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Cellular Encapsulation in *Spodoptera litura* (Fabricius) Infected with Potential Entomopathogens

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ABSTRACT

Background: Entomopathogens are foreign agents which elicits immune responses in insects. The insect innate immune system can identify and overcome these foreign-agents through cellular and humoral processes.

Methods: The current study reports the cellular encapsulation in *Spodoptera litura* to different test entomopathogens including *Beauveria bassiana*, *Metarhizium anisopliae*, *Bacillus thuringiensis* and Nuclear Polyhedrosis virus (*Sl* NPV). Third instar larvae were treated with the test entomopathogens and haemolymph was collected at different time intervals *i.e.*, 1, 3, 6, 12, 24, 48 and 72 hours after infection (HAI) to study the cellular encapsulation responses.

Result: The immediate response of larvae to entomopathogens infection was a significant increase in the number of encapsules formation when compared to the untreated control *i.e.*, *B. bassiana*: 51±2.52^{c}, *M. anisopliae*: 42±1^{d}, *Bt*: 95±1.53^{a}, *Sl* NPV: 61±2.52^{b} and control: 4±1.15^{e}. However, after 3 days from the immune challenge, the number of encapsules formed was significantly lower *i.e.*, *B. bassiana*: 11±1.53^{b}, *M. anisopliae*: 9±1^{b}, *Bt*: 21±1.53^{a}, *Sl* NPV: 10±1^{b} and control: 0.

Key words: *Bacillus thuringiensis, Beauveria bassiana*, Encapsulation, *Metarhizium anisopliae*, Nuclear polyhedrosis virus (*Sl* NPV), *Spodoptera litura*.

INTRODUCTION

Tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) larvae, is a destructive polyphagous pest that infests a variety of crops, including cotton, tomato, capsicum, potato, soybean, okra, clover and onion (Saleem *et al.,* 2016) and is known to cause extensive damage to crops. Insecticides are the major means of managing the *S. litura*. However, continuous use of insecticides causes health hazards and environmental pollution, besides development of insecticide resistance among the populations (Reddy *et al.,* 2021). Entomopathogens are the best alternative for the *S. litura* management (Pegu *et al.,* 2017: Ojha *et al.,* 2018; Qubbaj *et al.,* 2022). In addition to its devastating nature as a pest to the crops, *S. litura* is also an ideal insect for demonstrating the cellular and humoral mechanisms of defence to insect pathogens.

Entomopathogens are usually recognized as harmful foreign agents that cause immunological reactions which are managed by different immune cells and tissues in insects. The insect innate immune system can identify and get rid of specific diseases and parasites through cellular and humoral processes (Gupta, 2019). Haemocytes are considered as the main immune cells in insects (Hillyer and Strand, 2014). Insects use haemocytes, which are present in haemocoel, for numerous cellular and humoral defence systems to protect themselves against harmful microorganisms. Granulocytes (GRs) and Plasmatocytes (PLs) are the important haemocytes that are involved in immune mechanism of lepidopteran insects (Li *et al.,* 2022).

Encapsulation is a cellular immune response mechanism used against pathogens. Commonly employed by dipteran and lepidopteran larvae in response to infection with entomopathogens. In Lepidoptera, encapsulation is initiated when granulocytes attach to form a layer of cells that surrounds a pathogen. This cell adhesion is dependent on the binding of integrins to specific sites defined by an Arg-Gly-Asp (RGD) sequence. Encapsulation enables immune isolation by creating a physical barrier between the host immune system and the foreign biological body. Depending on the insect species and properties of the target, capsules may be continually formed over 2 - 24 h. These processes are complex mechanisms that include a wide range of cellular and humoral immune reactions (Eleftherianos *et al.,* 2021).

In the present study, we used histopathology to describe the relationship between entomopathogens and *S. litura* and characterized the cellular encapsulation of the

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entomopathogenic infection *in vitro*. The relative role of individual immunity in interaction with behavioral mechanisms in tobacco caterpillar is also discussed. Insect immunology is a developing subject of interest among the researchers. This article provides information to the researchers on how insect immune mechanisms with respect to encapsulation are responding when particular entomopathogens inti their body.

