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Combined effects of tomato cultivars and ingested insecticides on digestive α-amylase activity and biological parameters of *Helicoverpa armigera*

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ABSTRACT

The cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera; Noctuidae) is a polyphagous plant pest which causes great damage to tomato crops worldwide. This study evaluated the combined effects of four tomato cultivars (Monalisa, Super-Chef, King-Star, and Falat-111) and four gut-acting insecticides (spinosad, chlorantraniliprole, indoxacarb, and lufenuron), on digestive α-amylase activity and selected biological parameters of *H. armigera*. Larvae from the third to sixth instars were fed leaf discs treated with LC_{30} concentrations of each insecticide, and α-amylase activity, larval mortality, larval developmental period, final-instar weight, and adult fecundity were assessed. Notably, the highest inhibition of larval digestive α-amylase activity (75.02%) was recorded in the Super-Chef×chlorantraniliprole combination at the 3rd instar, whereas the lowest (19.38%) was observed in the Monalisa×lufenuron treatment at the 6th instar. Similarly, the combination of the Super-Chef cultivar with chlorantraniliprole and spinosad exhibited the strongest effects on the biological traits. In the Super-Chef×spinosad treatment, final larval weight was reduced to 98.37 mg compared with 341.77 mg in the Monalisa×control, while adult fecundity declined from 426.33 to 84.67 eggs in the Super-Chef × chlorantraniliprole treatment. The larval developmental period was extended to 29.12 days in Super-Chef×spinosad, while the shortest duration was observed in Monalisa×control. The highest level of larval mortality was recorded in Super-Chef×chlorantraniliprole, whereas the lowest was observed in Monalisa×lufenuron. Overall, the combination of the Super-Chef cultivar with chlorantraniliprole or spinosad provided the strongest suppression of *H. armigera*, indicating that integrating resistant tomato cultivars with effective insecticides can enhance pest control and support sustainable integrated pest management programs.

Keywords: Cotton bollworm, Enzyme inhibition, Gut-acting insecticides, Host plant resistance.

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INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a globally distributed and highly polyphagous pest causing severe yield losses in many major crops. Due to its growing resistance to chemical insecticides and transgenic crops, future control strategies must rely on integrated pest management (IPM) that combines biological, chemical, and physical approaches for sustainable suppression (Devi et al., 2024; Stavrakaki et al., 2024).

Alpha-amylases, key digestive enzymes in insects, hydrolyze starch and glycogen into glucose, supporting energy needs, including during non-feeding stages like pupation (Da Lage et al., 2018; Wang et al., 2023). These enzymes, critical for digestive efficiency, are prime targets for disruption (Saadati et al., 2007). Host plant quality and diet composition significantly affect α-amylase activity in polyphagous species like *H. armigera*, which exhibits enzymatic plasticity to optimize nutrient use across diverse hosts, influencing survival and growth (Sarate et al., 2012; Da Lage et al., 2018).

Insecticides significantly influence digestive enzyme activity. With diverse chemical structures, they exhibit toxicity through multiple mechanisms and can interact with both target and non-target biomolecules such as receptors and enzymes (Gupta et al., 2019). Exposure to different pesticides can differentially alter activities of digestive enzymes, such as α-amylases, proteases, lipases, and sucrases, potentially influencing insect growth, survival, and overall fitness (Gupta et al., 2019; Xu et al., 2021). Interactions between host plants and insecticides increase the complexity of pest management by influencing pesticide efficacy and pest susceptibility. Plant phytochemicals such as alkaloids, flavonoids, and tannins can enhance or reduce insecticide toxicity, highlighting the need to consider plant–insecticide interactions in developing sustainable control strategies (Hanson & Koch, 2018; Guo et al., 2022).

The physiological complexity of the digestive system, coupled with its capacity to regulate enzyme activity according to developmental stage and diet, underpins its polyphagous nature and allows it to overcome challenges posed by host plant defenses and insecticidal inputs (Zhang et al., 2024a; Dar et al., 2024). Although previous studies have separately examined the effects of host plants or insecticides on α-amylase activity, the combined effects of these factors remain poorly understood. Therefore, the present study aimed to investigate the interactions among digestive enzymes, host plants, and insecticides, to identify the least suitable tomato cultivar for larval feeding and the most effective insecticidal treatment. To this end, α-amylase activity in the

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midgut of third- to sixth-instar larvae fed on different tomato cultivars treated with various insecticides was evaluated.

MATERIALS AND METHODS

Plant and insect rearing

Tomato seeds of various cultivars, sourced from the Seed and Plant Improvement Institute (Karaj, Iran), were germinated in the University of Tabriz's Department of Plant Protection greenhouse. Seedlings were transplanted to experimental plots at Khalat-Poushan Research Station with 50 cm spacing, maintained with manual weeding and weekly irrigation. Four cultivars—Monalisa, Super-Chef, King-Star, and Falat-111—were chosen as larval host plants.

Helicoverpa armigera larvae, collected from the field, were reared in a greenhouse. To prevent cannibalism, fourth-instar larvae were individually housed in 40 mL aerated plastic cups. Pupae were placed in dark containers to mimic soil conditions. For adult emergence, 15 moth pairs were kept in mesh-covered cylindrical cages (24 cm×16.5 cm) for oviposition. Larvae were fed a chickpea-based artificial diet (Hamed & Nadeem, 2008), and adults received 10% honey solution. All bioassays and insect rearing procedures were conducted under controlled laboratory conditions at 28 ± 2 °C, 50 ± 5% relative humidity, and a 16:8 h (L:D) photoperiod.

