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Toxicity of insecticides on stingless bee under laboratory condition

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Abstract
The toxicity of insecticide on stingless bees under laboratory condition was conducted at Department of Agricultural Entomology, College of Agriculture, Dapoli during 2022-2023 with eight treatments and three replications. Results revealed that at 7th day of spraying, no mortality of stingless bees was recorded in treatment T_5- Neem oil 0.5% EC @ 5 ml and was significantly superior over the treatments T_4- Spinosad 45 SC @ 0.3 ml (16.9%), T_2- Emamectin benzoate 5 SG @ 0.4 g (17.4%) and T_6- Lambda cyhalothrin 5 EC @ 0.6 ml (34.3%). It was on par with the treatments, T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml (0.56%) and T_3- Spinetoram 11.7 SC @ 0.9 ml (1.12%). Maximum mortality of stingless bees was noticed in T_7- Imidacloprid 17.8 SL @ 0.3 ml (38.2%).

Keywords: Corrected mortality, insecticide, neem oil, stingless bee, toxicity

1. Introduction
Stingless bees are important not only in honey production but also in specialized practice like pollination of crops. No. of pesticidal sprays are taken on the crop against different pests. Pesticides are primarily used to kill pest insects, they also can harm non-target species such as pollinators. Its contamination poses considerable risks to non-target organisms. Among non-intentional organisms, the honey bee is a significant agro environmental, economic and scientific insect. During the pollination activity, stingless bees can also be exposed directly or indirectly to the insecticidal products. Hence the bees population is decreasing day by day. In the Konkan region, number of stingless bee colonies have been found in electric pole, plumbing pipe, crevices in wall, door, window, live and dead trees and other concealed places (Wankhede *et al.* 2022) [11] and playing a major role in pollination of different crops. The toxicity of every chemical employed in pest control to organisms other than the intended target should be thoroughly investigated. Prior evaluations of pesticide toxicity to honey bees have primarily been conducted using insecticides of technical grade. Farmers may not always receive enough information regarding formulated insecticides from such experiments. Bee safety must be taken into consideration while making pest management decisions that use insecticides to maintain crop productivity. Pollinator management needs to be considered carefully when managing pests.

2. Materials and Methods
The toxicity of insecticide on stingless bees under laboratory condition was conducted at Department of Agricultural Entomology, College of Agriculture. Dapoli during 2022-2023. The experiment was laid out in a completely randomised design with three replications and eight treatments *viz*., T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml, T_2- Emamectin benzoate 5 SG @ 0.4 g, T_3- Spinetoram 11.7 SC @ 0.9 ml, T_4- Spinosad 45 SC @ 0.3 ml, T_5- Neem oil 0.5% EC @ 5 ml, T_6- Lambda cyhalothrin 5 EC @ 0.6 ml, T_7- Imidacloprid 17.8 SL @ 0.3 ml and T_8- Untreated control.

Collection and acclimatization of stingless bees
The foraging worker bees were used in the study of toxicity. Bees from particular colony were

used for entire experiment, as the susceptibility of different colonies may vary from each other. Colony of stingless bee was maintained near the experimental site. The bees were collected from the entry hole of the hive or trap using wide mouthed plastic containers. The open end of the container was placed near the entry hole of colony. Air was blown into hive/ trap gently holding the container in slanting position to facilitate trapping of bees. After collecting the required number of bees, the open end of container was closed with clean, muslin cloth secured with rubber band. The collected bees were then taken to the laboratory and kept there for few minutes to acclimatize them.

Assessment of toxicity of different insecticides to stingless bee under laboratory condition

The insecticides were evaluated for their toxicity to stingless bee by thin film technique using standard procedure followed by Boli (2013) [2]. The formulation of the test insecticides at the recommended dose were prepared as described in Table 1. Round glass jars were washed thoroughly and dried. Whatman No. 1 filter paper was cut into round discs and kept at inner lower surface of the glass jar. One ml of the insecticide solution was transferred each jar using one ml pipette. One glass jar served as one replication. The glass jar and Whatman filter paper with insecticide solution were rotated in both ways, so that the solution coated the inner surface of jar uniformly. The glass jar was rotated till the water was dried and a thin dry coat of the insecticide only remained. The collected bees were as described above kept inside a refrigerator for two minutes to reduce their activity. As their all activities were slower down then they were taken out and immediately transferred to the insecticide treated glass jar. Twenty bees were released each glass jar. One ml of 40 per cent honey solution was pipetted out and spread on the filter paper inside the glass jars which served as food for them. The mouth of the glass jars was covered with the muslin cloth and tied with rubber band which provide enough aeration to the bees. The glass jar treated with water alone, having twenty bees served as control.

Observations recorded

Mortality counts of treated stingless bees were taken at 2, 4 and 6 hours. The observations were recorded at 1st, 3rd, 7th and 10th day.

