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The Effects of Entomopathogenic Fungus, *Tolypocladium cylindrosporum* on Cellular Defence System of *Galleria mellonella*

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ABSTRACT

The entomopathogenic-fungus-life cycle is associated with the synthesis and secretion of a number of toxic metabolites, including extracellular enzymes and the low-molecular weight compound (toxin). The potential for a successful pathogen relies on the ability to overcome the various host-defence systems. Interaction between the fungus, *Tolypocladium* (Deuteromycetes), its secondary metabolite, and its host cellular defence were investigated using *in vivo* and *in vitro* studies. *In vitro* studies showed that toxins (efrapeptins) inhibit phagocytic activity of *Galleria mellonella* (Lep: Pyralidae) haemocytes. The effect of efrapeptins on phagocytosis was in a dose-dependent manner i.e. the amount of phagocytosis in a treated cell-culture with 0, 3, and 30 μg efrapeptins per well was about 12, 7.5, and 4.5 %, respectively ($P<0.05$). *In vivo* studies showed that injection of insects with 0, 0.25, and 0.025 μg toxin rendered percentages of phagocytosis of 13, 11.5, and 7.2, respectively ($P<0.05$). There was no significant reduction in the total haemocyte count (THC) when larvae were injected with *Tolypocladium cylindrosporum* spores until 24 hours following injection. However, THC was suppressed at 48 hours post-treatment of larvae with spores. Considering that toxin suppresses phagocytosis, nodule formation, but not THC, this study suggests that efrapeptins may interfere with the ligand-receptor interactions that are likely to occur in the plasma membrane of specific haemocytes.

Keywords: Cellular defence system, Efrapeptins, Entomopathogenic fungi, Galleria mellonella, Interactions.

INTRODUCTION

Tolypocladium species exist as saprotrophs as well as insect pathogens ((Bisset, 1983) and *Tolypocladium cylindrosporum* strains are considered potential agents for the control of mosquitoes and other insect pests (Lam *et al*., 1988). With regard to biological control, entomopathogenic fungi are of special relevance since the approximately 1,000 known species from 100 genera are key regulatory factors in insect pest populations which occur worldwide and infect a wide range of host insects (St. Leger *et al*., 1991; Clarkson and Charnley, 1996). The infection process is initiated by the adhesion and germination of conidiospore on host cuticules. The chitinous integument is then actively penetrated by enzymatic digestion and physical mechanisms (Hajek and St. Leger, 1994). Upon reaching the haemocoel, penetrated fungal cells are exposed to the potential cellular and humoral defence system of the host (Dunn, 1986; Nappi and Sugumaran, 1993; Marmaras *et al*., 1996). Once the pathogens gain entry into the haemocoel of the insects they differentiate and propagate by the formation of hyphal bodies which lack a well developed cell wall (blastospore) required for their recognition by the host is immune system (Pendland *et al*., 1993; Clarkson and Charnley, 1996; Gillespie *et al*., 1997). This unicellular phase of growth

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and dissemination leads to insect death by some combination of mechanical damage produced by fungal growth, nutrient exhaustion and toxicosis (Hajek and St. Leger, 1994).

The initial haemolymph response of insects to foreign particles is mediated by circulating haemocytes. Insect haemocytes are extremely efficient at removing foreign particles such as bacteria, fungi, nematodes, and eggs of endoparasites from haemocoel, by phagocytosis, nodule formation or encapsulation (Dunn, 1986; Pathak, 1993; Nayar and Knight, 1997). Infection in insects stimulates a complex defensive response. Recognition of pathogens may be accomplished by plasma or haemocyte proteins that bind specifically to bacterial or fungal polysaccharides (Gillespie *et al*., 1997). Several morphologically distinct haemocyte cell types cooperate in the immune responses. Haemocytes attach to invading organisms and then isolate them using phagocytosis, by trapping them in haemocyte aggregates called nodules, or by forming an organised multicellular capsule around large particles (encapsulation).

Two classes of haemocytes have been implicated as effector cells in these responses: plasmatocyts and granulocytes. Granulocytes and plasmatocytes are phagocytes, and plasmatocytes are the predominant cells in capsules (Anggraeni and Ratcliffe, 1991; Vilcinskas *et al*., 1997).

Growth of *Beauveria bassiana*, *Metarhizium anisopliae* and *Tolypocladium niveum* in the haemolymph of the host larvae is associated with the secretion of toxins (secondary metabolites) by the pathogen (Mazet *et al*., 1994; Clarkson and Charnley, 1996; Bandani *et al*., 2000a). These secondary metabolic peptides of entomogenous fungi, such as destruxins and efrapeptins, are considered to be important virulence determinants (Huxham *et al*., 1989; Vilcinskas *et al*., 1997; Bandani *et al*., 2000a). They are believed to suppress the host's immune system. The exact mechanism(s) have not been fully explained but studies show that destruxins, secondary metabolites of *M. anisopliae*, inhibit haemocyte activity (Huxham *et al*., 1989; Vilcinskas *et al*., 1997). The aim of this study was to determine the interaction between the fungus *Tolypocladium* and efrapeptins, a major insecticidal metabolite of the fungus, on the cell-mediated defences of *Galleria mellonella*.

