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Effect of insecticides on domesticated species of honey bee (*Apis mellifera* and *Apis cerana indica*) on safflower

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Abstract
The investigation on effect of neonicotinoides, organophosphate, and spinosyn class of insecticides on visits of honey bee species on safflower crop were conducted on research farm of Department of Agricultural Entomology, College of agriculture, V.N.M.K.V. Parbhani (MS). The present result indicate that the frequency of visits of both bee species i.e. *Apis mellifera* and *Apis cerana* were uniform before spraying. However the intensity of bee visits falls down significantly on one day after spraying in each chemical treatment. Exposure of clothianidin 50% WDG found to be most hazardous to bee species which affected specificity of long term memory as well as affect successful returning of flights. Insecticide like imidacloprid 17.8% SL showed symptoms like impair honey bee navigation, decreased foraging activity on food sources and also had negative effect observed on olfactory learnt discrimination task. Symptoms like increase in responsiveness of water by *Apis mellifera* was observed when bees comes in contact with flowers sprayed by acetamiprid 20% SP. Thiamethoxam reduced foraging rates to nests, impair navigation, flight activity or both. The acetamiprid 20% SP found to be less toxic insecticide as compared to other insecticides for bee species.
The visits of *Apis cerana* was recorded more as compared to *Apis mellifera*. It's because *Apis cerana* is Indian origin bee which is well adopted in Indian environment and has internal body structure which resist insecticides more as compared to *Apis mellifera.*

Keywords: Domesticated, species, *Apis mellifera, Apis cerana indica*, safflower

Introduction
Neonicotinoid, a class of long-term persistent neurotoxic insecticides permanently bind to acetylcholine nicotine receptors, blocking them and thereby restricting the impulses of the passing nerve. The mode of action allows the insects to be managed and targeted by the root and neck, as well as feeding on the aerial part of the plant. The neonicotinoids, acting on contact, are especially suitable for controlling many insects with swallowed mouth parts piercing and sucking form. Neonicotinoids function on the nicotinic insect receptor (nAChR) (acetylcholine).
Neonicotinoids function on the nicotinic acetylcholine receptor (nAChR) of insects. Some neonicotinoids, in particular honey bees, demonstrate high toxicity to many pollinating insects. The symptoms of neonicotinoids, such as orientation disorders, behavioural changes and impairment of social activities, can be easily detected (Guez *et al.* 2001; Bortolotti *et al.* 2003) [5, 1].
Spinosyns are a class of insecticides with a broad range of action against many insect pests belonging to different orders, noxious to a wide variety of agricultural crops; spinosyns were also used against insects of sanitary interest. Spinosyns are derivative of biological active substances produced by soil Actinomycete Saccharopolyspora spinosa; being of biological origin, they are considered to have a low environmental impact and they are not much aggressive against non-target species. They act as allosteric activators of nicotinic acetylcholine receptors. (L. bacci *et al* 2016) [6].
Organophosphates poison insects and other animals, including birds, amphibians and mammals, primarily by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. The result is a loss of available AChE so that the effector organ becomes overstimulated by the excess acetylcholine (ACh, the impulse-transmitting substance) in the nerve ending. The enzyme is critical to normal control of nerve impulse transmission from nerve fibers to smooth and skeletal muscle cells, secretory cells and autonomic ganglia, and within the central nervous system (CNS). Once a critical proportion of the tissue enzyme mass is inactivated by phosphorylation, symptoms and signs of cholinergic poisoning become manifest. (Du Bois KP 1971) [3].

Materials and Methods

The experiment was carried out in *Rabi* 2019-20 at the farm of Department of Agricultural Entomology College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (MS) in randomised Block Design with three replications and eight insecticidal treatments. PBNS-86 variety of safflower was sown on 19th Nov 2019 with a spacing of 45x20cm (row x plant) distance. Parbhani is located at 19° 16' North Latitude and 73° 47' East longitude and 408.50 M above the mean sea level (MSL) altitude and has a subtropical climate. The position in the northern transitional zone (zone-8) receives an annual precipitation of approximately 800-900 mm in hot and dry summer and cool winter. Single bee hive of *Apis mellifera* and *Apis cerana* each were kept in the experimental field. Observations were recorded on 1st, 5th, 10th, 14th, and 21st days after application of chemical treatments.

Results and Discussion

The data on effect of insecticides on domestic species of honey bee on safflower were presented in Table no (01) and graphically depicted in fig no (01).

