Pakistan J. Zool., vol. 44 (3), pp. 753-758, 2012.

Prevalence and Effectiveness of *Metarhizium anisopliae* Against *Spodoptera exigua* (Lepidoptera: Noctuidae) in Southern Punjab, Pakistan

Shoaib Freed*, Mushtaq Ahmad Saleem, M. B. Khan and Muhammad Naeem
University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan

Abstract.- Entomopathogenic fungi are an efficient tool for the biological control of a wide range of insect pests particularly related to agricultural crops. The soils from diverse sources (agricultural crops, vegetables, orchards and plantation) were collected from different locations of Multan and Bahawalpur Districts for the isolation of different insect pathogenic fungi by using semi selective medium. The soils from the cotton fields harbor more number of *Metarhizium anisopliae* than other soil sources with recovery percentage of 18.12 and 7.5 for *Isaria fumosorosea*. The efficiency of *M. anisopliae* was evaluated against 3rd instar larvae of *Spodoptera exigua* in laboratory and semi field/green house conditions. Fungal isolate Ma002 proved to be more effective both in laboratory and semi field conditions showing 87.5 and 81.25% mortality of *Spodoptera exigua*. The presence of these fungi in the soils of different fields requires further work to be done for their efficacy against different insect pests and particularly Ma002 for finding the characters that make it different from the other isolates from same place or geographical origins.

Keywords: *Metarhizium*, insect pathogenic fungi, armyworm, biocontrol agents, destruxins, *Spodoptera exigua.*

INTRODUCTION

Insect pathogenic fungi embrace a distinctive place in microbial pathogenesis. Natural epizootics of diseases caused by fungi are rather frequent in insects, and their impact on insects exposes the potential of microbial pest management (Carruthers and Soper, 1987). This information was documented in the latter part of the 19th century and concluded in the different endeavors to utilize the fungus *Metarhizium anisopliae* for insect management (Gillespie, 1988). Regardless of these and other early achievements, imitated chemical insecticides have been the bastion of insect pest management for existence. However, the preamble of insecticide resistance and concern over the ecological effect of different inputs focuses consideration on biologically based pest control approaches.

Metarhizium is one of the finest known genera of insect pathogenic fungi, commonly known as "green muscardine fungus". The genus *Metarhizium* presently includes several morphologically distinctive species including *M. album*, *M. anisopliae*, *M. flavoviride*, *M. cylindrosporae*, *M. guizhouense* and *M. pingshaense* (Driver *et al*., 2000; Guo *et al*., 1986; Rath *et al*., 1995), that cause diseases in different insect species and are marketed as bio-control agents (Gilliespie and Claydon, 1989).

Fungi are exclusive among the entomopathogenic microbes as these needs not ingestion and can assault their prey directly all the way through the skin. Therefore, they can also transmit disease to immobile stages of insects. The incursion is often through mouthparts, non-sclerotised cuticle at intersegrnental folds or from spiracles, where elevated moisture encourages germination and incursion (Hajek and St. Leger, 1994). The insect pathogenic fungus *M. anisopliae* generates peptide toxins *i.e*., destruxins (DTX). Up to now about thirty toxins have been spotted in different media of this fungus; all include five amino acids and a hydroxyl acid (Suzuki *et al*., 1970; Pais *et al*., 1981). Destruxins are secreted throughout dynamic fungal development (Amiri-Besheli *et al*., 2000) and destruxins A, E and B be likely to prevail in the medium. Destruxins have also been described from *Aschersonia* sp. (Krasnoff *et al*., 1996). Introduction of DTX into larvae of lepidoptera and adults of Diptera roots instantaneous muscular paralysis, subsequent flaccidity; insects recuperate from little doses whereas elevated doses

* Corresponding author: shbfkh@gmail.com or sfareed@bzu.edu.pk
0030-9923/2012/0003-0753 $ 8.00/0

are deadly (Samuels, 1998).

