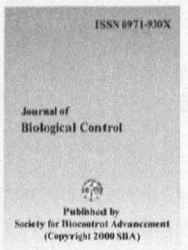

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Research Note

Cellular immune response of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) during mycosis of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT: *Beauveria bassiana*, one of the most common and ubiquitous entomopathogens, is known to be a highly potential bioagent for the control of insects belonging to various orders. The cellular immune response was examined in *Spodoptera litura* larvae infected with *B. bassiana*. Third instar larvae were treated with a fungal suspension @ 2.03×10^8 spores ml^{-1}. Haemolymph of treated and untreated larvae were taken after 2, 12, 24, 48, 72, 96 and 120 hours after inoculation. Plasmatocytes and granulocytes swelled 2 hours after inoculation (HAI) followed by lysis of plasma membrane. Within 24 HAI, apposition was observed. The encapsulation process started after 48 hours and continued for up to 72 hours. After 96 hours of inoculation the granulocytes and plasmatocytes were observed to form nodules. With increasing inoculation time, the size of clump increased, forming multiple layers at 120 HAI. However, despite these insect defense responses, growth of fungal hyphae was observed after 120 hours of inoculation which ultimately resulted in the death of the host.

KEY WORDS: *Beauveria bassiana, Spodoptera litura,* cellular immune response, encapsulation, immunity, hemolymph, hemocytes

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Insects have no specific defense mechanism such as antibodies of vertebrate animals, however, they can effectively protect themselves from infection of micro–organisms due to cellular and humoral immunity (Dunn, 1986). Insect cellular immunity is associated with haemocytes circulating freely in the haemocoel. They have the ability to attack foreign targets and form overlapping sheaths that cause melanization, encapsulation, disintegration, phagocytosis and nodule formation (Choi *et al.*, 1997). Insect humoral immunity depends on the amplification or production of number of antibacterial proteins in response to the invasion of pathogens like bacteria (Boman *et al.*, 1991). Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hyphocreales) have the capacity to produce toxins that act as inhibitors of the host defense reactions, destroying the haemolymph and nucleus of cells and also cause reduction of energy because of utilization of the nutrients of the haemolymph by the fungus (Harris *et al.*, 2000). *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), commonly known as tobacco caterpillar, is one of the most destructive pests of tobacco, castor, cauliflower, groundnut, tomato, cabbage and other cruciferous crops and has been reported to cause huge crop losses in various parts of India (Venkateswarlu *et al.*, 2006). Because of development of insecticide resistance in this pest, emphasis is being laid on microbial control. Effectiveness of *B. bassiana* has been reported against a number of lepidopteran pests. However, the cellular immune response of *S. litura* has not been well studied in relation to pathogenesis. The present study was carried out to investigate the effect of *B. bassiana* on the haemocytes of *S. litura.*

The studies were conducted in the Insect Biocontrol laboratory, Department of Zoology, Guru Nanak Dev University, Amritsar (Punjab), India. The larvae of *S. litura* were collected from the fields around Amritsar and reared on castor leaves in battery jars (15 cm × 10 cm) at 25±2°C and 60-70% relative humidity. The pupae were transferred to pupation jars. On emergence adults were shifted to oviposition jars lined with filter paper to facilitate egg laying. A cotton swab soaked in sugar solution was hanged with a muslin cloth for providing food to adults. Further culture was maintained on artificial diet as recommended by Koul *et al.* (2004) for experimental purposes.

