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Laboratory Evaluation on Insecticidal toxicity to Indian Honey bee, *Apiscerana indica* F. (Hymenoptera: Apidae)

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ABSTRACT

The honey bees are crucial for pollination of a wide range of plants, which is vital for the development and maintenance of biodiversity. Honey bees are critical pollinator around the world, yet it is increasingly vulnerable to illnesses, pesticides, and biotic stresses. Agricultural pesticides are a major cause of pollinator decrease around the world. The relative toxicity of seven insecticides to *Apiscerana indica* was determined under laboratory conditions. In the laboratory experiment, three methods were evaluated to assess the toxicity of insecticides. Topical and oral bioassay methods revealed similar mortality percentage for all insecticides. Honey bee mortality was reported to be substantially higher in topical and oral bioassays than in indirect filter paper disc bioassay tests. Insecticides *viz.*profenoFos50EC, thiodicarb 75WP, imidacloprid 17.8SL, fipronil 5SC, emamectin benzoate 5SG caused100 percent mortality in all the evaluation methods. Chlorantraniliprole 18.5SC and acetamiprid 20SP were found to be moderately and less toxic to honey bees respectively. In all the three methods the minimum LC_{50} value was obtained in emamectin benzoate 5SG followed by imidacloprid 17.8SL, fipronil 5SC, profenophos 50EC, thiodicarb 75WP, chlorantraniliprole 18.5SC and acetamiprid 20SP. The toxicity of these recommended insecticides varied greatly from extremely toxic to moderately toxic, and *A. ceranaindica* was killed at all recommended insecticide dosages except in acetamiprid and chlorantraniliprole. Hence, these insecticides should be handled with extreme caution because they kill non-target insects like pollinators.

Key words : *Indian honey bee, Laboratory toxicity, Bioassay methods.*

Introduction

The most essential pollinator of agricultural crops is the honey bee. Honey bees, like other living animals, are constantly subjected to a variety of biological and non-biological stressors, such as environmental influences, that can interact and affect the insects' health and survival (Gonza'lez-Varo *et al*., 2013). Insecticides are used to manage a wide range of pests on a number of agricultural crops. While insects pests are the primary target of insecticides, non-target species such as pollinators may also be affected. Insecticides are often used to kill insects, however they can also kill non-targeted organisms. The honey bee is an important agro-environmental, economic, and scientific insect among non-intentional organisms (Srinivasan, 2011).

Honey bees come into contact with many pollutants during their foraging activities, they are an ideal bioassay agent for investigating heavy metals and pesticide toxicity in both rural and urban regions (Porrini *et al*., 1996). Pollen and nectar from

flowers are collected by honey bee foragers to enhance colony survival and optimal brood development (Winston, 1987). Pesticides in the environment could potentially be passed on to juvenile bees (brood) through pollen, wax, or brood food contamination. Pollen, for example, is a main food source for both adult and immature honey bees; as a result, consumption of pollen can expose the entire colony to chemicals. (Chauzat *et al*., 2006). Pesticides have the potential to harm foraging honey bees reduce the lifespan of worker bees, Queen bee survival and weight are being reduced and have an impact on colony vitality (Beliën *et al*., 2009). Insecticides such as neonicotinoids and phenylpyrazoles differ from traditional insecticides in that they become systemic in the plant and can be identified in nectar and pollen throughout the blooming season.

The toxicity of pesticides to honeybees can be determined by suitable laboratory tests, but the hazard from the formulated pesticide is associated with specific circumstances in the field which must be considered in estimating the potential danger to honeybees and other non-target species.

Materials and Methods

This study was carried out in the Post Graduate laboratory of the Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli. The foraging worker bees used in this experiment were obtained from the apiary of the ADAC&RI. Through a laboratory bioassay approach, the toxicity of many regularly used insecticides belonging to various classes were assessed on honeybee at their field recommended dosages. For laboratory bioassays, various chemical substances such as organophospahates (profenofos), carbamates (thiodicarb), neonicotinoids (imidacloprid, Acetamiprid), anthranilicdiamide (chlorantraniliprole), phenyl pyrazole (fipronil), and macrocyclic lactones(emamectin benzoate) were chosen. The field recommended dose of each insecticides that is currently being used in the field was tested, and comparisons were made. Field recommended concentrations (ppm) of several insecticides being prepared in analytical grade acetone prior to the assays.