MATERIALS AND METHODS

Maintenance of Fungal Cultures, Preparation of Inoculum and Efrapeptins

Details of the maintenance of *Tolypocladium* cultures and purification of efrapeptins are given in Bandani *et al.* (2000a). Conidia were harvested from 14-day-old sporulating cultures and suspended in sterile 0.03% v/v aqueous Tween 80, sieved through cheesecloth to remove hyphal fragments then pelleted by centrifugation (3,000 g for 5 minutes). The conidia were resuspended and washed three times with sterile saline (172 mM KCl, 68 mM NaCl, 5 mM $NaHCO_3$, adjusted to pH 6.1 with HCl) and the final concentration adjusted to1×10^7 conida ml^{-1} before use in assays.

Insect Culture

Galleria mellonella were reared on an artificial diet composed of 15.4% dried skimmed milk, 30.8% wheat germ, 30.8% baby rice, 11.5% honey, 11.5% glycerine at 30°C in the dark.

Injection of Insects with Toxin and Conidia

Last instar *Galleria* larvae (230±20 mg weight) were chilled on ice for 15 minutes, the surface sterilised with 70% alcohol and then injected with 5 µl of the test sample using a. 1 ml Burkard syringe and microinjector (Burkard, UK) with a 30 gauge sterile disposable needle. After injection, larvae were transferred to a Petri dish (9 cm diam.)

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with an artificial diet to follow the course of the assay. Bleeding of the insects was performed at 4°C. Efrapeptins were dissolved in dimethyl sulfoxide (DMSO) (Sigma) and further diluted using saline. DMSO concentration in solution was 1% or less.

Growth and Labelling of Bacterial Cells

Bacillus cereus (B34) for phagocytosis experiments, were grown in nutrient broth (3% beef extract, 5% peptone; Difco Ltd, UK) with constant shaking for 18 hours at 35°C. The bacteria were labelled according to the methods of Rohloff *et al*. (1994) except that fluorescein-isothiocyanate (FITC) (Sigma) was used at $1/10^{th}$ of the recommended concentration. A concentration of 2.5×10^6 bacteria ml^{-1} was subsequently used to overlay cell monolayers to give a bacteria: haemocyte ratio of about 50:1 (Anggraeni and Ratcliffe, 1991).

In vitro Effects of Efrapeptins on Phagocytosis

Larvae were kept at 4°C for 15 minutes, swabbed with 70% alcohol then bled by proleg puncture with a sterile 26G needle. Haemolymph was collected using a 50 µl sterile glass capillary tube (Sigma) and emptied into a 1.5 ml Eppendorf tube containing 1 ml of ice-cold anticoagulant buffer. The latter consisted of 93 mM NaCl, 0.1 M glucose, 30 mM trisodium citrate, 26 mM citric acid and 10 mM EDTA at pH 4.6 and with an osmolarity of 440 mOsm kg^{-1} (Leonard *et al*., 1985). Haemolymph from two larvae was pooled in each Eppendorf tube and subsequently centrifuged at 800 g for 5 minutes at 4°C. The supernatant was discarded while the pellet was suspended in 1 ml Grace's insect medium (GIM, Sigma). The number of haemocytes was counted with an improved Neubauer haemocytometer and adjusted to 5×10^4 cells ml^{-1}. Fifty µl of the cell suspension was pipetted onto sterile, 5 mm diam. circular glass coverslips and placed at the bottom of the well of a flat bottomed microtitre plate (Nunc, Roskilde, Denmark). The plate was placed in a moist chamber for ca. 15 minutes at 29°C to allow the cells to attach to the coverslip before conducting the phagocytosis assays (Anggraeni and Ratcliffe, 1991). To test the effect of efrapeptins on the phagocytosis, efrapeptins were mixed with flouorescently-labelled bacteria and the mixture added to each well. The final concentration of toxin was either 30 or 3 µg per well. The controls consisted of (1) FITC-labelled bacteria without toxin and (2) FITC-labelled bacteria with 1% DMSO in saline to make sure that phagocytic activity of the cells was not affected by DMSO. There were two replicates per treatment and the whole experiment was repeated twice.

Haemocytes were incubated at 29°C for one hour to permit phagocytosis to take place, and then washed once with GIM. Fifty µl aliquots of 0.2% Trypan Blue in GIM was added to each well and incubated for 5 minutes to quench the FITC of non-phagocytosed bacteria. After quenching, only the ingested bacteria retained their fluorescence. The haemocyte monolayer was rinsed three times with GIM then fixed with 4% formaldehyde dissolved in GIM for 30 minutes. Coverslips were mounted on glass slides using Kaiser's glycerine jelly mountant (Merk) and sealed with clear nail varnish. Glass slides were stored in the dark at 4°C until needed. Phagocytic activity was determined by counting the cells with or without ingested bacteria under a fluorescence microscope. To quantify phagocytic activity, the percentage of phagocytosis in the control and treated monolayer was compared.

In vivo Effects of Efrapeptins on Phagocytosis

Twenty *Galleria* larvae were injected with 0.25 or 0.025 µg efrapeptins then bled 12 hours after injection to prepare the haemocyte monolayers as described earlier.

