Punjab University Journal of Zoology

35(2): 167-172 (2020)
https://dx.doi.org/10.17582/journal.pujz/2020.35.1.167.172

Research Article

Mortality Responses of *Helicoverpa armigera* (Lepidoptera: Noctuidae) against Different Insecticides under Laboratory Conditions

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Article History
Received: April 08,2020
Revised: May 6, 2020
Accepted: June 27, 2020
Published: July 14, 2020

Authors' Contributions
MUA designed, conducted research, recorded data, drafted manuscript and analyzed the data. SHS executed the experiment, collected data and wrote the manuscript. RM finalize experimental plan, supervised the project and proof read the manuscript. SAS helped in execution of experiment and data collection.

Keywords
Amercian bollworm, Comparison, Gram, Insecticides, Mortality

Abstract | Toxicity of five different insecticides viz., Uniron 10 EC, Bio Care TM 16000 IU/mg, Voliam Flexi 300 SC, Neomed 3 SC and Coragen 20 SC was recorded against 3rd instar larvae of *Helicoverpa armigera* under laboratory conditions at their low, recommended and high doses by leaf dip method. The results concluded that significantly high toxicity was shown after treatment with Neomed 3 SC which caused 100 % to the insect at its recommended (200 ml) and high dose (400 ml) after 24 hours of application followed by Voliam Flexi 300 SC causing 66.66 % and 75% mortality on its recommended (80 ml) and high dose (160 ml), respectively. After 72 hours, 83.33% mortality was observed in Voliam Flexi on its recommended dose whereas percent mortality was reached to 100% on its high dose. Uniron 10 EC was also found effective and statistically at par with Voliam Flexi 300 SC causing 100 % mortality at its recommended dose after 96 hours of exposure. However, Bio Care^{TM} 16000 IU/mg consisting of *Bacillus thuringiensis* was least effective at its recommended dose with percent mortality of 25% and 41.66% after 72 and 96 hours, respectively. It is thus concluded that Neomed 3 SC is the most effective insecticide against 3rd instar larvae of *H. armigera* as compared to other tested insecticides.

Novelty Statement | This study reported the efficacy of newer synthetic insecticides in comparison with biopesticide containing *Bacillus thruingiensis* against *Helicoverpa armigera*.

To cite this article: Asif, M.U., Soomro, S.H., Muhammad, R., Soomro, S.A., 2020. Mortality responses of *Helicoverpa armigera* (Lepidoptera: Noctuidae) against different insecticides under laboratory conditions. *Punjab Univ. J. Zool.*, **35**(2): 167-172. https://dx.doi.org/10.17582/journal.pujz/2020.35.1.167.172

Introduction

The genus *Helicoverpa* in the family Noctuidae consists of many significantly important pest species including *H. zea*, *H. assulta*, *H. armigera* and *H. punctigera* (Behere *et al*., 2007). Annual economic damage to crops due to the feeding of *H. armigera* is estimated to be in millions of dollars (Tabashnik *et al*., 2013; Smith-Pardo, 2014). This pest feeds on more than 180 plant species belonging to 45 different families including fibrous and food crops (Rauf *et al*., 2019). Successive generations due to high productiveness in addition to strong migratory potential, facultative diapause, resistance development phenomenon against insecticides enabled *H. armigera* to become most threatening and cosmopolitan pest of agriculture (Ahmad *et al*., 2004; Wakil *et al*., 2012).

Use of insecticides is the most efficient method of insect pest management all over the world. Worldwide usage of pesticide has increased manifold since the 1960s (Sheikh *et al*., 2011). In Pakistan, the dependence on pesticides is very much apparent from its fast growing trend of utilization,

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i.e. the import of pesticides increased from 13,030 tons in 1990 to 36,180 tons in 2011 (GOP, 2012).

Economic importance of crop yield compelled the growers to support the repeated usage of insecticides on alternate days, occasionally almost double the recommended doses (Babar *et al*., 2016). Now a day, synthetic chemicals like pesticides are being consumed in huge amount for the management of insect pest as compared to past (Rauf *et al*., 2019). This non-judicious and indiscriminate consumption has resulted into resistance development in numerous insect pests especially in *H. armigera* (Hamed *et al*., 2006).