Enzyme extract preparation

For each three treatment, three independent biological replicates were prepared. In each biological replicate, midguts (30, 20, 10, 5 from 3rd to 6th instar larvae) were dissected under a stereomicroscope (JENUS, SZM-45-B8T, China) and homogenized in 500 µL distilled water using a homogenizer (Ultra turax T8, IKA, Germany). The homogenates were centrifuged (Universal 320R, Hettich, Germany) at 10,000 rpm for 30 minutes at 4 °C, and the supernatants were stored at −20 °C as enzyme sources. Protein content was estimated using the Bradford (1976) method and adjusted to 4 mg/mL by distilled water.

Alpha-amylase activity assays

Larval digestive α-amylase activity was measured using a modified Bernfeld (1955) method. For each three biological replicate, enzyme activity was measured in three technical replicates. Enzyme extract (10 µL) was mixed with 65 µL universal buffer (pH 10) and 25 µL 1% starch, incubated at 37 °C for 30 min, then mixed with 100 µL DNS reagent and heated in a boiling

water bath for 10 min. Absorbance of 190 µL samples was read at 540 nm in a microplate reader (BioTek Instruments, Winooski, VT, USA).

In vitro α-amylase inhibition assay

In preliminary tests, the effects of various insecticides on α-amylase activity in third-instar larvae were assessed. Insecticides showing significant reduction in α-amylase activity - indoxacarb, lufenuron, chlorantraniliprole, and spinosad- were selected. For enzyme preparation, midgut extracts were obtained from 30 untreated third-instar larvae homogenized in 500 µL distilled water, as described above. For each insecticide concentration (50, 150, 450, 1350, and 4000 µg mL^{-1}), 10 µL of enzyme extract was incubated with the insecticide solution for 30 min at 37 °C prior to substrate addition. Enzymatic activity was then determined using the α-amylase assay described previously. Each concentration was evaluated using three biological replicates, and the entire experiment was repeated three independent times, resulting in a total of nine biological observations per concentration. Enzyme activity measurements within each biological replicate were performed in triplicate to ensure analytical accuracy. In parallel, control reactions without insecticide were prepared and used for calculation of enzyme inhibition.

Inhibition percentage was calculated relative to the control using the following formula:

$$\text{Inhibition } \% = \left[1 - \left(\frac{y' - y}{x' - x}\right)\right] \times 100$$

Where: X = control without enzyme and inhibitor, X′ = control with enzyme and without inhibitor, Y = control without enzyme and with inhibitor, and Y′ = treatment with enzyme and inhibitor.

Toxicity bioassay and LC_{30} estimation

Preliminary bioassays were conducted to determine the concentration ranges required for LC_{10}–LC_{90} estimation in the tested population of *Helicoverpa armigera*. Toxicity bioassays were performed using the leaf-dip method according to IRAC Susceptibility Test Method No. 007 (IRAC, 2009) with five logarithmically spaced concentrations of each insecticide (Table 1) and an untreated control. For each concentration, three replicates of 10 third-instar larvae were used (30 larvae per concentration). The entire bioassay was repeated three independent times. Treated leaf discs were prepared using the tomato cultivar ‘Petopride’, which provided suitable feeding conditions for larval development. Individual third-instar larvae were allowed to feed on treated leaf discs, and mortality was assessed 24 h after exposure. Mortality data were subjected to

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probit analysis to estimate lethal concentrations (LC values) (Finney, 1971), and the LC_{30} values obtained for each insecticide were subsequently used in the biological assays. Mortality data from five concentrations, each tested in three replicates and repeated in three independent experimental runs (45 observations per insecticide), were subjected to probit regression. Goodness-of-fit of the model was evaluated using Pearson's chi-square test. Degrees of freedom were calculated as the number of observations minus the number of estimated model parameters (intercept and slope)).

Table 1. Logarithmically spaced concentrations of insecticides used in main experiments.

Insecticides	Concentrations (µg/mL)				
chlorantraniliprole	25	52	110	235	500
spinosad	10	30	100	300	1000
indoxacarb	125	250	500	1000	2000
lufenuron	500	780	1220	1920	3000

Biological assays using LC_{30} concentrations

The LC_{30} concentrations of the selected insecticides were applied to leaf discs of the tested tomato cultivars using a fine brush. Each treated leaf disc was placed in an 8-cm Petri dish containing a single 24-h-old third-instar larva. For each treatment combination (cultivar × insecticide), 50 larvae were used per replicate. For each cultivar × insecticide combination, three biological replicates were used, and the entire experiment was repeated independently three times. Therefore, each treatment combination consisted of nine independent observations that were included in the statistical analyses. All larvae were maintained under controlled laboratory conditions at 28 ± 2 °C, 50 ± 5% relative humidity, and a 16:8 h (L:D) photoperiod. Larvae were inspected daily, and mortality and molting events were recorded every 24 h. Larvae were monitored throughout their development, and the following biological parameters were recorded: larval mortality, larval developmental period (was defined as the number of days from egg hatching to pupation), final-instar larval weight (was measured 24 h after molting to the sixth instar), and adult fecundity. For fecundity assessment, seven pairs of newly emerged adults (<24 h old) from each treatment were used per replicate. Each pair was maintained in a transparent plastic cup (6 cm diameter × 8 cm height) covered with a fine-mesh net that served as an oviposition substrate. Adults were provided daily with 10% honey solution and maintained under the same environmental conditions described above. The oviposition net was replaced every 24 h, and the number of eggs deposited on the net was counted under a stereomicroscope. Egg production was monitored throughout the lifetime of each female, and the total number of eggs

