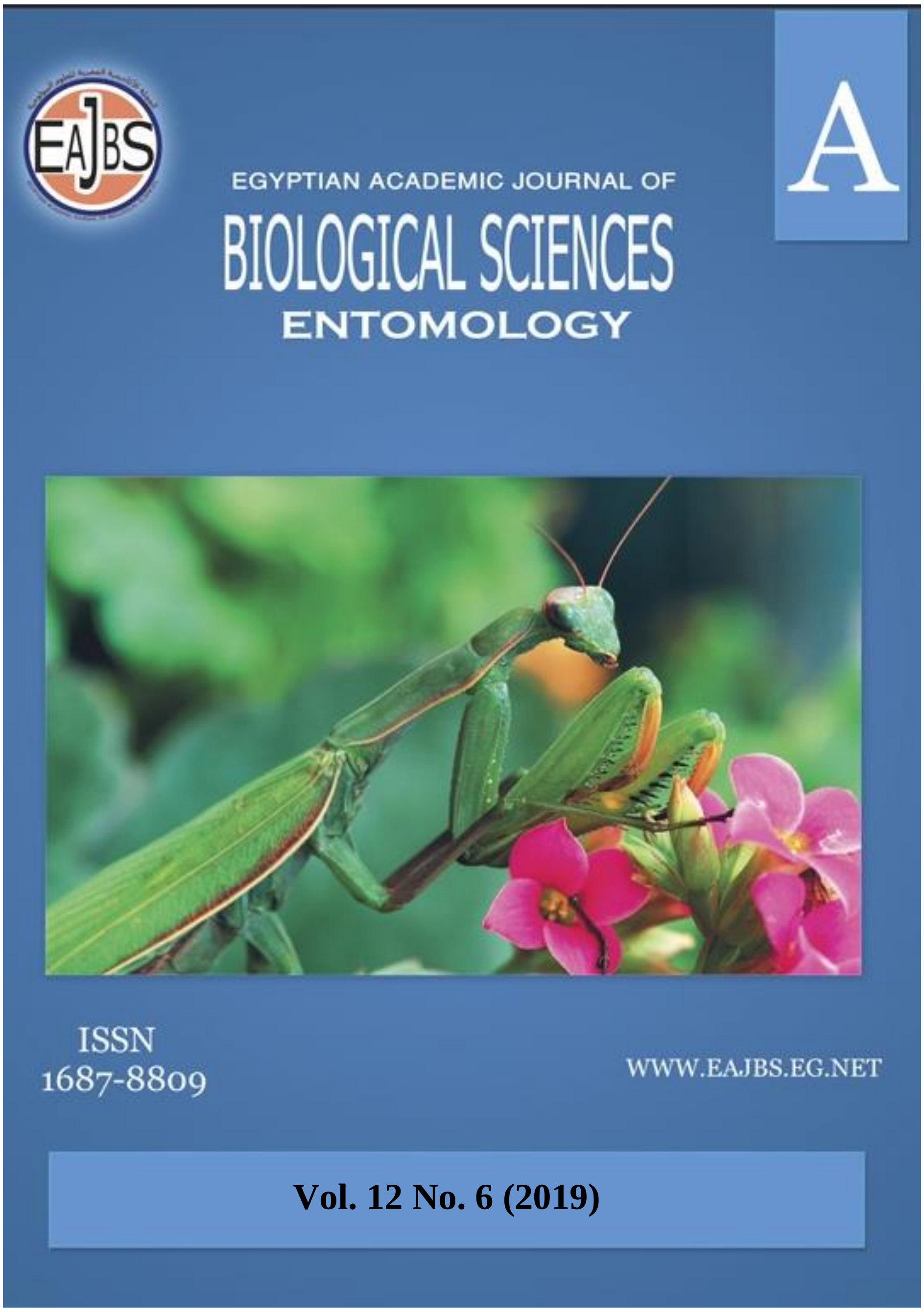

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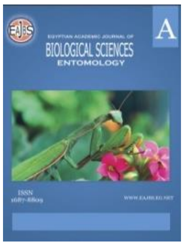

