Impact of two pathogenic fungal crude metabolites on mortality, biology and enzymes of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae)

K. Sahayaraj* and Majesh Tomson

ABSTRACT

Saprophytic fungi plays a crucial role in the pest management programme. Culture and mass production of the fungi is a tedious, laborious, time consuming and cost effective process. To minimize the use of chemicals, the metabolic products of the fungi have been utilized in the pest management progamme for the past one decade. A study was designed to evaluate the insecticidal activity of *Beauvaria bassiana* (Balasmo) Vuillimin.(BB) and *Metarhizium anisopliae* (Metchnikoff) Sorokin. (MA) crude metabolic extracts and fungal spores against *Dysdercus* cingulatus (Fab.) under *in-vitro* conditions. The toxicity bioassay revealed that MAF1 treated cotton seeds fed *D. cingulatus* showed high mortality (44.44%). Irrespective of the metabolic fractions and fungal spores, body weight of *D.cingulatus* gradually diminished when the nymph grew older. Maximum body weight reduction was recorded in BBF2 (44.3%) category followed by BBF1 (45.4%) and the metabolites showed higher activity than fungal spores. Treatments also reduced total body protein content. Maximum reduction was recorded in BBF2 (0.09mg/g) followed by MAF1 (0.102mg/g). Amylase level was highly reduced by MAF2 (0.036µg/mg) followed by BBF2 (0.085 µg/mg). Higher protease activity resulted in BBF2 ($9.1x10^{-5}$ µg/mg) followed by BBF1 ($6.5x10^{-5}$ µg/mg). The detoxification enzyme, glutamate oxalate transaminase (GOT) activity was highly reduced in BBF2 ($1.8x10^{-5}$ µg/mg) followed by MAF2 ($2.5x10^{-5}$ µg/mg). These observations indicated the potential of *B. bassiana* and *M. anisopliae as* the simple, inexpensive and accessible source of bioinsecticide to manage sucking pests like *D.cingulatus*.

Key words: *Beauvaria bassiana, Metarhizium anisopliae, Dysdercus cingulatus,* fungal metabolites

INTRODUCTION

Cotton, *Gossypium hirsutum* (Linn.) is the most economically important natural fiber material in the world and it is widely known as "The King of Fibers". Man has been utilizing cotton for his benefits since ancient times (Fryxell, 1992). Cotton is a multipurpose crop that supplies five basic products such as lint, oil, meal, seed hulls and linters. Lint is the most important product of the cotton plant and provides much of the high quality fiber for the textile industry. The economy of many countries like India, Pakistan, Egypt, Sudan etc. depends up on cotton and its products. In India, cotton is an important industrial crop, 24% of the total cotton production is cultured in India.

In recent years, yield of cotton has become static rather it is declining due to the infestation of insect pests and diseases. One of the major factors of low yield was the infestation of insect pests. Sucking pests are deleterious during early season of the cotton plant growth and development. Cotton stainer, jassids, aphids, white flies and thrips are constituted as important pests of cotton (Uthamasamy, 2004).

Dysdercus cingulatus (Fab.) (Hemiptera : Pyrrhocoridae) is a serious pest of cotton distributed in all the cotton growing regions of India (David and Ananthakrishnan, 2004; Sahayaraj and Ilayaraja, 2008). It is commonly known as red cotton bug and is an important pest of lady's finger, sambhal, hollyhock *etc*. Nymphs and adults of *D. cingulatus* feed mainly on developing or mature cotton seeds. It has been controlled by many synthetic insecticides. However they failed to controll this insect. because, both the nymphs and adults move from place to place very rapidly. Hence it is essential to find out an alternative method for the management of this economically important pest. For the past two decades, extension workers and pest management workers have been using fungal pathogens in pest management programme where *Beauvaria bassiana* and *Metarhizium anisopliae* have been play an important role (Arnold, 2005).