Biocontrol offers a striking substitute or addition to the application of insecticides in crop security. Mainly because they are safer for plants, animals and the environment relative to conventional pesticides (Khetan, 2001). Furthermore, such fungi have also been evaluated as biological control agents for more than 200 economically important insect species (Tanada and Kaya, 1993; Maurer *et al.*, 1997; Zurek and Keddie, 2000). Fungal biological control plays a significant part in agricultural and horticultural insect pest control (Lacey and Goettel, 1995). Presently, sixty six products representing at least thirty eight varied species of fungi have been developed or are being developed for the management of insect pests of different crops (Liu and Li, 2004). Insect pathogenic fungi propose an ecosystem affable substitute to chemicals, though their utilization is inadequate chiefly owing to the comparatively slow speed of killing the insect pests (St. Leger *et al.*, 1996). *M. anisopliae* and *I. fumosorosea* are at present being assessed as vital insect pathogenic fungi.

Keeping in view all the problems associated with control of lepidopterous and related pests and for the selection of a proficient strain for use in the field as an effective microbial control agent, an experiment was planned with the following objectives. To isolate *M. anisopliae* and *I. fumosorosea* from soils of different locations of Southern Punjab (Multan and Bahawalpur) and to check their efficacy against *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) on cauliflower under laboratory and semi field conditions.

MATERIALS AND METHODS

Sampling sites

The soil was sampled from diverse habitats including, agricultural (different crop fields) and forest/vegetation from different locations of Southern Punjab (Multan and Bahawalpur). Three soil samples from each field were taken from 10 cm deep under the earth from different areas of the above described locations. Then these samples were mixed together into a single sample. After the collection of the samples, these were preserved at 4°C until used for isolation of insect pathogenic fungi.

Screening of soil samples for pathogenic fungi

The soil samples collected from diverse sites were used for the isolation of *M. anisopliae* and *I. fumosorosea* on semi-selective medium depicted by Veen and Ferron (1966) and Freed *et al.* (2011a). The medium comprised glucose (10 g), peptone (10 g), bile (15 g) (Sigma), rose bengal and agar (30 g) in 1L of water. The medium was altered with 0.002 gL^{-1} dodine (American Cyanamid), 0.12 gL^{-1} cycloheximide **(**BDH**)** and 0.25 gL^{-1} chloramphenicol **(**BDH**).** A 10 g soil sample was put into sterile water (90 ml) and was mixed by a magnetic stirrer. The product was mixed three times with phosphate buffer (100 mM, pH7.0), 100 µl from every sample was spread on the media, and the plates were kept at 25°C in dark conditions. Cultures were inspected for seven days. Distinctive conidia of *Metarhizium* and *I. fumosorosea* were shifted onto PDA plates; the fungi were purified, and inspected under microscope for qualitative conidiogenesis.

The entire isolates were afterward reproduced from a single conidium. In order to carry out this, 100 µl of aliquot having a little concentration of conidia were spread on PDA plates. After 24 h of growth, a solitary conidium was spotted by using a dissecting microscope (20 times magnification), a part of the media surrounding only the marked conidium was detached and shifted onto PDA plates. After five days, morphological characters of *Metarhizium* and *I. fumosorosea* were verified by microscope, and the conidia were harvested and preserved in sterile 30% glycerol.

Insects

The larvae of *S. exigua* were collected from field and brought to laboratory. These larvae were reared in glass vials on cauliflower leaves under controlled laboratory conditions. The pupae were collected from the rearing glass vials on alternate days and kept in a plastic jar. Moths emerged from the pupae were shifted into glass jars with 1:1 male and female ratio. Adults were fed on 10% honey solution. Thirty pairs of moth were kept in one glass jar and tissue papers hanged to serve as egg laying substrate.

Laboratory bioassay

Ten different fungal isolates of *M. anisopliae*

from different locations (Table I) were used to check their efficiency against 3rd instar larvae of *S. exigua*. The conidia of each fungal isolates were harvested from the 7 days old cultures with 0.03% Tween 80 solutions and filtered by using a cheese cloth to eliminate conidial masses and hyphal remains. The conidial suspensions were altered to 1 x 10^8 conidia mL^{-1}. Forty 3rd instar larvae of *S. exigua* were dipped in the fungal suspensions. After dipping the larvae were allowed to move on the filter paper and each larva was shifted into the replicate petri dishes containing the cauliflower leaf discs. The petri dishes were fastened with parafilm and incubated at 25ºC. Cauliflower leaf discs and filter papers were changed after every 24 h. The larval mortality was recorded over seven days.