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laid per female was recorded as fecundity. Midgut extracts from surviving third-, fourth-, fifth-, and sixth-instar larvae were prepared as previously described, and α-amylase activity was determined. Enzyme inhibition was calculated relative to the untreated control and expressed as percentage inhibition.

Data analysis

Data normality was confirmed using the Kolmogorov–Smirnov test. Experiments followed a completely randomized design, assessing enzyme activity influenced by tomato cultivars, insecticides, and biological parameters. One-way ANOVA analyzed individual effects, while cultivar×insecticide interactions were evaluated using the General Linear Model (GLM) in SPSS (version 26.0; IBM Corp., Armonk, NY, USA). For α-amylase inhibition assays, percentage inhibition values were calculated relative to the untreated control, which was incorporated into the inhibition formula and therefore was not included as a separate treatment in the statistical analysis. The effects of tomato cultivar, insecticide treatment, and their interaction on α-amylase inhibition were analyzed separately for each larval instar using two-way ANOVA. For biological parameters (larval mortality, larval developmental period, final-instar larval weight, and adult fecundity), the untreated control was included as an independent treatment. Therefore, data were analyzed using two-way ANOVA with tomato cultivar and treatment (four insecticides plus control) as fixed factors.When significant differences were detected, means were compared using Tukey's honestly significant difference (HSD) test at ($P < 0.05$). Lethal concentrations (LC_{30}, LC_{50}, and LC_{90}) and their 95% confidence intervals were estimated by probit analysis according to Finney (1971). The goodness-of-fit of the probit models was evaluated using Pearson's chi-square test. Statistical significance was considered at $P < 0.05$ throughout the study. Differences in toxicity among insecticides were assessed by comparing the overlap of the 95% confidence intervals (95% CLs) of LC_{50} values.

RESULTS

***In vitro* α-amylase inhibition assay under insecticide treatments**

The results of α-amylase activity inhibition in third-instar larvae of *H. armigera* incubated by different insecticides revealed that chlorantraniliprole ($F_{(4,44)}$=193.36, $p<0.01$), spinosad ($F_{(4,44)}$=470.09, $p<0.01$), indoxacarb ($F_{(4,44)}$=529.87, $p<0.01$) and lufenuron ($F_{(4,44)}$=244.66, $p<0.01$) exhibited significant inhibitory effects, ranging from 57.63% with lufenuron to 74.43%

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with chlorantraniliprole. However, dose–response patterns varied significantly among treatments (Fig. 1). Chlorantraniliprole induced the highest α-amylase inhibition (74.43%) at 150 µg/mL, but inhibition gradually decreased with increasing concentrations up to 4000 µg/mL. Similarly, spinosad showed maximum α-amylase inhibition at 450 µg/mL, followed by a decline at higher concentrations. In contrast, indoxacarb and lufenuron displayed a different trend: α-amylase inhibition consistently increased up to 1350 µg/mL doses of these two insecticides.

Toxicity of the tested insecticides

The results of bioassays assessing the acute oral toxicity of the insecticides against third-instar larvae of *H. armigera* are presented in Table 2. Probit analysis revealed significant variation in toxicity levels among the four tested insecticides. Spinosad exhibited the highest toxicity, with the lowest LC_{50} value (58.92 µg/mL) and a relatively narrow confidence interval, whereas lufenuron showed the lowest toxicity (LC_{50}= 1408.06 µg/mL).