Testing Efficacy of Selected Insecticides against *Spodoptera litura* (Lepidoptera: Noctuidae) in Fodder Crops and Effects on Beneficial Insects

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ABSTRACT

The armyworm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) is an economically important insect pest of vegetables, ornamentals and other agricultural crops. Synthetic insecticides have remained primary means for the management of *S. litura* than any other management tool. We tested the effectiveness of some insecticides against different larval instars of *S. litura* in laboratory as well as in field on the prevailing instars in two fodder crops lucern/alfalfa, *Medicago sativa* L. and jantar/sesbania, *Sesbania sesban* L. In the laboratory, emamectin benzoate provided the most control 69-91% followed by lufenuron 70-85% of all larval instars after 48 hours of application. In the lucern field, emamectin benzoate reduced the larval population of *S. litura* up to 79.32% and with the application of lufenuron 73.55% reduction occurred. Similarly, in jantar crop, lufenuron and emamectin benzoate proved to be more toxic against *S. litura* population. The abundance of ladybird beetle, *Coccinella septempunctata* L. (Coleoptera; Coccinellidae), honeybee, *Apis mellifera* L. (Hymenoptera; Apidae) and green lacewing, *Chrysoperla carnea* Stephen (Neuroptera; Chrysopidae) were found higher in emamectin and lufenuron treated plots compared to methoxyfenozide and chlorpyrifos. These results indicate that new chemistry insecticides; emamectin benzoate and lufenuron have a potential for the management of *S. litura* and are relatively safer for beneficial insects as compared to conventional insecticides, methoxyfenozide, and chlorpyrifos.

INTRODUCTION

Armyworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), a generalist herbivore is one of the most important agricultural insect pests. It has the potential to cause losses up to 100% during flowering to the premature fruiting stage of various agricultural crops (Qin *et al.*, 2004). Immature larvae mainly feed on the epidermal leaf tissue and make holes in leaves, a distinctive damage symptom of armyworm (Sarwar, 2014). It has a wide host range of more than 100 plant species (Xue *et al*., 2010). It is a polyphagous insect pest and is found in several countries such as China, Brazil, Argentina, USA, and Pakistan (Wan *et al*., 2008), causing economic losses in various crops such as soybean, *Glycine max* L. Merrill, maize, *Zea mays* L., cotton, *Gossypium hirsutum* L., barseem, *Trifolium alexandrinum* L., and beans, *Phaseolus vulgaris* L., (Sharma *et al*., 2010; Nagoshi *et al*., 2010; Ahirwar *et al*., 2015). The presence of diverse host plant species within the migration range of this pest plays a major role in its outbreak and makes management more complex (Liu *et al*., 2004). In the last decade, a heavy infestation of *S. litura* is noticed on the fodder crops in Pakistan (Ahmad *et al*., 2013).

Alfalfa (locally known as lucern), *Medicago sativa* L., a perennial herbaceous legume with high nutritional quality, is considered one of the most important legumes worldwide that serves as a major source of protein for the livestock (Martí *et al*., 2009; Radovic *et al*., 2009). *Spodoptera litura* severely affects this crop by feeding on tender leaves and shoots. As a result of its chewing nature, rapid dehydration of plants occurs resulting in brown patches. Another short durational fodder crop called jantar (*Sesbania sesban* L.) is also cultivated in most parts of Pakistan (Mahmood *et al*., 2008). *S. litura* also damages this crop up to maturity and decreases the quality and quantity by feeding on the leaflets and plant is left only with sticks in the field (Ahmad *et al*., 2013).

