



## Effect of pyriproxyfen and diofenolan on the metamorphosis and enzymatic activities in tobacco cutworm, *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae)

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**Abstract.** *Spodoptera litura* (Lepidoptera: Noctuidae) is a polyphagous pest of agricultural crops and causes severe economic loss. In the present study, the biological effect and enzymatic activity of two insect growth regulators, pyriproxyfen and diofenolan was evaluated against *S. litura* larva. The penultimate instar larvae (0, 24, and 48-hours old) were treated topically at the last three abdominal tergites with sublethal doses (1, 2, and 4 µg/µl) of both the juvenile hormonal analogues (JHAs). Several effects were observed such as prolongation in larval-larval and larval-pupal ecdysial duration, larval and pupal mortality, eclosion failure, formation of intermediates, low pupation and reduced adult emergence. Along with these metamorphic catastrophe effects, both JHAs had inhibitory effects for acetylcholinesterase (AChE) but excitatory effects for glutathione -S-transferase enzyme (GST). Whereas, Log IC<sub>50</sub> and IC<sub>50</sub> values for adult emergence inhibition rate were found to be -0.98 and 0.1 µg/ µl for 24-hours old larvae (Pyriproxyfen); 0.005 and 1.01 µg/ µl for 48-hours old larvae (Pyriproxyfen); -0.085 and 0.82 µg/ µl for 24-hours old larvae (Diofenolan); -0.09 and 1.23 µg/ µl for 48-hours old larvae (Diofenolan). Our result manifests the insecticidal potency of both the IGRs against *S. litura*.

**Keywords:** Acetylcholinesterase, glutathione -S-transferase enzyme, juvenile hormonal, ecdysial

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## Introduction

*Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) is a polyphagous pest infesting a wide range of economically important crops leading severe economic loss worldwide (Mallikarjuna *et al.*, 2004; Ramaiah & Maheswari, 2018). Its larva is an active gregarious feeder and feed variously upon the young leaves preferably causing defoliation of the twig (Dhir *et al.*, 1992; Nasr *et al.*, 2010; Singh & Kumar, 2015; Maes, 2022). Constant deployment of conventional chemical pesticides for the control of this pest has resulted in the resurgence of the more resistant variety in the wild (Ramesh & Singh, 2022; Li *et al.*, 2023).

The widespread use and engagement of first and second-generation chemical pesticides to manage agricultural pests have raised concerns for other non-targeted insects such as bees (Hamaidia, 2014) and also pose a threat to public health and the environment (Kanaan, 2021). Therefore, to control of these negative impacts of chemical pesticides the concept of third-generation (hormone-based) pesticides was introduced (e.g. Pyriproxyfen, fenoxycarb and diofenolan) (Singh & Kumar, 2015; Subramanian & Shankarganesh, 2016; Maddheshiya, 2021). Hormonal analogs for insect pest control mainly include juvenile or ecdysone hormone analogs (JHAs/ EHAs). These insect growth regulators (IGRs) generally disturb the homeostasis of the endocrinological system in insects and cause developmental arrest (Hoffmann and Lorenz, 1998; Minakuchi & Riddiford, 2006). Pyriproxyfen and diofenolan are very stable non-terpenoidal aromatic JHAs and possess a broad spectrum of insecticidal activity and a shorter residual effect in the wild (Dhadialla *et al.*, 1998; Webb *et al.*, 2011). Therefore they are categorized as green pesticides (Smith *et al.*, 2021; Singh & Maddheshiya, 2022).

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Insects gain resistance against these insecticides due to certain enzymatic activities such as peroxides (POD), catalases (CAT), superoxide dismutase (SOD), glutathione  $\gamma$ -S-transferase enzyme (GST) and many others (Wu *et al.*, 2020). Superoxide dismutase is one of the major enzymes that provide defence against different insecticides (Wang *et al.*, 2011; Zhou *et al.*, 2018). Acetylcholinesterase (AChE) is also an important enzyme catalysing the hydrolysis of the key neurotransmitter acetylcholine (ACh) in the body organs. AChE concentration is affected by insecticides and other xenobiotics (Zibae *et al.*, 2009). GST is activated due to insecticidal toxicity in the hemolymph (Hu *et al.* 2019) In this study, the efficacy of two juvenile hormone analogs namely pyriproxyfen and diofenolan on metamorphosis and development of *S. litura* along with their effect on detoxifying enzymes (AChE and GST) was observed.

## Materials and methods

### Insect rearing

Egg masses of *S. litura* were collected from infected castor leaf and reared in the laboratory at  $27\pm 1^\circ\text{C}$ ,  $70\pm 5\%$  RH and 10L: 14D photoperiod in a 6-inch wide petridish (Fig. 1 A, B, and C). Naïve larvae were reared till the fifth instar stage on the fresh castor leaves. After the larvae reached penultimate instar, they were shifted to 6-inch wide glass trough containing 2-3 inches of sawdust for the purpose of pupation. The emerging adults were fed on 10% honey solution and fresh castor leaf was provided for oviposition (Singh & Kumar, 2015).

