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IMPACT OF NEEM OIL ON DEVELOPMENTAL STAGES OF HONEY BEE *APIS MELLIFERA* L.

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ABSTRACT

Toxicity of neem oil on developmental stages of *Apis mellifera L.* was evaluated by applying three concentrations within the marked comb cells with micropipette as single (applied only once) and multiple exposures (at one day interval up to capping of cells) on different age groups of larvae (1-2, 3-4 and 5-6 days). Results revealed that maximum survival was observed in controls (both negative and positive) and at par with 0.05% (T1) and 0.1 % (T2) concentrations. Minimum survival of larval stage was found in 1% (T3). Same trend was also observed in emerged pupae (CD=17.9) and in mortality of adult bees (CD=15.3). Significant difference in the survival of larval ($p<0.05$), emerged pupae ($p<0.05$) and mortality of adult ($p<0.05$) honey bees was found within stages originating from treated larvae. Both in single or multiple doses, it was found that larvae treated at 1-2 days age group was significantly affected as compared to other stages.

Key words: *Apis mellifera*, neem oil, developmental stages, comb cells, brood, hives, single exposure, multiple exposure, pupae, adult, larvae, emergence, survival

Insect pollinators play a vital role in agricultural and horticultural crops as they enable transfer of pollen and nectar from one flower to another (Haldhar et al., 2018). Among the insect pollinators, most valuable pollination services are provided by western honey bee (*Apis mellifera* L.). Unfortunately, population of insect pollinators is declining at an alarming rate (Sandilyan, 2014). Bees come in contact with pesticide residue on plant and get harmed by taking the contaminated dew and pollen grains (Pandey, 2010). Due to these harmful effects of insecticides, there is increased interest in botanical insecticides as these are cost effective and have no side effects on environment (Hikal et al., 2017). Products obtained from neem are used for controlling various mites such as *Acarapis woodi, Varroa destructor* or various pathogenic bacteria and fungi (Gomez et al., 2016). Seed kernels of neem yield about 90% of fixed oil (Windholz, 1987). Neem has dose dependent effect on honey bees and beneficial arthropods. It is not safe to honey bees at higher doses (Shawki et al., 2005). Most studies on the impacts of insecticides on honey bees was thus concentrated on adult workers. However, all the formative stages and castes may be conceivably influenced by residue of insecticides (Tome et al., 2012). The present study assesses the toxicity of neem oil on developmental stages of honey bee *A. mellifera*.

MATERIALS AND METHODS

The studies were carried out during spring season in PG Department of Agriculture, Khalsa College, Amritsar. Three concentrations of neem (99%) i.e. 0.05 (T1), 0.1 (T2) and 1(T3) % and two controls i.e. negative control (with acetone) and positive control (without acetone) were used. These concentrations were applied within the marked comb cells with micropipette. These were applied as single exposure (applied only once) on 1-2, 3-4 and 5-6 days old larvae and multiple exposure (applied repeatedly up to capping of cells) on 1-2 and 3-4 days age groups of worker larvae. The method of application was as described by Atkins and Kellum (1986). The experiment was laid out in a randomized block design (RBD) and each treatment was replicated thrice (in three hives with 8-10 bee frame strength colonies) and each replication contained ten treated and ten control larvae. For making proper emulsion of oils, acetone was added. For evaluating the impact of treatments, data was recorded 24 hr after application, on capping of brood (6 days after hatching of eggs) and at emergence of adult bees (21 days after hatching of eggs).

For recording survival, the larvae were selected in row and marked with colour pins. The survival

was observed as number of individuals survived and thereafter, the data were converted into %. Queen excluder was used to obtain same age group eggs or larvae of *A. mellifera*, two vacant (without brood) worker brood cell combs were placed in the selected colonies and carefully placed queen bee on these combs. Next day, eggs were obtained through regular monitoring of selected combs. Combs having one day old egg were replaced with other empty selected combs in order to obtain more eggs. This process continued up to fulfilling the requirement of selected age group larvae. The concentrations were applied with a micropipette and 1 µl was applied in each worker cell. The dose (1µl) was selected according to pollen and honey feed taken by larvae on daily basis (Babendreier et al., 2004). For morphometrics, small cages were used on capped brood cells to collect emergent adults which were later brought to the laboratory to observe variations. Morphometric evaluations were done under Magnus trinocular stereomicroscope with Magvision (Advance image analysis software). The statistical analysis was done using ICAR, WASP 1.0. Morphological characters were measured following El-Aw et al. (2012).

