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Pesticides use in plant protection with respect to honeybees: A review

Sapna Devi, Diksha Devi and Sawraj Jit Singh

Abstract
Honeybees are highly valued resource-insects that help in pollination, increasing fruit yield and also reduce fruit drop in many fruit crops. But in recent years, there has been a global decline in the honeybee population. Honeybees are exposed to different types of natural and artificial chemical substances such as pesticides during foraging. Over 98% of sprayed pesticides reach a destination other than their target species, including non-target species, air, water and soil. Compared to other insects, honeybees are extremely sensitive to pesticides, due to a deficiency in the number of genes encoding detoxification enzymes. Most pesticides are applied as sprays, droplets and dust, from the applications can fall directly on the bees that fly across the treated fields. Typical clinical symptoms of acute insecticide poisoning include cramping, disoriented behaviour of bees, and abnormal wing movements. After the intoxication with neonicotinoids symptoms such anomalous behaviour of worker bees as rolling, was observed in 71.4% of the affected hives. In the present review, we will discuss about all the aspects related to pesticide poisoning in honeybees.

Keywords: Pesticide, poisoning, honeybees, detoxification, symptoms

1. Introduction
In the recent years, honey production technology has been facing a huge global decline, in many European and North American countries. One unexpected finding from US showed that total summer loss was greater as compared to total winter loss as reported by some commercial beekeepers (Seitz *et al.*, 2015) [25]. When the honeybee colonies take the opportunity to use the plant's flowering season to reinforce themselves after the hard winter period, they may get exposed to potentially toxic pesticides by several routes.
Honeybees are exposed to different types of natural and artificial chemical substances. Pesticides are the second group of man-made chemicals in the world after the fertilisers (AAAS, 2013) [1]. Within the European Union there are 484 active substances approved for use as pesticides and 793 substances no longer approved for use as pesticides according to Regulation (EC) No 1107/2009. It was reported that overwintered, old and poorly fed bees are more vulnerable to toxic effect of pesticides as compared to strong and young ones. This is most likely true because such bees have a decrease of vitellogenin i.e. a hemolymph protein with some antioxidant properties (Johnson, 2015) [13]. Compared to other insects, honeybees are extremely sensitive to pesticides, due to a deficiency in the number of genes that encode for detoxification enzymes (Claudianos *et al.*, 2006) [7]. Intraspecific genetic differences also have an impact on the susceptibility to pesticides, e.g. imidacloprid (Suchail *et al.*, 2001) [30].
The extent of colonies damaged in confirmed honeybee poisoning incidents varied in the range of 10-90%. Beebread may be a reservoir of toxic systemic pesticides which when constantly eaten by wintering bees, leads to secondary sub-poisoning. Even similar and less intensive symptoms of acute poisoning (during the time when the poisoned bees could not be replaced), would lead to the perdition of honeybee social roles and to the rest of the honeybees removing themselves from their colony (Rueppell *et al.*, 2010) [24]. There are indications that reduced pollination-service delivery is not due to pesticide-induced changes in individual bee behaviour, but most likely due to effects at the colony level (Stanley *et al.*, 2015) [29]. This information is valuable for scientists, regulators, agrochemical companies, farmers, beekeepers, and enforcement authorities. These data could help improve risk assessment processes used during registration of PPPs and also help to establish accurate guidelines for inspection and controlling the risk of already approved pesticide uses, by standardisation of monitored systems.

2. Beekeeping (Or Apiculture)

It is the maintenance of bee colonies, commonly in man-made hives, by humans. Most such bees are honeybees belonging to genus *Apis*, but other honey-producing bees such as *Melipona* (stingless bees) are also kept. A beekeeper or an apiarist keeps bees in order to collect their honey and other products (including beeswax, propolis, flower pollen, bee pollen, and royal jelly), to pollinate crops, or to produce bees for sale to other beekeepers. A location where bees are kept is called an apiary or "bee yard."

2.1 World Scenerio Of Beekeeping

2.1.1 Change Of World Total Honey Output

Since 1970's, world honey production has developed constantly. In 1970-1978, world honey yield increased slowly, from 8,33,885 tonnes to 9,66,814 tonnes. In 1979 and 1980, world honey production declined greatly, only 8,27,964 tonnes in 1980, down 14.4% from 1978. Since 1981, honey production has showed an increase trend again. In 1985, world honey output exceeded 1 million tonnes for the first time. It reached to 1.21 million tonnes in 1990, top record of before. After 1990, world honey production maintained between 1.10 to 1.20 million tonnes. Output in 1996 is the lowest, only 1.10 million tonnes. From then on, honey production increased year after year. In 2000 it got to a high level of 1.22 million tonnes and in 2013 it is 13.88 lakh metric tonnes (Gu *et al.*, 2002) [11].

2.1.2 Distribution of Honeybee Colonies in the World

There are approximately 56.7 million colonies in the world now. Europe has more than 23 million hives, first of the continents. Russia has 3.58 million. Spain, France, Italy and Germany have 1.90, 1.12, 1.00, 1.60 million hives, respectively. In Asia, there are about 14.0 million hives. China has 6.51 million colonies and Turkey has 4.01 million. In Africa the number of all colonies reaches more than 13 million. Hives in North America are mainly distributed in America and Mexico, the number is 2.63 million and 2.00 million respectively. In South America, Argentina has 2.20 million colonies. The total amount of colonies in Australia and New Zealand, as well as in Oceania is near 1 million hives (Gu *et al.*, 2002) [11].

2.1.3 Distribution of Honey Producing Areas in the World

World honey production is mainly located in Asia, Europe and North America. Africa and South America also has some production. Output in Oceania is lower relatively. According to the statistics of FAO, we made a figure as below.