### Test Chemicals

Diofenolan (2-ethyl 4-[(4-phenoxyphenoxy) methyl]-1,3-dioxolane) and Pyriproxyfen [4-phenoxyphenyl (RS) 2-(2-pyridyloxy) propylether] were purchased from Sigma-Aldrich Chemicals Co. USA. Desired doses were prepared by dissolving a known amount of test chemicals in 1 ml of acetone (Merck).

### Experimental procedure

#### Morphological assay

Sublethal doses (1, 2, and 4  $\mu\text{g}/\mu\text{l}$ ) of pyriproxyfen and diofenolan were applied topically on last three tergites of the fifth instar larvae that were isolated from the same batch at 0, 24, and 48-hours (Lethal dose was 6 $\mu\text{g}/\mu\text{l}$ ). The control group received only diluent acetone as treatment. After treatment, larvae were transferred to containers with castor leaves. Regular inspections of both the control and treated batches were conducted every 24-hours until full emergence in control. For morphological analysis, dead or deformed larvae were fixed in Bouin's solution for 24-hours and then preserved in 70% ethyl alcohol.

#### Enzymatic Analysis

Hemolymph was collected after the cold anesthesia of 0, 24, and 48-hours old fifth instar larvae. Through a puncture in the cuticle, hemolymph was collected with a glass capillary and collected in cooled tubes with 4 mg/ml phenylthiourea added to stop melanin formation. The plasma component (cell-free) from the hemolymph was centrifuged at  $4^\circ\text{C}$  for 10 min at 11,000 rpm to ascertain the enzymatic activity.

#### GST Assay

The techniques used by Habig (1981) were adopted to conduct the GSTs assay. The collected sample was incubated with 0.1 M Na-phosphate buffer (pH 6.5), 1 mM DNFB, 1 mM glutathione, and 20  $\mu\text{l}$  of the solution for 5 minutes at  $25^\circ\text{C}$ . DNFB solution in acetone was used to start the reaction. At a wavelength of 340 nm, the concentration of 5-(2,4-dinitrophenyl) glutathione generated during the reaction was determined spectrophotometrically. A total of 10.0 nmol of CDNB can be conjugated with reduced glutathione using one unit of the enzyme every minute.

#### AChE Assay

Following the protocol used by (Ellman *et al.*, 1961), the activity of AChE was measured. The reaction mixture was made up of 50  $\mu\text{l}$  of sample solution, 100  $\mu\text{l}$  of 45  $\mu\text{M}$  5-5-dithiobis-(2-nitrobenzoic acid), 100 $\mu\text{l}$  of acetylthiocholine iodide, and 90  $\mu\text{l}$  of sodium phosphate buffer. A time duration of 40 minutes was spent tracking the change in absorbance at 405 nm. One milliunit of AChE activity is the amount of enzyme that will generate 1.0 nmol of choline per minute.

## Data Analysis

All measures were expressed as Mean  $\pm$  SEM and analyzed by one-way ANOVA followed by post-hoc Dunnett's multifactorial test using Graph Pad Prism 5.0. The alpha significance was set at  $P \leq 0.05$ . Photography was done using Nikon SMZ 1000 Binocular fitted with Nikon Digital Sight DS-U2 microscope and NIS-software (Nikon Corp., Japan).

## Results

### Developmental and morphological analysis

Topical administration of difenolan and pyriproxyfen has derailed the developmental path in the fifth instar larva of *S. litura*. Our result included prolongation in larval-larval and larval-pupal ecdysial duration, larval and pupal mortality, larval-pupal intermediate, ecdysial failure, reduced pupation and adult emergence. Both JHAs also severely affected the concentration of AChE and GST in the hemolymph.

Topical application of both IGRs to fifth instar larvae of *S. litura* resulted in delayed larval-larval and larval-pupal ecdysial duration (Fig. 2). The mean ecdysial duration due to pyriproxyfen and difenolan increased up to 1.6 and 1.2 days (0-hr. old larvae), 1.4 and 1.0 days (24-hrs. old larvae), and 0.9 and 0.5 days (48-hrs. old larvae) (Pyriproxyfen:  $r = 0.95^*$ ,  $0.96^*$  and  $0.97^*$  for 0, 24, and 48-hours old larvae respectively;  $P \leq 0.05$ ) (Difenolan:  $r = 0.95^*$ ,  $0.96^*$  and  $0.97^*$  for 0, 24 and 48-hours old larvae respectively;  $P \leq 0.05$ ).

