# OVIPOSITION RESPONSES OF *PLUTELLA XYLOSTELLA* TO ALPHAMETHRIN AND CHLORPYRIFOS UNDER PEST MANAGEMENT STRATEGIES

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## ABSTRACT

Cruciferous crops, especially cauliflower and cabbage, were significantly damaged by the Diamondback Moth, Plutella xylostella (P. xylostella) Linnaeus of the family Plutellidae; Lepidoptera. We evaluated the effect of insecticides alphamethrin and chlorpyrifos on the oviposition response of adults when applied to 2<sup>nd</sup> instar larvae. *P. xylostella* larva was collected from the field of cauliflower in Kushmahi, Gorakhpur, India. The sublethal doses of 10, 20, 40, and 60; ppm insecticide-treated larvae permit them to emerge up to adults in our designed insect rearing/oviposition chamber. Then, we used factorial mating design for mating experiments, and finally egg laving capacity and egg hatchability analysis were done by the nochoice oviposition assay method. We found that the highest concentration (60 ppm), fecundity was reduced to treated male with normal female, normal male with treated female, treated male with treated female: 145.83±3.48, 91.83, 43.66 ± 3.32 eggs for alphamethrin, and 140.67±2.16, 83.33±2.80,  $39.83\pm1.87$  eggs for chlorpyrifos, compared to  $281.17\pm4.70$  and  $279.16\pm2.48$  eggs in the control. Hatchability dropped significantly at all concentrations, with the highest reductions compared between 10 to 60 ppm in percentage of treated male with normal female, normal male with treated female, treated male with treated female as:  $52.35\pm1.48$ ,  $49.60\pm2.15$ ,  $45.64\pm1.88$  to  $37.49\pm2.24$ ,  $36.66\pm2.36$ ,  $34.74\pm4.28$ and 51.32±1.23, 48.32±1.38, 45.31±1.75 to 36.37±2.10, 34.54±3.55, 32.21±3.98 for both insecticides; respectively. The significant reduction in fecundity, fertility, and hatchability suggests that alphamethrin and chlorpyrifos have potent antifertility effects on *P. xylostella*, reducing its reproductive capacity by over 50% at sublethal doses. These insecticides could be a valuable tool in integrated pest management (IPM) strategies, although caution is advised to minimize environmental risks and prevent resistance development.

**Keywords**: Diamondback Moth, Alphamethrin, Chlorpyrifos, Oviposition control, Fecundity, Fertility, Sterility

# **INTRODUCTION**

*Plutella xylostella L. (P. xylostella)*, commonly known as the diamondback moth, is a notorious pest affecting cruciferous plants worldwide, including economically important crops like cabbage, broccoli, and cauliflower (Mason, 2022). This moth, belonging to the Plutellidae family, is distinguished by its small size and characteristic diamond-shaped marks on its wings (Ansari and Kumar, 2022). Its life cycle includes egg, larval, pupal, and adult stages, in which larvae being the most destructive phase (Mukembu, 2022). The larvae feed instably on the leaves of host plants, creating holes and skeletonizing the foliage, which significantly reduces photosynthetic capacity and, consequently, crop yields (Simmons *et al.*, 2018; Chandel *et al.*, 2022). The critical aspect of its life cycle that profoundly impacts both its ecological interactions and agricultural significance (Ramzan *et al.*, 2019). Severe infestations resultd in complete crop failure, especially in regions where cruciferous vegetables are a primary agricultural product. They

are active year-round in mild climates, especially from late spring to early fall (Zalucki *et al.*, 2012). But it can infest crops throughout the year in mild climates, with significant damage occurring during warmer seasons due to rapid lifecycle completion and multiple generations (Kays, 2020). Effective management of *P. xylostella* requires an integrated pest management (IPM) approach. This includes cultural practices such as crop rotation and intercropping, which disrupt the pest's life cycle. Biological control agents, like parasitoids and predators, play a crucial role in naturally regulating populations (Jaworski *et al.*, 2023; De Oliveira *et al.*, 2021).

