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REVIEW ARTICLE

TOXICITY OF INSECTICIDES AGAINST HONEY BEES IN LABORATORY CONDITION

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Abstract: Insecticides are harmful for all living organism. Many insecticides are harmful to honeybees, with neonicotinoids and some acaricides being particularly harmful, even at low dose, causing mortality, behavioral issues and weakened immune system. On the basis of toxicity, they are categorized in three groups i.e. highly toxic, moderately and nontoxic to honeybees. Toxic insecticides do not used during the flowering of the crop because bees died in the field and also died inside and outside of the hives after coming from the insecticides applied field. Insecticides should be applied during the evening hour or migrate the bee colony for safety of the bees. It has been observed that after 8 to 10 days of application of insecticides *Apis cerana indica* colonies were found mortality but *Apis mellifera* colonies were not observed.

Keywords: *Apis cerana indica*, *Apis mellifera, stingless bee, bumble bee,* Honey bee

INTRODUCTION

Honeybees are the very good pollinators owing to their important role in crop pollination and maintenance of wild plants communities and contribute global food worth 153 billion € annually Honeybees, mainly *Apis mellifera* L. and other bee species are the main source of pollination of many food crops. There are many sources of pollination, but honeybees play an important role by pollinating more than 75% of flowering plants across the world and almost from 100 valuable crops of the world, 70% are being pollinated by honeybees.

The use of agrochemicals put a key pressure on insect. Due to their indiscriminate use, colonies of honeybees are declining across the world. Agrochemicals can destabilize different types of pollinator communities before and after their application in the field crops. If the production of fruits and seeds are pollen limited then there will be certain effects on pollination due to low and variable pollinators which ultimately decreases the crop yield.

Insecticides also induce chronic toxicity in honeybees in terms of change in their foraging and learning behavior. Insecticides also affect their immune system and susceptibility to diseases. The decline of honeybee populations in the world is a serious problem. If this speed of their decline continues, the pollination of agricultural crops which depend upon bees will be badly affected. So, it can cause the world food production at risk, because they are the main source of pollination for different crops, fruits and vegetables, Shelke *et al*. (2024).

Insecticides are tool for integrated pest management which is widely used to control
insect pest population. Among different categories of pesticides, the insecticides are the most widely used compounds in terms of value. Traditionally insecticides have been categorized into five major chemical groups based on their chemical nature--organophosphate (e.g. chlorpyrifos, triazophos, profenofos); organochlorine (e.g. chlordane, endosulphan, DDT); carbamates (e.g. aldicarb, carbaryl, carbofuran); pyrethroids, the synthetic insecticides (e.g. allethrin, cymermethrin, pyrethrins) and neonicotinoids (e.g. imidacloprid, thiacloprid, thiomexotham) (IRAC, 2016). The insecticides are classified on the basis of chemical composition, mode of entry in insect, mode of action, toxicity and the stage of specificity. The toxicity of insecticides is expressed in quantitative terms, such as LD50 (Lethal Dose) and LC50 (Lethal Concentration) i.e. the dose and concentration which kill 50% of population within a specified period (Singh, 2012). Increases in land conversion and agricultural intensification due to insecticide use have accelerated the loss of soil biodiversity, resulting in a 60% decline in soil ecosystem services (Singh *et al*.,2019). In this review article the toxicity of insecticides on honey bee trying to awareness among the beekeepers,

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students, farmers and also for scientists on different species of bees.

(A) Toxicity of insecticides against Indian honey bee, *Apis cerana indica*

Insecticides can significantly harm honey bees through direct contact while foraging, exposure to contaminated pollen and nectar, or through systemic effects on their nervous and immune systems. Some insecticides, like chlorinated hydrocarbons, organophosphate, carbamate synthetic pyrethroides, oxadigene and neonicotinoids, are harmful to bees. While some insecticides are considered safer for bees.

Impact of Insecticides on Honey Bees:

- **Direct Contact:**

Bees can be killed by direct contact with insecticide spray on plants or blooms when foraging in treated areas.

- **Contaminated Food:**

Bees can ingest insecticide residues in pollen and nectar, which can weaken them and make them more susceptible to diseases and other stressors.

- **Systemic Effects:**

Some insecticides, particularly neonicotinoids, can disrupt the nervous system of bees, affecting their ability to navigate, forage, and communicate.

- **Weakened Immune System:**

Exposure to insecticides can weaken a bee's immune system, making them more vulnerable to infections and parasites.

Examples of Insecticides and Their Effects:

- **Neonicotinoids:**

Studies have linked neonicotinoids to widespread bee die-offs and population declines.

- **Organophosphates:**

Insecticides like coumaphos (organophosphate) and chlorpyriphos can also negatively effect on bees.

- **Endosulfan:**

While considered safer than some organophosphates, endosulfan still poses a risk to bees.

- **Etoxazole:**

This miticide is generally considered safer for bees.

