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Evaluation of pesticidal toxicity to Indian honey bee, *Apis cerana indica* F. (Hymenoptera: Apidae) through laboratory, confinement and field studies

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Abstract
Honey bees are essential for pollinating wide variety of plants and biodiversity conservation. In this study, we examined the toxicity of pesticides to *Apis cerana indica* by three tier assessment system (laboratory, confinement, field studies). Topical and oral bioassay method revealed similar mortality percentage for all pesticides. Average honey bee mortality was reported to be substantially higher in topical and oral bioassays than in indirect filter paper bioassay. Insecticides *viz*., profenofos, thiodicarb, imidacloprid, fipronil, emamectin benzoate causing 100 per cent mortality in all the methods at 48 HAT. Chlorantraniliprole was found moderately toxic and acetamiprid was found least toxic to bees. In fungicide treatments, none of the fungicides caused 100 per cent mortality to bees in all the experiment. Azoxystrobin and copper oxy chloride were found least toxic to bees. Difenoconazole, hexaconazole, tebuconazole, probiconazole were found slight to moderately toxic to bees. Carbendazim+mancozeb was found toxic to bees. In terms of biorationals, except 3G extract all are safe to bees. Particularly NPV and NSKE caused least mortality in all experiment. There is no significant difference between the confinement and laboratory studies experiment results both were having similar values. In field studies NPV treated plot having maximum number of bee count in all the days followed by NSKE, azoxystrobin, copper oxy chloride, acetamiprid. Upto 3DAS chlorantarniliprole treated plots had minimum bee visitation. Hence, all biorationals can use in the field without any restriction. The results of the field studies support the use of acetamiprid against sucking pests in blooming plants.

Keywords: *Apis cerana indica*, three tier assessment, bioassay methods, biorationals

1. Introduction
Beekeeping is becoming increasingly popular in rural India, where more than four native honey bee species (*Apis dorsata*, *Apis cerena*, *Apis florae*, and *Melipona irridipennis*) exist (Khanra and Mukherjee, 2018). Honey bees are significant not just for the honey they produce, but also for pollinating agricultural and horticultural crops. As a result, the health of honey bees has a significant economic influence over the world. Significant losses of bees from beehives and a fall in bee populations have been documented in recent years (Eva Forsgren, 2009) [12]. On a variety of agricultural crops, insecticides are used to control a wide range of pests. While pesticides are primarily used to kill pest insects, they can also harm non-target species such as pollinators (Velthuis and van Doorn, 2006). Insecticides are commonly used to kill insects, however they can also kill organisms that aren't intended to be killed. Among non-intentional organisms, the honey bee is a significant agro-environmental, economic, and scientific insect (Srinivasan, 2011). Due to a deficit in the number of genes producing detoxifying enzymes, honeybees are especially vulnerable to pesticides when compared to other insects (Claudianos *et al*., 2006) [7].

Honey bees are an effective bioassay agent for evaluating heavy metals and pesticide toxicity in both rural and urban areas since they come into touch with numerous contaminants throughout their foraging activities (Porrini *et al*., 1996) [23]. Honey bee foragers collect pollen and nectar from blooms in order to improve colony longevity and brood development (Winston, 1987). Pesticides in the environment could contaminate pollen, wax, or brood food, which could then be passed on to immature bees (brood). Because pollen is a primary food source for both adult and young honey bees, pollen eating can expose the entire colony to pollutants (Chauzat *et al*., 2006) [6]. Though there are a variety of ways for pesticide testing in non-target animals, particularly honeybees, Stanley *et al*. (2010) [27] uses a three evaluation scheme that includes early laboratory studies, semi-field studies, and field investigations. Neonicotinoids and phenyl pyrazoles are insecticides that become systemic in the plant and

can be detected in nectar and pollen throughout the flowering season, as opposed to standard insecticides (Cutler and Scott-Dupree, 2007).

With this in mind, an experiment was undertaken to evaluate commercially available pesticides used for pest management at their actual field dose to determine their toxicity to bees, *A. cerana indica*, by three - tier assessment, i.e. laboratory, confinement, and field studies.

2. Materials and Methods

This research was conducted in the Department of Plant Protection's Post Graduate laboratory and on the sunflower fields of an experimental form at Anbil Dharmalingam Agricultural College and Research Institute in Tiruchirapalli. The foraging worker bees utilised in this experiment taken from the ADAC&RI's apiary. To analyse the toxicity of pesticides, we used a three-tier evaluation system that included lab, confinement, and field investigations. We utilized seven different insecticides, fungicides, and biorationals for toxicity testing in *Apis cerana indica*. Organophospahates, neonicotinoids, carbamates, anthranilic diamide, macrocyclic lactones, and phenyl pyrazoles are some of the chemical compounds that have been chosen specifically for insecticides. The field recommended dose of each pesticide that is currently being used in the field was tested, and comparisons were made. Field recommended concentrations (ppm) of several insecticides, fungicides, and biorationals being prepared in analytical grade acetone in prior to the tests.

