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Research Article

ROLE OF CHITIN INHIBITORS IN ALTERING FITNESS PARAMETERS AND REPRODUCTIVE SUCCESS OF *CHILO PARTELLUS* (SWINHAE) (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Insect growth regulators (IGRs) are a promising alternative for controlling herbivorous pests. The current study investigated the effects of lufenuron and pyriproxyfen (at concentrations of 0.02, 0.04, and 0.08 ml) on the developmental duration from larva to adult, larval and pupal survival rates, fitness characteristics (larval and pupal length and weight), and reproductive parameters (longevity, fecundity, and fertility) of *Chilo partellus* (Lepidoptera: Crambidae), a major pest of fodder and cultivated crops. The results indicated significant reductions in developmental duration, survival rates, and fitness traits at higher concentrations of both IGRs. Specifically, at the 0.08 ml concentration, lufenuron and pyriproxyfen drastically shortened the larval (9.33 ± 0.19 days) and pupal (6.33 ± 0.19 days) durations compared to the control (16.22 ± 0.62 and 11.56 ± 0.59 days, respectively). Larval survival rates declined to 52.44% and 55.78%, while pupal survival rates dropped to 44.67% and 48.89%, respectively, at this concentration. Fitness assessments revealed significant reductions in larval and pupal lengths and weights following lufenuron and pyriproxyfen treatments. Adult longevity was also substantially reduced, with males and females exposed to lufenuron surviving for only 3.22 ± 0.29 and 4.67 ± 0.19 days, respectively, while those treated with pyriproxyfen lived for 5.33 ± 0.19 and 6.00 ± 0.19 days, compared to control values of 8.78 ± 0.48 and 11.89 ± 0.40 days, respectively. Furthermore, fecundity and fertility were significantly reduced at the 0.08 ml concentration, with lufenuron having the most pronounced effect (fecundity: 290.67 ± 19.00 eggs; fertility: $39.67 \pm 0.84\%$).

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INTRODUCTION

Maize (*Zea mays* L.), a member of the Poaceae family, is one of the most important cereal and fodder crops cultivated worldwide. It originated from Central America

and Mexico (Shiferaw et al., 2011; Hossain et al., 2016; Murdia et al., 2016; Seerat et al., 2022). The rising demand for maize, particularly in emerging nations, is projected to nearly triple by 2050, further underscoring

its role in global food security (CIMMYT, 2019). However, maize cultivation faces several biotic challenges, particularly from insect pests that threaten yield and crop quality (Nabeel et al., 2018).

The maize stem borer, *Chilo partellus* Swinhoe, is a highly destructive pest in Asia and Africa, causing severe crop losses ranging from 15% to as much as 75% under favorable conditions (Kumar and Ashwani, 2017). This pest not only reduces yield but also weakens the structural integrity of plants, leading to lodging, stem breakage, and the formation of dead hearts, all of which further diminish productivity (Davis and Pedigo, 1990; Chouraddi and Mallapur, 2017). *C. partellus*, originally from the Indian subcontinent, is particularly problematic during the Kharif season, when infestations can account for up to 90-95% of total damage in affected regions (Ashfaq and Farooq-Ahmad, 2002; Muhammad and Khawja, 2002).

Traditional pest control measures have relied on various chemical insecticides; however, the growing resistance of pests, environmental concerns, and health risks necessitate alternative management strategies (Khan et al., 2004; Ahmed et al., 2007; Javed et al., 2019a, b; Kassi et al., 2018, 2019; Muhammad et al., 2021a, b; Shehzad et al., 2021, 2022). In this context, chitin synthesis inhibitors (CSIs), a class of insect growth regulators (IGRs), offer a promising alternative for managing *C. partellus*. CSIs disrupt chitin synthesis, a critical component of the insect exoskeleton, thereby impairing molting, development, and overall fitness. By specifically targeting chitin synthesis, these inhibitors provide a selective, environmentally friendly approach to pest control while minimizing harm to non-target organisms. Recent studies have demonstrated the efficacy of CSIs in suppressing insect pest populations by disrupting their development and reproductive fitness (Berg, 2017; Oben et al., 2015).

The present research is novel in its focus on *C. partellus*, a major maize pest in Pakistan, and its evaluation of CSIs under field conditions. Although previous studies have investigated the efficacy of CSIs against other pests, this study is among the first to assess their impact on the fitness and reproductive parameters of *C. partellus* within the agroecological context of Tandojam, Pakistan. Specifically, it examines the effects of CSIs on infestation levels, larval mortality, and overall maize yield, providing valuable insights into integrated pest management strategies that could reduce reliance on conventional insecticides and promote sustainable maize production.