The present findings indicated that the intensity of visits of both *Apis mellifera* and *Apis cerana* were uniform before spraying but at one day after spraying, the frequency of visits of both species were drastically decreased in all chemically treated plots. However lowest frequency of bee visits were recorded in plots treated with Clothianidin 50% WDG (1.28 and 1.32/flower) followed by Imidacloprid 17.8% SL (1.37 and 1.47/flower) and Acephate 75% SP (1.55 and 1.59/flower) which found at par with each other. Other treatments according to their merits are Spinetoram 11.7%SC (1.85 and 1.88/flower), Thiamethoxam 25% WG (1.94 and 1.99/flower) and Acetamiprid 20% SP (1.98 and 2.10/flower) found at par with each other. Clothianidin found to be most poisonous to bee species as it affected successful returning of flights and long term memory. The neonicotinoids like Imidacloprid showed symptoms like negative effect on olfactory learning discrimination task, decreased foraging activity and impairing of navigation system.

On five days after spraying, slight improvement in visits of honey bee species were observed as compared to one day after spraying. The lowest frequency of visits of honey bee species were observed in plots treated with Clothianidin 50% WDG (1.41 and 1.55/flower) followed by Imidacloprid 17.8% SL (1.59 and 1.64/flower) and Acephate 75% SP (1.74 and 1.76/flower) which found at par with each other. Whereas the visits in plots treated with Spinetoram 11.7% SC (1.94 and 2.10/flower), Thiamethoxam 25% WG (2.01 and/flow 2.10/flower) and Acetamiprid 20% SP (2.10 and 2.19/flower) found at par with each other. Acetamiprid found to cause symptoms like increase in responsiveness to water. Thiamethoxam reduced flight activity, navigation and foraging rates to nests. Spinetoram was newly released insecticide hence its effect on bee species under field conditions were hardly known, however laboratory results showed that Spinetoram showed symptoms like paralysis and tremors in bee after exposure which correlates with the symptoms produced under field conditions.

The intensity of visits of both bee species started increasing from ten days after spraying. It showed that toxicity of insecticides started decreasing. However the lowest visits of *Apis mellifera* and *Apis cerana* were recorded in plots treated with Clothianidin 50% WDG (1.65 and 1.74/flower) followed by Imidacloprid 17.8% SL (1.74 and 1.88/flower) and Acephate 75% SP (1.92 and 1.98/flower) which found at par with each other. While treatments like Spinetoram 11.7% SC (2.10 and 2.22/flower), Thiamethoxam 25% WG (2.19 and 2.27/flower) and Acetamiprid 20% SP (2.25 and 2.27/flower) found at par with each other.

As compared to ten days after spraying the frequency of visits of bee species was recorded higher on fourteen days after spraying. However the intensity of visits of bee species in plots treated with Clothianidin 50% WDG (1.88 and 1.98/flower), Imidacloprid 17.8%SL (2.3 and 2.13/flower) and Acephate 75% SP (2.20 and 2.20 /flower) found at par with each other. Whereas visits in plots treated with Spinetoram 11.7% SC (2.37 and 2.47/flower), Thiamethoxam 25%WG (2.40 and 2.51/flower) and Acetamiprid 20% SP (2.50 and 2.56/flower) found at par with each other.

Highest frequency of visits of bee species was recorded on twenty one days after spraying in all chemically treated plots. The visits recorded in plots like Clothianidin 50% WDG (2.11 and 2.34/flower), Imidacloprid 17.8%SL (2.29 and 2.38/flower) and Acephate 75% SP (2.44 and 2.57 /flower) found at par with each other. While highest frequency of bee visits was recorded in plots treated with Acetamiprid 20% SP (3.10 and 3.16/flower).

The present results showed that intensity of visits of *Apis cerana* was higher as compared to *Apis mellifera* at the time of before spraying as well as after spraying of chemical treatments. That' s may be due to *Apis cerana* is originated in Indian environment and suitably adopted this climate as compared to *Apis mellifera* so it resist the toxicity of insecticides better than *Apis mellifera*.

Masal *et al.* (2017) [8] stated that honey bee visits were significantly poor for all insecticidal treatments, suggesting that all insecticides were significantly harmful to honey bees. Pooled data on 1 DAS showed that the lowest number of honey bee visits was found in plots treated with imidacloprid 17.8 SL (0.76 bees / plant / min) and clothianidin 50 WDG (0.89 bees / plant / min), followed by dinotefuran 20 SG (1.10 bees / plant / min) and visits from plots treated with acetamiprid 20 SP (2.11 bees / plant / min) and variations in their protection with bees. The order of toxicity of neo imidacloprid > clothianidin > dimethoate > dinotefuran > thiamethoxam > flonicamid > acetamiprid. Maximum honey bee visits were obtained at 14 DAS untreated plots (3.29 bees / plant / min) compared to treatments with clothianidin 50 WDG (1.84 bees / plant / min), imidacloprid 17.8 SL (2.03 bees / plant / min), dinotefuran (2.27 bees / plant / min) and diamethoate 30 EC variations in their bee toxicity. Maximum honey bee visits, however, were reported in plots treated with flonicamide 50 WG (2.63 bees / plant / min) and acetamipride 20 SP (2.63 bees / plant / min) and thiamethoxam 25 WG (2.41 bees / plant / min).

