Biological Forum — An International Journal, **2**(1): 9-11(2010) **ISSN : 0975-1130**

Effect of *Aspergillus fumigatus* infection on cellular and humoral immune responses in red cotton stainer, *Dysdercus similis* (Heteroptera: Pyrrhocoridae)

Karuna Singh and S.C. Pathak*

Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi (UP) INDIA
Department of Biosciences, Asterik Rani Durgavati University, Jabalpur (MP) INDIA

ABSTRACT : *Aspergillus fumigatus* Freseius caused aspergillosis in *Dysdercus similis* Freeman (Heteroptera: Pyrrhocoridae), which elicited both cellular and humoral immune responses in the insect. Cellular immune response, involves phagocytosis and cellular encapsulation. Humoral encapsulation was noted in infected insects of both sexes. Fungal conidia and hyphae were seen covered with the typical yellowish brown hyaline material. Infection was not externally manifested.

Keywords : Phagocytosis, Encapsulation, Haemocyte, *Aspergillus fumigatus*, *Dysdercus similis* Abbreviations : GRs (Granulocytes), PLs (Plasmatocytes), Cos (Coagulocytes)

INTRODUCTION

The defense of insects against infection in brought about by haemocytes. This defense may be cellular or humoral or both. Cellular defense comprises of phagocytosis, cellular encapsulation and nodule formation (Gupta 1985a; Lackie 1988). However, the number of papers published with reference to the humoral aspects of defense in insects are far less numerous as compared to those with reference to the cellular aspects. Present paper covers both cellular and humoral responses in the red cotton stainer *Dysdercus similis* Freeman (Heteroptera: Pyrrhocoridae) experimentally infected with *Aspergillus fumigatus.*

MATERIAL AND METHODS

(*a*) Insect culture. Experimental insects were obtained from the culture maintained in the laboratory. The adults and nymphs of *Dysdercus similis* were collected from the host Okra (*Abelmoscus esculantus*) and *Gossypium* plants in the months of February and March, from the experimental fields of Jawaharlal Nehru Agricultural University, Jabalpur and maintained at 30°C ±1°C and RH in the range of 70 to 80 in the laboratory. The insects were reared in sterilized containers and were fed on cotton seeds soaked in water.

(*b*) Isolation of Fungus. The isolate of *Aspergillus fumigatus* was obtained from the Medical Mycology laboratory of the Department of Biosciences, Rani Durgavati University, Jabalpur and sub cultured on Sabouraud's dextrose agar slants (Dextrose 40 g, Peptone 10 g, Agar 20 g in 1000ml of distilled water) for 48 hours at 28 ± 1°C. The fungal suspension was prepared in 5 ml of sterilized distilled water, homogenized and then filtered through sterilized muslin cloth to separate conidia from mycelia. Spore count was adjusted to 4.5×10^{-6}cfu/ml using haemocytometer. Slide cultures of the isolates were also prepared (Fig. 1a and b).

(*c*) Inoculation. A conidial suspension of *A. fumigatus* (conc. 4.5×10^{-6} cfu/ml) is used for inoculation. 2.5µl of this inoculums was injected into the haemocoel of the insect through arthrodial membrane in the $2^{nd}/3^{rd}$ or $3^{rd}/4^{th}$ abdominal sternae using 10µl Hamilton microsyringe. Three such inoculations were carried out for each sex. One insect of each sex was treated as control by injecting 2.5µl of distilled water in place of conidial suspension. To see the topical affect of fungus, the inoculum was applied topically on the dorsal and ventral body surfaces of the insects of both sexes by a paint brush.

(*d*) Preparation and staining of blood film. Liquid fixative viz 5% formaldehyde was used to fix the haemolymph for preparing blood film. The blood film was air dried and subjected to staining with Giemsa (Arnold and Hinks, 1979).

RESULTS AND DISCUSSION

Adults of *Dysdercus similis* with experimentally induced aspergillosis showed gradual decrease in activity. Male died generally after 75 hours of inculation but female generally lived up to 92 to 99 hours following inoculation. Topical application of fungal inoculum had no effect.

Phagocytosis was noticed in the Giemsa stained blood films of infected insects. The fungal hyphae and spores of *Aspergillus fumigatus* were seen engulfed by some granulocytes (GRs) (normal cell size ranged from 14.5µm × 10.1µm to 29µm × 21.7µm) (Fig. 1c, d, e) and coagulocytes (COs) (Fig. 1f). It was also observed that GRs increased in size (length 66.7µm and width 31.7 µm) (Fig. 1g) particularly in the females when phagocytosed fungal material. Plasmatocytes (PLs) also participated in the phagocytosis, although to a lesser extent. Cellular encapsulation was seen with some GRs/COs coming together with the fungal material. These cells lysate and produce dark colored core around which flattened PLs get arranged in multiple layers (Fig.1h). Gradually more and more layers of such cells added.