Similarly, larval-pupal ecdysis duration was also delayed due to the topical treatment (Fig. 3). The mean ecdysial duration due to pyriproxyfen and difenolan increased up to 1.6 and 1.5 days (0-hr. old larvae), 1.1 and 0.9 days (24-hrs. old larvae), and 0.7 and 0.5 days (48 hrs. old larvae) (Pyriproxyfen:  $r = 0.93$ ,  $0.9$  and  $0.87$  for 0, 24, and 48-hours old larvae respectively;  $P \leq 0.05$ ) (Difenolan:  $r = 0.93$ ,  $0.9$  and  $0.87$  for 0, 24 and 48-hours old larvae respectively;  $P \leq 0.05$ ). At all treatment stages, the use of pyriproxyfen and difenolan on penultimate instar larvae of *S. litura* have resulted in a dose-related larval mortality. At all stages, mortality was highest at the  $4\mu\text{g}/\mu\text{l}$  dosage treatment. Both the JHAs were more effective in the early age of larvae as the effect was lowered in the 48-hours old larvae in comparison to the 0-hour old larvae. Correlation coefficient between the pyriproxyfen doses applied was found to be  $0.99^*$ ,  $0.99^{**}$ , and  $0.97^*$  for 0, 24, and 48-hours old larvae respectively ( $P \leq 0.05$ ). Similarly,  $0.9^*$ ,  $0.97^*$ , and  $0.88$  was correlation for the larval mortality due to difenolan for 0, 24, and 48-hours old larvae respectively ( $P \leq 0.05$ ) (Table. 1, 2).



**Fig. 1.** Showing (A-C) Infestation of *Spodoptera litura* upon the castor leaves; (D-E) Ecdysial failure showing presence of larval exuviae and rectal prolapse; (F-G) Larval-pupal intermediates showing presence of larval prolegs, mouth parts, larval true legs and exuviae attached at the posterior end.

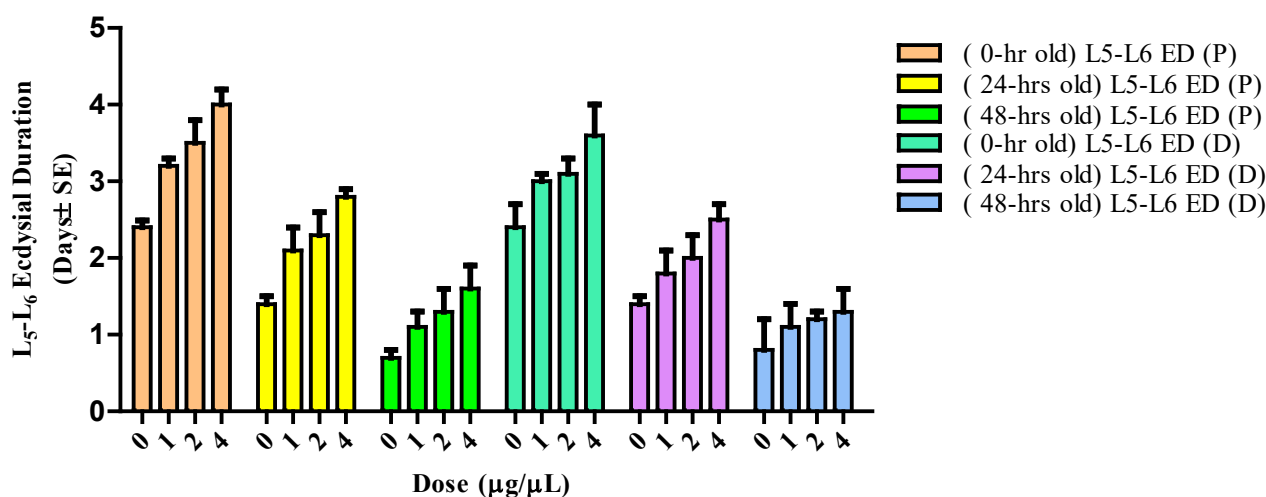
In all treated groups, the topical administration of both JHAs to *S. litura* fifth instar larvae resulted in ecdysial failure (Table 1, 2). Depending upon the degree of deformities, the ecdysial failure induced by pyriproxyfen in the penultimate larval instar of *S. litura* could be categorized as follows:

- (1) The larva was unable to molt completely into the next instar larva as the old larval exuvium was still attached to the posterior region of the body (Fig. 1D).
- (2) Ecdysial failure showing reduced body with larval exuvium attached to the body along with rectal prolapse (Fig. 1E).

**Table 1.** Effect of pyriproxyfen on the fifth instar larvae (0, 24, and 48-hours old) of *S. litura*. All data subjected to mean  $\pm$  SE ( $P \leq 0.05$ ).

