Research Article

Effect of Insecticides on the Longevity of *Apis mellifera* L. (Hymenoptera: Apidae)

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Abstract | Honey bee *Apis mellifera* L., are good crop pollinators, however, its population is decreased over the years due to various factors including spraying chemical insecticides for pest management. The present experiment was therefore, designed to look for the effect of some new insecticides (cyhalothrin, nitenpyram, emamectin benzoate) applied at flowering stage of sunflower (variety Hysun-33). After three days of pesticides application, *Apis mellifera* was exposed to insecticides, placing three honey bee colonies in the center of each plot in the field. Four frames from each colony and twenty cells from each frame were selected as samples for further studies. After egg hatching larval duration was recorded until pupal duration starts. The honey bees were caged after pupal duration starts and marked with permanent marker for adult data recording. The data was recorded daily for 30 days. The effects of different chemical insecticides (nitenpyram @75ml/acre- new chemistry, cyhalothrin; nok-out @200ml/ acre, tycon; emamectin benzoate @ 200ml/acre) on egg presence, egg hatching, larval duration, pupal period and adult longevity were examined. Results revealed that the nitenpyram was found statistically significant which means it was affecting the following stages in honey bees; eggs presence (less number of eggs) df=3, F=8.97, P=<.001, egg hatching (less number of eggs hatched) df=3, f=11.94, p <.001, day to pupation (F=3, df = 5.611, p< 0.001) and less adult emergence rates in the selected cell. The cyhalothrin was also found statistically significant and affecting the honey bees at egg hatching (decreased egg hatching as compared to control) and pupation (less pupation as compared to the control) stages and less toxic at day to adult emergence and day to pupation. While the emamectin benzoate was found affecting on egg hatching with average (0.71) and at pupation rate with average (0.86). The knowledge from this study describes the effects of chemical compounds on honey bees and potentially facilitates beekeeping farmers to avoid the use of chemical compounds for their colonies to reduce colony problems.

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Introduction

Honey bee, *Apis mellifera* L., are efficient crop pollinators (Klein *et al.*, 2007) and play an important role in pollinationof various important crops and fruit trees. However, worldwide population of honeybees are affected and decreased over the years, as a result of habitat devastation by application of pesticides, various bee pathogens, climate change and a mixture of these factors (Aizen and Harder, 2009: vanEngelsdorp and Meixner, 2010). Simultaneously, the crops cultivation also increased which are heavily dependent on the insect for pollination. Keeping in view the high losses faced by the beekeepers over the time particularly in winter, the aptitude to make available enough colonies to cope up this expected demand is questionable. The colonies which dying with the condition called colony collapse disorder (CCD) make up a prominent proportion of recent overwintering losses worldwide. These colonies are frequently contaminated with greater variety and higher dose of pesticides and disease agents than vigorous colonies (Johnson *et al*., 2009; vanEngelsdorp *et al*., 2009a; Cox-Foster *et al*., 2007). This implies that some factors or combination of the factors may be waning honey bees by making them susceptible to the contamination (vanEngelsdorp *et al*., 2009a).

The ability of the bees to fight against infection, interaction among disease agents, mites parasitism, meager nutrition and lethal and sub-lethal exposure to different compounds are being affected by the numerous biotic and abiotic factors (Johnson *et al*., 2009; vanEngelsdorp *et al*., 2009a). The systemic compounds like imidacloprid pose a novel exposure route through pollen and nectar and have been verified to have harmful consequence on honey bees learning (Decourtye *et al*., 2004). With the increase in synthetic compounds usage, the contamination risks for the honeybees amplified in the countries such as USA, China, Pakistan and India that is much alarming for the apicultural industry. The environmental risks gravity produced by the synthetic compounds is generally appraised by two ways i.e, dimension of pesticides exposure and effect of pesticides particularly on living organisms (Vander Werf, 1996).