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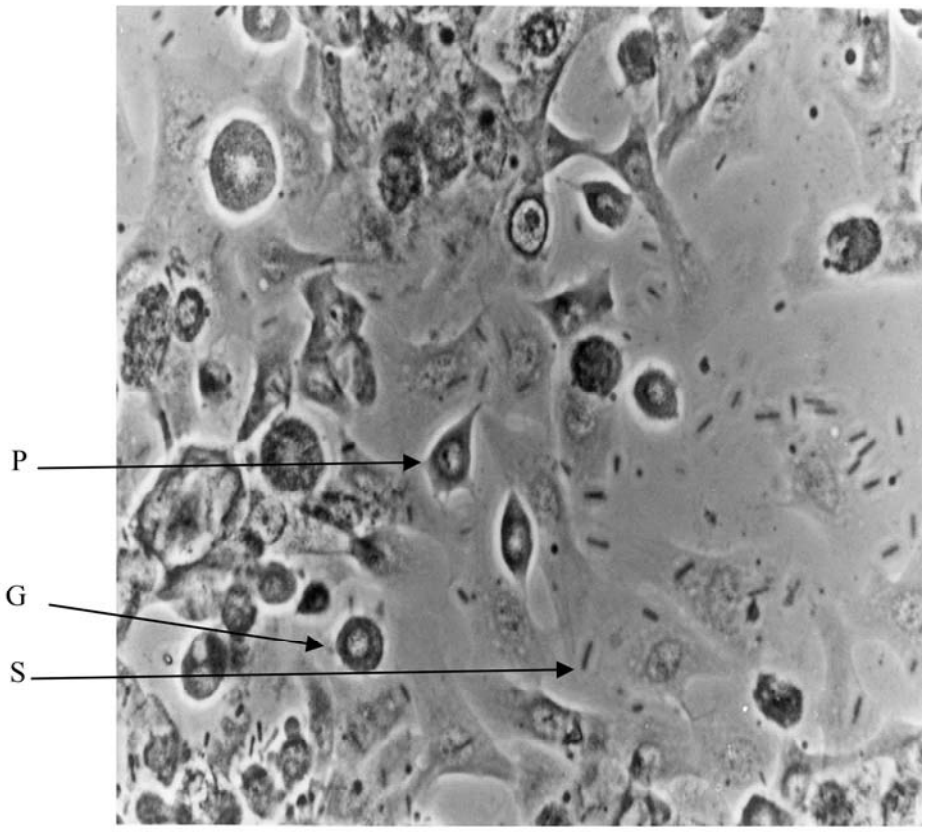
Phagoyctic activity was determined as described above.

The Effect of Efrapeptins on Nodule Formation

This was carried out by the method of Huxam *et al*. (1986). Zymosan A (Sigma) 10 mg ml^{-1} was suspended in saline, vortexed and then centrifuged at 1,500 g for 5 minutes. This step was repeated twice. Ten µl supernatant of Zymosan A or 5 µl of conidia (1×10^7 ml^{-1}) in saline were used for injection. Five insects were used for each treatment and the experiment repeated four times.

The effects of efrapeptins on nodule formation were investigated by injecting the larvae 3 hours before zymosan injection with either 0.25 µg of efrapeptins in 1% DMSO in saline. Controls consisted of 1% DMSO in saline or saline alone. The numbers of nodules formed six hours following injection were determined using the method of Hung *et al*. (1993). Briefly, the pseudoproleg was cut with scissors and 10 µl of haemolymph was pippetted onto glass microscope slides. The entire field was examined at 20 X to quantify the total number of nodules. Data were analysed using an analysis of variance.



P: Plasmatocyte
G: Granulocyte
S: Bacterial spore

Figure 1. Haemocyte monolayer of *Galleria mellonella* larvae after one hour incubation with flourescently-labelled spores of *Bacillus cereus*. Scale bar 50µm.

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The Effect of *Tolypocladium* and Efrapeptins on Haemocyte Numbers

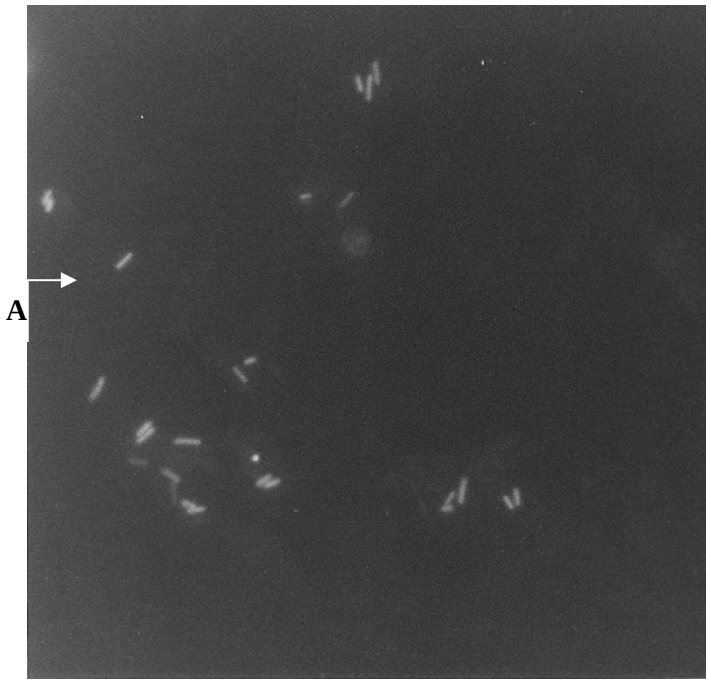
To determine if the injection of toxin or conidia caused any changes in the total haemocyte counts (THC), larvae (230±20 mg weight) were injected with 0.25 µg toxin, 5 µl of conidia (1×10^7 conidia/ml), or saline only (control). Haemolymph was collected 1, 6, and 12 hours post injection. Additional samples were taken 24 and 48 hours post-injection from larvae treated with conidia. Insects were bled into Eppendorf tubes containing ice-cold anticoagulant buffer (five larvae were bled into 1 ml of buffer). The tubes were gently inverted 5-7 times to facilitate mixing and haemocytes counted using an improved Neubauer haemocytometer. For each treatment, 20 larvae were used and the experiment repeated twice. Analysis of variance was used to analyse the data.