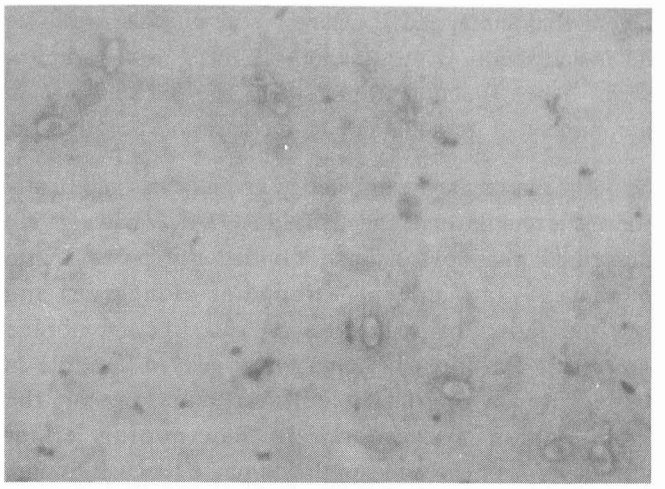

Fig. 1. Haemocytes of untreated larvae

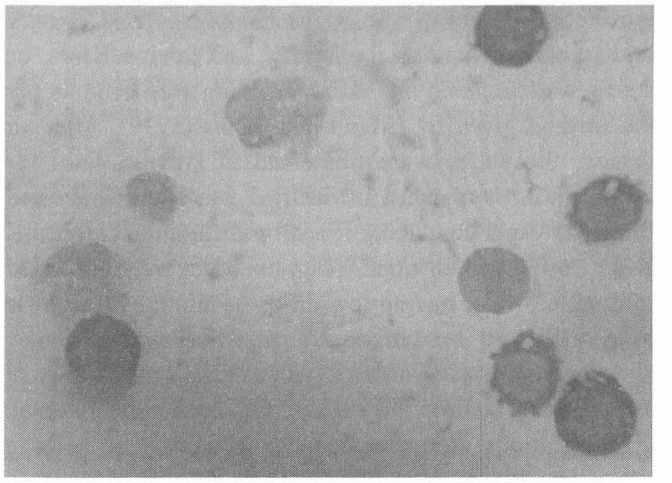

Fig. 2. Cell lysis

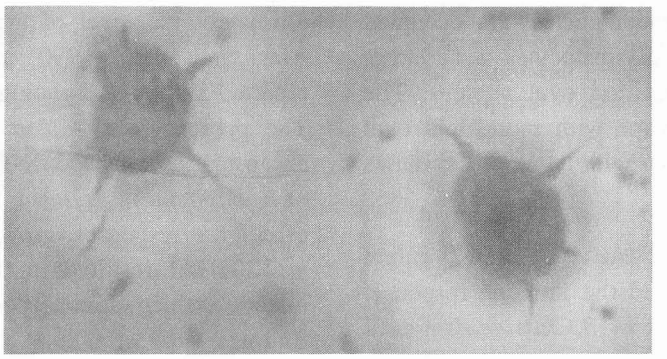

Fig. 3. Filopodial elongation

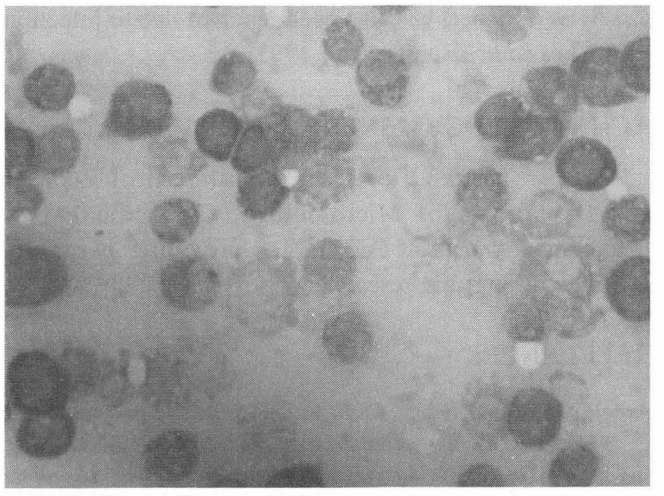

Fig. 4. Vacuolization

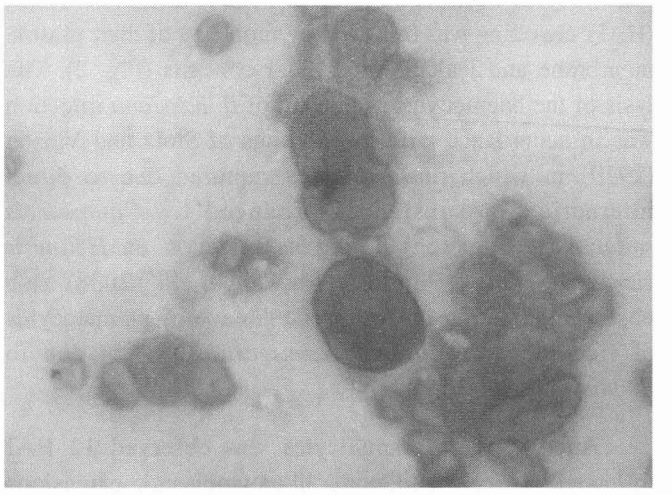

Fig. 5. Encapsulation

The culture of *B. bassiana* (PDBC- Bb-5a) was procured from Project Directorate of Biological Control (PDBC), Bangalore, India and maintained on PDA slants. Three weeks old culture was used for experimental purpose. A suspension was prepared by adding 10 ml of distilled water and a drop of 0.01 per cent Tween 80. Spore count was determined using a haemocytometer.