RESULTS AND DISCUSSION

On single exposure application of neem oil to different age groups of larvae (1-2, 3-4, and 5-6 days) of *A. mellifera* larvae it was found that these caused significant impact on the survival of all developmental stages. Significant differences were observed within concentrations- 1-2 days old larvae (CD= 17.9) (Table 1). In the larval stage, % brood survival was found maximum in controls with mean 86.29 in negative and 86.66 in positive control and was at par with 0.05%

Table 1. Toxicity of neem oil on lifestages of *A. mellifera* - single and multiple exposed workers

Treatments	Survived brood*(%)						
	Multiple exposure				Single exposure		
	1-2 day	3-4 day	5-6 day	Mean	1-2 day	3-4 day	Mean
Negative control	90.00± 5.77	83.33± 11.54	85.55± 1.92	86.29	90.00± 5.77	83.33± 11.54	86.665
Positive control	86.66± 15.27	86.66± 11.54	86.66± 15.27	86.66	86.66± 15.27	86.66± 5.77	86.66
0.05 (T1)	83.33± 15.27	76.66± 5.77	83.33± 5.77	81.10	76.66± 20.81	80.00± 17.32	78.33
0.1 (T2)	83.33± 5.77	80.00± 17.32	83.33± 15.27	82.22	73.33± 15.27	70.00± 17.32	71.665
1 (T3)	63.33± 5.77	66.66± 5.77	73.33± 15.27	67.77	50.00± 10.00	50.00± 10.00	50
CD (0.05)	17.99	N.S	N.S		25.46	NS	
Capped brood*(%)							
	Multiple exposure				Single exposure		
Treatments	1-2 day	3-4 day	5-6 day	Mean	1-2 day	3-4 day	Mean
Negative control	81.11± 3.84	80.00± 10.00	78.88± 7.69	79.99667	81.11± 3.84	78.88± 10.00	79.995
Positive control	83.33± 15.27	83.33± 15.27	83.33± 15.27	83.33	83.33± 15.27	83.33± 11.54	83.33
0.05 (T1)	76.66± 11.54	66.66± 11.54	76.66± 15.27	73.32667	73.33± 15.27	76.66± 20.81	74.995
0.1 (T2)	73.33± 5.77	70.00± 10.00	70.00± 10.00	71.11	60.00± 10.00	63.33± 5.77	61.665
1 (T3)	56.66± 5.77	56.66± 15.27	56.66± 15.27	56.66	50.00± 10.00	46.66± 15.27	48.33
CD (0.05)	N.S	N.S	N.S		21.2	NS	
Emerged brood*(%)							
	Multiple exposure				Single exposure		
Treatments	1-2 day	3-4 day	5-6 day	Mean	1-2 day	3-4 day	Mean
Negative control	80.00± 3.33	70.00± 10.00	77.77± 6.93	75.92333	80.00± 3.33	77.77± 10.00	78.885
Positive control	83.33± 15.27	76.66± 20.81	83.33± 15.27	81.10667	83.33± 15.27	83.33± 10.00	83.33
0.05 (T1)	70.00± 10.00	60.00± 10.00	73.33± 15.27	67.77667	70.00± 20.00	76.66± 20.81	73.33
0.1 (T2)	63.33± 5.77	60.00± 10.00	70.00± 10.00	64.44333	56.66± 5.77	53.33± 15.27	54.995
1 (T3)	46.66± 11.54	46.66± 15.27	53.33± 11.54	48.88333	50.00± 10.00	46.66± 15.27	48.33
CD (0.05)	17.91	N.S			22.9	NS	
Mortality*(%) of treated larva							
	Multiple exposure				Single exposure		
Treatments	1-2 day	3-4 day	5-6 day	Mean	1-2 day	3-4 day	Mean
Negative control	22.22± 1.92	20.00± 10.00	22.22± 6.93	21.48	22.22± 1.92	22.22± 10.00	22.22
Positive control	23.33± 20.81	23.33± 10.00	16.66± 15.27	21.10	23.33± 20.81	16.66± 10.00	19.995
0.05 (T1)	30.00± 10.00	40.00± 20.81	26.66± 15.27	32.22	30.00± 20.00	23.33± 20.81	26.665
0.1 (T2)	36.66± 5.77	40.00± 10.00	30.00± 10.00	35.55	43.33± 5.77	36.66± 5.77	39.995
1 (T3)	53.33± 11.54	53.33± 15.27	46.66± 11.54	51.10	50.00± 10.00	0.00± 0.00	25
CD (0.05)	15.3	N.S	N.S		15.08	NS	

(contd.)

