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Research Article

Seasonal Population Dynamics of *Helicoverpa armigera* and Comparative *In Vivo* Production of Entomopathogenic Nematodes Using *Galleria mellonella* as a Host

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ABSTRACT

The gram pod borer, *Helicoverpa armigera*, is a major pest of chickpea, causing significant yield losses. This study evaluated its seasonal incidence and assessed the *in vivo* mass production potential of selected entomopathogenic nematodes using different hosts and inoculum levels. Field observations conducted during the Rabi seasons of 2023-24 and 2024-25 revealed that larval activity commenced in Standard Meteorological Week (SMW) 49 and persisted until SMW 12 in both seasons. The pest population exhibited three major peaks, with the highest densities recorded during SMW 9-11. Maximum larval populations reached 2.3 larvae per plant in 2023-24 and 2.4 larvae per plant in 2024-25. Correlation analysis indicated a weak and non-significant association of temperature and relative humidity with pest incidence in 2023-24, whereas a significant positive relationship with temperature ($r = 0.691$-0.788; $P < 0.05$) and moderate correlation with relative humidity ($r = 0.504$; $P = 0.046$) were observed in 2024-25. In the bioassay studies, *Galleria mellonella* demonstrated higher variability but proved more suitable for nematode production than *H. armigera*. Among the tested EPN species, *Steinernema pakistanense* produced the highest infective juvenile (IJ) yield, followed by *S. balochiense* and *S. abbasi*. IJ production increased significantly with inoculum density, and *G. mellonella* yielded approximately 9-11% more progeny than *H. armigera*. In conclusion, *H. armigera* peaks during SMW 9-11, and *G. mellonella* is the most suitable host for efficient mass production of EPNs, especially *S. pakistanense*, supporting its use in sustainable pest management.

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Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume cultivated in many countries due to its high seed protein content and substantial nutritional value (Shahzaman et al., 2015; Didinger and Thompson, 2022; Koul et al., 2022). It belongs to the family Fabaceae and is commonly known as gram or referred to as the "King of Pulses." Pulses, defined as edible legumes, have been cultivated for thousands of years and serve as a nutritionally balanced food source worldwide. In India, pulses are traditionally regarded as the "poor man's meat and the rich man's vegetable," reflecting their importance as an affordable protein source. In recent years, the value of plant-based proteins has gained widespread global recognition (Bahadur, 2018).

The cotton bollworm, *Helicoverpa armigera* (Hübner)

(Lepidoptera: Noctuidae), is a highly polyphagous and adaptable pest that feeds on a wide range of crops, including chickpea, cotton, soybean, maize, tomato, vegetables, and fruits (Kassi et al., 2018, 2019; Aslam et al., 2019; Nagachandrabose, 2022). The larval stage is the most destructive phase of its life cycle, primarily feeding on the reproductive parts of plants; especially from the third instar onward (Das et al., 2022). This pest is considered one of the most damaging insect pests of chickpea worldwide (Volp et al., 2024). Although chemical pesticides remain the primary method for managing *H. armigera*, their extensive use has led to the development of resistance, contributing to recurrent pest outbreaks (Bird et al., 2023).

Entomopathogenic nematodes (EPNs) (Rhabditida: Heterorhabditidae and Steinernematidae) are soil-inhabiting, insect-pathogenic nematodes distributed across diverse ecosystems worldwide. Their symbiotic bacteria, belonging to the genera *Photorhabdus* and *Xenorhabdus*, play a crucial role in pathogenicity and are carried into insect hosts by the infective juvenile stage (Rahoo et al., 2011; Sajnaga, 2024). EPNs are widely used in biological control programs against insect pests (Rahoo et al., 2017, 2018a, b, 2019a, b, 2022, Gulzar et al., 2020). They may exhibit either facultative or obligate parasitism: facultative parasites can survive independently while opportunistically infecting hosts, whereas obligate parasites depend entirely on their hosts to complete their life cycle (Koppenhöfer and Kaya, 2001).

The mutualistic association between EPNs and their symbiotic bacteria represents an environmentally friendly alternative to chemical pesticides, particularly for managing soil-dwelling pests (Tomar et al., 2024). Under field conditions, EPNs exhibit high mobility and persistence in the soil, enabling them to actively locate and infect target hosts. Consequently, they are considered effective and environmentally safe biological control agents, often demonstrating performance comparable to or exceeding that of chemical pesticides (Javed et al., 2025). Infection occurs when nematodes enter the insect host through natural openings such as the mouth, anus, or spiracles, or by penetrating the cuticle, depending on the species. Once inside, they release their symbiotic bacteria, which proliferate rapidly and kill the host within 48-72 h (Yuksel et al., 2022).