Although external manifestation of unusual melanization or pigmentation was not observed in the infected male and female of *Dysdercus similis*, the transparent, flocculent and hyaline material was observed encapsulating the fungal spores (Fig.1i). A unique combination of cellular and humoral immune responses was also seen. Initiation of humoral encapsulation by a number of GRs that got attached to various growing points of a fungal colony was noticed (Fig.1j).

Both cellular and humoral responses are known to occur in insects facing challenge. Cellular immune response involves phagocytosis and cellular encapsulation, and is widely studied against various foreign bodies, both animate and inanimate. However, there are few reports of this response against antigens of fungal origin. Speare (1920) was perhaps the first worker to note that low dose of an entomopathogenic fungus *Sorosporilla uvella* in a non-susceptible host viz. *Bombyx* elicited phagocytic response, but a large dose of the same failed to induce this process. Phagocytosis against *Aspergillus flavus* (a pathogen) and *A. niger* (non-pathogenic to human), in *Hyalophora cecropia* and found higher in former case (Sussman, 1952).

The cell types invoved in phagocytosis are PLs, GRs and COs (Wago, 1980; Brehelin and Hoffmann, 1980; Guzo and Stoltz, 1987; Gunnarsson, 1988a). Phagocytosis had been noted in GRs of *Nezara viridula* infected with *Curvularia lunata* (Singh *et al*., 1991) and *Blattella germanica* infected with *Aspergillus flavus* (Kulshrestha and Pathak, 1997). In *Dysdercus similis*, bulk of phagocytic activity is carried out only by GRs while, COs and PLs also performed this function to a small extent. Cellular encapsulation involves COs and GRs in contact with the surface of the foreign material and then releasing these cells to lysate which is highly pigmented in the beginning but becomes dark later. This conglomerate then gets surrounded by multiple layers of flattened PLs. An outermost layer of rounded cells may also be present in the capsule (Ennasser and Nappi, 1984; Lackie *et al*., 1985; Lackie, 1988; Pathak, 1993) The typical multilayered cellular capsule was not seen in *Supella,* but initiation of cellular encapsulation by GRs and their lysate around the spores and hyphae of *Aspergillus fumigatus* were recorded (Shrivastava, 2001). In *Dysdercus* too, the formation of the core of the capsule involving fungal material and GRs/COs was observed.

