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EFFECT OF FENVALERATE, λ-CYHALOTHRIN, QUINALPHOS AND THIAMETHOXAM ON LARVAL SURVIVAL IN HONEY BEE *APIS MELLIFERA* L.

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ABSTRACT

This study evaluates the effects of some insecticides on the survival of larvae of *Apis mellifera* L. The pyrethroids (fenvalerate and λ-cyhalothrin) caused maximum mortality at highest concentration (12.5 ppm), when compared to quinalphos and thiamethoxam. Fenvalerate was observed to be extremely toxic in its maximum concentration, as none among 1-2 days old treated larvae (after multiple exposures) survived after 24 hours (i.e., after 4th exposure. Lavae were observed to be tolerant to thiamethoxam as 66.67% survival was observed till emergence under similar conditions.

Key words: *Apis mellifera*, larvae, insecticides, pyrethroids, fenvalerate, λ-cyhalothrin, thiamethoxam, survival, pollen, honey, toxicity, survival, emergence, brood, multiple exposer

Honey bees are reliable and effective pollinators in many cultivated crops (McGregor, 1976; Klein et al., 2007). It is estimated that > 80% of flowers are pollinated by honey bees, thus are important for food production and for maintenance of wild plant ecosystems (Ollerton et al., 2011). Honey bees also provide us high value products such as honey, beeswax, royal jelly, propolis, pollen and bee venom (Tautz, 2008). The population of honey bees is however found to decline globally (Potts et al., 2010; González-Varo et al., 2013). Pesticides, pathogens and parasites are considered to be some of the major reasons behind this decline (Neumann and Carreck, 2010; Johnson et al., 2010; Goulson, 2013). During foraging, bees often collect pesticide contaminated pollen and nectar, which is finally brought to the hive (Bonmatin et al., 2005; Kievits, 2007), leading to bee deaths (Van Engelsdorp et al., 2008), and thus a serious hazard (OECD, 1998; EFSA, 2014). Neonicotenoids are considered to be the main reasons behind the decline of pollinators worldwide (Goulson, 2013; Van der Sluijs et al., 2013). These are the neurotoxins which act against nicotinic acetylcholine receptors in insects (Matsuda et al., 2001; Elbert et al., 2008). However, thiamethoxam is a poor agonist of nAChRs in insects (Nauen et al., 2003; Tan et al., 2007) and is a full agonist at cercal afferent/ giant interneuron synapses (Thany, 2011). The LD_{50} for thiamethoxam against bees is 4-5 ng/ bee (Iwasa et al., 2004; Decourtye and Devillers, 2010; Laurino et al., 2011).

The synthetic pyrethroids are generally known to interfere with sodium gate in nerve membrane (Narahashi, 1962). Honey bees show variation in tolerance to different pyrethroid insecticides (Johnson et al., 2006). Fenvalerate is extremely toxic to honey bees with its LD_{50} of 0.0063 µg/ bee (Abrol and Andotra, 2003) and λ-cyhalothrin on the other hand is considered to be highly toxic to honey bees with its LD_{50} of 83 ng/ bee (Johnson et al., 2006). Organophosphates (OPs) are poisonous to insects because of their capability to inactivate enzyme, acetyl cholin estrase (Fukuto, 1990). Quinalphos is regarded to be moderately toxic to honey bees with its LD_{50} of 0.0292 µg/bee (Abrol and Andotra, 2003). Since less data is available regarding toxicity of insecticides to larvae of *A. mellifera*, this study focused on the larval stages as success of larval period is considered to be critical for maintenance honey bee colony (Godfray et al., 2014).

MATERIALS AND METHODS

The present study was carried out at the apiary situated in Khalsa College, Amritsar during 2018-2019. Three strong colonies of *A. mellifera* comprising 8-10 frames were chosen, with three replications having queens of the same age along with homogeneous circumstances (brood composition, capped larvae, nectar, pollen, free from diseases and pests etc.). For treatment, *A. mellifera* queen was first separated using queen excluder and restricted to two or three vacant frames to obtain larvae of same age group. Frames containing eggs were separated on the same day when eggs were placed in sufficient number by the queen by frequent surveillance of these frames. To achieve more eggs, these frames were substituted with other chosen frames and the process continued until the specified age group requirement was met. Ten larvae constituted a replication to accomplish requirement of larvae for control and for all the five concentrations from or close to the middle region of single frame with proper space for cage installation prior to emergence to record the rate of emergence.