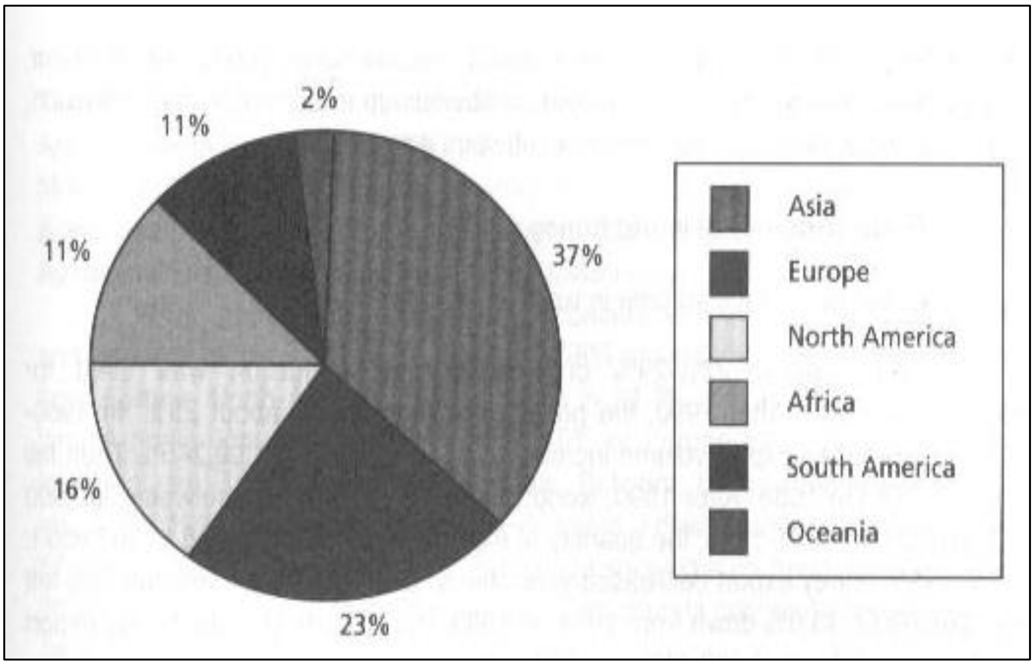

Fig 1: Proportion in world honey production of each continent (Gu *et al.*, 2002) [11]

Highest honey producing countries in the world are: China, Turkey, United states of America, Iran, Russia, India, Mexico, Brazil, Ukraine, New Zealand. One of the largest honey producing country in the world is New Zealand.

2.2 Beekeeping in India

Production of honey has been the major aim of the industry. Modern beekeeping also includes production of beeswax, collected pollen, bee venom, royal jelly, propolis, package bees, queen bees and nucleus colonies. All these are possible only with a proper management of bees utilizing the local plant resources and adapting to the local climatic conditions. Modern beekeeping makes use of bee keeping equipments as well as honey processing plant. By the advent of modern beekeeping in India many new organizations such as TNAU, the YMCA, KHADI, COPERATIVE SOCIETIES, and NGO"S have come forward in order to increase the scope in the field of apiculture (Gu *et al.*, 2002) [11].

2.2.1 Honey Production in India

Beekeeping as a cottage or a village industry has been promoted in India since the beginning of this century. Many states governments and Non-government organizations prepare and execute development programmers for the development of beekeeping in India. Table 1 shows the production of honey in India during the period from 2001-2002 to 2017-2018. In the year 2001 to 2002 honey production was 10 metric tons. It increased to 52 metric tons 2005 to 2007. This study shows that an increase in honey production was very little over the years. That is total honey production increased to 112 metric tons in 2010-2011.

Table 1: Variation in honey production in India during different years (NBB, 2018) [16]

Year	IN 000'MT
2001-02	10
2002-03	10
2003-04	10
2004-05	10
2005-06	52
2006-07	51
2007-08	65
2008-09	65
2009-10	65
2010-11	112
2011-12	84
2012-13	92
2013-14	76
2014-15	81
2015-16	89
2016-17	94.5
2017-18	35

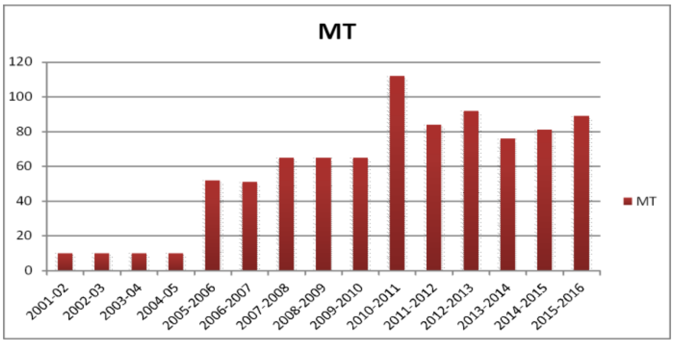

Fig 2: Graphic representation of honey production in India (NHB, 2016) [17]

Only 4 states *viz*., West Bengal, Uttar Pradesh, Punjab and Bihar contributed about 61% to the country's honey production in 2015-16. Based on potential of production the sates categorized under High Potential" are Punjab, WB, Bihar, Kerala, Karnataka, UP, TN &Uttarakhand. The National Commission on Agriculture had visualized the need for deploying about 150 million Bee colonies for pollinating 12 major agricultural crops in the country. Presently, 200 million Bee colonies are required for enhancing their yield which will provide employment to 215 lakh persons and produce 10 million tons of honey and increase in crop production.

Table 2: Honey export and import in India (NHB, 2016) [17]

Year	Export (IN 000"MT)	Import (IN 000"MT)
2000	2	1.2
2001	3	1.1
2002	7	3.4
2003	7	0.2
2004	10	2.6
2005	17	0.5
2006	8	1
2007	12	2.5
2008	16	2.8
2009	13	1.1
2010	23	2.4
2011	29	0.09
2012	25	1.3
2013	30	0.3
2014	28	0.3
2015	30	0.8
2016	38	0.4

Export and import of Indian honey to foreign countries in the year 2000 to 2016 is given Table above. India is one of the major exporters of honey. Major destinations included USA, Saudi Arabia, UAE, Morocco, Bangladesh; Canada etc. In 2015-16 India exported 38.2 thousand MT of honey valued at 706 corers.