Despite their potential, the large-scale commercial application of EPNs is constrained by high production costs, necessitating improvements in mass production techniques (Oliveira-Hofman et al., 2023). Nevertheless, EPNs can be mass-produced, applied using conventional equipment, and possess the unique ability to actively search for hosts (Mathew et al., 2020).

The present study was conducted to investigate the population dynamics of *H. armigera* on chickpea and its relationship with abiotic factors, including temperature (°C) and relative humidity (%). Moreover, entomopathogenic nematodes were mass-produced on *Galleria mellonella* and *Helicoverpa armigera* to compare their *in vivo* production efficiency.

Materials and Methods

Meteorological data and seasonal frequency

The present study on the population dynamics of the gram pod borer, *H. armigera* (Hübner), was conducted under field conditions at Naseerabad, Balochistan, Pakistan, during two consecutive chickpea cropping seasons (Rabi 2023-24 and 2024-25). The chickpea (*Cicer arietinum* L.) variety 'Lalri (Desi)' was sown in five experimental plots, each measuring 5 × 4 m. A uniform spacing of 30 cm between rows and 15 cm between plants was maintained.

To allow natural pest infestation, no insecticides were applied throughout the experimental period. All recommended agronomic practices were followed to ensure normal crop growth. For monitoring *H. armigera* populations, 10 plants per plot were randomly selected and permanently tagged. Weekly observations were recorded during early morning hours, starting from the initial appearance of larvae until crop maturity. Larval density (number of larvae per plant) was used as an indicator of pest incidence.

Weekly meteorological data, including maximum and minimum temperature (°C), relative humidity (%), and total rainfall (mm), were obtained from the Department of Meteorology located near the Naseerabad Power Plant. These abiotic factors were used to evaluate their relationship with pest population fluctuations.

In vivo mass production of entomopathogenic nematodes

Laboratory experiments were conducted to evaluate the *in vivo* production potential of three *Steinernema* species viz., *S. pakistanense*, *S. balochiense*, and *S. abbasi*. These cultures were obtained from the Entomopathogenic Nematode Storage Unit at the National Nematological Research Centre (NNRC), University of Karachi.

For *in vivo* mass production, last-instar larvae of two

host insects were used: the greater wax moth, *Galleria mellonella* L., and field-collected larvae of *H. armigera*. *G. mellonella* larvae were reared on an artificial diet following Shahina et al. (1998), consisting of bran (150 g), yeast granules (10 g), and ground grains of wheat, maize, and rice (65 g), mixed with a solution of 80 ml warm honey and 100 mL glycerol.

Experimental setup

Prior to experimentation, all larvae were surface-sterilized with 0.1% sodium hypochlorite and rinsed with sterile double-distilled water (DDW) to remove external contaminants. Twenty healthy fifth-instar larvae of each host species were placed separately in sterilized, labeled plastic containers (28 × 16 × 8 cm) containing 500 g of moist, sterilized sandy soil.

Aqueous suspensions of infective juveniles (IJs) of *S. pakistanense*, *S. balochiense*, and *S. abbasi* were prepared at three concentrations: 50, 150, and 250 IJs per larva in 2 ml of sterile distilled water. The concentrations were standardized using a nematode counting dish under a stereomicroscope, and densities were adjusted by serial dilution. Suspensions were gently agitated before application to ensure uniform distribution.

Each IJ suspension was evenly applied to the soil surface in the containers. The containers were covered with perforated lids to allow aeration and incubated at 25 ± 2°C with relative humidity above 70%. All treatments were replicated three times.

Infection and harvesting

After 24 h, insect cadavers were collected, rinsed thoroughly with sterile DDW, and surface-sterilized with 0.1% sodium hypochlorite. The cadavers were then transferred to White traps (White, 1927) for harvesting infective juveniles.

Each cadaver was placed on an inverted plastic cavity block (4.5 × 4.5 cm) lined with moist filter paper inside a plastic container (28 × 16 × 8 cm). Distilled water (approximately 1 cm depth) was added to facilitate nematode migration. The containers were covered and incubated at 30 ± 5°C.