Decourtye *et al.* (2004) [2] contamination of imidacloprid or deltamethrin syrup caused a decline in both the food source foraging activity and the activity at the entrance to the hive. In an olfactory learning discrimination task, negative effects of imidacloprid were also observed. Free-flying foragers were taken from the infected feeder and subjected, under laboratory

conditions, to a conditioned proboscis extension response (PER) assay. No effect of deltamethrin on the learning output of restrained individuals was found in the PER procedure, as with free-flying bees, whereas major effects were found with imidacloprid in both semi-field and laboratory conditions.

Laurino *et al*. (2011) [7] reported that the honey bees displayed strong signs of poisoning during the trials, such as trembling and tremors, uncoordinated and uncontrolled movements, stumbling, failure to take the correct position of the body, and prolonged frenetic movement of the legs and rotation while in the supine position. Direct observation of the activity of the honey bees in cages revealed that it was transitory at field concentrations for acetamiprid and thiacloprid and at lower concentrations for clothianidin and thiametoxam. In addition, the highest concentrations of clothianidin and thiametoxam induced severe vomiting in the honey bees in ingestion trials.

You Meng *et al.* (2017) found that while the mass of the body of *A. Cerana* is markedly lower than *A. Mellifera*, the susceptibility of the two animals to neonicotinoids, depending on the chemical structure of neonicotinoids, is not correlated with their body mass. To dinotefuran, the two species showed the similar sensitivity. To acetamiprid, *A. mellifera* was less sensitive than *A. cerana*. However, to imidacloprid and thiamethoxam, *A. mellifera* was more sensitive than *A. cerana*. These results suggested that the sensitivity of honey bees to neonicotinoids is closely associated with the structure of pesticides, but not with body mass of bees.

Fischer *et al.* (2014) [4] concluded that application of sub-lethal doses of the three neonicotinoids imidacloprid, clothianidin and thiacloprid interfered with honeybee navigation, although it did not affect per se flight efficiency or the willingness of the bees of return to the hive.

Simon tosi *et al.* (2017) [9] found that since bee flight power is associated with muscle temperature, this effect of thiamethoxam on thoracic flight muscles may inhibit flight. Following chronic exposure to the neonicotinoid thiacloprid (4.5 ppm), it showed reduced honey bee foraging. We also predict that bees foraging on neonicotinoid treated fields for only one or two days would then fly more slowly and in a reduced area, based on our findings. The pollination service given for the collection of seeds, nectar and pollen for the colony and the nutritional biodiversity of collected pollen for the colony should be decreased by this behavioral alteration.

The present findings are in close agreement with findings of scientist as mentioned above.

Table 1: Effect of neonicotinoides, organophosphate and spinosyn class of insecticides on visits of honey bee species on safflower

		Apis mellifera						***Apis cerana***					
Tr.no	**Treatments**	**1DAS**	**5DAS**	**10DAS**	**14DAS**	**21DAS**	**Mean**	**1DAS**	**5DAS**	**10DAS**	**14DAS**	**21DAS**	**Mean**
1	Acetamiprid 20% SP	3.10 (1.98)	3.44 (2.10)	4.10 (2.25)	5.33 (2.50)	8.66 (3.10)	4.92 (2.41)	3.44 (2.10)	3.84 (2.19)	4.66 (2.37)	5.66 (2.56)	9.11 (3.16)	5.34 (2.49)
2	Spinetoram 11.7% SC	2.44 (1.85)	2.77 (1.94)	3.44 (2.10)	4.66 (2.37)	6.22 (2.68)	3.90 (2.19)	2.66 (1.88)	3.10 (2.01)	3.99 (2.22)	5.22 (2.47)	6.88 (2.79)	4.36 (2.27)
3	Clothinidin 50% WDG	0.66 (1.28)	1.10 (1.41)	1.77 (1.65)	2.55 (1.88)	3.55 (2.11)	1.92 (1.68)	0.77 (1.32)	1.44 (1.55)	2.10 (1.74)	2.99 (1.98)	4.66 (2.34)	2.39 (1.82)
4	Acephate 75% SP	1.44 (1.55)	2.10 (1.74)	2.77 (1.92)	3.88 (2.20)	5.00 (2.44)	3.03 (2.00)	1.55 (1.59)	2.22 (1.76)	2.99 (1.98)	3.99 (2.20)	5.77 (2.57)	3.30 (2.04)
5	Imidaclporid 17.8% SL	0.99 (1.37)	1.55 (1.59)	2.10 (1.74)	3.22 (2.03)	4.33 (2.29)	2.43 (1.84)	1.22 (1.47)	1.77 (1.64)	2.55 (1.88)	3.66 (2.13)	4.77 (2.38)	2.79 (1.93)
6	Thiamethoxam 25% WG	2.77 (1.94)	3.10 (2.01)	3.88 (2.19)	4.99 (2.40)	6.66 (2.75)	4.26 (2.21)	3.10 (1.99)	3.44 (2.10)	4.33 (2.27)	5.44 (2.51)	8.10 (3.01)	4.88 (2.42)
7	Six framed *Apis mellifera*	13.77 (3.83)	20.32 (4.67)	16.44 (4.17)	17.66 (4.30)	15.66 (4.05)	16.77 (4.14)	-	--	--	--	--	--
8	Seven framed *Apis cerana indica*	--	--	--	--	-	--	14.99 (4.07)	15.88 (4.10)	17.88 (4.33)	19.77 (4.44)	16.44 (3.99)	16.99 (4.23)
	SE ±	0.14	0.16	0.13	0.14	0.15	0.16	0.16	0.13	0.13	0.15	0.14	0.13
	CD @5%	0.43	0.50	0.40	0.44	0.47	0.47	0.51	0.42	0.40	0.46	0.44	0.37
	CV %	13.30	13.90	10.79	10.72	10.43	16.73	14.94	11.72	10.21	10.82	9.53	12.76