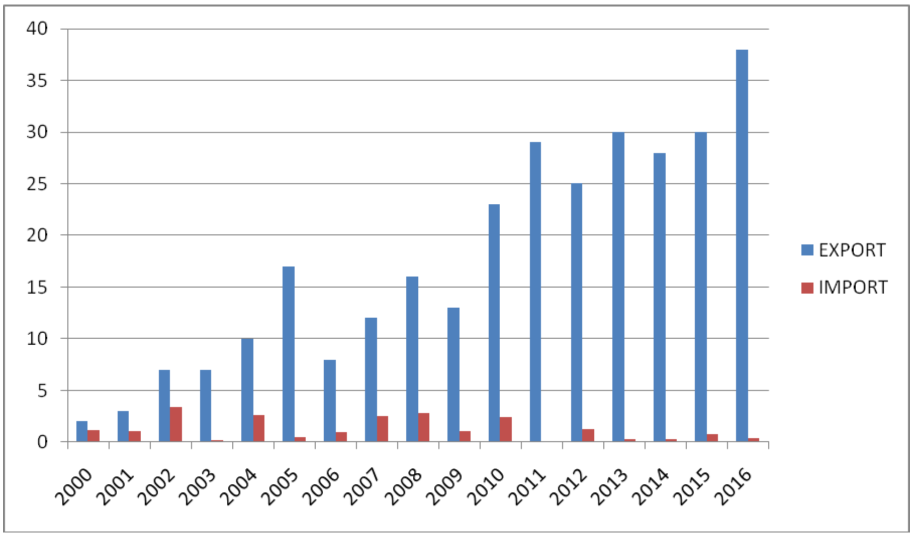

Fig 3: Graphic representation of import and export of honey in India (NHB, 2016) [17]

There are about 14 lakh colonies in India by 2.50 beekeepers giving employment to 1.50 lakh persons. Average yield of honey is 20.11kg/ hive.

2.3 Beekeeping In Himachal Pradesh

There are over 85,000 families in Himachal Pradesh engaged in beekeeping and the state produces 1600 tonnes of honey annually. The state is becoming the biggest producer of Himalayan honey. Under the beekeeping programme, 530.64 MT of honey has been produced upto 31.12.2013 in the State.1 "Himachal Pradesh", owing to its varied agro-climate, has a great variety of bee forage sources that provide the basis for development of beekeeping industry in the state. Scientists in the horticulture department trace the state's history of beekeeping to 1934, when it was first started in Kullu valley and then in Kangra in the year 1936. *Apis cerana indica*, the Indian honeybee, was reared initially, while *A. mellifera*, an Italian bee, was introduced at Bee Research Station, Nagrota (Kangra) in 1961. The fruit producing areas of Kangra, Chamba, Kullu, Mandi, Sirmaur and Shimla in Himachal Pradesh are catering to the growing demand, transforming the state's rural economy and giving locals a popular vocation in beekeeping. The production of honey has already increased to 1600 MTs against 700 MTs in 2005-2006. Some of the private beekeepers have started exporting honey to the UK, Maldives and Kuwait, owing to increasing demand for pure bee honey. The state government helps small entrepreneurs, providing incentives and marketing facilities. Himachal Pradesh will become biggest producer of Himalayan honey. In Kangra alone, 30,000 farmers are engaged in beekeeping, producing 1,200 MT honey annually for the last few years. Blessed with immense flora and fauna, Himachal's quality of honey is of very high grade.

2.4 The Importance of Bees and Pollination in Enhancing Crop Productivity

It has been estimated that over three quarters of the world's crops and over 80% of all flowering plants depend on pollinators, especially honeybees. Previous studies proved that bee pollination had increased yield as well as quality of produce in many horticultural crops e.g. in apple (Verma and Dulta, 1987) [31], strawberry (Partap, 2000a; Partap and Partap, 2000) [20, 22]. Bee pollination not only increased the fruit set but also reduced fruit drop in apple, peach, plum and citrus (Verma and Dulta, 1987; Partap and Partap, 2000) [31, 22]. Various reports have also indicated an increase in fruit juice and sugar content in citrus fruits (Partap, 2000a) [20]. In strawberry, bee pollination reportedly reduces the percentage of misshapen fruits (Partap, 2000b) [21]. Impact of honeybee pollination on fruit productivity of different horticultural crops has been studied below:

Table 3: Impact of honeybee (*Apis cerana*) pollination on fruit productivity

Crop	Increase in fruit set (%)	Increase in fruit weight (%)	Increase in fruit size (%)		References
			Length	Diameter	
Apple	10	33	15	10	Verma and Dulta, 1987 [31]
Peach	22	44	29	23	Partap and Partap, 2000 [22]
Plum	13	39	11	14	Partap and Partap, 2000 [22]
Citrus	24	35	9	35	Partap, 2000a [20]
			Also, premature fruit drop decreased by 46%, increased juice by 68% and sugar contents in juice by 39%		
Strawberry	112	48	Misshapen fruits decreased by 50%		Partap, 2000b [21]

According to Banik (1990) [3], the pollination activity of wind has little effect (10%) on onion pollination because of its sticky pollen, and other pollinators involved were only 3%, while honeybees were 87% in onion pollination. The honeybees were effective pollinators of onion because both pollen and nectar are available from the plant (McGregor, 1976) [14]. From the above, it emerges out that to increase or retain higher productivity of onion, effective pollination by various insect pollinators, particularly honeybees, needs to be ensured and its impact be analyzed.

Table 4: Effect of honeybee pollination in Onion (Padamshali and Mandal, 2018) [19].

