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Efficacy of Different Insecticides on Biology of Fall Armyworm (*Spodoptera frugiperda* J.E. Smith Lepidoptera: Noctuidae)

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ABSTRACT

Maize is considered an important cereal crop after wheat and rice and is grown in all provinces of the country. Many biotic and abiotic factors affect the yield of maize. Among the biotic factors fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a key pest of maize crop all over the world. Laboratory bioassay was carried out by using five different insecticides against FAW. Two different lethal concentrations LC10 and LC20 were used against 3rd larval instar of FAW. The study was carried out under Completely Randomized Design (CRD) with three replications. The data regarding mortality were recorded after 24 hours of application until start of pupal stage and were analyzed by using Statistix 8.1 software at 0.05% level of significance while the Tukey's HSD test was used for comparison of treatments means. The results showed that chlorantraniliprole was extremely potent against 3rd instar larvae. Spinetoram and chlorpyrifos, on the other hand, require a minimum concentration 0.025, 0.0003, 0.0083 ppm and 0.039, 0.0046, 0.0012 ppm to kill 50, 20 and 10% of the population, respectively. Pyriproxyfen proved the least effective insecticides and required more quantity of insecticide to kill 3rd instar larvae.

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INTRODUCTION

Maize is an important crop of the world and have wide range of applications in the food and feed sectors. Maize contributes 3.4% to the value added in agriculture and 0.6% to GDP of Pakistan and grown on 1418 thousand hectares of area with an average production of 8.5 million tones (Kumar *et al.*, 2022; GOP., 2020-21). Khyber Pakhtunkhwa (KPK) and Punjab account for 99% of total production of the country. KPK accounts for 51% of total land area with 31% of total production and Punjab accounts for 48% of total area with 69% maize production. The rest 1% is produced in Balochistan and Sindh. Maize is the third most important cereal of Pakistan after wheat and rice (Ali *et al.*, 2020). In Pakistan, maize products are used in the poultry feed

sector, wet milling and other remainder utilized as nourishment for humans and animals (Reddy and Jabeen, 2016). By increasing the population of the world, the eating patterns have been changed and demand for food production has been increased by 70%. Therefore, large quantity of food supply has been required. (Cordova *et al.*, 2006). The production of crop is linked with management practices against pests and diseases which cause losses (Lahm *et al.*, 2009). *Spodoptera frugiperda* is one of the most important pests of maize crops, resulting in significant damage and yield losses. It has been estimated that it results about 58% of the yield loss (Kumar *et al.*, 2022; Kenis *et al.*, 2022). *S. frugiperda* is a native pest from tropical to sub-tropical regions of United States of America, Argentina and

Mexico. This pest has entered in different areas of Africa in 2016 and gained the status of invasive pest (Tambo *et al.*, 2020). It has been reported as an aggressive key pest of infected fields of maize. *S. frugiperda* has been reported in April 2019, from province of Sindh, Pakistan (Carvalho *et al.*, 2013). This pest can also attack cotton, soyabean rice and sorghum (Desneux *et al.*, 2007). In addition, it is considered as severe pest of some vegetable crops like potato, tomato and cabbage (El-Sheikh, 2015). It has been reported that this pest has caused 100% damages in fodder crop of maize in district of Shaheed Benazirabad, 70% in Tando Allahyar and 60% of grain crop in Hyderabad from Sindh Pakistan (Gilal *et al.*, 2020). *S. frugiperda* caterpillars damage the forage grasses and cereals (Seth *et al.*, 2004). A neonate larva usually eats up the new leaves and feed on upper side of shoot and leaves creating a characteristic windowing effect (Sisay *et al.*, 2019). The destruction of leaves, stems and flowers of the crop is done by caterpillars through heavy feeding, and it happens mostly in the last three stages of caterpillar (Wang *et al.*, 2013).

Chemical insecticides are routinely employed to control *S. frugiperda* infestations when defoliation is found in the plant crop (Carvalho *et al.*, 2013). Chemical insecticides have great ability to control various insect pests including *S. frugiperda*. Ability to migrate and due to a vast host range, no other control approach was found as effective as insecticides against *S. frugiperda*. It was recently reported that control of *S. frugiperda* is fully dependent on chemical insecticides (Assefa and Ayalew, 2019). The application of insecticides is one of the most significant aspects of the IPM method against *S. frugiperda*. Synthetic insecticides which belong to different groups of insecticides has been broadly used as an emergence response to slow down the dispersion and minimizing the percentage damage of crops and these insecticides play an important role for managing the *S. frugiperda*. Still not any insecticide has been registered for *S. frugiperda* control in any country. The emergence, label and suggestion to urgent need for screening the synthetic insecticides against *S. frugiperda*. The focus of this study was based on the toxicological effect of spinetoram, pyriproxyfen, chlorpyrifos, chlorantraniliprole and lufenuron against nutritional efficiency of third instar larvae of *S. frugiperda*.

