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Dose-Response and mortality dynamics of *Steinernema abbasi* PN-1 Against *Achroia grisella* (Lepidoptera: Pyralidae): Implications for sustainable beekeeping

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Abstract

This study evaluates the effectiveness of *Steinernema abbasi* PN-1 as a biological control agent against *Achroia grisella* larvae, a major pest in beekeeping. By testing a range of nematode concentrations from 25 to 600 infective juveniles per milliliter (IJs/ml), the research examines the relationship between nematode dose and larval mortality over a 54-hour period. The results demonstrate a clear dose-dependent response, with higher concentrations leading to increased mortality rates and shorter median lethal times (LT₅₀). Specifically, complete mortality (100%) was observed at concentrations of 600 IJs/ml and 400 IJs/ml within 48 hours, underscoring the nematode's rapid and potent control capabilities. Additionally, as nematode concentration increased, LT₅₀ values significantly decreased, indicating faster larval mortality at higher doses. A table presenting the lethal concentrations (LC₅₀ and LC₉₅) and dose-response parameters for *S. abbasi* against *A. grisella* larvae underscores the nematode's effectiveness. The LC₅₀ was determined to be 34.14 IJs per insect, while the LC₉₅ was 904.99 IJs per insect, reflecting substantial control potential. These findings suggest that *S. abbasi* PN-1 could serve as a promising and environmentally sustainable alternative to chemical pesticides for managing *A. grisella* infestations in beekeeping. Future research should prioritize field trials and optimization of application strategies to enhance practical use.

Keywords: *Steinernema abbasi*, *Achroia grisella*, biological control, entomopathogenic nematodes, pest management, beekeeping

1. Introduction

Honey bees play an indispensable role in global agriculture, serving as key pollinators for a wide array of crops, thus supporting biodiversity and contributing to food security (Topno, 2021) [19]. In addition to their critical pollination services, honey bees are also valued for producing honey, beeswax, bee pollen, royal jelly, and propolis, which have significant economic and nutritional importance (Solanki *et al.* 2020) [17]. However, honey bee populations worldwide are increasingly threatened by various stressors, including habitat loss, pesticide exposure, climate change, parasites, and diseases. These factors collectively contribute to colony decline and pose significant challenges to the beekeeping industry (Solanki *et al.* 2020) [17]. Among the less recognized yet concerning threats to honey bee colonies is the infestation by the lesser wax moth (*Achroia grisella* Fabricius) (Lepidoptera: Pyralidae) (Suyal and Pandey, 2024) [18]. Although not as detrimental as other stressors, wax moth infestations can lead to severe consequences if not properly managed (Topno, 2021) [19]. Lesser wax moths are particularly problematic in poorly managed colonies, where they exploit weakened hive conditions, leading to extensive damage to the wax comb (Trinh *et al.* 2021). The larvae of *A. grisella* burrow through the comb, spinning silk and leaving behind a mass of webbing and frass, which can quickly overwhelm a hive (Mahapatra *et al.* 2023) [9]. Severe infestations disrupt the normal functioning of the colony, halting brood rearing and foraging activities. This can lead to a decline in colony strength and, in extreme cases, cause the bees to abscond, abandoning the hive altogether (Ebadi *et al.*, 1980; Gulati and Kaushik, 2004) [4, 7]. The susceptibility of honey bee colonies to wax moth infestations is often exacerbated by beekeeping practices that neglect proper hive management and stress mitigation.

