

## Journal of Plant Protection and Pathology

Journal homepage & Available online at: [www.jpmp.journals.ekb.eg](http://www.jpmp.journals.ekb.eg)

### The Latent Impacts of Oxadiazine Insecticide, Indoxacarb, on Varying Biological Characteristics of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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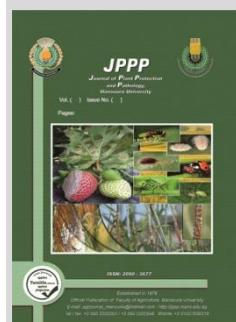
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#### ABSTRACT

The oxadiazine insecticide, indoxacarb, known for its potent efficacy against numerous Lepidopterous species, is widely marketed under various trade names in the pesticide industry. This study evaluated the acute toxicity and sublethal effects of indoxacarb on different life stages of *Spodoptera frugiperda*. The larval stage proved most susceptible to indoxacarb via ingestion; the 96-hour LC<sub>50</sub> value for larvae was 0.064 ppm, significantly lower than the 24-hour LC<sub>50</sub> of 0.57 ppm, demonstrating increased toxicity with prolonged exposure. Adults exhibited higher tolerance, with LC<sub>50</sub> values ranging from 6.87 to 14.61 ppm, while the egg stage was the most tolerant, showing an LC<sub>50</sub> of 322.21 ppm. Sublethal exposure (96-hour LC<sub>25</sub>) significantly impacted larval development and adult reproduction. Cumulative larval mortality reached 54%, and only 47% of treated larvae successfully pupated, compared to 88% in the control. Larval and pupal durations were significantly prolonged to 14.22 and 17.19 days, respectively, versus 11.25 and 14.69 days in the control. Daily food consumption and larval weight gain were also significantly reduced. Furthermore, adult females emerging from treated larvae laid significantly fewer eggs, and egg hatchability dropped to 61.65% compared to 96.14% in the control. These findings underscore indoxacarb's potent larvicidal activity and its significant sublethal effects on development and reproduction. Therefore, indoxacarb is a valuable tool for *S. frugiperda* management, particularly when applied to early larval instars, and should be integrated into comprehensive IPM strategies to maximize its effectiveness against this key pest.

**Keywords:** Lepidopterous species, Sublethal exposure, larvicidal.



#### Article Information

Received 6 / 9 / 2025

Accepted 4 / 10 / 2025

#### INTRODUCTION

The Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is a highly destructive pest of maize, posing a significant threat to global maize production. This polyphagous migratory pest is capable of causing severe economic losses in over 80 crops (Bakry and Abdel-Baky, 2023; Anandhi *et al.*, 2020; Caniço *et al.*, 2020; Maruthadurai and Ramesh, 2020; Goergen *et al.*, 2016), making it one of the most damaging maize pests (Anjorin *et al.*, 2022). Its rapid spread has particularly impacted Africa (Goergen *et al.*, 2016), where maize is among its preferred crops (Prasanna *et al.*, 2018). FAW is now regarded as the most harmful pest affecting maize in Egypt and globally due to its extensive dispersal (Bakry and Abdel-Baky, 2023). This widespread distribution is attributed to its strong migratory and dispersal abilities (Meagher *et al.*, 2004; Kumela *et al.*, 2018). With several generations annually, adult FAW can fly up to 100 kilometers per night (FAO, 2017), marking it as a nocturnal, invasive, and potentially dangerous pest threatening maize production and food security (Yigezu and Wakgari, 2020; Caniço *et al.*, 2020; Sun *et al.*, 2021). FAW attacks maize at all developmental stages, resulting in significant yield losses due to reduced photosynthesis, delayed growth, and reproductive setbacks (Chimweta *et al.*, 2020). Females lay 150–200 eggs per group on both upper and lower leaf surfaces, covering them with brownish hairs (Tendeng *et al.*, 2019). Newly hatched larvae scrape chlorophyll, leaving a transparent film that progresses

to white spots, elongated lesions, and bullet holes. Early instars feed on leaf tissue from one side while sparing the epidermal layer. These activities cause maize yield losses, with studies estimating reductions of 17–18% when 20% of plants are infested during the mid-whorl stage (Cruz *et al.*, 1996) and losses reaching 58% in severe cases (Cruz *et al.*, 1999; Lima *et al.*, 2010; García-Gutiérrez *et al.*, 2012). In Brazil, annual losses are valued at \$400 million (Figueiredo *et al.*, 2005). FAW was first reported in Africa in 2016, causing widespread maize damage and economic losses. In Egypt, its presence was confirmed in May 2019 in a maize field in Kom Ombo, Aswan Governorate (APC, 2019). According to the FAO, FAW likely entered Africa via ships or planes in 2016, subsequently invading more than 40 countries and threatening food security for 300 million people. Understanding FAW biology is crucial for developing effective Integrated Pest Management (IPM) strategies. FAW management is complicated by its polyphagy, migratory nature, and high reproductive potential, with larvae inflicting severe foliage and cob damage (Kumela *et al.*, 2019; Tambo *et al.*, 2019).