(Table 1 contd.)

S.No.	Treatment	Affected stage	p (0.05)
Single exposed larvae			
1		Larvae	0.7542
2	Within different age group of larvae	Capped pupae	0.9097
3	(df=2)	Emerged pupae	0.5387
4		Mortality	0.6858
5		Larvae	0.00025*
6	Within different concentrations of neem oil	Capped pupae	-
7	(df=2)	Emerged pupae	0.00016*
8		Mortality	0.00006*
Multiple exposed larvae			
1		Larvae	0.8937
2	Within different age group of larvae	Capped pupae	0.9817
3	(df=2)	Emerged pupae	0.8533
4		Mortality	0.1226
5		Larvae	-
6	Within different concentrations of neem oil	Capped pupae	-
7	(df=2)	Emerged pupae	-
8		Mortality	-
Difference between single and multiple doses			
1.	Difference between single and multiple doses in larvae (df=1)	1-2 days treated larvae	0.4976
2.		3-4 days treated larvae	0.5489
3.	Difference between single and multiple doses in capped pupae (df=1)	1-2 days treated larvae	0.5725
4.		3-4 days treated larvae	0.8542
5.	Difference between single and multiple doses in emerged pupae (df=1)	1-2 days treated larvae	0.9441
6.		3-4 days treated larvae	0.5992
7.	Difference between single and multiple doses in adult mortality (df=1)	1-2 days treated larvae	0.934
8.		3-4 days treated larvae	0.105
9.		Larvae	0.516
10.	Mean difference between single and multiple doses (df=1)	Capped pupae	0.707
11.		Emerged pupae	0.819
12.		Mortality in adults	0.303

concentration (81.10%) and T2 (0.1%) (82.22%) concentrations. Minimum survival of larval stage was found in T3 (1%) concentration. Same trend was also observed in emerged pupae (CD= 17.9) and in adult bees mortality (CD= 15.3). According to Xavier et al. (2015), neem oil has an acute toxicity to both larvae and adult workers of *A. mellifera*. Extracts of neem oil were acutely toxic to immature stages of honey bees (Rembold et al., 1980). The death of *A. mellifera* pupae exposed to neem oil might be due to effect on the insect ecdysis (Riba et al., 2003, Pineda et al., 2009, Mhazo et al., 2011). Significant difference in the survival of larvae (Table-1) ($p<0.05$, $p=0.00025$), emerged pupae ($p<0.05$, $p=0.00016$) and mortality of adult ($p<0.05$, $p=0.00006$) was also found within stages originated from larvae treated different concentrations. In larval stage, minimum survival was found in T3 (1%) dose and maximum in controls. Same trend was observed in survival of emerged pupae. In the mortality of adult bees, it was seen that with increased concentration, the mortality also increased. Minimum mortality was observed in controls and maximum was found in T3 concentration. However, in capped pupa, significant difference was not found within concentrations. According to Xavier et al. (2015), adult workers originated from larvae fed on a diet with botanical insecticide residues depicted significant differences among treatments. Neem oil caused mortality at the starting of the larval stages and at the emergence of adult bees.

It was observed that each treated larval age group respond equally to single exposure of neem oil, however, significant concentration dependent effect was observed. T3 (1%), the maximum concentration, resulted in minimum survival of larvae, capped pupae, emerged pupae and mortality of adult. Small or recommended concentration of neem oil has no adverse effect, but increased concentration can lead to adverse effects on developmental stages. After multiple exposure to different age groups of larvae significant difference within different concentrations was found in 1-2 days old treated larvae. Maximum survival was reported in controls and minimum was found

Table 2. Morphometrics of emerging bees after neem oil treatment (on 1-2 and 3-4 day treated worker larvae of *A. mellifera*)

