surroundings [13,14]. This could result in soil contamination months or years after application [15]. The impact of pesticides not only affects soil fertility but also affects insects and pollinators, such as honey bees [16]. The effect of pesticides depends upon the concentration of the pesticide and the impact level of pesticides upon honey bees [17]. There are several classes of pesticides that are hazardous only if present in a certain amount, whereas some of the classes of pesticides are toxic even if they are present in small quantities or low concentrations [18,19]. This review thus includes the exposure of pesticides in honey bees and ultimately to humans. The concentration of exposure to pesticides through different pathways has been discussed. Accumulation in honey bees and possible measures to prevent pesticide exposure have also been proposed.

1.1. Historical and current perspectives of pesticides found in Honey Bee.

In the past few years, exposure to pesticides remains in practice. In the year of 1995, *N. ceranae* pesticides were first found in honey bee colonies in the United States. Then in 1998, the same pesticides were found in bee hives along with Neonicotinoids (potent agonists of the Acetylcholine receptors) [20]. In France, a survey was carried out from 2002 to 2005 to study honey bee colonies (*Apis mellifera L.*) In relation to pesticide residue found in honey bees, various pesticides were detected, such as imidacloprid and 6-chloronicotinic acid residues [21]. Then, in 2007, the pesticide residue in bee bread was analyzed. Unprecedented amounts of fluvalinate at high frequencies have been detected in pollen (bee bread Brood nest wax) [22] bees. The most frequent pesticides and insecticides were detected in all samples, i.e., bifenthrin, herbicides thiobencarb, and metolachlor [13]. Pesticides detected in overall samples included the herbicides pendimethalin and propanil and the fungicides carbendazim, boscalid, and fluopyram [23]. The insecticide bifenthrin and the herbicides hexazinone, metolachlor, propanil, and thiobencarb were detected in samples for wild bees. For honey bees, the insecticide bifenthrin, the herbicide thiobencarb, and the fungicide carbendazim and fluopyram were detected on 30th March 2022 [24]. Pesticide residue was detected in a wide range of concentrations, and thus, it causes toxicity in honey bee larvae, as shown in Figure 1.

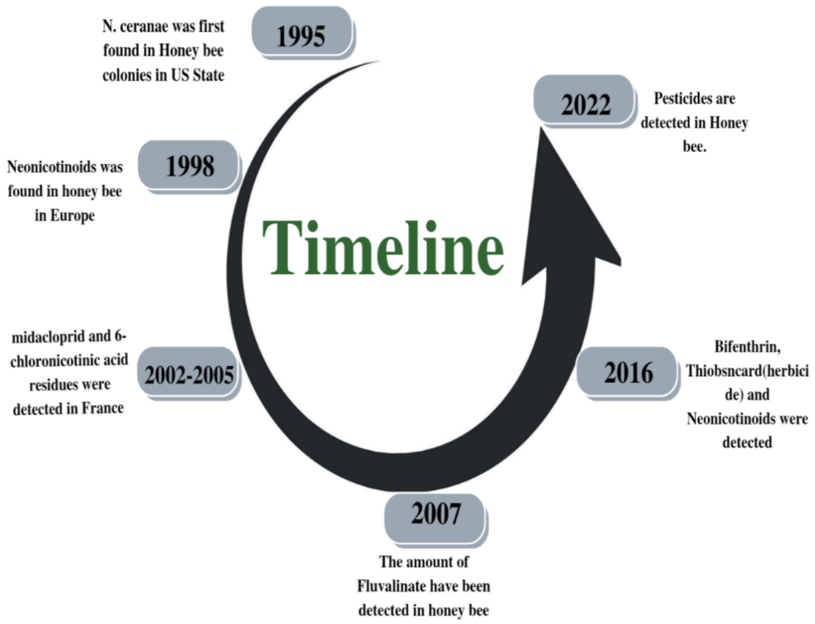

Figure 1. Timeline for identification of various pesticides found in honey bee.

