

Journal of Biological Control, 38(4): 406-412, 2024, DOI: 10.18311/jbc/2024/45792

Research Article

Bio-efficacy of a native strain of entomopathogenic nematode, *Steinernema siamkayai* against fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT: The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a serious pest known for its ability to feed on various crops, particularly maize, inflicting significant reduction in maize yield. Farmers have traditionally dependent on chemical insecticides to manage this pest. These insecticides are frequently ineffective because the pest's hidden feeding habits hinder the chemicals from reaching their intended target. In response to this challenge, an *in vitro* bioassay was performed to evaluate the virulence of an entomopathogenic nematode *Steinernema siamkayai* against third-instar larvae of *Spodoptera frugiperda* and wax moth (*Galleria mellonella*) at different concentrations of infective juveniles. Mortality rates were monitored from 24 to 96 h post-inoculation, with *S. siamkayai* demonstrating notable effectiveness against both insect species. For the fall armyworm, mortality rates risen progressively, beginning at 16.66% for 100 IJs larva^{-1} after 24 h, reaching 83.33% at 48 h, and achieving 100% by 96 h for doses of 30 IJs larva^{-1} or more. Wax moth larvae, on the other hand, showed greater susceptibility, with 22.91% mortality at 5 IJs larva^{-1} within 24 h and 100% mortality at 100 IJs larva^{-1} by the 48-hour mark. After 96 h, complete mortality was recorded in both species at doses of 30 IJs larva^{-1} or higher. Probit analysis indicated lower LC_{50} values for *G. mellonella* (1.83 and 0.87 IJs larva^{-1} at 72 and 96 h) compared to FAW (9.83 and 5.47 IJs larva^{-1}). These findings suggest that *S. siamkayai* is a highly efficient bio-control agent, especially for managing *S. frugiperda*, and shows potential for integration into pest management programs.

KEYWORDS: EPN, *Galleria mellonella, Spodoptera frugiperda, Steinernema siamkayai*, virulence

(Article chronicle: Received: 09-09-2024; Revised: 11-12-2024; Accepted: 14-12-2024)

INTRODUCTION

Maize (*Zea mays* L.) is a highly adaptable crop with diverse applications, often referred to as the 'Queen of Cereals' and the 'Miracle Crop' because of its remarkable genetic yield potential. In India, maize ranks as the third most important food crop after rice and wheat, contributing around 10% to the nation's total food grain production (Shukla *et al*., 2018). Besides being a staple food for humans and a nutritious feed for animals, maize also serves as a crucial raw material for various industrial products such as starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceuticals, cosmetics, textiles, gum, and packaging and paper products. The production of maize in India is currently facing a significant reduction due to pest infestations. These pests cause an estimated 22-67% economic loss of the crop across the world, which negatively impacts maize yields (Baudron *et al*., 2019). The pests affecting maize are categorized into four types: borers, sap feeders, ear head feeders, and leaf feeders. Key pests in India include the spotted stem borer (*Chilo partellus*), the pink stem borer (*Sesamia inference*), and the fall armyworm (*Spodoptera frugiperda*). Of these, the fall armyworm has emerged as a significant threat to maize cultivation.

A wide range of chemical insecticides have been tested to control *S. frugiperda*, but they have not provided satisfactory results. Chemical control has proven ineffective, as *S. frugiperda* has rapidly developed resistance to major classes of commonly used insecticides (Zhu *et al*., 2015) and cry proteins from *Bacillus thuringiensis* (Bt) (Murúa *et al*., 2019). Considering the issues associated with resistance and the potential adverse effects of repeated pesticide use on human health and environmental safety,

it is essential to develop effective and sustainable control strategies. Incorporating biological control measures, such as natural enemies including predators, parasitoids, and pathogens, is a key approach. Biological control agents are strongly recommended as eco-friendly alternatives to hazardous chemical pesticides, providing a sustainable and environmentally safe strategy for managing *S. frugiperda*.