2.1. Topical bioassay

Foraging worker bees of *A. cerana indica* were retrieved from the apiary by shaking the hive frames in a plastic cover. The bees were cooled in the refrigerator for two minutes at 40°C before treatment to for calmness. The calmed bees were topically dosed with 1µl drops of different insecticides formulated in acetone on their thorax. In total, thirty bees were used per treatment, with three replications of ten bees each. The only treatment administered to the control bees was acetone. The bees were then transferred into a plastic container (9 cm x 13 cm) and given tissue paper cubes soaked in sugar solution as a feeding supplement. The open end of the plastic container was capped with muslin cloth to prevent bees from escape and ensure adequate aeration. 24 and 48 hours after treatment, honeybee mortality was measured (HAT). Moribund bees were assumed to be dead as well.

2.2. Filter paper bioassay

A specific quantity of prepared solution (500µl) was diffused uniformly over a 9 cm diameter what man No.1 filter paper placed over a glass petri-dish of identical dimensions using an eppentorf 1ml micropipette. The filter paper should be left in air temperature for 10 minutes before being put into a petri dish for drying purposes. Amount of *A. Cerana indica* bees were collected from an apiary and immobilised in a refrigerator for 2 minutes at 4 degrees Celsius. To ensure proper aeration, the honey bees were placed in glass Petri plates with treated filter paper and a plastic cover with pores the same size as the glass Petri plate. The method was done three times with 30 bees each time. The bees were permitted to contact with the filter paper for a half-hour. The bees were then placed in 9 cm by 13 cm plastic jars and given cotton-tissue paper cubes saturated in sugar solution. The mortality of honeybees was assessed 24 and 48 hours after treatment (HAT).

2.3. Oral bioassay

Before being treated with pesticides, bees were anaesthetized for handling during bioassay techniques by cooling (40C for no longer than 2 minutes). Each treatment consisted of three replicas of a plastic container housing ten bees, each covered with nylon mesh, for a total of 30 honey bees in each concentration (three repetitions with ten bees per replication). The insecticide solution (20ml) was mixed with cotton bed and then attached to the upper surface of each container's nylon mesh cover (three replicates per concentration), where the bees were permitted to feed for 24 hours by lapping off the cotton wool fibres. Bees were fed a 50 percent (w/v) sucrose solution as a control. Data on bee death was collected at 24 and 48 HAT, and the % mortality was computed.

2.4. Confinement studies

Sunflower plants were grown in a three cents space at the ADAC&RI bee cafeteria and used in confinement research on pesticide toxicity to honeybees. For each treatment, fifteen plants were grouped together and sprayed with the appropriate pesticide at the field recommended concentration using a hand sprayer during full blossoming of the crop. The plants were sprayed until they were totally soaked with the spray liquid. The spray deposits were allowed to dry for 1 h after the plants were treated. Five plants were used in each replication of each treatment. These plants were completely encased in mosquito netting. *A. cerana indica* was collected from the hive and released into a mosquito net that was properly sealed on all side to avoid the bees from escaping. Thirty bees employed in total per treatment, with three replications with 10 bees each. Bees were collected from each quadrate after 1 h and placed in separate container with cotton-tissue paper cubes that were dipped in sugar solution. The death rate of bees were measured at 1, 24 and 48 h after treatment, and the percent mortality was calculated.

2.5. Field studies

The insecticides that were proven to be safer in laboratory and confinement experiments were then evaluated in sunflower fields for honeybee repellency. The experiment used foliar pesticide treatment on a sunflower crop. Recommended agronomic procedures were used to develop the sunflower crop at 60 × 45 cm spacing in the plots (4×5 m). With three replications, the experiment was designed in RBD. Three replication of a blooming (50 per cent flowering) sunflower crop were sprayed with the recommended pesticide dose. Only water sprayed on the control plots. During peak activity, bees were observed foraging on sunflowers, and then mean number of bees visited per five blooms every 5 minutes was calculated. The observation were made one day before, day on spray, one day after and two, three, five, seven and nine days after the pesticides were sprayed.(DBS- day before spray, DOS- Day of spray, DAS- Day after spray).

2.6. Statistical analysis

Laboratory and confinement studies mortality data obtained were converted to arc-sine values and subjected to Completely Randomised Design using Agres-agdata package. In field studies, data obtained were converted to square root values and subjected to Randomised Block Design using Agres-agdata package.

