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PATHOGENICITY OF INDIAN ISOLATES OF ENTOMOPATHOGENIC FUNGI AGAINST IMPORTANT INSECT PESTS AND NATURAL ENEMIES

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ABSTRACT

Entomopathogenic fungi (EPF) are one of the best alternatives to chemical pesticides and crucial component of IPM. Eight isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin, were obtained from Indian Type Culture Collection (ITCC) and National Centre of Integrated Pest Management, New Delhi. They were screened against adults of, *Bactrocera dorsalis,* larvae of *Corcyra cephalonica* (Stainton) and *Spodoptera litura* (Fab). By exposing the insects to 2-3 weeks old culture of (EPF) The pathogenicity of four isolates was proved, using contact method. Three isolates ITCC No. 6628, ITCC No. 6645 and B. NCIPM were found pathogenic to fruit flies; whereas, in case of *C. cephalonica,* pathogenicity of first two isolates in addition to (M. NCIPM) was proved. Mortality of adult fruit flies was 100% within 5-6 days of exposure, however, in case *C. cephalonica* (31-98%) within three weeks. Subsequently the pathogenic isolates were tested against *Coccinella septumpunctata* (L.) and *C. cephalonica.* Significant differences were observed among isolates, and the isolate B. NCIPM was found relatively safer to *C. septumpunctata.*

Key words: Biological control, entomopathogenic fungi, *Beauveria bassiana*, natural enemies, *Bactrocera dorsalis*, *Coccinella septumpunctata* and *Corcyra cephalonica.*

As an alternative to chemical control or as part of IPM programs, there is a resurgence of interest in the use of microbial insecticides for biological control of insect pests. Fungal agents are among the most promising group of biological control agents against insect pests (Reithinger *et al.,* 1997). Over 500 species of fungi are known to have insect pathogenic properties. Interestingly, *Beauveria* and *Metarhizium* (Deuteromycotina, Hyphomycetes) represent the most frequently used genera (Burges and Hussey, 1971) and are known to infect a broader range of insect pests of crops belonging to Lepidoptera, Homoptera, Hymenoptera, Coleoptera and Diptera. Most research on fungi has been directed to *Beauveria* and *Metarhizium* (Greathead and Prior, 1990; Whitten and Oakeshott, 1991). Entomopathogenic fungi (EPF), compared to other entomopathogenic microbial organisms can infect their host via contact i.e., invade via epicuticle of integument, and do not need to be ingested by the insect to cause infection (Goettel *et al.,* 2005; Ali *et al.,* 2010).These fungi are cosmopolitan and do not leave undesirable residues hence can be used, even close to harvest. Besides that, these are compatible with other pest management tactics. Additionally, their production is easy and economical and do not require high input technology (Prior, 1988). Commercially, *Beauveria bassiana* (Balsamo) Vuillemin and *Beauveria brongniartii* (Saccardo) are produced by more than 14 companies, and *Metarhizium (M. anisopliae* Metchnikoff) by more than 10 companies world-wide. Virulence is the most important indicator to measure the potential of fungi against pests and the basis of choosing highly virulent fungi in laboratory bioassays (Li *et al.,* 2012). Hence this study evaluates the pathogenicity of strains of *B. bassiana* and *Metarhizum anisopliae*, on some important insect pests of crops and natural enemies.

MATERIALS AND METHODS

Larvae of the Oriental fruit fly, *Bactrocera dorsalis*

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(Hendel) (Diptera: Tephritidae) were obtained from Biological Control Laboratory, Division of Entomology, Indian Agricultural Research Institute, New Delhi (IARI). The larvae were reared on ripe bananas whereas adult flies were maintained on sugar and yeast autolysate. These were kept in ventilated acrylic cages (20x20x20 cm) at 27±1^{0}C, 65±5% RH and 12:12 photoperiod. Water was supplied in vials with cotton wicks.