This pest targets cruciferous plants, as the primary sites for oviposition. The female moths prefer laying eggs on the underside of leaves near the veins, which offers the developing larvae a protected environment with ample food resources (Banjare, 2017). Several factors influence the oviposition behavior, especially plant chemistry, surface texture, and environmental conditions (Li *et al.*, 2016). For instance, the presence of glucosinolates, chemical compounds found in cruciferous plants, acts as a stimulant for oviposition (Huang and Renwick, 1994). Additionally, the moths tend to avoid laying eggs on plants that are already infested or treated with certain insecticides, which can act as deterrents (Sarfraz *et al.*, 2006). Environmental factors like temperature and humidity also play a critical role to enhance reproductive activity and egg-laying rates (Talekar and Shelton, 1993). The oviposition response of *P. xylostella* is crucial for developing targeted pest management strategies, such as using resistant plant varieties, applying oviposition deterrents, or insecticide agents to disrupt the moth's lifecycle and reduce crop damage (Peterson *et al.*, 2016).

Oviposition inhibition refers to the process of preventing or reducing the laying of eggs by female insects. This can be achieved through various methods, including the use of synthetic insecticide agents, natural extracts, or physical barriers that deter females from depositing their eggs on a particular substrate, such as a plant surface (Sarfraz *et al.*, 2006). Alphamethrin (pyrithroid) acts as a potent neurotoxin that disrupts the nervous system of insects upon contact (Jeschke *et al.*, 2020). This chemical's mode of action often triggers immediate avoidance behaviors in *P. xylostella* females, leading to reduced oviposition near treated surfaces and avoidance of areas with residual insecticide (Greene *et al.*, 2017). While chlorpyrifos (organophosphate) exhibits a broader spectrum of activity and longer residual effect, presenting a different set of challenges to *P. xylostella* oviposition behavior (Jeschke *et al.*, 2020). The oviposition response study of *P. xylostella* against these insecticides is still needed in our geographical area.

The present study has been designed and conducted to evaluate the effect of alphamethrin and chlorpyrifos on the oviposition response of *P. xylostella* adults when applied to  $2^{nd}$  instar larvae.

# MATERIALS AND METHODS

#### Field collection and identification of Plutella xylostella larvae:

The larvae, pupae, and adults of DBM were collected from the cauliflower field of Kushmhi, Gorakhpur, India, at the end of December and kept in a box lined with blotting paper. A small transparent bottle was used to capture the adults through the crop foliage. Then, they were transported to our departmental laboratory for further experiments. Collection activities were carried out during early morning and late evening hours to coincide with the peak activity periods of the moths. We have also collected the eggs, were gently detached from the leaves by fine paintbrush and transferred to petri dishes lined with moist filter paper.

We identified and collected the *P. xylostella* larvae, pupa, and adults with given key characteristics (as **Figure 1**). Adult *P. xylostella* were characterized by their cylindrical, greenish brown bodies, measuring about 6-8 millimeter (mm) in length with a wingspan of approx. 12–15 mm. When at rest, the wings were folded rough like over the body, with hindwings partially visible, giving the appearance of a diamond-shaped pattern along the back (Talekar & Shelton, 1993). Though its larvae are pale green with a tapered body, growing up to 12 mm in length and mainly presented on the lower side of the cauliflower leaves.

They exhibit a unique wriggling motion when disturbed, often dropping from the plant on a silk thread (Furlong *et al.*, 2013). The pupal stage occurs within a loosely woven cocoon, usually attached to the foliage of host plants.