Table I.- *M. anisopliae* isolates from different locations.

S. No.	*M. anisopliae* isolates	Origin	Source
1	Ma002	Makhdoom Rasheed, Multan	Cotton field
2.	Ma010	Band Bosan, Multan	Cotton field
3.	Ma012	B.Z.U. Farm, Multan	Cotton field
4.	Ma017	Nag Shah, Multan	Okra field
5.	Ma018	Aadhi Bagh, Multan	Cotton field
6.	Ma022	Kotli Nijabat, Shujaabad	Cotton field
7.	Ma024	Raja Ram, Shujaabad	Vegetable field
8.	Ma028	Rukan Hati (Bait), Shujaabad	Cotton field
9.	Ma031	Musafir Khana, Bahawalpur	Sugarcane field
10.	Ma033	Sama Satta, Bahawalpur	Cotton Field

Semi field/green house trials

Semi field trials were conducted on cauliflower plants for the control of *S. exigua*. The plants were grown in semifield/green house conditions. For this purpose fungal suspensions were prepared by scraping the conidia of isolates (Ma002, Ma018, Ma024 and Ma033) from the petri dishes into the Tween 80 (0.05%). The conidial suspension was shaken well for the proper mixing of the conidia. After that this suspension was sieved for the debris and conidial clumps. The fungal suspension at a concentration of 1x10^8 conidia mL^{-1} was sprayed onto the cauliflower plants. There were five treatments including control and each treatment was replicated four times. The data of mortality of the larvae was recorded continuously for seven days and calculated according to Abbot (1925). An Excel 2000 software (Microsoft, Seattle, USA) was employed to analyse data.

RESULTS

Prevalence of fungi

Multan is the District of Southern Punjab famous for its orchards and taken as the centre for the cotton and wheat production. Different locations of Multan, Makdoom Rasheed, Band Bosan, B. Z. University Agri. Farm, Tawakal Town, Nag Shah and Aadhi Bagh, Shujaabad etc. were sampled. The soil samples were taken from a wide range of soils including (Orchards, crop fields, vegetables, urban areas, vegetations). Out of total 160 samples taken from different sites of Multan and Shujaabad, 29 isolates of *M. anisopliae* were recovered, while 12 isolates of *I. fumosorosea* were isolated (Fig. 1). The soils from the cotton fields harbor more number of *M. anisopliae* than other soil sources. To check the prevalence of insect pathogenic fungi in Bahawalpur 80 soil samples were collected from different locations (Musafir Khana, Khanka Shareef, Noshehra Jadeed, Islamia University Agri. Farm, Sama satta, Kilanj wala etc.). Out of these soil samples only nine isolates of *M. anisopliae* and one of *I. fumosorosea* were recovered.

Mortality of 3rd instar larvae of S. exigua

Laboratory conditions

The chronic toxicity (mortality within 7 days) associated with fungal isolates applied on cauliflower leaf discs was assessed. The toxicity of the ten fungal isolates of *M. anisopliae* was evaluated against third instar larvae of *S. exigua* via oral administration. There was a significant effect of treatment with the addition of spores on the leaf discs with the mortality of the insect ranging from 3.12% to 87.5%, compared with the control treatment that showed only 3.12 percent mortality (Fig. 2). Among the isolates of *M. ansiopliae*, four isolates (Ma002, Ma024, Ma033, Ma018 and Ma010) showed significant oral toxicity to *S. exigua* larvae with a mortality percentage of 87.5, 84.37, 78.12, 75.00 and 71.87 after seven days of fungal application. Notably the most active fungal isolate was Ma002, which showed 87.5% mortality in

cauliflower leaf discs assay. The isolates Ma012, Ma017, Ma022, Ma028 and Ma031 showed somewhat same percent mortality *i.e.*, 56.25, 43.75 and 68.75, 43.75 and 40.62% after seven days of fungal treatment (Fig. 2).