To study the cellular immune response of *S. litura*, 3rd instar larvae were treated with 2.03×10^8 spores ml^{-1} of *B. bassiana*. Ten ml of fungal suspension was sprayed on 40 larvae. Similarly control larvae were sprayed with distilled water containing a drop of 0.01 percent Tween 80. Haemolymph of 5 treated and untreated larvae was taken at 2, 12, 24, 48, 72, 96 and 120 hours after

inoculation. Haemolymph of the larvae was bled directly on to a slide by cutting the foreleg with microscissors. Its smear was prepared and dried. Staining was done as per the method given by Arnold and Hinks (1979). After air drying, the slides were immersed in Giemsa stain for 20 minutes, then rinsed in distilled water and immersed briefly in water containing few drops of lithium carbonate. After rinsing with distilled water, the slides were immersed in distilled water having few drops of dilute hydrochloric acid followed by rinsing in distilled water. These slides were observed under Olympus CX13 microscope at 100x having a drop of xylene. For each time interval, 20 replications were prepared.

Prohaemocytes, plasmatocytes and granulocytes were observed in the hemolymph. The prohaemocytes were smaller in size and had almost round nucleus that occupied most of the cell volume. The plasmatocytes were larger and polymorphous, with round and oval nucleus. The granulocytes were round in shape with round and oval nuclei occupying central position with numerous granules in their cytoplasm.

Beauveria bassiana was found to be quite virulent against *S. litura* as it suppressed the immune response. Granulocytes and plasmatocytes of *B. bassiana* infected larvae showed morphological changes whereas no change was observed in control (Fig. 1). Plasmatocytes and granulocytes were found swollen 2 hours after inoculation (HAI). Swelling was followed by rupturing of their plasma membrane and leakage of cellular contents (Fig. 2). The lysis of the haemocytes as a result of *B. bassiana* infection was in accordance with the findings of Stolz and Vinson (1979) in which plasmatocytes ruptured due to direct interaction or substances produced by *Campoletis sonorensis* (Cameron) during its infection on *Heliothis virescens* (Fab.). Similarly Phukan *et al.* (2008) also observed rupturing of plasma membrane of plamatocytes of rice hispa, *Dicladispa armigera* (Olivier) due to *B. bassiana* infection.

Apposition of granulocytes was observed 12 HAI followed by fine pseudopodia like cytoplasmic extensions after 24 hours of inoculation (Fig.3). Within 48 hours of infection, small vacuoles appeared followed by a decrease in the number of granules in granulocytes (Fig. 4). The encapsulation process started after 48 hours (Fig. 5) followed by vacuolization with concomitant decrease in number of granules at 72 HAI. Nodule formation was recorded after 96 hours of inoculation. Phukan *et al.* (2008) also reported encapsulation in *D. armigera* initiated by the attachment of granulocytes to the target after 6 hour of inoculation. Plasmatocytes then formed concrete layers that entrapped the target and ultimately led to its melanization. The granulocytes of *D. armigera* lost their shape. The granulocytes also showed decrease in the number of granules due to increase in vacuolization.

The plasmatocytes adhered to granulocytes and finally formed a multilayered capsule at 120 HAI. Vinson (1990) described phagocytosis and divided the process into 3 phases – attachment, filopodial elongation and internalization by extension of veil like membrane processes. The filopodial elongations play a key role in triggering or regulating cellular reactions by the granulocytes, particularly in determining either phagocytosis or early encapsulation phase (Pech & Strand, 1996). Tiwari *et al.* (1999) also reported that granulocytes played an important role in encapsulation reaction and ultimately the level of granulocytes decreased. Earlier Rizki and Rizki (1990) and Russo *et al.* (2001) also reported similar morphological alteration in haemocytes. The present results also demonstrated phagocytosis, encapsulation and nodule formation against the invasion of *B. bassiana* in *S. litura.* However, despite the presence of defense responses, growth of fungal hyphae observed at 120 HAI resulted in further progression of fungal infection which ultimately led to the death of insect. These findings suggest that *B. bassiana* is quite virulent to suppress the cellular defense mechanism of the insect. Similar suppression of cellular defence mechanism in *S. exigua* (Hübner) has also been reported due to infection by *B. bassiana* (Hung *et al.*, 1993).