Treatments	Parameters					
	No. of Umbels/Sq.m	Av. no. of Pods/ Umbel	Av. no. of seeds/ umbel	1000 seed wt. (gm)	Seed yield (kg/ha)	Germination (%)
Without any Pollinator	46.14 (6.83)	6.33 (2.69)	18.72 (4.37)	2.67 (1.91)	5.91	14.00 (21.84)
With honeybee	47.14 (6.93)	220.78 (14.86)	802.21 (28.32)	3.23 (2.05)	457.72	76.00 (60.75)
With open pollination	47.43 (6.95)	232.14 (15.19)	851.29 (29.11)	3.65 (2.16)	518.22	71.00 (57.49)
SE(m)	2.91 (0.22)	13.52 (0.45)	32.94 (0.63)	0.22 (0.06)	12.97	(1.39)
CD (5%)	N/A	42.12 (1.42)	102.61 (1.95)	0.69 (0.17)	40.41	(4.35)

As in Table 4, among three treatments, open pollination gave highest yield of onion seeds (518.22 kg/ha) followed by honeybee pollination (457.72 kg/ha). Only 5.91 kg of seeds/ha was obtained in plots without any pollinator. Both honeybee pollination and open pollination were on a par with respect to seed yield. An increment of 77.45% in seed yield of onion was obtained by placing honeybee (*A. mellifera*) colonies in onion fields in comparison to that without any pollinator, whereas, in open pollination the increment was 87.68%. Seed germination was highest (76.00%) in honeybee pollinated crop closely followed by open pollination (71.00%), while it was very low (14.00%) in plots without any pollinator. The two former treatments, however, showed no significant difference among them in respect of seed germination (Padamshali and Mandal, 2018) [19].

2.5 Benefits of Beekeeping

Beekeeping has many benefits starting with its main product i.e. honey. The benefits of honey are many, starting with the fact that it is an excellent natural sweetener full of nutrients including niacin, riboflavin, pantothenic acid, calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. Also, it doesn't spoil, and can be used for a variety of health benefits, such as soothing coughs, boosting memory, treating wounds, potentially preventing low white blood cell count caused by chemotherapy, relieving seasonal allergies, killing antibiotic-resistant bacteria, providing fuel for the body, and resolving scalp problems and dandruff.

Wax is another product of bees. One can utilize the wax made in your hive for making candles, cosmetics, creams, lipstick, and lip balm. Bees can help make your plants healthy as they pollinate, as well as fruit trees in nearby orchards which helps the local economy. Moreover, bees can also enhance the productivity of agricultural crops. Beekeeping provides employment and income to youth (Morse and Flottum, 1978) [15].

3. Pesticide Usage History

Any substance intended for preventing, destroying, repelling, attracting or controlling any pest including unwanted species of plants or animals causing harm. The first known pesticide was elemental sulfur dusting used in ancient Sumer about 4,500 years ago in ancient Mesopotamia. The Rig Veda, which is about 4,000 years old, mentions the use of poisonous plants for pest control. By the 15th century, toxic chemicals such as arsenic, mercury, and lead were being applied to crops to kill pests. In the 17th century, nicotine sulfate was extracted from tobacco leaves for use as an insecticide. 19th century saw the introduction of two more natural pesticides, pyrethrum, which is derived from chrysanthemums, and rotenone, which is derived from the roots of tropical vegetables. Until the 1950s, arsenic-based pesticides were dominant Paul Mullar discovered that DDT was a very effective insecticide. Organochlorines such as DDT were dominant. Since then, pyrethrin compounds have become the dominant insecticide.

Herbicides became common in the 1960s, led by triazine and other nitrogen-based compounds. Some sources consider the 1940s and 1950s to have been the start of the "pesticide era". Pesticide use has increased 50-fold since 1950 and 2.3 million tonnes (2.5 million short tons) of industrial pesticides are now used each year. In the 1960s, it was discovered that DDT was preventing many fish-eating birds from reproducing, which was a serious threat to biodiversity is now banned under the Stockholm Convention on Persistent Organic Pollutants.

4. Pesticides and Their Formulations

Table 5: Classification of pesticides (Morse and Flottum, 1978) [15]

Sr.no.	Based on organism	Based on mode of entry	Based on mode of action	Based on chemical nature
1	Insecticide: Endosulphan malathion	**Stomach poisons:** Malathion	**Physical poisons:** Activated clay	**Inorganic compounds:** Sulphur, Zinc phosphite, Arsenicals
2	Rodenticide: Zinc phosphite	**Contact poisons:** Fenvelarate	**Protoplasmic poisons:** Arsenicals	**Organic compounds:** Nicotene, pyrethrum, rotenone
3	Acaricide: Dicofol	**Fumigants:** Aluminium phosphite	**Respiratory poisons:** Hydrogen cyanide	
4	Rodenticide: Zinc phosphite	**Systemic poisons:** Dimethoate	**Nerve poisons:** Malation	
5	Avicide: Zinc Anthraquinone		**Chitin inhibitors:** Diflubenzuron	
6	Mollusicide: Metaldehyde			
7	Nematicide: Ethylene dibromide			
8.	Fungicide: Copper oxychloride			
9.	Bactericide: Streptpmycin sulphate			
10.	Herbicide: 2,4-D			

5. Pesticides Banned In India

A number of pesticides have been banned for use, manufacture, import and export in India due to their toxic effect on humans and honeybees. A list of those pesticides is given below.

Table 6: List of banned pesticides in India (CIBRC, 2019) [6]

S. No.	Pesticides banned for	Pesticides
1.	Pesticides banned for manufacture, import and use (27)	Aldrin, Benzene Hexachloride, Calcium cyanide, Chlordane, Copper Acetoarsenite, Cibromochloropropane, Endrin, Ethyl Mercury Chloride, Ethyl parathion, Hepatchlor, Menzaone, Nitrifen, Paraquat Dimethyl Sulphate, Pentachlorophenol, Phenyl Mercury Acetate, Sodium Methane Acetate, Tetradifon, Toxafen, Aldicarb, Chlorobenzilate, Dieldrin, Malei Hydrazide, Ethylene dibromide, TCA, Metoxuron, Chlorofenviphos
2.	Pesticide formulations banned for used and but their manufacture is allowed to export (2 no.)	Nicotin Sulfate, Capatfol 80%
3.	Pesticide formulations banned for import, manufacture and use (4 no.)	Methomyl 24% L, Methomyl 12.5% L, Phosphamidon 85% SL, Carbofuron 50% SP
4.	Pesticides withdrawn (7 no.)	Dalapon, Ferbam, Formothion, Nickel Chloride, PDCB, Simazine, Warfarin
5.	Pesticides refused registration	Calcium Arsonate, EPM, Azinphos Methyl, Lead Arsonate, Mevinphos, 2,4,5-T, Carbophenothion, Vamidothion, Mephosfolan, Azinphos Ethyl, Binapacryl, Dicrotophos, Thiodemeton, Fentin acetate, Fentin Hydroxide, Chinomethionate, Ammonium Sulphamate, Leptophos
6.	Pesticides restricted for use in India	Aluminium phosphide, DDT, Lindane, Methyl bromide, Methyl Parathion, Sodium cyanide, Methoxy Ethyl Mercuric Chloride, Monocrotophos, Endosulphan, Fenitrothion, Diazinon, Fenthion, Dazomet