2. Routes of exposure to different pesticides in honey bees

Honey bees are exposed to a variety of pesticides during the process of forging. Pesticides enter the colony because of the contaminated food that causes contamination in the beehive matrices, such as honey, beebread, and wax [13,25-27]. To shield the plants or a part of it from various elements such as weeds, pathogens, predatory insects, rodents, etc., various types of formulations of pesticide travel over the plant in numerous ways.[28,29] Three main application techniques are frequently applied to treat crops, depending on the type of pesticide being used: direct spray, which is frequently used in and near homes; soil administrations; and seed sprays, which are typically used in major biological treatment [30]. These various application techniques are essential in ensuring these pesticides are exposed to the insect pollinators approaching a field [31].

Thus, based on the type of application method and persistence of different natural pesticides, the bees get exposed to various pesticides through these major routes: During the forging process, direct contact on the treated crop field; Particles of pesticide formulations contain pesticides that adhere to feeding bees or the entire colony after drifting through the environment; Pesticidal residue runoff from the treated field crop to near; Pesticides spread to forage plants that are not sprayed but are blooming close to the sprayed crop [12]; A residual amount of pesticides in pollen is present during seed treatment, as shown in figure 2 [32].

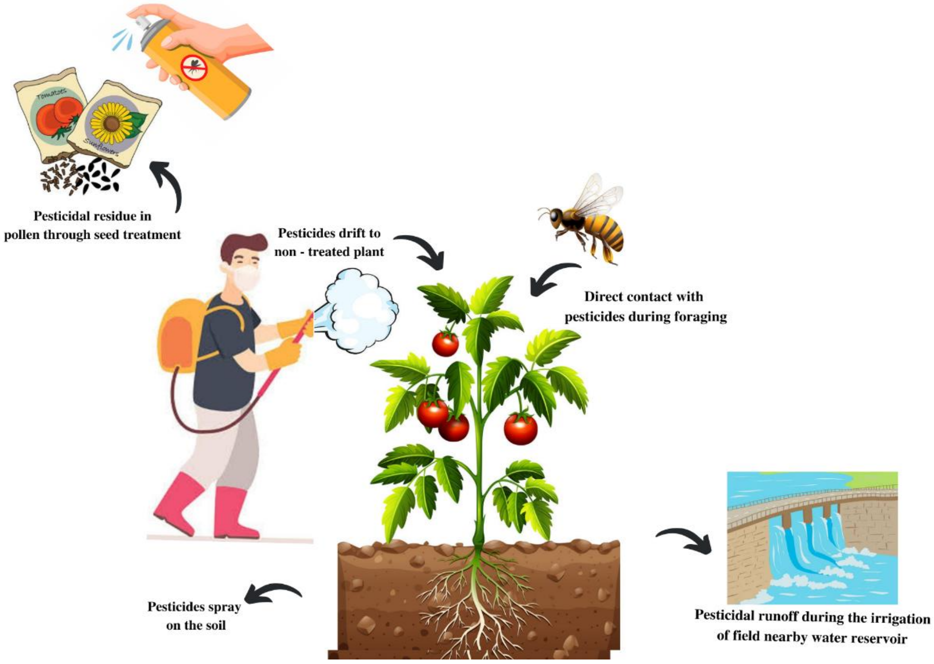

Figure 2. Routes of exposure to different pesticides in Honey Bees.

However, there are several various ways, including oral, respiratory, and cutaneous ingestion, by which these toxins are consumed by the foraging bees from the fields. These multiple routes for pesticide exposure to honey bees make it easier for them to enter a honey bee colony system. Here is a description of how these substances can be consumed. This pesticidal exposure to honey bees could have lethal and sub-lethal effects [33]. These insecticides are the most often used for insect pest management on vegetable and horticultural crops, and they can be applied directly or indirectly to plant canopy, as shown in Table 1 [26,34]. Pathways have the most effect on the intake concentration of pesticides, as seen in Figure 3.

