Influence of addition of host larval extract to medium on the virulence of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin against *Spodoptera litura* Fab.

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ABSTRACT

Bio assays were conducted with MUCL 38502 strain of *Beauveria basiana* (Balsamo) Vuillemin and MUCL 8237 of *Metarhizium anisopliae* (Metschnikoff) Sorokin cultured on SDA and SDA supplemented with host insect larval extract against third instar larvae of *Spodoptera litura* Fab. The trials were conducted at the Department of Entomology, College of Agriculture, GBPUA&T, Pantnagar to determine the effect on biological properties and virulence of the fungi. Each fungus responded differently to the supplementation. However, in both the fungi the bio mass, linear growth, conidial count and viability of the conidia were increased with the supplementation of larval extract. The LC_{50} and LT_{50} values of MUCL 38502 grown on SDA medium and medium supplemented with larval extract were 14.85 and 9.65 $X10^5$ conidia ml^{-1} and 123.02 and 113.95 h at $5X\ 10^7$ conidia ml^{-1} respectively. The LC_{50} and LT_{50} values recorded with MUCL 8237 were 45.23 and 21.32 $X10^5$ conidia ml^{-1} and 138.72 and 130.93 h at $5X10^7$ conidia ml^{-1}. The virulence of both the fungal isolates increased when grown on SDA medium supplemented with larval extract.

Key words: *Beauveria bassiana*, *Metarhizium anisopliae*, larval extract, *Spodoptera litura*, medium, bioassay

INTRODUCTION

Entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, infect hosts through the cuticle and are classic biocontrol agents used against insect pests with either chewing or sucking mouthparts (Feng *et al.,* 1994; De Faria and Wraight, 2007; Roberts and St. Leger, 2004; Thomas and Read. 2007). However, their action on target pests is quite slow due to a latent period of several days. This slow action is an obstacle to commercial development of mycoinsecticides (St. Leger *et al.,* 1996; Lacey *et al*., 2001). Fungal infection starts from the adhesion of conidia to the host cuticle, followed by germination and cuticle penetration into the hemocoel, where fungal cells are propagated by budding until the mycosis-affected host dies of nutrition depletion (Feng *et al*., 1994). Conidia ingested by foliage feeders, such as caterpillars, rarely cause substantial infection before excretion due to the lack of intestine-specific virulence factors (St. Leger *et al.,* 1996). For this reason, fungal agents are often not very effective against leafingesting insects, which are still able to cause considerable damages before dying of mycosis. Thus, efforts have been increasing to enhance fungal virulence by genetic manipulation. Fungal infection of aphids was accelerated by overexpressing a silkworm chitinase or a hybrid chitinase in *B. bassiana* (Fan *et al.,* 2007). Integration of a scorpion neurotoxin in *M. anisopliae* for specific expression in insect hemolymph after cuticle penetration resulted in increases of fungal toxicity to the insects. Enormous informations were available about the mass production of *M. anisopliae* (Jagadeesh Babu *et al*., 2008; Karthick Raja Namasivayam and Vinoth Kumar, 2009; Vijayavani *et al.,* 2010). Recently Qin *et al*. (2010) incorporated Insecticidal Protein Vip3Aa1 into *Beauveria bassiana* to enhances the fungal virulence to *Spodoptera litura* larvae.

The tobacco caterpillar, *Spodoptera litura* Fab. is an important polyphagous pest. Like other lepidopterans, *S. litura* is known to be susceptible to almost all groups of entomopathogens (Ranga Rao *et al.,* 1993). The indiscriminate and excess use of insecticides for the management of this pest resulted in the development of resistance to the commonly used insecticides. The recognition of deleterious effects of pesticides has prompted the development of microbial control agents (Dayakar and Kanaujia, 2003). To incorporate the microbial agents in pest management strategies, mass multiplication of the virulent strain is an essential component. However, attenuation and enhancement of virulence of entomopathogenic fungi in general was observed by repeated sub culturing followed by passaging through insect host (Dayakar and Kanaujia, 2004). Evlakhova

(1966) attributed the loss of virulence of spores to lack of or less quantity of certain specific nutrients such as chitin components. To overcome the problems of loss of virulence after every 4 to 5 successive subcultures it should be passed through insect host (Dayakar and Kanaujia, 2004) to maintain the virulence of the fungi. Incorporation of lard (animal proteins) into the nutrient substrates (Schaerffenberg, 1964), extract of the integument (Mierzejewska, 1982) and larval water extracts of *Trichoplusia ni* (Getzin, 1961) for the maintenance of virulence and germination of spores of muscardine fungi. Keeping these points in view the bio assays were conducted to find out the effect of supplementation of host larval extracts on the biological properties and virulence of entomogenous fungi *B. bassiana* and *M. anisopliae* against *S. litura*.