MATERIAL AND METHODS

Study area

The present study was conducted at the Laboratory of Pesticide Toxicity and Application Technology, Sindh Agricultural University, Tandojam, to evaluate the effectiveness of different insect growth regulators against the maize stem borer.

Insect rearing

A laboratory colony of *C. partellus* was established using eggs collected from maize plants at Latif Farm, Sindh Agricultural University. The eggs were transported to the Pesticide Toxicity Laboratory and maintained under controlled conditions of $24\pm2^{\circ}\text{C}$ temperature, $75\pm5\%$ relative humidity, and a 16:8 light/dark photoperiod.

Eggs were placed on flat surfaces for incubation, and upon hatching, the larvae were fed fresh maize leaves, which were regularly replaced to ensure an adequate food supply. As the larvae progressed through 5-6 instars, they were transferred to larger containers to accommodate their growth. Pupation was induced by providing moist soil or sand for burrowing. After 7-10 days, the emerging adults were allowed to mate and lay eggs, thus completing the life cycle.

Temperature, humidity, food availability, and overall colony health were essential for maintaining the colony and were regularly monitored. The provision of fresh maize leaves and proper sanitation ensured healthy larval development.

Treatments

The experiment included three treatments, each with three replications:

T1 = Lufenuron

T2 = Pyriproxyfen

T3 = Control (Distilled Water)

Tested insect growth regulators

The selected insect growth regulators and their common names, trade names, manufacturers, and IRAC modes of action, are listed in Table 1.

Preparation of stock solution concentrations

Stock solutions of lufenuron and pyriproxyfen (10,000 mg/L) were diluted to obtain the final working concentrations of 0.02 ml, 0.04 ml, and 0.08 ml per liter for laboratory applications. Specifically, for the lowest concentration (0.02 ml/L), 0.02 ml of the stock solution was added to 1 L of water; for the medium concentration (0.04 ml/L), 0.04 ml was added; and for the highest concentration (0.08 ml/L), 0.08 ml was used.

In real-world agricultural and pest control applications,

insecticides like lufenuron and pyriproxyfen are typically diluted to much lower concentrations, often in the range of 10-50 mg/L.

Fitness observation

Twenty newly molted third-instar larvae of *C. partellus* were continuously fed maize leaves and soft stems sprayed with three different concentrations of lufenuron and pyriproxyfen (0.02 ml/L, 0.04 ml/L, and 0.08 ml/L), along with a control treatment (distilled water). The larvae were assessed at 24, 48, and 72 h post-treatment for their length and weight, and the same parameters were recorded for pupae. Observations continued until adult emergence.

Effects on reproduction

At least five adult pairs (one male and one female per pair) were provided with a 15% honey solution to assess fecundity and fertility. Fecundity was determined by Table 1. Details of the IGRs used against *C. partellus*.

Common Name	Trade Name	Manufacturer	Mode of Action (MOA)
Lufenuron	Match 5.2% w/w	Syngenta	Insect Chitin Inhibitor
Pyriproxyfen	Century 11.3% w/w	Suncrop	Insect Chitin Inhibitor

RESULTS

Effect of lufenuron and pyriproxyfen on larval, pupal, adult male and female development

Insect growth regulators (IGRs), by interfering with normal growth and development, often result in the retardation of an insect's development or may cause mortality before it reaches adulthood. A highly significant difference was recorded between various concentrations of lufenuron (0.02 ml, 0.04 ml, 0.08 ml) and pyriproxyfen (0.02 ml, 0.04 ml, 0.08 ml) for a larval, pupal, adult male, and adult female, respectively and control to retard the development of *C. partellus* in duration days. Graphical data shows that overall, the lufenuron at 0.08 retarded with the highest developmental growth in all developmental stages, followed by 0.04 and 0.02, while in control, metamorphosis gradually increased with an increase in days. Figures 1 and 2 show the results regarding the effect of different concentrations (0.02, 0.04, 0.08) of lufenuron in comparison to control (distilled water) on developmental duration of immature stage (larval), resting stage (pupal) and adult (male and female) of *C. partellus*.