Insect rearing/oviposition chamber

Figure 1: Representative picture of (A) collection of DBM: Diamondback Moth (*Plutella xylostella L.*) adults and larva from cauliflower field, (B) adult male and female DBM, (C) larvae collection of rearing on cauliflower leaf, (D) leaf disc method for treatment of larvae from diluted concentrations of alphamethrin and chlorpyrifos, (E) survived larva converted into cocoon, (F) rearing of DBM in insect rearing/oviposition chamber, (G) mating the adults as per our experiment setup and (H) oviposition bioassay on cabbage leaf for fecundity, fertility and hatchability

# Culture of P. xylostella

We used our own designed insect rearing/oviposition chamber for culture and observation the oviposition inhibition of eggs. Both adult male and female insects under standard conditions (temperature  $27\pm2^{\circ}$ C, relative humidity  $70\pm5\%$  and photoperiod 12:12 h). The oviposition chambers (23 x 23 x 23 cm) were used as rearing units. The containers were covered with fine mesh to allow air circulation while preventing the escape of moths. Fresh cruciferous leaves were also placed in the containers to encourage oviposition. These leaves were thoroughly washed and dried to eliminate any pesticide residues before use. Adults were allowed to mate and lay eggs, which were subsequently collected for continuous culture (as shown in **figure 1**). They were allowed to feed on a 10% sugar solution with the help of cotton swabs and provided twice a day.

# Structure and preparation of used synthetic insecticides:

We tested two insecticides alphamethrin (10% E.C.) and chlorpyrifos (20% E.C.), that are commonly used in agricultural practices for pest control. The chemical structure and source of commercial availability are mentioned in **figure 2.** Both studied for their effects on oviposition inhibition, which is critical in controlling pest populations by preventing egg-laying and subsequent larval damage. In which alphamethrin, a synthetic pyrethroid, is known for its high efficacy in insect control. It acts as a neurotoxin, disrupting the normal function of the insect nervous system. Whereas chlorpyrifos, an organophosphate insecticide, works by inhibiting acetylcholinesterase, an enzyme essential for proper

nervous system function in insects. Its application not only kills adult insects but also exhibits oviposition deterrent properties. We prepared solutions of alphamethrin and chlorpyrifos sublethal concentrations as 10, 20, 40, and 60; ppm and assessed their effects (Mishra and Tiwari, 2024).



Figure 2: Source and chemical structure of synthetic insecticides; (A) Alphametrin, (B) Chlorpyrifos

# Estimation of egg-laying capacity and hatchability:

The adults emerged from untreated and treated sublethal doses that permit the *P. xylostella* larvae to grow, develop, and emerge but cause effect in the immediately sexed and used for mating experiments for evaluation of fecundity, fertility, and sterility variations. In order to do this, the following four kinds of crosses were created:

1) Normal male x Normal female (serving as control) (N $^{\wedge}_{\circ}$  x N  $^{\bigcirc}_{+}$ )

2) Treated male x Normal female (T  $\bigcirc$  x N  $\bigcirc$ )

3) Normal male x Treated female (N  $\stackrel{\scriptstyle <}{_{\scriptstyle O}}$  x T  $\stackrel{\scriptstyle \bigcirc}{_{\scriptstyle O}}$ )

4) Treated male x Treated female (T  $\stackrel{\frown}{\odot}$  x T  $\stackrel{\bigcirc}{\rightarrow}$ )

#### **Oviposition Bioassay**:

Firstly, we have used the leaf disc method for the treatment of 2<sup>nd</sup> instar larvae (Tabashanik,1987). In which, cauliflower leaves were treated with streptomycin for the removal of foreign organisms. Then these leaves were dipped in our diluted concentrations of alphamethrin and chlorpyrifos for 30 seconds and allowed to dry at room temperature for 1-1.5 hours (h). For the controls, the leaf disc was only immersed in distilled water and stapled in Petri dishes. On drying, the leaf discs were placed in individual petri dishes containing moistened filter paper in our insect rearing chamber. Larval survivability was assessed after 24, 48, 72, and 96; h respectively. We replicated six times including controls for 2<sup>nd</sup> instar larvae. Larvae were considered dead if they couldn't show any type of movement.