Beauvaria bassiana (Balasmo) Vuillumin (Ascomycota : Hypocreales) is one of the most ubiquitous and extensively studied entomopathogenic fungi (Dias *et al.*, 2008). Entomopathogens as biological agents are attracting increased attention because they provide environmentally safe insect control (E1-Mandarowy, 2005). *B. bassiana* infects larvae, pupae and adults of many insects successfully and at the time of insect death nearly all the internal organs of the insect are utilized by the fungus (Sabbahi *et al.*, 2008). *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycotina : Hyphomy cetates) is a rather common agent causing infection in natural insect populations (Borisov *et al.*, 2001; Serebrov *et al.*, 2007). It has a very broad host range and is used as a good pest control agent for several pests (Borgio and Sahayaraj, 2007). Spontaneous variability of *M. anisopliae* should be considered as a reserve for selection of this biocontrol agent on high virulence towards pest insects (Serebrov *et al.*, 2007). Very little information is available on the metabolic products of the fungi on insects and none has studied their impact on *D. cingulatus*. The present study is aimed to investigate the impact of crude metabolic fractions of *B. bassiana* and *M.anisopliae* on *D.cingulatus*

MATERIALS AND METHODS

Pest Collection and Rearing

Adults and nymphs of *Dysdercus cingulatus* were collected from cotton field in Peikulam Tirunelveli District, Tamil Nadu, India. Collected insects were maintained under laboratory condition (27±2° C temperature, 70-75 RH, 11L:13D) on its natural host cotton. The third nymphal instars of *D. cingulatus* were used for the present study.

Fungal source culture and preparations of fractions

Isolated *Metarhizium anisopliae* and *Beauvaria bassiana* were obtained from CPRC, St. Xavier's College, Palayamkottai and used for the present study. The isolated fungi were cultured using standard potato-dextrose broth. 10 ml of 7 day-old fungal culture were taken in a test tube and centrifuged at 5000 rpm for 30 minutes. Separate the supernatant from the pellets. This supernatant was considered as fraction I (BBF1 and MAF1). The weight of the pellet was recorded using monopan balance. Add required amount (1 mg pellet /3 ml phosphate buffer) of phosphate buffer to the pellet, mix well and transfer into another test tube. Heat the mixture at 70 - 72° C for 20 minutes in a water bath. Centrifuge the sample at 5000 rpm for 30 minutes, and separate the supernatant and consider it as fungal fraction II (BBF2 and MAF2).

Bioassay

One gm of cotton seeds were soaked in 10 ml of water for 10 minutes shade dried for 5 minutes. Take 10 ml of fraction I in a beaker (50 ml capacity) and introduce the cotton seeds into the beaker and allow them for 40 minutes. These seeds were as food for *D. cingulatus*. Every day the cotton seeds provided were replaced and provided fraction I treated seeds continuously for four days. Then they were fed with water soaked cotton seeds till their death. The seeds soaked in phosphate buffer are treated as control. Similar procedure was followed for fraction II. The 10^8 fungal spores of *B. bassiana* and *M. anisopliae* were inoculated, and the cotton seeds were dipped in the culture and supplied to the animal. Mortality was observed during the nymphal stages. Moreover, weight of the nymphs has been recorded every day using monopan balance. Nymphal total life time was recorded. Irrespective of the treatments, 3 day-old adults were used for biochemical analysis.

The whole body protein quantities of insects were estimated by Bradford (1976) method. The enzymes were estimated by using standard procedures, the enzyme source was prepared by the freshly dissected gut homogenized with 1ml of phosphate buffer (pH 7.2). Add 3ml of distilled water to it and keep it in an eppondorf. Centrifuge the samples at 5000 rpm for 20 minutes and take the supernatant for enzyme studies. Amylase, protease and glutamate oxaloacetate tranraminase (Bernfield, 1955) enzymes were quantified. The whole body protein profile of the insect was analyzed by SDS-PAGE method (Laemmli, 1970).

Statistical Analyses

Experimental data was compared with control category data by Tukey's Multiple Range Test and their significance was expressed at 5 % level

Table 1. Impact of *M. anisopliae* (MA) and *B. bassiana* (BB) crude metabolic fractions on the corrected mortality (in %) of *D. cingulatus*

Treatments	Days after Exposure			
	Fifth day	Sixth day	Seventh day	Eighth day
MAF1	5.0	20.0	42.1	44.44
MAF2	10.0	25.0	31.58	33.33
BBF1	20.0	25.0	31.58	38.88
BBF2	15.0	25.0	26.32	33.33
B. bassiana	10.0	16.66	30.0	33.33
M. anisopliae	10.0	23.33	26.32	30.0

Table 2. Impact of different fractions of fungi on the body weight (in mg.) of *D. cingulatus* nymphs