Chemical insecticides, such as indoxacarb, are widely used for control (Day *et al.*, 2017). In 2020, indoxacarb was recommended by Egyptian Ministry of Agriculture (Hafez *et al.*, 2020) as an effective oxadiazine insecticide targeting various lepidopteran, coleopteran and sucking pests (Wing *et al.*, 2000). Indoxacarb is metabolized into DCJW (N-decarbomethoxylated JW-062), a sodium channel blocker causing paralysis and death (Adhikari *et al.*, 2020). While it is most effective when ingested, its contact activity is limited

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DOI: 10.21608/jppp.2025.421274.1382

(Hou *et al.*, 2021). Indoxacarb's safety, environmental compatibility, and quick action make it a valuable tool for sustainable pest management. Easo WG-30%, which contains indoxacarb as the active ingredient works by disrupting sodium ion channels in insect nerve cells, causing paralysis and death. It rapidly stops insect feeding (2-8 hours) and kills within 1-2 days. Effective through both ingestion and contact, it targets most larval stages. Notably, it's safe for many beneficial insects and remains effective in hot climates (Cui *et al.*, 2018). This study was conducted to evaluate the acute and sublethal effects of indoxacarb on different life stages of *S. frugiperda*, a major agricultural pest. Due to increasing resistance to conventional insecticides and limited data on indoxacarb's impact on this species, the study aimed to determine LC<sub>50</sub> values for eggs, larvae, and adults, and assess sublethal effects on larval development, food consumption, and reproduction. The findings support the use of indoxacarb, especially against early larval stages, as part of integrated pest management (IPM) programs.

## MATERIALS AND METHODS

Armyworm egg, larvae, and adults used in this study were sourced from a laboratory colony maintained under controlled conditions, free from exposure to any insecticides. All experiments were conducted at a constant temperature of  $25 \pm 2^\circ\text{C}$  in the laboratory of the Plant Protection Department, Faculty of Agriculture, Minia University.

The insecticide tested was Easo WG-30%, which contains indoxacarb as the active ingredient, manufactured by Shoura Chemicals. It is used at a rate of 15 grams per 100 liters of water to control armyworm in maize. For this study, newly laid eggs (0–24 hours old), newly emerged adults (0–24 hours old), and 4<sup>th</sup> instar larvae (7 days old, with an average weight of 14.6–14.8 mg) were used to assess the toxicity of indoxacarb across these life stages. Toxicity was measured based on LC<sub>50</sub> and LC<sub>90</sub> values.

To evaluate the ovicidal activity of indoxacarb, maize (*Zea mays* L.) leaves containing freshly laid egg masses were dipped for 60 seconds in various concentrations of the insecticide diluted with water. Five days after treatment, the percentage of hatching was recorded and corrected for natural mortality in the untreated control using Abbott's formula (Abbott, 1925). Mortality data were transformed to probit units, plotted against the log of the concentrations, and analyzed using Finney's probit method (Finney, 1971) to generate LC-P line data.

Newly emerged adults were offered a 10% sugar solution mixed with different concentrations of indoxacarb. The adults were allowed to feed on this contaminated solution for four days. Mortality was recorded daily, corrected for control mortality, and analyzed using probit analysis as described by Finney (1971).

For the larval stage, the fourth instar larvae were provided with maize leaves treated with varying concentrations of indoxacarb. Larvae were allowed to feed on the treated leaves for four days, and mortality was recorded daily up to four days post-treatment. Mortality rates were corrected for untreated control mortality and subjected to Finney probit analysis as described above.

To investigate the effects of indoxacarb on certain biological parameters of the Fall armyworm, newly molted fourth instar larvae were individually isolated in plastic vials containing fresh maize leaf discs. The vials were covered with

perforated lids for ventilation. Two groups, each consisted of 100 larvae were prepared for the study: one group received untreated leaves as a control, while the second one was fed maize leaves treated with indoxacarb at a concentration corresponding to the 96-hour LC<sub>25</sub>, determined in a previous study (Smith *et al.*, 2021). Each group was further divided into 10 replicates each with ten larvae. Daily observations were made to monitor food consumption, larval weight, mortality, and the duration of the fourth larval stage until pupation. Surviving larvae were weighed daily, and the amount of food consumed was calculated. The pupal duration was also recorded and then adult sex was determined. For both treated and control groups, 10 pairs of adults were placed in jars (500 g capacity) containing branches of *Nerium oleander* (Tafla) and a piece of cotton soaked in a 10% sugar solution. The jars were covered with muslin cloth secured with a rubber band. The jars were observed daily to collect egg masses deposited on the leaves. These leaves were placed in Petri dishes after counting the eggs. For each replicate, the total number of eggs laid and the percentage of viable eggs were recorded. Mean egg numbers and hatching percentages were used to compare fecundity and fertility between control and treated groups using an independent *t*-test (Statistical Program).