The primary management strategy of this pest in Pakistan is the use of synthetic insecticides. More than 34 insecticides belonging to different groups are being applied alone or in combination to control lepidopteron pests including *Spodoptera* spp., (Mushtaq *et al*., 2008). Due to the extensive use of insecticides, it has developed resistance (Ahmad and Arif, 2010; Abbas *et al*., 2014) and the chemical control has become useless during its heavy outbreak (Shad *et al*., 2012; Tariq *et al*., 2007; Mushtaq *et al*., 2008). Besides managing *S. litura*, pesticides also pose a threat to natural enemies in the field by inhibiting their life span; affecting the fertility rate (Cloyd and Bethke, 2011; Desneux *et al*., 2007) and their movement behavior in searching prey and reduction in the sex ratio (Cloyd, 2006; Chandrayudu, 2014). This suggests the need to evaluate the effective insecticides for the sustainable control of *S. litura* with no harmful effects on the beneficial insects in the field.

Insect growth regulators (IGRs) are more appropriate due to their selectivity, compatibility~~,~~ and safety to the environment (Tunaz and Uygun, 2004) and could play an important role in the pest control. The objective of this study, therefore, is to evaluate some selected insecticides having the potential to control *S. litura* under laboratory conditions and in fodder crops and to determine their effect on beneficial insects.

MATERIALS AND METHODS

Test Insect:

The egg batches of *S. litura* were collected from unsprayed lucern and jantar field at Sargodha district (E, 32.0740° N, 72.6861°: E, 32.1340° N, 72.6868°) and brought to the Entomology Laboratory, University of Sargodha. For a homogeneous population, field-collected larvae were reared on a wheat-based semi-synthetic diet at 25±5°C temperature and 65±5% relative humidity with 14:10 hours light: dark photoperiod (Ahmad *et al*., 2005). Diet was replaced on a daily basis, and pupae were collected on alternate days. Newly emerged adults were kept in Perspex oviposition cages (40 × 40 × 40 cm) with two sides sealed with muslin for the purpose of ventilation and fed on the water-honey solution (9:1) presented on a soaked cotton wool ball. *S. litura* population were reared in the laboratory for the 3rd generation to obtain a susceptible population of insects for bioassays.

Insecticides:

Commercial formulation of four insecticides; chlorpyrifos, 750ml/acre (lorsban® 40EC, Arysta Life Sciences), lufenuron 200ml/acre (match® 5EC, Syngenta Pakistan), emamectin benzoate 200ml/acre (proclaim1.9EC, Syngenta Pakistan) and methoxyfenozide 90-100ml/acre (runner® 24SC, Dow Agro Sciences) were tested against *S. litura* in both laboratory and field conditions.

Laboratory Bioassay:

Unsprayed leaves from lucern and jantar were collected from the research area and washed with distilled water to remove contamination and dust particles. The leaves were immersed in a test solution for 15 seconds with slow agitation and allowed to dry at room temperature. Test solutions of insecticides were freshly prepared in distilled water. Leaves were immersed in distilled water comprised of the control treatment. The leaves were then placed in individual Petri plates containing moistened filter paper in order to avoid desiccation. Each treatment was replicated three times including control. Fifteen 3rd, 4th and 5th instar larvae were exposed to each insecticide in each replication and thus the total number of tested larvae per treatment for each larval stage was 45. The bioassays were kept at 25±5 °C temperature and 65±5% relative humidity with a 14:10 h (L:D) photoperiod. Mortality was assessed at 24 and 48 hours of exposure to insecticides (Khan *et al*., 2011). Percent corrected mortality was calculated by the following formula (Abbott, 1925);

$$\text{Percent corrected mortality} = 1 - \frac{\text{n in T after application}}{\text{n in Co after treatment}} \times 100$$

Where, n = number of insects, T = treatment and Co = control.

