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Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against tomato fruit borer, *Helicoverpa armigera*

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Tomato (*Lycopersicon esculantum* Mill.) is one of the important and most commonly grown vegetable crops throughout the country. In India fruit borer, *H. armigera* is a major pest of tomato crop across the country (11), causing an annual loss of over Rs. 2000 crores, in spite of expenditure of Rs. 500 crores on insecticides (5). Development of resistance to most of the chemical insecticides leading to large upsurges to this pest during the last decade necessitated the search for alternatives to chemical control. In the present study several isolates of *B. bassiana* and *M. anisopliae* were bioassayed against *H. armigera* to select the virulent isolates for biocontrol efficacy.

Some of the *B. bassiana* and *M. anisopliae* strains used in the present experiment were originally isolated from the larval cadavers of *H. armigera.* Cultures available in the Indian Type Culture Collection (ITCC), New Delhi were also utilized. Pure cultures of the fungi were maintained on potato dextrose agar (PDA) slants.

The culture of *H. armigera* was maintained in the laboratory on chickpea flour based semi-synthetic diet as suggested by Nagarkatti and Prakash (3) at a temperature of 27 ± 1°C and 60 ±10 percent relative humidity. Field collected larvae of *H. armigera* constituted the initial culture and were reared individually in small round plastic vials (3.5 x 2.0 cm) till pupation. Pupae were sexed and kept separately (4). After emergence about 10 adults (5 males + 5 females) were released into a rearing jar (20 x 158 cm) for oviposition. A cotton swab submerged with 10 percent sucrose solution containing vitamin E served as food source for moths. The moth laid shining yellow-white, spherical flat based (dome shaped) eggs. The eggs generally hatched in 2-4 days. The larval period was between 15-16 days under laboratory conditions. F_1 population was used for various experiments.

The fungal pathogens were cultured on PDA medium and the Petri dishes were incubated for 7-10 days at 25 ± 1°C. The fungal spores were harvested in 25-30 ml of sterilized distilled water (SDW) containing 0.05% Tween 20 (Polyoxyethelene sorbitan monolauriate). The spore count of this stock suspension was estimated with an improved Neubaur haemocytometer. The spore concentration of the isolates were adjusted to 10^1, 10^3, 10^5, 10^7 and10^9 spores ml^{-1} by adding measured quantity of SDW. To establish Koch's postulates, 10^7 spores (conidia) ml^{-1} were taken and the pathogens reisolated from the treated dead larvae of *H. armigera* were used for further experiments.

Newly molted second instar larvae (approximately 7-10 mm size) of *H. armigera* were bioassayed for their susceptibility to fungal pathogens. Ten larvae were taken in a Petri dish, which was lined by a filter paper at the bottom for absorbing excess moisture. Ten ml of conidial suspension of 10^7 spores/ml^{-1} concentration was directly sprayed on the larvae using a hand atomizer. Four replicates of ten larvae were used in each case. Four lots of 10 larvae sprayed with 10 ml of sterilized distilled water with 0.05 percent Tween 20 served as control. The larvae were air dried by keeping them in laminar airflow for 5 minutes and

carefully transferred to individual clean sterile plastic vials containing freshly prepared diet. These vials were then kept inside the BOD incubator at 25 ± 1^0C. The larval mortality was recorded at 24 h interval until ten days of treatment. The percentage larval mortality due to mycosis was calculated. The results of the assay were subjected to probit analysis and the median lethal time (LT_{50}) was calculated from mortality and time. The median lethal concentration (LC_{50}) for the most virulent isolates was also calculated. For this, five different concentrations *viz*., $1x10^1$, $1x10^3$, $1x10^5$, $1x10^7$, and $1x10^9$ conidia ml^{-1} were used. Ten ml of conidial suspension of different concentrations were directly sprayed on the larvae using hand atomizer. Three replications of 30 larvae were used for each concentration. The larval mortality was recorded up to ten days.

The pathogenicity studies showed the differential percent mortality with respect to entomopathogenic fungi and their isolates. All the eighteen isolates of *B. bassiana* were found pathogenic to the tested insect pest (Table 1). The percent larval mortality ranged from 40.0- 90.0 % for different isolates, the maximum being with isolate HBB-2 (90.0%) followed by DBB-1 (87.5%) and HBB-1 (75.0 %). The least larval mortality (40%) was observed with isolates ITCC-4521, ITCC-4668 and ITCC-4795. No larval mortality was observed in the control. Among the isolates, the least LT_{50} value was observed in HBB-2 (2.2 days) followed by DBB-1 (2.3 days) and HBB-1 (3.3 days). The maximum LT_{50} value was for the isolate ITCC-4795 (13.3 days). The inoculated larvae, which escaped death, had abnormal metamorphosis and development. Deformities were noticed in pupae and a few of them failed to emerge into adults.