## RESULTS AND DISCUSSION

### 1. Assessment of Acute Toxicity of indoxacarb on Different Life Stages of *Spodoptera frugiperda*

#### Larval stage

Among the three life stages tested, the larval stage exhibited the highest sensitivity to indoxacarb through ingestion. As the duration of feeding by 4<sup>th</sup> instar larvae on indoxacarb-treated maize leaves increased, both LC<sub>50</sub> and LC<sub>90</sub> values significantly decreased (as indicated by the overlapping of upper and lower confidence limits in Table, 1). The 24-hour LC<sub>50</sub> value [0.57 (0.45–0.67)] was approximately 9 times greater than the 96-hour LC<sub>50</sub> value [0.064 (0.054–0.081)]. A similar trend was observed for LC<sub>90</sub> values, with the 24-hour LC<sub>90</sub> recorded at 1.62 (1.36–1.85), which significantly decreased to 0.35 (0.25–0.46) after 96 hours of feeding on treated leaves. The significant acute toxicity of indoxacarb to armyworm larvae observed in this study aligns with previous research. Divya (2021) reported that indoxacarb was highly toxic to armyworm larvae based on LC<sub>50</sub> values. Similarly, Zhang *et al.* (2024) determined that the LC<sub>50</sub> of indoxacarb for *Spodoptera frugiperda* was 0.0061 ppm, making it the most toxic insecticide among those evaluated. Furthermore, determined the LD<sub>50</sub> value of indoxacarb to be 0.0019 µg/g larvae. Field studies corroborate the effectiveness of indoxacarb against armyworm larvae. For instance, Gutierrez-Moreno *et al.* (2019) evaluated the toxicity of various insecticides, including indoxacarb, and found that it was one of the most potent compounds against larval populations of *S. frugiperda* collected from maize fields. Their results indicated low LC<sub>50</sub> values for indoxacarb, consistent with our data, especially under prolonged exposure. Furthermore, Hardke *et al.* (2023) conducted bioassays on *S. frugiperda* and reported that indoxacarb showed high efficacy against late instars. Their study demonstrated that the ingestion route was more toxic than topical application, suggesting that indoxacarb acts effectively through feeding a mode of action that correlates

well with the ingestion-based exposure tested in our study. Another relevant study by Yu (2022) examined the susceptibility of *S. frugiperda* populations from different geographical regions to various insecticides. Indoxacarb consistently produced low LC<sub>50</sub> values, particularly in regions where resistance to other insecticides like pyrethroids was already evident. This suggests that indoxacarb remains an effective alternative for managing resistant larval populations. The high sensitivity of the larval stage may be attributed to the specific mode of action of indoxacarb. As a pro-insecticide, indoxacarb is metabolically activated within the insect to block voltage-gated sodium channels, resulting in feeding cessation, paralysis, and eventual death (Wing *et al.*, 2000). 4<sup>th</sup> instar larvae, being voracious feeders, are likely to ingest a lethal dose more quickly, thereby exhibiting higher mortality rates. Moreover, the importance of exposure duration in toxicity was also noted by Leite *et al.* (2023), who demonstrated that indoxacarb exhibited delayed but

progressive mortality in *S. frugiperda* larvae, with significant increases in mortality observed after 72–96 hours of continuous feeding on treated foliage. Collectively, the evidence from this study and Adhikari *et al.* (2020) research reinforces the utility of indoxacarb as a valuable component in the integrated pest management (IPM) of *S. frugiperda*, particularly targeting the larval stages. Its ingestion-based activity and delayed mortality allow treated plants to act as toxic baits, disrupting feeding and development while minimizing immediate pest damage. Additionally, Susanto *et al.* (2021) confirmed that indoxacarb is one of the newer insecticides effectively controlling ARW larvae infestations in maize fields. The LC<sub>50</sub> values observed in the current study were higher than those reported in earlier studies, likely due to variations in the armyworm strain used, differences in application methods, or formulations. Adjuvants, which enhance the physical properties of insecticides, may also contribute to variations in toxicity.

**Table 1. LC-P line data derived from the toxicity assays of Indoxacarb on newly molted 4<sup>th</sup> instar larvae (leaf disc feeding assay), newly emerged adults (sugar solution feeding assay), and 0-24hr old eggs (egg-dipping assay) of *Spodoptera frugiperda*.**

Tested stage	Time post treatment	Calculated Slope ± SE	C. $\chi^2$	T. $\chi^2$	df	LC <sub>50</sub> as ppm (95% CL)	LC <sub>90</sub> as ppm (95% CL)
Larval stage	24hr	2.62 ± 0.48	3.89	19.58	4	0.57 (0.45-0.67) <sup>a</sup>	1.62 (1.36-1.85)
	48hr	1.85 ± 0.31	6.72	24.43	7	0.29 (0.16-0.33) <sup>b</sup>	0.89 (0.77-0.99)
	72hr	1.89 ± 0.33	2.93	22.57	6	0.099 (0.073-0.12) <sup>c</sup>	0.55 (0.47-0.66)
	96hr	1.92 ± 0.41	0.36	18.58	4	0.064 (0.054-0.081) <sup>d</sup>	0.35 (0.25-0.46)
Adult stage	24hr	1.93 ± 0.27	3.73	18.58	4	14.61 (11.97-17.93) <sup>a</sup>	59.88 (48.49-83.85)
	48hr	1.55 ± 0.28	6.38	18.58	4	12.49 (9.47-16.59) <sup>a</sup>	47.15 (36.97-56.56)
	72hr	2.18 ± 0.38	1.73	20.63	5	10.27 (8.69-12.17) <sup>a</sup>	35.95 (28.85-44.91)
	96hr	2.77 ± 0.62	3.40	16.38	3	6.87 (3.68-9.53) <sup>b</sup>	18.23 (15.29-22.70)
Egg stage	120hr	1.27 ± 0.24	1.33	24.43	7	322.21 (213.31-453.23)	3025.95 (2035.51-5536.35)

For each stage, the LC<sub>50</sub> values followed by the same letter are not significantly different.

C.  $\chi^2$  means calculated Chi-square and T.  $\chi^2$  means tabulated Chi-square. When tabulated  $\chi^2$  is greater than calculated  $\chi^2$ , the differences are insignificant and this expresses the great fitness of probit data toward the toxicity lines which increase the confidence in the established probit data.