RESULTS

In vitro and *In vivo* Effects of Efrapeptins on Phagocytosis by *G. mellonella* Haemocytes

Efrapeptins significantly decreased the amount of phagocytosis by *Galleria* haemocytes compared with the controls ($P<0.05$) (Figure 1). The effect was dose-related (Figure 2 and 3). At 30 µg and 3 µg efrapeptin per well, the percentage of haemocytes that had phagocytosed labelled bacteria was 4.5% and 7.5%, respectively. In the controls, the number was 11.2% (saline only) and 12.5% (1% DMSO in saline).

Microscopic studies showed that haemocytes exposed to efrapeptins tended to withdraw their pseudopodia and become



A: Flouorescently-labelled spores of *Bacillus cereus* which have been ingested by cells. Note that after quenching with 0.2% Trypan Blue only the ingested bacteria retain their fluorescence whereas non ingested bacteria become coated with Trypan Blue.

Figure 2. Haemocyte monolayer of *G. mellonella* larvae after one hour incubation with flourescently-labelled spores of *B. cereus* and quenching with 0.2 % Trypton Blue.

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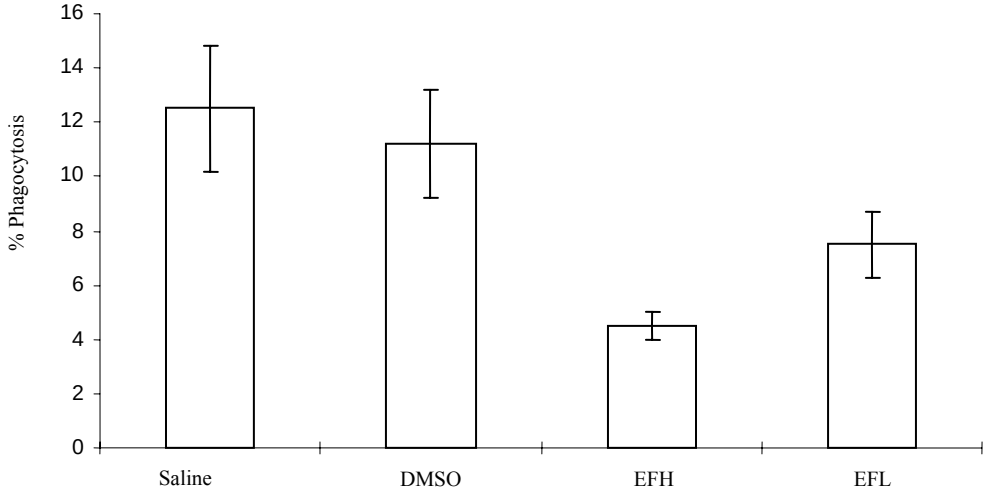

Saline: Haemocyte monolayer treated with saline (as a first control).
DMSO: Haemocyte monolayer treated with1 % DMSO in saline (as a second control).
EFH: Haemocyte monolayer treated with 30 µg efrapeptins per well.
EFL: Haemocyte monolayer treated with 3 µg efrapeptins per well.
There were significant differences (P<0.05) between controls and treatments.

Figure 3. In vitro effect of efrapeptins on the percentage phagocytosis of bacterium, *Bacillus cereus* by *Galleria mellonella* monolayer.

rounded. This was particularly evident at the highest efrapeptin concentrations (i.e. 30 µg per well).

Similar observations were made when efrapeptins were injected into the haemocoel of *Galleria* larvae; here phagocytosis was significantly depressed (P<0.05) (Figure 4). In insects injected with 0.25 µg and 0.025 µg efrapeptins, the percentage of haemocytes containing labelled bacteria was 7.2% and 11.5%, respectively (Figure 4). Haemocytes recovered from control insects had ingested marginally more bacteria (12-13%).

The Effect of Efrapeptins on Nodule Formation

Efrapeptins significantly (P< 0.05) affected nodule formation in *Galleria* larvae following injection with zymosan supernatant (Figures 5 and 6). There were no significant differences between the 1 % DMSO or saline controls (P>0.05).

The Effect of *Tolypocladium* and its Efrapeptins on Haemocyte Numbers

The total haemocyte count (THC) of saline-injected larvae ranged from $3.8\pm6\times10^7$ cells ml^{-1} after one hour and up to $3.5\pm6.0\times10^7$ cells ml^{-1} after 48 hours post-injection.

There were no significant differences in the THC of saline, spore, and toxin-injected larvae during the first 24 hours (P>0.05) (Table 1). However, after 48 hours, larvae injected with conidia had significantly (P<0.05) fewer circulating haemocytes (ca. $2.1\pm5.2\times10^7$ haemocytes ml^{-1}). This decrease corresponded with an increase in fungal hyphal bodies.