Age	Dose	L-L ecdysial duration	L-P ecdysial duration	Larval mortality	EF	LPI	Pupation		Adult emergence
							mortality	normal	
0-hour	0	2.4 $\pm$ 0.09	2.7 $\pm$ 0.01	0	0	0	0	100	100
	1	3.2 $\pm$ 0.14	3.6 $\pm$ 0.1	30	10	0	20	40	0
	2	3.5 $\pm$ 0.3	3.9 $\pm$ 0.3	50	10	10	20	0	0
	4	4.0 $\pm$ 0.2	4.3 $\pm$ 0.3	80	0	0	20	0	0
	r	0.95*	0.93	0.99*	-0.17	0.10	0.68	-0.85	-0.68
24-hours	0	1.4 $\pm$ 0.11	1.8 $\pm$ 0.1	0	0	0	0	100	100
	1	2.1 $\pm$ 0.26	2.5 $\pm$ 0.2	10	20	0	0	70	10
	2	2.3 $\pm$ 0.3	2.6 $\pm$ 0.3	20	30	10	10	30	10
	4	2.8 $\pm$ 0.1	2.9 $\pm$ 0.1	50	20	10	20	0	0
	r	0.96*	0.90	0.99**	0.58	0.85	0.97*	-0.98*	-0.75
48-hours	0	0.7 $\pm$ 0.1	0.9 $\pm$ 0.1	0	0	0	0	100	100
	1	1.1 $\pm$ 0.2	1.4 $\pm$ 0.3	0	10	0	0	90	50
	2	1.3 $\pm$ 0.3	1.4 $\pm$ 0.2	10	10	0	0	80	20
	4	1.6 $\pm$ 0.3	1.6 $\pm$ 0.1	30	20	0	20	30	0
	r	0.97*	0.87	0.97*	0.96*	-	0.88	-0.97*	-0.93

L-L = Larval-larval; L-P = Larval-Pupal; EF = Ecdysial failure; LPI = Larval pupal intermediates; r = Correlation coefficient.



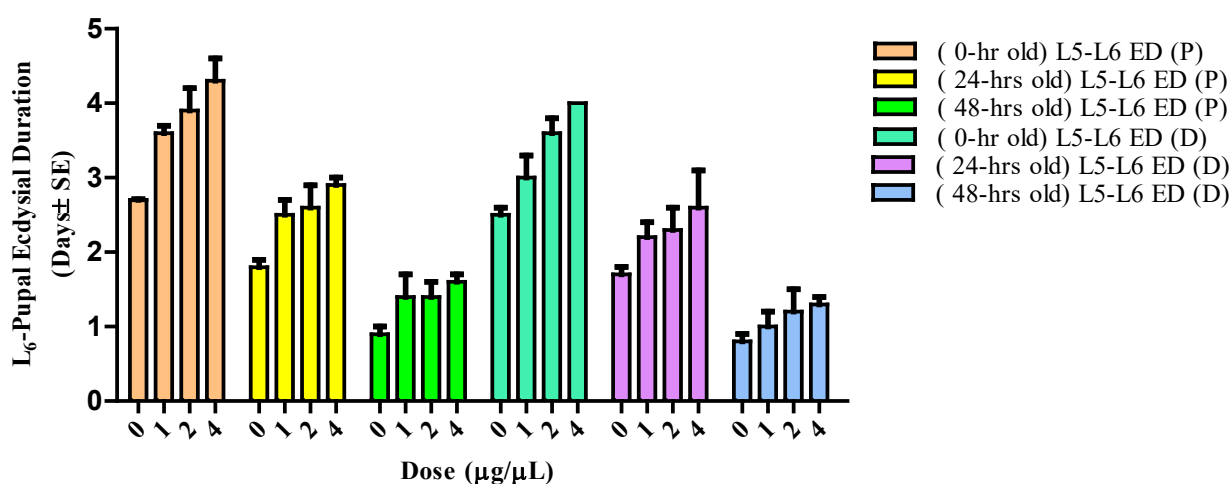
**Fig. 2.** Effect of pyriproxyfen and diofenolan on the larval-larval ecdysial duration of the fifth instar larvae of *S. litura*. All data subjected to mean  $\pm$  SE ( $P \leq 0.05$ ). ( $L_5$  = Fifth instar larvae;  $L_6$  = Sixth instar larvae; ED = Ecdysial duration; P & D = pyriproxyfen and diofenolan respectively).



**Table 2.** Effect of diofenolan on the fifth instar larvae (0, 24, and 48-hours old) of *S. litura*. All data subjected to mean  $\pm$ SE ( $P \leq 0.05$ ).