In addition to the consistency of our results with prior studies on the same insect species, our findings also correspond with research involving other Lepidoptera pests. Several earlier studies have investigated the acute toxicity of indoxacarb, with a particular focus on its significant effectiveness, as determined through probit analysis. For example, Ahmad *et al.* (2021) demonstrated that indoxacarb was the most effective treatment for *Spodoptera litura* larvae, outperforming other insecticides such as lufenuron, spinosad, and emamectin benzoate. Samad *et al.* (2022) determined the LC<sub>50</sub> values of indoxacarb for 2<sup>nd</sup> and 4<sup>th</sup> instar larvae after 48 hours to be 0.005 and 0.008 ppm, respectively. In a more recent study by Soliman *et al.* (2023), it was found that indoxacarb was the most potent larvicidal product against *Spodoptera frugiperda*, with an LC<sub>50</sub> value of 0.01 mg/kg, followed by spinosad, methoxyfenozide, and chlorpyrifos-methyl, whose LC<sub>50</sub> values ranged from 0.2 to 0.7 mg/kg. Bird (2017) established the LD<sub>50</sub> of indoxacarb on beet armyworm (*Helicoverpa armigera*) to be 0.04 µg/larva, while Cui *et al.* (2018) tested the compound using both topical and feeding methods. Their results showed the LD<sub>50</sub> for indoxacarb to be 3.86 × 10<sup>-3</sup> µg/larva.

#### Adult stage

The mortality of adults exposed to sugar solution contaminated with indoxacarb did not show a significant increase during the first 72 hours post-treatment. However, a significant increase in mortality was observed on the fourth day of continuous exposure to the treated sugar solution. The LC<sub>50</sub> values remained statistically similar during the first three

days, but significant differences began to appear on the fourth day (Table 1). The LC<sub>50</sub> and LC<sub>90</sub> values for 24 hours (expressed as mg active ingredient per liter) were 14.61 (11.97–17.93) and 59.88 (48.49–83.85), respectively. Extending the feeding period to four days on the treated sugar syrup led to a reduction in both LC<sub>50</sub> and LC<sub>90</sub> values, which decreased to 6.87 (3.68–9.53) and 18.23 (15.29–22.70), respectively. In this study, adult insects exhibited a 25.6-fold (based on the 24-hour LC<sub>50</sub>) to 107.3-fold (based on the 96-hour LC<sub>50</sub>) greater tolerance to indoxacarb than the larvae. The larval LC<sub>50</sub> ranged from 0.064 ppm at 96 hours post-treatment to 0.57 ppm at 24 hours post-treatment. In contrast, the corresponding LC<sub>50</sub> values for adults ranged from 6.87 to 14.61 ppm. According to a study by Worku *et al.* (2019), it was found that adults of *Spodoptera frugiperda* in their research were more sensitive to indoxacarb compared to the adults of *S. frugiperda* used in the current study. Additionally, Wu *et al.* (2022) reported that indoxacarb was highly toxic to wild *Spodoptera frugiperda* males, with LC<sub>50</sub> values (95% confidence limits) of 0.718 (0.532–0.878), 0.525 (0.316–0.751), and 0.182 (0.06–0.294) ppm for 24, 48, and 72 hours of exposure, respectively.

#### Egg stage

The egg stage was identified as the most tolerant, with an LC<sub>50</sub> (expressed as ppm) of 322.21 (213.31–453.23) (Table 1). The corresponding LC<sub>90</sub> value was significantly higher, reaching 3025.95 (2035.51–5536.35). Studies on the ovicidal activity of indoxacarb are limited. These findings align with the broader understanding that insect eggs often exhibit greater tolerance to

insecticides due to their protective chorion, which acts as a physical barrier, limiting the penetration and internal activity of chemical agents (Shelton *et al.*, 2001). In the case of indoxacarb, which relies on metabolic activation within the insect's body to exert its toxic effects, this limited penetration can greatly diminish its efficacy on non-feeding stages such as eggs. Previous research on *S. frugiperda* also supports this observation. For instance, studies by Leite *et al.* (2023) and Gutierrez-Moreno *et al.* (2019) primarily focused on the larval stages due to their higher susceptibility and economic importance in crop damage. These studies either did not include the egg stage or reported minimal ovicidal activity of indoxacarb, reinforcing the notion that this insecticide is less effective at controlling *S. frugiperda* at the egg stage. Similarly, Hardke *et al.* (2023) observed that indoxacarb's toxicity was highly stage-dependent, with ingestion by actively feeding larvae leading to significantly greater mortality. Since eggs do not consume external nutrients and possess low metabolic activity, the pro-insecticide nature of indoxacarb may result in reduced bioactivation, further contributing to its limited ovicidal action. This reduced sensitivity of eggs also implies that the timing of application is crucial when using indoxacarb in pest management programs. Targeting larval stages shortly after hatching may yield better results than attempting to suppress pest populations at the oviposition phase. This approach aligns with the recommendations of Yu (2022), who emphasized that chemical control of *S. frugiperda* is most effective when applied against feeding stages. In Contrast, Wu and Wang (2023) reported no ovicidal activity when metaflumizone mixed with chlorantraniliprole or indoxacarb was applied against the eggs of the fall armyworm, *Spodoptera frugiperda*. Zhao *et al.* (2020) noted that indoxacarb and emamectin produced noteworthy LC<sub>50</sub> values, approximately 2 mg/kg, which contrasts with the findings of the current study. This discrepancy is likely due to differences in application methods. Ganiger *et al.* (2018) examined the ovicidal activity of indoxacarb on *Spodoptera frugiperda* eggs, reporting the highest percentages of unhatched eggs (94.44%) with indoxacarb (0.01%), followed by emamectin benzoate (0.005%). Kuate *et al.* (2019) attributed the high ovicidal activity observed in their research to differences in application methods. This contrasts with the current findings, where the egg stage demonstrated high tolerance to indoxacarb, as reflected by the LC<sub>50</sub> and LC<sub>90</sub> values.