DISCUSSION

This study shows for the first time that efrapeptins produced by the insect pathogenic fungus *Tolypocladium* do affect the insect immune system. However, the effects appear to be restricted primarily to haemocyte activities, and were observed at

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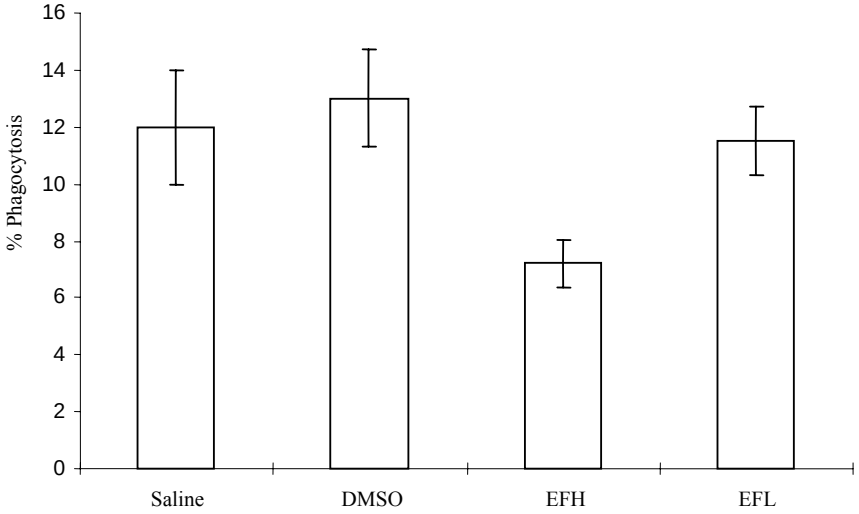

Saline: Saline-injected larvae (as a first control).
DMSO: 1% DMSO in saline-injected larvae (as a second control).
EFH: Larvae injected with high dose of efrapeptins (0.25 µg per larva).
EFL: Larvae injected with low dose of efrapeptins (0.025 µg per larva).
There were significant differences (P<0.05) between controls and treatments.

Figure 4. In vivo effect of efrapeptins on the percentage phagocytosis of bacterium, *Bacillus cereus* per *Galleria mellonella* monolayer.

efrapeptin concentrations higher than detected in naturally infected larvae (Bandani *et al*., 2000a). This implies that efrapeptins work in concert with other pathogenicity determinants in suppressing the host immune system. Indeed, *Tolypocladium* species do secrete a wide range of secondary metabolites (.e.g. cyclosporin) which could have an affect on the host's immune system. Similar observations have been reported for toxins of other entomogenous fungi. For example, destruxins of *M. anisopliae* inhibited phagocytosis of *Galleria* plasmatocytes (Vilcinskas *et al*., 1997) and toxins from *B. bassiana* suppressed phagocytosis and the spread of *Spodoptera exigua* haemocytes *in vivo* (Hung and Boucias, 1993). Destruxins are also known to interfere with haemocyte function and can prevent nodulation (Huxham *et al*., 1989). Our own observations show that, as the fungus colonises the haemocoel the number of circulating haemocytes and nodules decreases. This could partly be attributed to the metabolites (enzymes and toxins) se-

Table 1. The effect of *T. niveum* and efrapeptins on the total hemocyte count (cells ml^{-1}) of *G. mellonella*.

Treatment	Post injection (h)				
	1	6	12	24	48
Saline	$3.8 \pm 2.5 \times 10^7$	$4.2 \pm 2.0 \times 10^7$	$3.9 \pm 3.0 \times 10^7$	$4.5 \pm 3.7 \times 10^7$	$3.5 \pm 6.0 \times 10^7$
Spore	$4.5 \pm 3.0 \times 10^7$	$3.1 \pm 3.0 \times 10^7$	$3.7 \pm 2.3 \times 10^7$	$2.6 \pm 2.0 \times 10^7$	$2.1 \pm 2.0 \times 10^7$
Toxin	$3.6 \pm 2.5 \times 10^7$	$4.5 \pm 2.8 \times 10^7$	$4.0 \pm 3.5 \times 10^7$	----------	----------

There were no significant differences (P>0.05) between toxins, spores and saline injection at 1, 6, 12, and 24 hours post-injection.
However, There were significant differences between spore and saline injection at 48 hours post-injection (P< 0.05).

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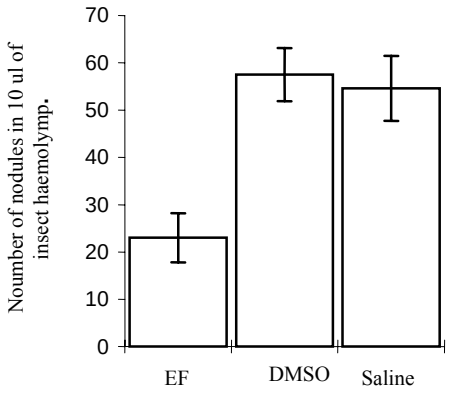

EF: Larvae injected with efrapeptins.
DMSO: Larvae injected with 1% DMSO in saline (as a first control).
Saline: Larvae injected with saline alone (as a second control).
There were significant differences (P<0.05) between controls and treatment.

Figure 5. Effect of efrapeptins on zymosan-induced nodule formation in the *G. mellonella larvae*.

creted by *Tolypocladium* and supports our earlier suggestion that these metabolites, although produced in relatively low amounts, probably work synergistically.