Four insecticides viz., thiamethoxam, λ-cyhalothrin, fenvalerate and quinalphos were evaluated, with the concentrations decided on the basis of residue reported in pollen and nectar (Johnson et al., 2010). However, approach towards higher concentrations was also made to compare toxicity. Five concentrations *viz*. 0.02, 0.1, 0.5, 2.5 and 12.5 ppm were included, and done using micropipette (10µl) through which 1 µl of the insecticide solution was applied in each cell in the marked region containing larva. Two controls were used viz. negative control (without solvent) and positive control (with solvent) for comparison. Insecticides were applied as single and multiple doses, in the latter case, applied more than once with a waiting period of 24 hr after each; thus applied four times in case of 1-2 days old larva and 2 times with 3-4 days old larva. The survival of worker brood was recorded after 24 hr of application of insecticides. The number of larvae capped among survived were also recorded. The data obtained was statistically analysed in randomized block design (RBD) through SPSS software (version 21).

RESULTS AND DISCUSSION

The results revealed a dose dependent reduction in survival of larvae of different age groups with application of insecticides; and mortality can occur at any stage of development after exposure; and 1-2 days old larvae were more susceptible compared to 3-4- and 5-6-days old ones. One time exposure of insecticides on survival of *A. mellifera* larva revealed high susceptibility towards fenvalerate. At highest concentration tested i.e.

Table 1. Effect of single exposure of insecticides on survival of larvae of *A. mellifera*