Emerging infective juveniles migrating into the surrounding water were collected daily until emergence ceased. The harvested nematodes were quantified using a nematode counting dish under a stereomicroscope.

Data analysis

Data were analyzed using SPSS (Version 26.0). Means and standard errors were calculated for larval populations and infective juvenile yields. Pearson's correlation analysis was performed to determine the relationship between *H. armigera* incidence and abiotic factors.

Laboratory data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of host species and inoculum density on IJ yield. Treatment means were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

Results

Incidence of gram pod borer (*H. armigera*) on chickpea

The seasonal incidence of the gram pod borer on chickpea is summarized in Tables 1 and 2 for the Rabi 2023-24 and 2024-25 seasons, respectively. During the 2023-24 season, larval activity commenced in SMW 49 (03-09 December 2023) with an initial mean density of 0.6 ± 0.15 larvae per plant and persisted until approximately SMW 12 (25 March 2024). The population exhibited three distinct peaks: the first in SMW 9 (29.18°C; 48.98% RH; 0.00 mm rainfall) with 2.1 ± 0.14 larvae per plant; the second and highest peak in SMW 10 (30.56°C; 53.28% RH; 0.00 mm) with 2.3 ± 0.13 larvae per plant; and a third peak in SMW 11 (33.43°C; 67.83% RH; 0.00 mm) with 2.2 ± 0.13 larvae per plant. A minor peak was observed in SMW 4 (27.84°C; 58.53% RH; 0.00 mm) with 1.2 ± 0.12 larvae per plant. The minimum infestation (0.4 ± 0.09 larvae per plant) was recorded in SMW 49 (24.37°C; 39.72% RH; 0.00 mm). Overall, the larval population ranged from 0.4 to 2.3 larvae per plant, with a seasonal mean of 1.39 ± 0.12.

In the 2024-25 season, infestation was again first recorded in SMW 49 (03-09 December 2024) at 0.6 ± 0.10 larvae per plant and continued until approximately SMW 12 (25 March 2025). Three major peaks were observed: the first and highest in SMW 9 (29.45°C; 32.3% RH; 0.00 mm) with 2.4 ± 0.15 larvae per plant; the second in SMW 10 (32.34°C; 33.2% RH; 22.35 mm rainfall) with 2.3 ± 0.14 larvae per plant; and the third in SMW 11 (32.82°C; 29.4% RH; 0.00 mm) with 2.2 ± 0.13 larvae per plant. The minimum infestation (0.6 ± 0.10 larvae per plant) was recorded in SMW 49 (28.37°C; 29.72% RH; 0.00 mm). The population ranged from 0.6 to 2.4 larvae per plant, with an overall mean of 1.18 ± 0.11.

Correlation analysis indicated that, in 2023-24, temperature showed a weak and non-significant positive correlation ($r = 0.146$-0.434; $P > 0.05$), while relative humidity also exhibited a weak positive association. In contrast, during 2024-25, temperature showed a significant positive correlation (r

= 0.691-0.788; P < 0.05) with larval population. Relative humidity exhibited a moderate positive correlation (r = 0.504; P = 0.046), suggesting that moderate humidity levels enhanced pest activity.

For bioassay comparisons, *G. mellonella* exhibited a higher standard error (211.29) and least significant difference (LSD = 443.90) compared to *H. armigera*, which showed a lower standard error (67.121) and LSD (141.02).

Table 1. Seasonal incidence of *H. armigera* during 2023-2024.

SMW	Date	Temperature (Min-Max °C)	Mean larvae/plant ± SE	Average precipitation (mm)	Relative Humidity (%)
49	03-09 Dec	11.82-24.37	0.4	0	39.72
50	10-16 Dec	10.64-22.89	0.8	0	40.54
51	17-23 Dec	9.27-23.68	0.7	0	38.51
52	24-31 Dec	6.85-22.46	0.8	10.3	45.12
1	01-07 Jan	5.62-20.81	0.9	0	38.05
2	08-14 Jan	6.85-23.77	1.1	0	40.21
3	15-21 Jan	9.23-26.71	1	16.5	42.46
4	22-28 Jan	8.34-27.84	1.2	0	58.53
5	29 Jan-04 Feb	8.31-27.12	1.2	0	49.62
6	05-11 Feb	11.54-28.54	1.6	0	57.35
7	12-18 Feb	12.19-29.26	1.8	30.8	54.34
8	19-25 Feb	12.31-28.98	1.7	0	22.18
9	26 Feb-04 Mar	12.76-29.18	2.1	0	48.98
10	05-11 Mar	16.34-30.56	2.3	0	53.28
11	12-18 Mar	17.19-33.43	2.2	0	67.83
12	19-25 Mar	19.32-36.62	1.7	0	60.17

Table 2. Seasonal incidence of *H. armigera* during 2024-2025.