Rearing of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), and *Spilarctia obliqua* (Walker) (Lepidoptera; Arctiidae) was carried out as per the protocols given in Gautam (2008). *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), *Pieris brassicae* (L.) (Lepidoptera: Pieridae) and *Drosicha mangiferae* (Green)*;* Hemiptera: Monophlebidae) were collected from the IARI fields, mustard crop, cabbage crop and mango tree, respectively. *C. septempunctata* were fed with *Brevicoryne brassicae* (L.) (Homoptera: Aphididae). While *P. brassicae* and *D. mangiferae* were reared on cabbage and mango, respectively. Ten insects were used for *D. mangiferae* for each isolate. Adults of *C. septumpunctata* and full grown larvae (30 days old) of *C. cephalonica* (100 insects) were used in five replications in completly randomized design. Whereas, 50 3rd and 4th instar larvae were used for each *S. litura* and *P. brassicae*. Temperature was maintained at 27±1^{0}C and 60-65% R.H., respectively. Experiments were carried out in 2011 and 2012 with eight fungal isolates (Table 1), with six of these obtained from Indian type culture collection (ITCC), Division of Plant Pathology, IARI, New Delhi; and two from National Centre for Integrated Pest Management IARI Campus, Pusa, New Delhi-110012. The fungi were grown on potato dextrose agar (PDA) in petri dishes and maintained at 27±1^{0}C in B.O.D. incubator for 15 to 21 days.

Preliminary screening was conducted on adults of *B. dorsalis*. Petri dish without lid (15x90mm) containing two weeks old culture of isolate kept in plastic jars (13x 10cm) containing water saturated sand at 27±1°C and 85±5% R.H. Twenty adults (three replications) of *B. dorsalis* were released and provided with banana fruits. The experiment lasted for 9 days and repeated twice. For other insects these were kept in each petri dish (15x90mm) shaken for 3 min. to get full coverage by conidia powder and then transferred into small jars and given food as described in rearing method. For the data of *C. cephalonica* and *C. septumpunctata* OPSTAT statistical programme was used, in which the data were subjected to square root transformation. For the rest results were restricted to (+ve) and (-ve) which means susceptible/not susceptible respectively, however % mortality was estimated. For calculating LT_{50} values, EPA Probit Analysis Program (Version 1.5), was used.

RESULTS AND DISCUSION

The eight isolates of entomopathogenic fungi (Table 1, Fig. 1), when screened against *B. dorsalis* (4-5 days old), revealed that three were found pathogenic (Table 3), with 100% mortality within 4-6 days and the cadavers were found fully covered with white mycelium within nine days (Fig. 2). This indicated that the inoculums picked up while the adults were walking

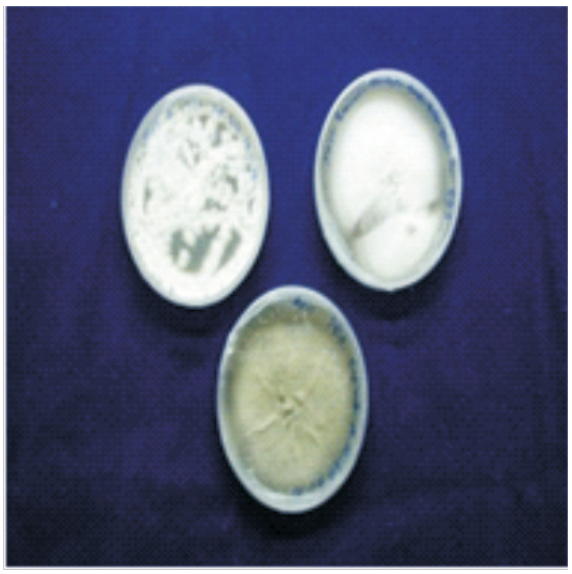

Fig. 1. Entomopathogenic cultures

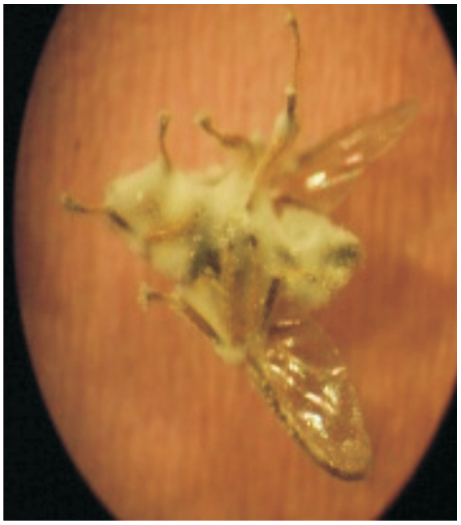

Fig. 2. *Bactrocera dorsalis*

Table 1. Details of fungal isolates tested

Isolates No	Isolate name	Accession numbe
1	*Beauveria bassiana* (Balsamo) Vuillemen	ITCC No. 6552
2	*Beauveria bassiana* (Balsamo) Vuillemen	ITCC No. 6628
3	*Beauveria bassiana* (Balsamo) Vuillemen	ITCC No. 6645
4	*Beauveria bassiana* (Balsamo) Vuillemen	ITCC No. 4512
5	*Metarrhizium anisopliae* (Metschin) Sorokin	ITCC No. 4514
6	*Metarrhizium anisopliae* (Metschin) Sorokin	ITCC No. 6377
7	*Beauveria bassiana* (Balsamo) Vuillemen	*Beauveria bassiana* N
8	*Metarrhizium anisopliae* (Metschin) Sorokin	*Metarrhizium anisopliae*