Treatments	Head*		Thorax*	Fore wing*		Hind wing*		Hind leg*	Abdomen*		Weight (g)
	Height	Width	Length	Length	Width	Length	Width	Length	Length	Width	
					1-2 day						
NC	3.47± 0.20	3.62± 0.10	4.30± 0.06	9.23± 0.03	3.01± 0.01	6.46± 0.01	1.80± 0.01	10.22± 0.02	6.05± 0.02	5.83± 0.10	0.02± 0.02
C	3.61± 0.11	3.63± 0.11	4.31± 0.06	9.20± 0.03	2.98± 0.01	6.59± 0.26	1.72± 0.03	10.12± 0.11	6.00± 0.01	5.74± 0.15	0.03± 0.01
0.05 (T1)	3.70± 0.01	3.70± 0.01	4.35± 0.02	9.24± 0.00	3.02± 0.00	6.46± 0.02	1.81± 0.00	10.12± 0.12	6.06± 0.00	5.70± 0.24	0.02± 0.01
0.1 (T2)	3.46± 0.00	3.70± 0.01	4.25± 0.04	9.10± 0.08	3.01± 0.00	6.34± 0.02	1.80± 0.01	9.94± 0.03	6.02± 0.03	4.76± 0.07	0.04± 0.01
1 (T3)	3.22± 0.01	3.46± 0.00	4.02± 0.03	8.67± 0.04	2.78± 0.00	6.05± 0.02	1.59± 0.01	9.72± 0.07	5.75± 0.04	4.73± 0.14	0.02± 0.01
CD (0.05)	0.21	NS	0.1	0.08	0.01	0.24	0.03	0.17	0.05	0.27	NS
					3-4 Day						
NC	3.21± 0.03	3.61± 0.13	4.36± 0.02	9.23± 0.03	2.99± 0.01	6.46± 0.01	1.81± 0.01	10.20± 0.02	6.04± 0.02	4.73± 0.07	0.03± 0.01
C	3.19± 0.03	3.62± 0.14	4.24± 0.04	9.20± 0.03	3.00± 0.01	6.41± 0.04	1.79± 0.01	10.22± 0.01	6.01± 0.03	4.70± 0.33	0.02± 0.01
0.05 (T1)	3.21± 0.01	3.70± 0.02	4.36± 0.01	9.24± 0.00	3.70± 0.02	6.46± 0.03	1.81± 0.00	10.20± 0.05	6.06± 0.00	4.76± 0.23	0.02± 0.01
0.1 (T2)	3.20± 0.00	3.69± 0.02	4.28± 0.07	9.10± 0.08	3.69± 0.02	6.33± 0.046	1.80± 0.01	9.96± 0.06	5.97± 0.02	4.10± 0.28	0.03± 0.02
1 (T3)	2.95± 0.01	3.45± 0.01	4.01± 0.04	8.67± 0.04	3.45± 0.01	6.09± 0.05	1.61± 0.02	9.67± 0.17	5.72± 0.02	4.10± 0.21	0.02± 0.01
CD (p=0.05)	0.03	NS	0.06	0.08	0.02	0.06	0.03	0.17	0.05	0.47	NS

NC= Control without any solvent; C= Control with solvent; NS- Non-significant; Mean± SD (mm)*

at highest (T3) dose. Same trend was also observed with capped pupae and emerged adults. Significant variation within doses was also seen in adults emerged from 1-2 days old treated larvae. Single and multiple exposure of neem oil did not show significant difference in larval survival, capped pupae, pupae survival and mortality of adult bees. However, 1-2 days old larvae are most susceptible. According to Gomez et al. (2016), adequate concentrations of neem oil may control *Varroa destructor* without affecting the colonies of bees. However, mortality of the brood increased with increased the concentration of neem oil and most susceptible phases of honey bees were eggs and first instar larvae. Morphometric variation was observed in adult bees originating from 1-2 days old treated larvae and significant variation was found in all morphological parameters except head width. Similar trend was also observed in 3-4 days old exposed larvae (Table 2). These results coincide with the results of Melathopoulos et al. (2000) that neem oil formulations might have negative effect on the development of *A. mellifera* larvae. According to Savarimuthu et al. (2004), this observation can be attributed to this botanical insecticide, which reduced the food conversion rates in the worker larvae. Thus, neem oil has no adverse effect on *A. mellifera* at recommended concentration.

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