Topical bioassay

By shaking the hive frames in a plastic cover, foraging worker bees of *A.ceranaindica* were obtained from the apiary. Before treatment, the bees were chilled in the refrigerator for two minutes at 4 ^{0}C for calmness. On their thorax, the calmed bees were topically dosed with 1 µl drop of the insecticides on their thorax which were prepared in acetone. Thirty bees were used per treatment with three replications

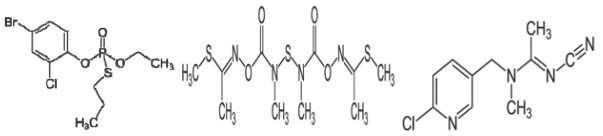
Table 1. List of insecticides used along with field recommended doses.

Insecticides	Field dose	Concentration (ppm)	Class	Mode of action
Profenofos 50EC	2ml/l	1000	Organo phosphates	Acetyl chlolineesterase (AChE) inhibitors
Thiodicarb 75WP	0.75g/l	562.5	Carbamates	
Acetamiprid 20SP	0.3g/l	60	Neonicotinoids	Nicotinic acetylcholine receptors (nAChr) agonists-competitive modulator post synaptic blockage of nAChR
Imidacloprid17.8SL	0.3ml/l	53.4		
Fipronil 5SC	2ml/l	100	Phenyl Pyerazole	GABA- gated chloride channel blockers
Chlorantraniliprole 18.5SC	0.3ml/l	55.5	AnthranilicDiamide	Ryanodine receptor modulators.Attack Ryanodine receptors and leak Ca2+ ions from receptors leading to paralysis and death
Emamectin benzoate 5SG	0.3g/l	15	Macrocyclic Lactones	Glutamate-gated Chloridechannel (GluCl) activators-Allosteric Modulators

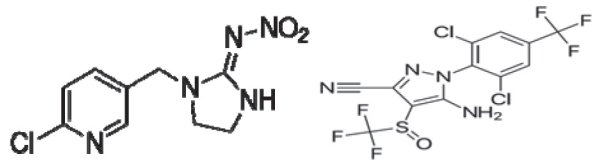
(10 bees/replication). A control was maintained with bees treated with acetone alone. Then bees were released in plastic containers (9 cm × 13 cm) and for feeding supplement the bees were provided with

Insecticide structure

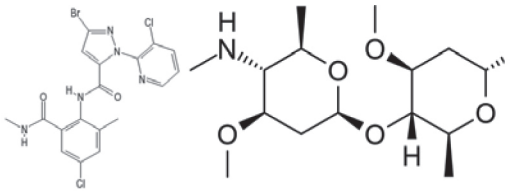
Profenofos Thiodicarb Acetamiprid



Imidacloprid Fipronil



Chlorantraniliprole Emamectin benzoate



tissue paper cubes which were dipped in sugar solution. To prevent the bees from escaping and to guarantee sufficient aeration, the open end of the plastic containers were covered with muslin cloth. Honeybee mortality was recorded 24 and 48 h after treatment (HAT). Bees that were moribund were also counted as dead (Stanley *et al*., 2014).

Filter paper bioassay

With an eppentorf 1ml micropipette, a fixed quantity of prepared solution (500µl) was dispensed uniformly over a 9 cm diameter whatman No.1 filter paper laid over a glass Petridish of identical dimensions. Before transfering into a Petridish the filter paper was kept in air temperature for 10 minutes for drying purpose. *A.ceranaindica* required for conducting the assay were obtained from apiary and kept in a refrigerator for 2 min at 4^0C to immobilize them.The honey bees were then placed into glass Petriplates with treated filter paper and a plastic cover with holes the same size as the glass Petriplate to guarantee proper aeration. The procedure was repeated three times, each time with 30 bees. The bees were allowed to come into contact with the filter paper. The bees were then transferred to plastic jars (9cm × 13cm) and were provided withcotton-tissue paper cubes soaked in sugar solution.The mortality was recorded 24 and 48 h after treatment (HAT) (Stanley *et al*., 2014).

Oral bioassay

Bees were sedated for handling during bioassay techniques by chilling (4^0C for 2 minutes) prior to treatment with insecticides. Each treatment consisted of a plastic container containing ten bees, each covered with nylon mesh and replicated thrice. The cotton bed was dipped inainsecticide solution (20 ml) and then attached to the upper surface of the nylon mesh cover of each container, and the bees were allowed to feed for 24 h by lapping off the cotton wool fibres. In control, the bees were only fed with 50 per cent (w/v) sucrose solution.Mortality of bees was recorded at 24 and 48 HAT (Badawy*et al*., 2014).