- **Emamectin Benzoate:**

Some studies suggest emamectin benzoate 5 WG may be safer for bees than some standard insecticides.

What Makes an Insecticide Bee-Safe?

- **Low Toxicity:** Insecticides with low toxicity to bees are preferred.
- **Contact vs. Systemic:** Contact insecticides, when used properly, may pose less risk than systemic insecticides that are taken up by plants.
- **Targeted Application:** Applying insecticides at times when bees are not actively foraging can minimize exposure.

Way and Synge (1948) determined the effects of DDT and benzene hexachloride (BHC) on honey bess and on several wild bee species. Laboratory experiments showed that BHC a powerful contact and stomach poison and results of field experiments with commercial preparation confirm its danger to the foraging bee population.

Weaver (1950) conducted to ascertain the toxicity of BHC, DDT, chlordane, and toxaphane to honey bees. The safest of the insecticides in the field was 20 per cent toxaphane,40 per cent sulphur, 10 per cent DDT, 40 per cent sulphur and 10 per cent chlordane, 40 per cent sulphur were more toxic, but deaths were still not high. Bees were repelled for about 3 hours following applications of cyclodyne compound like γ BHC and DDT and also by sulphur with recorded of slight mortality.

Anderson and Atkins (1958) found and tested 33 compounds for relative toxicity to honey bees. Ten of these compounds were found to be more toxic than the DDT standard treatment, four were about as toxic as DDT and all the rest (mostly phosphates) were proactively nontoxic to honey bees. Sabadilla at dosages comparable to those used in citrus groves was nontoxic to bees, while dosages comparable to those used on vegetable crops were highly toxic. DDT, toxaphane and sulphur alone and in various combinations, showed that DDT was moderately toxic to bees and that sulphur and toxaphane were only slightly toxic.

Clinch (1967) adopted a spray tower to enable insecticide sprays to be applied to the flowers of white clover (*Trifolium repens* L.) held in jars of water. Honey bees were enclosed with the flowers for an hour (3, 18 or 42 hours after application) to determine the residual contact toxicities of the sprays. Comparative tests on artificial flowers made of foil indicated that they cannot be used as a substitute for clover flowers.

Singh *et al*. (1974) tested 15 insecticides and reported that menazon and endosulfan were the least toxic, with LC50 values of 0.4826 and 0.4503 per cent, respectively. It was considered nontoxic to *A. cerana* at their recommended application rates of 0.01 per cent and 0.05 per cent, respectively. Bai and Reddy (1977) tested the comparative topical toxicity of 16 insecticides to *Apis cerana indica* under laboratory conditions at the dosage recommended in India for agricultural purposes, and all were lethal to *A. c. indica*. Organophosphates (op) and carbamates (c) showed a similar toxicity, which was greater than that of chlorinated hydrocarbons (ch). Toxicity, assessed by the speed with which all bees were killed, was found to be in the following order, starting with the most toxic: dichlorvos, methyl parathion, phosphamidon, quinalphos (all op); carbaryl (c); fenitrothion, monocrotophos, oxydemeton-methyl, dimethoate (all op); carbofuran (c), carbophenothion (op), malathion (op); heptachlor, DDT, endrin, and BHC (all ch).

Wilksniec (1980) tested six pesticides in the laboratory,3 were highly toxic to honeybees (Fenthoate, Permethrin, Diazinon). Dimilin 25PM (diflubenzuron) applied at 0.24 kg ha^{-1} was not toxic, but it caused increased mortality at higher dose Mitac 20 EC (Amitraz) at 2.4 l ha^{-1} and Croneton EC 500 (Ethiofencarb) at 0.6 l ha^{-1} were not toxic to honeybees.

Lingappa *et al*. (1985) reported the intrinsic toxicity of seven insecticides including bromophos, bromophos-ethyl and deltamethrin for the Indian hive bee, *Apis cerana indica*. Bromophos was safer than endosulfan while deltamethrin was the most toxic.

Kumar *et al*. (1986) evaluated by water distillation of seeds of ajwan (*Trachyspermum ammi* L.) and leaves of chenopodium (*Chnlopodium ambrosioides* L.), citronella (*Cymbopogon nardus* L.), eucalyptus (*Eucalyptus spp*.) and lantana (*Lantan camara* L.). Oils were evaluated in the laboratory for gustatory repellency (CCR 50) and stomach toxicity (LD 50) to worker honeybees of *Apis cerana indica* using benzaldehyde as a reference standard. Oils of ajwan, citronella, chenopodium, lantana and eucalyptus respectively were 6.49,5.95,3.20,1.31 and 0.69 times as repellent and 3.74,1.15,2.15, 1.10 and 1.36 times as toxic to bees as benzaldehyde. The order of safety to bees (rati of LC50 to CCR50) was citronella (11.36)>ajwan (3.81)> chenopodium (3.27)> lantana (2.62)> benzaldehyde (2.19)> eucalyptus (1.11), respectively.