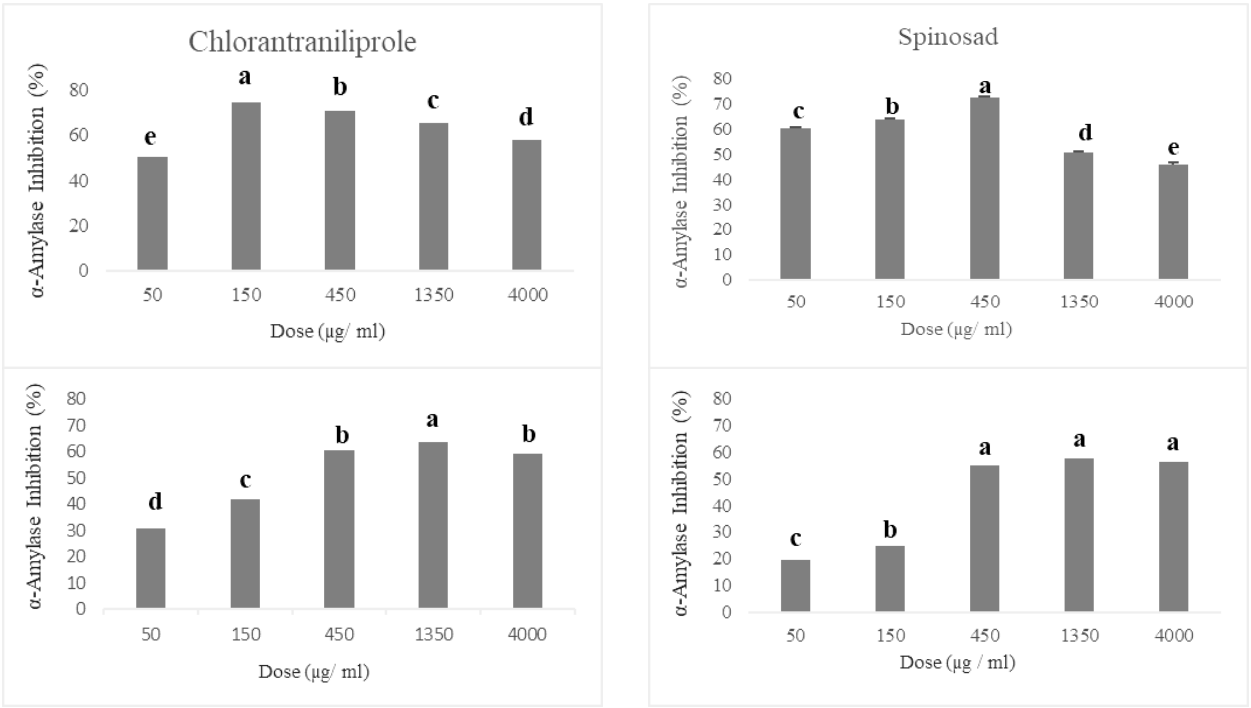

Fig. 1. The inhibitory effect of different concentrations of insecticides on α-amylase activity in the midgut of third instar larvae of *Helicoverpa armigera* under laboratory (*in vitro*) conditions. Data represent mean ± standard error of three replicates. Statistical comparisons among treatments were performed using ANOVA followed by Tukey's test, and different letters indicate significant differences at $P<0.01$.

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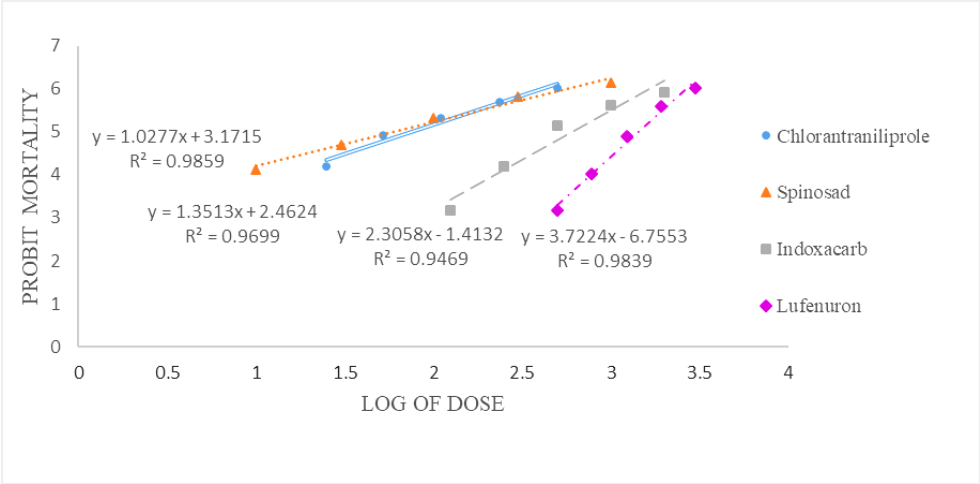
220 **Table 2.** Probit analysis for the toxicity of four insecticides against *Helicoverpa armigera.*

Treatments	χ^2 (df=43)	n	Sig.	Slope ± SE	Lethal concentration (µg/mL 95% FL*)		
					LC_{30}	LC_{50}	LC_{90}
Chlorantraniliprole	20.69	450	0.998	1.36 ± 0.15	30.35 (20.36 - 40.45)	73.96 (58.04 - 91.54)	651.90 (445.97 - 1135.12)
Sinosad	12.25	450	0.999	1.02 ± 0.10	17.97 (11.12 - 25.87)	58.92 (43 - 78.66)	1073.12 (658.59 - 2119.20)
Indoxacarb	35.70	450	0.777	2.18 ± 0.18	326.83 (272.33 - 381.11)	568.31 (492.85 - 656.40)	2196.68 (1737.05 - 2997)
Lufenuron	18.38	450	0.999	3.64 ± 0.30	1010.00 (907.60 - 1107.45)	1408.06 (1290.60 - 1540)	3171.60 (2748.37 - 3826.85)

221 * FL fiducial limits.

222 Examination of dose–response slopes (Fig. 2) indicated that lufenuron had a steeper slope,
223 suggesting a more uniform mortality response across concentrations. In contrast, spinosad
224 showed a shallower slope, reflecting greater variability in larval responses across doses.
225 Moreover, comparison of the 95% fiducial limits (95% FL) for LC_{50} values demonstrated partial
226 overlap between spinosad and chlorantraniliprole, indicating no significant difference in toxicity
227 between these two compounds. Conversely, the fiducial limits of indoxacarb and lufenuron did
228 not overlap with those of spinosad or chlorantraniliprole, confirming statistically significant
229 differences in their toxicities.