6. Effects of Pesticides on Honeybees

Honeybees are exposed to different types of natural and artificial chemical substances. Pesticides are the second group of man-made chemicals in the world after the fertilisers, as concerns the amount applied and the extent of use (AAAS, 2013) [1].

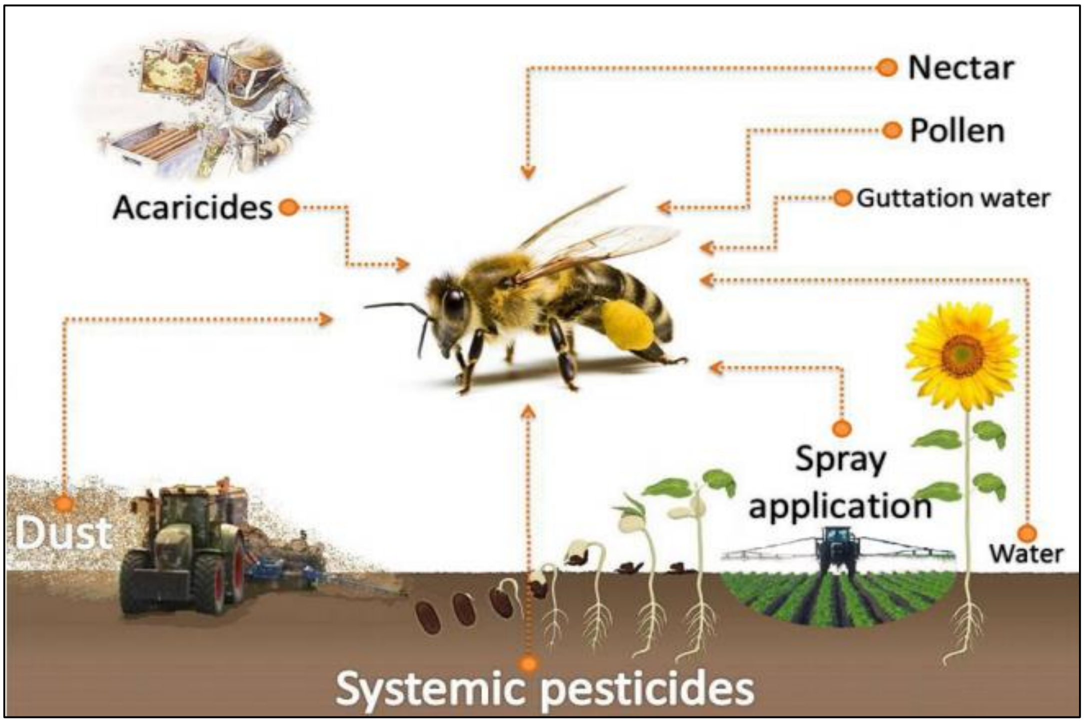

Fig 4: Route of exposure of honeybees to pesticides

6.1 Mode of Action of Pesticides on Honeybees

The sub-lethal effects of pesticides on cognitive, behavioural, and physiological functions of honeybees have already been studied by various workers (Desneux *et al.*, 2007; Belzunces *et al.*, 2012) [8, 4]. Neonicotinoids act on different subtypes of the nicotinic acetylcholine receptors (nAChRs) which are located entirely in the central nervous system of honeybees (Ensley, 2012) [9]. Neonicotinoids, such as imidacloprid, clothianidin, and thiamethoxam, are more toxic to bees than cyano substituted ones. Phenylpyrazole fipronil block gamma-aminobutyric acid (GABA) receptors and furthermore block glutamate-activated chloride channels (GluCls) (Narahashi *et al*., 2010) [18]. Fipronil also causes paralysis and death. Organophosphorus insecticides inhibit ace-tylcholinesterase (AChE), the enzyme that catalyzes the hydrolysis of neurotransmitter acetylcholine. Pyrethroids are able to alter the sodium channel function of cells in insect neuronal membranes, thereby disrupting electrical signalling in the nervous system (Soderlund, 2010) [28]. Moreover, pesticides can affect honeybees by acting as:

1. Contact poison: Absorbed through the integument.
2. Stomach poison: Absorbed through the alimentary canal when taken internally through feeding or cleaning activities.
3. Fumigation: Absorbed through the spiracles affecting respiratory system.

6.2. Symptoms of Bee Poisoning

As an effect of pesticides usage, honeybees showed a number of symptoms that lead to bee poisoning. Some of these symptoms have been listed below:

- Dead bees near the entrance of hive, colonies or top of frames
- Lack of recognition of guard bees
- Abdomen become distended
- Regurgitation of gut contents
- Aggressiveness
- Fighting among bees
- Queen stops laying eggs or lay eggs in irregular pattern
- Paralyzed bees crawling on near- by objects
- Sudden decline in food storage and brood rearing
- Poor recognition of pollen and nectar by bees
- Depleted population of the colony
- Finally results in contamination of bee products (Morse and Flottum, 1978) [15]

Table 7: Different pesticides and their symptoms

S. No.	Pesticides	Symptoms
1.	**Organophosphorus**	▪ Regurgitation ▪ Disorientation ▪ Irritability ▪ Perhaps distended abdomen ▪ Erratic attempts to clean selves ▪ Tumbling about ▪ Paralyses and ultimately die ▪ Wings held away from body but usually remaining hooked together ▪ High percentage of poisoned bees die near the colony