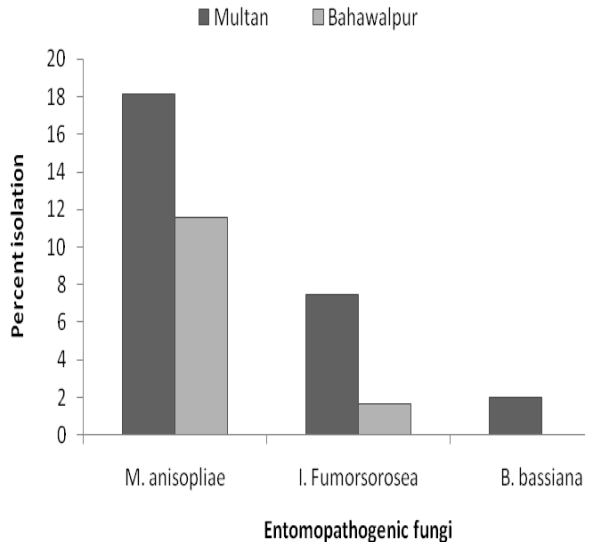

Fig. 1. Prevalence of entomopathogenic fungi in different locations.

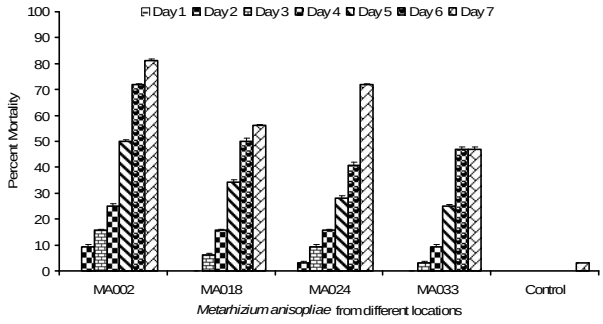

Fig. 2. Mortality of 3rd instar larvae of *S. exigua* induced by *M. anisopliae* ($1x10^8$ conidia/ml) under laboratory conditions. Means (±SE) of five replicates are presented.

Green house/semi field conditions

The chronic toxicity (mortality within 7 days) associated with fungal isolates applied on cauliflower leaves were assessed under semi field conditions. The toxicity of four laboratory assessed fungal isolates of *M. anisopliae* (Ma002, Ma018, Ma024 and Ma033) that had given promisive percent mortality in lab. bioassay were evaluated against 3rd instar larvae of *S. exigua* via oral administration on cauliflower plants under semi field conditions. There was a noteworthy effect of treatment with the addition of spores (1×10^8 conidia mL^{-1}) on the leaves with percent mortality varied from 3.12 to 81.25, compared with the control treatment that showed only diminutive percent mortality of 3.12 (Fig. 3). Among the four isolates of *M. anisopliae* tested Ma002 showed considerable oral toxicity to *S. exigua* larvae with a maximum percent mortality of 81.25 after seven days of treatment. The isolate Ma024 was also potent as it showed 71.87 % mortality of the tested larvae after seven days of treatment. Minimum mortality was recorded in Ma033 which showed 46.87% mortality after seven days of fungal treatment (Fig. 3).

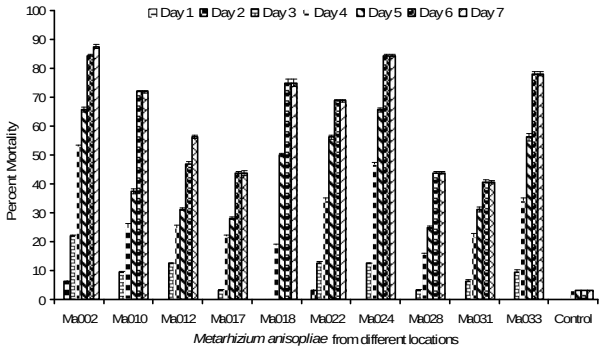

Fig. 3. Mortality of 3rd instar larvae of *S. exigua* induced by *M. anisopliae* ($1x10^8$ conidia/ml) under semi-field conditions. Means (±SE) of five replicates are presented.