We collected the survived male and female pairs and then placed them in separate, custom-designed oviposition chambers to mate and lay eggs. The eggs laid were collected daily until the females died. These eggs were transferred to a designated hatching site, where they were allowed to hatch. The total number of eggs laid by each pair (oviposition rate) and the number of eggs that successfully hatched (hatchability) were recorded. The percentage of eggs hatched and the percentage of sterility were calculated based on our data. To determine the corrected sterility rate, Chamberlain's formula (1962) was applied. Which are: -

Corrected sterility =  $100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in control - percentage hatch in treated}}{100 \text{ X} \frac{\text{Percentage hatch in treated}}{100 \text{ X} \frac{\text{Percen$ 

Percentage hatch in control

For each concentration, six pairs of male and female moths were placed in separate mating/oviposition chambers for experimentation.

#### Statistical analysis

Survival activity of larvae against insecticides was carried out for sub-lethal concentrations by the POLO Plus program (LeOra Software version 2.0). We conducted the experiments in six replicates, with percentages of fecundity, fertility, hatchability, observed and corrected sterility expressed as mean $\pm$ S.D. P value < 0.01 as a significant and statistical analysis for oviposition bioassay was performed using the student's t-test (Zhang, 2022).

## RESULTS

# Effect of alphamethrin on the oviposition rate of adult P. xylostella

The data on the effect of alphamethrin on the  $2^{nd}$  instar larvae of *P. xylostella* can be understood through the parameters of fecundity, hatchability, and sterility (**Table 1**). Fecundity measures the number of eggs laid per female and showed a clear decline with increasing concentrations of alphamethrin in our study. In the control group, where neither males nor females were exposed to the insecticide, the fecundity was high at an average of  $281.17\pm4.70$  eggs per female. However, as the concentration of alphamethrin increased, there was a notable reduction in egg production. For instance, at 10 ppm, the average number of eggs shown by treated male with normal female, normal male with treated female and treated male with treated female were  $258.16\pm5.30$ ,  $209.33\pm3.32$  and  $164.66\pm4.08$ , respectively. Whereas at 20 ppm of alphamethrin, the fecundity of *P. xylostella* showed a notable decline, with the number of eggs laid per female  $225.83\pm4.66$ ,  $176.33\pm4.41$  and  $122.33\pm3.72$  depending on the same above mating combination. At 40 ppm, the negative impact of alphamethrin on these parameters was even more pronounced. Fecundity continued to decline, with the average number of eggs laid per female further reduced to  $189.83\pm3.43$ ,  $132.66\pm3.93$  and  $93.83\pm3.06$ . This trend continued at higher concentrations, with a significant drop to  $145.83\pm3.48$ ,  $91.83\pm3.92$  and  $43.66\pm3.32$  eggs per female at 60 ppm for the group with the same above given treatment pattern.

Hatchability also declined as alphamethrin concentrations increased. In the control group, percentage of hatchability was very high at 97.38 $\pm$ 1.85, indicating a strong viability of eggs under natural conditions. However, exposure to even the lowest tested concentration of 10 ppm showed the average number of eggs treated male with normal female, normal male with treated female, and treated male with treated female ranged significantly reduced hatchability, with rates 52.35 $\pm$ 1.48, 49.60 $\pm$ 2.15 and 45.64 $\pm$ 1.88; respectively. Whereas at 20 ppm hatchability was similarly affected. It decreased significantly at this concentration, with 47.08 $\pm$ 1.57, 46.21 $\pm$ 2.21 and 43.87 $\pm$ 1.96 indicating that fewer eggs were able to develop into larvae. Hatchability at 40 ppm continued to fall, with rates 43.10 $\pm$ 1.52, 43.71 $\pm$ 2.32 and 39.78 $\pm$ 2.61 showing a substantial decrease in the viability of eggs. The concentration increased and the hatchability continued to decline, reaching as low as 37.49 $\pm$ 2.24, 36.66 $\pm$ 2.36 and 34.74 $\pm$ 4.28 at 60 ppm in the group at the same-above given treatment criteria.