Source : Catalogue of Fungal & Bacterial Cultures 1936-2012 (VIII Edition), IARI, New Delhi

Table 2. Mortality of adult *Bactrocera dorsalis* due to entomopathogenic fungi (within 5-6 days)

Isolate accession number	Per cent mortality
ITCC No. 6552	0
ITCC No. 6628	100
ITCC No. 6645	100
ITCC No. 4512	0
ITCC No. 4514	0
ITCC No. 6377	0
B. NCIPM	100
M. NCIPM	0
Control	0

was sufficient to kill the insect within the mentioned time. These results are in accordance with Dimbi *et al.,* (2003) who found adult mortality in *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa var. fasciventris* (Karsch) treated with *B. bassiana* and *M. anisopliae.* The mycelium appeared on the abdomen as well as mouth parts and joints of the leg (Fig. 3). These results are in conformity with the study of Muñoz (2000), on 16 strains of *B. bassiana* against *C. capitata* adults. Also Quesada Moraga *et al.,* (2006) reported 30-100% mortality after 20 days, while testing 10 isolates of *B. bassiana* and five isolates of *M. anisopliae* against adult fruit fly. Sookar *et al.,* (2008) reported the pathogenicity of seven isolates of *M. anisopliae*, five isolates of *B. bassiana* and two isolates of *Paecilomyces fumosoroseus* (Wise) in adults of *Bactrocera zonata* (Saunders) and *B.cucurbitae* (Coquillett). Since the three isolates ITCC No. 6628, ITCC No. 6645 and B. NCIPM were found not pathogenic to *Drosicha mangiferae*, this proved thier seleetinity.

All isolates given in Table 1, except NCIPM isolate were tested against full grown larvae of *C. cephalonica* and found non-pathogenic. However, ITCC No. 6628, ITCC No. 6645 and M. NCIPM were virulent and registered average mortality of 17, 19.6 and 3.4, respectively (Table 4). Significant difference between treatments and control, among themselves was observed.

The symptoms observed on treated larvae were growth of white mycelium on the intersegmental parts (Fig. 4), pink to reddish colour cadavers (Fig. 5). *C. cephalonica* larvae colour indicated secretion of a metabolite called oosporein registered by infections caused by ITCC No. 6645 and 6628. These findings

Fig. 3. *Bactrocera dorsalis*

Fig. 4

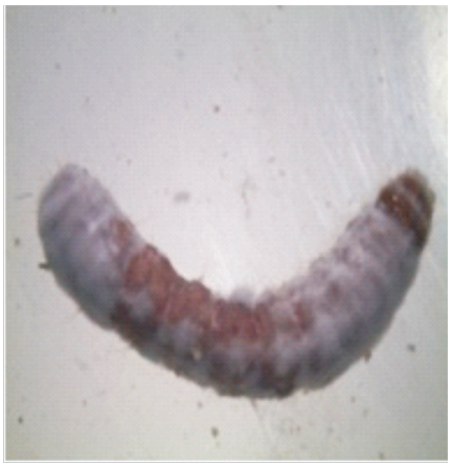

Fig. 5

Table 3. Pathogenicity and percentage mortality of entomopathogenic fungi against the tested insects

	Fungal isolates								
Insect species	ITCC No. 6552	ITCC No. 6628	ITCC No. 6645	ITCC No. 4512	ITCC No. 4514	ITCC No. 6377	M. NCIPM	B. NCIPM	mortality %
Bactrocera dorsalis	(-ve)	(+ve	(+ve	(-ve)	(-ve)	(-ve)	(-ve)	(+ve)	100
Corcyra cephalonica	(-ve)	(+ve)	(+ve)	(-ve)	(-ve)	(-ve)	(+ve)	——-	31-98
Coccinella septempunctata	——-	(+ve)	(+ve)	——-	——-	——-	——	——-	38-100
Spilosoma obliqua	——-	(+ve)	(+ve)	——-	——-	——-	——	———	30-50
Spodoptera litura	(-ve)	(+ve)	(+ve)	(-ve)	(-ve)	(-ve)	——	(+ve)	60
Pieris brassicae	——-	(+ve)	(+ve)	——-	——-	——-	——	——-	60
Drosicha mangiferae	——-	(-ve)	(-ve)	——-	——-	——-	——	(-ve)	0