3. Results and Discussion

The per cent mortality of stingless bees after the application of different insecticides under laboratory condition were represented in Table 1 and graphically depicted in figure 1.

Corrected mortality of stingless bees on 1st day of spraying:

The data depicted in table 1 indicated that the lowest mortality of stingless bees was recorded in treatment T_5- Neem oil 0.5% EC @ 5 ml (20.7%) and was found significantly superior over rest of treatments *viz*. T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml (38.5%), T_3- Spinetoram 11.7 SC @ 0.9 ml (64.2%), T_2- Emamectin benzoate 5 SG @ 0.4 g (69.8%), T_6- Lambda cyhalothrin 5 EC @ 0.6 ml (77.1%) and T_4- Spinosad 45 SC @ 0.3 ml (79.32%). Efrom *et al*. (2012) [6] found a significant increase in the mortality of adult workers *A. mellifera* with an increased exposure time of the bees to different concentrations of neem oil. Similarly, Xavier *et al*. (2015) [12] reported that neem oil showed an acute toxicity to both adult and larva workers of bees. The results of present study is in partial conformity with those of Boli (2013) [2] reported that mortality of stingless bees observed in treatment chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1} was 32.2 per cent at six hours after treatment under laboratory condition and was safe. The maximum mortality of stingless bees was noticed in treatment T_7- Imidacloprid 17.8 SL @ 0.3 ml (83.8%).

Corrected mortality of stingless bees on 3rd day of spraying:

The data at 3rd day showed that the treatment T_5- Neem oil 0.5% EC @ 5 ml was recorded minimum mortality (9.77%) of stingless bees. The present results corroborate with those of Naumann and Isman (1996) [7] and Riedl *et al*. (2006) [10] described that botanical pesticide like neem oil as safe for honey bees. It was found significantly superior over T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml with (36.8%), T_3- Spinetoram 11.7 SC @ 0.9 ml (43.7%), T_2- Emamectin benzoate 5 SG @ 0.4 g (54.0%), T_7- Imidacloprid 17.8 SL @ 0.3 ml (64.9%) and T_6- Lambda cyhalothrin 5 EC @ 0.6 ml (66.1%). The T_4- Spinosad 45 SC @ 0.3 ml (66.7%) treatment was recorded the maximum mortality of stingless bees. The observations on the toxicity of spinosad to the bees observed in the present study corroborate with those of Cleveland *et al*. (2002) [3] who showed that spinosad was acutely toxic to bees under laboratory condition. The results of present study are in agreement with Rabia *et al*. (2005) [9] and Pastagia and Patel (2007) [8] reported highest mortality of Indian bees (*Apis cerana indica*) when exposed to imidacloprid. In the present study, the mortality caused by imidacloprid and spinosad was found to be higher than that of lambda cyhalothrin at 24 HAT to both *A. cerana indica* and *T. iridipennis*.

Corrected mortality of stingless bees on 7th day of spraying:

At 7th day of spraying, no mortality of stingless bees was recorded in treatment T_5- Neem oil 0.5% EC @ 5 ml and was significantly superior over the treatments T_4- Spinosad 45 SC @ 0.3 ml (16.9%), T_2- Emamectin benzoate 5 SG @ 0.4 g (17.4%) and T_6- Lambda cyhalothrin 5 EC @ 0.6 ml (34.3%). It was on par with the treatments, T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml (0.56%) and T_3- Spinetoram 11.7 SC @ 0.9 ml (1.12%). The results of the study are in agreement with the findings of several other research workers. The safety of chlorantraniliprole to honey bees observed in the present study confirms to the report of Dinter *et al.* (2009) [5] who reported its safety to *A. mellifera* and the bumble bees *B. terrestris* at 0.005 microgram/ bees through contact toxicity. Maximum mortality of stingless bees was noticed in treatment T_7- Imidacloprid 17.8 SL @ 0.3 ml (38.2%).

Corrected mortality of stingless bees on 10th day of spraying:

The no mortality of stingless bees was recorded in treatment T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml, T_3- Spinetoram 11.7 SC @ 0.9 ml, T_4- Spinosad 45 SC @ 0.3 ml and T_5- Neem oil 0.5% EC @ 5 ml. It was followed by the treatments *viz*., T_2- Emamectin benzoate 5 SG @ 0.4 g (0.56%) and T_6- Lambda cyhalothrin 5 EC @ 0.6 ml (2.81%). It was found significantly superior over the treatment T_7- Imidacloprid 17.8 SL @ 0.3 ml (5.64%). The results derive support from Bailey *et al*. (2005) [1] and Deepika *et al*. (2022) [4] that the order of toxicity of insecticides to *Apis mellifera* by direct contact assay was clothianidin > carbofuran > imidacloprid > spinosad > lambda-cyhalothrin > Bt. carbosulfan caused less mortality to both species of bees at 24 HAT.