Sterility, expressed both as observed and corrected sterility, also increased with the concentration of alphamethrin. Observed sterility was relatively low in the control group as  $2.62\pm0.23$ . However, this value increased significantly at 10 ppm, where observed sterility for eggs treated male with normal female, normal male with treated female, treated male with treated female was  $47.65\pm1.56$ ,  $50.40\pm1.59$  and  $54.36\pm1.86$ . Whereas on the same parameter, observed sterility is  $52.92\pm2.17$ ,  $53.79\pm2.01$  and  $56.13\pm1.58$  at concentration of 20 ppm. At 40 ppm,  $56.10\pm2.09$ ,  $57.29\pm2.78$  and  $60.22\pm2.88$  and it rose

further to  $62.51\pm2.39$ ,  $63.34\pm2.21$  and  $65.26\pm1.97$  at 60 ppm with the above given treatment criteria. Corrected sterility, which adjusts for the natural sterility observed in the control group, followed a similar increasing trend, highlighting the genuine impact of the insecticide (**table 1**). This reflects a dramatic reduction in reproductive success across these concentrations.

Alphamethrin		Fecundity≠	Fertility≠	%	%	%
concentration	Crossing	(Eggs laid/	(Eggs	Hatchability	Observed	Corrected
(parts per	sets	female)	Hatched)	¥	Sterility≠	Sterility≠
million) ppm						
Control	$\mathbf{N} \stackrel{\mathcal{A}}{\odot} \mathbf{x} \mathbf{N} \stackrel{\mathcal{Q}}{=}$	$281.17 \pm 4.70$	$273.83 \pm 2.48$	97.38±1.85	$2.62\pm0.23$	-
10	$\mathbf{T} \stackrel{\mathcal{A}}{\odot} \mathbf{x} \mathbf{N} \stackrel{\mathcal{Q}}{\rightarrow}$	258.16±5.30**	135.16±2.63	52.35±1.48*	47.65±1.56	$46.24 \pm 2.37$
	$N \stackrel{<}{\mathrel{\circ}} x T \stackrel{\bigcirc}{\mathrel{\circ}}$	209.33±3.32*	$103.83 \pm 4.18$	49.60±2.15*	$50.40 \pm 1.59$	$49.06 \pm 2.83$
	T ♂ x T ♀	164.66±4.08*	75.16±2.48	45.64±1.88*	$54.36 \pm 1.86$	53.13±2.64
20	$\mathbf{T} \stackrel{\mathcal{A}}{\odot} \mathbf{x} \mathbf{N} \stackrel{\mathcal{Q}}{\rightarrow}$	225.83±4.66*	$106.33 \pm 2.80$	47.08±1.57*	$52.92 \pm 2.17$	$51.65 \pm 2.43$
	$N \stackrel{<}{\mathrel{\circ}} x T \stackrel{\bigcirc}{\mathrel{\circ}}$	176.33±4.41*	81.33±3.32	46.21±2.21*	$53.79 \pm 2.01$	$52.54 \pm 2.88$
	T ♂ x T ♀	122.33±3.72*	53.67±1.75	43.87±1.96*	56.13±1.58	$54.94 \pm 2.69$
40	$T \circ x N $	189.83±3.43*	81.17±2.48	43.10±1.52*	56.10±2.09	55.74±2.39
	$\mathbf{N} \stackrel{\mathcal{A}}{\odot} \mathbf{x} \mathbf{T} \stackrel{\mathcal{Q}}{\rightarrow}$	132.66±3.93*	56.67±2.58	42.71±2.32*	$57.29 \pm 2.78$	$56.14 \pm 2.97$
	T ♂ x T ♀	93.83±3.06*	37.33±2.13	39.78±2.6*	$60.22 \pm 2.88$	59.14±3.20
60	$T \circ x N $	145.83±3.48*	54.67±3.01	37.49±2.24*	62.51±2.39	$61.50\pm2.91$
	$N \stackrel{<}{\bigcirc} x T \stackrel{<}{\bigcirc}$	91.83±3.92*	33.67±1.63	36.66±2.36*	63.34±2.21	62.35±3.01
	T ♂ x T ♀	43.66±3.32*	15.17±1.47	34.74±4.28*	65.26±1.97	$64.40 \pm 4.66$

Table 1:	Effect	of alphamethrin	on the	e fecundity,	hatchability	and	their	sterility	of	Р.	xylostella
exposed a	as 2 <sup>nd</sup> in	star larvae									

 $\neq$ Values are represented as mean  $\pm$  standard deviation (S.D) of six replicates and significantly different \*P< 0.001 and \*\*P < 0.01 compared with controls when student t-test applied.