3. Results

3.1. Topical bioassay

The insecticides like profenofos 50 EC, thiodicarb 75 WP, imidacloprid 17.8 SL, fipronil 5 SC , emamectin benzoate 5 SG caused 100 per cent mortality to *A. cerana indica* at their field recommended doses at 48 HAT. In 24 HAT only this chemicals were showed 100 per cent mortality to bees. Anthralic diamide, chlorantraniliprole 18.5 SC caused 46.6 per cent mortality in bees at 48 HAT. Among the seven insecticides tested only acetamiprid caused least mortality of 26.6 per cent at 48 HAT to bees (Table 1). For fungicides assessment we have took six different fungicides and one combined fungicides (carbendazim+mancozeb). None of the fungicides showed 100 per cent mortality to *A. cerana indica* at 48 HAT in all the methods. In fungicides carbendazim+mancozeb combination caused highest mortality of 83.3 per cent at 48HAT to bees. Followed by difenoconazole 25 EC caused 53.3 per cent mortality in bees. hexaconozole 5 SC, tebuconazole 25.9 EC, probiconazole 25 EC caused similar mortality of 40, 46.6 and 40 per cent respectively at 48 HAT. Copper oxy chloride and azoxystrobin caused least mortality of 33.3 and 26.6 per cent respectively at 48 HAT (Table 2). Compare to insecticides and fungicides the biorationals were showed very least mortality rate in all the treatments in all the three methods. Upto 24 HAT also all the biorationals showed less than 30 per cent mortality in all the methods. In this biorationals 3G extract showed highest rate of mortality of 46.6 per cent at 48 HAT. Followed by azadirachtin, buprofesin, *beavaria*, *Bt* in the per cent mortality of 40, 26.6,30 and 26.6. Among the biorationals NSKE and NPV showed very least mortality of 20 per cent to *A. cerana indica* at 48HAT (Table 3).

3.2. Filter paper disc bioassay

Filter paper disc bioassay also revealed profenophos 50 EC, thiodicarb 75 wp, imidacloprid 17.8 SL, fipronil 5 SC , emamectin benzoate 5SG caused 100 per cent mortality in *A. cerana indica* at their field recommended concentration. At 24 HAT profenophos, thiodicarb did not showed 100 per cent mortality but increasing the time of exposures upto 48 h caused 100 per cent mortality to bees. Chlorantraniliprole and acetamiprid caused 40 and 23.3 per cent mortality to bees at 48 HAT (Table 1). In fungicides carbendazim+mancozeb showed highest mortality of 73.3 per cent at 48 HAT. Followed by difenoconazole 25 EC, hexaconozole 5 SC, tebuconazole 25.9 EC, probiconazole 25 EC, copper oxy chloride and azoxystrobin caused mortality of 46.6%, 40%, 36.3%, 30%, 23.3% and 23.3% at 48 HAT (Table 2). For biorationals at 48HAT 40 per cent mortality occurred in 3G extract to *A. cerana indica*. azadirachtin, buprofesin, *beavaria*, *Bt* caused less than 33.3 per cent mortality to bees. Less than 20 per cent mortality occurred in NSKE and NPV at 48HAT (Table 3).

3.3. Oral bioassay

The same five chemicals (profenophos 50 EC, thiodicarb 75 WP, imidacloprid 17.8 SL, fipronil 5 SC , emamectin benzoate 5 SG) caused 100 per cent mortality to bees at 48 HAT. Oral bioassay and topical bioassay methods showed similar mortality percentage in *A. cerana indica*. Chlorantraniliprole and acetamiprid showed 46.6 and 20 per cent mortality in bees at 48 HAT at their field recommended concentration (Table 1). In fungicides, compare to other 2 methods in oral bioassay the carbendazim + mancozeb mixtures showed highest rate of mortality of 90 per cent at 48 HAT. Difenoconazole 25 EC, hexaconozole 5 SC, tebuconazole 25.9 EC, probiconazole 25 EC showed average level of mortality in the range of 40 to 50 per cent to bees. Copper oxy chloride and azoxystrobin caused least level of mortality of 30 and 26.6 per cent respectively at 48 HAT (Table 2). In biorationals 3G extract showed highest mortality of 50 per cent to bees. Among all the biorationals moderate level of mortality occurred in azadirachtin, buprofesin, *beavaria*, *Bt* in the range of 30 to 40 per cent. Least mortality recorded in NSKE and NPV in the mortality of 20 per cent each at 48 HAT (Table 3).