Statistical analysis

Mortality data obtained were converted to arc-sine values and subjected to Completely Randomised Design using Agres-agdata package. To correct the mortality in control, Abbott's formula (Abbott, 1925) was utilised and median lethal doses (LC_{50}) were calculated using probit analysis (Finney, 1971).

Results

In topical method 48 h after application of insecticides viz., profenofos 50EC, thiodicarb 75 WP, imidacloprid 17.8 SL, fipronil5SC ,emamectin benzoate 5SG caused 100 per cent mortality to *A.ceranaindica* at their field recommended doses. At 24 HAT these insecticides caused 100% mortality to bees. Anthralicdiamidechlorantraniliprole 18.5 SC caused 46.6 per cent mortality of bees at 48HAT. Among the seven insecticides tested, acetamiprid caused the minimum mortality of 26.6 per cent at 48HAT to bees.

In filter paper method of application profenofos 50EC, thiodicarb 75 WP, imidacloprid 17.8 SL, fipronil5SC, emamectin benzoate 5SG caused 100 per cent mortality in *A.ceranaindica*. At 24HAT profenofos, thiodicarb did not shows 100 per cent mortality but increased time of exposures upto 48 h caused 100 per centmortality. Chlorantraniliprole and acetamiprid caused 40 per cent and 23.3 per cent mortality to

Fig. 1. A-Topical bioassay, **B**-Filter paper bioassay, **C**- Oral bioassay

bees,respectively at 48 HAT.

In oral bioassay profenofos 50EC, thiodicarb 75 WP, imidacloprid 17.8 SL, Fipronil 5SC, emamectin benzoate 5SG caused 100 per cent mortality in bees at 48HAT. In Oral bioassay and topical bioassay methods resulted similar bee mortality observed in *A.ceranaindica*. Chlorantraniliprole and acetamiprid showed 46.6 per cent and 20 per cent mortality in bees at 48 HAT.

Probit analysis

In topical bioassay, the toxicity of insecticides to *A.ceranaindica* varied considerably among the insecticides. emamectin benzoate 5SG was found to be the most toxic with LC_{50} of 5.87 ppm, followed by imidacloprid 17.8SL (24.21 ppm), fipronil 5SC (46.28 ppm), profenofos 50EC (226.02ppm), thiodicarb 75WP (258.60 ppm), chlorantraniliprole 18.5SC (467.86 ppm) and acetamiprid 20SP (759.81 ppm) at

Table 2. Laboratory evaluation on the acute toxicity of insecticidesto honey bees

Treatments	Mortality (%)					
	Topical bioassay		Filter paper bioassay		Oral bioassay	
	24 h	48 h	24 h	48 h	24 h	48 h
Profenofos 50EC	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 75WP	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 20SP	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 17.8SL	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 5SC	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 18.5SC	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 5SG	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 amean of three replications.
Figures within parentheses are arcsine transformed values
Means followed by common alphabets are not significantly different at 5% level by LSD.

24 h after treatment.

Honey bee mortality was found to be substantially higher in topical and oral bioassays than in filter paper disc bioassay tests. The same order of toxicity of insecticides was found in filter paper bioassay alsoemamectin benzoate 5SG was found more toxic with LC_{50} of 6.74 ppm followed by imidacloprid 17.8SL, fipronil 5SC, profenofos 50EC, thiodicarb 75WP, chlorantraniliprole 18.5SC and acetamiprid 20SP with LC_{50} of 26.33 ppm, 52.61 ppm, 269.81 ppm, 286.56 ppm, 661.49 ppm respectively and highest LC_{50} of 921.84 ppm at 24 h after treatment.

Topical and oral bioassay method showed more or less similar mortality for all insecticides. Similarly in oral bioassay, emamectin benzoate 5SG was found more toxic with LC_{50} of 5.98 ppm followed by imidacloprid 17.8SL, fipronil 5SC, profenofos 50EC, thiodicarb 75WP, chlorantraniliprole 18.5SC and acetamiprid 20SP with LC_{50} of 25.28 ppm, 48.83 ppm, 239.03 ppm, 256.31 ppm, 386.93 ppm respectively and maximum LC_{50} of 683.66 ppm at 24 h after treatment.