In this regard, neonicotinoids insecticides (neurotoxins) with lethal or sub-lethal concentrations substantially increased globally over last decades and represent threat to honeybee's growth, survival and development. The neonicotinoid actas agonists of nicotinic acetylcholine receptor by distract the neuronal cholinergic signal transmission, lead to abnormalities, emplacement, distressing in commemoration, immobility and ultimately cause of carnage of the target organisms (Matsuda *et al*., 2001; Tomizawa and Casida, 2005; Elbert *et al*., 2008). In the field, the forager bees exposed to the neonicotinoids-infected pollen and nectar, bring them to the hive and disseminate the contaminated pollen and nectar to the brood and nurse bees, bee bread and in honey itself which are equally suffered by their hazardous impacts (Genersch *et al*., 2010; Blacquière *et al*., 2012).

Elbert *et al*. (2008) assessed the neonicotinoids as most prevalent and speedily affecting chemical compounds on honey bees through their systemic effects. Jay (1964) assessed the effects of pesticides on larva and adults bees and on their development and growth, therefore, putting colony survival at risk. Various pesticides like Dimethoate, melathion, carbaryl and fungicide (captan®) are described as having morphogenetic effects on the health of adults bare as larvae. Generally, the adult honey bees suffered from immature growth, stunted body, super small as compared to normal size, wing deformity, infrequently wingless, and deformed legs and wings. All factors affect the capabilities and functioning of adult bees and make them incapable to continue them in-colony and out-colony tasks, foraging activities appropriately and etc (Atkins and Kellum, 1986).

The chemical compounds mostly affect the longevity of the bees and contribute in diminution of life span up to 20 % through their detrimental exposure of diazenon (MacKenzie and Winston, 1989). Smirle (1984) found the pesticides effects on life span and described that most probably the age reliance effect assumed by slight level of detoxifying enzymes and this progression of tasks adversely affect the longevity of honey bees. The direct pesticides exposure put adverse affects on the health of honey bees' colony and mainly weakens colony by causing mortality. This research was planned to assess the higher trophic effects of some insecticides on the larval, pupal and adult honey bees.

Materials and Methods

The experiment was conducted in the apiary of College of Agriculture, University of Sargodha in spring 2017. Sargodha is situated at 32.08° North latitude, 72.67° East longitude and 193 meters elevation above the sea level. Four different plots of about 500 sq. meters each were prepared for this experiment. After proper land preparation, sunflower cultivar "Hysun-33" was grown on all experimental plots as per recommended methods. All culture practices like hoeing weeding, fertilization and irrigation were performed accordingly. The stock solutions with a given concentration of each chemical were prepared. At flowering stage, three different insecticides were sprayed as per their recommended doses at each plot as given below, except one plot which was kept as control (untreated) to check the effect on egg presence, egg hatching, number of days to hatch, pupation, day to pupate, emergence, day to emergence, day to die of the bees. The insecticides used in the experiment were (nitenpyram-New Chemistry @10% SL/Acre, lambda-cyhalothrin- Pyrethroids 200 ml/Acre, emamectin benzoate @ 0.34 ml/Acre), Control (no insecticide). The honey bee colonies were equalized before experiment. The bee colonies were provided with sugar food (mixture of sugar and water) in the hive at equal rates before a week to start the experiment. The managed honeybee colonies each with 8 frames were brought from apiary after sunset time of the day, and placed three colonies at each plot. The strength of brood in the brought colonies was assured. The experimental plots were located at the distance of 500 meters from the apiary so that the queens of the apiary may not disturb. Out of eight, four frames from each colony and 20 cells per frame were selected randomly for data collection regarding egg hatching, larvae and pupae duration. After the egg hatching, data for larval duration was starts until pupal duration starts. The bees were caged after pupal duration and marked with permanent marker for adult data recording. The adult data were recorded up to the marked bees' death. The data were recorded two times at 8:00 am and 4:00pm daily. The experiment was carried out under completely randomized design (CRD).

Results and Discussion

Effect of insecticide on the egg presence

The results revealed that the insecticides greatly affected egg stage in the colony (df=3, F=8.97, P=<.001). The Maximum eggs were counted in control treatment (0.89 eggs) and minimum eggs were counted in nitenpyram and were found the most toxic to the eggs of honey bees. The lambda-cyhalothrin and emamectin benzoate was almost discovered affecting with same ratio since they got very small difference in average values 0.79 and 0.75 respectively as compared to control treatment (Figure 1).