3.4. Confinement studies

Confinement studies results were also shown to be equivalent to lab investigations (Table 4).

3.4.1. Insecticides

Imidacloprid and profenofos caused highest mortality in the range of 100 per cent to *A. ceran indica* at 48 HAT. Thiodicarb, fipronil, emamectin benzoate were found toxic to bees with 90-100 per cent mortality range at 48 HAT. Chlorantraniliprole caused 50 per cent mortality to bees at 48 HAT. Acetamiprid were found least toxic to honey bees with 30 per cent mortality at 48 HAT.

3.4.2. Fungicides

Highest mortality (83.3%) observed in combined fungicide mixture (carbendazim + mancozeb). Difenocozole, hexaconozole 5 SC, tebuconazole 25.9 EC, probiconazole 25 EC caused 50 to 70 per cent mortality to bees at 48 HAT. Copper oxy chloride caused 43.3 per cent mortality to bees. Azoxystrobin caused least mortality of 26.67 per cent to *A. cerana indica* in this confinement studies.

3.4.3 Biorationals

Except 3G extract all other biorationals caused less than 50 per cent mortality to honey bees at 48 HAT. NSKE and NPV were found less toxic compared to other biorationals with the less than 30 per cent mortality at 48HAT.

3.5. Field studies

The pesticides that caused the lowest mortality percent value in the first and second tier assessments were taken forward and utilized for field treatment at their field recommended concentration. I have been chosen 2 number of chemicals each from insecticides, fungicides and biorationals treatments which were having less mortality percentage values in all above assessments.

Table 5. shows the results of the field experiment. Before spray, there was no significant difference between the treatments in terms of the number of *A. cerana indica* per five heads per five minutes. On the day of spray the data revealed that , with the exception of untreated check all treatments had a significant reduction in foraging activity. Among all other treatment chlorantraniliprole treated field plot only had minimum bee visitation. The chlorantraniliprole repellent effect on foraging bees lasted upto three days. Except Chlorantraniliprole all other treatment had a repellent effect on foraging bees only on the day of spraying. On third day of spray normal foraging bee activity was restored in case of NPV treated plots. After biorationals treatement plots, the fungicide treated plots attract more bees in all the days. On third day after spraying foraging bee visitation in NPV treated plots almost equal to untreated check plots. After 7 days of treatment the foraging bee visiting number significantly

decreased in all the treatments.

4. Discussion

This research demonstrated that insecticides represent significantly different risks to *A. cerana indica*, and that this knowledge can be used to choose between selective and non-selective insecticides for honey bees, as well as the safest insecticides for field use.

In our research, imidacloprid (neonicotinoid) was found to be the most toxic to honeybees, whereas acetamiprid, another neonicotinoid, was determined to be the least toxic. These data are compatible with Stanley *et al*. (2015) laboratory and semi-field investigations on the topical contact toxicity of imidacloprid and acetamiprid. The structure of chemical compounds may have an impact on honey bee sensitivity to insecticides. According to Iwasa *et al*. (2004) [15], imidacloprid's increased toxicity may be related to the presence of a nitro group in the neonicotinoid, whereas acetamiprid's lower toxicity to bees may be due to cyano substitution. Profenofos, an OP chemical, and thiodicarb, a carbamate, caused the maximum mortality in *A. cerana indica* in a laboratory bioassay. Bee poisoning has been linked to the use of pyrethroid and OP insecticides in several crops (Kearns *et al*., 1998) [16]. Carbamates and OP compounds are more harmful to *A. cerana indica* as compared to organochlorine. Six organophosphates (dichlorvos, methyl parathion, posphamidon, quinalphos, fenitrothion (monocrotophos) and carbaryl, (carbaryl) were shown to be extremely poisonous to *A. cerana indica* (Kasturi Bai *et al*., 1977).

Fipronil, a phynyl pyrazole, has been found to be extremely harmful to honey bees. Fipronil is also efficient against insects such as crop pests at minimu doses (Balanch and De visscher, 1997). However, according to Tingle *et al*. (2003) [28], fipronil is very harmful to non-target insects and has a very low LD50 on honey bees. Fipronil is a neurotoxic insecticide that affects the honeybee's gustative perception, olfactory learning, and motor activity by inhibiting the gamma-aminobutyric acid receptor. Fipronil was found to be hazardous to honeybees even at extremely low doses, and it can cause a variety of physiologic disorders. (for example, sight and olfactory impairment), resulting in aberrant behaviour and possibly death (Roat *et al*., 2013) [24].