Bhat (1987) determined the toxicity of endosulfan, phosalone, dichlorvos and toxaphene to *Adoretus spp*., pests of cherry trees and to *A. cerana* workers, in laboratory tests. The beetles were exposed to sprayed leaves and the bees to sprayed flowers. Phosalone and toxaphene were the most toxic of the 4 insecticides to *Adoretus* both immediately and 12hrs after application. Phosalone and dichlorvos 12hrs after application were the least toxic of the treatments to *A. cerana*, followed by phosalone immediately after application.

Atallah *et al*. (1988) tested the topical applications of 5 concentrations of Decis (Deltamethrin), Nurelle (Cypermethrin), Cybolt (flucythrinate), Meothrin (fenpropathrin) and Sevin(carbaryl) to mouthparts or the abdomens of honeybees showed that, generally, Decis was the most toxic and Sevin the least toxic of the insecticides and that bees were more sensitive to abdominal applications. Oral applications, using the recommended concentrations of the insecticides, revealed the following order of toxicity: Sevin > Decis > Meothrin > Nurelle > Cybolt.

Panda *et al*. (1989) determined the contact toxicity of phosalone, endosulfan, malathion, phosphamidon, cypermethrin, fenvalerate, decamethrin, and neem oil on *Apis cerana indica* Fabr. foragers was tested in the laboratory. Based on LC50 values phosphamidon was highly toxic followed by endosulfan and phosalone whereas the Neem oil was the least toxic.

Deshmukh *et al*. (1989) tested in laboratory on the efficacy of a new pyrethroid insecticide cyhalothrin at Gujarat. Cyhalothrin was tested on *Apis cerana indica* for both 30 and 60 min where LC50 values were 21.05 and 244.61, respectively.

Gromisz and Gromisz (1996) studied the laboratory investigations on the sensitivity of bees to harmful effects of Bulldock formula, the insecticide Bulldock belongs to synthetis pyrethroids which contains 2.5 per cent betaciflutrin as an active ingredient. It was highly toxic to bees when ingested the lethal dose of the active ingredient, betaciflutrin, at 0.2-0.3 ppm dose per single bee.

Gromisz and Gromisz (1997a) tested on 144 and 36 bees in laboratory of the harmful effects of Sanmite 20 WP to oral and contact toxicity. This agent comprised 20 per cent of piridabene (biologically active ingredient). Doses of piridabene higher than1 microgram per bee increased the rate of mortality by 10-95 per cent. Doses comprising 1 microgram of the agent or less substantially increased the rate of mortality of bees under laboratory conditions and about 50 per cent of bees died during 17 to 25 days whereas control bees attained this mortality rate during 30 days. In the contact toxicity testing the base contaminated with the agent caused a 78 per cent mortality rate in bees even after 24 days.

Singh *et al*. (1997) studied in laboratory with 8 insecticides and among these endosulfan was found to be the least toxic and cypermethrin the most toxic to *Apis cerana indica* forager.

Reddy (1997) studied the relative toxicity of some insecticides to honey bees (*Apis cerana indica* Fab.). Cypermethrin and permethrin were highly toxic to foraging workers of *Apis cerana indica* with LC50s of 0.0002 and 0.00001 per cent, respectively. Methyl demeton and phosphamidon were moderately toxic (LC50=0.008 and 0.019 per cent, respectively), while endosulfan was the least toxic (LC50=0.41 per cent).

Gromisz and Gromisz (1998) evaluated the toxicity of the agent Aztec 140 EW in bees under laboratory condition. It was recently accepted by the Polish Pesticide Regulation, contains 140 g of biologically active triazamat per litre. This agent was used for aphid control in fruit cultivation. Bees exposed orally to concentrations of 0.0375 to 0.15 per cent demonstrated a moderate toxic response. However, no direct and significant increase in the mortality rate was found as a result of the treatment.

Rajeswaran *et al*. (2003) determined the relative contact toxicity of carbosulfan against Indian honey bee, *Apis cerana indica* Fab. Three doses of carbosulfan 25 EC were tested along with the standard check methyl-o-demeton for their contact toxicity under laboratory conditions. Carbosulfan 25 EC caused significant mortality at all the doses tested. The mortality ranged from 19.00 to 30.00,

35.00 to 59.60, 64.00 to 75.00 and 90.60 to 100.00 per cent at 3,6, 12, 24 hours after exposure, respectively. The level of mortality in methyl-o-demeton 25EC was comparable to that of the lowest dose of carbosulfan 25 EC.

Gour and Pareek (2005) tested the contact toxicity of 9 insecticides against adults of Indian honey bee, *A. cerana indica* in the laboratory. Among them Cypermethrin was observed most toxic, dimethoate, imidacloprid and acephate as moderately toxic and cartap hydrochloride, malathion, neem extract and ethofenprox as less toxic to bee adults as against standard endosulfan. The order of toxicity of bees was Cypermethrin > dimethoate > imidacloprid > acephate > cartap hydrochloride > malathion, neem extract > ethofenprox > and endosulfan.