Vilcinskas *et al*. (1997) suggested that the infection process of the entomopathogenic fungus, *Metarhizium anisopliae*, include an intracellular phase within phagocytic cells, which are probably used as a vehicle for dispersion of infective cells after penetration of the host integument. It is known that V-ATPases mediate the acidification of most intracellular organelles including phagocytic cells, which is vital for microbial degradation (Grinstein *et al*., 1992). It may be concluded that production of efrapeptins by *Tolypocladium* species, which has been detected inside the mycosed-insect, is a strategy for survival of hyphal bodies of the fungus, once ingested by phagocytic cells. Efrapeptins are known to inhibit the V-ATPase of a variety of organisms including insects (Bandani *et al*., 2000b).

Nodule formation, which is primarily a mechanism for sequestering particulate materials that enter the haemocoel, is also induced by the injection of soluble molecules such as β-1, 3-glucan from fungal cell wall, bacterial polysaccharides and certain glycoproteins (Ratcliffe *et al*., 1984; Lackie and Vasta, 1988). The suppression of glucan-induced nodule formation by fungal secondary metabolite makes sense in terms of the fungal strategy of immune suppression (Huxham *et al*., 1989). In *G. mellonella* at least two classes of haemocytes, plasmatocyte and granular cell, are involved in nodule formation (Dunn, 1986). These subpopulations of insect haemocytes distinguish between self and non-self structures. Their ability to recognize intruders is mediated by endogenous molecules that bind to particular sites on the foreign surface. Such mediators are either dissolved in the haemolymph or are exposed on the cell membrane of haemocytes. Insect immunity requires the recognition of non-self elements which is likely to involve the binding of pathogen cell surface oligosaccharides. One component of the humoral immune system, the lectins, are thought to act as opsonins (Pendland and Boucias, 1996). Phagocytosis of microbial cells may involve interactions between lectins on phagocytic cells and sugars on microbial surfaces (Nayar and Knight, 1997). Numerous lectins that bind to carbohydrate components on microbial cell walls have been identified in insect haemolymph.

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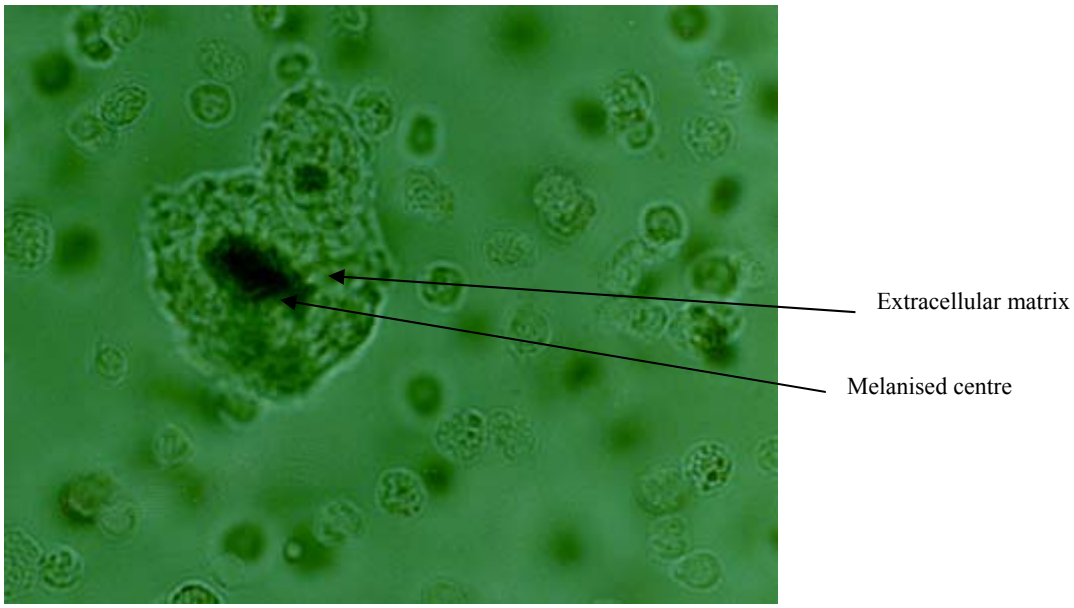

Figure 6. Induction of nodule formation in *G. mellonella* larvae with 10µl zymosan injection. Scale bar 30µm.

Recognition of fungal cell wall in insects is mediated by lectins that bind to polysaccharides such as β-1,3 glucans which are a major component of fungal cell walls (Boucias and Pendland, 1991). Several β-1,3 glucan binding proteins with different molecular weights have been identified in a variety of insects. Interactions between fungal pathogens and host defences are an important regulatory factor in fungal disease. Presentation of specific carbohydrates on the fungal cell wall is likely to be important in activating components of the insect immune response (Pendland and Boucias, 1996). Studies indicated that the hyphae cell walls of *M. anisopliae* contain mannose-rich glycoproteins and that cell walls rich in carbohydrate residue are more antigenic and can elicit the insect's immune response (Gillespie *et al*., 1997).

Adsorption of insect lectins to fungal cell walls has been shown to remove haemagglutination activity in the haemolympth of *Spodoptera exigua* (Pendland and Boucias, 1996). This suggests that the binding of carbohydrate-specific lectins to the oligosaccharide side-chains of glycoproteins on haemocytes and fungal surfaces is important in mediating insect immunity. Susceptibility to phagocytosis, agglutination and encapsulation will depend on the presence of specific lectin receptors on the fungal cell wall which are absent from the surfaces of early forms (protoplast and blastospore) of the fungus (Clarkson and Charnley, 1996). Therefore, although efrapeptins are produced inside the insect host at the sublethal level, that may be enough to interfere with the function of specific receptors e.g. β-1,3-glucan-specific protein of the insect immune system (Chen *et al*., 1995; Chen *et al*., 1998). It has been known that the recognition protein located on the cell surface of reactive tissue may interact with microbial ligands and subsequently initiate both cellular and humoral reactions, including phagocytosis, cell degranulation, encapsulation and biochemical proteolytic cascades, such as coagulation and proPO activation and the production of antimicrobial peptides (Hoffmann, 1994).