MATERIALS AND METHODS

A study was undertaken to identify and ascertain the cellular encapsulation response in *Spodoptera litura* (Fabricius) to the entomopathogens was carried out during 2022-23 in Insect Pathology Laboratory, Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad. The materials used and procedures followed for the study were as under.

Insect rearing

The *Spodoptera litura* larvae were collected from the college farm of college of agriculture, professor jayashankar telangana state agricultural univesrity (PJTSAU) and farmer fields in rajendranagar, Hyderabad. The collected larvae were maintained on the artificial diet (Gupta *et al.,* 2005) with relative humidity 70-75% and temperature 20±2Úc under laboratory conditions. The larvae which were in the stage to pupate were transferred into the tray containing soil. After pupation, the pupae were collected in a tray and covered with a muslin cloth. The emerged adults with a male and female ratio of 2:3, were transferred into the cages containing 15% sucrose solution (Santharam, 1985). The egg masses were collected and maintained in the Insect Pathology Laboratory, Department of Entomology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad.

Entomopathogens and inoculation procedure

Commercial formulations of different test entomopathogens *i.e.*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Bacillus thuringiensis* and Nuclear Polyhedrosis virus (*Sl* NPV) (Table 1) used in the present study were sourced from a noted agri biotech company, AgriLife (India) Private Limited, Hyderabad. Leaf dip method for *Bt* and *Sl* NPV and topical application for entomopathogenic fungi (Table 1) were used for inoculating the larvae. Once *S. litura* larvae moulted to 3rd instar, they were starved for 24 hours before inoculating with the leaf dip method. For inoculating the larvae with *Bt* and *Sl* NPV, the castor leaves were dipped in suspensions of *Bt* for treatment three and in viral suspensions for treatment four as described in Table 1. The treated castor leaves were then dried for 30 seconds and the dried leaves were placed on the filter paper within a petri dish (one leaf per petri dish). Two larvae per petri dish were released and four such replications for each treatment were maintained. Then the larvae were allowed to feed on the infested leaves. Since oral infection is not the primary infection route for entomopathogenic fungi, for treatment with *B. bassiana* and *M. anisopliae* the third instar larvae were topically exposed by adding 10 µl of the solution with a sampler device, following which they were placed on filter paper for 30 seconds and transferred two larvae per petri dish and four such replications were maintained for the treatment. For control, castor leaves were dipped in 10 µl of Tween 80 (0.05%) solution, then dried for 30 seconds and placed in the petri dish. Two larvae per petri dish were released.

Collection of haemolymph from infected larvae

Haemolymph was collected by cutting the tip of a proleg of the infected larvae from each treatment, previously anesthetized on ice using fine scissors and needle (Rosenberger and Jones, 1960). Gentle pressure was applied on the insect abdomen for getting more quantity of haemolymph (Barkat *et al.,* 2002). The collected haemolymph from the infected larvae was used to prepare blood smears, by spreading a drop (20 µl) of haemolymph on a glass slide and smeared by drawing a second slide across the first at an angle of 45° and then dried at room temperature. The air-dried haemolymph smear slides were then dipped into methanol two times and air dried. Slides were then stained with Giemsa stain (diluted five times with phosphate-buffer saline (PBS) and filtered before use) for 20 min and then rinsed in distilled water. The smear was washed in 0.02% acetic acid followed by rinsing in distilled water. After drying, permanent microscopy slides were prepared using permount or canada balsam.

Cellular encapsulation

Immediately after the haemolymph extraction, a 3 mm long piece of nylon monofilament was fully placed into the punctured wound of each larva to reduce the risk of rupturing the midgut. Larvae that survived were fed for 24 hours. The remaining larvae were then frozen and after the death of the larvae, the nylon monofilament was removed and placed on a slide and captured in a photograph. ImageJ, GIMP, photoshop and other software were used to quantify the degree of melaninization and the area of cell cover. The larval samples with gut ruptures and that were failed to recover the nylon filament during the dissection were discarded (Black *et al.,* 2022).