Sharma and Abrol (2005) tested the contact toxicities of five insecticides *viz*- malathion, demeton-s-methyl, cypermethrin, deltamethrin and fenvalerate applied to foragers of honeybee *A. cerana*. Each insecticide was evaluated at three levels of concentrations for contact toxicity under laboratory conditions. The mortality of honeybees increased with increase in concentration and exposure time. No quick knockdown effect was observed in all insecticides tested. The order of toxicity of different insecticides after 8 hour of treatment was in the order of Malathion > demeton-s-methyl > cypermethrin > fenvalerate and deltamethrin. All the insecticides were highly toxic to honeybees.

Muranjan *et al*. (2006*)* conducted the laboratory studies in Pune on foraging honey bee, *A. cerana indica* by using the contact filter paper technique to assess the acute toxicity of four ready mix formulations *viz*., endosulfan 32% + deltamethrin 0.8% (Decidan, 32.8 EC), acephate 25% + fenvalerate 3% (Koranda, 28 EC), chlorpyriphos 50%, + cypermethrin 5% (Nurelle D-505, 55 Ec), and triazophos 35% + deltamethrin 1% (Spark, 36 EC) alongwith seven individual comprising ad-mixed insecticides. The LC_{50} values for Decidan, Konanda, Nurelle D and Spark were found to be 0.01462, 0.002354, 0.0014903 and 0.004306 per cent, respectively while the values for endosulfan, acephate chlorpyriphos, triazophos, deltamethrin, fenvalerate and cypermethrin were 0.040123, 0.01362, 0.0009789, 0.002189, 0.0003238, 0.008443 and 0.0004493 per cent, respectively. Relatively safe ones for field use were judged on the basis of computed safety indices wherein, all the formulations were predicated to be safer except acephate, chlorpyriphos and Koranda which were predicated to be slightly toxic and Nurelle D to be toxic.

Pastagia and Patel (2007) tested the relative toxicity of insecticides against worker bees of *Apis cerana indica* with ten insecticides replicated thrice. The toxicity of insecticides was tested using dry film method. Counting the mortality of worker bees at 24 hours after exposure, the treatments of profenofos @0.05 per cent and indoxacarb @ 0.075 per cent recorded cent per cent mortality which was on par with carbosulfan @ 0.005 per cent (93.03 per cent). The treatment carbosulfan was also comparable in terms of mortality per cent with the treatments, thiamethoxam @ 0.005 per cent (85.67 per cent), imidacloprid 2 0.005 per cent (80.67 per cent) ethofenprox @ 0.005 per cent (76.81 per cent) and Fenobucarb @ 0.1 per cent (73.77 per cent). Endosulfan @ 0.07 per cent recorded significantly minimum mortality (56.70 per cent) among all the insecticides tested which was on par with fipronil @ 0.005 per cent (56.99 per cent) and acetamiprid @ 0.004 per cent (73.47 per cent).

Dhanasekaran (2007) tested against the honey bee, *Apis cerana indica* on four combination insecticides, cypermethrin 5% + monocrotophos 25% OC, cypermethrin 5% + dimethoate 20% EC, cypermethrin 3% + acephate 57% DF and monocrotophos 25% + dichlorvos 11% SL. The relative toxicity of these combination insecticides were worked out based on the LC< sub>50</ sub> value of endosulfan 35% EC. The order of toxicity for *A. cerana indica* was cypermethrin 5% + dimethoate 20% EC > cypermethrin 5% + monocrotophos 25% OC > monocrotophos 25% + dichlorvos 11% SL > cypermethrin 3% + acephate 57% DF > endosulfan 35% EC.

Kumar *et al*. (2010) evaluated the contact toxicity of spirotetramat 150 OD against Indian honeybees @ 45,60 and 75 g a.i. ha^{-1} caused 3.33, 10.00 and 20.00 per cent mortality respectively. After 6 HAT and it increased to 10.00 and ,16.67 and 30.00 per cent respectively after 24 HAT. But chloronicotinyl compounds, imidacloprid and acetamiprid and conventional insecticides, monocrotophos and methyl demeton caused increased mortality of 46.67,43.33, 56.67 and 63.33 per cent, respectively. Similar trend was observed in Italian bees, little bees and stingless bees. Hence, spirotetramat was considered to be relatively safer to honeybees than imidacloprid, acetamiprid, monocrotophos and methyl demeton.

