Journal of Biological Control, 32(3): 203-208, 2018, DOI: 10.18311/jbc/2018/16055

Research Article

Biocontrol potentiality of *Beauveria bassiana* Balsamo (Vuillemin) against *Chilo partellus* (Swinhoe) under controlled conditions

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ABSTRACT: Eight different isolates of *Beauveria bassiana* Balsamo (Vuillemin) were evaluated for their efficacy or biocontrol potentiality against *Chilo partellus* (Swinhoe) under controlled conditions at Punjab Agricultural University, Ludhiana. The results of the present studies revealed that all the eight isolates irrespective of their original host were pathogenic to *C. partellus*. The virulence of the isolates was increased with increase in spore concentration. As compared to other isolates, the native isolate of *B. bassiana*, PAU Bb and Bb 4668 caused higher mean mortality of *C. partellus* (68.75 and 56.25 %) at the concentration of 1×10^{10} spores/ml. These isolates were 56.49 and 7.17 times more pathogenic than the commercially available isolate of *B. bassiana.* Under screen house conditions, the efficacy of B. *bassiana* isolates, against *C. partellus* was comparatively lower than Decis 2.8 EC deltamethrin. However, the application of *B. bassiana* resulted in lower incidence of *C. partellus* than infested control.

KEY WORDS: *Beauveria bassiana*, bioassay, biocontrol, *Chilo partellus*, maize

(Article chronicle: Received: 08-05-2018; Revised: 15-08-2018; Accepted: 07-09-2018)

INTRODUCTION

Maize stem borer, *Chilo partellus* (Swinhoe) which is the key pest of *Kharif* maize, scraps in the leaf whorls of growing plants to produce characteristic 'window-paning', 'pin-holes' and under severe infestation conditions it results in formation of dead hearts (Panwar, 2005). It caused yield losses from 26.7 to 80.4 per cent in different agro climatic zones of India (Chatterji *et al.,* 1969). Although, the deployment of chemical control at right time is fairly successful for the management of this pest, but excessive use of chemicals can lead to many ecological problems. Therefore, an alternative control measure such as biological control with *Beauveria bassiana* Balsamo (Vuillemin) which has been reported to naturally regulate the populations of *C. partellus* in laboratory bioassays as well as in field experiments (Maniania,1993; Devi *et al*., 2001; Tefera and Pringle, 2004b) must be evaluated against *C. partellus*. But different isolates of *B. bassiana* differ in their host specificity and virulence (Mc Coy *et al*.,1988) and it has been reported that the native species of natural enemies always most fit as they are adapted for the local agro climatic conditions and ecosystem (Smith, 1996). Bidochka *et al.* (2002) have also reported that the selection of *B. bassiana* must be made primarily on the basis of same habitat. Keeping this in view, the present studies were planned to evaluate the efficacy of local and different isolates of *B. bassiana* against *C. partellus* under laboratory and screen house conditions.

MATERIAL AND METHODS

Fungal cultures

The six isolates of *Beauveria bassiana* were obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi. The details of these fungal isolates are as follows:

Sr. No.	Accession no.	Host of isolate	Year of isolation
1	Bb 4644	Oil palm insect	1996
2	Bb 4668	*Helicoverpa armigera*	1996
3	Bb 7126	*Helicoverpa armigera*	2012
4	Bb 7130	Shoot borer	2012
5	Bb 5408	*Helicoverpa armigera* on tomato	2002
6	Bb 5412	Tomato	2002

The native isolate of *B. bassiana* designated as PAU Bb was obtained from Insect Molecular Biology Laboratory, Department of Entomology, PAU, Ludhiana. *B. bassiana* isolate obtained from its commercial formulation was compared

with these seven other isolates. Each isolate was sub-cultured on potato dextrose agar (PDA) medium.

Mass multiplication and preparation of conidial suspension

The mass multiplication of each isolate was done on moistened and sterilized wheat grains and conidial suspensions were prepared as per the procedure followed by Sahayaraj and Namasivayam (2008).

Insect cultures: The larvae and pupae of *Chilo partellus* were regularly collected from maize fields and the insect culture was maintained on the green-gram based artificial diet (Kanta and Sajjan, 1992) prepared with the method followed by Siddiqui *et al*. (1977). Before the conduct of bioassay, the *C. partellus* larvae were allowed to feed on two week old maize leaves for 2 days.