The data in Table 2 indicated that all three tested concentrations of lufenuron and pyriproxyfen significantly inhibited the growth of larvae, pupae, adult males, and

counting the total number of eggs laid by each female within the first 10 days of oviposition. Pairs that failed to reproduce were excluded from the analysis. Fertility was assessed by calculating the proportion of eggs that hatched based on eggs collected six days after the start of oviposition.

Data analysis

A minimum of three replicates per treatment group was used to ensure reliable results, with the exact sample size determined based on prior studies and the number of treatments compared. Data analysis was conducted using Statistix 8.1 software, with the least significant difference (LSD) test applied at $P < 0.05$ to compare mean differences. This approach ensured that the study maintained statistical robustness, with sufficient replication to detect meaningful biological differences while minimizing experimental errors.

adult females compared to the control group. In the control group, the durations of the larval, pupal, adult male, and adult female stages were recorded as 16.22 ± 0.62 , 11.56 ± 0.59 , 9.00 ± 0.19 , and 10.78 ± 0.48 days respectively. The shortest recorded durations were observed at a lufenuron concentration of 0.08, reducing the developmental periods to 9.33 ± 0.19 days for larvae, 6.33 ± 0.19 days for pupae, 4.00 ± 0.51 days for adult males, and 4.00 ± 0.19 days for adult females. In comparison, other concentrations of both lufenuron and pyriproxyfen resulted in durations of 9.33 ± 0.19 , 7.56 ± 0.40 , 5.22 ± 0.29 , and 4.22 ± 0.29 days for larvae, pupae, adult males, and adult females, respectively.

Effect of lufenuron and pyriproxyfen on larval and pupal survival (%) of *C. partellus*

Figures 3 and 4 illustrated the significant impact of lufenuron and pyriproxyfen on larval and pupal survival. The lowest larval survival rates were recorded at $52.44 \pm 0.59\%$ and $55.78 \pm 0.59\%$ for the 0.08 concentration of lufenuron and pyriproxyfen, respectively. In contrast, the highest survival rate of $90.56 \pm 0.40\%$ was observed in the control group. Similarly, the lowest pupal survival rates of $44.67 \pm 0.51\%$ and $48.89 \pm 0.80\%$ were observed at the 0.08 concentration of lufenuron and pyriproxyfen, respectively, while the highest survival rate of $87.44 \pm 1.39\%$ was recorded in the control group (Table 3).

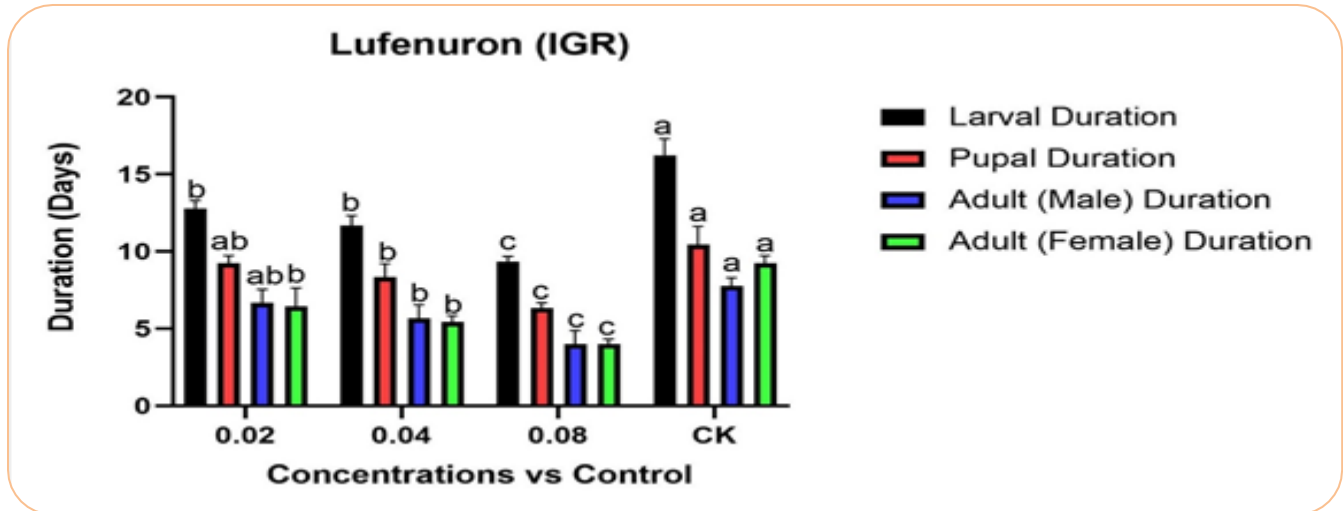


Figure 1. Effect of different concentrations of lufenuron on the duration of larval, pupal, adult male, and adult female stages of *C. partellus*.