Painkra *et al*. (2015) the order of contact toxicity of different insecticides to the forager bees *Apis cerana indica* was phosphamidon > monocrotophos > chlorpyriphos > profenophos > cypermethrin > fipronil > ethofenprox > fenvalerate > flubendiamide > cartaphydrocloride> imidacloprid > indoxacarb > thiamethoxam > neemoil. cypermethrin was observed to be most toxic whereas dimethoate, imidacloprid and acephate as moderately toxic and cartap hydrochloride, malathion, neem extract and ethofenprox as less toxic to bees adults as against standard check endosulfan the toxicity of different insecticides after 8 hour of treatment and the order of toxicity was malathion > cypermethrin > demeton s-methyl > fenvalerate > deltamethrin for *Apis mellifera* and

malathion > demeton-s- methyl > cypermethrin > fenvalerate > deltamethrin for *Apis cerana indica*. All the insecticides were highly toxic to honey bees. *Apis cerana indica* worker bees at 24 hours after exposure to different treatments and reported that profenophos and indoxacarb were highly toxic. Thiamethoxam, imidacloprid, ethofenprox and fenabucarb were moderately toxic and endosulfan was least toxic. Carbosulfan was highest toxicity whereas methiocarb and carbaryl were comparatabily less toxic. The spirotetramet 150 OD at 45,60 and 75 ai./ha caused 3.30, 10.00 and 20.00 per cent mortality to Indian bees after 6HAT, thereafter, it increased to 10.00, 16.67 and 30.00 per cent after 24 HAT.

Painkra *et al*. (2016) After 6 hour of exposure, the mortality per cent was observed maximum in phosphamidon 500 g.a.i./ha (90.0%) and it was statistically superior over control followed by monocrotophos 400 g.a.i./ha (85.0%). However, 12hrs after treatment per cent mortality of honey bees was recorded higher in cartap hydrocloride 300 g.a.i./ha (60.0%) followed by profenophos 500 g.a.i./ha Whereas the mortality in monocrotophos 400 g.a.i./ha was 12.5 per cent followed by phosphamidon 500 g.a.i./ha (10.0%), neem oil 2500 g.a.i./ha (2.5%). After the exposure of 24 hour, the per cent mortality was observed highest in fenvalerate 200 g.a.i./ha (77.5%). Whereas, less mortality was recorded in monocrotophos 400 g.a.i./ha (2.5%) as well as in fipronil 50 g.a.i./ha (2.5 %), neem oil 2500 g.a.i./ha (2.5%) but no mortality was noticed in control treatment.

(B) Toxicity of insecticides against Italian honey bee, *Apis mellifera*.

Murray (1985) investigated acute and residual toxicity to honeybees (*Apis mellifera)* of a new pyrathroid insecticide WL-85871 under laboratory condition. For acute toxicity tests technical grade and emulsifiable concentrate formulations of WL-85871 or dimethoate were administered orally in sucrose or by topical application to workers of *A. mellifera*. Residual toxicity was assessed by the application of typical field dose rates of emulsifiable concentrate formulations of WL-85871 at 15 gai ha^{-1}, dimethoate at 500 g ai ha^{-1} and phosalone at 1200 g ai ha^{-1} to batches of flowering *Phacelia campanularia* plants mounted in the top of cages containing workers of *A. mellifera*.

Danka *et al*. (1986) studied the contact toxicities for acetone formulations of azinphosmethyl, carbaryl, methyl parathion and permethrin applied to workers of Africanized and European honey bee (*Apis mellifera* L.) types. For each insecticide 95 per cent fiducial limits at the LC-50 levels for the two bee types did not overlap. Africanized bees showed greater tolerance to all the chemicals except carbaryl difference in tolerance to each of the four chemicals were all about 2-fold.The order of toxicity of the compounds on the Africanized bee was permethrin> carbaryl> azinphosmethyl> methyl parathion whereas on the European honey bees the order of toxicity was permethrin > azinphosmethyl > carbaryl > methyl parathion.

Fiedler (1987) the chronic toxicity of eight insecticidal organophosphorus compounds (acephate, methamidophos, dimethoate, omethoate, parathion-ethyl, paraoxon-ethyl, parathion-methyl and paraoxon-methyl) to honeybees *(A. mellifera)* was investigated by a simple laboratory method. Caged bees were provided with sugar syrup containing 0.25-2.00 ppm insecticide for 14 days. The intake of small amounts of insecticide resulted in increased mortality and reduced syrup consumption; parathion-ethyl was damaging at a level of 0.50 ppm, all other compounds at 0.25 ppm. For acephate, methamidophos and dimethoate, the accumulated dose resulting in a corrected mortality of 50 per cent was even lower than the acute oral LD_{50} (for 24 hrs). For the other compounds this accumulated dose was higher than or almost equal to the LC50.

Arzone and Patetta (1989) tested in laboratory ingestion and indirect contact. The organophosphates azinphosmethyl, omethoate and diazinon were highly toxic to honeybees. Dithianon, a fungicide, was not toxic on ingestion but highly toxic on contact. Hexythiazox and propergite were not toxic in these tests.

Buchler and Drescher (1989) tested the toxicity of insecticides against *Apis mellifera* in laboratory, which enables the influence of the leaves of various plant species and other test substrates were considered. Using paper as a substrate, highly significant differences in the toxicity of residues were observed when different application methods were used. A new device that simulates pesticide spraying under various field conditions used for the laboratory testing of the toxic effects of pesticides residues for honey bees. Bees were exposed to the leaves of different plants that had been sprayed with thiodan and the number of dead bees were counted and also, the reduction in food consumption by surviving contaminated bees were determined.

