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IMPACT OF INSECTICIDES ON FEW COLONY STRENGTH PARAMETERS OF *APIS MELLIFERA* (HYMENOPTERA: APIDAE)

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ABSTRACT

The honeybees (Apis *mellifera* L.) are broadly renowned beneficial organisms with respect to biological, ecological and scientific significance by generating economically important products like royal jelly, pollen, propolis, honey and wax. These critical pollinators are being affected by the chemical compounds used for controlling the insects –pests of agricultural crops. We performed experiments by sowing of sunflower crop at (500 sq. m) field and applied the pesticides early in the morning at flowering stage on crop and the bees were exposed to the pesticides this way. Here we hypothesized that insecticide's exposure affects the colony strength parameters of honey bees. To test this hypothesis we examined the effects of different chemical compounds from different groups (Nitenpyram @10%SL/acre), (Cyhalothrin @200ml/ acre-), (Emamectin benzoate @ 200ml/acre) on the adult bees, guard bees, forager bees, honey production, egg presence, egg hatching, larval duration, pupal period and adult lifespan. The data for the, forager bees was recorded two times a day 11:00 am and 2:00 pm and guard bees were collected once a day at 12:00 pm while he data for honey production was done after one month. The adult bees, guard bees and forager bees were observed dying greatly due to emamectin benzoate as compared to the other insecticides. The honey production in the treated colonies was being affected more by the insecticide cyhalothrin. The nitenpyram and cyhalothrin were more toxic for the egg presence and eggs hatching. The emamectin benzoate and cyhalothrin found toxic for pupal stage of honey bees. The timely adult emergence, new emerging bees and adult longevity were being affected extensively by nitenpyram.

Keywords: Insecticides, Pesticides, Colony strength and Honey bees

INTRODUCTION

Honeybees offer imperative services to the crops and many wild plants mainly contributing in increasing food security and biodiversity (Klein *et al.,* 2007). Recently, all around the world these valuable pollinators are afflicting as compared to increasing pollination demand to deal with the food production (Biesmeijer *et al.,* 2006; Potts *et al.,* 2010a; Cameron *et al.,* 2011). However, the economical condition (Klein *et al.,* 2007) of the world for pollination is facing a lot of intimidation. Of many intimidations, most existing ones are the hazardous chemicals often used in the field for controlling insects pests of various crops. In the agricultural crop pollination, western honey bees (*Apis mellifera*) are considered as ever imperative managed pollinators around the globe (Klein *et al.,* 2007). At present, unfortunately honey bee's population is suffering from severe decline worldwide (vanEngelsdorp and Meixner, 2010).

The detrimental effects of chemical compounds are evidenced and may not be subjected to killing and damage of non-target organisms with lethal doses, but abnormalities in their behavior and functioning induced with sub-lethal doses. The researchers agreed by statement that "as an example of imperative non-target organism, it is super decisive to appraise sub-lethal impacts of pesticides which mainly concerning their behavior and functioning of bees both as individual and colony" (Mayer and Lunden, 1986; Porrini *et al.,* 1996; Stone *et al.,* 1997; Mayer *et al.,* 2001; Decourtye *et al.,* 2004; Desneux *et al.,* 2007; Costa *et al.,* 2014). Honey bees are often performed their foraging tasks and pollen and

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nectar collection for colony survival and exposed to contaminants in field. Sub-lethal doses put detrimental effects on bees functioning, growth and making harder the colony survival (Decourtye *et al.,* 2005; Smirle *et al.,* 1984; Currie 1999). High pesticides doses carnage the forager bees and severely affect their functioning (Atkins *et al.,* 1981; Currie, 1999).

As insecticides residues become conscious through samples of nectar, pollen, plant tissues and soils (Schmuck *et al.,* 2001, Bonmatin *et al.,* 2003), it is supposed that foraging activity of bees may ruthlessly be affected by insecticides deposits collected from treated crops, thereby make colony survival vulnerable. Yang *et al.,* (2008) described that sub lethal doses of imidacloprid significantly affect foraging behavior of honey bees that is may be the setback and delay in their visit to the foraging location and this delay particularly based on imidacloprid concentrations.

