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Nithya PR
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Manimegalai S
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Nakkeeran S
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Mohankumar S
Director, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Jayarajan Nelson S
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
Nithya PR
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Virulence of indigenous isolates of white muscardine fungus, *Beauveria bassiana* on diamondback moth, *Plutella xylostella* under laboratory conditions

Nithya PR, Manimegalai S, Nakkeeran S, Mohankumar S and Jayarajan Nelson S

Abstract
Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is the most serious pest of crucifers, difficult to be managed using a single tactic. Entomopathogenic fungi, being an important component of IPM has been employed for managing this key pest. In the present investigation, two indigenous isolates of *Beauveria bassiana* were evaluated against second instar larvae of *P. xylostella* reared under controlled conditions. Bioassay was carried out by leaf dip method with concentrations ranging from 1 x 10^5 to 1 x 10^9 conidia/ml. Results of bioassay revealed that among the two isolates of *B. bassiana,* isolate TM MH590235 registered lowest values of LC_{50} (2.4 x 10^7 conidia/ml) and LT_{50} (3.62 days) compared to the isolate, BR MK918495 and thus can be employed for managing *P. xylostella* after formulation development and field evaluation.

Keywords: *Plutella xylostella, Entomopathogenic fungi*, *B. bassiana,* bioassay, mortality, LC_{50}, LT_{50}

Introduction
The reign of chemical pesticides faced a setback in the past few decades due to higher concern on the ill effects on natural enemies of insect pests, other non-targeted organisms and environment. In World scenario, problems of pest resurgence, pesticide residue and resistance development necessitated for the search of other alternate measures and entomopathogenic fungi are one among them (Roy *et al*., 2010) [1]. Nearly 700 species of insect pathogenic fungi belonging to 90 genera has been described (Inglis *et al*., 2001; Roberts and Humber, 1981) [2, 3]. Better integration of fungal biocontrol agents with other management measures can potentially reduce the use of chemical insecticides and their subsequent residual side effects in agricultural environment.

The white muscardine fungus, *B. bassiana* (Balsamo) Vuillemin is the most studied entomopathogenic fungi widely applied against agricultural insect pests due to broader host range (Wraight *et al*., 2000) [4].

Diamondback moth (DBM), *Plutella xylostella,* a pest of cruciferous vegetables around the world has developed resistance to almost all groups of insecticides (Zhao *et al.,* 2006; Sparks *et al.,* 2012) [5, 6]. Various commercial formulations of *B. bassiana* significantly suppressed the DBM populations in screened enclosures and field condition (Shelton *et al*. 1998; Vandenberg *et al*. 1998; Becker 1999) [7, 8, 9]. Under favourable conditions of moisture and humidity, these fungal pathogens can cause epizootics among the pests (Carruthers and Soper, 1987) [10].

Pathogenicity and virulence are intrinsic characters of fungal isolates (Sandos *et al.,* 2018) [11]. Indigenous fungal isolates are reported to survive and grow well under conditions of stress (high temperature, UV radiation, low humidity) due to their adaptability to specific growth habitat of the native (Abdulhai *et al.,* 2010; Kryukov *et al.,* 2012) [12, 13]. Use of native isolates can also overcome the problems encountered on employing exotic isolates such as adverse effects on non-target organisms (Gulsar Banu *et al*. 2004) [14]. Taking into consideration afore said facts, the present investigation was attempted to evaluate the efficacy of native isolates of entomopathogenic fungi, *B. bassiana* against DBM, *P. xylostella.*

Materials and methods

Insect culture for bioassay

Culture of DBM, *P. xylostella* were maintained on cauliflower plants for generations in cages under controlled condition (28±2 °C, 65 ±10% RH and 12:12 h (L:D) photoperiods. Initial culture was established from field collected larvae from Narasipuram, Tamil Nadu, India. Pupae were collected and placed in cages of 40 X 35 cm containing mustard seedlings for oviposition by the emerging adults. Cotton soaked in 10 per cent sugar fortified with few drops of multivitamin drops was provided as adult nutrition. Larvae that hatched from eggs laid on mustard seedlings were collected by placing cauliflower leaves over mustard seedlings. These larvae were maintained in plastic rearing trays of 30 x 20 x 12 cm covered with khada cloth by providing fresh feed every day.

Maintenance of *B. bassiana*

Two strains of *B. bassiana* (MH590235, MK918495) were obtained from Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India. Pure cultures of the isolates were maintained at 28±5 °C on potato dextrose agar (PDA) medium for carrying out the study.

Bioassay with conidial suspensions of *B. bassiana*

The isolates of *B. bassiana* were grown in sterile Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 28±5 °C and a photoperiod of 12:12 h (L:D) for 14 days. Spores were harvested in distilled water containing 0.01% Tween-80 using sterile scalpel. Spore count was assessed using an improved Neubauer haemocytometer.