# Effect of chlorpyrifos on the oviposition rate of adult P. xylostella

Fecundity decreases remarkably with increasing chlorpyrifos concentrations across all mating combinations, as per **table 2**. In the control (normal male with normal female), the fecundity was  $279.16\pm2.48$  eggs per female. At 10 ppm, fecundity declines across the treated male with normal female laid  $251.66\pm4.41$  eggs, normal male with treated female laid  $192.83\pm3.57$  eggs, and treated male with treated female laid  $143.83\pm2.23$  eggs. This decline process continues at 20 ppm and 40 ppm also. At the highest concentration of 60 ppm, the fecundity reaches its lowest, with  $140.67\pm2.17$  eggs laid per female for treated male with normal female,  $83.33\pm2.80$  for normal male with treated female, and only  $39.83\pm1.87$  for treated male with treated female, highlighting the significant impact of the insecticide on reproductive output.

Hatchability also declined with increasing chlorpyrifos concentration. The control exhibits a high hatchability of  $97.19\pm1.07$ . At 10 ppm, hatchability drops significantly in the treated groups as per table 2:  $51.32\pm1.23$ ,  $48.32\pm1.38$  and  $45.31\pm1.75$  for treated male with treated female. This trend continues at 20 and 40 ppm, with the same decline in hatchability rates. The lowest hatchability rates are recorded at 60 ppm, showing significant reductions to  $36.37\pm2.10$   $34.54\pm3.55$  and  $32.21\pm3.98$  indicating a severe decrease in the viability of eggs due to higher chlorpyrifos concentrations.

Sterility increases correspondingly with rising chlorpyrifos concentrations as compared to the control. It increases at 10 ppm as our groups from  $48.68\pm1.59$ ,  $51.68\pm1.64$  to  $54.69\pm1.98$ . Observed sterility continues to rise in the same pattern at 20 and 40 ppm also. At 60 ppm, observed sterility is at its highest, with values of  $63.63\pm2.43$ ,  $65.46\pm2.49$  and  $67.79\pm1.97$  in our groups, showing a substantial proportion of

eggs failing to hatch. Corrected sterility, which adjusts observed sterility for natural sterility levels seen in the control. The highest corrected sterility values are observed at 60 ppm, with  $62.58\pm2.36$  for treated male with normal female,  $64.46\pm3.71$  for normal male with treated female, and  $66.86\pm4.13$  for treated male with treated female.