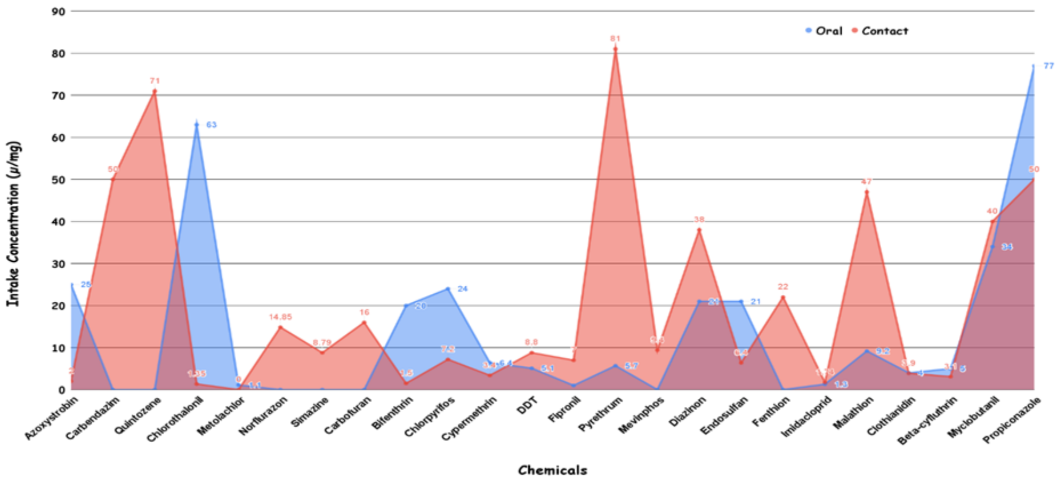

Figure 3. Comparison of pesticide intake concentration through oral and contact exposure in honey bees for different pesticides (Intake Concentration (µ/mg) was multiplied by 100 for values below 0 and was divided by 100 for values above 99 to facilitate the visualization of the content) [11].

3. Mode of intake of toxicant through honey bees

The main source of the transportation of pesticides into the plant body is ground or aerial spraying procedures [12]. The exposure also depends upon the method of application of pesticides used by the farmer in the field (e.g.:-foliar spray or seed treatment) [35,36]. It also depends upon the physio-chemical properties of the pesticide used in the field and the properties seen, such as –mobility of pesticide in soil [37], solubility of pesticide in soil, and dissipation of pesticides [38]. Transportation also depends upon abiotic factors such as pH, soil composition, temperature, and moisture content and depends upon physiological properties (e.g.:-, plant growth, plant transpiration rate, cultivation technique, varieties of fruits and vegetables) [11,39,40]. Depending upon the type of pesticide used and its chemical and physiological properties, it can reach the plant as residue [41], to which plant pollinators like honey bees can be exposed [42].

3.1. Dermal contact.

The major mode of intake of toxicants by bees is that they are exposed to direct contact with pesticides in the fields; during foraging, they come in contact with different pesticides, which are even lethal in trace amounts. Numerous insecticides have been shown to have skin toxicity when applied topically, and the honey bee thorax is commonly quoted as a primary site of pesticide dermal exposure. Insect wings have been recognized as a more harmful exposure route to bees than the thorax [43,44].

3.2. Oral intake.

This is facilitated by the forger bees. The forger bees come in contact with the pesticides from the external environment [45] (using pollen and nectar); then this contamination accumulates In the hive and then fended to the larvae and other bees, and the contamination reaches the honey bee via oral way [46-48].

3.3. Respiratory intake.

The farmer uses some pesticide formulations, such as dust and fumigation are sprayed into the air from where they reach the respiratory system of bees and can be retained on the body surface of foraging worker bees or absorbed through the trachea [38,49].

4. Ramifications of Pesticide Accumulation in Honey Bees

4.1. On the life cycle of honey bee.

Pesticides are toxic chemicals that are specifically designed to interrupt specific metabolic pathways to control the target group of organisms, as seen in Figure 3. However, each type of pesticide’s toxicity does not only affect the species it is intended to kill; Other organisms with similar metabolic rates are also affected [50,51]. Thus, when spread to crop plants, these pesticides cause many lethal and sub-lethal effects on honey bees and other organisms [51]. The mechanism and toxicity of pesticides are discussed in Table 1.