MATERIALS AND METHODS

S. frugiperda larvae were collected from different maize field at Entomology Research area of University of Agriculture Faisalabad. These larvae were reared under controlled conditions at $25 \pm 2^\circ\text{C}$ temperature, $65 \pm 5\%$ of relative humidity (RH) and photoperiod of 14:10 h (L:D). For mass rearing of *S. frugiperda* method described by Sisay *et al.* (2019) and Chapwa 2022. The adults were fed upon 10% honey solution. For eggs deposition, adult females were provided shoots dipped in water inside the adult cage. Eggs were collected by cutting the specific area of the leaves and kept in small sized box. On the emergence of first instar, they will be shifted to other cleaned plastic boxes and fed upon the soft leaves of maize. Feeding beds were prepared for each egg batch. These feed beds were changed after every 24 hours, and this practice extended up to the third larval instar. To prevent the cannibalism, third instars were kept separate in each small plastic container. Larvae were regularly fed by fresh maize leaves collected from the research area fields. It was made sure that no systemic pesticide was applied to field from where the leaves were taken. The leaves were washed and then dried before feeding them to larvae. Leaves were renewed every day. After completion of larval cycle, the pupae were kept within same rearing cages separated on basis of sex. The pupae will be observed daily until adult moths emerged. After the emergence of moth, they were transferred to rearing cages. The adults were fed with 10% honey solution, renewed at every day. Additionally, fresh maize leaves and stems were provided to facilitate oviposition. Mass rearing of FAW was carried out to get homogenous population for bioassays. The bioassay experiment was done to check the efficacy of different insecticides (Sisay *et al.*, 2019, Hina *et al.*, 2024).

Bioassay

Leaf dip bioassay of Tukaram *et al.*, (2014) was employed to establish three lethal concentrations as treatment LC₁₀, LC₂₀ and LC₅₀ in ppm on 3rd instar larvae. Three replications were used of each treatment on 20 Nos. of 3rd instar larvae under CRD. Because 3rd instar larvae were more susceptible, larger than 1st and 2nd instar, easier to handle and most active feeders. The experiment was conducted to check the efficacy of different insecticides such as Pyriproxyfen, Lufenuron, Chlorpyrifos, Spinetoram and Coragen on 3rd instar larvae of fall armyworm. The leaves were treated with the different concentration of each insecticide. The size of maize leaves was 15cm and dipped for 3 seconds in

stock solution and other concentrations made from stock solution of each insecticide. Treated leaves were placed on tissue paper to make them a little dry. Larvae were placed on treated leaves in separate small glasses. Leaves were renewed on a regular basis until the larval cycle was completed. Moulting was also observed from 3rd instar to 4th, 5th and 6th instar. The larval mortality was observed after every 24 hours until pupation. Sub lethal effects of insecticides were also examined against pupae and adults. In total 20 larvae and pupae were kept in petri dishes for adult emergence after seven days of pupation and male or female pupae were placed separated on sex basis. Pupae were considered dead if adults were not developed after 12 days. The adults were considered deformed if they were unable to shed their pupal exuvium or have abnormal wings. Pupae were placed in rearing chambers for adult moth emergence. The moths were fed upon 10% honey solution and renewed every 24 hours. To check mortality, both pupae and adult stages were tested on regular basis. To check fertility total number of eggs laid by each female was counted after first 12 days of oviposition. The number of eggs hatched in the control and treated were also recorded. The data were recorded

and analyzed using Statistix 8.1 at 0.05% level of significance. Tukey's HSD test was used to compare the treatment means. Initial larval weight, treated leaf weight, larval weight gain, weight of leaf consumed and feces weight were taken for the whole larval, pupal and adult period (Salem *et al.*, 2023). Probit analysis were carried out at 95% of confidence interval.