Discussion

This study proved that insecticides pose substantially different hazards to *A. cerana indica* and that this information can be utilized to choose between selective and non selective insecticides for honey bees as well as the safest insecticides for usage in fields.

In our study the neonicotinoids,imidacloprid was more hazardous to honeybees, whereas acetamiprid, another neonicotinoid, was found to be the least toxic. These findings are in line with observations on the topical contact toxicity of imidacloprid and acetamiprid in laboratory and semi field studies (Stanley *et al*., 2015). The structure of chemical compounds may influence the sensitivity of honey bees to insecticides. According to Iwasa *et al*. (2004) the higher toxicity of imidacloprid may be owing to the presence of a nitro group in the neonicotinoid, whereas the decreased toxicity of acetamiprid to bees may be due to cyano substitution.

Table 3. Dosage - mortality response of *A. ceranaindica* to insecticides (topical bioassay method)

S. No	Treatments	LC_{50} [a] (ppm)	Fiducial limits (95%) (ppm)		Y=bx+a	R^2	(χ^2)[b]
			Lower	Upper			
1.	Profenofos 50EC	226.02	154.13	331.44	Y=2.21x-0.22	0.988	0.998
2.	Thiodicarb 75WP	258.60	201.58	331.75	Y=3.36x-3.12	0.896	0.894
3.	Acetamiprid 20SP	759.81	368.33	1567.35	Y=1.08x+1.86	0.948	0.995
4.	Imidacloprid17.8SL	24.21	19.13	30.62	Y=3.70x-0.12	0.979	0.982
5.	Fipronil 5SC	46.28	31.77	67.41	Y=2.13x+1.46	0.922	0.945
6.	Chlorantraniliprole 18.5SC	467.86	232.99	939.52	Y=1.15x+1.91	0.965	0.998
7.	Emamectin benzoate 5SG	5.87	4.17	8.27	Y=2.35X+3.18	0.856	0.820

a Lethal concentration causing 50 % mortality after 24 h with 95 % confidence limits
b Chi square

Table 4. Dosage - mortality response of *A.ceranaindica* to insecticides (Filer paper disc bioassay method)

S. No.	Treatments	LC_{50} [a] (ppm)	Fiducial limits (95%) (ppm)		Y=bx+a	R^2	(χ^2)[b]
			Lower	Upper			
1.	Profenofos 50EC	269.81	181.57	400.95	Y=2.31x-0.50	0.964	0.999
2.	Thiodicarb 75WP	286.56	223.12	368.03	Y=3.29x-3.12	0.954	0.984
3.	Acetamiprid 20SP	921.84	490.56	1732.30	Y=1.30x+1.12	0.940	0.984
4.	Imidacloprid 17.8SL	26.33	19.24	36.02	Y=2.52x+1.41	0.895	0.969
5.	Fipronil 5SC	52.61	37.74	73.35	Y=2.48x+0.74	0.891	0.983
6.	Chlorantraniliprole 18.5SC	661.49	350.10	1249.83	Y=1.36x+1.16	0.960	0.994
7.	Emamectin benzoate 5SG	6.74	5.07	8.95	Y=2.90x+2.59	0.916	0.958

a Lethal concentration causing 50 % mortality after 24 h with 95 % confidence limits
b Chi square

In the laboratory bioassay,profenofos an OP compound and thiodicarbancarbamate caused maximum mortality to *A.ceranaindica*. In many crops, exposure to pyrethroid and OP insecticides has been linked to bee poisoning (Kearns *et al.*,1998). Compared to organochlorine, carbamates and OP compounds are highly toxic to *A.ceranaindica*. Six organophosphates (dichlorvos, methyl parathion, posphamidon, quinalphos, fenitrothion (monocrotophos), and carbamates - carbaryl, were very highly toxic to *A. ceranaindica* (Kasturi Bai *et al.*, 1977).

Fipronil, a phynylpyrazole was found highly toxic to honey bees. Fipronil is also effective at low doses against insects such as insect pest of crops However Tingle *et al*. (2003) reported fipronil was highly toxic to non target insects and LD_{50} on honey bees is very low. Fipronil is a neurotoxic insecticide that inhibits the gamma-aminobutyric acid receptor and can affect gustative perception, olfactory learning, and motor activity of the honeybee. Results showed that even at very low concentrations, pronil was harmful to honeybees and can induce several types of injuries to honeybee physiology (e.g., disruption of visual and olfactory capability), thus leading to abnormal behavior and possibly death (Roat *et al*., 2013).