230

231 **Fig. 2.** Dose-response curves of *Helicoverpa armigera* larvae exposed to different
232 insecticides.
233
234 **Interaction between tomato cultivars and insecticides on α-amylase activity**

235 The LC_{30} concentration of the four insecticides significantly affected α-amylase activity in
236 third-instar larvae ($F_{(3,143)}$=161.28, p<0.01). Tomato cultivar also significantly affected activity
237 ($F_{(3,143)}$=83.89, p<0.01), with a significant insecticide-cultivar interaction ($F_{(9,143)}$=2.79, p<0.01).
238 The strongest α-amylase inhibition in third-instar larvae was seen with
239 chlorantraniliprole×Super-Chef (75.02%) and spinosad×Super-Chef (72.23%), while

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lufenuron×Falat-111 showed the weakest effect. In fourth-instar larvae, α-amylase activity was significantly influenced by insecticide treatment ($F_{(3,143)}$=388.106, p<0.01), tomato cultivar ($F_{(3,143)}$=47.164, p<0.01) and their interaction ($F_{(9,143)}$=16.381, p<0.01). The lowest inhibition was recorded for lufenuron×King-Star (40.3%), while chlorantraniliprole×Super-Chef produced the highest inhibition (69.95%). For fifth-instar larvae, α-amylase activity showed a significant response to insecticide ($F_{(3,143)}$= 282.53, p<0.01), cultivar ($F_{(3,143)}$=184.25, p<0.01) and thier interaction ($F_{(9,143)}$=8.12, p<0.01). At the fifth instar, the lowest α-amylase inhibition was observed with lufenuron×Monalisa (21.51%), indoxacarb×Monalisa (23.42%), and lufenuron×Falat-111 (24.51%). The highest inhibition occurred with chlorantraniliprole×Super-Chef (57.36%), spinosad×King-Star (56.45%), spinosad×Super-Chef (55.59%), and chlorantraniliprole×King-Star (53.33%). Similarly, In sixth-instar larvae, insecticide ($F_{(3,143)}$= 195.91, p<0.01), cultivar ($F_{(3,143)}$=120.02, p<0.01) and their interaction ($F_{(9,143)}$= 5.41, p<0.01) significantly affected α-amylase activity. The lowest inhibition was observed in lufenuron×Monalisa (19.38%), lufenuron×Falat-111 (21.06%), indoxacarb×Falat-111 (23.75%) and indoxacarb×Monalisa (25.82%). The highest inhibition was associated with chlorantraniliprole×Super-Chef (54.99%), spinosad×Super-Chef (54.02%), and chlorantraniliprole×King-Star (49.83%). These results indicate that insecticide efficacy on α-amylase inhibition is strongly influenced by host-plant cultivar. Mean α-amylase activity reduction (%) across third- to sixth-instar larvae is shown in Table 3.

Table 3. The *Helicoverpa armigera* third- to sixth-instar larval midgut α-amylase inhibition ratio [Mean±SE (n=9)], which fed on different tomato cultivars treated with LC_{30} value of various insecticides.

Cultivar	Insecticide	Total larvae per treatment (N)	3rd instar	4th instar	5th instar	6th instar
Monalisa	Chlorantraniliprole	150	64.26 ± 0.82 bcd	59.51 ± 1.20 de	46.28 ± 1.59 b	36.71 ± 1.43 bcde
	Spinosad	150	62.62 ± 1.25 cd	59.13 ± 0.71 e	35.85 ± 1.70 cd	40.74 ± 1.02 bc
	Indoxacarb	150	46.17 ± 1.19 gh	47.05 ± 0.91 gh	23.42 ± 1 f	25.82 ± 1.19 f
	Lufenuron	150	44.47 ± 1.52 gh	43.24 ± 0.80 hi	21.51 ± 0.88 f	19.38 ± 1.10 f
Super-Chef	Chlorantraniliprole	150	75.02 ± 0.61 a	69.95 ± 0.72 a	57.36 ± 1.10 a	54.99 ± 2.04 a
	Spinosad	150	72.23 ± 0.91 a	60.96 ± 0.92 cde	55.59 ± 1.56 a	54.02 ± 0.97 a
	Indoxacarb	150	62.61 ± 2.38 cd	58.59 ± 0.86 e	43.12 ± 1.35 b	42.49 ± 0.95 b
	Lufenuron	150	54.84 ± 2.38 ef	48.35 ± 0.93 fg	32.73 ± 1.66 de	34.13 ± 1.28 de
King-Star	Chlorantraniliprole	150	70.71 ± 1.60 ab	57.61 ± 1 e	53.33 ± 0.82 a	49.83 ± 1.11 a
	Spinosad	150	69.77 ± 1.70 abc	63.75 ± 0.76 bcd	56.45 ± 0.64 a	40.71 ± 2.54 bcd
	Indoxacarb	150	63.57 ± 1.92 bcd	52.71 ± 1.11 f	46.25 ± 1.21 b	35.07 ± 1.48 cde
	Lufenuron	150	49.17 ± 1.54 fg	40.30 ± 1.24 i	31.9 ± 1.33 de	32.57 ± 1.37 e
Falat-111	Chlorantraniliprole	150	58.54 ± 0.78 de	64.60 ± 0.78 bc	42.18 ± 0.65 b	42.60 ± 0.75 b
	Spinosad	150	60.70 ± 0.96 de	66.37 ± 0.75 ab	40.22 ± 1.22 bc	40.44 ± 0.76 bcd
	Indoxacarb	150	47.65 ± 1.82 fgh	47.39 ± 1.03 gh	27.28 ± 1.40 ef	23.75 ± 1.09 f
	Lufenuron	150	40.34 ± 1.23 h	45.24 ± 0.75 gh	24.51 ± 0.83 f	21.06 ± 1.02 f