Field Application:

The experiment was conducted in the research area of University of Sargodha. Lucern and jantar crops were grown on a plot size of 675.25 ft^2. Each crop was sown in 5 plots with a plot-plot distance of 3 ft. Each plot was further divided into 3 blocks and a randomized complete block design (RCBD) with 3 replications was performed for the experiment. The population of *S. litura* larvae was monitored daily. The selected insecticides were sprayed at field recommended doses using knapsack sprayer. The application of water was done in control treatment. A total 15 plants (5 from each block) were selected randomly from each plot. The selected plants were examined from the upper, middle and lower parts for larval counts of *S. litura*. A total number of larvae were counted before and after 24, 72 and 168 hours of insecticides application. Percent reduction of larvae in the field was calculated by the following formula; (Singh and Kumar, 2015)

$$\text{Percent reduction} = \frac{\text{No. of larvae after application} - \text{No. of larvae before application}}{\text{No. of larvae before application}} \times 100$$

Effect of Insecticidal Application on Beneficial Insects:

To assess the non-target effect of insecticides on the beneficial insects, an abundance of coccinellids, honeybees, and *Chrysoperla carnea* were recorded from the experimental field before and after the application. Similarly, a total of 15 randomly selected plants were counted for the abundance of these beneficial insects. The immature and adult population of *C. carnea* and coccinellids was recorded and visiting honeybees were counted. After the insecticidal application, the abundance data were recorded at 24 hrs interval for one week.

Statistical Analysis:

Data for percent larval mortality, percent reduction and abundance of beneficial insects were analyzed using one-way analysis of variance (ANOVA) to check the significance of treatment/insecticides. Means were separated by the least significant difference (LSD) test at 5% level of significance. All the analyses were performed using SPSS 20.0 software.

RESULTS

Laboratory Bioassay of Insecticides against *S. litura*:

The insecticides showed a significant effect ($P < 0.001$) on all larvae instars after 24 and 48 hrs of application. Percent mortality of 3rd instar larvae of *S. litura* ranged from 69.3-91.4% after application of emamectin benzoate, while, lufenuron controlled 70.0-85.1% larvae at 48 h of application. Chlorpyrifos and methoxyfenozide were found least effective against all larval instars of *S. litura* (Table 1).

Table (1): Effect of different insecticides on the percent mortality (mean±SE) of various larval instars of *Spodoptera litura* under laboratory conditions

Insecticides	3rd instar		4th instar		5th instar	
	24 HAA*	48 HAA	24 HAA	48 HAA	24 HAA	48 HAA
Lufenuron	59.03±2.89b	85.15±2.75b	64.34±2.77b	76.56±2.71b	63.53±2.88a	71.53±2.52a
Emamectin benzoate	68.33±3.54a	91.41±3.58a	77.36±3.98a	89.47±3.59a	61.75±3.59a	69.69±3.23a
Methoxyfenozide	14.86±1.45c	37.06±1.25d	10.29±1.21d	17.19±1.13d	29.22±1.21b	38.33±2.11b
Chlorpyrifos	16.97±1.75c	45.03±1.38c	26.42±1.63c	33.12±1.79c	27.84±2.13b	31.11±1.15c
Control	0.0000 d	0.0000e	0.0000e	0.0000e	0.0000c	0.0000d
F-value	*10.33*	*8.16*	*7.45*	*9.27*	*9.13*	*7.72*
P-value	*0.0000*	*0.0008*	*0.0001*	*0.0000*	*0.0001*	*0.0002*

*HAA=Hours after application, means sharing similar letters within a column are not significantly different at P > 0.05

Field Evaluation of Insecticide against *Spodoptera litura*:

Among insecticides, a significant difference was found for the reduction of *S. litura* larvae in lucern crop after 24 hr (F = 171.0, $P < 0.001$), 72 hr (F = 102.0, $P < 0.001$) and 168 hr (F = 189.0, $P < 0.001$) of exposure. The larval reduction was higher (79.32%) with the application of emamectin benzoate followed by lufenuron (73.55%) after 168 hrs of application in lucern crop. While minimum reduction was recorded (43.5-56.3%) with the application of chlorpyrifos and methoxyfenozide (Fig. 1).