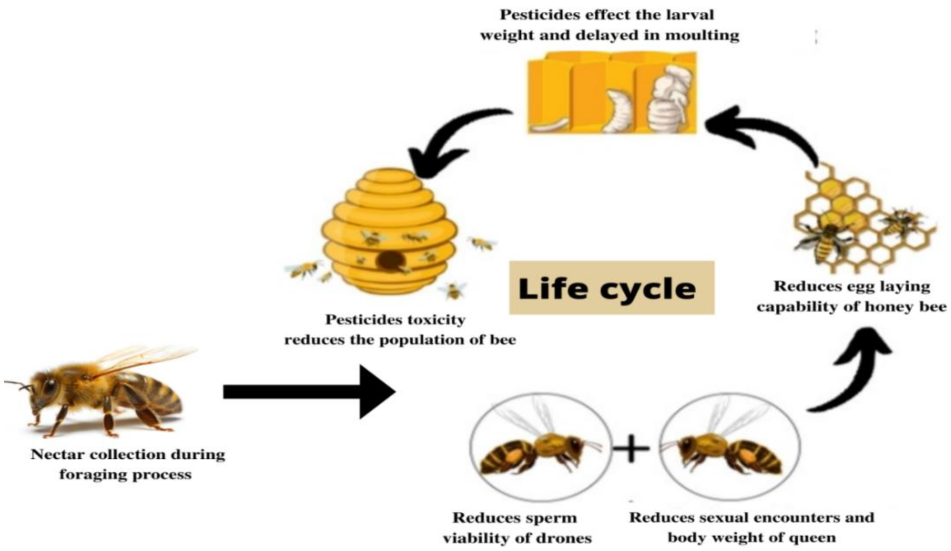

Figure 3. Harmful effects of pesticides on the life cycle of honey bee.

4.1.1. Effects of pesticides on the reproductive system and development phase.

Exposures of queens to lethal doses of pesticides [52], like Thiamethoxam during development, can slow down the reproductive cycles, reduce body weight, and lower the probability of queen success [53]. And queens exposed to neonicotinoids have reduced mating capacity [54]. Pesticides have also been connected to abnormal larval development. They cause abnormal developmental stage progression, skipping some stages, decreased larval weight, delayed molting, and imperfect development because some proteinsaren't produced appropriately [55].

Table 1. Various Pesticides along with their toxic mechanism and structure. (Adapted and Modified from [56].

S.No.	Pesticide	Chemical Safety	Toxic mechanism	Comment	Structure
1	**Azoxystrobin**	Acute toxic, Environmental hazard	It significantly alters amino acid metabolism and also induces oxidative stress and mitochondrial impairment	It is a broad-spectrum fungicide that protects against many kinds of pathogenic fungi by preventing their ATP production.	$C_{22}H_{17}N_3O_5$
2	**Carbendazim**	Health Hazard, Environmental hazard	It has significantly disturbed the immune system of honey bees	Carbendazim is a type of fungicide that is widely used to control fungus in various nectareous crop	$C_9H_9N_3O_2$
3	**Quintozene**	Environmental hazard, Irritant	It affected the gut microbiome of newly emerging bees and decreased the survival of honey bee larvae.		$C_6Cl_5NO_2$
4	**Chlorothalonil**	Corrosive, Acute toxic	It affected the gut microbiome of newly emerging bees and decreased the survival of honey bee larvae.	One of the most frequently found pesticides in bee hives is the nonsystemic fungicide chlorothalonil.	$C_8Cl_4N_2$
5	**Metolachlor**	Acute toxic, Irritant	Significant increases in syrup consumption were observed in honey	Pre-emergent herbicide metolachlor is used to suppress some broadleaf weed species as well as annual grassland weeds.	$C_{15}H_{22}ClNO_2$