Sub-lethal doses of pesticides or insecticides are considered to create disturbance in honeybees' behavior (Haynes K.F, 1988), which further lead to deplorable performance in different in-colony and out-colony tasks and cause population fluctuation disorders in the bee hives (MacKenzie K.E, 1989). Hence, numerous insecticides and pesticides are found toxic and responsible for attenuation the off-spring production in the apiaries (Bariola, 1984; Kwan and Gatehous, 1978).

Various in-vitro, in field or and semi-field conditions studies have been carried out to evaluate the impacts of hazardous chemicals particularly of imidacloprid on the honeybees. The results of most of these studies revealed that the imidacloprid exposure to the honeybees exert negative impact on their health, reducing commemoration, positioning, learning capabilities, affecting hypopharyngeal glands and disrupting in the respiratory functioning of the honey bees (Decourtye *et al.,* 2003; Hatjina *et al.,* 2013).

Four compounds like (fluvalinate, coumaphos, chlorothalonil, and chloropyrifos), with field recommended doses were exposed either singly or in combination significantly increased the mortality at larval stage (Zhu *et al.,* 2014). Some sort of synergistic effects were found in binary mixture however, Ops, Coumaphos and Chlorpyrifos mixture showed the additive toxicity (Zhu *et al.,* 2014).

There is a significant covenant of anxiety over the turn down of honeybees around the globe. On the other hand, insecticides influence the tremendous foraging behavior of honeybees verily. Well known fact about direct exposure of insecticides to the colony as their diet, cause mortality, and weaken the overall colony and brought behavioral changes in bees at low dose. But the higher trophic effect of insecticides on colony strength parameters is still unknown. Ignorance about sub lethal aptitude of conventional lethal feathers like LD_{50} or LC_{50} is resulted irony of constant effect on beneficial and non-target insects. The direct exposure of the chemical compounds to bees possesses serious threat and cause mortality. Hence, the current research study was conducted to evaluate the effects of the insecticides on the colony strength parameters of honey bees.

MATERIALS AND METHODS

Experimental field and insecticidal spray

The present field study was performed at the apiary of College of Agriculture, University of Sargodha, Punjab Pakistan. The sunflower cultivar Hysun-33 was grown in April during summer season. Four plots were prepared with plot size of $500m^2$. The distance between plots was 200 m. Seed bed of the plots were prepared in the spring season on the soil containing pH 6.0 (pH was measured with portable pH meter) by keeping planting depth between 1-3 inches and row to row distance around 30 inches through conventional system consist of chisel plough to invert residues and several other field operations. The field doses of insecticides Table 1) were sprayed by using Knapsack sprayer in the morning at flowering stage of the crop to see their effect on the honeybees. The insecticides were applied exclusively to check the effect on the bees and not for any insect. Each plot was treated with only one insecticide. A control plot was sprayed with water only to rationalize the treatments in all plots. The control plot was maintained at a distance of 200 m from the treated plots. At each plot, three honey bee colonies were placed.

Three colonies of *A. mellifera* L. (8 frames/ colony) were moved from the apiary located at a distance of 2 Km away from the trial site in the evening and placed within the centers of the plots after spraying. The bee colonies were brought from the apiary and placed in the centre of the sunflower plot on the same day when insecticides were sprayed in the evening approximately around 8 hours after spray. The queen right (containing queen in the colony) colonies were used for this experiment.

The bees entering into the colony after forging were considered as foragers and the bees sitting and hovering around the entrance were considered as the guard bees. The colonies were placed almost one foot up the ground or rest of the field level to save it from water, rain and any other pest attack and also placed a thick layered cloth under the lid of each colony for protection during research trail. The bees were released manually from the hive for foraging and other operations for colony survival near the crop field to expose them to the insecticides and to check insecticidal effect on the worker bees (guard, foragers) and honey production.

Honey bees colonies were set aside the field i.e.2km away from the apiary, so that the queens of the original apiary colonies don't get disturbance. Only the selected colonies containing queens (containing queens) for the experiment were shifted from the apiary to the trail site and rest of the colonies (containing workers, drones and queens) were left.

Experimental design

The experimental design was Randomized Complete Block Design (RCBD). There were three replications (three colonies were placed at each plot) and four treatments (3insecticides and 1control) and four experimental units (sunflower plots).