Age	Dose	L-L ecdysial duration	L-P ecdysial duration	Larval mortality	EF	LPI	Pupation mortality	Adult emergence	
								normal	
0-hour	0	2.4 $\pm$ 0.3	2.5 $\pm$ 0.1	0	0	0	0	100	100
	1	3.0 $\pm$ 0.1	3.0 $\pm$ 0.3	20	0	0	20	60	0
	2	3.1 $\pm$ 0.2	3.6 $\pm$ 0.2	40	0	10	30	20	0
	4	3.6 $\pm$ 0.4	4.0 $\pm$ 0.0	70	0	0	20	10	0
	r	0.95*	0.93	0.99*	-0.17	0.10	0.68	-0.85	-0.68
24-hours	0	1.4 $\pm$ 0.1	1.7 $\pm$ 0.1	0	0	0	0	100	100
	1	1.8 $\pm$ 0.3	2.2 $\pm$ 0.2	0	0	0	0	100	40
	2	2 $\pm$ 0.3	2.3 $\pm$ 0.3	20	20	10	10	40	20
	4	2.4 $\pm$ 0.2	2.6 $\pm$ 0.5	40	20	10	20	10	0
	r	0.96*	0.90	0.99**	0.58	0.85	0.97*	-0.98*	-0.75
48-hours	0	0.8 $\pm$ 0.4	0.8 $\pm$ 0.1	0	0	0	0	100	100
	1	1.1 $\pm$ 0.3	1.0 $\pm$ 0.2	0	0	0	0	100	70
	2	1.2 $\pm$ 0.1	1.2 $\pm$ 0.3	0	0	0	10	90	40
	4	1.3 $\pm$ 0.3	1.3 $\pm$ 0.1	20	10	0	20	50	20
	r	0.97*	0.87	0.97*	0.96*	-	0.88	-0.97*	-0.93

L-L = Larval-larval; L-P= Larval-Pupal; EF= Ecdysial failure; LPI= Larval pupal intermediates; r= Correlation coefficient.

**Fig. 3.** Effect of pyriproxyfen and diofenolan on the larval-pupal ecdysial duration on the fifth instar larvae of *S. litura*. All data subjected to mean  $\pm$  SE ( $P \leq 0.05$ ). (L6 = Sixth instar larvae; ED = Ecdysial duration; P & D = pyriproxyfen and diofenolan respectively).

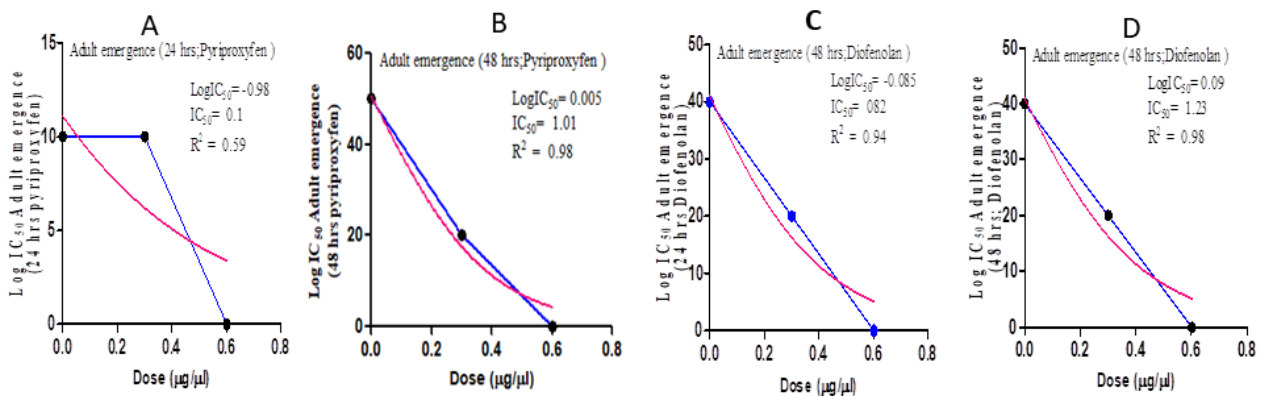
Administration of both JHAs to the fifth larval instar of tobacco cutworm resulted in the formation of larval-pupal intermediates (LPI) at the all the duration except 48 hours old larvae. The correlation coefficient for both the JHAs was positively correlated (Pyriproxyfen:  $r = 0.1$  and  $0.85$ ; 0 and 24-hours old larvae;  $P \leq 0.05$ ) (Diofenolan:  $r = 0.01$  and  $0.85$ ; 0 and 24-hours old larvae respectively;  $P \leq 0.05$ ) (Table. 1,2). Depending upon the presence of both the larval and pupal characters, larval-pupal intermediates produced in the penultimate instar larvae of tobacco cutworm could be classified as:

- (1) Intermediates with pupal abdomen and larval mouthparts with exuvium attached at the dorsal thoracic region. Larval prolegs are also present on the sclerotized pupal abdomen (Fig. 1 F).
- (2) Intermediates showing pupal abdomen and presence of larval head along with exuvium attached at the posterior region. Larval prolegs are also present on the abdomen but slightly reduced. The thoracic region shows the presence of larval legs (Fig. 1 G).