Treatments	Observation After Treatments (in days)			
	Fifth day	Sixth day	Seventh day	Eighth day
Control	62.3 ± 0.7	63.2 ± 0.4	64.6 ± 0.5	66.5[a] ± 1.0
MAF1	61.8[NS] ± 1.3	60.2* ± 0.4	56.7* ± 1.6	48.6 ± 0.7
MAF2	58.6* ± 1.8	57.6* ± 1.8	52.4* ± 1.3	48.0 ± 0.9
BBF1	61.3[NS] ± 0.5	54.4* ± 0.7	52.8* ± 0.7	45.4 ± 0.6
BBF2	60.5 [NS] ± 1.0	56.1* ± 1.2	51.4* ± 0.8	44.3 ± 1.4
B.bassiana	62.1 [NS] ± 0.6	61.8 [NS] ± 0.5	60.4 [NS] ± 0.6	58.2 * ± 0.6
M.anisopliae	61.4 [NS] ± 0.6	59.5 [NS] ± 0.3	57.6* ± 0.8	56.2* ± 0.9

NS – Not significant, * Significant at 5% level by TMRT

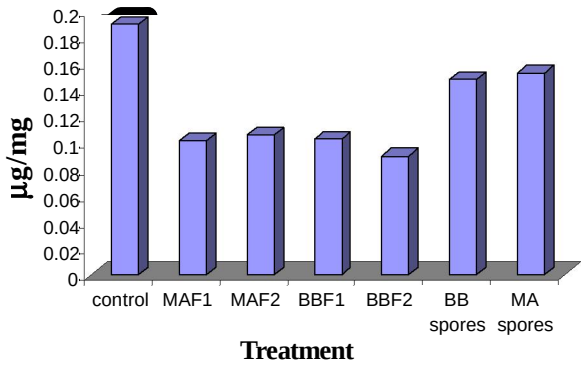

Figure 1. Protein quantity (µg/mg) of *D. cingulatus* fed with fungus and fungal fractions treated cotton seeds

RESULTS

Mortality

The percent mortality of *D. cingulatus* showed that the fungal crude fractions caused a moderate (44.44% for MAF1) or low mortality (33.33% for MAF2 and BBF2) to nymphal stages of insect. The fungal spores also showed moderate mortality (33% for *B.bassiana* and 30% for *M. anisopliae).* The toxic effect increased with increase in the exposure time.

Body weight

Dietary utilization of *D. cingulatus* was severely affected, when it was fed with cotton seeds treated with fungal metabolites and spores (Table 2). In control the weight of the nymphs gradually increased and it attained a maximum weight of 66.5 mg on the eighth day of adult life. It was significantly ($P < 0.05$) reduced when the nymphs fed with BBF-2 (44.3mg) followed BBF-1, MAF-1 and MAF-2.

Nymphal development

Table 3 shows the nymphal period of *D. cingulatus* during the test period. It showed that insignificantly the fungal fractions and spores extend the nymphal period. However the fungal fractions and spores seriously affect the morphology of *D.cingulatus* during its molting.

Total body Protein

The total body protein of control *D. cingul*atus was 0.1896 µg/mg. Reduction of total bodyprotein was observed in fungal fractions treated *D. cingulatus* when compared with control category. Figure 1 shows the quantity of protein in animals decreased during the test period. The fraction of *B.bassiana* showed the higher activity than *M. anisopliae* in protein reduction. At the end of the eighth day, the maximum reduction was recorded in BBF-2, followed by MAF1, BBF-1, MAF2, *M. anisopliae* and *B. bassiana*.

SDS protein profile

SDS protein profile of *D. cingulatus* adults after feeding fungal metabolites showed in Plate 1b. In normal pest, four polypeptides were recorded (11832 Kd to 38128 Kd). It was reduced by *B.bassiana* fractions (Table 4). However, both MAF-1 and MAF-2 increased the molecular weight of the polypeptides. (Fig 2)

Enzyme Activity

Two digestive enzymes (amylase and protease) and a detoxification enzyme (GOT) were quantified. First fraction of both the tested fungi highly reduced amylase activity, whereas BBF2 increased the activity, in order to the fungal spores the enzyme activity is low. In contrast, invariably fractions and spores of these two fungi reduced protease level. In the case of GOT, higher activity was recorded in MAF1 followed by BBF1.