In an effort to alleviate insecticide resistance, various tactics have been proposed and evaluated in the field and these novel techniques must be substantiated with pest control and economic data to satisfy growers. Key elements of resistance management include minimizing pesticide use, avoiding tank mixes, avoiding persistent chemicals, and using long-term rotations of pesticide from different chemical classes (Gressel *et al*., 1996; Bethke, 2010). With such strategies, resistance may be delayed or reduced by conserving adequate population of susceptible individuals through the usage of available pesticides at lower rates and avoidance in the selection of recessively resistant heterozygotes. Contrarily, use of pesticide at high dose is also suggested but only for reducing or eliminating occurrence of heterozygotes where resistance is dominant (Helps *et al*., 2017). Numerous studies have been carried out in this aspect with rodenticides and fungicides, while some were conducted against insects for resistance management by high/low dose strategy (Denholm and Rowland, 1992).

So far, this strategy has not been evaluated on the resistant population of *H. armigera* collected from field. The present studies report the comparative efficacy of different insecticides at low and high recommended field dose rate against *H. armigera*.

Materials and Methods

Insects rearing

Experiments were performed in maintained laboratory conditions at 25 $\pm$ 2^0 C, 60 $\pm$ 5% RH and 14-10 h (L:D) photoperiod. The larvae collected from chickpea crop grown at experimental farm of Nuclear Institute of Agriculture, Tandojam were brought to the laboratory and kept singly in the petri dishes (9 cm x 1.5 cm). Fresh leaves of gram were provided daily until pupation in clean petri dishes to avoid microbial contamination. Pupae were transferred to adult rearing jars (17 cm x 9 cm) for emergence. The adults on emergence were paired in the jars and covered with muslin cloth for mating and egg laying. The adults were supplied with 10% sugar solution. The muslin cloth was also provided at the surface of plastic jar to facilitate the females for egg laying. After hatching, larvae were transferred individually in petri dishes and provided fresh leaves of gram. Insecticidal treatments were applied on the homogeneous population of 3rd instar larvae acquired from F-1 generation.

Insecticides

Five different insecticides were tested against 3rd instar larvae of *H. armigera.* Three dosages i.e. recommended (mentioned on label), high (double of recommended) and low (half of recommended dose) of each insecticide were made by serial dilutions (Table 1).

Bioassay

Toxicity of five different insecticides was determined by using leaf dip method (Busvine, 1971). Fresh leaves of gram were immersed in insecticides solution for ten seconds. After air drying, treated leaves were put in petri dishes containing moistened blotting paper below to avoid dryness. Twelve third instar larvae of *H. armigera* were placed singly on treated leaves in petri dishes with four larvae per replicate. The treatments were replicated thrice. In control treatment, larvae were placed on leaves treated with water.

Statistical analysis

The data regarding percent mortality were recorded after 24, 48, 72 and 96 hours of treatment. Insects were considered dead with no apparent sign of life even when touched with a needle. Data on percent larval mortality were analyzed through one way ANOVA and means were compared by using LSD (Least Significant Difference) test at 5% probability level.

Table 1: Details of insecticides.

Tr.	Insecticides	Active ingredient	Doses (per acre)		
			Low	Recommended	High
T_1	Uniron 10 EC	Novaluron	150 ml	300 ml	600 ml
T_2	Bio Care 16000 IU/mg	Bacillus thuringiensis	125 gm	250 gm	500 gm
T_3	Voliam Flexi 300 SC	Thiamethoxam + Chlorantraniliprole	40 ml	80 ml	160 ml
T_4	Neomed 3 SC	Emamectin benzoate + Lufenuron	100 ml	200 ml	400 ml
T_5	Corgaen 20 SC	Chlorantraniliprole	25 ml	50 ml	100 ml
T_6	Control				

Results

Percent mortality at recommended doses

The results revealed significant difference in the mean percent mortality of the 3rd instar larvae of *H. armigera* after exposure to recommended doses at various time intervals (Table 2). After 24 hours, the maximum percent mortality of 100% was observed in Neomed followed by Voliam Flexi with percent mortality of 66.66%. However, no mortality was observed for Uniron, Bio Care and Coragen after 24 hours. After 48 hours, 41.66% and 8.33% mortality was recorded in Uniron and Coragen, respectively whereas it was constant in case of Voliam Flexi. Mortality after 72 hours increased significantly in four insecticides as 83.33 % for Volim Flexi and Uniron, 66.66% for Coragen and 25% for Bio Care. Uniron showed complete mortality (100%) after 96 hours followed by Voliam Flexi and Coragen with percent mortality of 91.66% and 83.33%, respectively. The lowest mortality of 41.66% was caused by Bio Care at its field recommended dose after 96 hours.