While *Apis mellifera* colonies are somewhat resilient to wax moth attacks, *A. cerana* colonies are particularly vulnerable, especially during periods of natural swarming (Mahapatra *et al.* 2023) [9]. The tendency of beekeepers to overlook the early signs of wax moth infestation, often mistaking them for minor issues, can result in significant colony losses and economic damage (Mahapatra *et al.* 2023) [9]. Recognizing the need for effective management strategies, recent research has focused on biological control methods as sustainable alternatives to chemical insecticides (Pavan *et al.* 2024a; Pavan *et al.* 2024b; Pavan *et al.* 2024c) [10-12]. Entomopathogenic nematodes, particularly those from the genera *Steinernema* and *Heterorhabditis*, have emerged as promising biocontrol agents (Shamseldeen *et al.* 2024) [16]. These nematodes are known for their ability to infect and kill a wide range of insect pests, making them an attractive option for managing wax moth populations (Pervez, 2022) [13]. *Steinernema abbasi*, in particular, has shown potential in targeting lesser wax moth larvae, offering a non-toxic and environmentally friendly solution to this persistent problem (Georgis and Manweiler, 1994) [6]. The current study was undertaken to assess the virulence of *S. abbasi* against *A. grisella* larvae (Bandaru *et al.* 2020) [2]. The goal is to develop an effective biological control strategy that can be integrated into existing beekeeping practices, reducing the impact of wax moth infestations and promoting the health and productivity of honey bee colonies. By exploring the use of entomopathogenic nematodes as a tool for pest management, this research aims to provide beekeepers with practical solutions to enhance colony resilience, ensuring the sustainability of the beekeeping industry in the face of growing environmental challenges.

2. Materials and methods

2.1 Rearing of *A. grisella*

Fully grown larvae and pupae of *A. grisella* were carefully separated from infested combs collected from the apiary and placed in glass jars to facilitate adult emergence. To eliminate natural enemies of *A. grisella*, uninfested combs were treated by spraying with a 0.4% formalin solution and then stored in airtight polythene covers for two days. After treatment, the combs were shade-dried and subsequently used as a food source for the moth culture. Emerging adult

moths were transferred to a glass container, where a piece of comb was provided for egg-laying. The adult moths were fed with cotton soaked in a 20% sugar solution. Combs containing eggs were removed daily and placed in separate transparent boxes for incubation. The larvae that hatched from these eggs were then separated and used for further experimental studies.

2.2 EPN Culture

The nematode culture of *S. abbasi* PN-1 was obtained from the Insect Pathology Laboratory at the College of Agriculture, Pantnagar. The mass multiplication of *S. abbasi* was conducted using last-instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) as the host. The larvae were placed in Petri dishes lined with Whatman No. 1 filter paper, and 1 ml of a suspension containing 1000 infective juveniles (IJ) of *S. abbasi* was added to each dish. After the larvae succumbed to the nematode infection, they were transferred to a White trap setup and kept in an incubator. The freshly emerged infective juveniles (J3) were then washed twice with distilled water to ensure purity and stored in sterile distilled water in tissue culture flasks. These flasks were maintained in an incubator at 25±2 °C for use in subsequent experiments.

2.3 Virulence of *Steinernema abbasi* Against *A. grisella* Under Laboratory Conditions

The virulence of the entomopathogenic nematode *S. abbasi* against *A. grisella* was evaluated using the Petri dish method. To assess the pathogenicity, 1 ml of sterile distilled water containing varying concentrations of *S. abbasi* infective juveniles (IJs) was evenly distributed on a 9 cm diameter Whatman No. 1 filter paper placed in the lid of a 9 cm diameter Petri dish. The concentrations tested included 0, 25, 50, 100, 200, 400, and 600 IJs per larva. Ten last instar *A. grisella* larvae were placed on the treated filter paper in each Petri dish, which was then covered with the inverted Petri dish lid. Each treatment was replicated three times, and the experimental setup was arranged in a Completely Randomized Design (CRD). The bioassay was monitored daily for four days to record larval mortality, providing insight into the effectiveness of *S. abbasi* at various concentrations (Fig 1.).