Effect of insecticide on the egg hatching

Egg hatching is significantly affected by treatment application (df=3, f=11.94, p <.001). Results revealed that maximum egg hatching was recorded in control as compared to others with average value as (0.86). Insecticides, lambda-cyhalothrin and nitenpyram were found more harmful among the tested insecticides and affecting hatched eggs at the same ratio with average values (0.66), (0.65) respectively. However, emamectin benzoate was comparatively less toxic for hatched eggs with average value as (0.71) (Figure 2).

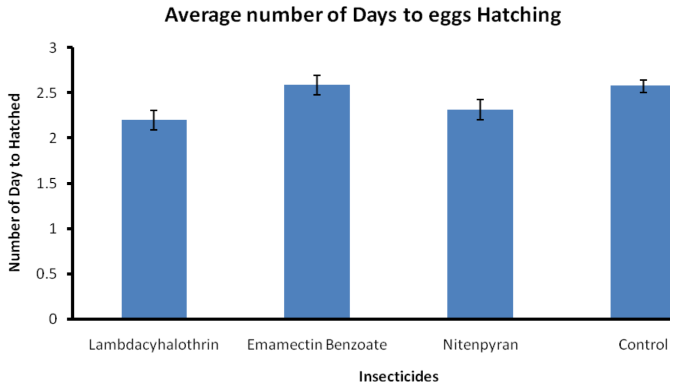

Figure 1: *Average number of (mean ± SEM) egg presence of Apis mellifera in each insecticide application.*

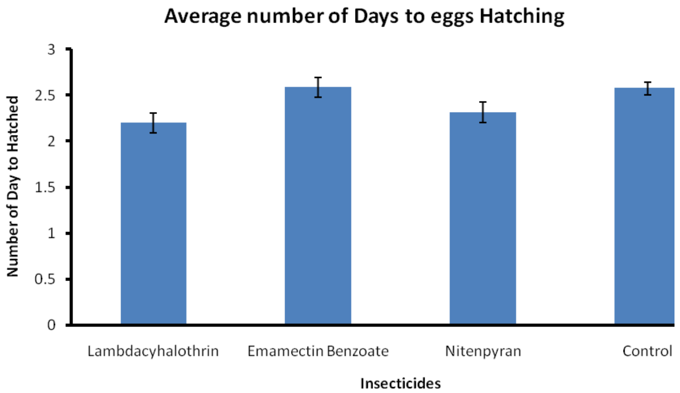

Figure 2: *Average number of (mean ± SEM) egg hatching of Apis mellifera in each insecticide application.*

Effect of insecticide on the day to egg hatch

The result of insecticides' effect on "hatching day" depicted that emamectin benzoate was toxic to egg hatching since it causes delay in egg hatching with average value as (2.58). However, the nitenpyram and lambda-cyhalothrin were not affecting the hatching duration with average values as (2.31) and (2.20) respectively, as compared to the control treatment. The analysis of variance was calculated and the results showed that the model was significant at 5% level of significance with F=3, df= 3.642, p< 0.001 and explained variation in the dependent variable (Figure 3).

Effect of insecticide on the pupation

The average values of nitenpyram and control treatments for effect of insecticides on the pupation were (0.83), (0.83) respectively; hence nitenpyram was considered safe at pupation stage. However, the lambda-cyhalothrin and emamectin benzoate were found toxic at pupation stage due to less pupation rate as compared to control with average values as (0.67) and (0.86) respectively. The analysis of variance for pupation revealed that model was significant with the values; F= 3, df = 10.06, P<0.001 (Figure 4).