RESULTS

Population response of *S. frugiperda* fed on treated maize leaves

Table 1 presented the lethal concentrations of different insecticides after applying on the 3rd instar larvae of *S. frugiperda*. These findings clearly indicated that coragen was very effective and highly potent against 3rd instar larvae. Moreover, minimum amount concentration is required to kill 50, 20 and 10 percent (0.016, 0.0072 and 0.00014 ppm, respectively) population, followed by spinetoram ($LC_{50} = 0.025$, $LC_{20} = 0.0003$ and $LC_{10} = 0.00083$ ppm) and chlorpyrifos ($LC_{50} = 0.039$, $LC_{20} = 0.0046$ and $LC_{10} = 0.0012$ ppm). Pyriproxyfen regarded as least potent and required maximum amount of insecticide ($LC_{50} = 1.37$, $LC_{20} = 0.267$ and $LC_{10} = 0.0148$ ppm).

Table 1. Probit regression analysis of different insecticides against 3rd instar larvae of *S. frugiperda*.

Insecticide	LC_{50} (ppm)	LC_{20} (ppm)	LC_{10} (ppm)	Slope \pm SE
Spinetoram	0.025	0.0003	0.00083	0.26 ± 0.016
Chlorpyrifos	0.039	0.0046	0.0012	0.42 ± 0.027
Lufenuron	0.152	0.023	0.0034	0.37 ± 0.075
Pyriproxyfen	1.37	0.267	0.0148	0.51 ± 0.033
Coragen	0.016	0.0072	0.00014	0.34 ± 0.019

Table 2 shows the initial and gained average larval weight, weight of leaf consumed and weight of feces after the application of LC_{20} of different insecticides. Initial maximum larval weight of 3rd instar larvae was recorded (0.050g) followed (0.048 g) whereas the minimum weight was recorded (0.042g). In case of fresh leaf weight, the maximum leaf was (0.715 g) while minimum leaf weight was (0.512g). Maximum larval weight gained was 0.007g by 3rd instar larvae after consuming LC_{20} of pyriproxyfen treated leaves whereas the minimum weight gained (0.004 g) was recorded in coragen treated leaves. The data indicated that the maximum pyriproxyfen treated leaves (0.298g) were consumed while the minimum (0.238g) of coragen treated leaves. The maximum feces (0.007g) were

excreted after the consumption of pyriproxyfen treated leaves while the minimum (0.003 g) after the consumption of coragen treated leaves by the 3rd instars larvae. The maximum larval weight gained by 4th instar larvae were (0.022 g) after consuming LC_{20} of pyriproxyfen treated leaves while the minimum (0.011g) in LC_{20} of coragen treated leaves. The data also exhibited that the maximum pyriproxyfen treated leaves have been consumed (0.405g) while the minimum (0.355g) of LC_{20} of coragen treated leaves. The maximum weight of feces (0.025g) were excreted by 4th instar larvae after consumption of LC_{20} pyriproxyfen treated leaves while the minimum (0.011g) after the consumption of LC_{20} of coragen treated leaves. The results indicated that maximum 5th instar larval weight gain was (0.064g) after

the consumption pyriproxyfen treated leaves while the minimum larval weight gain was (0.036g) after the consumption of coragen treated leaves were consumed. Maximum pyriproxyfen treated leaves were consumed with the value of (0.413g) while the minimum (0.369g) by the consumption of coragen treated leaves. Maximum feces weight (0.063g) were excreted by 5th instar larvae while the minimum (0.051g). The results indicated that maximum larval weight gained was (0.101g) by 6th instar larvae while the minimum (0.067 g). Maximum pyriproxyfen treated leaves were consumed (0.585g) while the minimum (0.464g) of coragen treated leaves. Maximum feces weight (0.710g) were excreted by 6th instar larvae by the consumption of coragen treated leaves while the minimum (0.117g) by the consumption of chlorpyrifos treated leaves.