Table 3. Impact of fungal metabolites on nymphal life time (in days) of *D. cingulatus*

Treatments	Third instar	Forth instar	Fifth instar	Total nymphal period
Control	2.5 ± 0.12	3.6 ± 0.14	5.0 ± 0.3	18.2 ± 0.9
MAF1	2.6 ± 0.13	3.6 ± 0.14	5.6 ± 0. 3	18.3 ± 0.9
MAF2	2.5 ± 0.12	3.6 ± 0.14	5.6 ± 0. 3	18.8 ± 0.9
BBF1	2.6 ± 0.13	3.6 ± 0.14	5.6 ± 0. 3	18.3 ± 0.9
BBF2	2.5 ± 0.12	3.6 ± 0.13	5.6 ± 0.3	18.8 ± 0.9
B. bassiana	2.5 ± 0.12	3.6 ± 0.14	5.6 ± 0.3	18.8 ± 0.9
M. anisopliae	2.5 ± 0.12	3.6 ± 0.14	5.6 ± 0.3	18.8 ± 0.9

Table 4. Enzyme Levels of *D. cingulatus* in relation to various fungal fractions

Treatments	Amylase	Protease	GOT
Control	3.4×10^{-2}	4.3×10^{-5}	4.8×10^{-6}
MAF1	1.6×10^{-2}	5.5×10^{-5}	3.3×10^{-5}
MAF2	3.6×10^{-3}	6.0×10^{-5}	2.5×10^{-5}
BBF1	1.5×10^{-2}	6.5×10^{-5}	2.6×10^{-5}
BBF2	8.5×10^{-3}	9.1×10^{-5}	1.8×10^{-5}
B. bassiana	2.4×10^{-2}	1.2×10^{-4}	3.7×10^{-5}
M. anisopliae	3.9×10^{-2}	1.4×10^{-4}	3.9×10^{-5}

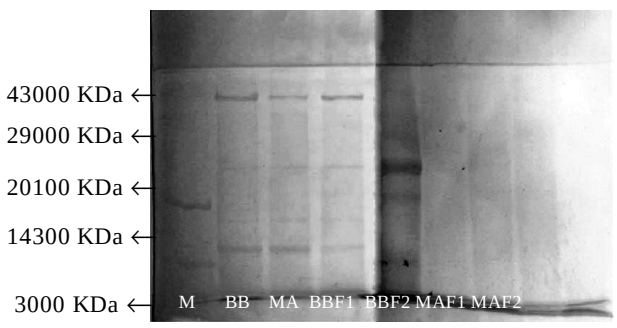

Figure 2. SDS - PAGE profile of *D. cingulatus* treated with fungal spores and the fractions

DISCUSSION

The development of pest control measures using microorganisms especially entomopathogens has attracted widespread attention in recent years. Fungi have considerable epizootic potential and can spread quickly through an insect population and cause its collapse. Because fungi penetrate the insect's body, they can infect sucking insects such as aphids and whiteflies that are not susceptible to bacterial and virus attacks. Our study reveals that two tested funguses are having the capacity to kill another sucking pest *D. cingulatus* (Borgio and Sahayaraj, 2007). However, recent scenario is to find out the insecticidal compounds form microorganism (Dowd *et al*., 1992; Essien, 2004). The mycopesticides based on deuteromycetous fungi *B. bassiana* is a common soil borne fungus that occurs worldwide and has been reported as a suppressive agent for several insect pests (Borisov and Serebrov, 2001) particularly pests having sucking type of mouthparts.

In the present study the metabolites of *B. bassiana* fraction 2 (BBF2) interfere with the digestive process, and the pest undergoes starvation, as a result the weight was reduced to 33.34 per cent when compared to the control. Simple mechanism proposed earlier that fungal metabolites can bind to a hydrophobic site remote from the catalytic site (Cheng and Ling, 1991). Weight reduction might be due the interference of these metabolic products affecting gut physiology events (*ie*., ion transport), reduce the palatability of the cotton seed to suck leading to the starvation of *D. cingulatus*, causing more body reduction which leads to death. It is essential to isolate and identify the chemical structure of these metabolic products and to integrate them in sucking pests management. Between the two digestive enzymes, crude fungal metabolites interfere more with the protease enzymes of *D. cingulatus* than with amylase. However, detoxication enzyme level was not much affected by the fungal metabolites, as observed by the action of botanicals in lepidopteran larvae of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) (Sahayaraj and Nirupa Antony, 2006).

The results revealed that the insect death mainly occurred at the time of moulting. We recorded different kinds of deformities like curling of wings, deformities in legs, retarded growth of the body, and haemolymph and/or body fluid oozing out from the abdomen. These changes affect further growth of the pests. Cadavers also showed the fungal hyphal growth of *B. basiana*. Although the exact mode of toxicity was not determined in the present study, earlier investigation have shown that fungi, particularly the aspergillus possess the ability to elaborate harmful metabolites which can induce acute and chronic mortality in insects (Dowd *et al*., 1992). This study also indicated that first fraction of *Beauveria bassiana* and *Metarhisium anisopliae* showed more impact than the second fractions. Hence these first fractions can be used for the sucking pest management either alone or in combination.