Different letters within each column indicate significant differences among treatments according to two-way ANOVA followed by Tukey's test (P< 0.05).

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Interaction between host-plant cultivars and insecticides on the biological parameters

Table 4 shows that the Super-Chef cultivar combined with chlorantraniliprole and spinosad had the strongest impact on *H. armigera* biological traits. Insecticide ($F_{(4,59)}$=39.81, p<0.01), and cultivar ($F_{(3,59)}$=47.16, p<0.01) significantly affected larval developmental period, with a significant insecticide-cultivar interaction ($F_{(12,59)}$=3.84, p<0.01).

The larval developmental period was longest in Super-Chef×spinosad (29.13 days) and shortest in Monalisa×control (17.83 days). Insecticide ($F_{(4,59)}$=538.08, p<0.01), and cultivar ($F_{(3,59)}$=110.06, p<0.01) significantly affected fecundity, with a significant insecticide×cultivar interaction ($F_{(12,59)}$=6.33, p<0.01). Fecundity dropped to 84.67 eggs in Super-Chef×chlorantraniliprole, compared to 426.33 eggs in Monalisa×control. Larval mortality was significantly influenced by insecticide ($F_{(4,59)}$=246.98, p<0.01), and cultivar ($F_{(3,59)}$=23.34, p<0.01) but their interaction was not significant ($F_{(12,59)}$=1.04, p=0.432). Highest mortality occurred in Super-Chef×chlorantraniliprole (46.67%), and lowest (excluding controls) in Monalisa×lufenuron (20%). Across cultivars, lufenuron caused the lowest mortality, while chlorantraniliprole caused the highest, explaining the non-significant interaction. Final-instar larval weight was significantly affected by insecticide ($F_{(4,59)}$=365.24, p<0.01) and cultivar ($F_{(3,59)}$=52.65, p<0.01), but not their interaction ($F_{(12,59)}$=1.42, p=0.196). Weight was lowest in Super-Chef×spinosad (98.37 mg) compared to 341.77 mg in Monalisa×control.

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Table 4. Mean ± SE of Larval mortality, larval Period, Final instar larval weight and fecundity of *Helicoverpa armigera* reared on different tomato cultivars treated with various insecticides.

Cultivar	Insecticide	Total larvae (N)	Larval mortality (%) ± SE	Larval period (day) ± SE	Final-instar weight (mg) ± SE	Fecundity (Mean no. of egg per female) ± SE
Monalisa	Chlorantraniliprole	150	37.33 ± 1.76 bcd	19.82 ± 1.11 efg	158.80 ± 4.35 ef	126.71 ± 4.67 ghi
	Spinosad	150	28.67 ± 1.76 defgh	21.10 ± 0.91 defg	161.01 ± 7.54 ef	141.76 ± 6.69 gh
	Indoxacarb	150	26.67 ± 1.76 efgh	22.02 ± 0.07 cdef	218.57 ± 6.94 bcd	208.57 ± 4.95 de
	Lufenuron	150	20 ± 1.15 h	19.50 ± 0.94 efg	259.12 ± 4.06 b	262.76 ± 5.67 c
	Control	150	3.33 ± 0.67 i	17.83 ± 0.30 g	341.77 ± 5.44 a	426.33 ± 21.53 a
Super-Chef	Chlorantraniliprole	150	46.67 ± 0.67 a	27.55 ± 0.58 ab	124.67 ± 14.84 fg	84.67 ± 3.20 i
	Spinosad	150	38.67 ± 1.76 abc	29.13 ± 0.57 a	98.37 ± 2.65 g	94.57 ± 4.06 hi
	Indoxacarb	150	34.67 ± 1.76 bcde	25.37 ± 0.43 abc	153.77 ± 10.79 ef	99.05 ± 2.67 hi
	Lufenuron	150	28.67 ± 2.91 defgh	24.20 ± 1.07 bcd	182.11 ± 4.61 de	112.38 ± 3.40 ghi
	Control	150	6 ± 1.15 i	18.85 ± 0.89 efg	304.67 ± 3.32 a	320.62 ± 15.37 b
King-Star	Chlorantraniliprole	150	40 ± 1.15 ab	24.34 ± 0.85 bcd	126.64 ± 12.78 fg	102.43 ± 1.79 hi
	Spinosad	150	34 ± 3.46 bcdef	27.34 ± 0.44 ab	126.98 ± 3.75 fg	115.43 ± 3.18 ghi
	Indoxacarb	150	29.33 ± 0.67 defg	24.34 ± 0.39 bcd	189.93 ± 4.89 de	135.05 ± 7.45 gh
	Lufenuron	150	25.33 ± 1.33 fgh	22.18 ± 0.97 cdef	218.76 ± 4.19 bcd	154.05 ± 6.93 fg
	Control	150	4.67 ± 0.67 i	19.31 ± 0.36 efg	307.85 ± 4.80 a	349.24 ± 23.18 b
Falat-111	Chlorantraniliprole	150	34 ± 1.15 bcdef	22.69 ± 0.61 cde	163.10 ± 4.62 ef	123.67 ± 3.24 ghi
	Spinosad	150	3 ± 2.31 cdefg	22.47 ± 1.28 cdef	159.97 ± 6.99 ef	142.76 ± 3.91 gh
	Indoxacarb	150	26.67 ± 0.67 efgh	20.21 ± 0.73 defg	209.93 ± 5.74 cd	195.52 ± 4.07 ef
	Lufenuron	150	22 ± 2 gh	18.30 ± 0.42 fg	244.09 ± 7.78 bc	256.71 ± 5.73 cd
	Control	150	3.33 ± 1.76 i	18.73 ± 1.20 efg	329.56 ± 16.77 a	399.86 ± 10.06 a

