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Survey of *Beauveria bassiana* from cadaver of *Indarbela quadrinotata* and its virulence against the *Plutella xylostella* (Diamond back moth) in various places of Allahabad and Koushambi District

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Abstract
The entomopathogenic fungus *Beauveria bassiana* is one of the most promising bio control agent against *Plutella xylostella*. Laboratory bioassays were conducted to study the virulence of ten isolates of *B. bassiana* against 3rd instar larvae of the *P. xylostella*. The *B. bassiana* isolates used in the study were obtained from cadaver of *Indarbela quadrinotata*. Initially, a survey was carried out in ten guava orchards located at SHUATS, Mahewa, Jhalwa, Preetam Nagar, Khusrubagh, Meza, George Town, Johnstonganj and Allahpur of Allahabad district, and Kaushambi in September to October during 2013-2015 and total 20 isolates were obtained. Maximum 4 isolates were collected from Jhalwa. Isolate Bb 3 from Jhalwa (63.33%) recorded maximum mean per cent mortality. All this isolate showed promise for use as bio control agents against *P. xylostella*.

Keywords: *Beauveria bassiana*, entomopathogenic fungus, isolates, mortality, *Plutella xylostella*, Survey

Introduction
The diamond back moth, *Plutella xylostella* (Lepidoptera: Plutellidae), is an important and cosmopolitan pest of cruciferous crops in many parts of the world. DBM is one of the principal pests of brassicas, capable of reducing leaf area, devaluing products, slowing growth and causing plant death if left uncontrolled. Among the factors that favour the occurrence and consequent infestation of the diamondback moth, its elevated biotic potential is considered relevant since the lifecycle takes approximately 16 days and each female's deposits about 140 eggs (temperature of 25 ºC and relative humidity of 70%). This pest can be controlled through biological agents such as the entomopathogenic fungus *Beauveria bassiana* that is virulent to *P. xylostella* and is effective at reducing the pest population and increasing cabbage production (Godonou *et al*., 2009) [7]. DBM has been controlled by various chemical pesticides. In recent years, resistance to most of the conventional insecticides has developed (Sun *et al*. 1986) [15]. The rapid development of resistance is probably associated with the very rapid reproduction of DBM i.e. more than 25 generations per year in the tropics (Keinmeesuke *et al*., 1985) [8]. The problems of insecticide resistance as well as the environmental and consumer health hazards associated with insecticide residues in plant material have focused attention on alternative methods for the control of DBM; hence the search for bio control agents for incorporation into IPM programmes against this insect is a dire need.

The entomo pathogens, fungi are of significant importance because, unlike bacteria and viruses which need to be ingested for causing diseases, fungus enters the insect by penetrating its cuticle, although infection through the digestive tract occurs with some species. Although many major fungal entomopathogens have basic diagnostic characters which make them quickly identifiable, species such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* are complex and their resolutions will depend on correlating molecular, morphological, patho-biological and other characters (Humber, 1997). Entomopathogenic fungi, *Beauveria bassiana* has been widely used to control *P. xylostella* (Loc and Chi, 2007) [9], producing over 50% mortality (Furlong and Pell, 2001) [6]. Keeping in view, this study was undertaken to survey and isolate *B. bassiana* from different locations.

Materials and Methods

Rearing of diamond back moth

Diamond back moth, larvae were collected from the SHUATS cabbage field. Larvae were reared in the laboratory on young and fresh cabbage leaves in cylindrical cages (25×25×35 cm) and incubated under the following conditions: 25±2 °C, 55±5% (RH) under a photoperiod of 12:12 (L:D). The adults were continuously supplied with honey diluted in distilled water (1:10, honey: water) in a piece of cotton hung in the central of the cage.

A Survey of *Beauveria bassiana* from cadaver *Indarbela quadrinotata* was carried out in ten guava orchards viz SHUATS, Mahewa, Jhalwa, Preetam Nagar, Khusrubagh, Meza, George town, Johnson gang Allahpur in Allahabad district and Kaushambi in September to October during 2013-2015.

Collection of sample (fungus)

Samplings were carried out by the method of Reay *et al* (2008) [10]. At each orchard five trees were selected along a randomly located transect. All samples were stored individually in plastic bags in an icebox until they were processed in the laboratory. Before each sampling procedure, all sampling tools were sterilised with 70% ethanol. These sampling tools included a smooth forceps for sampling cadaver of *I. quadrinotata.*

Isolation of Fungus

B. bassiana isolate the cadavers of *I. quadrinotata* in laminar flow chamber, inoculated in Petri plates containing the medium SDA. After which white cushiony fungal growth was observed. The fungus was further re-isolated into SDA test tube slants in order to purify the culture. Slides were prepared from the pure culture in order to identify the spores of *B. bassiana* based on its morphology.