Bioassay

The direct spray bioassay method was used to test bioefficacy of the *Beauveria bassiana* isolates at four different spore concentrations i.e. 1×10^6, 1×10^7, 1×10^8 and 1×10^{10} spores/ml along with control against 3rd instar larvae of *C. partellus* in the laboratory. The concentration-mortality response (with the same bioassay method) of two most virulent isolates namely PAU Bb, Bb 4668 and one that was isolated from commercial formulation was used to calculate LC_{50} values against 3rd instar larvae of *C. partellus*. The experiment was replicated four times. Each replication consisted of 20 *C. partellus* larvae placed in single sterile Petri plate and the respective conidial suspensions were applied with the help of automated hand sprayer. The control treatment consisted of 20 larvae of *C. partellus* sprayed with sterile distilled water containing Tween 80 (0.1% v/v). The Petri plates containing treated larvae were labeled, sealed with paraffin tape and incubated at 25 ± 2°C. The mortality data was recorded daily upto 15 days. The frass and leaf debris in each Petri plate was removed and dead larvae were transferred to another Petri plate lined with moist filter paper to encourage mycosis in the dead cadavers.

Screen house experiment

The experiment was conducted by following Completely Randomized Design (CRD) with five replications and each replication consisted of 5 potted maize plants of maize hybrid, PMH-1. These maize plants were artificially infested with 20 black headed eggs of *Chilo partellus* at 13 days after germination and the respective treatments were applied after 24 hours of artificial infestation. The treatments consisted of spore suspension of PAU Bb, Bb 4668 and isolate of *B. bassiana* from commercial formulation @ 1×10^{10} spores / ml, respectively. Decis 2.8EC @ 200 ml/ha was applied as standard check. The control treatments consisted of infested and un-infested unsprayed maize plants, separately. The leaf injury and dead hearts incidence was recorded at 21 days after the artificial infestation of plants. Severity of infestation was recorded on 1–9 scale at 25 days after the artificial infestation of plants (Panwar, 2005).

Data analysis

The data on mortality of *C. partellus* caused by different isolates of *B. bassiana* was corrected (Abbott, 1925) and was subjected to ANOVA, the different treatment means were subjected to Least Significant Difference (LSD) at p = 0.05 (Gomez and Gomez, 1984). The LC_{50} values were also determined by using probit analysis (Finney, 1971). The data on leaf injury and dead-hearts incidence of *C. partellus*, Interpolated Transformed Values (ITV) of non parametric leaf injury rating (LIR) data and maize grain yield was analyzed using ANOVA and the different treatment means were separated by least significant difference test (LSD) at p = 0.05 (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results of the present study revealed that all the eight isolates were pathogenic to *Chilo partellus* (Table 1). The virulence of different isolates of *Beauveria bassiana* increased with increase in spore concentration. Similar results were also obtained by Tefera and Pringle (2004a). The PAU Bb isolate of *B. bassiana* at all the test concentrations caused maximum mortality of *C. partellus* as compared to other test isolates. All the isolates caused mortality of *C. partellus* larvae, but it was maximum by PAU Bb isolate at 1×10^{10} spores/ml concentration i.e., 68.75 percent followed by Bb 4668 which caused 56.25 per cent. Similarly, Devi *et al*., (2001) had reported that all the 20 pathogenic isolates of *B. bassiana* (procured from national and international culture collections) varied in their pathogenicity against *C. partellus*. This can be explained on the basis that the native isolate has better adaptation to local environment which governs its pathogenicity (Bidochka *et al*., 2002). Indigenous isolate of *B. bassiana* was found most effective against diamondback moth, *Plutella xylostella* Linnaeus (Godonou *et al.,* 2009).

In the present study variable efficacy of seven isolates of *B. bassiana* (collected from insect hosts other than *C. partellus*) against *C. partellus* was seen. Similarly, Devi *et al*., (2001) had reported that the most pathogenic isolate of *B. bassiana* against *C. partellus* was isolated from coleopteran host and that isolated from lepidopteran insects including the one from *Chilo* were moderately aggressive. On the contrary Samuels *et al.* (1989) had reported that in most of the cases the fungal isolates are pathogenic to their original host or to a closely related species. Therefore our studies support the evidence that entomopathogenic fungi are also pathogenic towards insect pests other than its original host.