Similarly, all the isolates of *M. anisopliae* tested were found to be pathogenic to the larvae of *H. armigera*. The percent larval mortality ranged from 50.0 - 92.5 % for the ten isolates of *M. anisopliae*. No larval mortality was observed in control. The maximum mortality was observed with isolates, UTMA-1 (92.5%) followed by HMA-2 (90.0%) and UTMA-2 (80.0 %). The least larval mortality was observed with isolate ITCC-4710 (50.0%). The least LT_{50} value was observed with isolate UTMA-1 (2.3 days), followed by HMA-2 (2.4 days) and DMA-1 (3.3 days). The maximum LT_{50} value was observed for the isolate ITCC-4710 (8.0 days).

It is evident from Table 1 that our own isolates showed significantly higher mortality than the cultures taken from Indian Type Culture Collection (ITCC), New Delhi (collected from different host and countries), perhaps because they were obtained from cadaver of the larvae of *H. armigera* itself. The other possible reason for ITCC cultures being less pathogenic may be its continued sub culturing on artificial media for years. Rockwood (6) had demonstrated that *Beauveria* and *Metarhizium* lost their virulence after one year of growth on artificial media. Besides virulence, the mycelial growth, sporulation, germination and toxin production may be reduced progressively due to successive sub-culturing on artificial media (12). Sundararababu (10) reported that first four successive sub-culturing of *M. anisopliae* on carrot medium did not affect the growth, sporulation and infectivity, however, a gradual reduction in these traits were noticed beyond fourth sub-culturing and infectivity was lost beyond eighth sub-culturing. In the present studies, the strains taken from ITCC were passed through the host *H. armigera* (larvae) repeatedly to revive their pathogenicity before the final bioassay, however, their virulence always lagged behind our own isolates.

The LT_{50} value for *B. bassiana* were in close agreement with the value reported by Butt *et al.* (1) for *B. bassiana* against cruciferous pests at 1×10^6 conidia ml^{-1}. Samuael *et al.* (7) reported that LT_{50} value for *M. anisopliae* against *N. lugens* ranged from 5 days (highly pathogenic) to 14 days (non-pathogenic) at $1x\ 10^6$ spores ml^{-1}. During the present bioassay, the LT_{50} for highly pathogenic isolate (UTMA-1) of *M. anisopliae* was found to be 2.3 days against second instar larvae of *H. armigera*. Difference in the level of virulence of isolates may be due to the fact that the microbes undergo selection, recombination and mutation depending upon the ecological situations that ultimately influence their genetic make up. Sikura and Bevzenko (8) found variations in toxin production in different strains of *B. bassiana*, which could be correlated with the virulence.

The epizootics caused by the fungi, *B. bassiana* and *M. anisopliae* resembled closely. During the first 24 hours the larvae showed no changes,

Table 1. Virulence of different isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to II[nd] instar larvae of *Helicoverpa armigera**

Isolates	% Mortality up to 10 days**	LT_{50} (Days)	Fiducial limit (95%)		Slope (± S.E.)
			Lower	Upper	
B. bassiana					
DBB-1	87.5	2.3	1.6	3.2	2.1 (0.41)
DBB-2	70.0	3.4	2.4	4.8	1.4 (0.37)
HBB-1	75.0	3.3	2.4	4.4	1.7 (0.38)
HBB-2	90.0	2.2	1.6	3.0	2.3 (0.24)
HBB-3	67.5	3.5	2.4	5.1	1.3 (0.37)
UBB-1	70.0	3.6	2.6	4.9	1.5 (0.38)
RBB-1	62.5	5.6	4.5	6.8	2.0 (0.40)
RBB-2	62.5	5.6	4.5	6.9	1.9 (0.40)
ITCC-913	47.5	9.3	5.4	13.2	1.2 (0.39)
ITCC-4512	60.0	6.4	5.3	7.7	2.4 (0.43)
ITCC-4513	45.0	9.7	6.7	14.1	1.6 (0.42)
ITCC-4521	40.0	11.1	7.5	16.6	1.8 (0.45)
ITCC-4644	70.0	4.4	3.5	5.4	2.0 (0.39)
ITCC-4668	40.0	12.5	6.9	22.6	1.3 (0.41)
ITCC-4704	42.5	13.2	5.6	14.4	0.8 (0.38)
ITCC-4705	45.0	10.3	6.5	16.4	1.4 (0.40)
ITCC-4795	40.0	13.3	6.9	16.0	1.1 (0.40)
ITCC-4796	52.0	7.1	5.2	9.8	1.4 (0.39)
Control	00.0	-	-		
M. anisopliae					
DMA-1	77.5	3.3	2.6	4.3	2.0 (0.39)
HMA-1	75.0	4.0	3.2	4.9	2.2 (0.39)
HMA-2	90.0	2.4	1.8	3.2	2.5 (0.43)
UTMA-1	92.5	2.3	1.8	3.1	2.6 (0.43)
UTMA-2	80.0	4.1	3.4	4.8	2.8 (0.42)
ITCC-4514	77.5	3.9	3.1	4.7	2.3 (0.40)
ITCC-4707	70.0	4.5	3.7	5.6	2.1 (0.39)
ITCC-4708	52.5	7.9	6.2	10.1	2.0 (0.43)
ITCC-4709	55.0	7.2	5.9	8.9	2.3 (0.45)
ITCC-4710	50.0	8.0	6.0	10.7	2.7 (0.41)
Control	00.0	-	-		