Chemical and Conc. used (in ppm)		1-2 days old larvae			3-4 days old larvae			5-6 days old larvae		
		Survived brood (1 DAT)	Capped brood	Emerged bees	Survived brood (1 DAT)	Capped brood	Emerged bees	Survived brood (1 DAT)	Capped brood	Emerged bees
Fenvalerate	NC	10.00^{a}	9.33^{a}± 0.58	8.67^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
	PC	9.33^{a}± 0.58	9.33^{a}± 0.58	9.00^{a}± 0.00	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
	0.02	10.00^{a}	9.67^{a}± 0.58	8.67^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
	0.1	9.67^{a}± 0.58	9.00^{a}± 0.00	8.67^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
	0.5	9.33^{a}± 0.58	9.00^{a}± 0.00	8.33^{a}± 0.58	10.00^{a}	9.33^{b} ± 0.58	9.00^{b}± 0.00	10.00^{a}	10.00^{a}	9.33^{a}± 0.58
	2.5	9.33^{a}± 0.58	9.00^{a}± 0.00	7.67^{a}± 1.15	10.00^{a}	9.00^{b}± 0.00	8.67^{b}± 0.58	9.33^{a}± 0.58	9.33^{a}± 0.58	9.33^{a}± 0.58
	12.5	4.67^{b}± 1.15	1.67^{b}± 1.53	1.00^{b}± 1.00	8.67^{b}± 0.58	8.00^{c}± 0.00	6. 67^{c}± 0.58	8.67^{a}± 1.15	8.67^{a}± 1.15	6.67^{b}± 0.58
λ-cyhalothrin	NC	10.00^{a}	9.67^{a}± 0.58	9.33^{a}± 0.58	10.00^{a}	10.00^{a}	9.67^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}
	PC	10.00^{a}	9.00^{a}± 0.00	9.00^{ab}± 0.00	10.00^{a}	9.67^{ab}± 0.58	9.67^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}
	0.02	10.00^{a}	10.00^{a}	9.33^{a}± 0.58	10.00^{a}	10.00^{a}	9.67^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}
	0.1	10.00^{a}	10.00^{a}	9.00^{ab}± 0.00	10.00^{a}	10.00^{a}	9.33^{a}± 0.58	10.00^{a}	10.00^{a}	10.00^{a}
	0.5	10.00^{a}	9.33^{a}± 0.58	8.67^{ab}± 0.58	10.00^{a}	9.33^{ab}± 0.58	9.00^{a}± 0.00	10.00^{a}	10.00^{a}	9.67^{ab}± 0.58
	2.5	9.33^{ab} ± 0.58	9.00^{a}± 0.00	7.67^{b}± 0.58	9.67^{a}± 0.58	8.67^{b}± 0.58	8.33^{a}± 0.58	10.00^{a}	10.00^{a}	9.00^{b}± 0.00
	12.5	8.67^{b} ± 0.58	3.67^{b}± 0.58	3.33^{c}± 0.58	9.00^{b}± 0.00	7.33^{c}± 0.58	6.67^{b}± 0.58	9.33^{b}± 0.58	9.33^{b}± 0.58	7.33^{c}± 0.58
Quinalphos	NC	10.00^{a}	9.67^{a}± 0.58	9.33^{a}± 0.58	9.67± 0.58	9.00^{a}± 0.00	9.00^{a}± 0.00	10.00^{a}	10.00^{a}	9.67^{a}± 0.58
	PC	10.00^{a}	9.33^{ab}± 0.58	9.00^{ab}± 0.00	9.33± 0.58	9.33^{a}± 0.58	9.33^{a}± 0.58	10.00^{a}	10.00^{a}	9.67^{a}± 0.58
	0.02	10.00^{a}	9.67^{a}± 0.58	9.33^{a}± 0.58	9.67± 0.58	9.67^{a}± 0.58	9.00^{a}± 0.00	10.00^{a}	10.00^{a}	9.67^{a}± 0.58
	0.1	8.67^{ab}± 0.58	8.67^{ab}± 0.58	8.67^{ab}± 0.58	9.67± 0.58	9.33^{a}± 0.58	9.00^{a}± 0.00	10.00^{a}	10.00^{a}	9.67^{a}± 0.58
	0.5	9.33^{ab}± 0.58	8.67^{ab}± 0.58	8.67^{ab}± 0.58	9.67± 0.58	9.00^{a}± 0.00	8. 67^{a}± 0.58	10.00^{a}	10.00^{a}	9.33^{a}± 0.58
	2.5	9.33^{ab}± 0.58	8.00^{b}± 0.00	7.33^{b}± 0.58	9.00± 1.00	8.67^{a}± 0.58	8.33^{a}± 0.58	10.00^{a}	10.00^{a}	9.00^{ab}± 0.00
	12.5	8.00^{b}± 1.00	6.33^{c}± 0.58	4.00^{c}± 1.00	8.33± 0.58	7.00^{b}± 1.00	6.67^{b}± 0.58	9.33^{b}± 0.58	9.33^{b}± 0.58	7.33^{b}± 1.15
Thiamethoxam	NC	9.67± 0.58	9.67^{a}± 0.58	9.67^{a}± 0.58	10.00	10.00^{a}	9.67± 0.58	10.00	10.00	9.67± 0.58
	PC	10.00	10.00^{a}	9.67^{a}± 0.58	10.00	10.00^{a}	9.67± 0.58	10.00	10.00	9.67± 0.58
	0.02	10.00	10.00^{a}	9.67^{a}± 0.58	10.00	10.00^{a}	9.67± 0.58	10.00	10.00	9.67± 0.58
	0.1	10.00	9.67^{a}± 0.58	9.33^{a}± 0.58	10.00	10.00^{a}	9.67± 0.58	10.00	10.00	9.67± 0.58
	0.5	10.00	10.00^{a}	9.33^{a}± 0.58	10.00	10.00^{a}	9.33± 0.58	10.00	10.00	9.67± 0.58
	2.5	10.00	9.33^{a}± 0.58	8.67^{ab}± 0.58	9.67± 0.58	9.67^{a}± 0.58	9.33± 0.58	10.00	10.00	9.67± 0.58
	12.5	9.67± 0.58	9.00^{b}± 0.00	7.33^{b}± 0.58	9.67± 0.58	9.00^{b}± 0.00	8.33± 0.58	10.00	10.00	9.00± 0.00

All values Mean± SD; NC- Negative control; PC- Positive control; DAT- Days after treatment; Values significant at p=0.05; Variables ($^{a, b, c..}$) significantly differ from each other (p=0.05)

Table 2. Effect of multiple exposures of insecticides on larvae of *A. mellifera*