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REFERENCES

1. Anggraeni, T. and Ratcliffe, N. A. 1991. Studies on Cell-cell Co-operation during Phagocytosis by Purified Haemocyte Populations of the Wax Moth, *Galleria mellonella*. *J. Insect Physiol.,* **37:** 453-460.
2. Bandani, A. R., Khambay, B. P. S., Faull, L., Newton, R., Deadman, M. and Butt, T. M. 2000a. Production of Efrapeptins by *Tolypocladium* Species (Deuteromycotina: Hyphomycetes) and Evaluation of Their Insecticidal and Antimicrobial Properties. *Mycol. Res.,* **104:** 537-544.
3. Bandani, A. R., Amri, B., Butt, T. M. and Gordon-Weeks, R. 2000b. Effects of Efrapeptins and Destruxin, Metabolites of Entomogenous Fungi on the Hydrolytic Activity of a Vacuolar Type ATPase Identified on the Brush Border Membrane Vesicles of *Galleria mellonella* Midgut and on Plant Bound Hydrolytic Enzymes. *Biochimica et Biophysica Acta*, **78029**: 1-11.
4. Bissett, J. 1983. Notes on *Tolypocladium* and Related Genera. *Canadian J. Bot.,* **61:** 1311-1329.
5. Boucias, D. G. and Pendland, J. C. 1991. The Fungal Cell Wall and Its Involvement in the Pathogenic Process in Insect Hosts. In: "*Fungal Cell Wall and Immune Response"*. (Eds.) Latge, J. P. and Boucias, D. J. Springer, Berlin Heidelberg, New York. pp. 303-316.
6. Chen, C., Durrant, H. F., Newton, R. P. and Ratcliffe, N. A. 1995. A Study of Novel Lectins and Their Involvement in the Activation of the Prophenoloxidase System in *Blaberus discoidalis*. *Biochem. J.,* **310:** 23-31.
7. Chen, C., Rowley, A. F. and Ratcliffe, N. A. 1998. Detection, Purification by Immunoaffinity Chromatography and Properties of β-1,3-glucan-specific Lectins from the Sera of Several Insect Species. *Insect Biochem. Molec. Biol.,* **28:** 721-731.
8. Clarkson, J. M., and Charnley, A. K. 1996. New Insight into the Mechanisms of Fungal Pathogenesis in Insects. *Trends Microbiol.,* **4:** 197-203.
9. Dunn, P. E. 1986. Biochemical Aspects of Insect Immunology, *Ann. Rev. Entomol.,* **31:** 321-329.
10. Gillespie, J. P., Kanost, M. R. and Trenczek, T. 1997. Biological Mediators of Insect Immunity. *Annu. Rev. Entomol.,* **42:** 611-643.
11. Grinstein, S., Nanda, A., Lukacs, G., and Rotstein, O. 1992. V-ATPase in Phagocytic Cells. *J. Exp. Biol,* **172:** 179-192.
12. Hajek, A. E. and St. Leger, R. J. 1994. Interaction between Fungal Pathogens and Insect Hosts. *Annu. Rev. Entomol.,* **39:** 293-322.
13. Hoffmann, J. A. 1994. Immune Response of Insects. *Develop. Comp. Immunol.,* **18(S1):** 37-38.
14. Hung, S. Y. and Boucias, D. G. 1992. Influence of *Beauveria bassiana* on the Cellular Defense Response of the Beet Armyworm, *Spodoptera exigua*. *J. Invertebrate Pathol.,* **60:** 152-158.
15. Hung, S. Y., Boucias, D. G. and Vey, A. J. 1993. Effect of *Beauveria bassiana* and *Candida albicans* on the Cellular Defence Response of *Spodoptera exigual*. *J. Invertebrate Pathol.,* **61:** 179-187.
16. Huxham, I. M., Lackie, A. M. and McCorkindale, N. J. 1989. Inhibitory Effects of Cyclodepsipeptides, Destruxins, from the Fungus *Metarhizium anisopliae*, on Cellular Immunity in Insects. *J. Insect Physiol.,* **35:** 97-105.
17. Lackie, A. M. and Vasta, G. R. 1988. The Role of a Galactosyl-binding Lectin in the Cellular Immune Response of the Cockroach *Priplaneta americana* (Dictyoptera). *Immunology,* **64:** 353-357.
18. Lam, T. N. C., Goettel, M. and Soares, G. G. Jr. 1988. Host Records for the Entomopathogenic Hyphomycete Tolypocladium Cylindrosporum. *The Florida Entomol.,* **71:** 86-89.
19. Leonard, C., Kenneth, S. and Ratcliffe, N. A. 1985. Studies on Prophenoloxidase and Protease Activity of *Blaberua craniifer* Haemocytes. *Insect Biochem.,* **15:** 803-810.
20. Mazet, I., Hung, S. Y. and Bocias, D. G. 1994. Detection of Toxic Metabolites in the Hemolymph of *Beauveria bassiana* Infected *Spodoptera exigua* Larvae. *Experentia,* **50:** 142-147.
21. Marmaras, V. J., Charalambidis, N. D. and Zervas, C. G. 1996. Immune Response in Insects: The Role of Phenoloxidase in Defense Reaction in Relation to Melanization and