Choi *et al*. (1989) investigated the acute and chronic toxicities of pesticides to honeybees, (*Apis mellifera*). The LC50 and the residual toxicity of 41 insecticides, 18 acaricides, 41 fungicides and 6 herbicides to honeybees. Among these, 30 insecticides had high contact and oral toxicity. Most of the acaricides, fungicides and herbicides had low acute toxicity, but dialifos,dinobuton, edifenphos + BPMC, isoprothiolane + BPMC, isoprothiolane, pyrazophos, streptomycin and 2,4-D had high acute toxicity to honeybees. The residual toxicity varied with the types of chemicals, but, in general, residual toxicity lasted 2-4 days.

Wael and Laere (1989) tested the pyrethroid insecticides Sumicidin (fenvalerate), Fastac (alpha-cypermethrin) and Danitol (fenopropathrin) were as

contact or oral poisons against honeybees in the laboratory. All three substances were highly toxic, with LD 50 values (oral toxicity) of 0.423, 0.056 and 0.046 µg/bee for fenvalerate, alphamethrin and fenpropathrin, respectively.

Hagler *et al*. (1989) tested the pyrethroid insecticide, permethrin, separately and in combination with pieronyl butoxide (PB) at ratios of 1 part insecticide to 4 and 9 parts piperonyl butoxide. Foraging honeybees, *Apis mellifera* L.were exposed to treated filter paper to determine toxicity and synergistic effects between the binary mixture. Mortality was recorded 48hrs after treatment. Permethrin used alone was highly toxic to the bees. Piperonyl butoxide synergized permethrin at both ratios, increasing the efficacy of permethrin 9-fold. Combination of permethrin and piperonyl butoxide (PB) may merit testing in field situations to determine the actual hazard to honeybees.

Gough *et al*. (1994) tested the contact and oral acute toxicity of technical dimethoate to worker honey bees (*Apis mellifera*) was determined between May and October for 12 consecutive years,1981-1992 (63 contact tests and 62 oral tests), using standard laboratory methods. The study indicated that the 24hrs LD50 values ranged from 0.11 to 0.26 (mean 0.16) µg a.i./bee for contact toxicity, and from 0.11 to 0.33 (mean 0.18) µg a.i./bee for oral toxicity. In the test period of 48hrs, LD50 values were similar to the 24hrs ones, indicating that there were no delayed toxic effects. There were no significant seasonal trends in contact or oral toxicity nor were there any consistent trends over the 12-year period.

Gromisz and Gromisz (1997b) conducted the oral and contact toxicity in laboratory of the harmful effect of Karate 25 EC (a pyrathroide containing 25g of Lamda-cyhalothrine) in 137 and 70 bees respectively. Karate 25 EC in sugar syrup at the concentration recommended in plant protection was consumed at dose levels of 53 µg to 180 µg of the active ingredient per bee. These doses correspondent to mortality rates of 5 per cent to 100 per cent. The contact testing revealed that Karate 025 EC damped on blotting paper retained toxic activities during 1 to 4 hrs.

Mayer and Lunden (1999) tested the toxicity of fipronil to adult female bees and was found least to the alkali bee, *Nomia elanderi* (LD50=1.130 µg/bee), intermediate to the honey bee, *Apis mellifera*, (0.013µg/bee) and greatest to the alfalfa leafcutter bee, *Egachile rotundata* (0.004 µg/bee), both in topical tests and tests involving fipronil residues on alfalfa (*Medicago sativa*) foliage. Adding an adjuvant to fipronil sprays changed the toxicity of fipronil to bees in residue bioassay studies with varying result with adjuvant and species of bee. Fipronil at 100 ppm and 500ppm in feeders containing a sucrose/honey syrup caused a significant reduction in honey bee visitation. Spraying fipronil at 0.014 or 0.028 kgai ha^{-1} on flowering canola (*Brassica nappus* cv.Legend) did not cause significant mortality of adult honey bees or reduce bee visitation.

Kakamand *et al*. (2008) investigated the effect of acute and oral toxicity of deltamethrin, malathion and thiamethoxam in different concentrations on the midgut tissue of forager workers of honey bee *Apis mellifera* L. in laboratory. The highest mortality among treated bees was 96.66 per cent that occurred by 12.5 ppm malathion after 12hrs from administration, followed by 93.33 per cent in 10.00ppm deltamethrin and 0.125ppm thiamethoxam after 12hrs from administration. The minimum concentration of each insecticide has little effect on the midgut tissue without damaging the entire tissue while lethal concentrations ingestion resulted in midgut tissue damage.