Chemical and Conc. used (in ppm)		1-2 days old larvae			3-4 days old larvae		
		Survived brood (1 DAT)	Capped brood	Emerged bees	Survived brood (1 DAT)	Capped brood	Emerged bees
Fenvalerate	NC	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	10.00^{a}	10.00^{a}	10.00^{a}
	PC	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	10.00^{a}	10.00^{a}	10.00^{a}
	0.02	$9.67^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	10.00^{a}	10.00^{a}	10.00^{a}
	0.1	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.00^{ab}\pm 1.00$	10.00^{a}	10.00^{a}	10.00^{a}
	0.5	$9.00^{a}\pm 1.00$	$8.67^{a}\pm 1.15$	$8.67^{ab}\pm 1.15$	$9.33^{a}\pm 0.58$	$9.00^{a}\pm 0.00$	$8.67^{b}\pm 0.58$
	2.5	$8.33^{a}\pm 0.58$	$8.00^{a}\pm 0.00$	$7.00^{b}\pm 1.00$	$9.33^{a}\pm 0.58$	$9.00^{a}\pm 0.00$	$8.00^{b}\pm 0.00$
	12.5	0.00^{b}	0.00^{b}	0.00^{c}	$7.00^{b}\pm 1.00$	$6.00^{b}\pm 1.00$	$5.00^{c}\pm 1.00$
λ-cyhalothrin	NC	10.00^{a}	10.00^{a}	$9.33^{a}\pm 0.58$	10.00^{a}	10.00^{a}	$9.33^{a}\pm 0.58$
	PC	$9.67^{a}\pm 0.58$	$9.67^{ab}\pm 0.58$	$9.33^{a}\pm 0.58$	10.00^{a}	$9.67^{a}\pm 0.58$	$9.33^{a}\pm 0.58$
	0.02	10.00^{a}	10.00^{a}	$9.33^{a}\pm 0.58$	10.00^{a}	10.00^{a}	$9.33^{a}\pm 0.58$
	0.1	10.00^{a}	10.00^{b}	$9.00^{a}\pm 0.00$	10.00^{a}	10.00^{a}	$9.33^{a}\pm 0.58$
	0.5	$9.33^{a}\pm 0.58$	$9.00^{ab}\pm 0.00$	$8.33^{ab}\pm 0.58$	10.00^{a}	$9.33^{a}\pm 0.58$	$9.00^{a}\pm 0.00$
	2.5	$9.00^{a}\pm 1.00$	$8.67^{b}\pm 0.58$	$7.33^{b}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$8.33^{a}\pm 0.58$
	12.5	$1.67^{b}\pm 1.53$	$0.67^{c}\pm 0.58$	0.00^{c}	$6.67^{b}\pm 0.58$	$5.67^{b}\pm 0.58$	$4.33^{b}\pm 1.53$
Quinalphos	NC	$9.33^{a}\pm 0.58$	$9.00^{a}\pm 0.00$	$9.00^{a}\pm 0.00$	10.00^{a}	$9.67^{a}\pm 0.58$	$9.00^{ab}\pm 0.00$
	PC	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{ab}\pm 0.58$	$9.33^{ab}\pm 0.58$	$9.33^{a}\pm 0.58$
	0.02	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.00^{a}\pm 0.00$	10.00^{a}	$9.00^{abc}\pm 0.00$	$9.00^{ab}\pm 0.00$
	0.1	$9.00^{a}\pm 0.00$	$8.67^{a}\pm 0.58$	$8.33^{ab}\pm 0.58$	$9.33^{ab}\pm 0.58$	$8.67^{abc}\pm 0.58$	$9.00^{ab}\pm 0.00$
	0.5	$9.00^{a}\pm 0.00$	$8.33^{a}\pm 0.58$	$8.00^{ab}\pm 1.00$	$8.67^{ab}\pm 0.58$	$8.33^{abc}\pm 0.58$	$8.33^{ab}\pm 0.58$
	2.5	$7.67^{a}\pm 0.58$	$7.33^{a}\pm 0.58$	$7.33^{b}\pm 0.58$	$8.33^{b}\pm 0.58$	$8.00^{bc}\pm 0.00$	$8.00^{b}\pm 0.00$
	12.5	$3.67^{b}\pm 2.31$	$2.67^{b}\pm 1.53$	$1.33^{c}\pm 0.58$	$8.33^{b}\pm 0.58$	$7.67^{c}\pm 0.58$	$4.67^{c}\pm 0.58$
Thiamethoxam	NC	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	$9.33^{a}\pm 0.58$	10.00	10.00	$9.33^{a}\pm 0.58$
	PC	10.00^{a}	$9.67^{a}\pm 0.58$	$9.67^{a}\pm 0.58$	9.67 ± 0.58	9.67 ± 0.58	$9.33^{a}\pm 0.58$
	0.02	$9.67^{a}\pm 0.58$	$9.67^{a}\pm 0.58$	$9.00^{a}\pm 0.00$	10.00	10.00	$9.33^{a}\pm 0.58$
	0.1	$9.33^{a}\pm 0.58$	$9.00^{ab}\pm 0.00$	$9.00^{a}\pm 0.00$	10.00	9.67 ± 0.58	$9.33^{a}\pm 0.58$
	0.5	$9.00^{ab}\pm 0.00$	$9.00^{ab}\pm 0.00$	$8.67^{a}\pm 0.58$	10.00	9.33 ± 0.58	$9.00^{ab}\pm 0.00$
	2.5	$9.00^{ab}\pm 0.00$	$8.67^{ab}\pm 0.58$	$8.33^{a}\pm 0.58$	9.67 ± 0.58	9.33 ± 0.58	$8.67^{ab}\pm 0.58$
	12.5	$8.00^{b}\pm 0.00$	$7.33^{b}\pm 1.15$	$6.67^{b}\pm 0.58$	9.33 ± 0.58	9.00 ± 1.00	$7.67^{b}\pm 0.58$