Different letters within each column indicate significant differences among treatments according to two-way ANOVA followed by Tukey's test ($p < 0.05$, n=3).

DISCUSSION

Alpha-amylase, crucial for starch hydrolysis and sugar release in insect growth, is inhibited by plant-derived inhibitors or insecticides, impacting larval development, weight, and reproduction (Wang et al., 2023). The insecticides chlorantraniliprole, spinosad, indoxacarb, and lufenuron significantly inhibited α-amylase activity in third-instar *H. armigera* larvae, with varying potency and dose effects. *In vitro* assays revealed that spinosad and chlorantraniliprole showed non-linear inhibition, with diminished effects at higher doses, likely due to inhibitor–activator duality and enzyme conformational changes (Robin et al., 2018). Thus, reduced inhibition at higher doses likely reflects intrinsic enzyme kinetics rather than larval compensatory mechanisms. This dose-dependent response is consistent with Bartling et al. (2024), who reported that high insecticide doses can induce compensatory adjustments such as increased enzyme synthesis, isoenzyme variation, and enhanced metabolism. Likewise, Zhang et al. (2024 b) found that carboxylesterase activity in *Spodoptera frugiperda* increases at higher doses, linking elevated enzymatic activity with greater detoxification capacity.

Digestive bioassays showed that the four insecticides exhibited differential toxicity against third-instar *H. armigera* larvae. spinosad and chlorantraniliprole had the highest toxicity with

lower LC_{50} values, while lufenuron showed the lowest efficacy. Chlorantraniliprole activates ryanodine receptors, and spinosad stimulates the central nervous system, causing strong metabolic stress even at low doses. In contrast, indoxacarb and lufenuron, an insect growth regulator (IGR), act by disrupting molting and development, thus requiring higher doses and longer exposure (Cordova et al., 2006; Sparks & Nauen, 2015). Similar trends were reported by Saleem et al. (2024) and Kaur (2016), confirming higher toxicity of chlorantraniliprole and spinosad compared to indoxacarb and lufenuron.

The study showed that α-amylase inhibition in *H. armigera* larvae depended on both insecticide type and tomato cultivar. The "Super-Chef" cultivar exhibited the strongest inhibition, especially with chlorantraniliprole, reaching 75.02% and 69.95% in third- and fourth-instar larvae. This may result from phenolic or alkaloid compounds in Super-Chef acting synergistically with insecticides. In contrast, Monalisa and Falat-111 showed weaker inhibition, particularly with lufenuron and indoxacarb (<25%). This cultivar×insecticide interaction suggests that larval metabolism is influenced by the host's secondary metabolites (Eigenbrode & Trumble, 1994; War et al., 2012). Chlorantraniliprole and spinosad consistently produced higher inhibition across cultivars, whereas indoxacarb and lufenuron required higher doses to be effective, likely due to differences in their modes of action (Sparks & Nauen, 2015; Aly & Ali, 2024). Overall, α-amylase inhibition is governed by the interaction between host cultivar and insecticide type, integrating dual selective pressures that better represent field conditions compared with single-factor studies (Naseri et al., 2009; Baghery et al., 2014; Ashouri et al., 2017; Hemmati et al., 2022).

Enzyme inhibition was higher in third- and fourth-instar larvae than in later instars, likely due to greater detoxification capacity and activation of compensatory enzymes (esterases, GSTs, P450s) in older larvae. Thus, targeting early instars is critical for effective population control. Similar stage-dependent variations in digestive enzyme activity and gene expression were also reported by Ashouri and Farshbaf (2021) and Mahajan et al. (2013).