Overall, while indoxacarb shows strong toxicity against *S. frugiperda* larvae and moderate effects on adults, its limited efficacy against eggs highlights the importance of integrated pest management (IPM) strategies that combine chemical, biological, and cultural controls to target all life stages effectively.

## 2. Sublethal Impacts of indoxacarb on Various Biological Traits of *S. frugiperda*

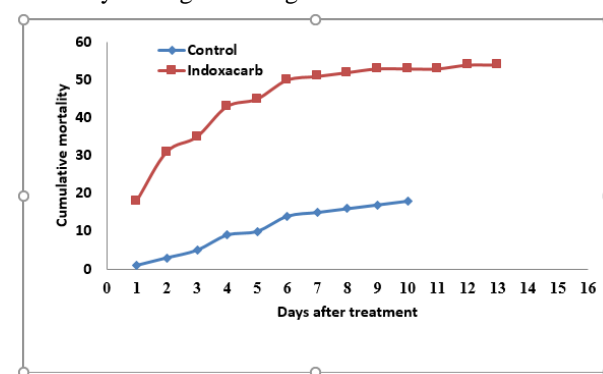
In this study, fourth instar larvae, freshly molted, were fed maize leaves treated with indoxacarb at an estimated concentration equivalent to the 96-hour LC<sub>25</sub>. The research investigated latent impacts on larval survival, daily food consumption, average larval weight, development durations of the larval and pupal stages, and adult fecundity and fertility.

### Accumulated mortality during the larval stage

As illustrated in Fig. (1), mortality was monitored until pupation. Cumulative mortality increased over time, reaching 54% in the chemical treatment group by the end of the larval stage, with only 47% of indoxacarb-treated larvae successfully pupating. In contrast, 88% of untreated larvae in

the control group successfully pupated. Except for the first two days post-treatment, the mean mortality percentages were significantly higher in the indoxacarb treatment group compared to the untreated control (unpaired t-test). These findings are consistent with earlier studies demonstrating the efficacy of indoxacarb against *S. frugiperda* and other Lepidopteran pests. For instance, Sayyed *et al.* (2008) reported high larval mortality in indoxacarb-treated *Helicoverpa armigera*, another economically important noctuid pest. Similarly, Yu and McCord (2021) observed significant suppression of *S. frugiperda* larval populations following indoxacarb exposure, with marked impacts on survival and development. Research by Boaventura *et al.* (2020) also emphasized indoxacarb's strong larvicidal activity against field populations of *S. frugiperda*, including those with varying degrees of insecticide resistance.

These studies reinforce the conclusion that indoxacarb has substantial larvicidal efficacy, acting through ingestion and contact, and disrupting sodium channel function, ultimately leading to feeding cessation and death.



**Fig. 1. Cumulative mortality percentages of newly molted 4<sup>th</sup> instar *S. frugiperda* larvae fed on maize leaves treated with Indoxacarb at the 96hr LC<sub>25</sub> concentration.**

### Amount of food consumed daily

Larvae fed on maize leaves treated with indoxacarb consumed significantly less food starting from the second day post-treatment (Fig. 2). With the exception of the 24-hour post-treatment period, food consumption was consistently lower in the indoxacarb-treated group compared to the untreated control. The highest food consumption in the indoxacarb group occurred on day 11, but it dropped sharply on days 12 and 13 as some larvae ceased feeding before entering the pre-pupal stage. By day 14, no food consumption was recorded as all surviving larvae had transitioned to the pre-pupal and pupal stages. In the control group, the peak food consumption was on day 9, followed by a significant decrease on day 10 as most larvae stopped feeding just before entering the pre-pupal stage. From day 11 onwards, no food consumption was observed in the control group, as all surviving larvae had converted to pre-pupae and pupae. Kokou *et al.* (2023) supported these findings, showing that sub-lethal concentrations of indoxacarb (LC<sub>5</sub>, LC<sub>10</sub>, and LC<sub>20</sub>) significantly reduced the growth and food intake of *Spodoptera frugiperda* larvae, along with a marked decrease in amylase activity in the midgut of larvae treated with indoxacarb. Similarly, Bharadwaj *et al.* (2020) reported significantly lower maize leaf consumption by *Spodoptera frugiperda* larvae exposed to indoxacarb, chlorantraniliprole, and spinetoram treatments compared to those fed untreated maize leaves. In the current study, total food consumption throughout the entire period (from the beginning of the 4<sup>th</sup> instar to the end of the 6<sup>th</sup> instar) was calculated (Fig. 3) and



compared between the control and treatment groups. Despite the significant reduction in daily food consumption in the indoxacarb treatment compared to the control, also, total food consumption was significantly different between the two treatments (Unpaired t-test). Furthermore, total food consumption was greater (1501.42 mg/larva) in the control than in the indoxacarb treatment (1296.03 mg/larva), though the difference was statistically significant. The application of indoxacarb appears to have a negative impact on the cumulative food amount when compared to the control.