Bioassay Procedure

Spore suspension was prepared from 15 days old culture of *B. bassiana* on SDA medium. The fungal mycelium was removed from the flasks and then it was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent (Rombach *et al*., 1986) [11]. The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005) [12]. Spore concentration of the filtrate was determined using a Neubauer Hemocytometer. This Spore suspension of *B. bassiana* maintained at 2.1×10^8 spores/ml was prepared by adding sterile 0.02% Tween 80 in distilled water. Viability of the conidia was checked by a germination test prior to the experiment and assures to be ≥ 90%for all isolates. Virulence test for all the 10 isolates (one isolate from each orchard) were carried out with a single concentration (2.1×10^8 spores/ml) of conidial suspension. Three replicates of 20 third instars larvae of *P. xylostella* each replicates were maintained for each isolate. Cabbage leaves dipped in the conidial suspension (2.1×10^8 spores/ml) for 10 second and transferred to separate Petri plates with larvae, for Control larvae it was similarly dipped in a solution of Tween 80 (0.05%) in sterile distilled water. Mortality of larvae was recorded after 24, 48 and 72 hours, per cent mortality for each of the isolates to select the highly virulent strains for further studies. Abbott's formula was applied to obtain the per cent mortality.

Results and Discussion

Distribution of *B. bassiana* as indicated by infected *I. quadrinotata* larvae exposed in guava orchards from different location showed that there were significant differences in frequency of fungi (number of infected insects) among locations. Most of the infected insects were obtained from Jhalwa (4), followed by SHUATS (3), Mahewa (2), Preetam Nagar (2), Khusrubagh (2), Meza (1), George town (2), Johnson gang (2), Allahpur (2), Kaushambi (2) and minimum no. of the infected insect *I. quadrinotata* by *B. bassiana* was found in Meza (1) and Kaushambi (1) (Table1). The presence of diverse fungal entomopathogens has been documented in tropical and temperate forests of Mexico and worldwide (Evans, 1982,) [5]. Sergio *et al.* (2011) [13] reported the entomopathogenic fungi *B. bassiana* and *M. anisopliae* were common in the sampled areas, but showed marked differences in abundance across habitats. All the 10 isolates were pathogenic to *P. xylostella* at 2.1×10^8 spores/ml and the mean per cent mortality ranged from 41.10 (Bb8 isolate) to 63.3% (Bb3 isolate) compared with the control treatment that showed only 8.88 percent mortality (Table 2). All the isolates caused significant per cent mortality. The isolates Bb 2, Bb 3 and Bb 5 caused 50.55%, 63.33% and 51.10% mortality respectively. Isolate Bb 3 from Jhalwa (63.33%) recorded maximum mean per cent mortality and was significantly superior to rest of the isolates. Above results are supported by Bai *et al* 2010 [2], and Eyal *et al*., 1994 [4], Strasser *et al.*, 2000 [14], reported *B. bassiana* isolates 7320, 7569 and 7771 isolated from the soil were identified as the most pathogenic isolates to adult houseflies, causing mortalities of 90 per cent. Ekesi *et al*., 2000 [3] also reported the entomopathogenecity of *B. bassiana* isolate to the apterous adult of the cowpea aphid, *Aphis craccivora* and recorded 58-91%, 64-93% and 66-100% mortality by 3 isolates of the two fungi, respectively after 7 days of treatment.

Conclusion

From our results we conclude that *Beauveria bassiana* prevails in different locations of Allahabad and Kaushambi and the isolate Bb3 contains some characters that can make it suitable to be effective against 3rd instars larvae of *Plutella xylostella* may be mass multiplied for the management of the insect pest population and ecofriendly. This fungus can also be included successfully in IPM.

Table 1: Survey of *B. bassiana* from *I. quarinotata* from guava trees at different locations of Allahabad and Kouusambi district.

Orchards	No. of trees surveyed in each orchards	No. of infected larvae of *I. quarinotata* / in each tree					Total infected larvae of *I. quarinotata* by *B. bassiana*
		1st Tree	2nd Tree	3rd Tree	4th Tree	5th Tree	
SHUATS	5	-	1	1	1	-	3
Mahewa	5	-	1	-	1	-	2
Jhalwa	5	1	-	1	-	2	4
Preetam Nagar	5	-	1	-	-	1	2
Khusrubagh	5	1	-	-	1	-	2
Meza	5	1	-	-	-	-	1
George town	5	-	1	-	1	-	2
Johnson gang	5	1	-	-	-	-	1

Allahpur	5	-	1	-	1	-	2
Kaushambi	5	1	-	-	-	-	1

Table 2: Evaluation of *B. bassiana* isolates on the per cent mortality of 3rd instar larvae of *P. xylostella* in lab.

Treatment	Spores/ml	Mortality Percent			Total Mean per cent mortality
		24 hrs	48hrs	72hrs	
Control	Distilled water	00.00	10.00	16.66	8.88 ^{h}
Bb 1	2.1×10^{8}	30.00	53.33	60.00	47.77 ^{de}
Bb 2	2.2×10^{8}	35.00	50.00	66.66	50.55 ^{cd}
Bb 3	2.1×10^{8}	41.66	68.33	81.66	63.33 ^{a}
Bb 4	2.1×10^{8}	30.00	46.66	65.00	47.21 ^{ef}
Bb 5	1.9×10^{8}	30.00	50.00	73.33	51.10 ^{c}
Bb 6	2.1×10^{8}	25.00	40.00	68.33	44.44^{f}
Bb 7	2.1×10^{8}	28.33	53.33	61.66	47.77^{de}
Bb 8	2.0×10^{8}	25.00	43.33	55.00	41.10^{g}
Bb 9	2.1×10^{8}	36.66	51.66	70.00	52.77^{bc}
Bb 10	2.3×10^{8}	36.66	53.33	73.33	54.44^{b}
CD (P = 0.05)					3.110
S. Ed (+)					1.49

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