In all three bioassay methods, emamectin benzoate-treated bees showed 100% mortality. Abdu-Allah *et al*. (2017) observed that the macro cyclic lactones class of insecticides were successful in eliminating hazardous insect pests and that emamectin benzoate, one of four macro cyclic lactones, was highly toxic to honey bees. When compared to its equivalent, abamectin, emamectin benzoate has a higher contact toxicity, which could be due to higher penetration and/or slower metabolic detoxification. According to Zoclanclounon *et al*. (2016) [34], the lowest dose of emamectin benzoate resulted in more than 90% bee mortality 48 hours after treatment. The findings are comparable with those of several other research involving bees and other insects, and avermectins have high absorption coefficients in general. Because of emamectin benzoate's high efficacy against target pests, pesticide managers should exercise caution when using it to safeguard agricultural pollinators.

Chlorantraniliprole, an anthranilic diamide, was found to be moderately hazardous to honey bees in this study. At 48 HAT, this insecticide killed less than half of the bees in all of the approaches. Our findings are consistent with those of Axel Dinter *et al*. (2010), Chlorantraniliprole and its produced compounds, Coragen and Altacor, have shown no inherent toxicity in honey bees and bumblebees. Cytochrome p450 in honey bees may have a role in chlorantraniliprole tolerance (Wade *et al*., 2019) [29]. This insecticide's lower acute toxicity in honey bee species is most likely owing to pollinator ryanodine receptor sensitivity to chlorantraniliprole (Yang *et al*., 2008) [33]. The rising use of diamide insecticides in agricultural and non-agricultural settings, as well as their unique method of action, necessitates investigation into the possible sublethal effects of exposures on overall pollinator production, safety, and fitness. (Williams *et al*., 2020) [30].

In comparison to other pesticides, acetamiprid showed very low mortality in *A. cerana indica* in all bioassay methods. Acetamiprid is a foliar spray that is a second-generation chloro neonicotinoids with contact and systemic activity (Devan *et al*., 2015) [8]. Like all neonicotinoids, acetamiprid is a selective agonist of nicotinic acetylcholine receptors in the central nervous system of insects (Shimomura *et al*., 2006) [25]. It is much less hazardous to honey bees than nitro-substituted neonicotinoids in terms of acute toxicity (Lundin *et al*., 2015) [19]. Because of its comparably more "bee-friendly" properties, acetamiprid is allowed to be sprayed on flowering crops (Godfray *et al*., 2014) [13].

Except carbendazim + mancozeb all the fungicide tested in the experiment caused less than 60% mortality to bees in the entire duration of the experiment. According to Mussen *et al*. (2004) [21], Most fungicides are not hazardous to honeybees in the quantities consumed or encountered while forage but they have been proven to prevent feeding cause hypothermia in adult bees in some situations. Our findings agree with those of Kubik *et al*. (1999) [18], who found that fungicides have a low toxicity for bees, allowing for crop spraying during bloom. He further claimed that while fungicides may not harm bees, residues can be found in pollen grains and nectar collected by bees from plants that have been treated.

In all the experiments biorationals azadiractin, *beavaria, Bt* were found less to moderately toxic to honey bees in our studies. These results collaborate with (Chella, 2019) who found *Bt* var k and azadiractin and *Beavaria bassiana* was found slight to moderately toxic to *Apis cerana*. NSKE caused very least moratlity in all the experiments. Nauman *et al.* (1994) [22] reported that the amount of foraging bees collected in neem-treated, solvent-treated, and untreated canola plots, on the other hand, showed no significant variations. Other pollinator species in the area were unaffected in a similar way. Their findings show that honey bees may be successfully utilized in blooming crops that have been treated with doses of NSE sufficient to control phytophagous insect pests.

In this study in all the experiments NPV was found very least toxic compound to honey bees and also in field studies maximum number of bees visitation observed in the NPV treated plots in all the days. The only NPV tested for safety to honeybees is also from *M. separata.* The inclusion bodies were found to cause no significant harm to colonies *of Apis cerana indica* (F.) when administered either orally and topically (Dhaduti and Mathad, 1980) [9]. Similarly, no adverse effects were detected in the honey bee, *Apis mellifera* L., when injected with wild-type or recombinant NPVs (Kevin *et al*., 1995). The NPV was safe to honey bees as well as parasitoids and predators, which were found abundantly in the sunflower ecosystem (Arora *et al*., 1998). Shabarish *et al*. (2018) reported that npv treated and control plots attracted maximum number of bees on the day of spray with 1.07bees/plant each. Chlorantraniliprole treated plots some

what repellent to bees with 0.7 bees/plant.

