



EGYPTIAN ACADEMIC JOURNAL OF  
**BIOLOGICAL SCIENCES**  
**ENTOMOLOGY**

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ISSN  
1687-8809

[WWW.EAJBS.EG.NET](http://WWW.EAJBS.EG.NET)

**Vol. 16 No. 2 (2023)**



**In Vitro and In Vivo Toxicity of Selected Insecticides Against 2<sup>nd</sup> Instar Larvae of *Spodoptera Frugiperda***

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**ARTICLE INFO**

**Article History**

Received:19/4/2023

Accepted:21/6/2023

Available:25/6/2023

**Keywords:**

Fall armyworm;

Invasive pest; Maize;

Crop production;

Insecticides; Integrated pest management.

**ABSTRACT**

*Spodoptera frugiperda*, a polyphagous pest that is a member of the Noctuidae family and the Lepidoptera order, has become a significant problem for the economies of several nations, particularly Pakistan. One of the main strategies for controlling the FAW infestation in areas where maize is grown is chemical control. The current study was conducted to check the efficacy of some insecticides against 3<sup>rd</sup> instar larva of FAW under laboratory and greenhouse conditions. The mean mortality of FAW larvae was significantly different among the treatments. The average mean percentage mortality of larvae after 24, 48 and 72 hours of coragen application was  $59.41 \pm 4.32$ ,  $73.11 \pm 4.72$ , and  $85.90 \pm 4.31$ , respectively. Among all tested insecticides, coragen was recorded as the most toxic chemical followed by tracer and imidacloprid. Coragen showed the highest larval mortality (80.95%) at 72 hours of application, followed by tracer (69.77%), and imidacloprid (55.33%). Only 1.20 percent mortality was recorded in the control treatment at 72 hours of application. The mortality of larvae was 30.3 and 44.11% at the first and second sprays of coragen, respectively, while 26 and 30.34% by the application of tracer in the greenhouse conditions. The outcome thus helped to highlight the important need to focus on screening newly recommended insecticides against *S. frugiperda* under controlled conditions and the potential to utilize each insecticide at farmers' fields conditions that some academician researchers did not take into consideration in the large scale of maize farm.

**INTRODUCTION**

Maize, *Zea mays* commonly known as queen of cereal is a high-yielding staple crop in the world. It is the main source of fodder for animals, feed for poultry and food for humans. It is considered an important source of raw materials in various industries from which

different products are prepared and used for different purposes. FAW has the power to cause 8.3 to 20.6 m tons of maize losses annually in African countries and become a risk to food security. In Pakistan, maize is planted in all four provinces on an area of 0.974 million hectares with 3.707 m tons annual production and an average yield of 3805 kg/ha. Its production is reducing due to many abiotic factors (irrigation, land, soil type and climate) and biotic factors (weeds, pests and diseases) (Sisay *et al.*, 2019; Ramzan *et al.*, 2019; Murtaza *et al.*, 2019; Kumari *et al.*, 2020).

Maize is highly infested with several insect species. Among reported species, an invasive alien insect species, fall armyworm (FAW) *Spodoptera frugiperda* is considered the top and most serious pest of maize in the globe especially in Asian countries including Pakistan. FAW larvae attack the tender parts of plants, mostly feeding on the young plant parts and even cob. The severe attack of larvae has caused 8-20 million tons per annum of maize yield losses in Africa. It is becoming a major threat to maize production in Pakistan and as polyphagous in nature, pest is spreading to other host plants (Ramzan *et al.*, 2020a; Akeme *et al.*, 2021).