*Means followed by the same letters are not significantly different at $P < 0.05$ (LSD test) across different concentrations of lufenuron and the control.

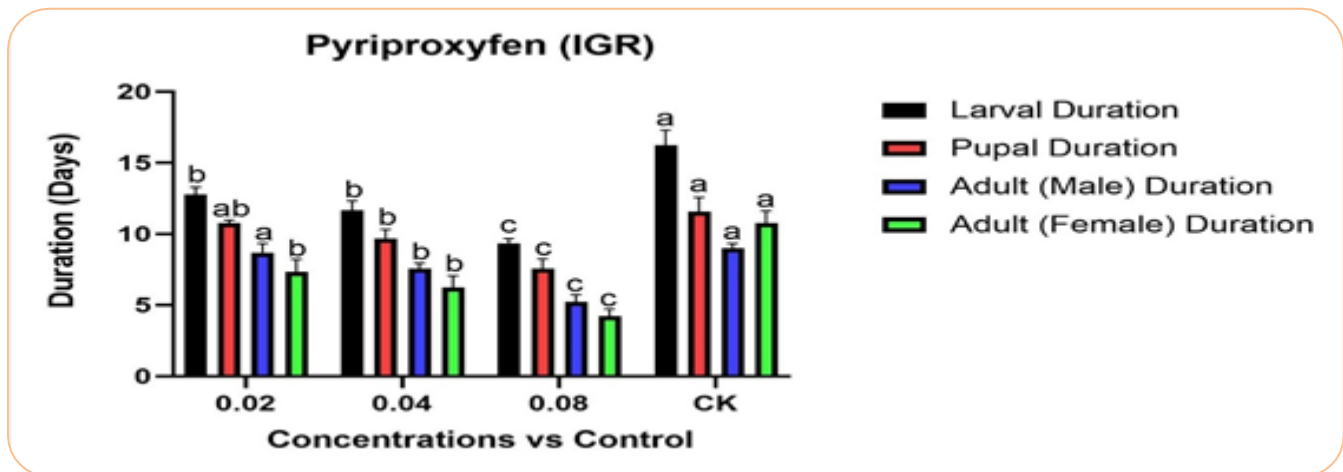


Figure 2. Effect of different concentrations of pyriproxyfen on the duration of larval, pupal, adult male, and adult female stages of *C. partellus*.

*Means followed by the same letter are not significantly different at $P < 0.05$, based on LSD values for variations in pyriproxyfen concentration and the control.

Table 2. Effect of different concentrations of lufenuron and pyriproxyfen on the duration of different developmental stages of *C. partellus*.

IGRs	Concentration (ml)	Larval Duration (Days)	Pupal Duration (Days)	Adult Male Duration (Days)	Adult Female Duration (Days)
Lufenuron	0.02	12.78 ± 0.29 b	9.22 ± 0.29 ab	6.67 ± 0.51 ab	6.44 ± 0.68 b
	0.04	11.67 ± 0.38 b	8.33 ± 0.51 b	5.67 ± 0.51 b	5.44 ± 0.22 b
	0.08	9.33 ± 0.19 c	6.33 ± 0.19 c	4.00 ± 0.19 c	4.00 ± 0.19 c
Pyriproxyfen	0.02	12.78 ± 0.29 b	10.78 ± 0.11 ab	8.67 ± 0.38 a	7.33 ± 0.51 b
	0.04	11.67 ± 0.38 b	9.67 ± 0.38 b	7.56 ± 0.22 b	6.22 ± 0.48 b
	0.08	9.33 ± 0.19 c	7.56 ± 0.40 c	5.22 ± 0.29 c	4.22 ± 0.29 c
Control (Distilled Water)		16.22 ± 0.62 a	11.56 ± 0.59 a	9.00 ± 0.19 a	10.78 ± 0.48 a