DISCUSSION

A detailed study of insect pathogenic fungi in the agro-ecological soil environment of different Districts (Multan and Bahawalpur) was performed. *M. anisopliae* is an insect pathogenic fungus with cosmopolitan nature. It has been screened from a range of environments *i.e.*, agricultural, forest and urban soils from diverse ecological sources (St. Leger *et al.*, 1992). From our results it is apparent that this fungus exists in different regions of Multan and Bahawalpur (Fig. 1), as the isolates of *M. anisopliae* were recovered from different soil sources of these Districts including agricultural and forest/vegetation. As the frequency of occurrence of

the insect pathogenic fungi is concerned, the maximum frequency was observed in Multan as compared to other locations. The frequency of the entomopathogenic fungi were varying from different latitude and altitude as experienced by Sun and Liu (2008) in China. The same was done by Freed *et al.* (2011a, b) who examined the incidence and inherent variation of the entomopathogenic fungi *M. anisopliae* and *I. fumosorosea* in Asian and a European country. The fungus prevailed extensively in agricultural and forest soils all through China particularly in the south and the south western provinces, with a utmost incidence of 81.6 %, whereas in Laos it was found profusely in just forest soils, the Netherlands also seemed to be an excellent source of pathogenic fungi showing a noteworthy prevalence. The areas for the isolation of the current study comes under which have usually high temperature with less rain fall, Under dry situations, the fungi may carry on in the hyphal phase, but not succeed in creating conidia on the exterior of the corpse, however below 10°C and over 35°C no sporulation happen. The most favorable temperature for sporulation is 25-30°C (Sun *et al.*, 2003) and in these areas the practice of mulching is not performed, that has antagonistic affect on the insect pathogenic fungi due to increased amount of organic matter in the soil (Fargues and Roberts, 1985; Studdert and Kaya, 1990).

Insect behavior may also effect the dispersal of entomopathogenic fungi in the soil ecosystem as the fungal conidia are spread by the insect activities, by minimizing the plant to plant distances can create mulch on the soil, which can in turn minimize the population of pests while these conditions become favorable for the insects living under the soil. (Schmidt *et al.*, 2004).

The second aspect of the study shows the effectiveness of *M. anisopliae* against 3rd instar larvae of *S. exigua*. Ten isolates of *M. anisopliae* from different locations (Multan and Bahawalpur Districts) were examined for their outcomes against *S. exigua* under laboratory and semi field/green house conditions. Fungal isolate Ma002 proved to be more effective both in laboratory and semi field conditions showing 87.5 and 81.25% mortality of *S. exigua* (Figs. 1, 2). As accounted by the preceding studies that *M. anisopliae* and its secondary metabolites are effective against lepidopteron and coleopterans (Pedras *et al.,* 2002; Quesda-Moraga *et al.,* 2006). Kershaw *et al.* (1999) described that all the isolates of *Metarhizium*, whether pathogenic or non-pathogenic, evoked melanization of the cuticle, and the growth from of the fungus in the haemolymph was clearly associated with the virulence towards the lepidopterous insect pests. The more virulent isolates tended to grow more profusely then the less virulent isolates. Our findings are in confirmation with the finding of Sabbour and Sahab (2005) and Yoon *et al.* (1999) who reported that the entomopathogenic fungi *M. anisopliae* and *B. bassiana* can be used as microbial control agent of the cabbage insect pest in green house as well as in field conditions. From our results we conclude that *Metarhizium* prevails in different areas of Multan and Bahawalpur and the isolate Ma002 contains some characters that can make it suitable to be effective against *S. exigua*. However more work is needed to be done for its efficiency and the characters that make it different from the other isolates from same place or geographical origins.

ACKNOWLEDGMENT

This research was funded by the Higher Education Commission, Islamabad, Pakistan.