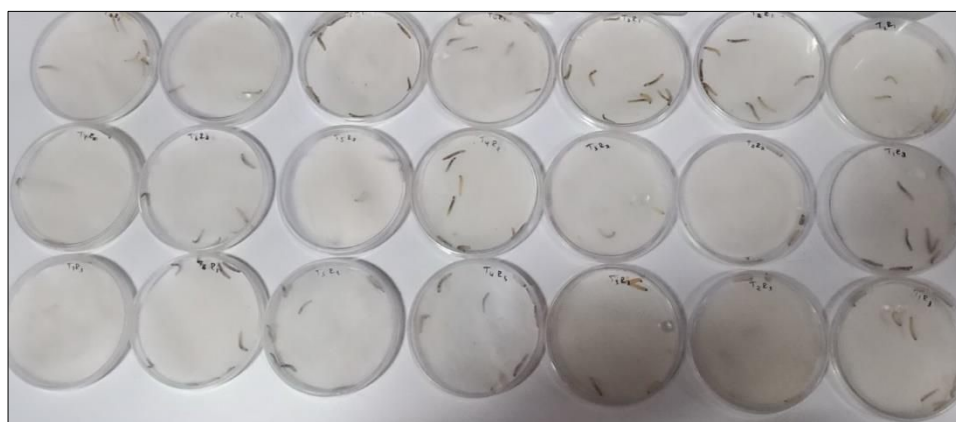


Fig 1: Virulence of *Steinernema abbasi* against *A. grisella*

3. Results

These results clearly demonstrate the strong potential of *S. abbasi* PN-1 as an effective biological control agent against *A. grisella* larvae, which are significant pests in beekeeping.

The marked increase in mortality with higher concentrations of nematodes and longer exposure times points to the nematode's ability to effectively penetrate and kill *A. grisella* larvae, disrupting their lifecycle and reducing their

impact on honeybee colonies. **Concentration-Dependent Efficacy:** The data reveal a direct correlation between nematode concentration and larval mortality. At the highest concentrations (600 and 400 IJs/ml), nearly complete mortality was observed within 36 hours, with all larvae succumbing by 48 hours. This indicates that *S. abbasi* can effectively control even large infestations of wax moths if applied at appropriate concentrations. The absence of mortality in lower concentrations and control treatments further validates the nematode's role in the observed larval deaths, ruling out environmental or incidental factors. **Time-Dependent Efficacy:** The results also highlight the importance of exposure time in the effectiveness of *S. abbasi*. Mortality rates increased significantly over time, with substantial differences observed between the 24-hour and 48-hour assessments. This suggests that while *S. abbasi* begins to act quickly, its full impact is realized over a slightly longer period, making it crucial for beekeepers to allow sufficient time for the nematodes to work after application. **Practical Implications:** The practical implications of these findings are significant for the beekeeping industry. Wax moth infestations, if not properly managed, can lead to severe damage to combs and ultimately result in colony absconding. The use of *S. abbasi* provides a sustainable and environmentally friendly alternative to chemical treatments, which can have adverse effects on bees and hive products. By incorporating *S. abbasi* into integrated pest management (IPM) strategies, beekeepers can effectively manage wax moth populations, protecting their colonies and reducing economic losses.

Table 1: Per cent mean mortality of *A. grisella* at different concentrations of *Steinernema abbasi* PN-1

Treatments (IJs/ml)	Mean per cent mortality at different hours			
	24 HAT	36 HAT	48 HAT	60 HAT
25	0	43.33	83.33	100
50	0	56.33	96.66	100
100	16.66	73.33	100	100
200	23.33	80	100	100
400	33.33	100	100	100
600	36.66	100	100	100
Control	0	0	0	0
CD	0.848	0.848	0.600	
SE(m)	0.272	0.272	0.192	