In our field studies second maximum number of bee visitation observed in NSKE treated plots in all the days. However, non target insects such as bees may be harmed by botanical insecticides (Xavier *et al*. 2010) [32]. Although Melathopoulos *et al*. (2000) they found no detrimental effects of neem on adult honey bees, they did find that it reduced the number of larvae in colonies and caused diverse abnormalities when the bees emerged from their cocoons at sub lethal dosages. According to Naumann *et al*. (1994) [22], Although foragers were precluded from feeding on sugar solutions containing extremely low concentrations of azadirachtin, no substantial reduction in foraging bees in canola fields sprayed with neem pesticide was observed.

The data differed in field studies due to the richness of the floral source, as well as changes in the formulations and doses employed in the field. The repellence impact of pesticide was also influenced by meteorological conditions. The mechanism behind this effect was not entirely known, although it incorporated visual, olfactory, gustatory, and chemical cues. Furthermore, the masking of floral odour by strong chemical odour cannot be overlooked. Some insecticides, on the other hand, may be considered harmless since they repel bees, however in some cases, the attraction of food may overcome the repellent effect.

Table 1: Laboratory evaluation on the acute toxicity of insecticides to honey bees

Treatments	Mortality (%)					
	Topical bioassay		Filter paper bioassay		Oral bioassay	
	24 h	48 h	24 h	48 h	24 h	48 h
Profenofos 50 EC	100 (88.84)^{c}	100 (88.84)^{d}	80 (63.93)^{c}	100 (88.84)^{d}	100 (88.84)^{d}	100 (88.84)^{d}
Thiodicarb 75 WP	100 (88.84)^{c}	100 (88.84)^{d}	90 (74.94)^{d}	100 (88.84)^{d}	100 (88.84)^{d}	100 (88.84)^{d}
Acetamiprid 20 SP	23.3 (29.27)^{b}	26.6 (31.49)^{b}	16.6 (21.64)^{b}	23.3 (29.27)^{b}	13.3 (21.64)^{b}	20 (26.56)^{b}
Imidacloprid 17.8 SL	100 (88.84)^{c}	100 (88.84)^{d}	100 (88.84)^{e}	100 (88.84)^{d}	100 (88.84)^{d}	100 (88.84)^{d}
Fipronil 5 SC	100 (88.84)^{c}	100 (88.84)^{d}	100 (88.84)^{e}	100 (88.84)^{d}	100 (88.84)^{d}	100 (88.84)^{d}
Chlorantraniliprole 18.5 SC	26.6 (31.49)^{b}	46.6 (43.57)^{c}	23.3 (29.27)^{b}	40 (39.64)^{b}	26.6 (31.49)^{c}	46.6 (43.57)^{c}
Emamectin benzoate 5 SG	100 (88.84)^{c}	100 (88.84)^{d}	100 (88.84)^{e}	100 (88.84)^{d}	100 (88.84)^{d}	100 (88.84)^{d}
Control	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}
SEd	1.5665**	2.2191**	4.0635**	2.0305**	1.7502**	2.3427**
CD(0.05)	3.3209	4.7044	8.6143	4.3046	3.7103	4.9665
CV%	3.03	4.19	8.68	3.86	3.43	4.44

Note: Each value is a mean of three replications.
Figures within parentheses are arc sine transformed values
Means followed by common alphabets are not significantly different at 5% level by LSD.

Table 2 : Laboratory evaluation on the acute toxicity of fungicides to honey bees

Treatments	Percent mortality					
	Topical bioassay		Filter paper bioassay		Oral bioassay	
	24 h	48 h	24 h	48 h	24 h	48 h
Copper oxy chloride 50 WP	26.6^{bc} (29.27)	33.3 (35.42)^{bc}	16.6 (24.35)^{b}	23.3 (29.27)^{b}	23.3 (29.27)^{b}	30 (31.49)^{bc}
Azoxystrobin 23 SC	23.3 (26.56)^{b}	26.6 (31.49)^{b}	13.3 (21.64)^{b}	23.3 (29.27)^{b}	23.3 (29.27)^{b}	26.6 (29.27)^{b}
Tebuconazole 25.9 EC	33.3 (35.71)^{cd}	40 (39.73)^{bc}	26.6 (31.28)^{bc}	36.3 (37.72)^{cd}	20 (27.06)^{b}	40 (39.64)^{de}
Hexaconozole 5 SC	36.6 (37.72)^{c}	46.6 (43.57)^{cd}	26.6 (31.28)^{bc}	40 (39.73)^{cd}	26.6 (31.28)^{b}	50 (45.49)^{de}
Probiconazole 25 EC	26.6 (29.27)^{bc}	40 (39.64)^{c}	23.3 (29.27)^{b}	30 (33.5)^{c}	26.6 (31.28)^{b}	33.3 (37.72)^{cd}
Difenoconazole 25 EC	40 (37.43)^{c}	53.3 (51.35)^{d}	26.6 (31.28)^{bc}	46.6 (43.57)^{cd}	23.3 (31.28)^{b}	40 (39.73)^{de}
carbendazim 12%+mancozeb 63%	63.3 (53.27)^{d}	83.3 (61.71)^{e}	40 (39.64)^{dc}	73.3 (59.50)^{e}	60 (51.35)^{c}	90 (72.06)^{f}
Control	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}
SEd	4.1773**	4.0540**	4.6007**	2.9929**	3.9358**	3.0216**
CD(0.05)	8.8556	8.5942	9.7531	6.3448	8.3437	6.4056
CV%	16.28	13.02	21.37	10.67	16.69	9.95