Among the various biological control methods, Entomopathogenic Nematodes (EPNs) have emerged as one of the most effective and attractive options for managing *S. frugiperda*. Entomopathogenic nematodes from the families Heterorhabditidae and Steinernematidae are highly virulent due to their symbiotic relationships with the bacterial genera *Photorhabdus* spp. and *Xenorhabdus* spp., respectively (Kavya *et al.*, 2006). However, the efficacy of native EPN strains must be assessed before incorporating them as key components in Integrated Pest Management (IPM) programs. Therefore, the present study was taken up to evaluate of the efficacy of a native strain of EPN, *Steinernema siamkayai* for the management of the larvae of *S. frugiperda* (third instar) and to compare its virulence with EPN susceptible host *Galleria mellonella.*

MATERIALS AND METHODS

Rearing of *Spodoptera frugiperda*

For the experiment larvae of *S. frugiperda* were collected from maize crops situated in the fields of ICAR-Central Institute for Cotton Research (ICAR-CICR), Nagpur, Maharashtra, India during 2023–24. Early-stage larvae were reared on maize stem before shifting them to a factitious diet (Prasanna *et al*., 2018). The larvae were maintained under controlled conditions of 27±1°C, 65±5% Relative Humidity (RH), and a 14-h light to 10-h dark photoperiod. Laboratory culture of *Galleria mellonella* was simultaneously maintained at ICAR-CICR on an artificial diet consisting of wheat and corn (Metwally *et al.*, 2012) under a similar controlled environment.

Nematode culture

The culture of EPN, *Steinernema siamkayai* was obtained from the Crop Protection Division of ICAR-CICR, RS Coimbatore. A late 5th instar of *G. mellonella* larvae was used to maintain this culture. The freshly emerged infective juveniles from larval cadavers of *G. mellonella* were trapped and collected using the White trap method (White, 1927) and kept in sterilized water.

In vitro bioassay

Virulence of the *S. siamkayai* was evaluated against the 3rd instar larvae of *Spodoptera frugiperda* using a larval bioassay. The third instar larvae are one of the most destructive stages among the various larval instars therefore the same has been targeted in the present study. To treat a single 3rd instar larva 40 µL of water (diluent) was calibrated and used. In the beginning, a range-finding bioassay was conducted with doses 2, 20, 100, 300, and 500 IJs larva^{-1} (data not shown) to ascertain the final dosages required in subsequent bioassays. Based on the preliminary data, seven different concentrations of IJs (5, 10, 20, 30, 40, 80 and 100 IJs larva^{-1}) were finalized for further studies.

Each treatment consisted of 48 larvae (12 larvae/ replication and replicated four times). The total number of larvae used during the experiment was 384, including a control group where water without IJs was applied. Individual larvae were kept in multi-cell plates containing 24-well (Genaxy Scientific Pvt. Ltd, India) to be fed on the cubes made up of a semi-synthetic diet (0.25 to 0.40 mL per well). The EPN doses were applied directly onto the larvae in each well.

Every plate was secured using the Parafilm to prevent loss of moisture. These larvae were then incubated in a BOD incubator at 25°C±2°C. Untreated control plates were maintained under identical conditions, except no IJs were introduced. Larval mortality was recorded at 24 h intervals till 120 h post-inoculation. To ascertain that the cause of observed mortality is nematode infection, the infested cadavers were dissected in Ringer's solution under a stereomicroscope to detect the nematodes. The larval mortality data, recorded was later used to calculate LC_{50} value by subjecting it to probit analysis (Finney, 1971) by employing the Polo Plus software (Version 2.0, LeOra).

Additionally, a similar experiment was also conducted against *G. mellonella* to compare the efficacy of *S. siamkayai* between FAW and wax moth. *Galleria* is the most susceptible host for EPN, therefore a present study was useful to check and understand the performance of *S. siamkayai* against FAW.

Statistical analysis

Percentage data were normalized using arcsine transformation, the analysis was undertaken on the transformed data. The number of IJs required for killing 50% Lethal Concentration (LC_{50}) and 90% (LC_{90}) of the FAW and greater wax moth larvae after 72 and 96 h post-inoculation was calculated using Probit analysis at 95% confidence intervals. The data on mortality were pooled from repetitive experiments and subjected to Analysis Of Variance (ANOVA) using SPSS software version 21.0 (IBM Corp, 2012). When ANOVA was significant, comparisons of relevant means were made using Tukey's significance test values at the 5% level of significance.