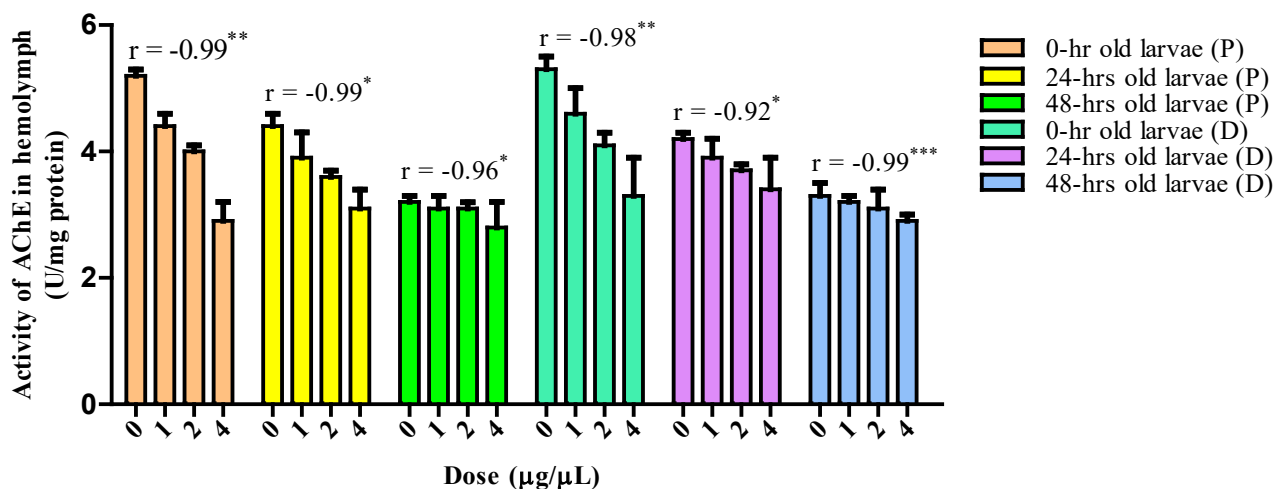
Application of pyriproxyfen and diofenolan to the 0, 24 and 48-hours old penultimate instar larvae resulted in low pupation at 1, 2, and  $4 \mu\text{g}/\mu\text{L}$  dose respectively as compared to 100% pupation in control (Pyriproxyfen:  $r = -0.85$ ,  $-0.92^*$ , and  $-0.95^*$  for 0, 24, and 48-hours respectively; Diofenolan:  $r = -0.85$ ,  $-0.98^*$ , and  $-0.97^*$  for 0, 24, and 48-hours respectively;  $P \leq 0.05$ ) (Table. 1, 2).

Topical administration of pyriproxyfen and diofenolan to *S. litura* fifth instar larvae resulted in the pupal mortality but the effect was notable in 0-hours old larvae (Pyriproxyfen:  $r = 0.68, 0.97^*$ , and  $0.88$  for 0, 24, and 48-hours respectively; Diofenolan:  $r = -0.6, -0.97^*$ , and  $-0.88$  for 0, 24, and 48-hours respectively;  $P \leq 0.05$ ) (Table 1, 2). Application of both JHAs has also resulted in reduced adult emergence. No adult emergence was observed in the 0-hour old larvae treated with pyriproxyfen and diofenolan in all the doses. The coefficient of correlation was negatively correlated in a dose dependent manner (Pyriproxyfen:  $r = -0.68, -0.75$ , and  $-0.93$  for 0, 24, and 48-hours respectively; Diofenolan:  $r = -0.68, -0.90$ , and  $-0.94^*$  for 0, 24, and 48-hours respectively;  $P \leq 0.05$ ) (Table 1, 2).

Log  $IC_{50}$  and  $IC_{50}$  values for adult emergence inhibition rate were found to be  $-0.98$  and  $0.1 \mu\text{g}/\mu\text{l}$  for 24-hours old larvae (Pyriproxyfen);  $0.005$  and  $1.01 \mu\text{g}/\mu\text{l}$  for 48-hours old larvae (Pyriproxyfen);  $-0.085$  and  $0.82 \mu\text{g}/\mu\text{l}$  for 24-hours old larvae (Diofenolan);  $-0.09$  and  $1.23 \mu\text{g}/\mu\text{l}$  for 48 hours old larvae (Diofenolan);  $P \leq 0.05$ ) (Fig. 4).



**Fig. 4.** Inhibitory effect of topical application of pyriproxyfen and diofenolan on larvae of *Spodoptera litura* against inhibition of adult emergence: (A & B) Log $IC_{50}$  graph of 24 and 48-hours old larvae treated with pyriproxyfen respectively, (C & D) Log $IC_{50}$  graph of 24 and 48-hours old larvae treated with diofenolan respectively. ( $R^2$  = coefficient of determination; IC = inhibitory concentration).