Note: Each value is a mean of three replications.
Figures within parentheses are arc sine transformed values
Means followed by common alphabets are not significantly different at 5% level by LSD.

Table 3: Laboratory evaluation on the acute toxicity of biorationals to honey bees

Treatments	Percent mortality					
	Topical bioassay		Filter paper bioassay		Oral bioassay	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Azadirachtin 5%	33.3 (33.5)^{e}	40 (39.64)^{cd}	20 (28.78)^{cd}	33.3 (35.71)^{ef}	26.6 (31.28)^{cd}	40 (39.74)^{cd}
NSKE	10 (18.93)^{b}	20 (26.55)^{b}	10 (16.04)^{abc}	16.6 (24.35)^{bc}	13.3 (21.64)^{bc}	20 (26.56)^{b}
Beauvaria bessiana	20 (26.56)^{bcd}	26.6 (31.28)^{bc}	23.3 (29.27)^{d}	30 (33.71)^{def}	23.3 (29.27)^{cd}	33.3 (35.71)^{c}
3G extract	26.6 (31.28)^{de}	46.6 (43.57)^{d}	26.6 (31.28)^{d}	40 (39.64)^{f}	33.3 (35.71)^{d}	50 (47.42)^{d}
NPV	13.3 (21.64)^{bc}	20 (27.06)^{b}	6.6 (13.33)^{ab}	13.3 (21.64)^{b}	10 (16.04)^{b}	20 (26.56)^{b}
Bt	23.3 (29.27)^{cde}	26.6 (31.28)^{bc}	13.3 (21.46)^{bcd}	20 (27.06)^{bcd}	23.3 (29.27)^{cd}	30 (33.71)^{bc}
Buprofezin 25 SC	16.6 (24.35)^{bcd}	30 (33.71)^{bc}	16.6 (24.35)^{bcd}	26.6 (31.28)^{cde}	20 (26.56)^{bcd}	36.6 (37.72)^{c}
Control	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}	0 (2.15)^{a}
SEd	4.1552**	4.1591**	6.0574**	3.4688**	5.2694**	3.8681**
CD(0.05)	8.8088	8.8171	12.8413	7.3535	11.1708	8.2002
CV%	21.69	17.32	35.56	15.76	26.89	15.19

Note: Each value is a mean of three replications.
Figures within parentheses are arc sine transformed values
Means followed by common alphabets are not significantly different at 5% level by LSD.

Table 4: Toxicity of pesticides to honey bees tested in confinement studies

Insecticides	24 h	48 h	Fungicides	24 h	48 h	Biorationals	24 h	48 h
Profenofos 50EC	83.34 (70.02)^{c}	100 (88.84)^{d}	Copper oxy chloride 50 WP	16.67 (24.35)^{bc}	43.34 (43.57)^{bc}	Azadirachtin 5%	6.67 (10.45)^{ab}	43.34 (41.65)^{cde}
Thiodicarb 75 WP	73.34 (59.70)^{c}	96.67 (83.24)^{d}	Azoxystrobin 23SC	6.67 (13.33)^{ab}	26.67 (31.28)^{b}	NSKE	0 (2.15)^{a}	26.67 (31.27)^{cb}
Acetamiprid 20SP	13.34 (18.75)^{ab}	30 (33.5)^{b}	Tebuconazole 25.9EC	33.34 (35.50)^{cd}	53.34 (47.41)^{bc}	*Beauvaria bessiana*	16.67 (24.35)^{cd}	46.6 (45.49)^{de}
Imidacloprid 17.8SL	86.67 (72.23)^{c}	100 (88.84)^{d}	Hexaconozole 5SC	16.67 (24.35)^{b}	53.34 (47.41)^{bc}	3G extract	26.67 (31.28)^{c}	70 (57.49)^{e}
Fipronil 5SC	66.67 (55.28)^{c}	93.34 (80.53)^{d}	Probiconazole 25EC	26.67 (31.28)^{bc}	56.67 (51.42)^{bc}	NPV	0 (2.15)^{a}	13.34 (18.75)^{b}
Chlorantraniliprole 18.5SC	26.67 (31.28)^{bc}	50 (45.49)^{b}	Difenoconazole 25 EC	23.34 (28.57)^{bc}	63.34 (55.48)^{c}	Bt	13.34 (18.75)^{abc}	43.34 (41.56)^{cde}
Emamectin benzoate 5SG	76.67 (61.71)^{d}	96.67 (83.53)^{d}	carbendazim 12%+mancozeb 63%	46.67 (43.57)^{d}	83.34 (44.70)^{c}	Buprofezin 25 SC	16.67 (24.38)^{c}	36.67 (39.64)^{cd}
Control	0 (2.15)^{a}	0 (2.15)^{a}	Control	0 (2.15)^{a}	0 (2.15)^{a}	Control	0 (2.15)^{a}	0 (2.15)^{a}
CD(0.05)	17.5698	13.2393	CD(0.05)	12.2260	17.9277	CD(0.05)	13.8375	13.1142
CV%	21.88	12.10	CV%	27.81	25.77	CV%	55.28	21.80