SMW	Date	Temperature (Min-Max °C)	Mean larvae/plant ± SE	Average precipitation (mm)	Relative Humidity (%)
49	03-09 Dec	17.10-28.37	0.6	0	29.72
50	10-16 Dec	18.32-28.89	0.9	0	25.54
51	17-23 Dec	11.51-25.28	0.7	0	31.51
52	24-31 Dec	8.23-19.46	0.8	28.76	30.12
1	01-07 Jan	7.42-18.81	1	0	29.05
2	08-14 Jan	7.43-20.41	1.1	0	34.21
3	15-21 Jan	8.56-22.14	1.2	0	32.46
4	22-28 Jan	7.43-23.84	1.4	0	35.53
5	29 Jan-04 Feb	7.23-24.12	1.8	15.67	39.62
6	05-11 Feb	8.52-26.17	1.5	20.87	42.2
7	12-18 Feb	8.15-29.51	1.6	0	31.7
8	19-25 Feb	9.43-28.32	2	0	28.6
9	26 Feb-04 Mar	12.82-29.45	2.4	0	32.3
10	05-11 Mar	14.63-32.34	2.3	22.35	33.2
11	12-18 Mar	17.53-32.82	2.2	0	29.4
12	19-25 Mar	17.62-34.34	1.8	0	33.2

In the 2024-25 season, infestation was again first recorded in SMW 49 (03-09 December 2024) at 0.6 ± 0.10 larvae per plant and continued until approximately SMW 12 (25 March 2025). Three major peaks were observed: the first and highest in SMW 9 (29.45°C; 32.3% RH; 0.00 mm) with 2.4 ± 0.15 larvae per plant; the second in SMW 10 (32.34°C; 33.2% RH; 22.35 mm rainfall) with 2.3 ± 0.14 larvae per plant; and the third in SMW 11 (32.82°C; 29.4% RH; 0.00 mm) with 2.2 ± 0.13 larvae per plant. The minimum infestation (0.6 ± 0.10 larvae per plant) was recorded in SMW 49 (28.37°C; 29.72% RH; 0.00 mm). The population ranged from 0.6 to 2.4 larvae per plant, with an overall mean of 1.18 ± 0.11.

Correlation analysis indicated that, in 2023-24, temperature showed a weak and non-significant positive correlation with larval population ($r = 0.146$-0.434; $P > 0.05$), while relative humidity also exhibited a weak positive association. In contrast, during 2024-25, temperature showed a significant positive correlation ($r = 0.691$-0.788; $P < 0.05$) with larval population. Relative humidity exhibited a moderate positive correlation ($r = 0.504$; $P = 0.046$), suggesting that moderate humidity levels enhanced pest activity.

For bioassay comparisons, *G. mellonella* exhibited a higher standard error (211.29) and least significant difference (LSD = 443.90) compared to *H. armigera*, which showed a lower standard error (67.121) and LSD (141.02).

In vivo mass production of entomopathogenic nematodes

The *in vivo* multiplication of *S. pakistanense*, *S. balochiense*, and *S. abbasi* was evaluated using two lepidopteran hosts, *G. mellonella* and *H. armigera*, at three inoculum densities (50, 150, and 250 IJs per larva). The results demonstrated a significant ($P < 0.05$) increase in infective juvenile yield with increasing inoculum density for all nematode species and both hosts. *G. mellonella* supported the highest IJ yield overall, confirming its superior suitability for EPN mass production.

Among the nematode species, *S. pakistanense* consistently produced the highest mean progeny, followed by *S. balochiense* and *S. abbasi*. Two-way ANOVA revealed that both host insect ($F = 12.67$, $P < 0.01$) and inoculum level ($F = 17.89$, $P < 0.01$) significantly affected IJ yield. The interaction between host and inoculum level (host × dose) was also significant ($F = 6.23$, $P < 0.05$), indicating that the response to inoculum density varied slightly between host species.