Table 3 shows the initial and gained average larval weight, weight of leaf consumed and weight of feces after the application of LC₁₀ of different insecticides. Initial maximum larval weight of 3rd instar larvae was recorded (0.049g) followed (0.048 g) whereas the minimum weight was recorded (0.044g). In case of fresh leaf weight, the maximum leaf was (0.640g) while minimum leaf weight was (0.468g). Maximum larval weight gained was 0.011g by 3rd instar larvae after consuming LC₁₀ of pyriproxyfen treated leaves whereas the minimum weight gained (0.006 g) was recorded in lufenuron and chlorpyrifos treated leaves. The data indicated that the maximum pyriproxyfen treated leaves (0.366g) were consumed while the minimum (0.282g) of coragen treated leaves. The maximum feces (0.010g) were excreted after the consumption of pyriproxyfen treated leaves while the minimum (0.005 g) after the consumption of coragen, chlorpyrifos and spinetoram treated leaves by the 3rd instars larvae. The maximum larval weight gained by 4th instar larvae was (0.021 g) after consuming LC₁₀ of pyriproxyfen treated leaves while the minimum (0.014g) in LC₁₀ of coragen and spinetoram treated leaves. The data also exhibited that the maximum pyriproxyfen treated leaves have been consumed (0.467g) while the minimum (0.403g) of LC₁₀ of coragen treated leaves. The maximum weight of feces (0.022g) were excreted by 4th instar larvae after consumption of LC₁₀ pyriproxyfen treated leaves while the minimum (0.015g) after the consumption of LC₁₀ of coragen and spinetoram treated leaves. The results indicated that maximum 5th instar larval weight gain was (0.070g) after the consumption pyriproxyfen treated

leaves while the minimum larval weight gain was (0.044g) after the consumption of spinetoram treated leaves. Maximum pyriproxyfen treated leaves were consumed with the value of (0.496g) while the minimum (0.384g) by the consumption of coragen treated leaves. Maximum feces weight (0.072g) were excreted by 5th instar larvae while the minimum (0.054g). The results indicated that maximum larval weight gained was (0.110g) by 6th instar larvae by the consumption of pyriproxyfen treated leaves while the minimum (0.078g) after the consumption of LC₁₀ spinetoram treated leaves. Maximum pyriproxyfen treated leaves were consumed (0.697g) while the minimum (0.527g) of coragen treated leaves. Maximum feces weight (0.515g) were excreted by 6th instar larvae after the consumption of spinetoram treated leaves while the minimum (0.102g) after the consumption of coragen treated leaves.

DISCUSSION

The study was conducted under laboratory conditions and recorded the response of *S. frugiperda* larvae against lethal concentrations of different insecticides. The results clearly indicated that coragen found very lethal followed by spinetoram, chlorpyrifos and lufenuron, while the pyriproxyfen ranked as least effective in terms of lethality. These results are in line with Hardke *et al.* (2011) who established a baseline dosages responses in *S. frugiperda* larval bioassay. They tested residual efficacy of selected insecticides against *S. frugiperda* under field conditions. In field trial experiments chlorantraniliprole caused significant greater mortality, negative effect on the growth and reduced the number of infected whorls as compare with non-treated and lambda-cyhalothrin-treated plots. Reduced number of infested whorls have been observed in the treated plots as compared with non-treated plots at seven DAT. Similarly, Salem *et al.*, 2023 observed the efficacy synthetic insecticides Methomyl, Chlorpyrifos and Spinosad against fall armyworm under laboratory bioassay. Bioassays were conducted on 4th instar larvae. The findings of the experiment indicated that A significant increase in the life duration of 4th, 5th and 6th instars larvae with the LC₅₀ concentrations of insecticides used. Accordingly, to this study the larval growth a significant increase in the total duration due to toxicity the effect the nervous tremors of the thoracic legs and mouthparts and the larva become was unable to feed.

Our findings included conventional as well as novel insecticides but in partial agreement with Wang *et al.* (2013) who used only Spinosad is a selective biological pesticide against *S. exigua*. Lethal and sub - lethal impacts should be included for a thorough examination of spinosad impact in order to create successful pest management tactics. However, few investigations on the sub - lethal impacts of spinosad on *S. exigua* have been published. By recording and evaluating numerous toxicological and physiological characteristics, this study seeks to evaluate the fatal and sub - lethal impacts of spinosad on this pest. The lethality of spinosad against *S. exigua* were tested in the lab using late second-instar larvae exposed to the substance orally. Spinosad LC₅₀ values for *S. exigua* were 0.317 and 0.293 mg x kg⁻¹, respectively, 48 and 72 hours after therapy. Spinosad, at sub - lethal dosages, considerably increased the developmental period of survival larvae and decreased the moist weight of the larvae. Reduced pupation ratio and pupal weight, longer prepupal and pupal durations, and lower emergence ratio, fertility, and lifespan of adults were all signs of post-exposure impacts. In the exposed spinosad groups, the net replacement rate was lower than in the unexposed spinosad groups. The high-dose group (0.365) had a much significantly lower rate of growth in population ($r(m)$) than the control (0.521) and reduced group (0.521), but the latter two did not vary significantly. These results suggested that the combination of fatal and sub - lethal effects of spinosad may have a major impact on *S. exigua* population trends by reducing survival and reproduction, as well as delaying development.