FAW is a polyphagous pest that belongs to the order Lepidoptera and Family Noctuidae and has become a serious issue for the economy of various countries of the globe. It is widely distributed in tropical and subtropical areas of the world. Before 2016, it was limited to American countries, while reported from Africa in 2016, Indian in 2018, Vietnam, Myanmar, Bangladesh, Pakistan, Thailand, China and Sri Lanka in 2019 (Goergen *et al.*, 2016; Prasanna *et al.*, 2021, 2018; Guo *et al.*, 2020; Wu *et al.*, 2019). The infestation of pests has been recorded on many crops including rice (Nagoshi *et al.*, 2019; Ramzan *et al.*, 2021b; Abid *et al.*, 2021), millet, maize, sorghum (Sun *et al.*, 2020; Ramzan *et al.*, 2020b), groundnut, cotton, wheat, beet, onion, tomato, soybean, potato, and several vegetable crops (Sharanabasappa *et al.*, 2018).

Therefore, quick actions are needed to control the current pest at an early stage to minimize the yield losses. Several management approaches have been adopted to control insect pests such as chemical, biological and botanicals throughout the globe. Each management strategy has some positive and negative effects. Chemicals (insecticides) are the best methods that give quick and positive responses against insect pests. This widely used method is adopted by farmers and researchers around the globe to minimize the pest population. In the current study, some insecticides are used to check their toxicity larvae of FAW under laboratory conditions.

## MATERIALS AND METHODS

Egg batches were collected from maize fields planted by the farmer nearby the study area. The collected batches of eggs were brought safely to the laboratory and kept in a plastic box for rearing under controlled conditions. The second instar larvae after hatching were shifted individually into Petri dishes for further population and protection from cannibalism. The larvae were applied to maize leaves until pupation. Each stage of the pest was kept separate and F1 was used in the bioassay study. The rearing procedure of early researchers was followed during the whole study period (Ramzan *et al.*, 2021a). Three insecticides (imidacloprid, chlorantraniliprole (Coragen 200 SC), and spinosad (Tracer 480 SC) were obtained from a nearby market and tested to check their toxicity in laboratory and field conditions. The maize leaves were collected separately for 1–2 h after being sprayed and fed to the larvae. The collected leaves were cut and weighed to 60 g (from our FAW rearing experience, 45–60 g maize leaves can feed approximately 10 to 15 larvae for 2–3 days). Each 50 g contains 4–5 pieces of leaves of 5–6 cm in length. The leaves were placed in a plastic jar with a perforated lid. Ten third-instar larvae were released into the plastic jars containing

the treated leaves. Leaves treated with sterile water were included as a control. The insect diets (maize leaves) were checked every day. Insect mortality was assessed 24, 48, and 72 h after treatment application. A larva was considered dead if it could not right itself after being placed on its dorsal surface. The mean percentage of larval mortality was calculated and statistically analyzed.

## RESULTS AND DISCUSSION

FAW is an insect pest that poses a significant threat to agricultural crops, particularly maize (corn), across many regions of the world. It belongs to the family Noctuidae and is native to tropical and subtropical regions of the Americas. The term "armyworm" refers to the insect's behavior of migrating in large numbers, devouring vegetation as they move, which gives the appearance of an army on the move. FAW has become a major concern for farmers and agricultural authorities due to its rapid spread and destructive feeding habits. It can cause substantial damage to maize crops, but it is also known to attack other crops such as rice, sorghum, sugarcane, cotton, and vegetables. The larvae of the armyworm feed voraciously on plant leaves and can cause complete defoliation, leading to reduced crop yields or even crop loss (Gebreziher, 2020; Ramzan *et al.*, 2020b).

One of the key reasons for the widespread impact of FAW is its ability to reproduce rapidly and disperse over long distances. The adult moths can migrate over hundreds of kilometers, aided by favorable weather conditions and wind patterns. They lay eggs on leaves, and within a few days, the larvae hatch and start feeding on the plants. The life cycle from egg to adult takes around 30 days, allowing for multiple generations within a single growing season. Controlling FAW infestations is challenging due to several factors. The larvae are highly adaptable and can develop resistance to insecticides. Additionally, their feeding behavior makes it difficult to target them effectively. Integrated pest management strategies are often employed, combining various approaches such as cultural practices, biological control agents, and judicious use of insecticides when necessary (Temple *et al.*, 2009; Viteri *et al.*, 2019).