RESULTS

Bio-efficacy of *Steinernema siamkayai* against *S. frugiperda*

An *in vitro* bioassay was carried out to assess the virulence of *S. siamkayai* against third instar larvae of the Fall Armyworm (FAW) using different concentrations of Infectiv#e juven#iles (IJs). Mortality was recorded at intervals from 24 to 96 h post-inoculation, revealing significant variations in mortality rates across the different IJ concentrations.

At 24 h, an initial mortality rate of 2.08% was observed when larvae were inoculated with 40 IJs larva^{-1}, while a higher dose of 100 IJs larva^{-1} resulted in 16.66% mortality. As the exposure time extended to 48 h, the treatment with the 100 IJs larva^{-1} dose provided the highest mortality, with a mortality rate of 83.33%, whereas the lowest mortality of 16.66% was seen at the 5 IJs larva^{-1} dose (Table 1).

By 72 h, there was a gradual increase in mortality, with the lowest mortality rate of 25% observed at the 5 IJs larva^{-1} and the highest mortality of 97.91% at the 100 IJs larva^{-1}. After 96 h, 100% mortality was recorded at the doses of 100, 80, 40, and 30 IJs larva^{-1}. A mortality rate of 91.66% was observed at the 20 IJs larva^{-1}, which was statistically similar to the higher doses, while the lowest mortality rate of 43.75% was recorded at the 5 IJs larva^{-1}. A minimal mortality rate of 2.08% was recorded in the control (Figure 1).

These results demonstrate that *S. siamkayai* is highly effective against FAW larvae, with higher doses leading to significantly increased mortality over time. The study highlights the importance of selecting an appropriate dose to achieve optimal pest control, particularly in integrated pest management programs where *S. siamkayai* could serve as a potent biological control agent.

Bio-efficacy of *S. siamkayai* against susceptible host *Galleria mellonella*

To compare the efficacy of *S. siamkayai* with FAW the same was evaluated against wax moth and found that all doses had significant effects on larval mortality. After 24 h of inoculation, a dose of 5 IJs larva^{-1} resulted in 22.91% mortality, while the highest dose of 100 IJs larva^{-1} achieved 100% mortality.

As the exposure time increased to 48 h, the mortality rates also rose, resulting in observed mortality of 100% at the highest dose, *i.e.*, 100 IJs larva^{-1}, contrarily, the lowest mortality of 70.83% was observed at the 5 IJs larva^{-1} dose. By 72 h, mortality across the doses ranged from 83% to 100%, with the lowest mortality at the 5 IJs larva^{-1} dose and the highest at 100 IJs larva^{-1} (Table 2). After 96 h, complete mortality (100%) was recorded at doses of 100, 80, 40, and 30 IJs larva^{-1} which was on par with treatments with 20 and 10 IJs larva^{-1} where mortality of 95.83 % and 93.75%, respectively were recorded. The lowest mortality rate of 89.58% was observed at the 5 IJs larva^{-1} dose.

Probit analysis of *S. siamkayai* against FAW indicated that the LC_{50} values for 3rd instar larvae were 9.83 and 5.47