Chlorpyrif		Fecundity	Fertility	%	%	%
OS	Crossing	(Eggs laid/	(Eggs	Hatchability	Observed	Corrected
concentrat	sets	female)	Hatched)		Sterility	Sterility
ion (ppm)						
Control	$N \partial x N Q$	279.16±2.48	271.33±1.76	97.19±1.07	2.81±0.26	-
10	T♂xN♀	251.66±4.41**	129.17±2.13	51.32±1.23*	$48.68 \pm 1.59$	47.19±1.63
	N♂ x T♀	192.83±3.57*	93.17±2.04	48.32±1.38*	51.68±1.64	50.28±1.75
	T♂xT♀	143.83±2.23*	65.17±2.31	45.31±1.75*	$54.69 \pm 1.98$	$53.38 \pm 2.05$
20	Tổ x N♀	222.33±2.58*	$103.33 \pm 2.58$	46.47±1.28*	53.53±2.23	52.18±1.67
	N♂xT♀	163.83±3.81*	75.17±2.85	45.88±2.04*	54.12±2.03	$52.79 \pm 2.30$
	T♂xT♀	$115.66 \pm 3.32*$	46.16±2.92	39.91±2.77*	60.09±1.72	58.93±2.97
40	T♂xN♀	175.16±3.71*	73.66±2.22	42.05±1.55*	57.95±2.19	56.73±1.88
	N♂xT♀	121.83±3.65*	50.17±2.13	41.18±2.14*	$58.82 \pm 2.09$	57.63±2.39
	T♂xT♀	72.66±2.16*	26.33±1.75	36.24±2.64*	63.76±3.02	62.71±2.84
60	Tổ x N♀	140.67±2.17*	51.17±2.85	36.37±2.10	63.63±2.43	62.58±2.36
	$\mathbf{N}_{\mathcal{O}} \mathbf{x} \mathbf{T}_{\mathcal{Q}}$	$83.33 \pm 2.80*$	$28.66 \pm 2.80$	34.54±3.55	$65.46 \pm 2.49$	64.46±3.71
	$\mathbf{T}_{\mathcal{O}} \mathbf{x} \mathbf{T}_{\mathcal{O}}$	39.83±1.87*	$12.83 \pm 1.47$	32.21±3.98	67.79±1.97	66.86±4.13

Table	2	Effect	of	chlorpyrifos	on	the	fecundity,	hatchability	and	their	sterility	of	<b>P</b> .	xylostella
expos	ed	as 2 <sup>nd</sup> in	nsta	ır larvae										

\*Values are represented as mean  $\pm$  standard deviation (S.D) of six replicates and significantly different \*P< 0.001 and \*\*P< 0.01 compared with controls when student t-test applied.

# DISCUSSION AND CONCLUSION

We have seen the effect of alphamethrin and chlorpyrifos on fecundity, fertility, and hatchability in *P. xylostella* from our results as table 1 and 2. It was noted that treated male with normal female, normal male with treated female, treated male with treated female had fecundity reduced significantly in comparison to normal male with normal female. There was also a correlation with observed a decrease in eggs hatched, and percent hatchability was found to be significantly reduced in comparison to control.

The percentage sterility was almost 55–65% in test females, which is a good sign of the reproductive inhibitory response of alphamethrin. There is a possibility that alphamethrin binds to the vitellogenins of insect eggs and agglomerates the egg fertilizing membrane, poisoning the egg cytoplasm, which results in the death of the developing embryo inside the eggs (Pandey *et al.*, 2024). This disruption could agglomerate the egg fertilizing membrane and poison the egg cytoplasm, ultimately leading to embryo mortality (Guedes *et al.*, 2016). Here one important outcome is that as soon as the exposure period increased, the cidal effect of alphamethrin exponentially slowed up to the 96 h. It seems to be efficient to achieve effective control and may help in test control more efficiently. It will assist in reduction in crop damage at very low levels of alphamethrin (Seth *et al.*, 2004). This suggests that alphamethrin can be highly effective in controlling *P. xylostella* populations over extended periods. This effect is primarily due to its repellency and sublethal effects, which can reduce mating success and alter behavior, deterring females from ovipositing on treated surfaces (Nadda *et al.*, 2005).

In the next experiment, similar process was followed for the determination of the effect of chlorpyrifos on fecundity, fertility, hatchability, and sterility. The fecundity and hatchability were decreased at 20 ppm

followed to 40 ppm and 60 ppm. Fertility was noted as continuously decreased when doses were increased. It is clear that, the fertility was significantly cut down up to 75–80% with the increase in dose and time period as previous study (Legwaila *et al.*, 2014). The percentage of observed sterility was significantly accelerated in treated than control insects up to 65%. Studies have shown that exposure to chlorpyrifos can lead to a significant reduction in egg-laying in *P. xylostella*. The reduction is attributed to the toxic effects on adult moths and potential disruption of sensory cues that females use to locate suitable oviposition sites (Sowmya, 2021).

