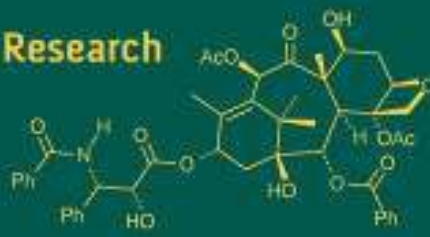
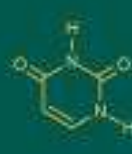


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Biocontrol potential of *Photorhabdus luminescens* isolate UASD_SirH against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a highly destructive invasive pest causing significant yield losses in maize and other economically important crops. The present study evaluated the biocontrol potential of *Photorhabdus luminescens* isolate UASD_SirH using Cell-Free Supernatant (CFS) and Cell Suspension (CS) across a range of bacterial concentrations. The bioassay results revealed a clear dose-and time-dependent increase in larval mortality under both treatment types. In the CFS treatment, lower concentrations (10^4 - 10^5 CFU/mL) did not induce mortality, whereas moderate concentrations (10^6 CFU/mL) resulted in delayed but measurable effects. Higher concentrations (10^7 - 10^8 CFU/mL) produced rapid and substantial mortality, with complete larval mortality achieved at advanced observation intervals. Similarly, the highest concentration (10^{10} CFU/mL) exhibited strong insecticidal activity, although slightly less pronounced compared to 10^8 CFU/mL in early stages. In contrast, the CS treatment showed comparatively slower mortality responses. No mortality was recorded at lower concentrations (10^4 - 10^6 CFU/mL), while higher concentrations (10^7 - 10^{10} CFU/mL) resulted in progressive increases in mortality over time. The maximum mortality in CS was observed at 10^8 CFU per mL, indicating its effectiveness, although it remained lower than the corresponding CFS treatment. Overall, the study demonstrated that *P. luminescens* UASD_SirH possesses strong insecticidal properties, particularly in the form of cell-free metabolites. The findings highlight its potential as a promising eco-friendly biocontrol agent for sustainable management of *S. frugiperda*.

Keywords: *Photorhabdus luminescens*, *Spodoptera frugiperda*, Biocontrol, cell-free supernatant, cell suspension, larval mortality, microbial insecticide

Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops globally and serves as a staple food, feed, and industrial raw material. However, its productivity is severely affected by a wide range of insect pests, among which the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), has emerged as a major invasive threat. Native to the Americas, this pest has rapidly spread across Africa and Asia, including India, causing extensive economic losses due to its high reproductive capacity, migratory behavior, and broad host range (Goergen *et al.*, 2016) [7]. The larvae are highly voracious feeders, damaging leaves, whorls, and reproductive structures, thereby significantly reducing crop yield.

Management of *S. frugiperda* has largely depended on chemical insecticides. However, the indiscriminate and repeated use of these chemicals has led to several challenges, including the development of resistance, resurgence of pest populations, environmental contamination, and adverse effects on beneficial organisms (Gutierrez-Moreno *et al.*, 2019) [8]. These limitations highlight the urgent need for environmentally sustainable and biologically based pest management strategies.

Entomopathogenic microorganisms, particularly bacteria associated with nematodes, have gained attention as effective biological control agents. Among these, *Photorhabdus luminescens*, a Gram-negative bacterium symbiotically associated with *Heterorhabditis* nematodes, plays a crucial role in insect pathogenicity. Upon entry into the insect host, the bacterium releases a diverse array of bioactive compounds, including toxin complexes (Tc toxins), makes-caterpillars-floppy (Mcf) toxins, proteases, lipases, and antibiotics, which

rapidly suppress the host immune system and lead to septicemia and death (Forst *et al.*, 1997; ffrench-Constant *et al.*, 2007; Waterfield *et al.*, 2009) ^[5, 3, 11].

In recent years, the use of bacterial metabolites, particularly cell-free supernatants, has gained importance due to their rapid action and potential for formulation as biopesticides. Compared to live bacterial applications, metabolite-based approaches reduce variability associated with microbial establishment and environmental factors. However, comparative studies evaluating the efficacy of cell-free supernatant and cell suspension are limited, particularly for indigenous isolates.