HAT = hours after treatment, IJs = infective juveniles

3.1 Determination of LT₅₀ of *Steinernema abbasi* PN-1

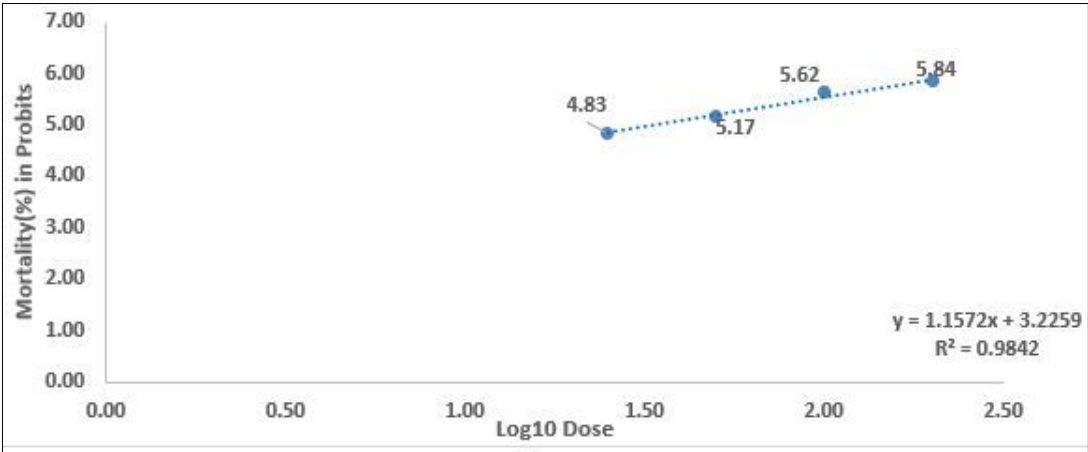


Fig 2: Probit Analysis of *Steinernema abbasi* Virulence Against *A. grisella* Under Laboratory Conditions

Against *Achroia grisella*

The median lethal time (LT₅₀) for last instar larvae of *A. grisella* was determined at various concentrations of *S. abbasi* PN-1. The LT₅₀ values were calculated as follows: 28.71 hours at 600 IJs/larva, 28.75 hours at 400 IJs/larva, 29.23 hours at 200 IJs/larva, 30.22 hours at 100 IJs/larva, 34.78 hours at 50 IJs/larva, and 38 hours at 25 IJs/larva (Table 2). The results indicate that higher concentrations of infective juveniles (IJs) required less time to achieve 50% mortality of the tested larvae. Specifically, the LT₅₀ values were inversely correlated with the concentration of IJs, meaning that as the concentration of infective juveniles increased, the time required to reach 50% mortality decreased. This negative correlation suggests that *S. abbasi* PN-1 is more effective in a shorter time frame when applied at higher concentrations, thereby enhancing its utility as a rapid biocontrol agent against *A. grisella*.

Table 2: Determination of LT₅₀ of *S. abbasi* PN-1 to *A. grisella* larvae

Treatments (IJ/ml)	LT ₅₀ (Hours)	Fiducial limit		Regression equation
		Lower	Upper	
25	38	34.13	42.13	7.8591x (-7.4153)
50	34.78	31.91	37.91	11.376x (-12.524)
100	30.22	27.52	33.17	11.207x (-11.584)
200	29.23	26.32	32.46	9.5179x (-8.9509)
400	28.93	24.75	33.45	5.3078x (-2.7566)
600	28.71	23.91	34.47	4.3788x (-1.3843)

3.2 Dose-Mortality Relationship

The LC₅₀ analysis for *S. abbasi* against *A. grisella* larvae provides insights into the efficacy of the nematode as a biological control agent. The LC₅₀, representing the concentration of infective juveniles (IJs) required to kill 50% of the larvae, is estimated at 34.14 IJs per insect (Table. 3). The 95% confidence interval for this value ranges from 16.66 to 69.99 IJs per insect, indicating a moderate degree of variability in the concentration needed to achieve this level of mortality. As the dose of *S. abbasi* increases (as shown by the increasing Log₁₀ Dose on the x-axis), the mortality of *A. grisella* also increases (as reflected by the increasing probit values on the y-axis). This indicates that higher concentrations of *S. abbasi* lead to greater mortality in *A. grisella*, which is expected in a dose-response experiment (Fig 2.).