Laurino *et al*. (2011) evaluated the effects of the neonicotinoid insecticides on *A. mellifera* L. in Italy. Thiametoxam, Clothianidin, Acetamiprid and Thiacloprid were tested in the laboratory. Commercial formulations, dispersed in sugar syrup and water, at the highest dose level marked on the label were used to carry out oral and indirect contact trials on each pesticide. Clothianidin and Thiamethoxam caused higher mortality than the untreated controls and were also tested at decreasing concentrations until mortality was statistically insignificant in comparison with that of the control, the acute oral Lethal Dose 50, the acute indirect contact Lethal Concentration 50, and the related Hazard Quotient were calculated at 24, 48, and 72hrs from test initiation. On the contrary, Acetamiprid and Thiacloprid caused higher mortality than the untreated controls only in oral toxicity tests when honey bees, which had starved for two hours, were used. Honey bees that died during the trials were analyzed and the quantity of residues of insecticides determined.

Shelke *et al*. (2024) evaluated the toxicity of insecticides against *Apis mellifera* L. (at half the recommended dose of insecticide + azadirachtin) by residual exposure method. consisted of seven treatments viz. Imidacloprid 17.80% SL + Azadirachtin 1500 ppm, Cypermethrin 10% EC + Azadirachtin ppm, Profenofos 50% EC + Azadirachtin 1500 ppm, Chlorantraniliprole 18.50% SC + Azadirachtin 1500 ppm, Emamectin benzoate 05% SG + Azadirachtin 1500 ppm, Chlorantraniliprole 09.30% + Lambda-cyhalothrin 04.60% ZC + Azadirachtin 1500 ppm and untreated control. Which revealed that at 72 hours, Chlorantraniliprole 18.50% SC + Azadirachtin 1500 ppm caused 50 percent cumulative mortality in honey bees. This was followed by Chlorantraniliprole 09.30% + Lambda cyhalothrin 04.60% ZC; + Azadirachtin 1500 ppm (70%). The treatment of Imidacloprid 17.80% SL + Azadirachtin 1500 ppm, Profenofos 50% EC +

Azadirachtin 1500 ppm, Emamectin benzoate 05% SG + Azadirachtin 1500 ppm and Cypermethrin 10% EC + Azadirachtin 1500 ppm recorded cent percent mortality in bees. The LT50 values revealed the time required to cause 50% mortality of honeybees. It was as follows: Chlorantraniliprole + Azadirachtin 1500 ppm (61.18 h) Chlorantraniliprole + Lambda cyhalothrin + Azadirachtin 1500 ppm (33.53 h), Imidacloprid + Azadirachtin 1500 ppm (10.83 h), Profenofos + Azadirachtin 1500 ppm (8.98), Emamectin benzoate + Azadirachtin 1500 ppm (7.79 h) and Cypermethrin + Azadirachtin 1500 ppm (6.25 h).

(C) Toxicity of insecticides against stingless bees- Macieira and Beraldo **(**1989) studied in the laboratory LD50 values of organophosphate, carbamate, organochlorine (including cyclodiene) and pyrethroid insecticides were determined for workers of *Trigona spinipes* by using the topical application techniques from which dose-mortality was derived using Bliss method. In order of decreasing toxicity, the LD 50 values for workers, in µg/bee were as follows-heptachlor, 0.0168; dieldrin, 0.0289; cypermethrin, 0.0704; permethrin, 0.0724, parathion, 0.0956; lindane, 0.1331; methomil, 0.1402; dicrotophos, 0.1685; endosulphan, 0.2097; malathion, 0.2649; acephate, 0.4234; carbaryl, 0.7472; fenvalerete, 1.0880. These insecticides tested were highly toxic to *T.spinipes*.

(D) Toxicity of insecticides against bumble bee- Tasei *et al*. (2000) conducted a laboratory test on queenless micro-colonies of three bumblebee workers (Bombus *terrestris* L.) and studied the effects of low doses of imidacloprid on pollen and syrup consumption, worker survival, brood size and larval development. Two doses were used viz. DI-10 µg AI kg^{-1} in syrup and 6 µg AI kg^{-1} in pollen, D2- was 2.5 times higher in syrup and 2.7 higher in pollen. During 85 days 27,30 and 29 micro-colonies were reared respectively for control, D1 and D2 treatments. The result indicated that the food consumption was not affected by either dose. During the 5 day pre-oviposition period the mean insecticide intake was 4.8 µg per day per worker in treatment D2.

Marletto *et al*. (2003) assessed the toxicity against the bumblebees, *Bombus terrestris* (L.) the insecticides were acephate, buprofezin, carbaryl, cartap hydrochloride, chlorpyrifos-methyl, cyfluthrin, cyromazine, dimethoate, heptenophos, imidacloprid, lamdacyhalothrin, methomyl, phosalone, pirimicarb, quinalphos, rotenone, and teflubenzuron, of the acaricides fenazaquin, fenpyroximate, hexathiazox, propergite and tebufenpyrad and of the insecticide-acaricides abamectin and amitraz was tested in the laboratory. Oral, topic contact and indirect contact trials were carried out for each pesticide, employing formulated compound in water at the highest field dose marked on the label.