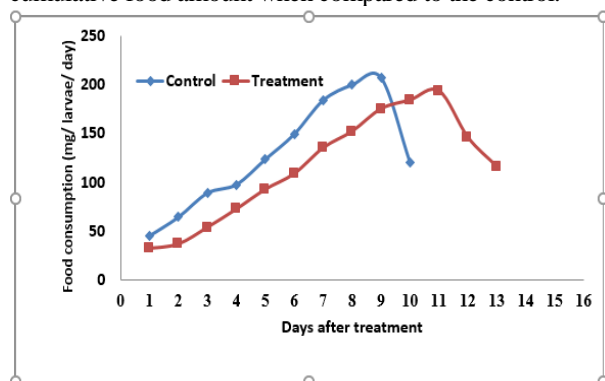


Figure 2. Daily food consumption (Mean  $\pm$  SD) by newly molted 4<sup>th</sup> instar *S. frugiperda* larvae fed on maize leaves treated with Indoxacarb at the 96hr LC<sub>25</sub> concentration.

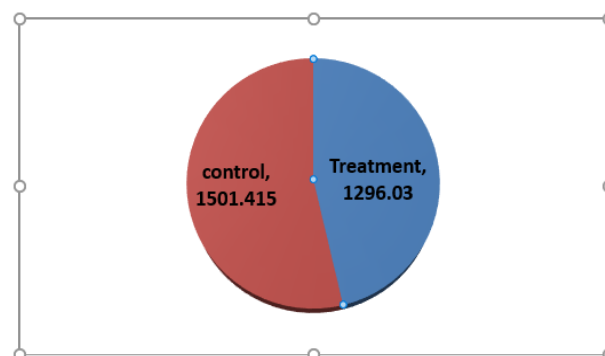


Fig. 3. Total food consumption (Mean  $\pm$  SD) from the 4<sup>th</sup> instar larvae to pupation, fed on untreated and Indoxacarb-treated leaves at the 96hr LC<sub>25</sub> concentration.

This suggests that indoxacarb may reduce the feeding rate or limit food accumulation in the treated larvae. Such an effect could be attributed to its influence on metabolic functions, appetite suppression, or alterations in feeding behavior. Consequently, indoxacarb may exert an inhibitory or suppressive effect on the overall growth and feeding activity of the target larvae. Similar findings have been reported by Al-Ayat *et al.* (2022), who investigated the effects of sublethal concentrations of indoxacarb (LC<sub>5</sub>, LC<sub>10</sub>, and LC<sub>20</sub>) on *S. frugiperda* larvae. Their findings revealed a noticeable reduction in both larval growth and food consumption. Additionally, they observed a significant decline in amylase activity within the larval midgut, indicating potential disruptions in digestive function. Kannan and Jeyalakshmi (2016) observed reduced food consumption and growth rate in *Spodoptera litura* after exposure to indoxacarb. Likewise, Brar *et al.* (2020) demonstrated that indoxacarb significantly reduced larval feeding and growth of *Helicoverpa armigera*, another economically important lepidopteran pest. These results suggest that indoxacarb may exert an inhibitory or suppressive effect on the overall growth

and feeding activity of the target larvae, making it an effective component in integrated pest management programs.

#### Larval weight on a daily basis

The changes in the mean weight of larvae over time are shown in Fig. (4). With the exception of the measurements taken on day 0 (just before treatment) and day 1 (24 hours post-treatment), the average weight of larvae in the chemical treatment group was significantly lower than that in the untreated control group. This difference can primarily be attributed to reduced food intake. The highest weight gain was recorded on day 10 for the control group and on day 13 for the chemical treatment group. This suggests that larvae in the chemical treatment group experienced slower growth, taking 13 days to reach the same weight that the control larvae achieved in 10 days. As anticipated, weight gain was closely linked to food consumption in both treatment groups (Fig. 5).

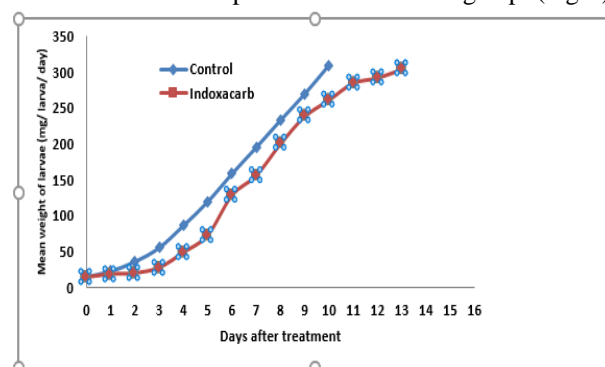


Fig. 4. Daily larval weight (Mean  $\pm$  SD) of newly molted 4<sup>th</sup> instar *S. frugiperda* larvae fed on maize leaves treated with Indoxacarb at the 96hr LC<sub>25</sub> concentration.

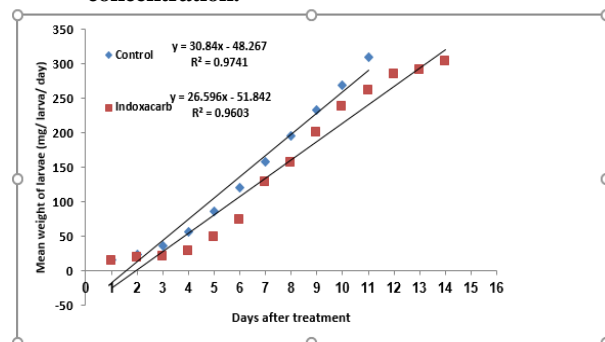


Fig. 5. Mean larval weight versus daily food consumption in 4<sup>th</sup> instar *S. frugiperda* larvae fed on untreated or Indoxacarb -treated maize leaves at the 96hr LC<sub>25</sub> concentration.