Similarly, insecticides significantly reduced the S. litura in Jantar crop after 24 h (F = 87.9, $P < 0.001$), 72 hr (F = 204.0, $P < 0.001$) and 168 hr (F = 326.0, $P < 0.001$) of application. Lufenuron showed higher reduction (87.24%) of *S. litura* larval population followed by emamectin benzoate (84.25%) after 168 hr of application. Chlorpyrifos and methoxyfenozide were found least effective against *S. litura* causing 58.24% and 43.59% of larval reduction respectively (Fig. 2).

Effect of Insecticides on Beneficial Insects:

A significant effect of insecticides was found on the abundance of beneficial insects; coccinellids ($F = 63.43$, $P < 0.001$), honeybee ($F = 79.08$, $P < 0.001$), and *Chrysoperla carnea* ($F = 47.41$, $P < 0.05$) in lucern crop. In control treatment, the highest number of coccinellids (3.18 number/plant), honeybees (2.48 numbers/plant) and *C. carnea* (2.83 number/plant) visited the field. Contrarily, with the application of emamectin benzoate; 2.74 number/plant of coccinellids, 2.04 number/plant honeybees, and 2.39 number/plant of *C. carnea* was recorded. A similar number of beneficial insects were recorded after the lufenuron application. While the population of these beneficial insects was found lowest; 1.31 number/plant of coccinellids, 0.61 number/plant honeybee and 0.72 number/plant *C. carnea* in chlorpyrifos and 1.27 number/plant coccinellids,0.3 number /plant honeybees and 0.92 number/plant *C. carnea* with the application of methoxyfenozide compared to the control (Fig. 3).

In jantar crop, insecticidal application also significantly affects the abundance of coccinellids (F = 59.13, $P < 0.001$), honeybees (F = 45.21, $P < 0.001$) and *C. carnea* (F =

58.31, $P < 0.001$). The population of all three beneficial insects was found higher (2.39-3.02 number/plant) in control treatment. However, the trend in abundance of the beneficial insects was found; 1.86-2.63 number/plant after application of emamectin benzoate followed by 1.61-2.38 number/plant by lufenuron. The least abundance of beneficial insects was found after the application of chlorpyrifos and methoxyfenozide insecticides in jantar crop (Fig. 4).

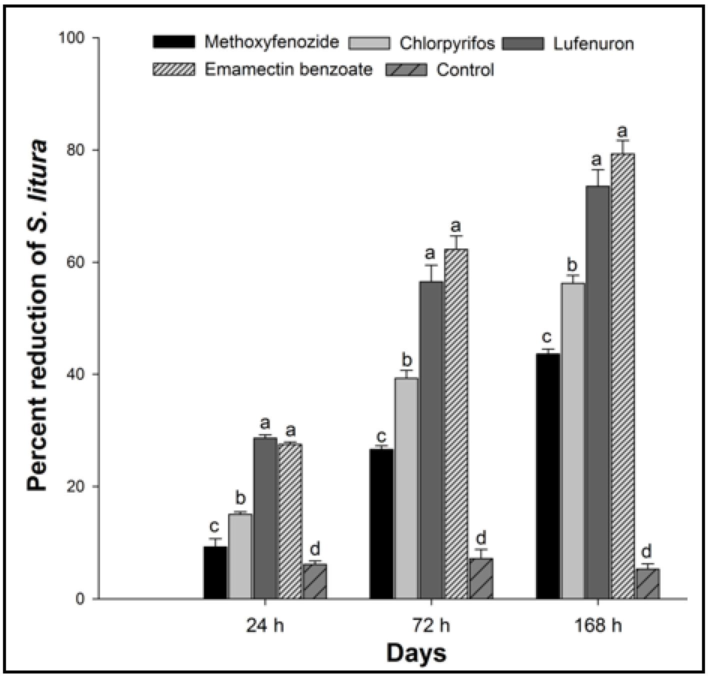

Fig. (1): Percent reduction (means± SE) of *Spodoptera litura* after application of insecticides in lucern crop at different time of exposure, means sharing similar letters for each time interval are not significantly different at P > 0.05