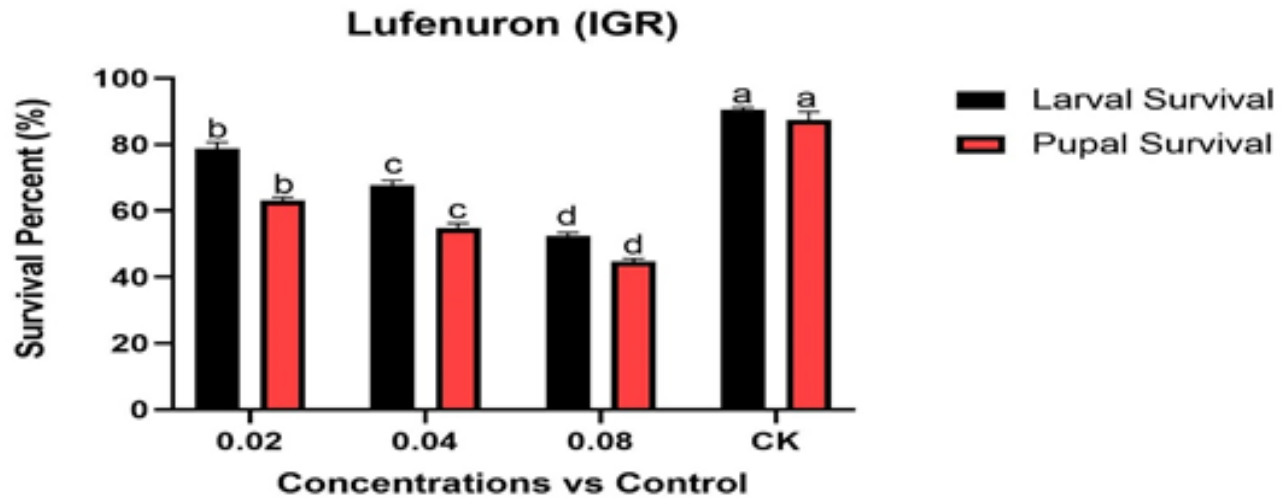


Figure 3. Effect of different concentrations of lufenuron on larval and pupal survival of *C. partellus*.

*Means followed by the same letters are not significantly different according to LSD at $P < 0.05$.

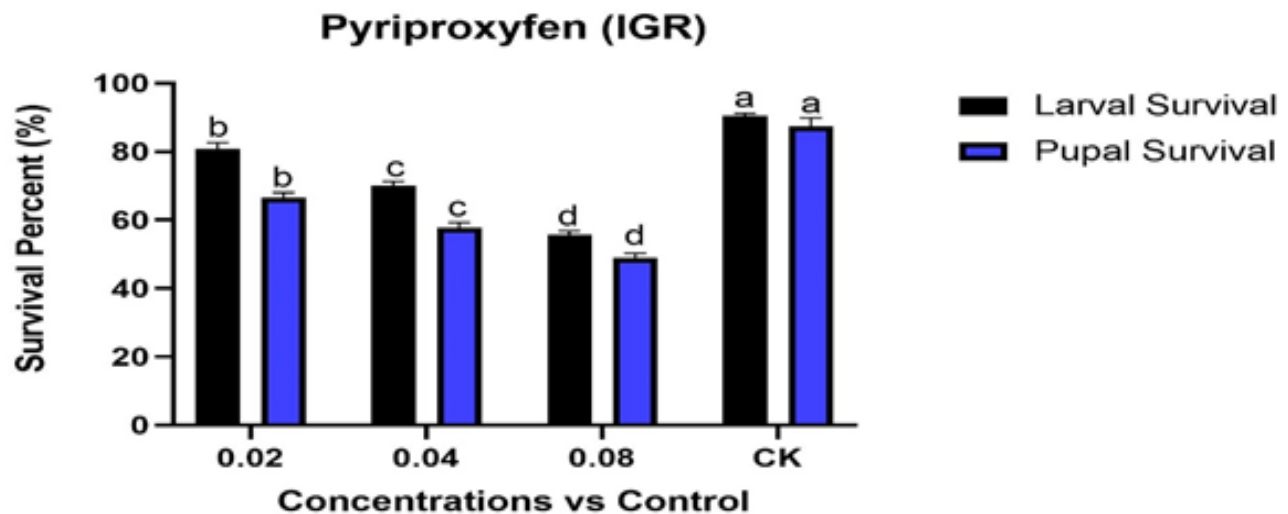


Figure 4. Effect of different concentrations of pyriproxyfen on the larval and pupal survival of *C. partellus*.