REFERENCES

ABBOT, W.S., 1925. A method for computing the effectiveness of insecticides. *J. econ. Ent*., **18**:265-267.

AMRI-BESHELI, B., KHAMBAY, B., CAMERON, S., DEADMAN, M.L. AND BUTT, T.M., 2000. Inter- and intra-specific variation in destruxin production by insect pathogenic *Metarhizium* spp., and its significance to pathogenesis. *Mycol. Res.*, **104**: 447-452.

CARRUTHERS, R. I. AND SOPER, R. S., 1987. Fungal diseases. In: *Epizootiology of Insect Diseases* (eds. J. R. Fuxa and Y. Tanada). Wiley, New York. pp. 357-416.

DRIVER, F., MILNER, R. J. AND TRUEMAN, W. H., 2000. A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. *Mycol. Res.*, **104**:134-150.

FARGUES, J. AND ROBERT, P. H., 1985. Persistence of conidia of 4 entomopathogenic Hyphomycetes in soil, *Beauveria bassiana* (Bals) Vuill *Metarhizium anisopliae* (Metsch) Sor, *Nomuraea rileyi* (F) Samson and *Paecilomyces fumosoroseus* Wize, in controlled

conditions. *Agronomie*, **5**: 73-80.

FREED, S., JIN, F. L. AND REN, S. X., 2011a. Determination of genetic variability among the isolates of *Metarhizium anisopliae* var. *anisopliae* from different geographical origins. *World J. Microbiol. Biotechnol.*, **27**: 359-370.

FREED, S., JIN, F. L. AND REN, S. X., 2011b. Phylogenetics of entomopathogenic fungi isolated from the soils of different ecosystems. *Pakistan J. Zool.*, **43**: 417-425

GILLESPIE, A. T. AND CLAYDON, N., 1989. The use of entomopathogenic fungi for pest control and the role of toxins in pathogenesis. *Pestic. Sci*., **27**: 203-215.

GILLESPIE, A. T., 1988. Use of fungi to control pests of agricultural importance. In: *Fungi in biological control systems* (ed. M. N. Burge). Manchester University Press, Manchester, UK. pp. 37-60.

GUO, H. L., YE, B. L., YUE, Y.Y., CHEN, Q. T. AND FU, C.S., 1986. Three new species of *Metarhizium. Acta Mycol. Sin*., **5**: 185-190.

HAJEK, A.E. AND ST. LEGER, R.J., 1994. Interactions between fungal pathogens and insect hosts. *Annu. Rev. Ent.*, **39**: 293- 322.

KERSHAW, M. J., MOORHOUSE, E. R., BATEMAN, R., REYNOLDS, S. E. AND CHARNLEY, A.K., 1999. The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insect. *J. Inverteb. Pathol.*, **74**: 213-223.

KHETAN, S.K., 2001. *Microbial insect control*. Marcel Dekker, Inc., New York. pp. 167-190.

KRASNOFF, S. B., GIBSON, D. M., BELOFSKY, K. G. N., GLOER, B. AND GLOER, J. B., 1996. New destruxins from the entomopathogenic fungus *Aschersonia*, sp. *J. Nat. Prod.*, **59**: 485-489.

LACEY, L. A. AND GOETTEL, M. S., 1995. Current developments in microbial control of insect pests and prospects for the early 21st century. *Entomophaga*, **40**:1-25

LIU, X. Z. AND LI, S. D., 2004. Fungal secondary metabolites in biological control of crop pests. In: *Handbook of industrial mycology* (eds. Z.Q. An). Dekker, New York. pp. 723-747.

MAURER, P., COUTEAUDIER, Y., GIRARD, P.A., BRIDGE, P.D. AND RIBA, G., 1997. Genetic diversity of *Beauveria bassiana* and relatedness to host insect range. *Mycol. Res.*, **101:**159-164.

PAIS, M., DAS, B.C. AND FERRON, P., 1981. Depsipeptides from *Metarhizium anisopliae. Phytochemist*,**20**:715-723

PEDRAS, M.S.C., ZAHARIA, I. L. AND WARD, D. E., 2002. The destruxins: synthesis, biosynthesis, biotransformation, and biological activity. *Phytochemistry*, **59**: 579-596.