Conidial suspensions were prepared in sterile distilled water containing Tween-80 (0.01%). Cauliflower leaf discs of 9 cm diameter were immersed in 10 mL of conidia suspension for 10s. The experiments consisted of six treatments (1 x 10^5, 1 x 10^6, 1 x 10^7, 1 x 10^8 and 1 x 10^9 conidia/ml) and untreated control with five replication. Larvae treated with distilled water containing 0.01 per cent Tween-80 served as a control. The bioassay was conducted in laboratory condition at 28±5 °C temperature and 65 ± 5% relative humidity. Observations were made for mortality of larvae up to seven days after treatment. The dead larvae were transferred to Petri dishes lined with moistened filter paper and incubated for five days. Only those larvae covered with white mycelia and spores were considered dead due to fungal infection. Percent mortality was calculated following Abbott's formula (1925) [15]:

$$\text{Abbott's corrected mortality} = \frac{\text{\% mortality in treatment} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

Statistical analysis

Linear relationship between mortality and doses were analysed using Probit analysis for LC_{50} and LT_{50} estimation of the isolates with 95% confidence limits (CL) (SPSS Statistics Data Editor ver.21, IBM software).

Results and Discussion

Studies on dose mortality and concentration mortality responses of *P. xylostella* to *B. bassiana* (MH590235) are presented in Table 2 and 3, respectively. Larval mortality was dose dependant and percentage mortality increased with increase in concentration and days after treatment. Mortality increased with increase in time of treatment. At three, five and seven days after treatment, mortality of larvae ranged from 4.00 to 58.00 per cent, 10.00 to 84.00 per cent and 34.00 to 96.00 per cent, respectively. Highest mortality of 96.00 per cent was recorded at 10^9 conidia/ml after seven days of treatment. Concentration mortality responses of *P. xylostella* to *B. bassiana* showed that LC_{50} value of 2.4 x 10^7 conidia/ml was obtained with the isolate, MH590235 at fiducial limit of 10^6 – 10^8 conidia/ml. LT_{50} value estimated for the isolate was 3.62 days at 1 x 10^8 conidia/ml (Table 6).

Similar linear relationships between the conidial concentrations and percent morality was determined for the isolate, MK918495. At three, five and seven days after treatment, mortality of larvae ranged from 2.00 to 44.00 per cent, 8.00 to 66.00 per cent and 26.00 to 88.00 per cent, respectively (Table 4). Highest mortality of 88.00 per cent was recorded at 10^9 conidia/ml after seven days of treatment. LC_{50} value of 1.03 x 10^8 conidia/ml was obtained with this isolate, MK918495 at fiducial limit of 10^7 – 10^8 conidia/ml (Table 5). LT_{50} value estimated was 4.59 days at 1 x 10^8 conidia/ml (Table 6).

Entomopathogenic fungi serve as a feasible alternative for safer and eco-friendly pest management and some products are already available commercially (Ekessi *et al*. 2001) [16]. Ubiquitous nature of these fungi has contributed to the occurrence of numerous isolates naturally (Feng *et al*. 2004) [17].

Results of the present study demonstrated that native isolates of *B. bassiana* are capable of causing infection and mortality against *P. xylostella via* contact. Sabbour and Sahab (2005) [18] demonstrated that *B. bassiana* exhibited larvicidal activity against *P. xyllostella.* Wraight *et al*. (2010) [19] compared virulence of *B. bassiana* isolates against lepidopteran pests of vegetable crops and demonstrated that all lepidopteran species were susceptible to *B. bassiana.*

In the present study, similarity existed in both the isolates with respect to environment condition and host factor such as host immunity, nutritive status of host and host cuticle morphology. External white mycelial growth from cadavers was evident within 48–72 h of death. The MH590235 isolate of *B. bassiana* has the highest virulence against *P. xylostella* larvae since it registered lower LC_{50} and LT_{50} values compared to the other isolate. Difference in susceptibility of *P. xylostella* might be due to variation in physiological response of *P. xylostella* to isolates of *B. bassiana.* Leland *et al*. (2005) [20] too reported that physiological and enzymatic properties of fungal isolate served as a pathogenicity determining factor.