*Means followed by the same letter are not significantly different at $P < 0.05$ according to the LSD test.

Table 3. Effect of different concentrations of lufenuron and pyriproxyfen on larval and pupal survival (%) of *C. partellus*.

IGRs	Concentration (ml)	Larval Survival (%)	Pupal Survival (%)
Lufenuron	0.02	78.78±1.09b	63.11±0.56b
	0.04	67.78±0.80c	54.67±0.88c
	0.08	52.44±0.59d	44.67±0.51d
Pyriproxyfen	0.02	80.78±1.06b	66.56±0.87b
	0.04	70.11±0.68c	57.78±0.89c
	0.08	55.78±0.59d	48.89±0.80d
Control	Distilled Water	90.56±0.40a	87.44±1.39a

Effect of lufenuron and pyriproxyfen on the fitness (larval length, weight, and pupal length, weight) of *C. partellus*

The mean larval length, larval weight, pupal length, and pupal weight of *C. partellus* were significantly affected after 24, 48, and 72 h following the application of different concentrations of lufenuron and pyriproxyfen to third-instar larvae. Moreover, lufenuron demonstrated a stronger inhibitory effect on the fitness of *C. partellus* compared to pyriproxyfen.

After 72 h of exposure at a concentration of 0.08, the minimum larval lengths recorded were 5.22 ± 0.29 mm for lufenuron and 7.78 ± 0.29 mm for pyriproxyfen. In contrast, the longest larval lengths observed were 11.56 ± 0.29 mm and 13.67 ± 0.19 mm, respectively, after 24 h of exposure to a 0.02 concentration of lufenuron and pyriproxyfen. The control group exhibited the maximum larval length (19.00 ± 0.19 mm).

A similar trend was observed for larval weight. After 72 h of exposure to a 0.08 concentration of both lufenuron and

pyriproxyfen, the lowest mean larval weights were recorded (9.89 ± 0.73 mg and 7.78 ± 0.29 mg, respectively). Measurements taken at 48 and 24 h showed intermediate values. The highest larval weight was recorded in the control group (30.44 ± 0.29 mg) (Table 4). The minimum pupal lengths (4.00 ± 0.51 mm and 6.11 ± 0.29 mm) were observed after 72 h at a concentration of 0.08 for lufenuron and pyriproxyfen, respectively, while the maximum pupal lengths (10.44 ± 0.29 mm and 11.00 ± 0.19 mm) were recorded after 24 h at a concentration of 0.02 for lufenuron and pyriproxyfen. However, the greatest pupal length (19.00 ± 0.51 mm) was observed in the control group.

A similar trend was noted for pupal weight, with the lowest mean pupal weights (29.22 ± 0.91 mg and 31.89 ± 0.48 mg) recorded after 72 h of exposure to a 0.08 concentration of lufenuron and pyriproxyfen, respectively, followed by weights measured at 48 h and 24 h. In contrast, the highest pupal weight (56.89 ± 0.40 mg) was recorded in the control group (Table 4).

Table 4. Effect of different concentrations of lufenuron and pyriproxyfen on the fitness (larval length and larval weight) of *C. partellus*.

IGR	Conc. (ml)	Larval Length (mm)		Larval Weight (g)	
		24 Hours	48 Hours	72 Hours	24 Hours
Lufenuron	0.02	$11.56 \pm 0.29d$	$10.67 \pm 0.38d$	$9.44 \pm 0.62e$	$21.56 \pm 0.40d$
	0.04	$10.67 \pm 0.38d$	$9.22 \pm 0.62e$	$7.67 \pm 0.58f$	$19.67 \pm 0.33e$
	0.08	$8.89 \pm 0.11e$	$7.33 \pm 0.19f$	$5.22 \pm 0.29g$	$16.67 \pm 0.38g$
Pyriproxyfen	0.02	$13.67 \pm 0.19d$	$12.44 \pm 0.29e$	$11.11 \pm 0.40f$	$23.78 \pm 0.29d$
	0.04	$12.56 \pm 0.29e$	$11.00 \pm 0.33f$	$9.56 \pm 0.48g$	$22.33 \pm 0.58e$
	0.08	$10.89 \pm 0.48f$	$9.67 \pm 0.51g$	$7.78 \pm 0.29h$	$19.89 \pm 0.99f$
Control	Distilled Water	$15.67 \pm 0.19c$	$17.11 \pm 0.29b$	$19.00 \pm 0.19a$	$26.33 \pm 0.19c$