Table 2: Effect of recommended doses of insecticides on the larvae of *H. armigera* after different time intervals.

Tr.	Insecticides	Mean percent mortality			
		After 24 hrs	After 48 hrs	After 72 hrs	After 96 hrs
T_1	Uniron 10 EC	0 c	41.66 bc	83.33 a	100 a
T_2	Bio Care 16000 IU/mg	0 c	0 d	25.00 b	41.66b
T_3	Voliam Flexi 300 SC	66.66b	66.66 ab	83.33 a	91.66a
T_4	Neomed 3 SC	100 a	100 a	100 a	100 a
T_5	Corgaen 20 SC	0 c	8.33 cd	66.66 a	83.33a
T_6	Control	0 c	0 d	0 b	0 c

Mean sharing similar letters in columns are not significantly different at p=0.05

Overall mean percent mortality comparison showed that maximum mortality (100%) was seen at the recommended dose of Neomed followed by Voliam Flexi and Uniron with percent mortality of 77.08% and 56.25%, respectively. However, Coragen and Bio Care caused 39.58% and 16.66% percent mortality, respectively and found least effective insecticides against larvae of *H. armigera* at their recommended dose rates (Figure 1).

Percent mortality at high doses

After 24 hours, the highest percent mortality of 100 % was recorded in Neomed followed by Voliam Flexi (75%). The lowest percent mortality was observed in Uniron (8.33 %) whereas no mortality was observed after treatment with Coragen and Bio Care. Mortality increased after 48 hours of exposure in four insecticide treatments as 83.33 % for Voliam Flexi, 58.33 % for Coragen, 50 % for Uniron and 25 % for Bio Care. Voliam Flexi showed complete mortality (100 %) after 72 hours followed by Coragen and Uniron with percent mortality of 91.66 % and 83.33 %, respectively. The lowest percent mortality of 33.33% was caused by Bio Care insecticide. After 96 hours, 100 % mortality was also reached in Uniron whereas Coragen and Bio Care showed 91.66 % and 41.66 %, respectively (Table 3).

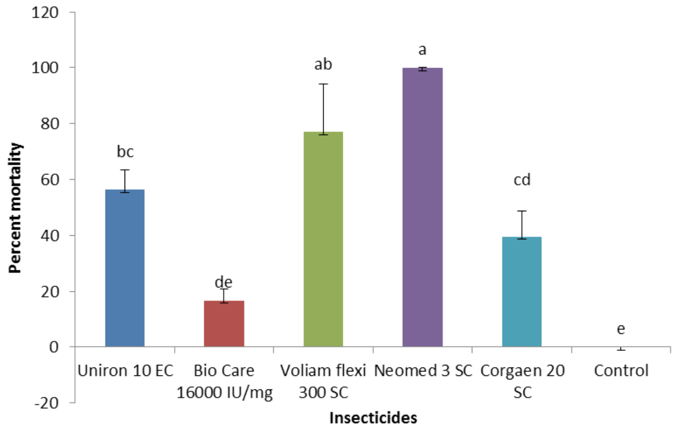

Figure 1: Overall mean percent mortality of *H. armigera* after exposure to recommended doses of different insecticides.

Table 3: Effect of high doses (double of recommended) of insecticides on the larvae of *H. armigera* after different time intervals.

Tr.	Insecticides	Mean percent mortality			
		After 24 hrs	After 48 hrs	After 72 hrs	After 96 hrs
T_1	Uniron 10 EC	8.33 c	50 cd	83.33 a	100 a
T_2	Bio Care 16000 IU/mg	0 c	25 de	33.33 b	41.66 b
T_3	Voliam Flexi 300 SC	75 b	83.33 ab	100 a	100 a
T_4	Neomed 3 SC	100 a	100 a	100 a	100 a
T_5	Corgaen 20 SC	0 c	58.33 bc	91.66 a	91.66 a
T_6	Control	0 c	0 e	0 c	0 c

Mean sharing similar letters in columns are not significantly different at p=0.05

Overall comparison of mean percent mortality at high dose revealed that Neomed was best by showing 100 % mortality of *H. armigera* followed by 89.58 % in Voliam Flexi, 60.41% in Coragen and Uniron, whereas Bio Care was least effective with 25 % mortality (Figure 2).