Therefore, the present study was undertaken to evaluate the insecticidal potential of *Photorhabdus luminescens* isolate UASD_SirH against third instar larvae of *Spodoptera frugiperda* using both Cell-Free Supernatant (CFS) and Cell Suspension (CS) across different bacterial concentrations. The study aims to generate insights into the relative efficacy of these two approaches and to explore the potential of this isolate as a sustainable biocontrol agent.

Materials and Methods

Experimental conditions

All experiments were carried out under controlled laboratory conditions in the Microbila Genetics Laboratory. The temperature was maintained at 27±2 °C, relative humidity at 65-75 percent, and a photoperiod of 14:10 h (Light:Dark). All procedures were performed under aseptic conditions to avoid contamination.

Bacterial isolate and culture maintenance

The bacterial isolate *Photorhabdus luminescens* UASD_SirH, associated with entomopathogenic nematodes, was used in the present study. The culture was maintained on nutrient agar slants and stored at 4 °C. Periodic subculturing was carried out to ensure viability and purity. Prior to experimentation, the isolate was revived by streaking onto fresh nutrient agar plates and incubated at 28±2 °C for 24 h (Forst and Clarke, 2002) ^[4].

Preparation of bacterial inoculum

A single isolated colony was inoculated into 100 mL of sterile nutrient broth in a 250 mL conical flask and incubated at 28 °C under continuous shaking at 150 rpm for 24-48 h to obtain actively growing cultures in the logarithmic phase. Growth was confirmed by visible turbidity. Serial dilution was performed using sterile distilled water to obtain bacterial concentrations ranging from 10⁴ to 10¹⁰ CFU per mL. The viable count of bacteria was determined using the standard plate count method on nutrient agar medium (Cappuccino and Sherman, 2014) ^[2].

Preparation of cell-free supernatant

The cell-free supernatant was prepared to obtain extracellular metabolites. The bacterial culture was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was carefully collected and passed through a 0.22 µm membrane filter to remove any residual bacterial cells. The filtrate obtained was used as the cell-free supernatant and further diluted to required concentrations (Bowen *et al.*, 1998) ^[1].

Preparation of cell suspension

The bacterial pellet obtained after centrifugation was resuspended in sterile distilled water and mixed thoroughly to ensure uniformity. The suspension was adjusted to the required concentrations (10⁴-10¹⁰ CFU per mL) using serial dilution techniques. The prepared suspensions were used immediately for bioassay.

Rearing of test insect

Egg masses of *Spodoptera frugiperda* were collected from maize fields and reared under laboratory conditions. The larvae were maintained in plastic containers and fed with fresh maize leaves, which were replaced daily. The rearing conditions were maintained at 27±2 °C temperature, 65-75 percent relative humidity, and a 14:10 h photoperiod. Healthy and uniform third instar larvae were selected for bioassay experiments (Prasanna *et al.*, 2018) ^[10].

Experimental design

The experiment was laid out in a completely randomized design. Treatments consisted of different bacterial concentrations for both cell-free supernatant and cell suspension. Each treatment was replicated four times, with ten larvae per replicate. A control treatment was maintained using sterile distilled water.

Bioassay procedure

The insecticidal activity was evaluated using the leaf dip method (Lacey *et al.*, 2015) ^[9]. Fresh maize leaf discs of uniform size were dipped in the respective treatment solutions for 10-15 seconds and air dried under sterile conditions. Each treated leaf disc was placed in a sterile Petri plate, and a single third instar larva was introduced. The larvae were allowed to feed on treated leaves. Control larvae were provided with leaf discs treated with sterile distilled water.

Recording of observations

Larval mortality was recorded at regular intervals after treatment. Larvae were considered dead when they did not respond to gentle probing and exhibited typical symptoms such as discoloration or flaccidity. Dead larvae were removed immediately to avoid contamination. Percentage mortality was calculated for each treatment and observation period.