The previous scientists including Bharadwaj *et al.* (2020), Zhou *et al.* (2020), Deshmukh *et al.* (2020), De Groote *et al.* (2020), Mondal *et al.* (2020), Huang *et al.* (2020) and Qiu *et al.* (2020) working in different parts of the world under laboratory and field conditions and

used novel insecticides spinetoram, coragen, spinosad, fubendiamide, chlorantraniliprole, emamectin benzoate, chlofenapyr, lufenuron and lambda-cyathrin against different life stages. They found that the LC₅₀ values of spinetoram on FAW in Dehong and Dongtai were 0.179 mg/L and 0.475 mg/L, correspondingly. The LC₅₀ values for chlorantraniliprole against FAW in Dongtai were from 0.849 mg/L in Xuzhou to 3.446 mg/L in Dongtai. Lufenuron LC₅₀ values for FAW in Dongtai were 1.169 mg/L and 7.956 mg/L. Field spray resulted the first spray, the mean larval population of *S. frugiperda* ranged from 2.51 to 38.16 larvae per 25 plants. Spinetoram revealed 2.51 larvae per 25 plants, followed by emamectin benzoate, chlorantraniliprole, chlorantraniliprole+lambda cyhalothrin, thiomethaxam + lambda cyhalothrin. The coragen found effective in reducing the population of fall armyworm and its related insect pest species. The findings of experiment showed that significant mortality percentage was observed by application of spinetoram, chlorantraniliprole, emamectin benzoate, chlorfenapyr, and lufenuron as compared to lambda-cyhalothrin and azadirachtin that caused low mortality against the larvae of FAW. Results showed that larvae were susceptible to indoxacarb that showed significant difference (10.0-fold for LC₅₀) through the different geographical populations.

The results from this experiment highlight the differential effects of five different insecticides on larval growth, leaf consumption, and feces production. Pyriproxyfen treatment consistently showed favorable outcomes across multiple instar stages as compare with coragen which was effective to reduce the larval growth of FAW. However, further studies would be needed to understand the underlying mechanisms driving to assess the long-term implications on larval development and population dynamics of FAW.

Table 2. Population response of *S. frugiperda* after the application of LC₂₀ of different insecticides.

	3 rd instar larvae					4 th instar larvae			5 th instar larvae			6 th instar larvae		
Insecticides (ppm)	Initial larval weight (g)	treated leaf weight (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)
Pyriproxyfen	0.042	0.680	0.007	0.298	0.007	0.022	0.405	0.025	0.064	0.413	0.063	0.101	0.585	0.145
Lufenuron	0.042	0.685	0.006	0.263	0.005	0.014	0.392	0.012	0.043	0.416	0.045	0.077	0.509	0.125
Chlorpyrifos	0.048	0.715	0.005	0.266	0.004	0.012	0.391	0.011	0.040	0.376	0.046	0.070	0.500	0.117
Spinetoram	0.044	0.575	0.005	0.252	0.004	0.012	0.365	0.011	0.040	0.376	0.044	0.066	0.501	0.320
Coragen	0.050	0.512	0.004	0.238	0.003	0.011	0.355	0.011	0.036	0.369	0.051	0.067	0.464	0.710

Table 3. Population response of *S. frugiperda* after the application of LC₁₀ of different insecticides.

	3 rd instar larvae					4 th instar larvae			5 th instar larvae			6 th instar larvae		
Insecticides (ppm)	Initial larval weight (g)	treated leaf weight (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)	Larval weight gain (g)	Weight of leaf consumed (g)	Weight of feces (g)
Pyriproxyfen	0.045	0.625	0.011	0.366	0.010	0.021	0.467	0.022	0.070	0.496	0.072	0.110	0.697	0.150
Lufenuron	0.044	0.640	0.006	0.309	0.007	0.016	0.431	0.017	0.057	0.459	0.063	0.092	0.656	0.146
Chlorpyrifos	0.049	0.662	0.006	0.296	0.005	0.015	0.442	0.018	0.054	0.435	0.060	0.081	0.584	0.120
Spinetoram	0.048	0.560	0.007	0.301	0.005	0.014	0.412	0.015	0.044	0.400	0.058	0.078	0.537	0.515
Coragen	0.049	0.468	0.007	0.282	0.005	0.014	0.403	0.015	0.046	0.384	0.054	0.081	0.527	0.102

CONFLICT OF INTEREST

The authors declared no conflict of interests.

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