G. mellonella yielded approximately 9-11% higher IJ production than *H. armigera*, further confirming its suitability as a standard *in vivo* host. *S. pakistanense* exhibited the highest reproductive efficiency as shown in Figure 1, suggesting superior host adaptation and fecundity. The increase in IJ yield with higher inoculum density indicates density-dependent infection success, a common characteristic of steinernematid nematodes.

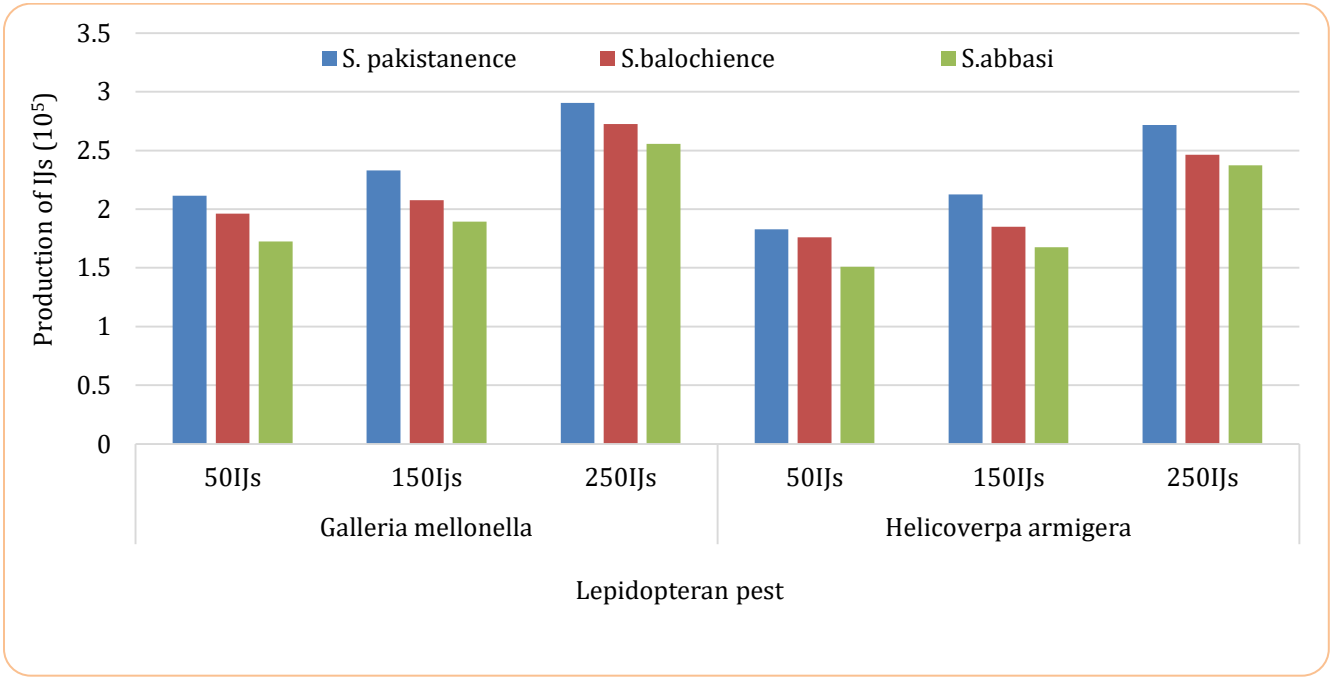

Figure 1. Comparative production of IJs in *G. mellonella* and *H. armigera*.

Discussion

The present study investigated the seasonal incidence of the gram pod borer, *H armigera*, on chickpea in Naseerabad, Balochistan, during the Rabi seasons of 2023-24 and 2024-25, and evaluated the *in vivo* mass production potential of three indigenous entomopathogenic nematodes viz., *S. pakistanense*, *S. balochiense*, and *S. abbasi* using two lepidopteran hosts, *G. mellonella* and *H. armigera*.

Larval activity in both seasons commenced around SMW 49 (early December) and persisted until SMW 12 (late March). During 2023-24, the mean larval density ranged from 0.6 to 2.4 larvae per plant (mean = 1.39 ± 0.12), whereas in 2024-25 it ranged from 0.4 to 2.3 larvae per plant (mean = 1.18 ± 0.11). In both seasons, the population exhibited three distinct peaks around SMW 9-11. These findings are consistent with previous studies conducted in chickpea-growing regions of India and Pakistan, where *H. armigera* populations peak during late winter to early spring (Rehman et al., 2017; Devi et al., 2024; Singh et al., 2025).