A strong linear correlation between daily larval weight and daily food consumption was observed in both treatments. Moreover, the high and significant correlation ( $R^2 = 0.960$  for indoxacarb and  $R^2 = 0.974$  for the control group) further suggests that the chemical may primarily influence feeding behavior, rather than affecting digestive enzyme activities. These findings are in agreement with previous studies on lepidopteran pests. For instance, Kannan and Jeyalakshmi (2016) reported that indoxacarb exposure significantly reduced larval weight gain in *Spodoptera litura*, attributing it to decreased feeding activity. Similar results were reported by Saini *et al.* (2020), who observed that indoxacarb caused sublethal effects on growth and development in *S. frugiperda*, including delayed weight gain and extended larval duration. Additionally, Brar *et al.* (2020)

demonstrated that reduced larval biomass in *Helicoverpa armigera* was associated with lower food consumption following treatment with indoxacarb, reinforcing the hypothesis that the compound primarily disrupts feeding behavior rather than metabolism or digestion directly. This finding contrasts with that of Sagar *et al.* (2020), who observed a significant decrease in amylase activity in the midgut of larvae treated with indoxacarb.

### 3. Developmental process and reproductive potential of insects Larval and pupal durations

The effects of indoxacarb on insect development and reproductive potential are summarized in Table (2). In the chemical treatment group, the duration of the larval stage was significantly extended. Larvae exposed to sub-lethal concentrations of indoxacarb exhibited slower growth, requiring an average of 14.22 days to reach the pupal stage, whereas the untreated control group took only 11.25 days (Table 2).

**Table 2. Development and reproductive potential of *S. frugiperda* when newly molted 4<sup>th</sup> instar larvae were fed on maize leaves treated with Indoxacarb at the 96hr LC<sub>25</sub> concentration.**

Measured parameter	Mean $\pm$ SD		P-value
	Control	indoxacarb	
Larval duration	14.69 $\pm$ 0.32	17.19 $\pm$ 0.23	0.054
%Pupation	88.0 $\pm$ 6.27	47.0 $\pm$ 4.27	0.008
Pupal duration	11.25 $\pm$ 0.52	14.22 $\pm$ 0.28	0.059
%Emergence#	100 $\pm$ 0.0	89.50 $\pm$ 12.28	0.88
%Emergence##	86.0 $\pm$ 6.27	45.0 $\pm$ 6.99	0.009
%Female	51.25 $\pm$ 16.94	49.26 $\pm$ 21.28	0.84
No. of eggs/female	1108.29 $\pm$ 61.86	858.50 $\pm$ 28.81	0.009
% egg hatching	96.14 $\pm$ 9.51	61.65 $\pm$ 6.42	0.009

#related to number of formed pupae

## Related to number of treated larvae

The difference in larval duration between the control and chemical treatment groups was found to be significant (Unpaired t-test). Additionally, pupal duration was 17.19 days in the chemical treatment group, compared to 14.69 days in the control group, with this difference also being statistically significant. Camara *et al.* (2018) observed that sublethal concentrations of insecticides, including diamides (which share some modes of action with indoxacarb), significantly extended the larval and pupal developmental periods. Their work highlights the metabolic stress induced by these compounds, leading to slower growth rates. Similarly, Abdel-Rahman (2014), working with *Agrotis ipsilon* (black cutworm, another lepidopteran pest), found that exposure to various insecticides at sublethal doses resulted in extended larval and pupal durations, mirroring our observations. This suggests a common physiological response across related insect species to insecticide exposure. Janssen and Van Loon (2012) demonstrated that sublethal doses of insecticides can prolong developmental times in Coleoptera (beetles), indicating that this phenomenon is not limited to a single insect order. These studies collectively reinforce the understanding that insecticide exposure, even at sublethal concentrations, can disrupt the normal developmental trajectory of insects, leading to prolonged immature stages. This has significant implications for pest management strategies, as longer developmental periods can affect population dynamics and the overall life cycle of the pest.

#### Percentages of pupation and adult emergence

Approximately 88.0% of untreated larvae successfully pupated, compared to only 47.0% in the chemical treatment group, with this difference being highly significant (Table 2). All pupae in the control treatment successfully developed into adults, whereas 89.50% of the

pupae in the chemical treatment group reached the adult stage. However, when adult emergence percentages were calculated based on the number of treated larvae, the difference between the chemical and control treatments was highly significant. This suggests that larvae in the indoxacarb treatment that successfully pupated did not experience prolonged toxic effects during the pupal stage. Furthermore, no significant difference was observed between the female ratios in the chemical and control treatments. Huang *et al.* (2009), studying *Spodoptera litura* (Oriental leafworm), found that sublethal insecticide doses significantly reduced both pupation rates and adult emergence. Their work suggests that physiological stress experienced during larval development can have lasting, negative carryover effects on subsequent life stages, even without immediate mortality. This directly supports our observation of decreased pupation percentages. Similarly, Li *et al.* (2018) reported a substantial decrease in successful pupation and adult eclosion in *Plutella xylostella* (diamondback moth) after exposure to various insecticides. They stressed that even if some larvae manage to survive and pupate, these developmental bottlenecks can severely impede overall population growth. Beyond Lepidoptera, these adverse effects on metamorphic success are a broader phenomenon across different insect orders. For instance, Mahjoor *et al.* (2019) showed that sublethal concentrations of certain insecticides reduced pupation and adult emergence rates in the Coleoptera species *Tribolium castaneum* (red flour beetle). While our study found no significant impact on female ratios, which is consistent with some literature suggesting sex determination might be less sensitive to these specific sublethal effects of indoxacarb, the overall reduction in the number of emerging adults from treated larvae remains a key factor in limiting population growth, regardless of the sex ratio.