Table 1: Toxicity of insecticides on stingless bees under laboratory condition

Tr. No.	Treatment	Formulation	Dose per litre	Per cent corrected mortality of stingless bees			
				Day 1	Day 3	Day 7	Day 10
T 1	Chlorantraniliprole	18.5 SC	0.25 ml	38.5 (38.4)	36.8 (37.3)	0.56 (4.29)	0.0 (0.71)
T 2	Emamectin benzoate	5 SG	0.4 g	69.8 (56.7)	54.0 (47.3)	17.4 (24.6)	0.56 (4.29)
T 3	Spinetoram	11.7 SC	0.9 ml	64.2 (53.3)	43.7 (41.4)	1.12 (6.07)	0.0 (0.71)
T 4	Spinosad	45 SC	0.3 ml	79.3 (62.9)	66.7 (54.7)	16.9 (24.2)	0.0 (0.71)
T 5	Neem oil	0.5% EC	5 ml	20.7 (27.0)	9.77 (18.2)	0.0 (0.71)	0.0 (0.71)
T 6	Lambda cyhalothrin	5 EC	0.6 ml	77.1 (61.4)	66.1 (54.4)	34.3 (35.8)	2.81 (9.65)
T 7	Imidacloprid	17.8 SL	0.3 ml	83.8 (66.3)	64.9 (53.7)	38.2 (38.2)	5.64 (13.7)
T 8	Untreated control						
SE±				1.63	1.41	0.71	0.07
C.D. @ 5%				4.90	4.22	2.14	0.20

*(Figures in parenthesis are arc sine transferred values)

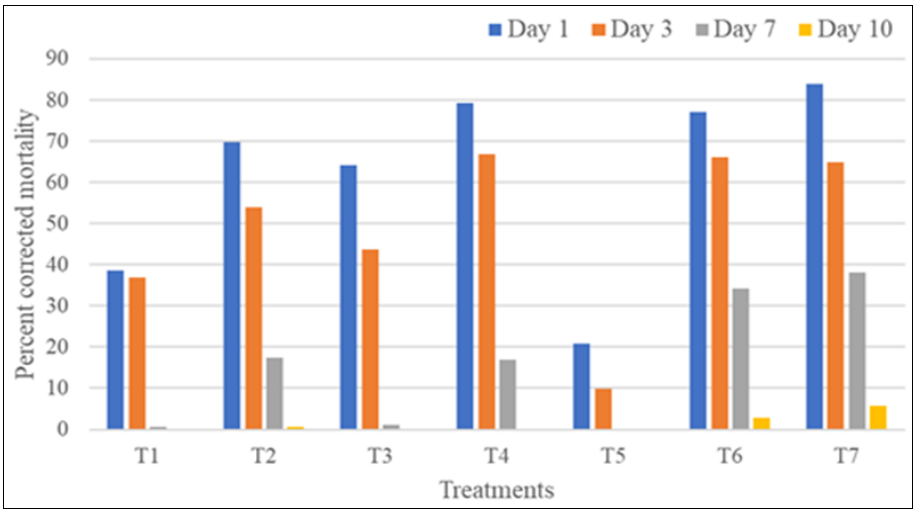

Fig 1: Toxicity of insecticides on stingless bees under laboratory condition

Fig 2: Experimental set

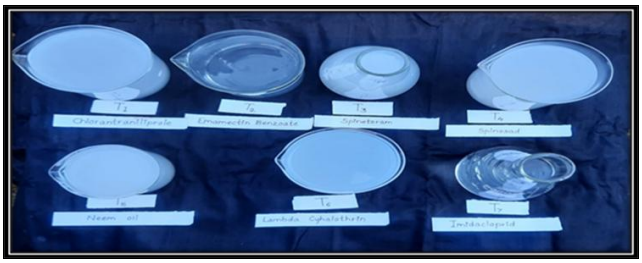

Fig 4: Prepared formulations of insecticides

Fig 3: Treatments

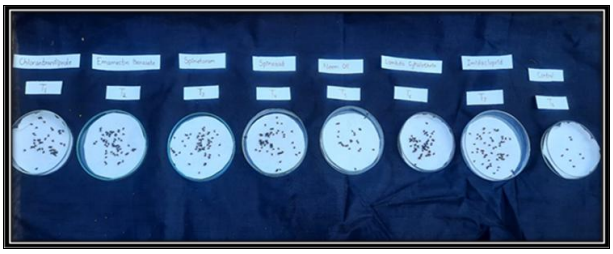

Fig 5: Mortality of treated stingless bees

4. Conclusion

The T_5- Neem oil 0.5% EC @ 5 ml treatment was recorded zero mortality of stingless bees under laboratory condition followed by the T_1- Chlorantraniliprole 18.5 SC @ 0.25 ml (0.56%) on seventh day of spraying. These are safe insecticides for stingless bees at seven days of expose.

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