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Sclerotization. *Arch. Insect Biochem. Physiol.,* **31:** 119-133.
22. Nappi, A. J. and Sugumaran, M. 1993. Some Biochemical Aspects of Eumelanin Formation in Insect Immunity. In: "*Insect Immunity"* (Ed.) Pathak, J. P. N. Oxford and IBH Publishing Co. PVT. LTD., New Delhi. pp. 131-147.
23. Nayar, J. K., and Knight, J. W. 1997. Hemagglutinind in Anopheles Quadrimaculatus Strains Susceptible and Refractory to Brugia Malayi and Their Role in the Immune Response to Filarial Parasites. *Com. Biochem. Physiol.,* **11(6B):** 109-117.
24. Pathak, J. P. N. 1993. Haemagglutinins (Lectins) in Insects. In: *Insect immunity*. (Ed.) Pathak, J. N. P. Oxford and IBH Publishing Co. PVT. LTD. New Delhi. pp.149-169.
25. Pendland, J. C. and Boucias, D. G. 1996. Phagocytosis of Lectin-opsonized Fungal Cells and Endocytosis of the Ligand by Insect *Spodoptera exigua* Granular Hemocytes: and Ultrastructural and Immunocytochemical Study. *Cell Tiss. Res.*, **285:** 57-67.
26. Pendland, J., Hung, S. Y. and Boucias, D. G. 1993. Evasion of Host Defense by in Vivo Produced Protoplast Like Cells of the Insect Mycopathogen Beauveria Bassiana. *J. Bacteriol.,* **175:** 18, 5962-5969.
27. Ratcliffe, N. A., Leonard, C. and Rowley, A. F. 1984. Prophenoloxidase Activaion: Nonself Recognition and Cell Cooperation in Insect Immunity. *Science,* **226:** 557-559.
28. Rohloff, L., Wiesner, A. and Gotz, P. 1994. A Fluorescence Assay Demonstrating Stimulation of Phagocytosis by Haemomymph Molecules of *Galleria mellonella*. *J. Insect Physiol.,* **40:** 1045-1049.
29. St. Leger, R. J., Goettle, M., Roberts, D. W. and Staples, R. C. 1991. Prepenetration Events during Infection of Host Cuticle by *Metarhizium anisopliae*. *J. Invertebrate Pathol.,* **58:** 168-179.
30. Vilcinskas, A., Matha, V. and Gotz, P. 1997. Inhibition of Phagocytic Activity of Plasmatocyes Isolated from *Galleria mellonella* by Entomogenus Fungi and Their Secondary Metabolites. *J. Insect Physiol.,* **43:** 475-483.

برهم کنش قارچ پاتوژن(*Tolypocladium cylindrosporum*) و سیستم د فاع سلولی کرم مومخوار

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چکیده

دوره زند گی قارچ های بیمارگر حشرات باسنتز وترشح تعدادی متابولیت های سمی از قبیل آنـزیم هـا و ترکیبات باوزن مولکولی پائین (توکسین) مرتبط می باشد. قارچی در فرایند بیمارگری موفق خواهد بود که قادر به غلبه بر سیستم های دفاعی بدن حشره میزبان باشد. در این مطالعه به طریق آزمایشگاهی و تزریق سم وکنیدی به داخل بدن حشره برهم کنش قارچ و متابولیت های ثانویه آن با سلولهای د فاعی کرم مومخـوار زنبورعسل مطالعه شدند.مطالعات آزمایشگاهی نشان داد که سم قارچ که بنـام افـراپپتین نـام دارد خاصـیت مهارکنند گی برای سلولهای قاگوسیتوزی حشره دارد و این خاصیت مهارکنند گـی وابـسته بـه دزسـم مـی باشد. برای مثال وقتی که کشت سلولی حشره درمعرض دزهای ۳۰ و ۳ میکروگـرم سـم قرارگرفـت میـزان فاگوسیتوزی به ترتیب ۴,۵ و ۷,۵ درصد بود در صورتیکه میـزان فاگوسـیتوزی در کنتـرل ۱۲ درصـد بـود.

وقتیکه سم به داخل بدن حشره تزریق گردید میزان فاگوسیتوزی در دزهای ۰,۲۵ و ۰,۰۲۵ میکروگرم و کنترل به ترتیب۷,۵، ۱۱,۵ و۱۳ درصد بودند. وقتی که اسپر قارچ به درون بده حشره تزریق گردید تعداد کل سلولهای خونی در بیست و چهار ساعت اولیه بعد از تزریق، تفاوت معنی داری با کنترل نداشت. امادر چهل و هشت ساعت بعد ازتزریق تعداد کل سلولهای خونی به مقدارقابل ملاحظه ای کاهش پیدا کردند. با توجه به اینکه توکسین میزان فاگوسیتوز و تشکیل نودول را تحت تاثیر قرار میدهد اماروی میزان کل سلولهای خونی بی اثر است میشود نتیجه گیری کرد که این سم در برهم کنشهای لیگاند-رسپتور که در سطح سلولهای خونی اتفاق می افتد اختلال ایجاد می کند.

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