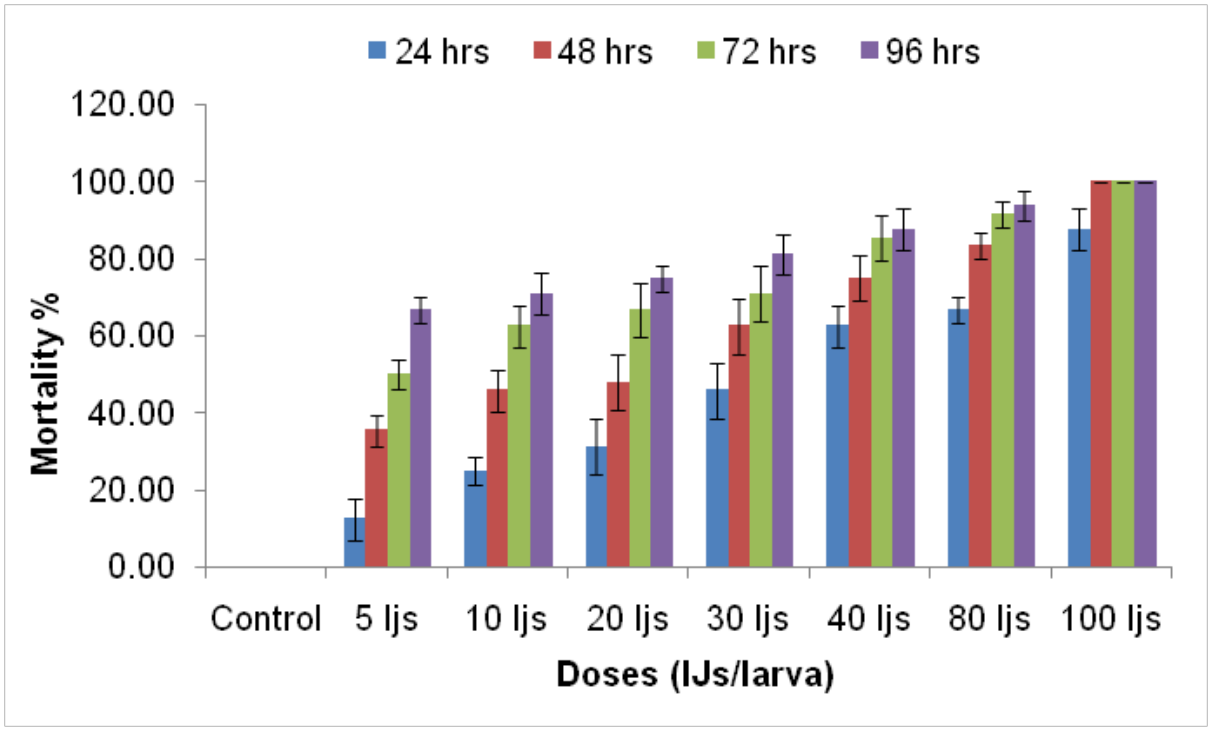


Figure 1. Bio-efficacy of *Steinernema siamkayai* against *S. frugiperda.*

Table 1. Percent larval mortality *of Spodoptera frugiperda* by *Steinernema siamkayai*

S. No	Concentration of EPN isolate used (IJs larva^{-1})	Mortality (%)			
		24 h	**48 h**	**72 h**	
1	5	0.00^{b} (0.0)	16.67^{d} (23.74)	25.00^{d} (29.09)	43.75^{d} (41.37)
2	10	0.00b (0.0)	31.25^{c} (33.87)	60.42^{c} (51.07)	83.33^{c} (66.26)
3	20	0.00b (0.0)	43.75^{bc} (41.33)	66.67^{c} (54.82)	91.67^{b} (75.46)
4	30	0.00^{b} (0.0)	52.08^{b} (46.32)	81.25^{b} (64.78)	100.00^{a} (89.49)
5	40	2.08^{b} (4.574)	56.25^{b} (48.63)	85.42^{ab} (70.84)	100.00^{a} (89.49)
6	80	4.17^{b} (8.641)	58.33^{b} (50.23)	91.67^{ab} (75.46)	100.00^{a} (89.49)
7	100	16.67^{a} (23.743)	83.34^{a} (69.36)	97.92^{a} (85.43)	100.00^{a} (89.49)
8	Control	0.00^{b} (0.0)	0.00^{e} (0.0)	0.00^{e} (0.0)	2.08^{e} (4.57)
F_{test}		sig**	sig**	sig**	sig**
F_{cal}		11.59	21.94	42.87	140.12
SE (m)		2.458	4.803	4.110	2.656

(Figures in parentheses are arcsine transformed values, **F test highly significant at 5% level of significance)

Table 2. Per cent larval mortality of *Galleria mellonella* by *Steinernema siamkayai*