#### Adult fecundity and fertility

Females that emerged from pupae treated during the larval stage laid significantly fewer eggs than those that emerged in the control treatment. Additionally, 61.65% of the eggs laid by females in the chemical treatment hatched, compared to 96.14% of the eggs in the control treatment. Exposure to indoxacarb at a sublethal concentration (96hr LC<sub>25</sub>) adversely affected larval survival, daily food consumption, average larval weight, prolonged larval and pupal durations, and reduced adult fecundity and fertility. These findings are consistent with those of Oumar *et al.* (2023), who fed 4<sup>th</sup> instar larvae of *Spodoptera frugiperda* maize leaves treated with the estimated LC<sub>25</sub> value of indoxacarb. They observed significant reductions in both female and male pupal weights, adult fecundity, and egg hatchability. Similarly, Kumar and Mohan (2020) reported that the fecundity of *S. frugiperda* was significantly decreased after treating 3<sup>rd</sup> instar larvae with sub-lethal concentrations of indoxacarb. Furthermore, Salem *et al.* (2023) found that newly emerged *S. frugiperda* adults, when exposed to sublethal concentrations of indoxacarb mixed with 2.5 M sucrose as a feeding stimulant, experienced reduced larval hatch rates and decreased mating frequency in females. Additionally, larval survival to the pupal stage was significantly reduced.

## CONCLUSION

This study assessed indoxacarb's toxicity on *S. frugiperda*'s life stages, finding larvae most susceptible, with increasing exposure significantly lowering LC<sub>50</sub>/LC<sub>90</sub> values. Adults showed higher tolerance, while eggs were the most

resistant stage, likely due to their protective chorion and the pro-insecticide's need for metabolic activation. Sublethal indoxacarb exposure severely impacted larval survival, reduced feeding and weight gain, prolonged developmental durations, and significantly impaired adult fecundity and egg fertility. These results emphasize indoxacarb's efficacy against larvae and the need for IPM strategies targeting all life stages due to varying susceptibilities. The findings highlight indoxacarb's utility for *S. frugiperda* control, particularly by targeting early larval instars and allowing sufficient time for full efficacy.

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## التأثيرات المتأخرة للمبيد الحشري إندوكسكارب التابع لمجموعة أوكساديازين على الخصائص البيولوجية المتنوعة لدودة الحشد الخريفية

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### المخلص

يُعد الإندوكسكارب، معروفاً بفعاليتّه العالية ضد العديد من أنواع حشرية الأجنحة. هدفت هذه الدراسة إلى تقييم السمية الحادة والتأثيرات تحت المميّة للإندوكسكارب على المراحل المختلفة من دورة حياة دودة الحشد الخريفية (*Spodoptera frugiperda*). وقد ثبت أن الطور اليرقي هو الأكثر حساسية للإندوكسكارب عند تناوله عن طريق الفم؛ إذ بلغ تركيز  $LC_{50}$  بعد ٩٦ ساعة ٠,٠٦٤ جزء في المليون. أظهرت الحشرات البالغة تحملاً أعلى، إذ تراوحت قيم  $LC_{50}$  لديها ٦,٨٧ جزء في المليون، في حين كان طور البيضة هو الأكثر تحملاً، حيث بلغ  $LC_{50}$  قيمة ٣٢٢,٢١ جزء في المليون. أثر التعرض للجرعة تحت القاتلة ( $LC_{25}$  بعد ٩٦ ساعة) بشكل كبير على نمو اليرقات وتكاثرها. حيث بلغت نسبة الوفيات التراكمية في الطور اليرقي ٥٤%، ولم تتج سوى ٤٧% من اليرقات المعاملة في التحول إلى العزراء، مقارنةً بـ ٨٨% في مجموعة الكنترول. كما طالت مدة الطور اليرقي والعزري بشكل ملحوظ لتصل إلى ١٤,٢٢ و ١٧,١٩ يوماً على التوالي، مقارنةً بـ ١١,٢٥ و ١٤,٦٩ يوماً في الكنترول. كذلك، انخفض كل من استهلاك الغذاء اليومي وزيادة الوزن اليرقي بشكل ملحوظ. علاوة على ذلك، فإن الإناث البالغة الناتجة عن اليرقات المعاملة وضعت عدداً أقل بكثير من البيض، كما انخفضت نسبة فقس البيض إلى ٦١,٦٥% مقارنةً بـ ٩٦,١٤% في الكنترول. تؤكد هذه النتائج الفعالية القوية للإندوكسكارب كمبيد يرقات، بالإضافة إلى تأثيراته تحت القاتلة على النمو والتكاثر. وبالتالي، يُعد الإندوكسكارب أداة قيمة لإدارة دودة الحشد الخريفية، خصوصاً عند استخدامه ضد الأطوار اليرقية المبكرة، وينبغي دمجه ضمن استراتيجيات الإدارة المتكاملة للآفات (IPM) لتحقيق أقصى فعالية في مكافحة هذه الآفة الرئيسية.