Note: Each value is a mean of three replications.
Figures within parentheses are arc sine transformed values
Means followed by common alphabets are not significantly different at 5% level by LSD.

Table 5: Field evaluation of pesticides on honey bees at peak time of foraging**.**

Sl.no	Treatments	Number of honeybees/5 flower heads/5 min DBS DOS 1DAS 3DAS 5DAS 7DAS 9DAS						
1.	Acetamiprid 20 SP	9.33 (3.29)^{a}	5.66 (2.47)^{c}	6.67 (2.66)^{c}	7.00 (2.72)^{d}	7.67 (2.85)^{ce}	6.34 (2.60)^{de}	3.67 (2.03)^{a}
2.	Chlorantraniliprole 18.5 SC	10.00 (3.23)^{a}	4.00 (2.11)^{d}	4.30 (2.19)^{d}	5.33 (2.40)^{e}	6.67 (2.66)^{e}	5.34 (2.47)^{e}	3.34 (1.95)^{a}
3.	Azoxystrobin 23 SC	10.67 (3.34)^{a}	7.00 (2.72)^{bc}	7.34 (2.78)^{bc}	8.00 (2.90)^{cd}	8.34 (3.01)^{d}	8.00 (2.90)^{bcd}	4.34 (2.19)^{a}
4.	Copper oxy chloride 50 WP	9.34 (3.13)^{a}	6.34 (2.60)^{bc}	7.34 (2.79)^{bc}	7.67 (2.90)^{cd}	9.67 (3.12)^{bd}	7.34 (2.78)^{cde}	4.00 (2.11)^{a}
5.	NPV	11.34 (3.43)^{a}	8.00 (2.90)^{ab}	8.34 (2.96)^{b}	11.00 (3.38)^{ab}	11.34 (3.43)^{ab}	9.67 (3.18)^{ab}	4.67 (2.26)^{a}
6.	NSKE 5%	9.66 (3.18)^{a}	7.34 (2.78)^{abc}	8.00 (2.90)^{bc}	9.00 (3.07)^{bc}	10.34 (3.23)^{ab}	9.00 (3.07)^{abc}	4.00 (2.19)^{a}
7.	Untreated control	10.00 (3.23)^{a}	9.34 (3.07)^{a}	11.00 (3.38)^{a}	11.66 (3.48)^{a}	12.00 (3.48)^{a}	10.34 (3.28)^{a}	4.34 (2.19)^{a}

SEd	0.174	0.1422	0.1295	0.1423	0.1543	0.1490	0.1288
CD(0.05)	0.3800	0.3099	0.2821	0.3100	0.3361	0.3246	0.2807
CV%	6.59	6.51	5.63	5.83	6.06	6.28	7.38
F statistics	NS	**	**	**	**	**	NS

Note: Each value is a mean of three replications.
Figures within parentheses are square root transformed values
Means followed by common alphabets are not significantly different at 5% level by LSD.

5. Conclusion

From this study we conclude that NPV is the safest compound to *A. cerana indica*. In terms of insecticides, acetamiprid can mostly be suggested for use in crop bloom during insect infestation without impacting honey bees. In further, more research into the sub lethal effects of these pesticides on honey bee populations can be done. Pesticides are ingested not only by foragers who visit the crops, but also by hive bees and larvae who feed on nectar and pollen held in the honeycomb. As a result, the impacts of pesticide exposure on bees at various phases of development can be investigated.

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