Iwasa *et al*. (2004) determined the contact honey bee toxicity of neonicotinoid insecticides. The most toxic to the honey bee in laboratory studies with LD50 values of 18 µg/bee for imidacloprid, 22 µg for clothianidin, 30 µg for thiamethoxam,75 µg for dinotefuran and 138 µg for nitenpyram.The cyano-substituted neonicotinoids exhibited a much lower toxicity with LD50 values for acetamiprid and thiocloprid of 7.1 and 14.6 µg/bee, respectively. Piperonyl butoxide,triflumizole and propiconazole increased honey bee toxicity of acetamiprid 6.0-,244- and 105- fold and thiacloprid 154-,1,141-and 559- fold, respectively but had a minimal effect on imidacloprid (1.70,1.85 and 1.52-fold, respectively). Aliouane *et al*. (2009) conducted bioassays to evaluate the effects on honeybee behavior of sublethal doses of insecticides chronically administered orally or by contact. Emergent honeybees received a daily dose of insecticide ranging from one fifth to one five hundredth of the median lethal dose (LD50). After exposure to fipronil (0.1 and 0.01 µg/bee), acetamiprid (1 and 0.1 µg/bee), or thiamethoxam (1 and 0.1 µg/bee) behavioral functions of honeybees were tested on day 12. Fipronil, used at the dose of 0.1 µg/bee, induced mortality of all honeybees after one week of treatment. As a result of contact treatment at 0.01 µg/bee, honeybees spent significantly more time immobile in an open field apparatus and ingested significantly more water. In the olfactory conditioning paradism,fipronil-treated honeybees failed to discriminate between a known and an unknown odorant.Thiamethoxam by contact induced either a significant decrease of olfactory memory 24hrs after learning at 0.1 µg/bee or a significant impairment of learning performance with no effect on memory at 1 µg/bee.

Stanley *et al*. (2009) tested the toxicity of diafenthiuron to four different bee species in the laboratory of the cardamom ecosystem. Diafenthiuron was found to be slightly harmful to *A.dorsata, A. cerana indica, A. florea* and moderately harmful to *T. iridipennis*. It was slightly to moderately toxic to bees on contact with treated surfaces in the laboratory. T. *iridipennis* and *A.dorsata* were highly susceptible to the insecticide. It was found that diafenthiuron was highly toxic when applied to the thorax of bees but it was less toxic on ingestion, ie. contact toxicity.

Akca *et al*. (2009a) studied the efficacy of three insecticides ie. Carbaryl, Carbosulfan, and Methiocarb in laboratory condition and lethal concentrations were determined against honey bees' adults. The highest toxicity based on LC50 values was observed in carbosulfan (1.3 mg Al L^{-1}). The LC50's (mg Al L^{-1}) for methiocarb and carbaryl in topical application method were 65.9 and 71.7, respectively.

Akca *et al*. (2009b) examined the acute toxicity of eight insecticides such as karate 5EC (Lamda-cyhalathrine), Deltanete 400 EC (Furathiocarb), Sevin 85WP (carbaryl), Sevin XLR plus (Carbaryl), Marshal 25 EC (Carbosulfan), Oncol EC 200(Benfurocarb), MesurolWP 50 (Metiocarb) and Neem Azal T/S (Azadirachtin) with three doses including ½ and ¼ recommended doses. The mortality ratio was counted 1,8,16 and 24hrs after each application. Results indicated that Marshall, Oncol, Deltanate, Mesurol, Karate, Sevin XLR and SevinWP 85 had the harmful effects on bee while Azadirachtin was environmentally eco-friendly for controlling hazelnut pests. Results also revealed that Sevi XLR contained the fastest insecticide toxic effects on bees.

CONCLUSION

It is concluded that the toxicity of insecticides on honeybee workers depends on several factors, for instance, the type of pesticide, the dose. Some pesticides are more toxic than others although low doses can have harmful effects on bees. It is important to use IPM practices that minimize the use of pesticides and promote alternative methods of pest control. It is also important to follow label instructions carefully when using pesticides and avoid applying them during times when bees are most active especially during flowering periods.

Whenever possible, do not treat crops in bloom

Honey bees are active primarily during the morning and early afternoon. Many pesticides can be effectively applied in the late afternoon or evening with relative safety to bees.

Honey bee hives should not be placed next to fields or orchards that are likely to be treated with pesticides toxic to bees. A small number of hives may be protected from pesticides by covering the colonies with wet burlap for a period of one to two days. In some cases, it may be practical to move hives to a less exposed site. Beekeepers should inform farmers of the location of hives. Beekeepers and growers will then be able to develop a plan for providing effective pest control and honey bee protection. Beekeepers are required by Connecticut law to register their hives with the town clerks in the towns where bees are kept.

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