Percent mortality at low doses

Table 4 also showed significant toxicity variations among the evaluated insecticides at their low doses after 24, 48, 72 and 96 hours of exposure. No mortality was recorded for Uniron, Bio Care and Coragen after 24 hours of treatment whereas it was significantly high for Neomed followed by Voliam Flexi with percent mortality of 91.66% and 58.33%, respectively. After 48 hours, Neomed caused 100% mortality whereas slight increase in mortality was observed in Voliam Flexi (66.66%) and Coragen (8.33%). Mortality further increased after 72 hours of exposure in three treatments as 83.33 % for Voliam Flexi, 58.33 % for Coragen and 8.33 % for Uniron. No larval mortality was observed for Bio Care after 48 and 72 hours. After 96 hours, Voliam Flexi and Coragen showed 83.33% mortality followed by 58.33% in Uniron whereas Bio Care was least effective with percent mortality of 41.66%.

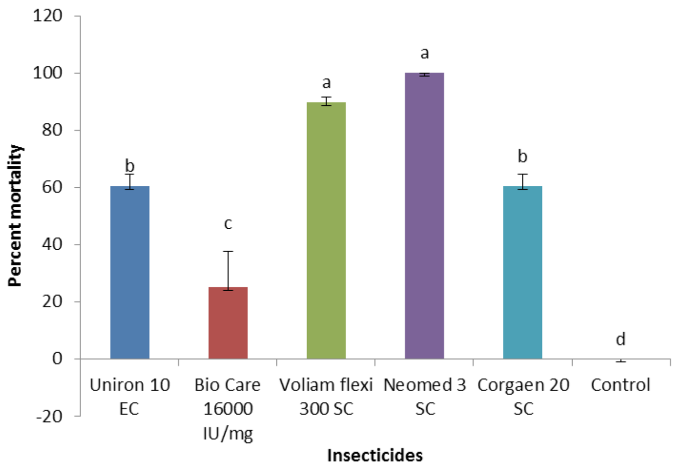

Figure 2: Overall mean percent mortality of *H. armigera* after exposure to high doses (double of recommended) of different insecticides.

Table 4: Effect of low doses (half of recommended) of insecticides on the larvae of *H. armigera* after different time intervals.

Tr.	Insecticides	Mean percent mortality			
		After 24 hrs	After 48 hrs	After 72 hrs	After 96 hrs
T_1	Uniron 10 EC	0 c	0 c	8.33 c	58.33bc
T_2	Bio Care 16000 IU/mg	0 c	0 c	0 c	41.66c
T_3	Voliam Flexi 300 SC	58.33 b	66.66b	83.33a	83.33ab
T_4	Neomed 3 SC	91.66 a	100 a	100 a	100 a
T_5	Corgaen 20 SC	0 c	8.33 c	58.33b	83.33ab
T_6	Control	0 c	0 c	0 c	0 d

Mean sharing similar letters in columns are not significantly different at p=0.05

The overall comparison of mean percent mortality at low doses of tested insecticides showed that Neomed was most effective against *H. armigera* by bringing about 97.91% mortality followed by Voliam Flexi (72.91%) and Coragen (37.5%). However, Uniron and Bio Care were least effective with percent mortality of 16.66% and 10.41%, respectively at their low dose rates (Figure 3).

Discussion

The present studies indicated that all the tested insecticides were found effective and caused significant mortality of 3rd instar larvae of *H. armigera*. Among the tested insecticides, Neomed 3 SC (emamectin benzoate + lufenuron) was the most effective insecticide by causing highest percent mortality followed by Voliam Flexi 300 SC (thiamethoxam + chloranraniliprole), Uniron 10 EC (novaluron) and Coragen 20 SC (chlorantraniliprole) at their recommended doses. Neomed is the combination of emamectin benzoate and lufenuron. Emamectin benzoate belongs to second-generation of avermectins having unique toxic potential against lepidopteran pests (Vargas *et al*., 1997). It acts as an activator of chloride channels by decreasing the neurons excitability. Soon after its exposure the insect stop feeding, larvae become paralyzed irreversibly and die (Cardwell *et al*., 2005). Lufenuron acts as a chitin synthesis inhibitor and effects the larval development of lepidopterans and also causes the infertile eggs production. Exposed insects fail to complete the moult due to the inhibition of the synthesis of new cuticle (Tunaz and Uygun, 2004). Our results are in agreement with Baikar and Naik (2016) who reported that emamectin benzoate caused highest percent mortality of *H. armigera* larvae and found best among the other treatments under laboratory conditions. Similarly, Babar *et al*. (2012) determined the larvicidal and ovicidal action of emamectin benzoate against *H. armigera* and recorded 96 % mortality in laboratory and reported it as the most effective larvicide. Likewise, Jat *et al*. (2016) recommended that for effective management of *H. armigera,* emamectin benzoate and spinetoram could be the first choice. Khatri *et al*. (2014) also observed lufenuron and flufenoxuron more effective compared to other two tested insect growth regulators against 3rd instar larvae of *H. armigera*. Akbar *et al*. (2018) reported significant mortality of larval population of *H. armigera* after application of emamectin benzoate (74.99%) and lufenuron (62.49%) with lower pod damage and enhanced yield. Iqbal *et al*. (2014); Babar *et al*. (2016) and Hakeem *et al*. (2017) confirmed the high toxicity of emamaectin benzoate and lufenuron against *H. armigera* in laboratory and field studies.