Table 3: Lethal Concentrations and Response Parameters of *Steinernema abbasi* Against *Achroia grisella*

LC ₅₀ IJ/insect	95% CI (Lower-Upper)	LC ₉₅ IJ/insect	95% CI (Lower-Upper)	Slope (±SE)	χ ²
34.14	16.66-69.99	904.99	441.49-1855.05	1.15(0.15)	0.98

4. Discussion

The results from this study on the efficacy of *S. abbasi* PN-1 against the last instar larvae of *A. grisella* align well with previous research on the performance of entomopathogenic nematodes (EPNs) in controlling various insect pests. The dose-response relationship observed, where higher concentrations led to increased mortality rates and shorter median lethal times (LT₅₀), is consistent with established findings in the literature. The effectiveness of different concentrations of *S. abbasi* PN-1 observed in this study parallels findings by Aasha *et al.* (2019) ^[1], who tested multiple isolates of *Steinernema* sp. against *G. mellonella*. They reported 100% mortality after 60 hours of exposure, which supports the effectiveness of EPNs in achieving complete pest control, similar to the 100% mortality observed in our study at higher concentrations by 48 hours. In agreement with our findings, Istkhari *et al.* (2016) ^[8] documented mortality initiation within 24 hours of infection with one strain of *Steinernema*, reaching 100% mortality by 60 hours at all tested concentrations. This underscores the rapid action of EPNs and their ability to cause significant pest mortality within a relatively short period, which is crucial for effective pest management. Bhat *et al.* (2021) ^[3] also reported a rapid onset of mortality with *S. abbasi* isolates. They noted that larvae of *G. mellonella*, *Helicoverpa armigera*, and *Spodoptera litura* showed mortality starting within 12 hours and complete mortality within 48 hours. This is consistent with our study, where significant mortality was observed within the same time frame, further validating the quick and effective control capabilities of *S. abbasi* PN-1. The similarity in results across these studies highlights the reliability of *Steinernema* species as effective biological control agents. The rapid and high mortality rates observed at various concentrations of *S. abbasi* PN-1 indicate its potential for managing *A. grisella* infestations in beekeeping environments. The ability of *S. abbasi* to provide complete pest control within 48 hours, especially at higher concentrations, aligns with the needs of beekeepers for quick and efficient pest management solutions.

5. Conclusion

This study demonstrates the promising potential of *S. abbasi* PN-1 as a biological control agent against *A. grisella*, a pest that poses significant challenges in beekeeping. The research confirms that different concentrations of *S. abbasi* are effective in managing the larvae of *A. grisella*, with higher concentrations resulting in increased mortality rates and shorter median lethal times (LT₅₀). Specifically, the nematode achieved 100% mortality at higher concentrations (600 IJs/ml and 400 IJs/ml) within 48 hours, showcasing its rapid and effective control capabilities. The observed dose-response relationship, where higher concentrations of *S. abbasi* led to faster and more complete pest control, aligns with similar findings from previous studies. The rapid onset of mortality and the high efficacy of *S. abbasi* PN-1 underscore its potential as a viable alternative to chemical pesticides, offering an environmentally friendly solution for managing wax moth infestations in beekeeping. These results provide a solid foundation for integrating *S. abbasi*

PN-1 into integrated pest management (IPM) strategies for beekeeping. Future research should focus on field trials to confirm laboratory findings, assess the impact of environmental conditions on nematode efficacy, and explore the interactions with non-target organisms. Optimizing application methods and concentrations will also be crucial for practical implementation. In summary, *S. abbasi* PN-1 emerges as a highly effective biological control agent with the potential to significantly improve pest management practices in the beekeeping industry, contributing to sustainable and environmentally friendly pest control solutions.

6. Author contributions

- **PV:** Methodology, Investigation Software analysis, Writing- Original draft preparation, Validation, Formal analysis.
- **JSP&MAP:** Conceptualization, Visualization, Methodology, Data curation, Supervision, Reviewing and Editing.
- **HDM:** Methodology, Data curation, Supervision, Reviewing and Editing.
- **HJ:** Investigation Software analysis, Formal analysis, Reviewing and Editing.

7. Data availability statement

All the data have been provided in the main text of the manuscript.

8. Ethics approval statement

Not Applicable

9. Consent to participate

Not Applicable

10. Consent to publish

Not Applicable

11. Reference

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