S.No.	Pesticide	Chemical Safety	Toxic mechanism	Comment	Structure
			bees exposed to metolachlor		
6	**Norflurazon**	Environmental hazard			$C_{12}H_9ClF_3N_3O$
7	**Simazine**	Environmental hazard, Health Hazard	It may have a mild therapeutic effect on honey bee		$C_7H_{12}ClN_5$
8	**Carbofuran**	Environmental hazard, Health Hazard	Bees exposed to this herbicide saw a persistent decline in adult bees and sealed brood, eventually resulting in death.	One of the most poisonous broad-spectrum pesticides, carbofuran is widely used in agriculture, the home, and industry as an insecticide, nematicide, and acaricide.	$C_{12}H_{15}NO_3$
9	**Bifenthrin**	Environmental hazard, Health Hazard, Acute toxic, Irritant	It has adverse effects on immunological function, development, neurobehavioral function, oxidative damage, and endocrine function.	Bifenthrin is a synthetic insecticide that has been commonly used for agricultural and domestic pest control	$C_{23}H_{22}ClF_3O_2$
10	**Chlorpyrifos**	Environmental hazard, Acute Toxic	It causes oxidative stress/damage, found increased in the nervous system of *Apis mellifera* that were exposed to chlorpyrifos	Chlorpyrifos is an insecticide, acaricide, and miticide made of organophosphates that is largely used to combat soil- and foliage-borne insect pests.	$C_9H_{11}Cl_3NO_3PS$
11	**Cypermethrin**	Enviromental hazard, Irritant	It causes hypoglucosemia and hypotrehalosemia, and some neurological disorders	Cypermethrin, a synthetic pyrethroid, is extensively employed as an insecticide. It poses significant harm to fish, bees, and aquatic insects, as highlighted by the National Pesticides Telecommunications Network. This substance is a common component in various household insect killers, including Raid and ant chalk.	$C_{22}H_{19}Cl_2NO_3$
12	**DDT**	Environmental hazard, Health Hazard, Acute Toxic	It's is not much toxic ut causes only slight mortality in honey bees	The chemical compound dichlorodiphenyltrichloroethane, also known as DDT, is an organochloride that is colorless, flavorless, and practically odorless. It was initially created as an insecticide but quickly gained notoriety for damaging the environment.	$C_{14}H_9Cl_5$
13	**Fipronil**	Environmental hazard, Health Hazard, Acute toxic	It reduces the honey bee mitochondrial activity and ATP production and also affects the central nervous system,	Fipronil is a type of broad-spectrum pesticide that kills pest and insects but has an effect in central nervous system of the insect and also affects the mitochondrial activity of insects.	$C_{12}H_4Cl_2F_6N_4OS$
14	**Pyrethrum**	Environmental hazard, irritant	It mainly affects honey bee walking and flying ability		$C_{43}H_{56}O_8$
15	**Mevinphos**	Environmental hazard, Acute Toxic	It causes physiological and neurological disorders in honey bee	Mevinphos is an organic phosphate insecticide that controls insects in various crops by acting as an acetylcholinesterase inhibitor. It is most frequently used to eliminate spider mites and chewing and sucking insects.	$C_9H_9N_3O_2$

4.1.2. The neurophysiological effects of pesticides on the behavior of honey bees.

The primary excitatory neurotransmitter in the honeybee brain, acetylcholine, regulates various behaviors that maintain the survival of the individual bees and the colony as a whole. The sub-lethal effects of the pesticide on honey bee behaviors responsible for the decline of the population of honey bee colony Neonicotinoid chemicals cause synaptic activation and momentarily block Acetylcholine's activity when they bind to nAchRs (Nicotinic acetylcholine receptor). This impacts olfactory learning and memory, which has been demonstrated to rely on cholinergic signaling.

4.1.3. Effects on the immune system of honey bees.

Pesticides can cause immunosuppression in bees, making them more susceptible to pathogens [57-59]. It reduces the global anti-microbial peptide (AMP) generation, thus compromising an already-developed immune system [60-62]. Exposure to pesticides like neonicotinoids impairs melanization responses due to a reduction in the activity of Phenoloxidase (PO) [63,64]. The consequences are decreased pathogen isolation and clearance and slower wound healing; these could increase viral load and systemic infection. Both of these could increase viral loads and systemic infections [63,65,66], as shown in Figure 3.

4.2. On human health.

Pesticides are a major cause of direct human intake of toxic compounds and a secondary source of inhalation of harmful substances through the mouth and nose [67-70]. These pesticides enter the body parts through contaminated agricultural products, and the concentration is high enough to cause clinical problems in human health [71-73]. Pesticides enter the bodies of humans through the skin, mouth, eyes, and respiratory system, where they induce serious illnesses such as headaches, nausea, vomiting, rashes on the skin, respiratory illnesses [74], convulsions, and coma [75]. Direct exposure may even cause death [76,77]. Cancer, asthma, dermatitis, endocrine disorders, reproductive issues, immunotoxicity, neurobehavioral disorders, and birth malformations are examples of chronic diseases [75,78]. Chronic illnesses could result from pesticides' primary effect of altering cellular homeostasis (disorders of enzymes, ionic channels, and receptors) [79,80], as represented in Figure 4.