*M. NCIPM= *Metarhizium anisopliae* B.NCIPM= *Beauveria bassiana* = not tested

Table 4 Average mortality of *Corcyra cephalonica* and *Coccinella septumpunctata*

Treatments	*Corcyra cephalonica*	*Coccinella septumpunctata*
	Average mortality	
ITCC No. 6628	17.0 (4.18)	20.0(4.4)
ITCC No. 6645	19.6 (4.48)	19.0 (4.3)
M. NCIPM	3.4 (1.94)	---
B. NCIPM	---	7.6(2.7)
CONTROL	1.8 (1.41)	2.2(1.4)
C.D.	(0.486)	(0.463)
SE(m)	(0.161)	(0.153)
SE(d)	(0.227)	(0.217)
C.V.	(11.542)	(9.999)

*Figures in parentheses are square root transformed values

Table 5. LT_{50} values of entomopathogenic fungi isolates against *Corcyra cephalonica*

Strains	Heterogenity	Regression equation	LT_{50}	Fiduciallimits	
ITCC No. 6628	2.162	Y=-7.083380 + 4.944646x	11.57 days	10.785	12.329
ITCC No. 6645	4.674	Y=-5.292781+ 4.562572x	7.51 days	7.51	6.785
M. NCIPM	6.193	Y=-17.107702 + 8.064309x	22.97 days	20.50	37.967

are in agreement with Zimmermann (2007), wherein presence of a major secondary metabolite dihydroxybenzoquinone, an extracellular secretion of, many isolates of *B. bassiana* was indicated. Samodra and Ibrahim (2006) noted up to 90% *C. cephalonica* larval mortality within 15 days when treated with isolates of formulated *B. bassiana*. Rice (1999) reported an isolate of *B. bassiana* to be pathogenic to adults of *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). Khashaveh *et al.,* (2011), reported the potential of a commercial product based on *B. bassiana* against adults of *S. granarius* and *Oryzaephilus surinamensis* and *T. castaneum*.

Results of the efficacy of three isolates of *B. bassiana* on *C. septumpunctata* and *C. cephalonica* are presented in Table 4. The highest average mortality was 20, 19 and 7.6 for the isolates ITCC No. 6628, ITCC No. 6645 and B. NCIPM, respectively. The latter showed minimum mortality which reflects its safety to *C. septumpunctata*. This is in accordance with Zimmerman, (2007) where he stated that host specificity is a strain-specific trait. Efficacy of virulent isolates was also obseved on *S. litura* (Fig. 6) and *Spilarctia obliqua* (Fig. 7).

It was also observed that the recently deposited (6-18 months) cultures were more virulent compared

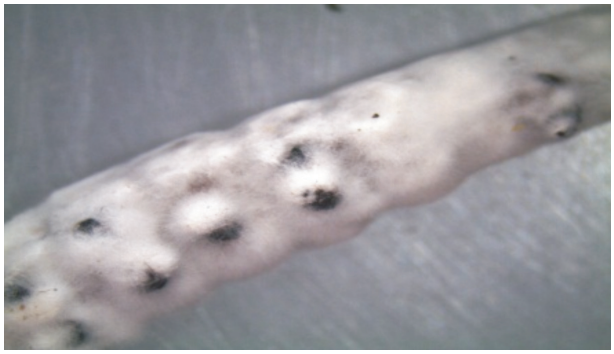

Fig. 6

Fig. 7

to those deposited long back (more than three years) (Table 1). The reason for losing the virulence needs to be worked out and appropriate measures taken to conserve it. Isolates which proved to be pathogenic to fruit fly and other insect pests if used later might not be virulent,due to longer storage period. This leads to loss of biological wealth, efforts and resources.