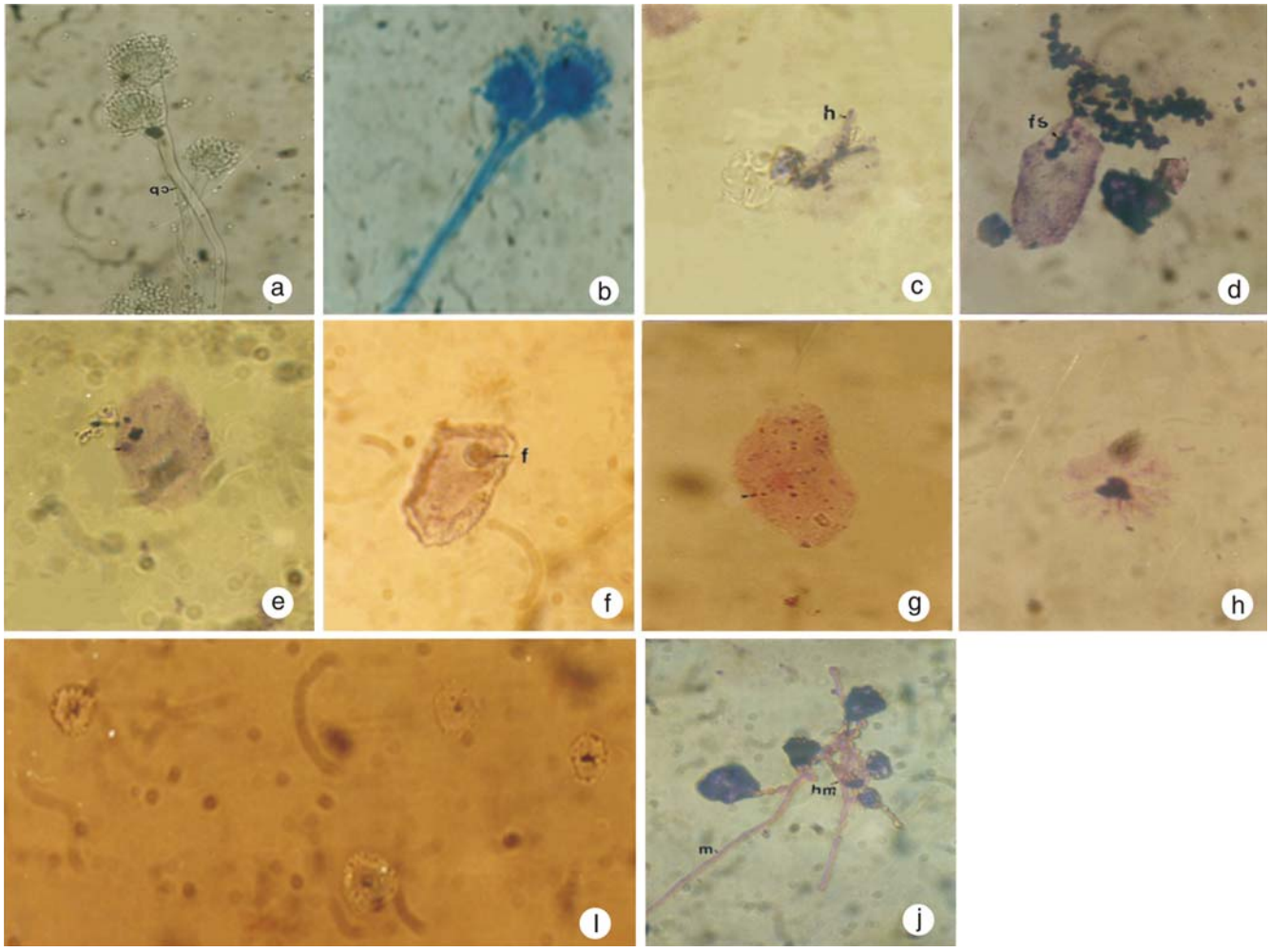

Fig.1. (a),(b) Stained and unstained conidium, conidiophore, (c) fungal hyphae, (d) fungal spore, (e) Phagocytosis of fugnal material, (f) Coagulocyte showing phagocytosis (g) An enlarged granulocyte (h) Cellular encapsulation of fungal material occurring in haemolymph, (i) Humoral encapsulation of fungal spores (j) Lysed humoral material encapsulation fungal hyphae, granulocytes are also seen

Humoral encapsulation has been noted in *Dysdercus similis* against the fungus *Aspergillus fumigatus*. Fungal conidia and hyphae were seen covered with the typical yellowish brown, hyaline material that partly covered the invader. This humoral encapsulation was caused by phenoloxidase present in the cuticle and haemolymph (Lackie, 1988). However, humoral encapsulation has so far been seen only in mosquitoes and chironomids larvae, parasitized by nematodes (Esslinger, 1962; Gotz, 1973; Vey, 1993). Gotz *et al*., (1977) had stated that humoral encapsulation occurred only in certain dipteran species that have a low THC (<6000 cells/mm^3). However, Butt *et al*., (1988) had later observed this phenomenon in a homopteran *Empoasca fabae* infected with a fungus *Erynia radicans*. Further binding of prophenoloxidase to the surface of haemocytes and its involvement in melanization has been reported by Ling and Yu (2005). This phenomenon has also been reported from crustaceans against the fungus *Aphanomyces astaci* (Soderhall, 1982). In chironomid larvae, humoral encapsulation had been observed against the fungi *Beauveria bassiana*, *Mucor hiemalis* and *Aspergillus niger* by Gotz and Vey (1974). An important role of the cuticle chemical composition, encapsulation and melanization reactions was demonstrated in mycoses (Leger *et al*., 1988; Hung *et al*., 1993; Lord and Howard, 2004). In *Supella,* PLs, COs and GRs were also seen accumulated on the surface of the foreign body engulfed by the lysate material (yellowish brown, hyaline). In *Dysdercus similis*, a number of GRs were seen adhering to various growing points of fungal colony, while initiating the process of humoral encapsulation.

ACKNOWLEDGENENT

Authors are thankful to Prof S.M. Singh, former Head of Department of Biosciences for providing fungal cultures.

REFERENCES

Arnold, J.W. and Hinks, C.F. (1979). Insect haemocytes under light microscopy technique. (Ed Gupta AP) *Insect Haemocytes,* Cambridge University Press, Cambridge.

Brehelin M. and Hoffman J. (1980). Phagocytosis of inert particles in *Locusta migratoria* and *Galleria mellonella*: Study of ultrastructure and clearance. *Journal of Insect Physiology*., **26:** 103-111.

Butt, T.M., Wraight, S.P., Galani-Wraight, S., Humber, R.A., Roberts, D.W., Soper, R.S. (1988). Humoral encapsulation of fungus *Erynia radicans* (Entomophorales) by the potato leaf hopper *Empoasca fabae* (Homoptera: Cicadellidae). *Journal of Invertebrate Pathology*., **52:** 49-56.