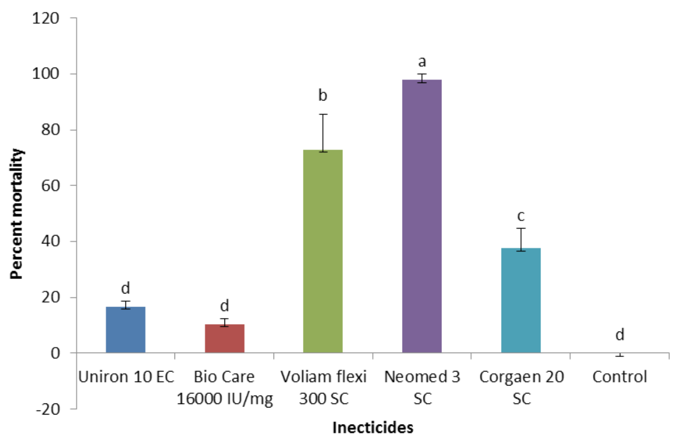

Figure 3: Overall mean percent mortality of *H. armigera* after exposure to low doses (half of recommended) of different insecticides.

The present study have indicated that Voliam Flexi 300 SC was the second most effective insecticide and caused more than 90% mortality of *H. armigera* at its recommended dose. Voliam Flexi is the combination of chlorantraniliprole and thiamethoxam. Chlorantraniliprole belongs to anthranilic diamides with novel mode of action and acts as an activator of ryanodine receptors of insects causing rapid muscle paralysis and dysfunction (Cordova *et al*., 2006, 2007; Lahm *et al*., 2005, 2007). Thiamethoxam is a broad-spectrum systemic insecticide. It is rapidly absorbed and transported to all parts of the plants including pollen where it acts to deter insect feeding. It enters the insect body through direct contact or through stomach after feeding and also through its tracheal system. The compound disrupts the transfer of information between nerve cells by interfering with nicotinic acetylcholine receptors and ultimately paralyzes the insects muscles (FAO, 2014). These results are in line with the Rizvi and Jaffar (2015), who conducted a study to compare the biopesticide and synthetic insecticide on the development and growth of three different pests belonging to Noctuidae family. They concluded that Voliam Flexi 300 SC and Delegate 25 EC caused highest percentage mortality of *H. armigera*. Likewise, Abbas *et al*. (2015) also reported 89 % mortality of the target pests with Voliam Flexi.

The present results showed that Uniron 10 EC (novaluron) did not cause any larval mortality after 24 hours of exposure. These results are completely in line with Jat *et al*. (2016) who also concluded that insecticide novaluron did not show any promising results after 24 h feeding of 3rd instar *H. armigera*. Bio insecticide, Bio Care ™ 16000 IU/mg that consists of *Bacillus thuringiensis* was found least effective with minimum mortality of tested insect. *B. thuringiensis* Cry and Cyt protein families are a diverse group of proteins with activity against insects of different orders-Lepidoptera, Diptera, Coleoptera, and also against other invertebrates such as nematodes. Their primary action is to lyse midgut epithelial cells by inserting into the target membrane and forming pores (Bravo *et al*., 2007). Similar to these findings, Baikar and Naik (2016) also observed only 11% mortality of *H. armigera* after treatment with *B. turingiensis*.

Conclusion

It can be inferred from the present study that insecticides Neomed 3 SC (emamectin benzoate + lufenuron) and Voliam Flexi 300 SC (thiamethoxam + chloranraniliprole) could be the first choice for management of *H. armigera* in field crops.

Conflict of interests

The authors have declared no conflict of interest.

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