S. No	Concentration of EPN used (IJs larva^{-1})	Mortality (%)			
		24 h	**48 h**	**72 h**	**96 h**
1	5	22.92^{d} (28.36)	70.83^{bc} (57.73)	83.33^{c} (66.26)	89.58^{b} (73.63)
2	10	27.08^{d} (30.56)	77.08^{bc} (61.64)	85.42^{bc} (68.09)	93.75^{ab} (79.53)
3	20	31.25^{d} (33.87)	81.25^{b} (64.94)	89.58^{bc} (73.63)	95.84^{a} (81.36)
4	30	52.08^{c} (46.19)	87.50^{b} (74.91)	93.75^{ab} (79.53)	100.00^{a} (89.49)
5	40	85.42^{b} (68.09)	97.92^{ab} (85.43)	100.00^{a} (89.49)	100.00^{a} (89.49)
6	80	95.84^{ab} (81.36)	100.00^{a} (89.49)	100.00^{a} (89.49)	100.00^{a} (89.49)
7	100	100.00^{a} (89.49)	100.00^{a} (89.49)	100.00^{a} (89.49)	100.00^{a} (89.49)
8	Control	0.00^{e} (0.0)	0.00^{d} (0.0)	0.00^{d} (0.0)	0.00^{c} (0.0)
	F_{test}	sig**	sig**	sig**	sig**
	F_{cal}	82.68	50.49	83.00	83.41
	SE (m)	3.35	4.15	3.29	3.41

(Figures in parentheses are arcsine transformed values, **F test highly significant at 5% level of significance)

Table 3. Dose-mortality response of third-instar larvae of *S. frugiperda* to *S. siamkayai* at 72 and 96 h expressed as lethal concentration (LC)

S. No.	Hours	DF	Slope ± SE	χ^2	LC_{50}	95% FL	LC_{90}	95% FL
1	72	5	1.75± 0.205	4.04	9.83	7.21-12.42	53.04	40.48-7.74
2	96	5	3.09±0.448	3.48	5.47	4.13- 6.65	14.23	11.75 -8.68

Table 4. Dose-mortality response of third-instar larvae of *G. mellonella* to *S. siamkayai* at 72 h expressed as lethal concentration (LC)

S. No.	Hours	DF	Slope ± SE	$\chi 2$	LC_{50}	95% FL	LC_{90}	95% FL
1	72	5	1.28±0.299	5.60	1.23	0.153-2.80	12.25	7.57-8.77
2	96	5	1.53±0.474	2.50	0.87	0.019- 2.34	5.95	2.07-9.42

IJs $larva^{-1}$, at 72 and 96 h of inoculation respectively (Table 3). *S siamkayai* was comparatively more virulent against 3rd instar larvae, with significantly lower LC_{50} values of 1.83 and 0.87 IJs $larva^{-1}$, at 72 and 96 h of inoculation respectively (Table 4).

DISCUSSION

The global success of EPN's employment in integrated pest management is widely documented (Kumar *et al*. 2022; Mandadi *et al*., 2024). Utilizing native strains of EPN is essential for effective pest control, as they are typically more efficiently adapted to the indigenous environmental conditions (Nikdel & Niknam, 2015). *In vitro* assessments play a crucial role in selecting EPN strains with the highest virulence for subsequent field applications (Thube *et al*., 2023).

EPNs are capable of targeting and eliminating a wide range of insect species and can be efficiently mass-produced. Their safety for humans and the environment makes them an effective biocontrol agent, particularly for soil-dwelling insect pests. These are vulnerable to ultraviolet light and desiccation, the feeding behaviour of FAW caterpillars, which typically reside deep within the maize whorl, provides a favourable environment for EPN application in the maize–FAW system. EPNs demonstrate remarkable virulence against FAW, capable of killing young caterpillars within just 24 h (Andalo *et al*., 2010; Acharya *et al*., 2020). This rapid action is significantly quicker compared to other Natural enemies, such as entomopathogenic fungi, viruses, or parasitoids.

This study explored the biocontrol potential of the native strain of *S. siamkayai* against FAW. Results indicated significant mortality of FAW within a shorter span of inoculation. The virulence of EPN species depends on various factors, such as host insect species, reproduction rates, and penetration efficiency (Kaya & Gaugler 1993). The *S. siamkayai* strains used in this study were isolated from the rhizosphere of cotton from ICAR- CICR, Regional Station, Coimbatore, Tamil Nadu, India.