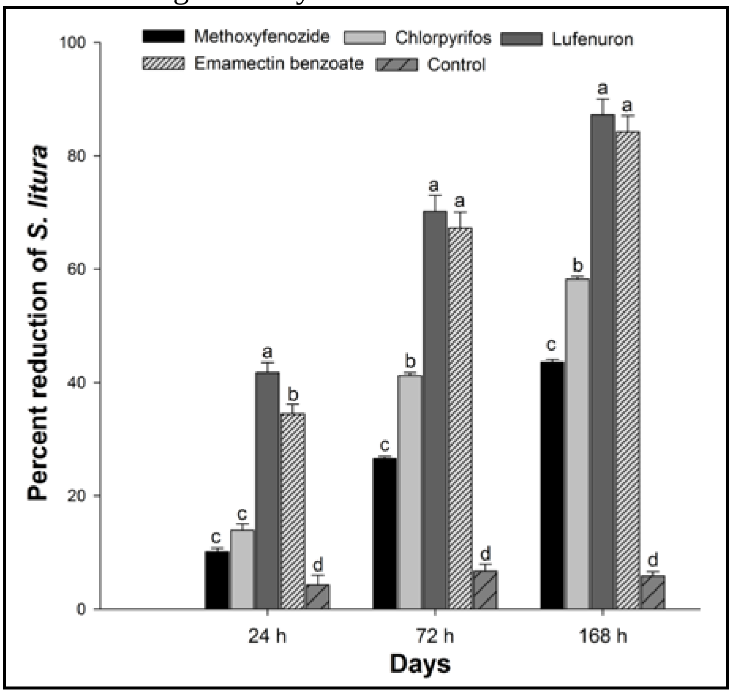

Fig. (2): Percent reduction (means± SE) of *Spodoptera litura* larvae after application of insecticides in jantar crop at different time of exposure, means sharing similar letters for each time interval are not significantly different at P > 0.05