Data collection

Data collection was started after three days of insecticide application on the sunflower crop. The bees moving and staying around the hive entrance were supposed as guard bees while the bees performing other out-colony tasks were considered as worker bees. The data for the guard bees was recorded through visual counting of bees present in alert position or patrolling, intercepting and wondering at colony entrance (Boch & Shearer, 1966) up to 15 minutes at11:00am once a day for one month (Delaplane *et al.,* 2013). The guard bees were counted once a day and the forager bees were measured as indirect measure of colony strength (Grüter *et al.,* 2011) twice a day. The data for forager bees from the queen-right colonies was recorded daily at 8:00 am and 4:00 pm for one month by counting the number of alive worker bees going outside and coming back from foraging tasks and entering into the hive as forager bees (Frazier et al, 2015). The forager bees were counted at the hive entrance by counting manually returning from the field to the hive for 15 minutes. The bees hovering in the field far from the hive were not counted since those may be from another apiary and were foraging there. The guard bees normally don't go far from the hive entrance and mix themselves with the foragers so, could be identified around the entrance. As well as the honey harvesting concerns, thirty days after the insecticides application, the honey was harvested from the frames of treated colonies with the help of honey extractor to check the effect of insecticides on honey production.

Statistical analysis

In order to determine the significance of difference in forager rate of change across 30 days, two linear mixed models were compared through chi-square test using analysis of variance (ANOVA) method in R software. Treatment, day and time were used as fixed factors in both models. Treatment × day interaction was an additional fixed effect in one model, the colony was used as a random effect in both models and number of foragers was dependent variable in both models. The analysis was repeated using number of guard bees instead of number of foragers as dependent variable to determine the treatment effect over time on guard bees present. The package LS means was used for post-hoc comparison of the estimated least-squared means of the significant treatment/day interactions. Method "lstrends" was used to test the significance in the change foraging rate in each treatment across days. P-values for multiple comparisons were adjusted using tukey's method for comparing family of 4 estimates by the method.

RESULTS

Effect of insecticides on number of forager honeybees

The forager honey bees were found maximum in the control treatment with an average value of 385.72 and the minimum foragers were observed in the treatment emamectin benzoate with average value as 340.05 which were more harmful for the forager bees. The number of forager bees in the treatment containing cyhalothrin was found with average of 365.38 and in nitenpyram with average as 373.79.

The below graph illustrates the number of forager bees in each treatment by showing the dots of different colors across the four lines. The amount of forager bees was recorded maximum in the control treatment and the minimum were found in the treatment with Emamectin benzoate. On the other hand, numbers of forager bees in cyhalothrin and Nitenpyram were nearly same but less than control treatment and more than emamectin benzoate. This shows that forager bees were affected by the pesticides exposure since the fluctuation in number of bees was found significant among treatments.

The results of the model were significant at 5% level of significance as $F(3, 716) = 3.493$; > 0.05. The results showed the variation among the means of different treatments (insecticides) significantly, which revealed that bees were being affected by the insecticides.

Effect of insecticides on number of guard honeybees

The results revealed that the maximum numbers of guard honey bees were calculated in the control treatment with average value 33.03 and the minimum number guard bees with average value 10.43 were calculated in the treatment containing Emamectin Benzoate and found more harmful (Hussain *et al.,* 2014). The numbers of guard bees in the cyhalothrin and Nitenpyram treatments were less than that of control with average value as 15.64 however, in Nitenpyram alone observed more than rest of all with average value as 20.26 and hence less toxic as compared to rest of the compounds. However, all the chemical compounds used found effective over the guard bees.

The above table shows the estimates for the effect of each treatment on average number of guard honey bees measured in 15 minute period compared to control treatment. The estimates were given to guard bees per day. The results revealed that there is no significant difference in the treatments as compared to control treatment.

Fig.4 shows the effect of insecticide on guard bees. Results depict that guard bees were suffering from insecticide contamination In this regard, according to the emamectin benzoate was most notorious for the guard bees in the experiment with minimum number of guard bees and hence, in comparison, cyhalothrin found harmful for guard bees. According to the results, the guard bees were being affected by the treatment with Nitenpyram insecticide as compared to control treatment. The maximum number of guard bees was recorded in the control treatment. The fluctuation in the number of guard bees indicated that the pesticides were harmful for the guard bees.

The results depicted that model was significant at 5% significance level. The calculated F value 3, 716 = 1094; $P<0.05$ and the model describes the explained variation among the dependent variables of the study as well. Variation in the model revealed that there is difference between means of all treatments (insecticides) and the guard bees those were affected by insecticides.