2.	**Chlorinated hydrocarbons**	▪ Erratic movements ▪ Tumbling ▪ Hind legs dragged as if paralyzed and wings held away from body but usually remaining hooked together ▪ High percentage of bees die in the field or near apiary
3.	**Carbamates**	▪ Aggressiveness ▪ Erratic movements ▪ Unable to fly ▪ Most bees usually die at the colony ▪ Queens often cease egg laying ▪ Hive bees initiate supercedure ▪ Rearing queens before egg laying resumes
4.	**Botanicals**	▪ Regurgitation from highly toxic Pyrethrins together with erratic movements then inability to fly followed by paralysis and death ▪ Bees often die between foraging area and colony
5.	**Dinitrophenyl**	▪ Similar to symptoms of chlorinated hydrocarbons ▪ Most affected bees usually die at colony
6.	**Other effects of pesticides**	▪ The use of herbicides eliminates the weed flora which serves as very good food source for bees especially during dearth period. ▪ Direct exposure to insecticidal sprays results in the death of bees and sometimes lead to the destruction of bee colonies. ▪ Contamination of water resources affect water carriers. ▪ Contamination of nectar and pollen causes brood mortality. ▪ Widespread use of chemicals also contaminates the hive products. ▪ Indiscriminate use of pesticides threatens the integrity of bee–flower mutualistic system.

6.3 Previous Studies Demonstrating the Effect of Pestcides on Beekeeping

A number of workers have studied the effect of various pesticides on honeybees, few of which have been described below:

6.3.1 Effect of thiamethoxam on hypopharyngeal gland development of honeybees

Thiamethoxam can affect the hypopharyngeal gland development in honeybees as it causes smaller acini and frequent modification of their shape (Renzi *et al.*, 2016) [23].

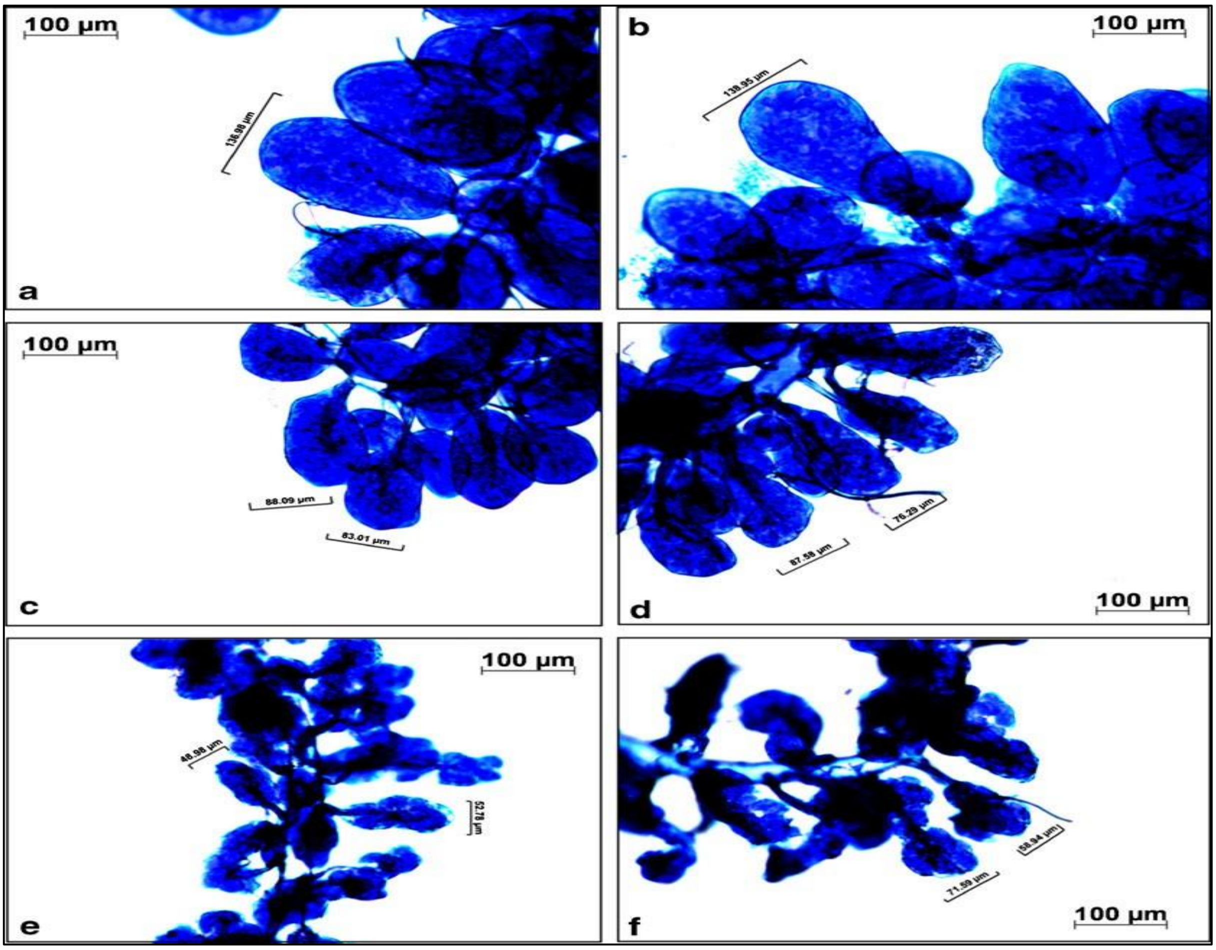

Fig 5: Different shapes of HPG acini observed. As the pesticide concentration increased and the diet quality decreased, the acini had a reduced size and acquired a more irregular outline, and the general aspect of the HPGs became less compact. a, b BHigh^ and BLow^ diet combined with 0 µg/L of thiamethoxam; c, d BHigh^ and BLow^ diet combined with 10 µg/L of thiamethoxam; and e, f BHigh^ and BLow^ diet combined with 40 µg/L of thiamethoxam

6.3.2 Assessment of sensitivity level of honeybee (*A. mellifera*) to neonicotinoid pesticides

Out of the four neonicotinoids used in the study, imidacloprid, thiacloprid and chlothianidin are most toxic pesticides with 100% mortality observed after 3 hours and 6 hours. Maximum mortality was observed under field dose and mortality level decrease with serial dilution of field dose (FD, FD10, FD100, FD1000 and FD10000). Acetamiprid shows less toxicity at recommended field dose with 86.6% mortality (Imran *et al*., 2018) [12].