QUESADA-MORAGA, E., CARRASCO-DIAZ, J. A. AND SANTIAGO-ALVAREZ, C., 2006. Insecticidal and antifeedant activities of proteins secreted by entomopathogenic fungi against *Spodoptera littoralis* (Lep., Noctuidae*). J. appl. Ent.*, **130**: 442-452.

RATH, A.C., CARR, C.J. AND GRAHAM, B.R., 1995. Characterization of *Metarhizium anisopliae* strains carbohydrate utilization (AP150CH). *J. Inverteb. Pathol.*, **65**: 152-161.

SABBOUR, M.M. AND SAHAB, A.F., 2005. Efficiency of some microbial control agents against cabbage insect pests in Egypt. *Pakistan J. biol. Sci.*, **8**: 1351-1356.

SAMUELS, R.I., 1998. A sensitive bioassay for destruxins, cyclodepsipeptides from the culture filtrates of the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorok. *Anas. Soc. Ent. Brasil*., **27**: 229-235.

SCHMIDT, M. H., THEWES, U., THIES, C. AND CHARNTKE, T., 2004. Aphid suppression by natural enemies in mulched cereals. *Ent. exp. Appl*., **113**: 87-93.

ST. LEGER, R. J., JOSHI, L., BIDOCHKA, M. J., RIZZO, N. W. AND ROBERTS, D.W., 1996. Chemical characterization and ultrastructural localization of two extracellular trypsins produced by *Metarhizium anisopliae* in infected insect cuticles. *Appl. enviorn. Microbiol*., **62**: 1257-1264

ST. LEGER, R. J., MAY, B., ALLEE, L. L., FRANK, D. C., STAPLES, R. C. AND ROBERTS, D. W., 1992. Genetic differences in allozymes and in formation of infection structures among isolates of the entomopathogenic fungus *Metarhizium anisopliae. J. Inverteb. Pathol*., **60**: 89-101.

STUDDERT, J. P. AND KAYA, H. K., 1990. Water potential, temperature, and claycoating of *Beauveria bassiana* conidia effect on *Spodoptera exigua* pupal mortality in 2 soil types. *J. Inverteb. Pathol*., **56**: 327-336.

SUN, B. D. AND LIU, X. Z., 2008. Occurrence and diversity of insect-associated fungi in natural soils in China. *Appl. Soil Ecol*., **39**:100-108.

SUN, J., JAMES, R. F. AND GREGG, H., 2003. Effects of virulence, sporulation, and temperature on *Metarhizium anisopliae* and *Beauveria bassiana* laboratory transmission in *Coptotermes formosanus. J. Inverteb. Pathol.*, **84**:38-46.

SUZUKI, A., TAGUCHI, H. AND TAMURA, S., 1970. Isolation and structure elucidation of three new insecticidal cyclodepsipeptides, destruxins C and D and desmethyl destruxin B produced by *Metarhizium anisopliae. Agric. BioChemist.*, **34**: 813-816.

TANADA, Y. AND KAYA, H. K., 1993. *Insect pathology*. Academic Press, San Diego.

VEEN, K.H. AND FERRON, P., 1966. A selective medium for the isolation of *Beauveria tenella* and of *Metarhizium anisopliae. J. Inverteb. Pathol.*, **8**:268-269.

YOON, C.S., SUNG, G.H., PARK, H.S., LEE, S.G. AND LEE, J. O., 1999. Potential of the entomopathogenic fungus, *Beauveria bassiana* strain CS-1 as a biological control agent of *Plutella xylostella* (Lep., Yponomeutidae). *J. appl. Ent.*, **123**:423-425.

ZUREK, L. AND KEDDIE, B.A., 2000. *Beauveria bassiana* (Balsamo) Vuillemin, a promising microbial control agent of the satin moth (Lepidoptera: Lymantriidae). *Biocont. Sci. Technol*., **10**: 641-644.

(Received 19 September 2011, revised 29 November 2011)