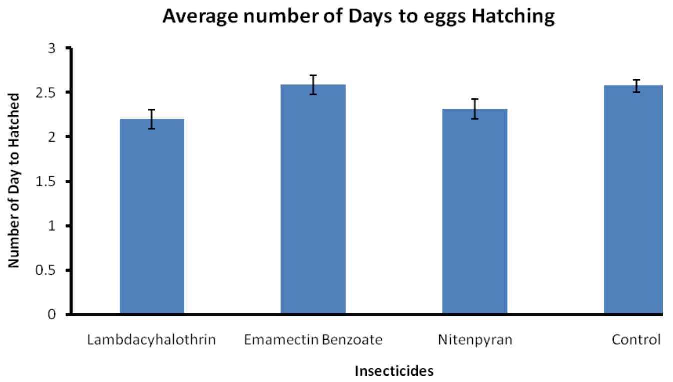

Figure 3: *Average number of day (mean ± SEM) to egg hatching of Apis mellifera in each insecticide application.*

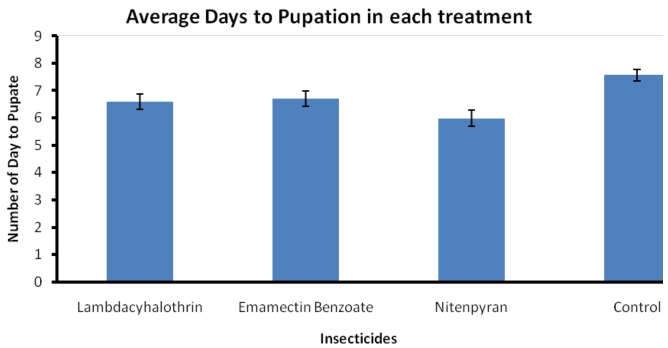

Figure 4: *Average number (mean ± SEM) of pupation of Apis mellifera from larvae to pupae in each insecticide application.*

Effect of insecticide on days to pupation

Results revealed that as compared to control treatment (7.57), nitenpyram was more toxic at this stage with average value (5.98) since the day to pupation found not enough F=3, df = 5.611, p< 0.001. It means that early pupation may causes problem in the normal growth and development of honeybees around their lifespan. However, lambda-cyhalothrin and emamectin benzoate were relatively less toxic for day of pupation with average values of (6.60) and (6.69) respectively (Figure 5).

Effect of insecticide on the emergence

Results revealed that maximum adults were found in the control treatment with average value (0.80) and minimum in nitenpyram (0.59 which means it was more toxic at the emergence stage as compared to control treatment F=3, df = 9.314; p<0.001 (Figure 6). Emamectin benzoate and lambda-cyhalothrin were also found toxic for bees but not as severe as nitenpyram. These were toxic almost at the same rate with average values (0.65) and (0.63) respectively.

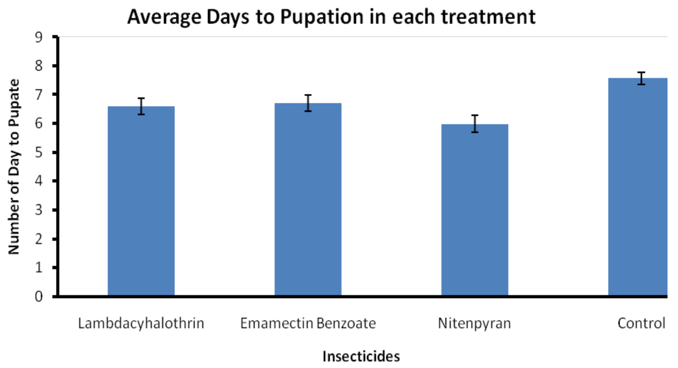

Figure 5: *Average number of days (mean ± SEM) to pupation of Apis mellifera from larvae to pupae in each insecticide application.*

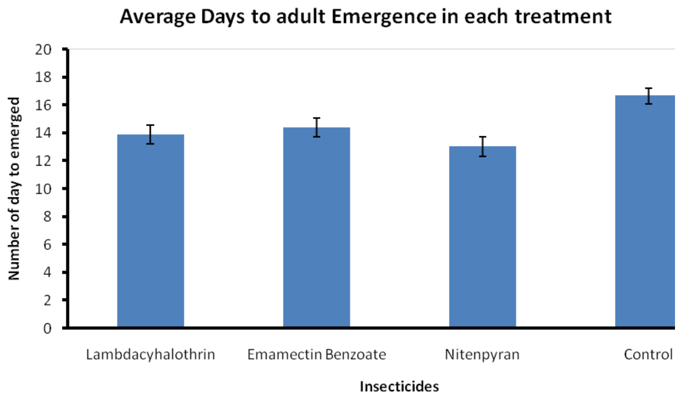

Figure 6: *Average of adult emergence (mean ± SEM) of Apis mellifera in each insecticide application from pupae to adult.*