Effect of insecticides on honey production by honeybees

The same pesticides were used to evaluate the honey production. Result revealed that the maximum average of the honey production recorded from control treatment with average value as 4.19kg and the minimum honey was produced in the treatment containing cyhalothrin with average value (2.93) which was found to be potentially more toxic for the honey production. However, comparatively with less toxic effects, the average honey production quantity in the treatment nitenpyram was recorded as 3.3kg3 while in the emamectin benzoate the average production of honey was calculated as 3.01kg.

The analysis of variance was calculated and the results revealed that model was insignificant at 5% level as $F(3, 8) = 1.941$; $P > 0.05$. The variation in the dependant factors showed that the bees were being affected by the use of different treatments (insecticides).

Table 1
Insecticides and their field doses

Insecticidal treatments			Dose/acre
Chemical Name	Brand name	Insecticide group	
Nitenpyram 10%SL	Capstar	Neonicotinoids	15-75ml/Acre
Lambdacyhalothrin2.5%EC	Nok-out	Pyrethroid	200 ml/ Acre
Emamectin benzoate1%EC	Proclaim	New Chemistry	0.34 ml/ Acre
Water	Control	-	

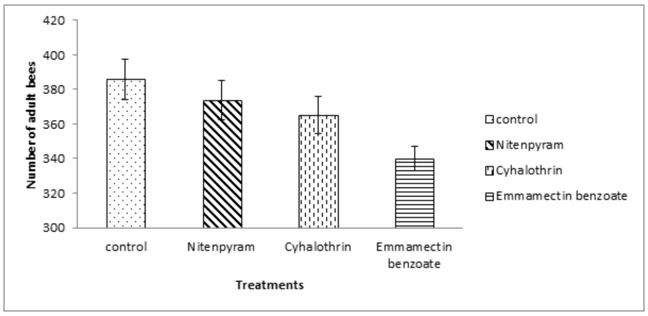

Fig 1.
Adult forager honey bees (Mean ± SE) mean followed by same letters is not significantly different for each temperature regime (Tuckey HSD, P≤0.05)

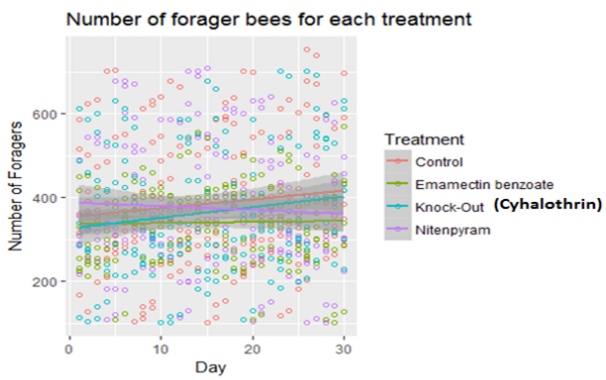

Fig 2.
Number of forager honey bees across 30 days for each treatment. Samples are indicated by circles. Linear trends for each treatment are also shown for comparison.

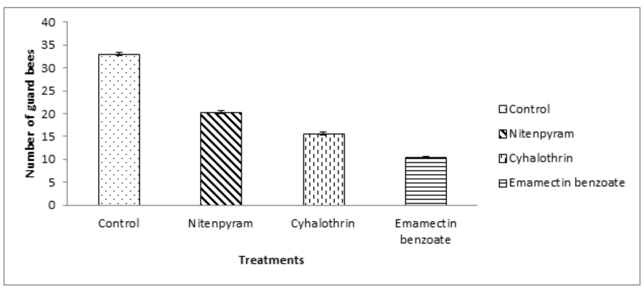

Fig 3.
Mean number of guard bees (Mean ± SE) mean followed by same letters is not significantly different for each temperature regime (Tuckey HSD, P≤0.05).