Emamectin benzoate treated bees showed 100 percent mortalityir respective of the method of bioassay. These results were supported by Abdu-Allah *et al*. (2017) that macro cyclic lactones class of insecticides were effective in controlling the harmful insect pests and also found among four macro cyclic lactones emamectin benzoate was highly toxic to honey bees. The increased contact toxicity of emamectin benzoate when compared to its analogue, abamectin, could be attributable to higher penetration and/or slower metabolic detoxification. Zoclanclounon *et al*. (2016) found that the lowest concentration of emamectin benzoate resulted in bee mortality of more than 90 per cent at 48 h after application. Avermectins have high absorption coefficients in general, and the findings are consistent with those of several other studies involving bees and other insects.considering strong efficacy of emamectin benzoate against target pests, pesticide managers should use caution while using it to protect crop pollinators.

In this study an anthranilic diamidechlorantraniliprole wasfound moderately toxic to honey bees. In all the methods this insecticide caused less than 50 per cent mortality to bees at 48 HAT. Our results are in conformity with Axel Dinter *et al*. (2010). Honey bees and bumblebees have shown little intrinsic toxicity to chlorantraniliprole and its manufactured products, Coragen and Altacor. Honey bee P450s may play a key role in chlorantraniliprole tolerance in honey bee (Wade *et al*., 2019). The less acute toxicity of this insecticide to honey bee species is most likely due to differences in ryanodine receptor sensitivity to chlorantraniliprole in pollinators (Yang *et al*., 2008). The increased usage of diamide insecticides throughout agricultural and nonagricultural ecosystems, as well as the unique mechanism of action of these insecticides, requires research into the possible sublethal consequences of exposures on overall productivity, safety, and fitness of these pollinators. (Williams *et al*., 2020)

In all the bioassay methods acetamiprid shown very less mortality to *A. ceranaindica* compare to other insecticides. Acetamiprid is a second-generation chloroneonicotinoids having contact and systemic activity that is used as a foliar spray (Devan *et al*., 2015). Acetamiprid, like allneonicotinoids, is a selec-

Table 5. Dosage - mortality response of *A.ceranaindica* to insecticides (Oral bioassay method)

Sl. No.	Treatments	LC_{50}[a] (ppm)	Fiducial limits (95%) (ppm)		Y=bx+a	R^2	(χ^2)[b]
			Lower	Upper			
1.	Profenofos 50EC	239.03	165.36	345.51	Y=2.21x-0.22	0.988	0.989
2.	Thiodicarb 75WP	256.31	202.42	324.55	Y=3.63x-3.75	0.924	0.966
3.	Acetamiprid 20SP	683.66	333.13	1403.01	Y=1.09x+1.90	0.910	0.986
4.	Imidacloprid 17.8SL	25.28	19.96	32.00	Y=3.64x-0.11	0.961	0.960
5.	Fipronil 5SC	48.83	32.70	72.90	Y=1.97x+1.66	0.963	0.949
6.	Chlorantraniliprole 18.5SC	386.93	206.65	724.48	Y=1.29x+1.64	0.967	0.994
7.	Emamectin benzoate 5SG	5.98	4.26	8.40	Y=2.37x+3.14	0.818	0.772

a Lethal concentration causing 50 % mortality after 24 h with 95 % confidence limits
b Chi square

tive agonist of nicotinic acetylcholine receptors in insects' central nervous system (Shimomura *et al*., 2006). It has a far lower acute toxicity to honey bees than nitro-substituted neonicotinoids (Lundin *et al*., 2015). Acetamiprid is allowed to be sprayed on flowering crops because of its comparatively more "bee-friendly" qualities (Godfray *et al*., 2014). At this point, acetamiprid can mostly be suggested for use in crop bloom during insect infestation without impacting honey bees.

Conclusion

The present investigation revealed that emamectin benzoate, imidacloprid,fipronil, profenofos, thiodicarbwere very toxic to honey bee and showed harmful side effects on honey bee workers.An anthranilicdiamide, chlorantraniliprole was found to be moderately toxic to *A. ceranaindica*. In contrast, acetamipridwas safe on honey bee workers and showed less harmful side effects comparedwith other tested insecticides. Thus, it is evident to create awareness and suggest mitigatory policies to protect honeybees in the agricultural environment and to minimize the impact of insecticides on honey bees.

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Conflict of interest

The authors declare that there is no conflict of interest.

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