Effect of insecticide on days to adult emergence

Results depicted that the nitenpyram found with minimum average value of (13.04) and the maximum in control treatment with average value as (16.67). The average value of emamectin benzoate treatment was (14.40) and of lambda-cyhalothrin was (13.90) which mean these were also affecting at this stage F=3, df = 5.487; p<0.001 (Figure 7).

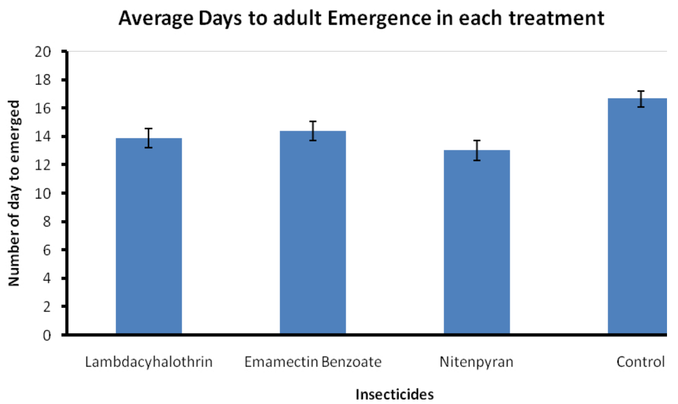

Figure 7: *Average number of days (mean ± SEM) to emergence of Apis mellifera from pupae to adult in each insecticide application.*

Effect of insecticide on number of day to die adult bees

The results depicted that maximum lifespan of bees was found in control treatment with average value as (36.82 days) and the minimum was in nitenpyram with average value as (20.42 days). The average values of emamectin benzoate was 22.17 and with a slight difference the lambda-cyhalothrin average was 20.44 that means the insecticides were toxic to the bees and caused early death, F=3, df= 52.97; p<0.001 (Figure 8).

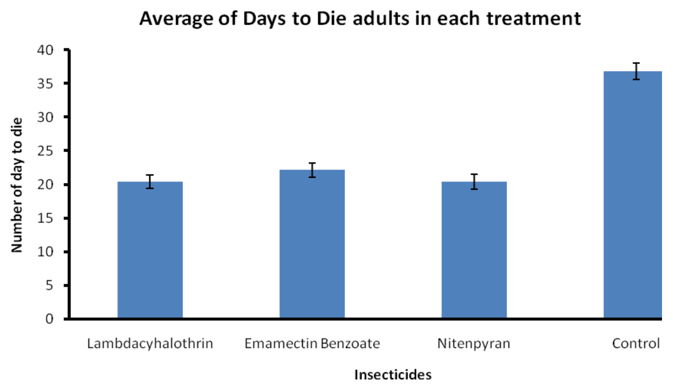

Figure 8: *Average number of days (mean ± SEM) to die of adults of Apis mellifera in each insecticide application.*

One-way ANOVA was performed to observe the effect of chemical compounds on honey bees. The results showed that there is statistically significant difference exists among the means of different parameters of chemical compounds on honey bees such as F < 0.05 alpha level. The detailed results are shown in Table 1.

Since the results are significant, therefore Post-hoc comparisons were performed by Tukey's HSD test for days to pupate, days to emerge and days to die factors in the study. The results from the Tukey's HSD test are shown in Table 2.

The results from the Table 2 show that there was no statistical significant difference among three pairs of Lambda-Cyhalothrin with Emamectin Benzoate (p=0.995), Lambda-Cyhalothrin with Nitenpyram (p=0.389) and Lambda-Cyhalothrin with Control (p=0.388) at "days to pupate stage". However, Control with Nitenpyram (p=0.000) pair of means has shown statistical significant difference at "days to pupate stage". Similarly, at "days to emerge stage" the pairs of Control with Lambda-Cyhalothrin (p=0.017) and Control with Nitenpyram (p=0.001) show statistically significant difference. On the other hand, at "days to die stage" all three pairs such as Control with Lambda-Cyhalothrin (p=0.000), Control with Emamectin Benzoate (p=0.000) and Control with Nitenpyram (p=0.000) show statistically significant difference.