Control = Sterilized distilled water containing 0.05% Tween 20

Concentration used for bioassay = 1 x 10^7 spores/ml

*4-5 days old larvae (approx. size = 7-10 mm) used for bioassay

** Data based on 4 replications of 10 larvae each

Mortality was recorded 2, 4, 6, 8, and 10 days after treatment

S. E. = Standard error

feeding normally and responding to external stimuli. After that the larvae became sluggish, lethargic and if made to lie on their back, crippled showing inability to straightening themselves. Finally, they undergo moribund stage and death occurs after sometime. After death they turned mummified. About 36-48 hours of mummification shiny white mycelial growth in the case of *B. bassiana* and

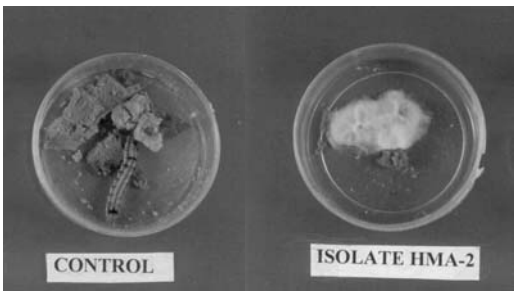

Fig. 1. Bioassay of *M. anisopliae* (isolate HMA-2) on second instar larvae of *H. armigera*

Susceptibility of the larvae was found positively associated with the spore concentration of both, *B. bassiana* and *M. anisopliae* (Table 2). In the case of *B. bassiana*, the highest percentage of larval mortality (96.6%) was noticed at the highest concentration (1 x 10^9 spores ml^{-1}) used in the assay and lowest (41.1%) at lowest concentration (1x10^1 spores ml^{-1}). No larval mortality was recorded in control, which is in close agreement with Devprasad *et al*. (2) who found a decreased mortality of *H. armigera* with an increase in the concentration

Table 2. Effect of concentration of spores of *Beauveria bassiana* and *Metarhizium anisopliae* on larval mortality of *Helicoverpa armigera*

Concentration (Spores/ml)	Mean larval mortality (%)*	LC_{50} value (Spores/ml)	Fiducial limit (95%)		Slope (± S.E.)
			Lower	Upper	
B. bassiana					
(Isolate HBB-2)					
1x 10^1	41.09	0.955 x 10^3	108.0	8362.6	0.15 (0.04)
1x 10^3	54.55				
1x 10^5	62.22				
1x 10^7	74.44				
1x 10^9	96.66				
Control	0.00				
M. anisopliae					
(Isolate HMA-2)					
1x 10^1	37.38	1.243 x 10^3	242.8	6365.1	0.20 (0.24)
1x 10^3	41.11				
1x 10^5	67.78				
1x 10^7	80.00				
1x 10^9	87.00				
Control	0.00				

*Three replications of 30 II^{nd} instar larvae each used for assay
Larval mortality recorded up to 10 days
S. E. = Standard error

initially white mycelial growth of *M. anisopliae* turning to herbage green (Fig. 1) appeared on the body of larvae which first emerged through the abdomen and joints of legs and soon covered the body completely. Abnormal metamorphosis and development was observed in the inoculated larvae escaping death. Deformities in pupae were noticed and a few of them failed to emerge into adults. Sikura and Gritsaenko (9) had also observed the effects like disturbance in fecundity and diapause of surviving images in *Carpocapsa pomonella* infected by an inoculum at a level distinctly lower than the optimum.

of the fungus. The mean LC_{50} value for *B. bassiana* (isolate HBB-2, ITCC No.-5411) and *M. anisopliae* (isolate HMA-2, *ITCC No.*-5415) against second instar larvae of *H. armigera* were found to be 0.955 x 10^3 and 1.243 x 10^3 spores ml^{-1} respectively. This was quiet lower than the estimated LC_{50} value (approx. 1x 10^6 conidia ml^{-1}) of both *B. bassiana* and *M. anisopliae* against cruciferous pest (1). The virulent isolates identified from the present studies can be exploited commercially.

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