Values Mean± S; NC- Negative control; PC- Positive control; DAT- Days after treatment
Values are significant at 5% level of significance, Variables ($^{a,\ b,\ c..}$) significantly differ from each other at 5% level of significance

12.5 ppm, survival reduced significantly ($p\leq 0.05$) to 10 and 66.67% in 1-2 days old and 3-4 and 5-6 days old larvae, respectively. The other insecticide observed to be highly toxic was λ-cyhalothrin- in 1-2 days old larvae survival reduced significantly ($p\leq 0.05$) to 33.33% at the time of emergence at highest concentration i.e. 12.5 ppm; in 3-4 and 5-6 days old larvae, survival observed at emergence was 66.67 and 73.33% at 12.5 ppm. With quinalphos, at highest concentration (12.5 ppm), the survival in 1-2, 3-4, and 5-6 days old larvae significantly reduced ($p\leq 0.05$) to 40, 66.67 and 73.33% at the time of emergence. In case of thiamethoxam, 1-2 days old larvae showed 73.33% survival at emergence stage at 12.5 ppm; in 3-4 days old larvae, 83.33% survival was observed; and survival rate was extremely high in 5-6 days old larvae; however, at emergence, survival at highest concentration reduced non-significantly to 90% (Table 1).

Multiple exposure of insecticides to *A. mellifera* larvae lead to higher mortality in comparison to single exposure; 24 hr after application of last dose (4th exposure) of fenvalerate (12.5 ppm), all the treated larvae died; and same trend was observed in 3-4 days old larvae when exposed twice; at emergence, survival was 50% at highest concentration i.e. 12.5 ppm. Against λ-cyhalothrin, 6.67% survival in 1-2 days old larvae was observed at capping stage; and none survived till emergence; and in 3-4 days old ones, survival reduced significantly ($p\leq 0.05$) to 43.33% at emergence at 12.5 ppm. For quinalphos, 13.33% larval survival was observed in 1-2 days old larvae; and 46.67% survival was observed in 3-4 days old ones at 12.5 ppm. After multiple exposures of thiamethoxam, survival of larvae significantly reduced ($p\leq 0.05$) to 66.67% in 1-2 days old larvae and to 76.67% in 3-4 days old ones 12.5 ppm (Table 2). Comparison of toxicity with data obtained after single as well as multiple exposure, revealed reduction in survival at highest concentration as follows: Fenvalerate> λ-cyhalothrin> Quinalphos> Thiamethoxam. Abrol and Andotra (2003) reported that toxicity of fenvalerate to *A. mellifera* was greater than that of quinalphos and Marletto et al. (2003) revealed that after 72 hr of exposure, λ-cyhalothrin was slightly more toxic to bumble bees than quinalphos.

The present study also showed that among the two pyrethroids, fenvalerate was more toxic to *A. mellifera* larvae; 24 hr after multiple exposures, all the larvae died at highest concentration (12.5 ppm); and with λ-cyhalothrin, also highly toxic, after multiple exposures, only 6.67% survival was observed at capping stage, whereas none survived till emergence. Lesser toxicity of λ-cyhalothrin as compared to fenvalerate might be due to difference in honey bee tolerance. Johnson et al. (2006) observed that honey bees showed variation in tolerance to pyrethroids and cytochrome P450 monooxygenases (P450s) plays an important role in detoxification. Among the insecticides evaluated, thiamethoxam was observed to be least toxic to larvae of *A. mellifera*. Earlier, however, thiamethoxam had been reported to be highly toxic to adult bees both via ingestion as well as through indirect contact (Iwasa et al., 2004; Laurino et al., 2011; Shah et al., 2020). It shows that the larval stage of honey bee is more tolerant to thiamethoxam. Lesser toxicity of some of the neonicotinoids to honey bee larvae is known (Yang et al., 2012; Tavares et al., 2015; Tavares et al., 2017). Although the reduction of survival was observed at higher concentrations tested, the survival was high at lower concentrations (residue level). Hence these insecticides can be considered as safe in case honey bee larvae, when exposed through residue in pollen or nectar.

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