Pesticide-exposed plants exert detrimental effects to the honey bee colonies. The emerged bees suffer more from the chemical compounds (Girolami *et al.*, 2009; Pohorecka *et al.*, 2012). The results identified the effects of lambda-cyhalothrin, nitenpyram and emamectin benzoate on the honey bees. Chemical compounds used in the study showed strong toxicity to the honey bees. We present the effects of chemical compounds on the larval, pupal and the adult lifespan. The results revealed that the chemical compounds were affecting the bees on different stages. The results were in line with (Fairbrother *et al.*, 2014) that the nicotinoid insecticide nitenpyram is hazardous for the honey bees and also in this study found toxic for the number of eggs as compared to the control treatment. The lambda-cyhalothrin has detrimental effects on the lifespan of bees (Liao *et al.*, 2018) and here also lambda-cyhalothrin found most toxic for the egg hatching of honey bees as compared to emamectin benzoate treated however, the emamectin benzoate

Table 1: *Analysis of variance (ANOVA).*

		Sum of squares	df	Mean square	F	Sig.
Days to pupate	Between groups	305.305	3	101.768	5.611	.001
	Within groups	17321.262	955	18.137		
	Total	17626.567	958			
Days to emerge	Between groups	1724.235	3	574.745	5.487	.001
	Within groups	100029.515	955	104.743		
	Total	101753.750	958			
Days to die	Between groups	45477.443	3	15159.148	52.965	.000
	Within groups	273328.680	955	286.208		
	Total	318806.123	958			

Tukey HSD Post Hoc test.

Table 2: *Multiple comparisons.*

Dependent variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Days to Pupate	Lambda-Cyhalothrin	Emamectin Benzoate	-.09583	.388	.995	-1.09	.90
		Nitenpyram	.61674	.389	.388	-.38	1.61
		Control	-.96667	.388	.063	-1.96	.03
	Emamectin Benzoate	Lambda-Cyhalothrin	.09583	.388	.995	-.90	1.09
		Nitenpyram	.71257	.389	.259	-.28	1.71
		Control	-.87083	.388	.113	-1.87	.12
	Nitenpyram	Lambda-Cyhalothrin	-.61674	.389	.388	-1.61	.38
		Emamectin Benzoate	-.71257	.389	.259	-1.71	.28
		Control	-1.58340*	.389	.000	-2.58	-.58
	Control	Lambda-Cyhalothrin	.96667	.388	.063	-.03	1.96
		Emamectin Benzoate	.87083	.388	.113	-.12	1.87
		Nitenpyram	1.58340*	.389	.000	.58	2.58
Days to emerge	Lambda-Cyhalothrin	Emamectin Benzoate	-.50042	.935	.950	-2.90	1.90
		Nitenpyram	.86208	.935	.793	-1.54	3.26
		Control	-2.76292*	.935	.017	-5.16	-.35
	Emamectin Benzoate	Lambda-Cyhalothrin	.50042	.935	.950	-1.90	2.90
		Nitenpyram	1.36250	.934	.463	-1.04	3.76
		Control	-2.26250	.934	.074	-4.66	.14
	Nitenpyram	Lambda-Cyhalothrin	-.86208	.935	.793	-3.26	1.54
		Emamectin Benzoate	-1.36250	.934	.463	-3.76	1.04
		Control	-3.62500*	.934	.001	-6.02	-1.22
	Control	Lambda-Cyhalothrin	2.76292*	.935	.017	.35	5.16
		Emamectin Benzoate	2.26250	.934	.074	-.14	4.66
		Nitenpyram	3.62500*	.934	.001	1.22	6.02
Days to Die	Lambda-Cyhalothrin	Emamectin Benzoate	-1.73750	1.544	.674	-5.71	2.23
		Nitenpyram	.01491	1.545	1.000	-3.96	3.99
		Control	-16.38750*	1.544	.000	-20.36	-12.41
	Emamectin Benzoate	Lambda-Cyhalothrin	1.73750	1.544	.674	-2.23	5.71
		Nitenpyram	1.75241	1.545	.669	-2.22	5.73
		Control	-14.65000*	1.544	.000	-18.62	-10.67
	Nitenpyram	Lambda-Cyhalothrin	-.01491	1.545	1.000	-3.99	3.96
		Emamectin Benzoate	-1.75241	1.545	.669	-5.73	2.22
		Control	-16.40241*	1.545	.000	-20.38	-12.42
	Control	Lambda-Cyhalothrin	16.38750*	1.544	.000	12.41	20.36
		Emamectin Benzoate	14.65000*	1.544	.000	10.67	18.62
		Nitenpyram	16.40241*	1.546	.000	12.42	20.38