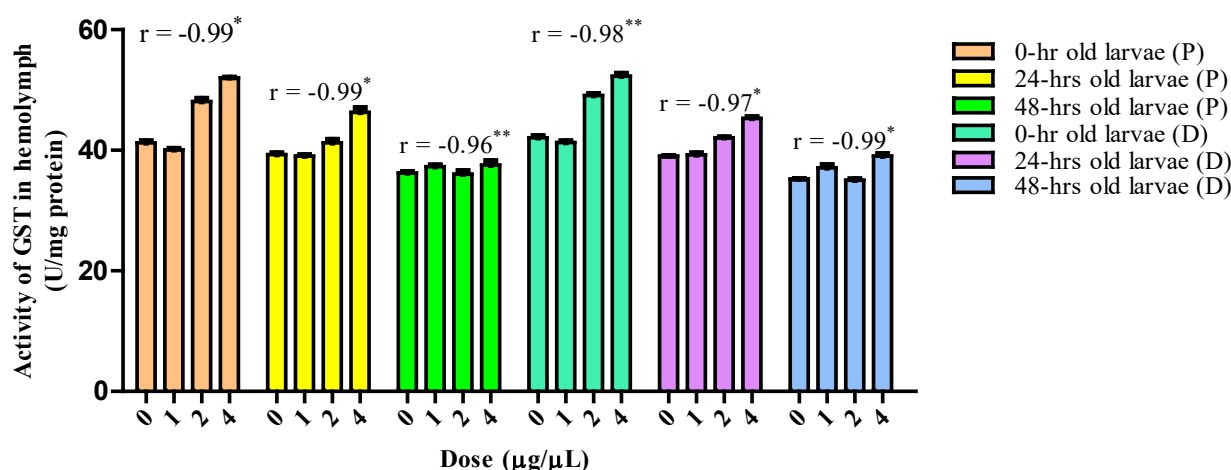


**Fig. 5.** Changes in the activity of acetylcholinesterase (AChE) in the fifth instar larvae (0, 24, and 48hours old) of *S. litura*. All data represent Mean $\pm$ SE value. \*, \*\* and \*\*\* represent significance levels at  $P \leq 0.05, 0.01$ , and  $0.001$  respectively after one-way ANOVA followed by Dunnett multivariate test.

### Biochemical Assay (Acetylcholinesterase and glutathione –S-transferase enzyme)

The AChE concentration in the hemolymph of tobacco cutworm was observed to decrease due to the application of both the JHAs in a time and dose dependent manner (Fig. 5). The correlation was found to be negatively significant at all the durations of exposure. The lowest concentration of AChE was observed at the highest dose concentration at all the treatment durations and in control highest concentration was observed in early phase larvae as compared to 48-hours larvae.

Exposure of the penultimate instar larvae of *S. litura* to pyriproxyfen and diofenolan resulted in an increased GST activity in the hemolymph (Fig. 6). The correlation was significantly negative at all the durations of larval treatment showing the inhibitory effect of both the JHAs on GST activity in the hemolymph. The increased in GST level affirms that these JHAs make tobacco cutworms more susceptible to them.



**Fig. 6.** Changes in the activity of glutathione-S-transferase (GST) in the fifth instar larvae (0, 24, and 48 hours old) of *S. litura* treated with pyriproxyfen and diofenolan. All data represent Mean  $\pm$  SE value. \*and \*\* represent significance level at  $P \leq 0.05$  and  $0.01$  respectively after one-way ANOVA followed by Dunnett multivariate test.

## Discussion

Application of diofenolan and pyriproxyfen topically on the fifth instar larvae (0, 24, and 48-hours old) of *S. litura* resulted in developmental metamorphic catastrophe and hampered the enzymatic concentration of hemolymph.

Both JHAs caused prolongation in larval-larval and larval-pupal ecdysial duration. Pyriproxyfen was more effective at the tested stage as compared to diofenolan. According to (Nijhout, 2015), this prolongation in developmental duration is mainly since exogenous application of JHAs overload the intrinsic level of JH or its mimic in hemolymph and this increased level of JH results in a “*status quo*” condition in an insect. Thus prolongation in the developmental stage is seen (Suzuki *et al.*, 2010; Singh & Maddheshiya, 2022).

Both JHAs have resulted in larval and pupal mortality but pyriproxyfen showed more pronounced results in comparison to diofenolan. Mortality was higher in the earlier stadial period as compared to later. High mortality is mainly due to the toxicity in the hemolymph or impairment of the vitally important homeostatic mechanisms due to an imbalance in JH titer in hemolymph and in other enzymes (Edwards & Abraham, 1985).

Among the latent effects due to the topical administration of JHAs was the formation of intermediates. According to (Riddiford *et al.*, 2018) intermediates are formed mainly due to the insensitivity of some epidermal structures or area to the exogenous JHAs due to which some regions have developed to the forward stage and some region development halted. Also, due to the overload of JH in the hemolymph and the endogenous presence of JHAs, may inhibit the PTTH and ecdysteroid release. These inhibitory actions of accumulated JH/JHAs in the hemolymph prevents the molting of the insect to advancement as a result insect dies or develop traits as mosaic or intermediate (Tunaz & Uygun, 2004).

Adult emergence was also severely affected due to pyriproxyfen and diofenolan. The most severe effect was observed in naïve fifth instar larvae causing zero emergence at all the treated doses. The adult emergence inhibition shows that both of these JHAs somehow interfere with the cellular differentiation and dedifferentiation therefore curtailing the pupal development marching towards adult eclosion (Maddheshiya, 2021). Reduced adult emergence is a major outcome that can be observed due to the engagement of JHAs as insecticide and it has been observed by many scholars in *Ephesia kuehniella*, *Parasarcophaga argyrostoma*, *Sarcophaga ruficornis* and *Chrysomya megacephala* (Maddheshiya, 2021).