The biological performance of *H. armigera* was significantly affected by the combination of host cultivar and insecticide type, influencing larval weight, development time, fecundity, and mortality. The highest mortality (46.67%) occurred in the "Super-Chef×chlorantraniliprole" treatment, while the lowest (20%) was recorded in "Monalisa×lufenuron." These differences likely result from synergistic effects of secondary metabolites (e.g., phenolics, alkaloids) in Super-Chef enhancing chlorantraniliprole toxicity, whereas nutrient-rich Monalisa allowed

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partial recovery from lufenuron's milder action as an IGR. Similar synergistic effects were reported by Wangari et al. (2020) and Godbold et al. (2023). Developmental duration extended to 29.13 days in "Super-Chef×spinosad," compared with 17.83 days in "Monalisa" control, due to reduced digestive efficiency and energy imbalance. This delay increases exposure to predators and reduces relative growth rate, ultimately lowering adult weight and fecundity (Naseri et al., 2009). Final larval weight in "Super-Chef×spinosad" declined to 98.37 mg versus 341.77 mg in control, reflecting both α-amylase inhibition and increased metabolic costs for detoxification. Such reductions produce smaller adults with lower reproductive potential (Scriber & Slansky, 1981; Borzoui et al., 2017). Neuro–muscular insecticides such as spinosad and chlorantraniliprole disrupt mating and fecundity in *H. armigera* (Wang et al., 2009; Carneiro et al., 2016). The highest fecundity occurred in the Monalisa control (426.33 eggs), while the lowest (84.67 eggs) was recorded in the "Super-Chef×chlorantraniliprole" treatment, reflecting nutritional limitations that reduce adult lipid and protein reserves (Kang et al., 2022; Chamani et al., 2025).

CONCLUSIONS

This study demonstrated that the interaction between tomato cultivars and insecticide type significantly affected α-amylase activity, larval development, weight, fecundity, and mortality in *H. armigera*. Chlorantraniliprole and spinosad showed the strongest inhibitory and biological effects, particularly in resistant cultivars like Super-Chef, whereas lufenuron and indoxacarb required higher doses to produce similar outcomes. Growing evidence indicates that *H. armigera* populations have developed resistance to conventional insecticides such as indoxacarb and lufenuron, threatening the long-term effectiveness and sustainability of pest management strategies (Bird et al., 2023; Saleem et al., 2024). Integrating these resistant cultivars with selective insecticides in IPM programs effectively suppresses *H. armigera* populations, minimizes chemical use, and promotes sustainable pest management.

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اثرات ترکیبی ارقام گوجه‌فرنگی و حشره‌کش‌های روده‌اثر بر فعالیت آنزیم گوارشی آلفا-آمیلاز و مولفه‌های زیستی *Helicoverpa armigera*

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چکیده:
کرم غوزه پنبه، *Helicoverpa armigera* Hübner (Lepidoptera:Noctuidae) آفتی چندخوار است که خسارت زیادی به محصولات گوجه‌فرنگی در سراسر جهان وارد می‌کند. در این مطالعه، اثرات ترکیبی چهار رقم گوجه‌فرنگی (مونالیزا، سوپرشف، کینگ استار و فلات 111) و چهار حشره‌کش روده‌اثر (اسپینوساد، کلرانترانیلیپرول، ایندوکساکارب و لوفنورون) بر فعالیت آنزیم گوارشی آلفا-آمیلاز و برخی مولفه‌های زیستی این حشره مورد ارزیابی قرار گرفت. لاروهای سنین سوم تا ششم با دیسک‌های برگی تیمار شده با غلظت LC_{30} هر حشره‌کش تغذیه شدند و فعالیت آلفا-آمیلاز، تلفات لاروی، دوره رشد لاروی، وزن سن آخر لاروی و باروری حشرات کامل اندازه‌گیری شد. قابل توجه است که بالاترین میزان بازدارندگی فعالیت آلفا-آمیلاز گوارشی لارو (75/02 درصد) در ترکیب رقم سوپر شف با کلرانترانیلیپرول در سن سوم لاروی ثبت شد، در حالی که کمترین میزان (19/38درصد) در تیمار مونالیزا با لوفنورون در سن ششم لاروی مشاهده گردید. به طور مشابه، ترکیب رقم سوپرشف با کلرانترانیلیپرول و اسپینوساد قوی‌ترین اثرات را بر صفات زیستی نشان داد. در تیمار سوپرشف همراه با اسپینوساد، وزن نهایی لارو به 98/37 میلی‌گرم در مقایسه با 341/77 میلی‌گرم در تیمار شاهد مونالیزا کاهش یافت، در حالی که باروری حشرات کامل در تیمار سوپرشف همراه با کلرانترانیلیپرول از 426/33 به 84/67 تخم کاهش پیدا کرد. دوره رشد لاروی در تیمار سوپرشف همراه با اسپینوساد به 29/12روز افزایش یافت، در حالی که کوتاه‌ترین مدت در تیمار شاهد مونالیزا مشاهده شد. بالاترین میزان تلفات لاروی در ترکیب سوپرشف با کلرانترانیلیپرول ثبت گردید، در حالی که کمترین میزان در تیمار مونالیزا با لوفنورون مشاهده شد. به طور کلی، ترکیب رقم سوپرشف با کلرانترانیلیپرول یا اسپینوساد قوی‌ترین مهار را بر کرم غوزه پنبه اعمال نمود که نشان می‌دهد ادغام ارقام مقاوم گوجه‌فرنگی با حشره‌کش‌های مؤثر می‌تواند کنترل آفت را افزایش داده و از برنامه‌های مدیریت تلفیقی آفات پایدار پشتیبانی کند.