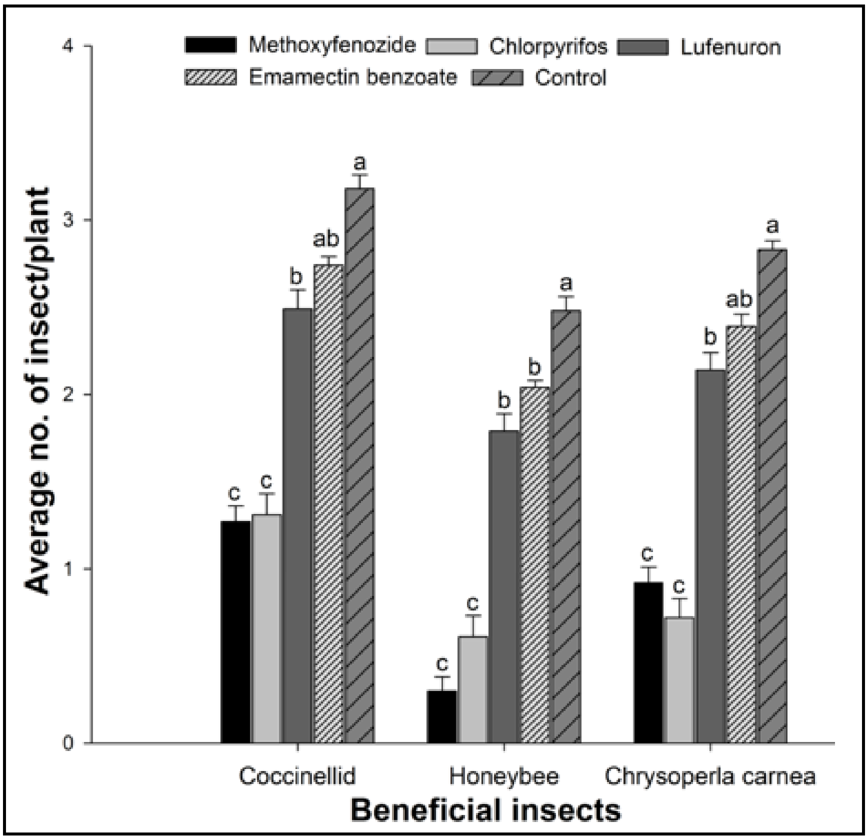

Fig. (3): Effect of insecticidal application on the abundance (means ±SE) of beneficial insects in lucern crop, means sharing similar letters for each insect are not significantly different at P > 0.05

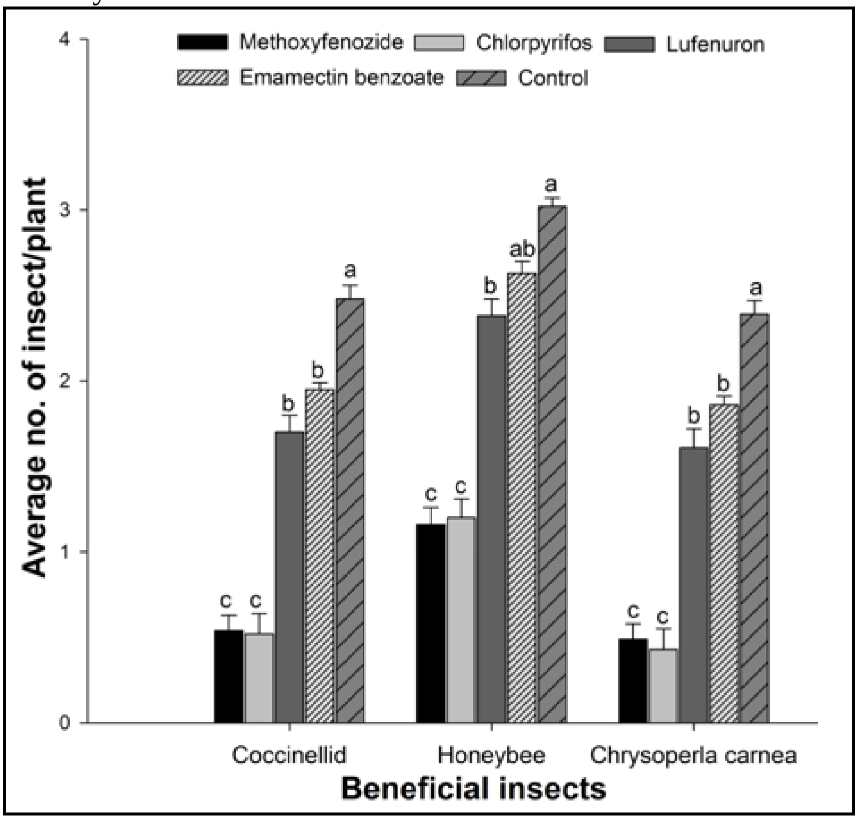

Fig. (4): Effect of insecticidal application on the abundance (means ±SE) of beneficial insects in jantar crop, means sharing similar letters for each insect are not significantly different at P > 0.05

DISCUSSION

The use of integrated pest management (IPM) protocols to minimize the pest infestations is among the effective pest management programs. We tested the efficacy of new insecticides emamectin benzoate and lufenuron in comparison to conventional insecticide, chlorpyrifos, and methoxy-fenozide to show the most effective insecticide against *S. litura*. The IGRs; emamectin benzoate and lufenuron were more toxic against all larval instars of *S. litura* in the laboratory study. Comparatively, emamectin benzoate was more toxic to 3rd and 4th instars larvae and lufenuron showed greater mortality of 5th instar larvae. Our results are in accordance to Saeed *et al*. (2012) who reported that emamectin benzoate was more toxic against 2nd instar larvae of *S. exigua*, and lufenuron was more effective in killing 2nd and 4th instar larvae of *S. littoralis.*