Table 8: Toxicity of four neonicotinoid pesticides against *Apis mellifera* at recommended field dose and its serial dilation by ingestion method with sugar solution

Insecticides	Time	Expose bees	FD	FD/10	FD/100	FD/1000	FD/10000	Control
Acetamiprid	3hrs	15	86.7	20.0	0.0	0.0	0.0	0.0
	6hrs	15	93.3	33.3	0.0	0.0	0.0	0.0
	24hr	15	100.0	60.0	53.3	46.7	20.0	33.3
	48hr	15	100.0	100.0	80.0	53.3	33.3	33.3
Imidacloprid	3hrs	15	100.0	100.0	66.7	26.7	6.7	0.0
	6hrs	15	100.0	100.0	73.3	40.0	13.3	0.0
	24hr	15	100.0	100.0	86.7	46.7	60.0	0.0
	48hr	15	100.0	100.0	100.0	100.0	80.0	26.7
Thiacloprid	3hrs	15	80.0	40.0	6.7	13.3	40.0	0.0
	6hrs	15	100.0	40.0	6.7	20.0	40.0	0.0
	24hr	15	100.0	73.3	20.0	60.0	60.0	0.0
	48hr	15	100.0	80.0	73.3	73.3	80.0	26.7
Clothianidin	3hrs	15	80.0	40.0	6.7	13.3	40.0	0.0
	6hrs	15	100.0	40.0	6.7	20.0	40.0	0.0
	24hr	15	100.0	73.3	20.0	60.0	60.0	0.0
	48hr	15	100.0	80.0	73.3	73.3	80.0	26.7

6.3.3 Comparative effects of different pesticides on honeybees: When applied as a direct spray, all the pesticides had significant impacts on mortality of honeybees, with slightly different time courses (Table 9). The order of cumulative acute direct toxicity was: Betacyfluthrin > Carbaryl > Betacyfluthrin + Imidacloprid > Ethiprole > Demeton-O-Methyl > Profenophos > Imidacloprid > Malathion (Sharma and Abrol, 2014) [26].

Table 9: Effect of direct spray of pesticides on honeybee mortality rate

Treatment	Concentration (%)	Mean per cent mortality after hours						
		1	2	4	8	12	24	48
Betacyfluthrin	0.007	100	100	100	100	100	100	100
Imidacloprid	0.0025	15	34.2	55.8	79.2	100	100	100
Betacyfluthrin +Imidacloprid	0.004	100	100	100	100	100	100	100
Carbaryl	0.10	100	100	100	100	100	100	100
Demeton-o- methyl	0.03	15	45	72.5	90.8	100	100	100
Ethiprole	0.07	24.2	50.8	70.8	95.8	96.7	100	100
Malathion	0.07	3.3	5.0	20.0	40.0	61.7	79.2	94.2
Profenphos	0.07	10.8	31.7	53.3	80.8	98.3	100	100
Control	-	0.0	0.0	0.0	0.0	0.8	2.5	4.2
CD		4.3	2.8	2.5	2.1	1.2	1.3	1.1

6.3.4 Effects of pesticides on sperm viability of drones

In 2017, it was found that drones reared in frames coated with wax exposed to miticides or agro-chemicals had lower sperm viability than those reared in pesticide-free frames ($F = 22.5$, $P<0.0001$). The researchers sampled sperm viability from 33 drones in the control group, 38 drones in the miticide treatment group, and 50 drones in the agro-chemical treatment group. Drones in the miticide treatment group, which consisted of a mixture of fluvalinate and coumaphos ("F+C" treatment), had an average sperm viability of 80.0% ± 2.87%, which was significantly lower than the viability of 96.9% ± 0.64% in the control group ($t = 5.73$, $P< 0.0001$; Fig). Drones exposed to the agrochemical treatment group, which consisted of a mixture of chlorothalonil and chlorpyrifos ("C+C" treatment), had an average sperm viability of 93.2% ± 1.16%. While sperm viability of drones in the C+C group was higher than the viability of drones in the F+C group, it was nevertheless significantly lower than the sperm viability of drones in the control group ($t = 2.43$, $P = 0.009$; Fig) (Fisher and Rangel, 2016) [10].

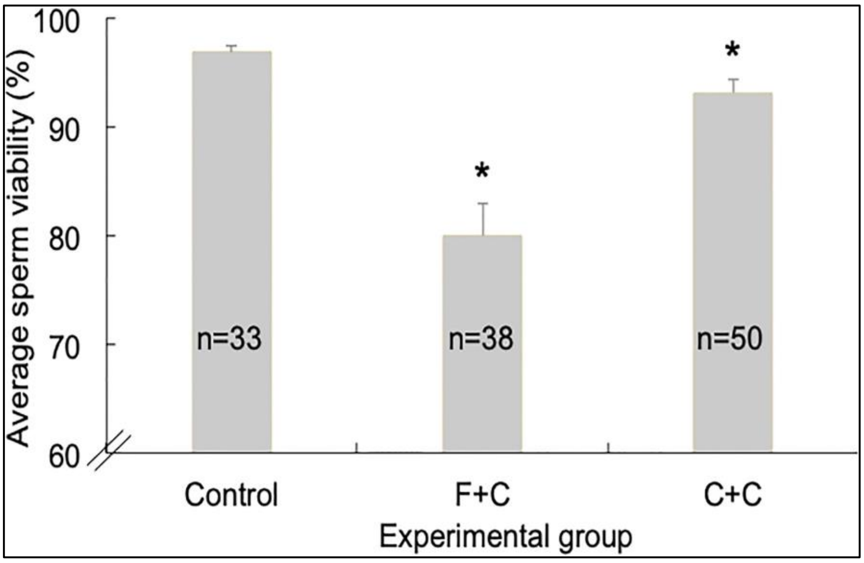

Fig 6: Sperm viability in drones exposed to miticides and agro-chemicals during development