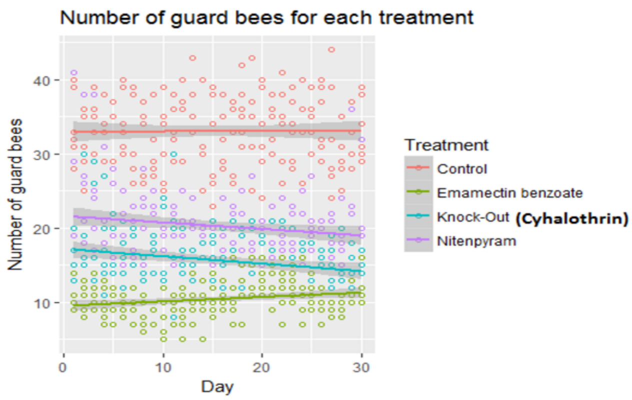

Fig 4.
Number of guard honey bees observed across 30 days for each treatment. Samples are indicated by circles. Linear trends for each treatment are also shown for comparison.

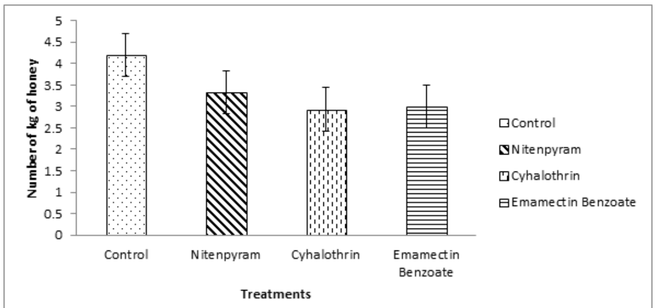

Fig 5.
Mean honey production (Mean ± SE) mean followed by same letters is not significantly different for each temperature regime (Tuckey HSD, P≤0.05).

Table 1
Analysis of variance for number of adult bees

Model	Df	SS	Mean Sq	F	Pr(>F)	*F crit*
Treatment	3	202151	67384	3.493	<.001	2.617342
Residuals	716	13811661	19290			

Table 2.
Estimates for effect of each treatment on average number of guard honey bees measured in a 15 minute period compared to control treatment. Estimates are in guard bees per day.

Treatment	Effect in forager bees per day (SE)
Emamectin Benzoate	0.056 (0.047)
Cyhalothrin	-0.102 (0.047)
Nitenpyram	-0.091 (0.047)

Table 3.
Analysis of variance for number of guard bees under different insecticides.

	Df	SS	MS	F	Pr(>F)	F crit
Treatment	3	50459	16820	1094	<.001	2.617342
Residuals	716	11005	15			

Table 4
Analysis of variance for honey production under different insecticides treatments.

	Df	SS	MS	F	Pr(>F)	F crit
Treatment	3	3.006	1.0019	1.941	0.202	4.066
Residuals	8	4.13	0.5163			

DISCUSSION

The susceptibility of honey bees to pesticides exposure is established on every caste and age. The adult bees could get exposed during the foraging activities and younger ones may get exposed in the hive from the contaminated nectar and pollen or pesticides applied in hive to control the *Varroa destructor* mites. In this study, the bees were exposed to different chemical compounds (emamectin benzoate, cyhalothrin, nitenpyram) to check the effect on forager bees, guard bees and honey production. The pesticides used were found to be affecting the forager and guard bees and contributing in the less honey production by the bees as well. However, statistically we did not find any significant difference during 30-days period of exposure to the different chemical compounds among three insecticides.

Our findings suggest that the pesticides are toxic for honey bees due to the constant decrease in the number of forager and guard bees. Under the emamectin benzoate, the number of forager bees and guard bees were computed less and hence, found more toxic. The treatments containing cyhalothrin and nitenpyram were also found toxic for the forager and guard bees but at slow rate as compared to the emamectin benzoate. Our findings are in line with that of Hussain *et al.,* (2014) regarding emamectin benzoate insecticide. In case of honey production, the cyhalothrin was found having most detrimental effects by the bees as compared to rest of the treatments. Though, there was no significant difference between treatments in average amount of honey produced. We found the significant difference guard bee models. The post-hock test shows the significant difference between Emamectin benzoate & cyhalothrin and Emamectin benzoate & Nitenpyram. However there was no significant difference between the control treatment and other treatments. On the basis of our findings we ought to be stated that all the pesticides in the experiment were harmful for both the forager and the guard bees and honey production as well.

Authors' contributions

HKS and MAA designed the study and wrote the manuscript with input from all authors. AMR and EA analyzed the data. MA gave their suggestions for improvement in the manuscript. All authors read and approved the final manuscript.

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