Pathogenicity of an entomopathogen is a complex process, dependent upon both host and pathogen attributes. The host cuticle has great impact on all stages of the infection process, *viz.,* adhesion, germination and aspersorium differentiation (Butt *et al*. 2001) [21]. Evn though relationships between enzyme activities and the virulence of *B. bassiana* toward *Galleria mellonella* and *Trichoplusia ni* (Hu¨bner) was demonstrated by Gupta *et al*. (1994) [22] and many other researchers, this may not be same in with other host and entomopathogenic fungi (Gillespie *et al*. 1998) [23]. Cuticle-degrading enzymes (CDEs) are not simply virulence determining factors but also the host specificity of fungal isolate (Gupta *et al*. 1994) [22]. Different insects vary in their cuticle types with respect to their protein composition and degree of sclerotization (Charnley, 2003) [24]. Most of the CDEs are induced by cuticular components (Paterson *et al*. 1994; Butt *et al*. 1998) [25, 26] and some are produced under nutrient- poor conditions and repressed by excess nutrients (St. Leger *et al*. 1992) [27]. Thus, the virulence of an

entomopathogen differs from host to host based on the variation in cuticle composition.

Table 1: Details of *B. bassiana* isolates used in the present study

Accession number	Substrate	Location (Country)
MH590235	*Helicoverpa armigera*	Dharmapuri, TamilNadu (India)
MK918495	*Leucinodes orbonalis*	Coimbatore, TamilNadu (India)

Table 2: Dose-mortality responses of *Plutella xylostella* treated with different spore concentrations of *B. bassiana* (MH590235)

Treatments (Spores mL^{-1})	Per cent mortality (Days after treatment)		
	3	5	7
10^9	58.00^a (49.67)	84.00^a (66.98)	96.00^a (82.08)
10^8	42.00^{ab} (40.33)	62.00^b (52.02)	82.00^{ab} (67.53)
10^7	22.00^b (27.60)	40.00^c (38.96)	56.00^b (48.51)
10^6	10.00^c (16.56)	22.00^d (27.60)	50.00^c (45.00)
10^5	4.00^d (7.92)	10.00^e (16.56)	34.00^d (35.62)
Control	0.00^e (0.91)	0.00^f (0.91)	0.00^e (0.91)
SE(d)	4.22	4.24	4.80
CD (0.05)	8.70	8.75	9.92

Figures in parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA.

Table 3: Concentration mortality response of *Plutella xylostella* to isolate of *B. bassiana* (MH590235)

Fungal isolate	Heterogeneity ($\chi 2$)*	Regression equation	LC_{50} (x10 ^{7} spores mL^{-1})	Fiducial limit (x10^6 to 10^8 spores mL^{-1})
Beauveria bassiana (MH590235)	0.06	y = 0.563x + 0.8578	2.4	4.7 –1.2

Table 4: Dose-mortality responses of *Plutella xylostella* treated with different spore concentrations of *B. bassiana* (MK918495)

Treatments (Spores mL^{-1})	Per cent mortality (Days after treatment)		
	3	5	7
10^9	44.00^a (41.54)	66.00^a (54.51)	88.00^a (80.46)
10^8	30.00^a (33.09)	52.00^b (46.15)	72.00^b (63.38)
10^7	12.00^a (18.18)	36.00^b (36.77)	58.00^b (49.67)
10^6	4.00^b (7.92)	10.00^c (14.68)	30.00^c (32.49)
10^5	2.00^c (4.41)	8.00^{cd} (14.93)	26.00^e (30.43)
Control	0.00^c (0.91)	0.00^d (0.91)	0.00^e (0.91)
SE(d)	4.42	4.57	6.00
CD (0.05)	9.13	9.43	12.39

Figures in parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA.

Table 5: Concentration mortality response of *Plutella xylostella* to isolate of *B. bassiana* (MK918495)

Fungal isolate	Heterogeneity ($\chi 2$)*	Regression equation	LC_{50} (x10 ^{8} spores mL^{-1})	Fiducial limit (x10^7 to 10^8 spores mL^{-1})
Beauveria bassiana (MH918495)	0.52	y= 0.494x + 1.0448	1.03	1.31 – 8.02

Table 6: LT_{50} values of isolates of *B. bassiana* isolates to larvae of diamondback moth, *P. xylostella* at 1 x 10^8 concentration.

Isolates of *B. bassiana*	LT_{50} (days)	Confidence limits (95%)
MH590235	3.62	2.30-5.69
MK918495	4.59	3.19-6.59

Conclusion

Laboratory bioassays are an initial and essential step for identifying virulent strain of any entomopathogenic fungi prior to field or large scale use. In the present study, the virulent nature of the indigenous isolates of *B. bassiana* was proved by dose and time mortality responses. Out of the two native isolates of *B. bassiana,* isolate MH590235 was found to be effective against DBM larvae compared to the isolate, MK918495. The findings of the present study ascertain the ubiquitous nature of *B. bassiana* and also being a native isolate, MH590235 can be included as a fundamental component of biological pest management strategies. The dose mortality responses of *B. bassiana* to DBM larvae can further be exploited for development of formulation and large scale application strategies. Basic idea of entomopathogenicity can also act as a pivotal point in terms of strain improvement studies. Further studies on the efficacy under field conditions are needed for advancement of this isolate for use in integrated management of *P. xylostella.*

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