The results presented herein are the first time reports of evaluation of pathogenicity of entomopathogenic fungi against fruit fly and *C. cephalonica* from India. The virulence of the three isolates (ITCC No. 6628, ITCC No. 6645 and B. NCIPM) has been proved but detailed studies needs to be conducted using these isolates on other insect pests. Incorporation of this important component is imperative in IPM programmes for fruit flies in India.

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REFERENCES

Ali, S., Huang, Z. and Ren, S. 2010. Production of cuticle degrading enzymes by *Isaria fumosorosea* and their evaluation as a biological agent against diamond back moth. *Journal of Pest Science,* **83**:361–370.

Burges, H.D. and Hussey, H.W. (eds.) 1971.Microbial control of insects and mites. In Control of insect pests and weeds, with special reference to developing countries. *FAO Plant Protection Bulletin,* **39:**155-181.

Dimbi, S., Maniania, N. K., Lux, A. S., Ekesi, S. and Mueke, K.J. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Wiedemann), *C. rosa*var. *Fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). *Mycopathologia,* **156**: 375-382.

Goettel, M.S., Eilenberg, J. and Glare, T.R. 2005. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, Latrou K, Gill S (eds) *Comprehensive Molecular Insect Science*, Vol **6**: Control. Elsevier/Pergamon, Oxford, pp 361–406.

Greathead, D.J. and Prior, C. 1990. The regulation of pathogens for biological control with special reference to locust control. In Somme, L., and Bie, S. (eds.). Proceedings of the workshop on health and envvironmental impact of alternative control agents for desert locust control. NORAGRIC Occasional Papers Series C: *Development and Environment,* **5**: 65-80.

Khashaveh. A., Ghosta. Y., Safaralizadeh, M. H. and Ziaee. M. 2011. The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. In Assays with Storage Grain Beetles. *Journal of Agricultural Science and Technology,* **13**: 35-43.

Li, M., Lin, H., Li, S., Chen, P., Jin, L. and Yang, J. 2012. Virulence of entomopathogenic fungi to adults and eggs of *Nilaparvata lugens* Stal (Homopera: Delphacidae). *African Journal of Agricultural Research,* **7** (14): 2183-2190.

Muñoz, R., J. A. 2000. Patogenicidad de *Beauveria bassiana* (Bals.) Bullí. sobre la moscadelmediterráneo, *Ceratitis capitata* (Wied.) en condiciones de laboratorio. Tesisde Licenciatura. Facultad de Ciencias Agrícolas. Univ. Aut.de Chiapas. Huehuetán, Chis., México. 55 p.

Quesada Moraga, E., Ruiz Garcia, A. and Santiago Alvarez, 2006, *Journal of Econmoic Entomol*ogy **99**:1955. In: S. Ekesi & N.K. Maniania (Eds.) *Use of entomopathogenic fungi in biological pest management. Research Signpost,* **37/661 (2):** 239-274.

Reithinger, R., Davies, C. R., Cadena, H. and Alexander, B. 1997. Evaluation of the fungus *Beauveria bassiana*as a potential biological control agent against phlebotomine sand flies in Colombian coffee plantations. *Journal of. Invertebrate Pathology,***70:** 131–135.

Samroda. H and Ibrahim.Y (2006) effectiveness of selected entomopathogenic fungi in packed rice grain at room temperature against *Corcyra cephalonica* Stainton. AJSTD **23**(3): 183-192.

Sookar, P., Bhagwant, S. and Awuor Ouna, E. 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae) *Journal of Applied Entomology* **132**: 778–788.

Tscharntke, T. Klein, A.M. Kruess, A. Steffan Dewenter, I. and Thies, C. 2005. Landscape perspectives on agricultural intensification and biodiversity ecosystem service management. *Ecological Lett*ers, **8**: 857- 874.

Whitten, M.J. and Oakeshott, J.G. 1991. Opportunities for modern biotechnology with special reference to locust control. In Proceedings of the workshop on health and environmental impact of alternative control agents for desert locust control (eds. Somme, L. and Bie, S.). *NORAGRIC Occasional Papers Series C: Development and Environment* **5**: 65-80.

Wraight, S.P., Jackson, M.A. and De Kock S.L. 2001. Production, stabilization and formulation of biocontrol agents. In: Butt, T.M., Jackson, C. and Magan, N., (eds). *Fungi as Biocontrol Agents, Progress, Problems and Potential*. CABI Publishing, New York. 253-288.

Zimmermann, G. (2007): Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*, *Biocontrol Science and Technology*, **17**(6): 553-596.

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