Data analysis

Each treatment was replicated four times and the data was analyzed by completely randomized design (CRD) using Web Agri Stat Package (WASP 2.0) software. The data was subjected to the standard statistical analysis using techniques of analysis of variance. Differences

Table 1: Entomopathogens used for experimentation.

Treatment	Dosage
T1 - Beauveria bassiana 1%WP	5 g / l
T2 - Metarhizium anisopliae 1%WP	5 g / l
T3 - Bacillus thuringiensis var. kurstaki 20SL	2 ml/l
T4 - Nuclear polyhedrosis virus (*Sl* NPV)	250 LE / ha
T5 - Control (Untreated check)	

Table 2: Number of encapsules formed in *S. litura* larval haemolymph after infection with selected entomopathogens.

Treatment	1 HAI	3 HAI	6 HAI	12 HAI	24 HAI	48 HAI	72 HAI
B. bassiana	12±0.58^{b}	26±2.31^{c}	35±2.08^{c}	42±1.53^{c}	51±2.52^{c}	29±2.08^{b}	11±1.53^{b}
M. anisopliae	-	23±1^{c}	31±2.08^{c}	39±1.53^{c}	42±1^{d}	17±1.15^{c}	9±1^{b}
Bt	25±1^{a}	49±0.58^{b}	67±2.52^{a}	84±2^{a}	95±1.53^{a}	126±1.53^{a}	21±1.53^{a}
Sl NPV	22±1.15^{a}	64±0.58^{a}	41±1^{b}	49±2.65^{b}	61±2.52^{b}	18±2.31^{c}	10±1^{b}
Control	-	1±0.58^{d}	4±1^{d}	6±1^{d}	4±1.15^{e}	1±0.58^{d}	-

*Different alphabets denote statistical differences among treatments (P≤0.05).

between samplings were considered statistically significant at a probability more than 5% ($p \leq 0.05$).

Results and Discussion

Infection of *Spodoptera litura* larvae with different test entomopathogens showed that maximum number of encapsules were observed in *Bt* (126±1.53^{a}) and minimum was observed in case of *M. anisopliae* (42±1^{d}). The order of encapsulation observed in different test entomopathogens was *Bt* > *Sl* NPV > *B. bassiana* > *M. anisopliae* (Table 2). Untreated control showed least encapsulation in comparison with any of the test entomopathogen.

The number of encapsules formed were increased up to 24 HAI in *B. bassiana* (51±2.52^{c}) and *M. anisopliae* (42±1^{d}). In case of *Bt* up to 48 HAI (126±1.53^{a}). On the other hand, *Sl* NPV showed highest encapsulation at 3 HAI (64±0.58^{a}). However irrespective of the entomopathogen tested, the number of encapsules formed were initially increased in the larval haemolymph and gradually decreased, this is due to the inability of the insect to counteract the entomopathogens for longer time.

Infection with entomopathogens initially increased the number of encapsules formed which gradually decreased later. Zhong *et al.* (2017) conducted similar studies on the effect of the entomopathogenic fungus, *Nomuraea rileyi* on the cellular immunological responses of *Helicoverpa armigera*. Their results showed that encapsulation was reduced over time after inoculation.

The experimental results showed that at various phases of infection, *S. litura* reacts differently to each main entomopathogenic group. Notably, among all the test entomopathogens, the immunological response of *S. litura* against Bt was the strongest. This was also shown by Black *et al.* (2022) stating that among all the entomopathogens tested *Helicoverpa zea* immune response to *Bt* was the highest.

Conclusion

In conclusion, our study demonstrated that the cellular encapsulation in *Spodoptera litura* to different test entomopathogens including *Beauveria bassiana*, *Metarhizium anisopliae*, *Bacillus thuringiensis* and Nuclear Polyhedrosis virus (*Sl* NPV) infection was a significant increase in the number of encapsules formation. However, after 3 days from the immune challenge, the number of encapsules formed was significantly lower.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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