In the present study, *S. siamkayai* demonstrated significant efficacy against third-instar larvae of *Spodoptera frugiperda*, achieving 100% mortality within 48 h when applied at a concentration of 100 IJs $larva^{-1}$. Additionally, the lower lethal concentration (LC_{50}) values were recorded at 9.83 IJs $larva^{-1}$ after 72 h and 5.47 IJs $larva^{-1}$ after 96 h, indicating a higher virulence against the target pest. Our results align with previous studies like Gowda *et al*., (2020) reporting 100% mortality of 3rd instar larvae of *S. litura* 48 h post-inoculation with *Heterorhabditis indica* (strain NBAIIH38) and *S. siamkayai* (strain IIVR JNC01) at a rate of 200 IJs $larva^{-1}$. This supports our findings, where 83% mortality was observed at 100 IJs $larva^{-1}$ against *S. frugiperda* at 48 h. Similarly, Wattanachaiyingcharoen *et al*., (2021) found that *H. indica* isolates AUT 13.2 and *S. siamkayai* isolate APL 12.3 showed 27.5% and 17.5% mortality in second-instar larvae, and 17% and 15% mortality in fifth-instar larvae of FAW, respectively, when inoculated at 50 IJs $larva^{-1}$. In a recent study two species of EPN *viz*., *Heterorhabditis indica* (1 NBAIIH38) and *Steinernema carpocapsae* (NBAIRS59) caused 100% mortality in third- and fourth-instar larvae of *S. frugiperda* (Patil *et al.*, 2022). Their results indicate that mortality rates increased with increasing concentrations and time which are consistent with our observation. Garcia *et al*. (2008) reported that the concentration of 280 IJs of *Steinernema* sp. was required to achieve complete mortality of third instar larvae of *S. frugiperda*, while a concentration of 400 IJs of *H. indica* caused 75% mortality. This contrasts with our study, where 100 IJs $larva^{-1}$ of *S. siamkayai* was sufficient to achieve 100% mortality. Furthermore, Salvadori *et al*. (2012) evaluated eight isolates of entomopathogenic nematodes (EPNs) against *S. frugiperda*, reporting 77% mortality with *Heterorhabditis* spp. and 70% with *Steinernema* spp. at 100 IJs $larva^{-1}$ for fifth-instar larvae over five days. Our study corroborates these results, with 100% mortality observed

in third-instar larvae at 100 IJs larva^{-1} within four days, suggesting that *S. siamkayai* may be particularly potent at lower inoculation rates compared to other species. Mohamed and Shairra (2023) further reported that *S. frugiperda* third-instar larvae exhibited 100% mortality when treated with *S. carpocapsae* at concentrations of 600–2400 IJs ml^{-1} after 48 h. Lower concentrations (150–300 IJs ml^{-1}), however, led to mortality rates of 44.44% and 83.33%, respectively. These findings highlight the superior performance of *S. siamkayai* in our study, as 100 IJs larva^{-1} were sufficient to cause complete mortality within 48 h, demonstrating its potential as an efficient biocontrol agent against FAW.

These findings carry a great significance for environmentally safer pest management strategies. Identifying the EPN strains with high virulence can guide the establishment of a highly targeted and potent biological control strategy against FAW damage. This has the potential to reduce dependence on hazardous insecticides and to support more ecologically safer agricultural practices. Further research to understand the underlying mechanisms driving the enhanced virulence of certain strains is crucial to maximize their effectiveness in IPM. The results of the present work demonstrated that *S. siamkayai* is a promising bio-control agent against FAW.

CONCLUSION

In conclusion, *S. siamkayai* exhibited high virulence against 3rd instar larvae of *S. frugiperda*, achieving 100% mortality at doses (30 IJs larva-1 onwards) within 96 h. Mortality rates increased significantly with higher doses of IJs and longer exposure times. The study highlights the potential of *S. siamkayai* as a virulent bio-control agent in integrated pest management of FAW. The lower LC_{50} values further support its efficacy, with comparable results observed in the wax moth, a highly susceptible host.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-Central Institute for Cotton Research (CICR) Nagpur, for providing the research facilities and the College of Agriculture, Nagpur for allowing them to conduct the research work at CICR. The authors are also thankful to the competent authority of ICAR-CICR, RS, Coimbatore for providing the EPN strain evaluated under this study.

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