**. The mean difference is significant at the 0.05 level.*

was affecting the more on day to egg hatching. The lambda-cyhalothrin and ememectin benzoate are most toxic tested chemical compounds affecting honey bees' (Abdelrazik, 2019) pupation in this study since the number of pupation found less as compared to control treatment. However, the emamectin benzoate is toxic for all stages in different castes of honey bees (Hussain *et al.*, 2014). The day to pupation was also being affected by the nitenpyram. The fact of pesticide exposure affecting the bees' lifespan has been discussed in many studies, and results of this study are consistent with these findings. Lambda-cyhalothrin also is known to exert negative effects on honey bees' health which may lead to mortality (Liao *et al.*, 2018). The lambda-cyhalothrin with sub-lethal effects decreases the lifespan of honey bees (Zhou et al., 2014). The lambda-cyhalothrin and emamectin benzoate were found to be less toxic as compared to nitenpyram for the adult emergence and the day to adult emergence. Because the lambda-cyhalothrin increase mortality in the honey bees (Johnson *et al.*, 2009, 2010), so, here in case of day to die of adult, the lambda-cyhalothrin and emamectin benzoate were found toxic since bees started foraging and exposed to the compounds in the field and got decreasing number of alive bees (Abramson *et al.*, 1999; Chauzat *et al.*, 2006).

On the basis of the findings, we ought to be stated that the potential problems could be reduced by not spraying on the flowering agricultural crops (Tomlin, 2003). The imprudent usage of chemical compounds for controlling various insects- pests causes decline in the pollinators particularly the honey bees and their residues in the flora for bees could be the supplementary mortality ground (Haq and Gardezi, 1983).

Conclusions and Recommendations

It is concluded from the results that the Nitenpyram was significantly affecting the eggs presence and less adult emergence rates in the selected cell in the honey bees. Moreover, Cyhalothrin was also affecting the honey bees at egg hatching and pupation stages and less toxic at day to adult emergence and day to pupation. The data from this study describes the effects of chemical compounds on honey bees and in future beekeepers will be able to avoid the use of chemical compounds for their honey bee colonies to reduce more complex issues.

Following are the few recommendations:

- Further research may be conducted by exposing bees to insecticides treated on citrus crop for foraging and then effects of insecticides will be measured.
- Desired information must be conveyed to beekeepers by organizing training sessions for better honey production in the country.

Novelty Statement

Effect of direct exposure of chemical compounds on honey bees in Pakistan is known however, higher trophic effects are still unknown**.** In this study, bees were exposed to insecticides treated sunflower crop for foraging and then effects of insecticides were measured. Knowledge from this study describes effects of chemical compounds on honey bees and potentially facilitates beekeeping farmers to avoid use of chemical compounds for their colonies to reduce colony problems.

Author's Contribution

Hafiz Khurram Shurjeel conceived the idea of the study and finalized the write up, Muhammad Anjum Aqueel supervised overall research process. Ejaz Ashraf helped in statistical analysis and interpretation. Asad Ali helped in reviewed and corrected the manuscript. Arooba Rubab helped in data collection and final submission.

Conflict of interest

The authors have declared no conflict of interest.

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