Several countries and international organizations have initiated efforts to monitor and manage FAW infestations. Early detection, timely reporting, and sharing of information among farmers, researchers, and agricultural extension services are crucial for effective control measures. Additionally, research is ongoing to develop improved crop varieties that are resistant to armyworm infestations. The impact of FAW extends beyond agricultural losses. It affects the food security, and livelihoods of farmers, and can lead to increased pesticide use, which can have negative environmental and health consequences (Kumela *et al.*, 2019; Maruthadurai and Ramesh, 2020).

Therefore, there is a need for sustainable and integrated approaches to combat this pest while minimizing the reliance on chemical interventions. Overall, the FAW is a significant agricultural pest that demands attention and concerted efforts to mitigate its impact on crops and the livelihoods of farmers. Collaboration among researchers, farmers, and policymakers is essential to develop and implement effective strategies for its control and management.

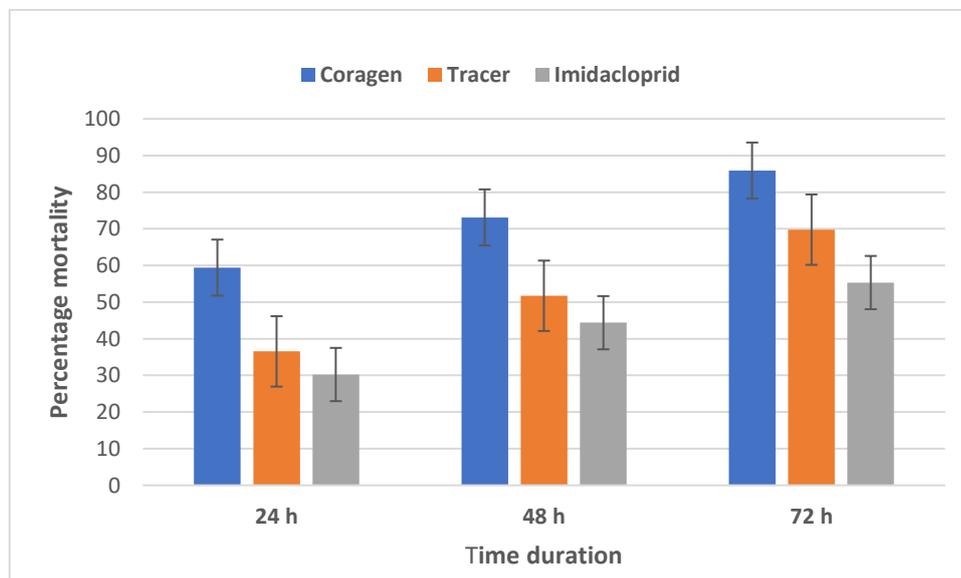
The current study was conducted to check the efficacy of some insecticides against 3<sup>rd</sup> instar larva of FAW under laboratory and greenhouse conditions. The mean mortality of FAW larvae was significantly different among the treatments. The average mean percentage mortality of larvae after 24, 48 and 72 hours of coragen application was  $59.41 \pm 4.32$ ,  $73.11 \pm 4.72$ , and  $85.90 \pm 4.31$ , respectively. Among all tested insecticides, coragen was recorded as the most toxic chemical followed by tracer and imidacloprid. Coragen showed the highest larval mortality (80.95%) at 72 hours of application, followed by tracer (69.77%), and

imidacloprid (55.33%). Only 1.20 percent mortality was recorded in the control treatment at 72 hours of application (Table 1). The percentage mortality of larvae after 24, 48 and 72 hours of insecticide application is shown in Figure 1.