MATERIALS AND METHODS

Third instar larvae of *S. litura*, obtained from the nucleus culture maintained in the laboratory, weighing 10 g were macerated separately in physiological saline (0.89 g Sodium Chloride in 100 ml of sterile distilled water). The extracts were filtered through a double muslin cloth. The two fungal isolates MUCL 38502 of *Beauveria bassiana* (Balsamo) Vuillemin and MUCL 8237 of *Metarhizium anisopliae* (Metschnickoff) Sorokin originally obtained from Belgian Coordinated Collection of Microorganisms (BCCM™), Belgium, were used for the bio assays. Sabouraud's Dextrose Medium enriched with 5% yeast extract was prepared with and without agar. Both the liquid media (SDY) and agar media were added with larval extract in the ratio of 1:10 in separate flasks before autoclaving. Sabouraud's dextrose with 5% yeast extracts (SDAY) media with and without larval extracts was plated. Sabouraud's dextrose with 5% yeast extract (SDY) medium with and without larval extract was poured in volumetric flasks for maintaining submerged cultures and seeded with the fungus and incubated at 25$\pm$ 2°C and 95 $\pm$ 5 per cent relative humidity for 14 days.

The biological properties such as biomass (g), conidial count (X 10^7 ml^{-1}) and viability of conidia (%) were measured from the submerged cultures cultured in volumetric flasks and the linear growth (mm) was recorded from the SDAY containing plates by following standard procedures in three replications (Dayakar and Kanaujia, 2004). The viability of conidia was examined before carrying out the bioassay using the micro culture method (De la Rosa *et al*., 1997). Different conidial concentrations ranging from 10^4 to 10^9 conidia ml^{-1} were prepared for both the fungal isolates from both the cultures to determine the LC_{50} value. The bioassays were carried out on third instar larvae of *S. litura*. Twenty third instar larvae were taken in Petri plate (90 by 1 mm) lined with sterilized filter paper were placed and sprayed under Potter's tower at a pressure of 40 $\pm$ 2 lbs $inch^{-1}$ with 2 ml of each concentrations. Observations were recorded at 8hr interval until the eighth day. To determine the mean lethal time (LT_{50}) the larvae were sprayed under Potter's tower with conidial concentration standardized at 5 x 10^7 conidia ml^{-1}. The check insects were treated only with sterile distilled water containing 0.02 per cent Tween-80. Each treatment was replicated thrice. Mortality was recorded at 24 hr interval up to 8th day. The cumulative data was used for the Probit analysis (Finney, 1964).

RESULTS AND DISCUSSION

Biological Properties

The results indicated that maximum biomass, conidial count (5.53 x 10^7conidia ml^{-1}) and viability of conidia (91.27 %) were produced by *B. bassiana* in larval extract containing media than the conidia with out larval extract (Table 1). Similar trend was also observed with linear growth. The linear growth was 46.60 mm and 39.20 mm in SDAY with larval extract and SDAY with out larval extract, respectively. While in case of MUCL 8237 of *M. anisopliae* the biomass, linear growth conidial count and viability of conidia were 0.716 and 0.681g, 42.03 and 37.63 mm, 5.26 and 4.94 x 10^7 conidia ml^{-1} and 84.98 and 78.96 per cent with media containing larval extract and media with out larval extract, respectively. The positive effect on biological properties by the larval extract containing medium is attributed to the availability of certain nutrients.

Table 1. Influence of larval extract on the biomass (in g), linear growth (in mm), conidial count (x10^7 conidia ml^{-1}) and viability (in %) of *B. bassiana* and *M. anisopliae*.

Culturemedium	Biomass	Linear growth	ureConidial count	Viability
B.bassiana				
Medium	0.691^{ab}	39.20^{c}	5.06^{c}	87.10^{b} (69.04)
Medium + larval extract	0.737^{a}	46.60^{a}	5.53^{a}	91.27^{a} (72.82)
M. anisopliae				
Medium	0.681^{b}	37.63^{d}	4.74^{d}	78.96^{d} (62.70)
Medium + larval extract	0.716^{ab}	42.03^{b}	5.26^{b}	84.98^{c} (67.19)

In a column, means followed by same letter are not significantly different by DMRT (p=0.05)

Table 2. Dosage mortality response of third instar *S. litura** to the fungi cultured on Sabouraud's medium (SM) with and with out *S. litura* larval extract (LE)

Fungal isolate	Medium	x^2	Regression equation	LC_{50}	Fiducial limits(95%)	Relative virulence
B. bassiana	SM	0.24	y = 4.7338+0.2272x	14.85	3.7726-58.4143	1.00
	SM + LE	0.26	y = 4.7700+0.2336x	9.65	2.4653-37.7799	1.53
M. anisopliae	SM	0.05	y = 4.6863+0.1895x	45.23	9.0474 – 226.1578	1.00
	SM + LE	0.10	y = 4.7112+0.2174x	21.32	5.1939-87.5175	2.12