Under the field condition, both IGRs also performed well in suppressing the *S. litura* population in both fodder crops. The larval population was recorded minimum in the plots where emamectin benzoate and lufenuron was applied in both fodder crops. In most of the region of Pakistan, emamectin benzoate is still considered as an effective tool for the management of *S. litura*. According to Ishtiaq *et al*. (2012), populations of *S. litura* is more susceptible to emamectin benzoate and lufenuron as compared to pyrethroid and organophosphorus insecticides. The effectiveness of emamectin benzoate was studied on different insect species and indicated high toxic effects to kill a wide range of lepidopterans (Firake and Pande, 2009; Argentine *et al*., 2002). Razaq *et al*. (2005) and Talikoti *et al*. (2009) reported that IGRs; emamectin benzoate and lufenuron yielded maximum mortality of lepidopterous insects by foliar application under the field conditions. IGRs are insecticides that mimic hormones in young insects and disrupt the growth and reproduction, egg-hatching and molting process in insects (Horowitz and Ishaaya, 2004). In both laboratory and field studies, the least effective chemicals against *S. litura* were chlorpyrifos and methoxyfenozide and the results are similar to the findings of Kitturmath (2007) who reported that chlorpyrifos and methoxyfenozide as a stomach poison caused less mortality of *S. litura* after the foliar spray. The less effectiveness could be due to either resistance of this pest (Gunning and Balfe, 2002; Chen *et al*., 2008) or other factors including the preponderance of ingestion versus contact mode of uptake, slow cuticular penetration, and its unique mode of action at GABA and nicotinic acetyl-choline receptors (Islam *et al*., 2015).

However, effective this emamectin benzoate and lufenuron in killing *S. litura* larvae, determination of their non-target effect on the beneficial insects in the field are also important. We also recorded the abundance of beneficial insects in the field after insecticidal application. The population of coccinellids, honeybees and *C. carnea* was found higher in the plots where emamectin benzoate and lufenuron were applied compared to chlorpyrifos and methoxyfenozide. In comparison to control plot of lucern field, emamectin benzoate and lufenuron reduced 13.5-28.0% population of beneficial insects, while 58.8-88.0% population was reduced with the application of chlorpyrifos and methoxyfenozide. Similarly, in jantar crop, 12.5-33.0% reduction of beneficial insects was found after emamectin benzoate and lufenuron application compared to control treatment. While 60.0-83.0% population reduction of visiting beneficial insects was found with the application of chlorpyrifos and methoxyfenozide. These findings indicate that the emamectin benzoate and lufenuron are comparatively safer to beneficial insects. Udikeri *et al*. (2004) reported that the emamectin benzoate and lufenuron are safer for natural enemies and pollinators. Thompson *et al*. (2000) reported that chlorpyrifos, spinosad, and methoxyfenozide are highly toxic to the *C. carnea*, *C. septumpunctata* including other beneficial arthropods. So, these natural enemies avoided the plots treated with such conventional insecticides (Rimoldi *et al*., 2012; Fernandes *et al*., 2016). Using emamectin benzoate and lufenuron in *S. litura* control will likely maintain the plant free from damage due to the compatibility of theses insecticides with other control techniques like biological control. In addition to mortality, evaluation of the effect of an insecticide on beneficial insects together with information about the residual activity of insecticides on the surface of the plant under field conditions is important for designing a management strategy for insect pests (Carmo *et al*., 2010).

CONCLUSION

Our study suggests that emamectin benzoate and lufenuron have potential to suppress the population of *S. litura* and are safer to the beneficial insects, thus these insecticides could be considered in integrated pest management program of this notorious pest. An important focus for future research will be to better understand the effect of these insecticides against lepidopterous insects in combination/ rotation with biological control agents; the two major tools in controlling plant pest species.

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