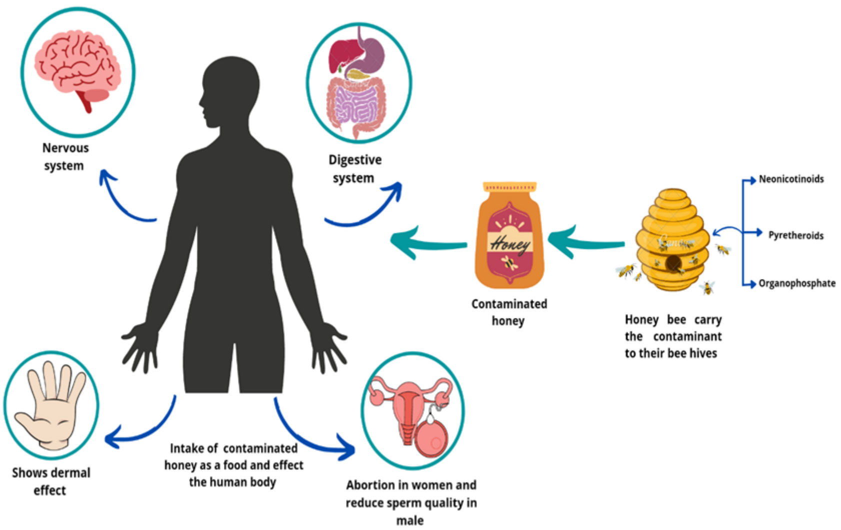

Figure 4. Pathway of pesticides from field to the human body and its effect on the human body.

5. Measures to protect honey bees from pesticide contamination

The selection of pesticides with the lowest toxicity rating to bees and pesticide applicators do not apply insecticides with long residual toxicity to honey bees and blooming plants, including weeds. Applying pesticides with residual toxicity when bees are not present or inactive form because they generally visit the fields during the daylight hours and may also visit the same crop at a specific time period. When temperatures are incredibly low or there is humidity after treatment, avoid applying pesticides. Under these circumstances, residue often lasts at least twice as long for bee toxicity. Avoid the tank mixing process of different fungicides and insecticides; such a specific mixture causes synergistic effects on honey bees. The farmers should minimize the use of spray drift. Avoid prophylactic application of pesticides, including fungicides, while bees are present. Supply sources of pollen and nectar far from treated crops. Pollen and nectar may be produced by blooms of any kind, including weedy plants that are not deemed noxious by the state agriculture department. Avoid using insecticides near red clover, cranberries, other berry crops, and nearby wild areas and fence rows. Bumble bees, an essential pollinator for many crops, can build their nests in these places.

6. Conclusion & future prospects

Previous research on this has been reported in foreign countries; no such detailed research has been done in India. Future perspectives could focus on the vast toxicity of honey bees and their impact on biodiversity on a larger scale, not only on the exposure of pesticides in the insect. Remediation techniques could be a developing possibility for treating pesticide exposure, and past studies have mainly focused on the exposure and toxicity pathways and not on their treatment or prevention. In this review, we have discussed the availability, mode of exposure, and various hazardous effects of pesticide contamination on honey bees. The pesticides are applied directly to bee-attractive fields because the amount and concentration of pesticides vary by plant species; bee foraging exposure must necessarily be linked to the plant pollinators, i.e., honey bees—pesticides found in floral, ariel, and food samples. Bees are exposed to far more chemicals, according to soil samples. The contamination of the pesticides is carried out by various pathways such as dermal and oral contact and direct contact of the pesticides with bees during the application of pesticides in the field. Exposure to pesticides affects the life cycle of honey bees; the contamination of pesticides in Honey bees reduces the sperm viability of the queen and reduces the weight of the larva, which skips larval development and then results in a decrement in the population of the Bee colonies. Reduction in the use of pesticides in agriculture fields needs to be done, which could decrease the contamination of the pesticides in Honey bees.

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Conflicts of Interest

The authors declare no conflict of interest.

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