DISCUSSION

Metamorphosis and molting are two important physiological processes in an insect's life. Both processes are regulated by the steroid 20-hydroxyecdysone and the sesquiterpenoid juvenile hormone (Palli, 2009). IGR compounds are used to control insect pests and are thus known as insect development inhibitors, as they prevent or delay the normal metamorphosis of larvae into adults. In the present study, treatment with various doses of lufenuron and pyriproxyfen resulted in a reduction in the duration of the larval, pupal, and adult stages in both sexes. Previous research has shown that ecdysone agonists increase larval mortality in later instars of treated insects. Although IGRs are generally selective,

they have potential drawbacks, such as non-target effects and the development of pest resistance. Non-target organisms, including beneficial insects and aquatic species, may be affected by IGR exposure, leading to ecological imbalances and disruption of natural pest control mechanisms (Desneux et al., 2007). Furthermore, the present study recorded a significant reduction in the survival rates of both larval and pupal stages when third-instar larvae were treated with different concentrations of both IGRs. Moreover, fitness parameters, such as the length and weight of larvae and pupae, were also suppressed compared to the control. Higher concentrations of IGRs resulted in greater suppression of fitness. These findings align with

previous research indicating that ecdysone agonists cause larvae to cease feeding, ultimately reducing their body weight (Pineda et al., 2006; Eizaguirre et al., 2007). Previous studies have associated several sublethal effects, such as larval weight reduction and deformities in both pupal and adult stages of survivors, with these substances. In the present study, third-instar *C. partellus* larvae treated with three different concentrations of lufenuron and pyriproxyfen IGRs exhibited significant effects on reproductive traits, including adult longevity, fecundity, and fertility. Similar outcomes were observed when *Platynota idaeusalis* was treated with tebufenozide (Biddinger et al., 2006). These findings are consistent with those of Ghoneim et al. (2014), who reported that treatment with novaluron led to a dose-dependent reduction in fertility in the penultimate or last instar larvae of *Spodoptera littoralis*. Regardless of the duration of treatment, novaluron had a diminishing effect on fertility when administered to larvae at various concentration levels. The effects of flufenoxuron on the fecundity and fertility of *S. littoralis* adults following the treatment of sixth-instar larvae by topical application were similar to those of methoxyfenozide, which also led to a dose-dependent reduction (Pineda et al., 2006; El-Sabrou, 2009).

Ecdysone agonists are toxic to lepidopteran pests; however, most research has focused on their effects on larval stages, with limited literature available on their impact on the reproductive traits of surviving individuals. In contrast to our findings, a reduction in the hatching rate of *Helicoverpa zea* eggs (Carpenter and Chandler, 1994) and a decrease in the number of eggs laid by female *P. idaeusalis* (Biddinger et al., 2006) were observed when individuals were exposed to tebufenozide as neonates or third instars, respectively. These discrepancies may be attributed to differences in exposure timing, as our study exclusively treated larvae during the fifth and sixth instars, whereas Carpenter and Chandler (1994), Adel and Sehnal (2000), and Biddinger et al. (2006) exposed larvae throughout their entire larval development.

Our study clearly demonstrates that lufenuron and pyriproxyfen at a concentration of 0.08 ml were effective against *C. partellus* in terms of developmental duration, survival, fitness, and reproductive traits. This suggests that the combined use of different IGRs may influence the population dynamics of *C. partellus* in the field.

CONCLUSIONS

Insect growth regulators generally inhibit insect growth and development. In the present study, two different IGRs, lufenuron and pyriproxyfen, were applied at three concentrations (0.02, 0.04, and 0.08 ml) to third instar larvae of *C. partellus*. The results indicated that both IGRs significantly affected survival, fitness, and reproduction. Lufenuron at 0.08 ml exhibited the strongest effects against *C. partellus*, followed by 0.04 ml and 0.02 ml, with pyriproxyfen showing comparatively lower efficacy. Based on these findings, lufenuron is recommended for field application as an IGR against *C. partellus* and other lepidopteran pests, as it not only inhibits insect growth but also contributes to population suppression.

AUTHORS' CONTRIBUTIONS

AA and KHD conceptualized the idea and designed the studies; AA and WBZ conducted research trials and arranged data; AAC and SSD fed the diet to *C. partellus*, AA and AR wrote the manuscript; AL and KF analyzed the data; JH proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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