**Table 1.** In vitro Percentage mortality of FAW larvae at 24, 48, and 72 h after insecticides applications.

Insecticides	%Age larval mortality		
	24 h	48 h	72 h
Coragen	59.41 ± 4.32ab	73.11 ± 4.72abc	85.90 ± 4.31abc
Tracer	36.54 ± 4.89bc	51.72 ± 6.91c	69.77 ± 6.65cd
Imidacloprid	30.23 ± 7.91bc	44.37 ± 6.88ab	55.33 ± 3.43a
Control	0.00b ± 00d	0.00b ± 00d	1.20 ± 0.52f
df	3	3	3
<i>p</i> <	0.001	0.001	0.001

The means within a column not sharing a common letter are significantly different at  $p < 0.05$  using Tukey's test.



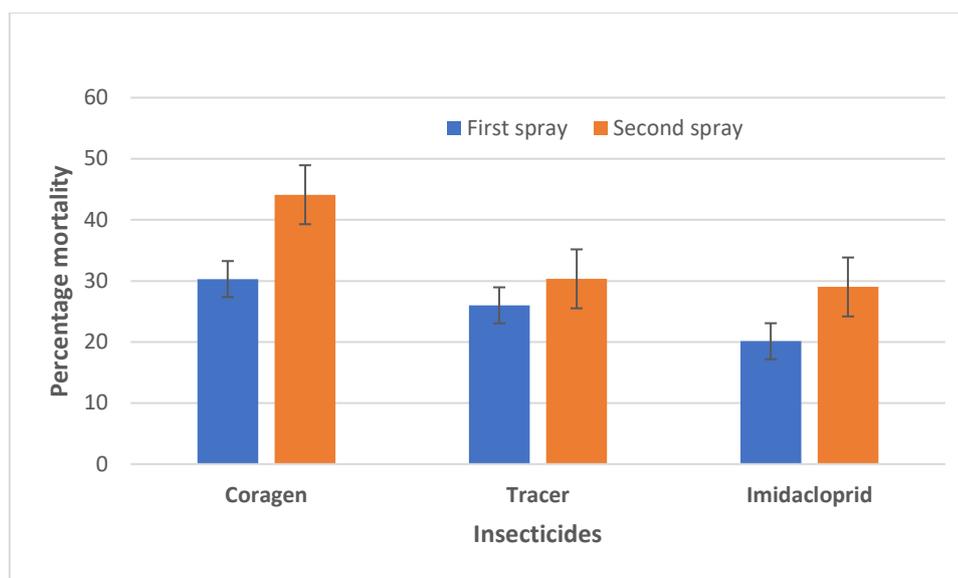
**Fig. 1.** Mortality percentage of larvae at 24, 48 and 72 hours of insecticides application.

The mortality of larvae was 30.3 and 44.11% at the first and second sprays of coragen, respectively, while 26 and 30.34% by the application of tracer in the greenhouse conditions as given in Table 2. The percentage mortality of larvae after 1<sup>st</sup> and 2<sup>nd</sup> spray of insecticides is shown in Figure 2.

**Table 2.** In vivo Percentage mortality of FAW larvae after 1<sup>st</sup> and 2<sup>nd</sup> spray of insecticides in the greenhouse.

Insecticides	%Age larval mortality	
	1 <sup>st</sup> spray	2 <sup>nd</sup> spray
Coragen	30.3 ± 4.23ab	44.11 ± 7.17abc
Tracer	26.0 ± 9.99ab	30.34 ± 5.98ab
Imidacloprid	20.12 ± 0.10ab	29.01 ± 39.14abc
Control	0.00 ± 0.00b	0.02 ± 0.00bc
df	3	3
<i>p</i> <	0.002	0.004

The means within a column not sharing a common letter are significantly different at  $p < 0.05$  using Tukey's test.



**Fig. 2.** Percentage mortality of larvae after 1<sup>st</sup> and 2<sup>nd</sup> spray of insecticides.

### Conclusion

In all situations, all pesticides that were tested were successful in controlling maize fall armyworms. When utilized in an integrated pest management program, these pesticides were crucial. Additional investigation was required to examine *S. frugiperda* ability to acquire resistance to each of the chosen insecticides over a five-year period.

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