B. bassiana and *M. anisopliae* have the potential use as biological control agents against insect pests because they were relatively safe on non target insects, such as natural enemies and beneficial soil insects.

REFERENCES

Arnold, A. E. and Lewis, L. C. 2005. Ecology and evolution of fungal endophytes, and their roles against insects, **In:** (Vega F. E., Blackwell, M. eds), Insect–Fungal Associations: Ecology and Evolution. Oxford University Press, New York, 74–96 **PP**.

Bern field, P. 1955. Amylase alpha and beta. **In:** (Colowick, S. P., Kalpan, N. O. eds.). *Methods in Enzymology*, Academic Press, New York, Vol. 1. 149 - 151**PP.**

Borgio, J. F. and K. Sahayaraj. 2007 Bioefficacy of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) on *Dysdercus cingulatus* (Fab.) (Hemiptera:

Pyrrhocoridae) eggs. **In:** Proceeding of the National Seminar on Technology and Management of Bioresearches (Narayanan, M., Sethuramalingam, T. and Sahayaraj, K. eds.) 29 **PP**.

Borisov, B. A. and Serebrov, V. V. 2001. Entomopathogenic fungi. Ascomycota and Deuteromycota. **In :** *Insect Pathogens - Structural and Functional Aspects*, (Glupov. eds.) Moscow, 352 – 427.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Annals of Biochemistry*, **72:** 248 – 254.

Cheng J. W. and Ling K. H. 1991. Territrem, an inhibitor of acetyl cholinesterase. In ROC – Japan seminar on Mycotoxins. *National Taiwan University. Tapei*. **24** : 22-23.

David, B.V. and Ananthakrishnan T. N. 2004 General and Applied Entomology. Tata Mc Graw - Hill publishing company Limited, New Delhi.

Dias, B. A., Neves, P. M. O. J., Furlancto – Maia, L. and Furlaneto, M. C. 2008. Cuticle – degrading proteases produced by to entomopathogenic fungus *Beauvaria bassiana* in the presence of coffee berry borer cuticle. *Brazilian Journal of Microbiology*, **39** : 39-306.

Dowd, P. F., Peng, F. C., Chen J. W. and Lingka 1992. Toxicity and anticholinesterase activity of the fungal metabolites territrems to the corn earworm, *Helicoverpa zea*. *Entomology Experimental Application*, **65**: 57-64.

El-Mandarawy, M. B. R. 2005. Efficacy of entomopa thogenic mematodes against potato Lubermoth *Pathorimaeae operculata* (Zella) (Lepidopliae: Gelechiidae) *Egt. German Society of Zoology Entomology*, **46E** : 93 – 104.

Essien, J. P. 2004. Insecticidal potential of an orally Administered Metabolic Extract of *Aspergillus niger* on *Chrysomya chloropyga* (Green bottle fly) larvae., *Applied Science Environmental management*, **8 :** 45 – 48

Freeman, P. 1947 A revision of the genous *Dysdercus boisdual* (Hemipera) excluding the American species *Royal Entomological Society, London*, **98**: 373-424

Laemmli, U. K. 1970. Cleavage of structural proteins during the assemble of the head of bacteriophage T_4. *Nature*, **227:** 680 - 685.

Sabbahi, R. I., Merzouki. A. and Guertin C. 2008. Efficacy of *Beauvaria bassiana* against to the tarnished plant bug, *Lygus lineolaris* L. in Strawberries. *Journal of Applied Entomology*, **132**: 124- 134.

Sahayaraj, K. and Ilayaraja, R. 2008. Ecology of *Dysdercus cingulatus* (Fab.). *Egyptian Journal of Biology*, **10**: 122 – 125.

Sahayaraj, K. and Nirupa Antony. 2006. Impact of five plant extracts on the digestive and detoxification enzymes of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Hexapoda*, **13** (1 & 2) : 53 - 57.

Serebrov, V.V., Maljarchuk, A. A. and Shternshis, M. V. 2007. Spontaneus variability of *Metarhizium anisopliae* (Metsch.) Sor. Strains as an approach for enhancement of insecticidal activity. *Plant Science*, **44**: 236-239

Uthamasamy, S., Kannan, M., Mohan, S. 2004. Impact of insecticides on sucking pests and natural enemy complex of transgenic cotton in India. *Current Science*, **86**: 726-729.

K. Sahayaraj* and Majesh Tomson

Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St.Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India, Phone: + 91 462 4264376, Fax: + 91 462 256 1765, E- mail: ksraj42@gmail.com