The observed moderate larval densities may reflect the influence of local agro-climatic conditions, crop management practices, host plant resistance, and natural enemy pressure (Ali et al., 2016). Correlation analysis revealed that during 2023-24, temperature showed a weak and statistically insignificant positive correlation with larval population ($r = 0.146$-0.434; $P > 0.05$), while relative humidity (RH) also exhibited a weak positive association. In contrast, during 2024-25, both temperature and RH were significantly and positively correlated with larval population (temperature: $r = 0.691$-0.788, $P < 0.05$; RH: $r = 0.504$, $P = 0.046$), suggesting that moderately humid conditions enhanced pest activity (Hussain et al., 2015). These results are in agreement with earlier reports indicating that *H. armigera* population dynamics are strongly influenced by temperature, humidity, rainfall, and crop phenology (Hossain et al., 2008., Bhagat et al., 2020).

From an integrated pest management (IPM) perspective, the consistent occurrence of population peaks around SMW 9-11 indicates that monitoring and control interventions should be strategically timed during this period. However, the variability in correlations between seasons suggests that reliance solely on temperature or RH thresholds may be inadequate. Instead, a holistic approach incorporating crop growth stage, sowing time, cultivar resistance, and natural enemy dynamics is recommended (Rehman et al., 2017; Devi et al., 2024).

The *in vivo* multiplication of *S. pakistanense*, *S. balochiense*, and *S. abbasi* was evaluated using *G. mellonella* and *H. armigera* as hosts at three inoculum densities (50, 150, and 250 infective juveniles per larva). Increasing inoculum density significantly enhanced IJ yield across all nematode species and hosts. *G. mellonella* supported approximately 9-11% higher IJ production compared to *H. armigera*. Among the tested species, *S. pakistanense* exhibited the highest reproductive efficiency, indicating superior adaptation and fecundity.

Previous studies have also demonstrated host-dependent variation in IJ yield. For instance, Fayyaz and Javed (2009) successfully cultured several Pakistani EPN strains on stored-grain insect hosts, while Javed et al. (2020) highlighted the importance of inoculum density and host quality in determining reproductive success under laboratory conditions. The present findings further demonstrate that locally available hosts such as *H. armigera* can serve as effective alternatives for EPN mass production, with only a marginal reduction (approximately 9-11%) in IJ yield compared to *G. mellonella*. These results are consistent with earlier laboratory studies conducted at NNRC, emphasizing the feasibility of large-scale propagation of indigenous EPNs for field application (Fayyaz and Javed, 2009; Javed et al., 2020).

Notably, *S. pakistanense* exhibited comparatively better performance under higher temperatures, suggesting greater thermal tolerance relative to the other species. This adaptability may enhance its effectiveness under field conditions where temperature fluctuations are common. Furthermore, the findings align with global research highlighting the importance of host selection, inoculum density, and host immune response in optimizing IJ production (Kaya and Gaugler, 1993; Shapiro-Ilan et al., 2022).

To evaluate the efficacy of IJs produced from different hosts, a bioassay was conducted using freshly emerged juveniles obtained from *H. armigera* and *G. mellonella*. The results demonstrated that IJs derived from *H. armigera* were equally effective as those obtained from *G. mellonella*, confirming the suitability of this pest as an alternative production host. Overall, integrating indigenous EPNs with optimized inoculum densities and suitable host systems provides a practical and environmentally sustainable approach for the biological control of *H. armigera* in chickpea and other cropping systems.

Conclusion

This study provides comprehensive insights into the temporal dynamics of *Helicoverpa armigera* on chickpea and demonstrates the feasibility of *in vivo* mass production of indigenous entomopathogenic nematodes, particularly *Steinernema pakistanense*. The identification of predictable pest population peaks, combined with efficient laboratory-scale nematode production, offers a strong foundation for developing sustainable, biologically based pest management strategies. Integration of these approaches within broader IPM programs can significantly reduce reliance on chemical insecticides while promoting environmentally safe and economically viable crop protection practices.

Author's contributions

MAM carried out the experiments, analyzed the data, and drafted the initial manuscript. SJ conceptualized and supervised the study, provided guidance on the experimental design, and critically reviewed and revised the manuscript for intellectual content. Both MAM and SJ contributed to the critical evaluation of the study, participated in editing and refining the manuscript, and enhanced its scientific clarity and overall presentation. All authors have read and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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