*@360 larvae per bioassay

B. bassiana performed better when grown on carrot medium supplemented with *S. litura* larval extract than the carrot medium alone against *S. litura* larvae (Prasad, 1989). Enriching the culture medium with suitable additives to compensate for certain nutrient deficiencies was emphasized by Smith and Grula (1981). In *in vitro* studies Campbell *et al.* (1983) indicated that *B. bassiana* has been found to grow and sporulate best on trehalose, a major carbon component of insect haemolymph. The performance of the fungus with regard to the biological properties shows positive influence on the larval mortalities. The present findings are in corroboration with the above reports and indicated the need to enrich the culture media with suitable additives to compensate for certain nutrient deficiencies.

Bioefficacy of entomogenous fungi

The results of bioassay indicated that addition of host larval exract to the culture medium decreased the LC and LT_{50} value against third instar larvae (Table 2). LC_{50} values of 9.65 and 14.85 x 10^5 conidia ml^{-1} were recorded with *B. bassiana* when cultured on sabouraud's medium with and with out *S. litura* larval extract respectively. Where as the same for *M. anisopliae* was 21.32 and 45.23 x 10^5 conidia ml^{-1}. Though the LC_{50} values were high in case of *M. anisopliae* compared to *B. bassiana*, the relative virulence was more pronounced with *M. anisopliae* against third instar *S. litura* larvae. The regression statistics also revealed a slight increase in slope function indicating higher response in the host larvae. There was a 1.53 fold increase in virulence of *B. bassiana* to *S.litura* third instar with the addition of host larval extract, while it was 2.12 fold with *M. anisopliae*.

The $?^2$ test showed homogeneity of the test population which indicates the good fit of the observed and expected response (Table 2). This ultimately accounted for the precision of the regression. A good fit of the regression also is a reflection of the precision of the technique and procedure adopted (Ignoffo *et al.*, 1982). The slope functions were very low in all the bio assays (<1.0) indicating that the dose dependent responses were not well pronounced. Slopes of the dose response lines for entomogenous fungi generally have been less steep than those for other entomopathogens as established by Ignoffo *et al.* (1982). The shallow dose mortality response seems to be typical for fungus insect interactions (Rombach and Gillespie, 1988). In the present study, the slope function for *B. bassiana* was maximum with larval extract containing medium indicating more virulence than the other medium. The slope functions of *M. anisopliae* were 0.2174 and 0.1895 for conidia obtained from medium with larval extract and without larval extract respectively. According to Lagunes (1991), the value of the slope produced by regression analysis indicates level of uniformity of the response of the insects to the fungus applied, the greater the value of the slope the greater the uniformity of the response. The slope functions recorded in the present study confirms the above reports.

Table 3. Time- mortality response of third instar *S. litura** to the fungi cultured on Sabouraud's medium (SM) with and with out *S. litura* larval extract (LE)

Fungal isolate	Medium	x^2	Regression equation	LT_{50} #	Fiducial limits(95%)	Relative virulence
B. bassiana	SM	2.27	y = 3.0928+2.6871x	123.02	109.7376 - 137.9160	1.00
	SM + LE	3.23	y =3.1635+2.7417x	113.95	101.3184 – 128.1552	1.07
M. anisopliae	SM	0.39	y = 3.1985+2.3644x	138.72	121.3608 – 158.5656	1.00
	SM + LE	1.54	y =2.8737+2.8858x	130.93	117.6264 – 145.7304	1.06

*@360 larvae per bioassay
at 5 x 10^7 conidiaml^{-1}

The LT_{50} values from the probit analysis of the time mortality data of entomogenous fungi cultured on medium with and with out larval extract evaluated against *S. litura* third instar larvae ranged from 113.95 to 138.72 hr (Table 3). *B. bassiana* cultured on larval extract containing medium recorded the lowest LT_{50} value 113.95 compared to the medium without larval extract, 123.02 hr. While, the LT_{50} values recorded with *M. anisopliae* were 138.72 and 130.93 hr with medium containing larval extract and with out larval extract, respectively. There was 1.07 and 1.06 fold increase in virulence of *B. bassiana* and *M. anisopliae*, respectively, when cultured on media containing larval extract against*S. litura.* Regression statistics also revealed a slight increase in the slope function with the addition of larval extracts in both the fungi indicating higher susceptibility of the larvae. Decrease in the LT_{50} value with the addition of larval extract could be attributed to the presence of nutrients in larval extract (Pandey and Kanaujia, 2004). The present findings are in agreement with the reports. In the era of WTO, the use of insecticides is given last preference and the use of potential alternates like microbials are of high value. To incorporate the microbials in bio-intensified pest management strategies, mass multiplication of fungi is necessary and hence the present studies have oppositive bearing in cost effective viable mass multiplication of entomopathogenic fungi.

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