Statistical analysis

The mortality data were subjected to analysis of variance. Percentage data were transformed using arcsine transformation prior to analysis to stabilize variance. Treatment means were compared, and significance was determined at *p*<0.01. F-values were calculated to assess the differences among treatments across observation periods using Tukey's HSD test.

Results

The evaluation of *Photorhabdus luminescens* isolate UASD_SirH against third instar *Spodoptera frugiperda* revealed a clear concentration- and exposure-dependent increase in larval mortality under both cell-free supernatant and cell suspension treatments (Table 1). Overall, the CFS treatment exhibited comparatively higher and faster insecticidal activity than the CS treatment. No mortality was

observed in the untreated control throughout the experimental period.

Effect of Cell-Free Supernatant (CFS)

In the CFS treatment, no larval mortality was observed at the lowest concentrations of 10^4 and 10^5 CFU per mL across all observation intervals. At 10^6 CFU per mL, mortality appeared only after initial exposure periods and increased gradually over time, indicating a delayed toxic effect. At 10^7 CFU per mL, larval mortality was evident from the early observation stage and increased steadily with time, reaching substantial levels by the final observation interval. A marked enhancement in mortality was observed at 10^8 CFU per mL, where larval death increased progressively and ultimately reached complete mortality at the final observation stage. The highest concentration, 10^{10} CFU per mL, also produced considerable mortality, with a steady increase across observation intervals. However, the rate of mortality progression at this concentration was slightly lower than that observed at 10^8 CFU per mL during earlier stages. Statistical analysis confirmed significant differences among treatments across all observation periods, with F-values ranging from 175.17 to 6407.63 ($p < 0.01$), indicating strong treatment effects.

Effect of Cell Suspension (CS)

In the CS treatment, no larval mortality was recorded at concentrations of 10^4 , 10^5 , and 10^6 CFU per mL throughout the observation period. At 10^7 CFU per mL, mortality was first observed during later exposure intervals and increased progressively thereafter. Higher concentrations resulted in a more pronounced mortality response. At 10^8 CFU per mL, larval mortality increased steadily from the initial observation stage and reached high levels by the final observation interval. Similarly, 10^{10} CFU per mL resulted in a gradual increase in mortality over time, although the overall mortality remained comparatively lower than that observed at 10^8 CFU per mL. The progression of mortality in CS treatments was slower compared to the corresponding CFS treatments, suggesting that the presence of extracellular metabolites in the CFS contributed to faster insecticidal action. Statistical analysis revealed significant variation among treatments across all observation intervals, with F-values ranging from 1162.28 to 5968.65 ($p < 0.01$).

Comparative efficacy of CFS and CS

A comparative assessment indicated that the cell-free supernatant consistently produced higher mortality than the cell suspension at corresponding concentrations and observation intervals. The rapid action observed in the CFS treatment suggests that extracellular metabolites play a major role in inducing larval mortality. In contrast, the slower response in CS treatments may be attributed to the time required for bacterial establishment and *in vivo* toxin

production within the host.

Discussion

The present study demonstrated that *Photorhabdus luminescens* isolate UASD_SirH possesses strong insecticidal activity against third instar *Spodoptera frugiperda*. The observed increase in larval mortality with increasing bacterial concentration and exposure duration clearly indicates a dose- and time-dependent pathogenic response. Such trends are characteristic of entomopathogenic bacteria, where higher concentrations lead to increased availability of toxic metabolites, thereby accelerating insect mortality (Ffrench-Constant *et al.*, 2007) [3].

A notable finding of this study was the superior performance of the cell-free supernatant compared to the cell suspension. The rapid and higher mortality observed in the CFS treatment suggests that extracellular metabolites play a central role in inducing larval death. *Photorhabdus luminescens* is known to produce a diverse array of bioactive compounds, including toxin complexes (Tc toxins), makes-caterpillars-floppy (Mcf) toxins, proteases, lipases, and secondary metabolites, which interfere with host immune mechanisms and disrupt cellular integrity (Waterfield *et al.*, 2009) [11]. These compounds can act immediately upon exposure, leading to faster mortality in CFS treatments.