In pest management, oviposition inhibition is an important strategy to control insect populations, especially those that are highly destructive to crops and more able to resistant (Sharma and Ortiz, 2002). By preventing insects from laying eggs, the subsequent generation of larvae, which often causes the most damage, can be significantly reduced (Hilker and Meiners, 2011). This approach is beneficial in IPM as an appropriate amount of chemical treatments to achieve more effective and sustainable pest suppression (Pretty and Bharucha, 2015). Overall, our study indicated that both alphamethrin and chlorpyrifos exert strong negative effects on the reproductive parameters of P. xylostella. The observed reduction in egg hatchability and increased sterility across various treatment groups suggest that even at sublethal doses, these chemicals impose significant antifertility effects. This effect was observed to impact more than 50% of the population, underscoring the potential of these insecticides as effective tools in IPM strategies. The results align with previous studies, which have demonstrated similar adverse effects of various insecticides on P. xylostella (Atwal and Dhaliwal, 2018; Desneux et al., 2007; Guedes et al., 2016). For instance, Bhagat et al., (2016) reported significant reductions in reproductive parameters following insecticide treatments, including decreased egg viability and increased sterility. Likewise, Jaleel et al., (2018) observed that exposure to certain insecticides resulted in diminished reproductive success and increased mortality rates in *P. xylostella* populations. Such findings highlighted the careful management and application of these chemicals to ensure their effectiveness, while minimizing potential negative impacts on non-target species and the broader ecosystem (Dhawan and Singh, 2018; Huang et al., 2011). All these studies have concordance with my study, which had primarily shown that there is a dosedependent decrease in fecundity, fertility, hatchability, and sterility by the exposure of alphamethrin and chlorpyrifos and severely impacts the reproductive output of these moths. This reduction was most severe in the group where both male and female were treated, highlighting the strong dose-dependent effect of the insecticides. The increase in sterility with higher concentrations of alphamethrin and chlorpyrifos indicates that the insecticide not only reduces the number of eggs laid and their ability to hatch but also significantly compromises the overall reproductive capacity of the moths.

However, these findings demonstrate the utility of alphamethrin and chlorpyrifos in pest control; they also raise concerns about the potential environmental impact, particularly on non-target organisms. The extensive use of these chemicals could lead to resistance development in pest populations and unintended harm to beneficial species, including pollinators and natural predators of pests (Zalucki *et al.*, 2012; Li *et al.*, 2016). Moreover, breeding for resistant crop varieties and the judicious use of chemical insecticides, when necessary, can help manage outbreaks while reducing the risk of resistance development (Abro and Shankar, 2014). So, commercially available synthetic insecticides are required, but their lowest dose-dependent duration is also assessed to reduce the resistance. Therefore, integrating these insecticides into IPM strategies should be done with caution, emphasizing the need for proper dosage management, rotation of different classes of insecticides, and incorporation of non-chemical control methods to sustainably manage pest populations (Desneux *et al.*, 2007; Zalucki *et al.*, 2012).

**In conclusions**, our study found that diluted concentrations especially 60ppm of alphamethrin and chlorpyrifos allowed larvae to grow, develop, and emerge but still caused significant disruptions to the nervous system of the insects. It was concluded that very low concentrations or sublethal doses applied to adult females imposed antifertility in more than 50 percent of the *P. xylostella*. These disruptions

impacted key reproductive parameters such as fecundity, fertility, and sterility, as well as altered the nervous biochemistry of the adult moths. Both insecticides are important tools in the inhibition of oviposition in *P. xylostella*, helping to reduce pest populations and protect crops from damage. Their use should be integrated with other control strategies to ensure sustainable pest management. The study underscores the effectiveness of alphamethrin and chlorpyrifos in reducing the reproductive success of *P. xylostella*, highlighting their potential role in IPM programs. However, to maximize their benefits while minimizing ecological risks.

# **CONFLICTS OF INTEREST**

TA and SK have filled patent application for insect rearing/oviposition chamber. Except this none of others declare any conflict of interest.

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