GST is one of the major enzymes involved in the detoxification process of electrophilic toxic compounds in the hemolymph of insects (Claudianos *et al.*, 2006). In insects such as, *S. exigua*, GST is co-expressed by ROS/Cnc pathway upon exposure to pesticides. There are different GST genes in insects as HvGST genes. such as HvGSTd1, HvGSTD2, SeGSTe9, and HvGSTe2. These genes get activated upon contact with insecticides and function as detoxificant through CncC/Maf pathway which in turn is activated by elevated reactive oxygen species (ROS) upon insecticidal stress (Hu *et al.*, 2019). Our finding showed that the administration of both JHAs topically increased the level of GST. This increase in the concentration of GST focuses on the fact that this enzyme is somehow involved in the process of detoxification in this insect (Kostaropoulos *et al.*, 2001). Similarly, AChE is the enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine at synapses (central and peripheral nervous system) (Lionetto *et al.*, 2013). JH or its analogues inhibits the AChE accumulation and further its induction is blocked (Cherbas *et al.*, 1989). Similar results were also obtained in the *Chilo suppressalis* and *Musca domestica* after exposure to fipronil (Xiao *et al.*, 2017; Kinareikina & Silivanova, 2023). Inhibition of AChE or acetylcholine accumulation in the body of the insects causes over excitation of neurons and ultimately leading to mortality (Casida & Durkin, 2013; Jankowska *et al.* 2018).

## Conclusion

The present study shows the efficacy of pyriproxyfen and diofenolan against the fifth instar larvae (0, 24, and 48-hours old) of *S. litura*. Both the JHAs were more potent in controlling the growth and development of tobacco cutworm when administered at the naïve fifth stage larvae. These effects were mainly due to the fluctuation in the internal hormonal level of the insect body. Based on the value of the inhibition of the adult emergence (IC<sub>50</sub>), we recommend the regular use of 0.1 µg/µl of pyriproxyfen and 0.82 µg/µl of diofenolan for the population growth and infestation control of *S. litura*. Based on our result we can recommend the judicious use of both the IGRs for curbing the population growth of this insect.

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## اثر پیری پروکسی فن و دیفنولان بر استماله و فعالیت‌های آنزیمی کرم جوانه خوار تنباکو *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae)

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### هکیده

کرم جوانه‌خوار تنباکو *Spodoptera litura* (Lepidoptera: Noctuidae)، آفتی چندخوار روی محصولات کشاورزی است که خسارت اقتصادی شدیدی به بار می‌آورد. در مطالعه حاضر، اثر بیولوژیک و فعالیت آنزیمی دو تنظیم کننده رشد حشرات، پیری پروکسی فن و دیفنولان علیه لارو *S. litura* مورد بررسی قرار گرفت. لاروهای سن ماقبل آخر (۰، ۲۴ و ۴۸ ساعته) با دوزهای کشنده (۱، ۲ و ۴ میکروگرم بر میکرولیتر) هر دو آنالوگ هورمونی جوانی (JHAs) به صورت موضعی تیمار شدند. اثرات متعددی از جمله طولانی شدن مدت پوست اندازی لارو به لارو و لارو به شفیره، مرگ و میر لارو و شفیره، خروج ناموفق حشره از مرحله شفیرگی، شکل بینابین، تشکیل تعداد کم شفیره و کاهش ظهور حشره بالغ مشاهده شد. علاوه بر این اثرات در پوست اندازی، هر دو هورمون جوانی دارای اثرات مهاری روی استیل کولین استراز (AChE) بودند اما روی آنزیم گلوکوتاتیون-S-ترانسفراز (GST) دارای اثرات تحریکی بودند. مقادیر  $IC_{50}$  و  $Log IC_{50}$  پیریپروکسی فن برای نرخ مهار ظهور بالغین، معادل ۰/۹۸- و ۰/۱ میکروگرم بر میکرولیتر برای لاروهای ۲۴ ساعته، ۰/۰۰۵ و ۱/۰۱ میکروگرم بر میکرولیتر برای لاروهای ۴۸ ساعته بود. این مقادیر در دیفنولان برای لاروهای ۲۴ ساعته، ۰/۰۸۵- و ۰/۸۲ میکروگرم بر میکرولیتر بود و در لاروهای ۴۸ ساعته، معادل ۰/۰۹- و ۱/۲۳ میکروگرم بر میکرولیتر، محاسبه شد. نتایج نشان داد که هر دو ترکیب تنظیم کننده رشد حشرات، روی لاروهای *S. litura* دارای قدرت حشره‌کشی هستند.

**کلمات کلیدی:** استیل کولین استراز، آنزیم گلوکوتاتیون ترانسفراز، هورمون جوانی، پوست اندازی

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