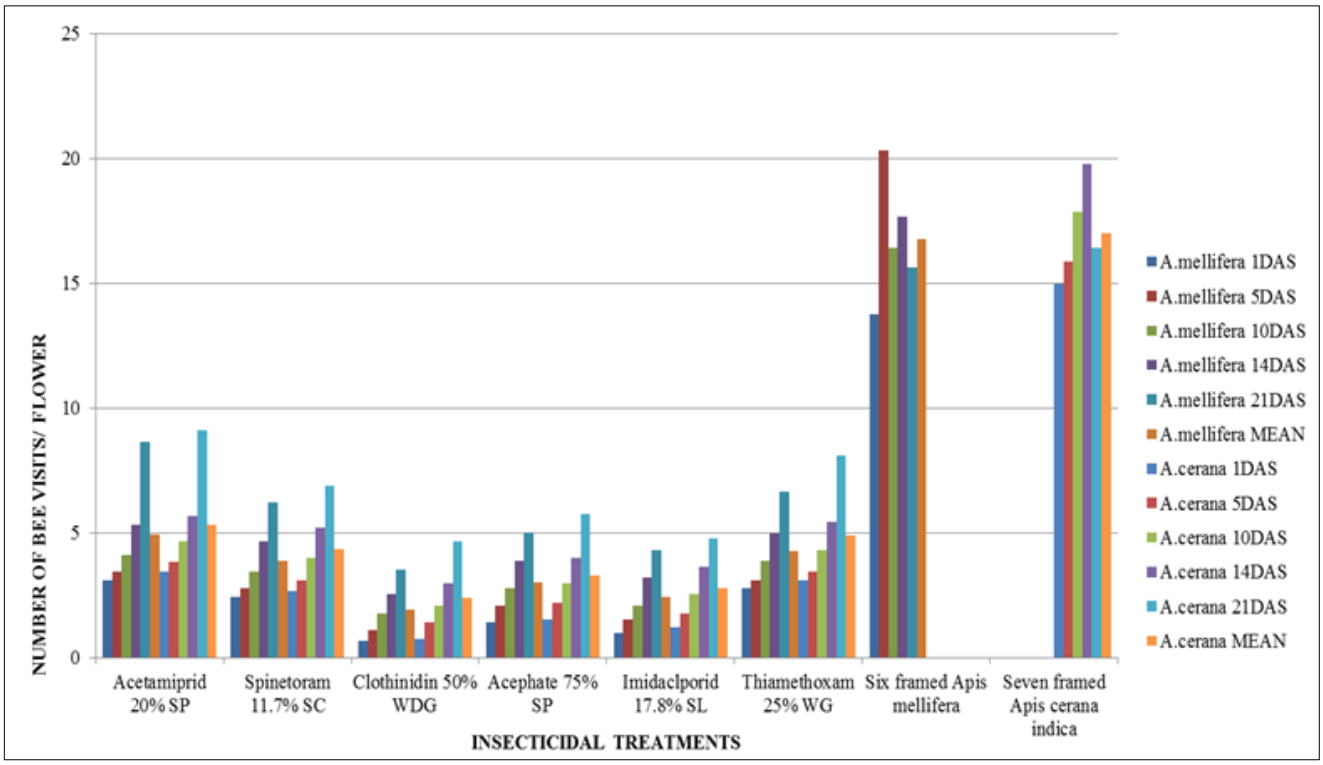

Fig 1: Effect of neonicotinoides, Organophosphate and spinosyn class of insecticides on visits of honey bee species on safflower

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