6.3.5 Effect of neonicotinoids on *A. mellifera* under field and semi-field conditions

In thiamethoxam treated mustard bloom under field conditions the average bee mortality was significantly higher on 1st (123.33 bees/DBT/day) and 2nd (41.33 bees) after spray in comparison to control. In general, average bee mortality of *A. mellifera* as per dead bee trap was significantly higher (20.21 bees/DBT/day) in thiamethoxam treated plots as compared to control (8.43 bees) (Sharma *et al.*, 2018) [27].

Fig 7: Dead bees inside hive

On the basis of LD_{50}, cypermethrin, imidacloprid, lambda cyhalothrin and spinosad were found to be highly toxic (0.001-1.99 µg/bee), endosulfan as moderatley toxic (LD_{50} 2.0-10.0 µg/bee) and azadirachtin, *Bacillus thuringiensis* subsp. *kurstaki*, benzoylphenyl urea, spiromesifen and thiacloprid as relatively non-toxic with $LD_{50} > 11.0$ µg/bee (Choudhary and Sharma, 2007) [5].

7. Factors Influencing Bee Poisoning

The fundamental symptom indicating that poisoning occurs is the high number of dead bees. The figures established by the FAO as guidelines for assessing the extent of pesticide poisoning are: that 100 dead bees per day is the colony's normal death rate; 200-400 dead bees indicate a low level of pesticide poisoning; 500-1000 dead bees indicate a medium level of pesticide poisoning; over 1000 dead bees indicate a high level of pesticide poisoning (Akratanakul, 1990) [2].

a. Pesticide formulation: Dust formulations are more hazardous to bees than sprays as these contaminate the atmosphere and can be carried to neighboring localities. Wettable powders have longer residual effect than emulsions.

b. Period of application: Bee visits plants during their flowering period, therefore pesticide application during flowering period result in harmful effects on honey bees.

c. Time of application: Bees are generally active during day time and attends pick intensity at afternoon. Pesticide applications during this period are therefore very hazardous to the bees.

d. Residual action of pesticide: Many pesticides are made so that they can retain their properties for long after application. Such chemicals with long residual action are harmful to the bees.

e. Location of colonies: Honey bees normally have a foraging range of 1 to 2 km from the colony. Application of pesticides within 2 km of the colonies, therefore proves detrimental.

f. Temperature: The most significant factor causing differences in the toxicity of pesticides. Immediate effect may be much greater at higher temperatures whereas, residual effects are likely to be less because the toxic materials breaks down more quickly.

g. Age and size of bees: Smaller bees have a higher surface to volume ratio and contact poison will be more toxic to them to larger bees.

8. Pesticide Management to Reduce Bee Losses

It is very important to manage the use of pesticides if one want to reduce bee losses. The following points to be considered while maintaining an apiary.

a) **Use pesticides only when needed:** Don't apply any pesticides unless the crop is heavily infested - If necessary, use those insecticides which are non-toxic or less harmful to the bees.
b) Avoid the application of a pesticide to a crop in bloom.

c) Use of safest formulation of a safest insecticide - Granular formulations are safest for bees. Dusts are more harmful than the sprays of the same insecticide. Emulsifiable and water soluble concentrates are safest for the bees, Adding solvent or an oily substance tends to make the sprays safest for the bees.
d) **Method of pesticide application:** Ground application is safer than the aerial application. Inject systemic insecticides into the ground where ever possible, not into the plant. Fine sprays are safer than the coarse ones. A combined application is often safer as well as cheaper than the application of separate insecticides at different times. Repellents may be used to discourage bees from foraging on the treated crop.
e) **The timing of application:** Never apply pesticides while the crop is in bloom or while interplant's or adjacent crops are in bloom. Never apply insecticides when bees are flying. Take early morning or late evening application of pesticides depending upon bee activities on the crop are relatively safe. Pesticide dusts and small granules should not be left open or thrown carelessly anywhere because bees are likely to collect such dusts during acute dearth periods.
f) **Early Warning To Beekeepers**

i. Providing sufficient space in hives
ii. Provide proper ventilation
iii. Shading hives
iv. Covering the hives with net absorbent matting
v. Provide water inside the hives
vi. Minimizing the period of confinement
vii. Use biocide applications as far as possible outside the blooming period
viii. Pesticides which have short residual effects are less hazardous to honeybees
ix. Broad spectrum pesticides should be avoided as they are more hazardous to bees than the selective pesticides
x. Both the orchardists and beekeepers should be educated properly about pesticide applications schedules and how to reduce poisoning in a particular area
xi. Evening or early morning application of pesticides is always desirable because foraging bees are at that time in the hive and out of danger
xii. Keep bee colonies away from the treated fields as far as possible
xiii. The order of toxicities of insecticide formulation: dust > wettable powder > Emulsifiable concentrate or soluble or liquid solution > Granular formulation
xiv. Remove all the flowering weeds from the field so that they do not act as a source of poison to bees.
xv. Primary emphasis should be on the use of an Integrated Pest Management programme which relies on biological, cultural or other non-chemical methods of insect pest control and minimize the use of poisonous chemical.

9. Conclusion
Since usage of these plant protection products cannot be stopped as they are necessary for agricultural production a rational approach must look at minimising the risk of such agrochemicals to bees .Farmers should be aware of the problems that pesticides exhibit for honey-bees. Farmers should minimize the contamination of landscape including water bodies with pesticides because not only the honeybees but a large array of pollinator species may also be affected. Simply banning the pesticide is clearly not the way forward and appropriate option.

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