Ennesser, C.A. and Nappi, A.J. (1984). Ultrastructural study of encapsulation response of the American cockroach, *Periplaneta americana*. *Journal of Ultrastructure Research*., **87:** 37-45.

Esslinger, J.H.Y. (1962). Behaviour of microfilariae of *Bruggia pahangi* in *Anopheles quadrimaculatus*. *American Society of Tropical Medicine and Hygiene*., **1:** 749-758.

Guzo, D., Stoltz, D.B. (1987). Observations on cellular immunity and parasitism in the tussock moth. *Journal of Insect Physiology*., **33:** 19-31.

Gunnarson, .SG.S. (1988). Effect *in vivo* of B1, 3-glucans from fungal cell wall on the circulatory haemocytes of the desert locust *Schistocerca gregaria*. *Journal of Insect Physiology*., **34:** 47-51.

Gotz, P. (1973). Immunoreaktionen bei Insekten. *Naturwiss Rundschau*., **26:** 367-375.

Gotz, P. and Vey, A. (1974). Humoral encasulation in Diptera (Insecta): Defense reactions of chironomous larvae against fungi. *Parasitology*., **68:** 193-205.

Gupta, A. P. (1985a). Cellular elements in the haemolymph. (Eds. Kerkut, G.A. and Gilbert, L.I.). *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Pergamon Press., **3:** 401-451.

Gotz, P., Roettgen, I. and Lingg, W. (1977). Encapsulement humoral en tant que reaction de defense chez les Dipteres. *Annual Parasitology*., **52:** 95-97.

Kulshrestha, V., Pathak, S.C. (1997). Aspergillosis in german cockroach *Blattella germanica* (L.) (Blattoidae: Blattellidae). *Mycopathologia*., **139:** 75-78.

Lackie, A. M. (1988). Haemocyte behaviour. Advanced Insect Physiology., **21:** 85-178.

Lackie, A. M., Tackel, G. and Tetley, L. (1985). Haemocytic encapsulation in the locust *Schistocerca gregaria* (Orthoptera) and in the cockroach *Periplaneta americana* (Dictyoptera). *Cell and Tissue Research*., **240:** 343-351.

Leger, R.J., St .Cooper, R.M. and Charnley, A.K. (1988). The Effect of Melanization of *Madhuca sexta* cuticle on growth and infection by *Metarhizium anisopliae. Journal of Invertebrate Pathology*., **52:** 459-470.

Ling, E., Yu, X-Q. (2005). Prophenoloxidase binds to the surface of hemocytes and is involved in hemocyte melanization in *Manduca sexta*. *Insect Biochemistry and Molecular Biology*., **35:** 1356-1366.

Lord, J.C. and Howard, R.W. (2004). A proposed role for the cuticular fatty amides of *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) in preventing adhesion of entomopathogenic fungi with dry conidia. *Mycopathologia*., **158:** 211-217.

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.

Pathak, J.P.N. (1993). Cell mediated defense reactions in Insects. (Ed. Pathak, J.P.N.), *Insect Immunity,* Oxford and IBH Publishing Co. Pvt. Ltd, pp 47-58.

Shrivastava, S. (2001). Studies on the immune system in domestic cockroach *Supella supellectilium* Serville with special reference to microbial antigens. Ph.D. Thesis, R.D University, Jabalpur, India.

Singh, S.M., Pathak, S.C., Kulkarni, N., Naidu, J. and Dubey, V. (1991). First report of phaeohyphomycosis of *Nezara viridula* Linn. (Insecta : Heteroptera) caused by *Curvularia lunata*. *Mycopathologia.*, **116:** 37-43.

Soderhall, K. (1982). Prophenoloxidase activating system and melanization a recognition mechanism of arthropods. *Developmental and Comparative Immunology*., **6:** 601-611.

Speare, A.T. (1920). Further studies of *Sorosporella uvella*, a fungus parasite of noctulid larvae. *Journal of Agricultural Research*. **18:** 399-439. Cited by Shapiro M. (1979).

Sussman, A.S. (1952). Studies on the insect mycosis III. Histopathology of an aspergillosis of *Platysamia cercopia* L**.** *Annals of the Entomological Society of America.*, **45:** 233-245.

Vey, A. (1993). Humoral encapsulation. (Ed JPN Pathak) *Insect Immunity,* Oxford & IBH Publishing Co. Pvt. Ltd, pp 59-68.

Wago, H. (1980). Humoral factors promoting the adhesive properties of the granular cells and plasmatocytes of the silkworm *Bombyx* mori and possible role in the initial cellular reactions to foreignness. *Cell Immunology*., **54:** 155-169.