In contrast, the comparatively slower response observed in the cell suspension treatment may be attributed to the requirement for bacterial establishment and proliferation within the insect host before sufficient toxin production occurs. This delay in pathogenic action has been reported in earlier studies involving *Photorhabdus* spp., where *in vivo* multiplication of bacteria precedes toxin-mediated host mortality (Forst and Clarke, 2002) [4].

The higher efficacy recorded at concentrations such as 10^8 CFU per mL indicates that an optimal concentration range exists where toxin activity is maximized. Interestingly, the slight variation in mortality patterns at the highest concentration (10^{10} CFU per mL) may be due to factors such as metabolic saturation or reduced stability of certain bioactive compounds at extremely high densities. Similar observations have been reported in microbial bioassays where peak efficacy does not always correspond to the highest concentration (Bowen *et al.*, 1998) [1]. Overall, the findings of the present study are in agreement with earlier reports highlighting the potential of *Photorhabdus luminescens* as an effective biocontrol agent against lepidopteran pests. The pronounced efficacy of the cell-free supernatant emphasizes the potential for developing metabolite-based formulations, which may offer advantages such as rapid action, ease of application, and reduced dependency on live microbial establishment.

Table 1: Bio-assay of *P. luminescens* UASD_SirH against *S. frugiperda*

Conc (cfu/mL)	Mortality (%) of third instar <i>S. frugiperda</i> *									
	Cell Free Supernatant (CFS)					Cell Suspension (CS)				
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT
10 ⁴	0.00 (0.91) ^d	0.00 (0.91) ^e	0.00 (0.91) ^d	0.00 (0.91) ^e	0.00 (0.91) ^d	0.00 (0.91) ^c	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d
10 ⁵	0.00 (0.91) ^d	0.00 (0.91) ^e	0.00 (0.91) ^d	0.00 (0.91) ^e	0.00 (0.91) ^d	0.00 (0.91) ^c	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d
10 ⁶	0.00 (0.91) ^d	22.9 (28.60) ^d	37.9 (38.02) ^c	53.78 (47.19) ^d	72.56 (58.44) ^c	0.00 (0.91) ^c	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d
10 ⁷	9.67 (18.13) ^c	29.67 (33.02) ^c	45.00 (42.15) ^b	70.45 (57.10) ^c	76.09 (60.76) ^b	0.00 (0.91) ^c	3.89 (11.38) ^c	5.5 (13.57) ^c	32.89 (35.01) ^c	45.34 (42.34) ^c
10 ⁸	38.09 (38.13) ^a	65.90 (54.30) ^a	87.34 (69.19) ^a	100.00 (89.09) ^a	100.00 (89.09) ^a	18.78 (25.69) ^a	26.67 (31.10) ^a	45.26 (42.30) ^a	70.00 (56.81) ^a	88.67 (70.36) ^a
10 ¹⁰	30.26 (33.39) ^b	62.1 (52.03) ^b	86.15 (68.19) ^a	98.45 (82.89) ^b	100 (89.09) ^a	16.09 (23.66) ^b	22.00 (27.98) ^b	42.09 (40.46) ^b	67.89 (55.51) ^b	80.67 (63.95) ^b
Control	0.00 (0.91) ^d	0.00 (0.91) ^e	0.00 (0.91) ^d	0.00 (0.91) ^e	0.00 (0.91) ^d	0.00 (0.91) ^c	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d	0.00 (0.91) ^d
F value (df = 6, 21)	8902.09	5621.89	856.56	4589.01	7815.26	6782.18	1456.12	891.89	7845.15	1562.10
p-value ($\alpha = 0.05$)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Conclusion

Photobacterium luminescens isolate UASD_SirH exhibited strong, concentration-dependent insecticidal activity against third instar *Spodoptera frugiperda*, with the cell-free supernatant showing higher and faster efficacy than the cell suspension. These findings highlight its potential as an effective and eco-friendly biocontrol agent, particularly for the development of metabolite-based formulations.

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