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REFERENCES

Arnold, J. W. and Hinks, C. F. 1979. Insect haemocytes under light microscopy techniques, pp. 531–538. In: Gupta, A. P. (Ed.). *Insect Hemocytes: Development, forms, functions and techniques*. Cambridge University Press, Cambridge, UK.

Boman, H. G., Faye, I., Gudmundsson, G. H., Lee, J. Y. and Lidholm, D. A. 1991. Cell free immunity in *Cecropia*: a model system for antibacterial proteins. *European Journal of Biochemistry,* **201**: 23–31.

Choi, C. S., Yoe, S. M., Kim, E. S., Chae, K. S. and Kim, H. R. 1997. Purification and characterization of antibacterial peptides, Spodopsin Ia and Ib induced in the larval haemolymph of the common cutworm, *Spodoptera litura. Korean Journal of Biological Science,* **1**: 457–462.

Da Silva, J. B., De Albuquerque, C. M., De Araujo, E. C., Peixota, C. A. and Hurd, H. 2000. Immune defense mechanism of *Culex quinquefasciatus* (Diptera: Culicidae) against *Candida albicans* infection. *Journal of Invertebrate Pathology,* **76**: 257–262.

Dunn, P. E. 1986. Biochemical aspects of insect immunology. *Annual Review of Entomology,* **31**: 17–21.

Harris, R. S., Harcourt, S. J., Glare, T. R., Rose, E. A. and Nelson, T. J. 2000. Susceptibility of *Vespula vulgaris* (Hymenoptera: Vespidae) to generalist entomopathogenic fungi and their potential for wasp control. *Journal of Invertebrate Pathoogy,* **75**: 251–258.

Hung, S.Y., Boucias, D.G. and Vey, A.J. 1993. Effect of *Beauveria bassiana* and *Candida albicans* on the cellular defense response of *Spodoptera exigua. Journal of Invertebrate Pathology,* **61**: 179–187.

Kurtz, J. and Sauer, K. P. 2001. Gender differences in phenoloxidase activity of *Panorpa vulgaris* hemocytes. *Journal of Invertebrate Pathology,* **78**: 53–55.

Koul, O., Singh G., Singh, R., Singh, J., Daniewski, W. M. and Berloecki, S. 2004. Bioefficacy and mode of action of some limonoids of Salannin group from *Azadirachta indica* A. Juss. and their role in a muticomponent system against lepidopteran larvae. *Journal of Biosciences,* **29**: 409–416.

Pech, L. L. and Strand, M. R. 1996. Granular cells are required for encapsulation of foreign targets by insect haemocytes. *Journal of Cell Science,* **109**: 2053–2060.

Phukan, M., Hazarika, L. K., Barooah, M., Puzari, K. C. and Kalita, S. 2008. Interaction of *Dicladispa armigera* (Coleoptera: Chrysomelidae) haemocytes with *Beauveria bassiana. International Journal of Tropical Insect Science,* **28**: 88–97.

Rizki, R. M. and Rizki, T. M. 1990. Parasitoid virus-like particles destroy *Drosophila* cellular immunity. *Proceedings of the National Academy of Sciences of the United States of America,* **87**: 8388–8392.

Russo, J., Brehelin, M. and Carton, Y. 2001. Insects, pp. 471–490. In: Ratcliffe. N. A. and Rowley, A. F. (Eds.). *Invertebrate Blood Cells*. Academic Press, New York, USA.

Stolz, D. B. and Vinson, S. B. 1979. Viruses and parasitism in insects. *Advances in Virus Research,* **24**: 125–171.

Tiwari, R. K., Shukla, R. S., Mishra, A. and Agarwal, D. P. 1999. Effect of feeding ethoprene and 20 hydroxy ecdysone treatments on total haemocyte count in starved larvae of the lemon-butterfly, *Papilio demoleus* (Papilionidae: Lepidoptera). *Journal of Advanced Zoology,* **20**: 32–38.

Venkateswarlu, U. T., Madhumathi, P. A. R. R. and Srinivaso Rao, V. 2006. Status of insecticide resistance in *Spodoptera litura* on cotton in Guntur and Prakasam Districts of Andhra Pradesh. *Indian Journal of Plant Protection,* **34**: 26–32.

Vinson, S. B. 1990. How parasitoids deal with the immune system of their host: an overview. *Archives of Insect Biochemistry and Physiology,* **13**: 3–27.