The results of differential pathogenicity of different fungal isolates at their different concentrations also differed significantly (Table 1). The most virulent, PAU Bb isolate of *B. bassiana* at its highest concentration i.e., 1×10^{10} spores/ml caused maximum mortality of *C. partellus* (68.75%) and it was followed by Bb 4668 @ 1×10^{10}

spores/ml (56.25%). The mortality of *C. partellus* caused by lowest concentration (1×10^6 spores/ ml) PAU Bb isolate (33.75 %) was at par with the mortality of *C. partellus* caused by the highest concentration of Bb 7130 (37.50 %) and Bb7126 (36.25 %) which again indicates that there are differences in the virulence of these isolates. It also shows that the lesser spore concentration of virulent isolate is needed to cause the same level of mortality as caused by the higher concentration of less virulent isolates.

The present results also show that the most virulent isolates of *B. bassiana* (PAU Bb and Bb 4668) caused higher mean mortality of *C. partellus* (33.75–68.75 and 25.00–56.25 %). Whereas, the least virulent isolates (Bb 7130 and Bb 7126) resulted in minimum mortality of *C. partellus* (11.25-37.50 % and 11.25-36.25 %).

The concentration-mortality response of the tested isolates reported that PAU Bb isolate caused 50 per cent mortality (LC_{50}) of *C. partellus* at minimum concentration i.e., 5.93×10^5spores/ ml (Table 3). This LC_{50} values worked out for Bb 4668 and isolate from commercial formulation were 4.67×10^6 and 3.35×10^7spores/ ml, respectively. This indicates that PAU Bb and Bb 4668 isolates were 56.49 and 7.17 times more virulent than commercial isolate. The LC_{50} value of *B. bassiana* isolate against European corn borer *Ostrinia nubilalis* Hubner ranged from 9.25×10^2 to 2.89×10^6 CFU/cm^2 (Feng *et al*.,1985). This variation in LC_{50} values may be because of the variation in virulence of different isolates.

Bioassay under screen house conditions

The leaf injury and deadhearts incidence recorded at 21 days and LIR recorded at 25 days after the artificial infestation of plants with *Chilo partellus* eggs revealed that leaf injury, deadhearts incidence and LIR differed significantly in all the treatments (Table 4). The incidence of *C. partellus* was not observed on any of the plants with uninfested control treatment showing that there was no external infestation of *C. partellus* in the screen house. Among other treatments, the application of Decis 2.8 EC resulted in minimum leaf injury and deadhearts incidence on the maize plants (12.00 and 8.00%, respectively) and the LIR of the infested plants was also found to be minimum (4.20). The leaf injury and deadhearts incidence due to *C. partellus* was maximum on plants sprayed with spore suspension of commercial isolate of *B. bassiana* (24.00 and 40.00%, respectively) which was further at par with the plants following the application of PAU Bb (20.00 and 28.00%,

Table 1. Mean mortality of *Chilo partellus* caused by different isolates of *Beauveria bassiana* at their different concentrations

Sr. No.	Treatments	Concentration of spore suspension (spores/ml)					Overall mean
		1×10^{10}	1×10^8	1×10^7	1×10^6	Control	
		Mean* per cent mortality of *C. partellus* (after 15 days)					
1	PAU Bb	68.75 (56.34)	50.00 (45.02)	42.50 (40.38)	33.75 (35.31)	0.00 (0.00)	39.00 (35.52)
2	Bb4668	56.25 (48.73)	42.50 (40.52)	33.75 (35.40)	26.25 (30.79)	0.00 (0.00)	31.75 (31.20)
3	Bb4644	48.75 (44.24)	25.00 (29.93)	20.00 (26.47)	18.75 (25.52)	0.00 (0.00)	22.50 (25.35)
4	Commercial isolate	43.75 (41.36)	25.00 (29.84)	18.75 (25.30)	17.50 (24.52)	0.00 (0.00)	19.75 (23.53)
5	Bb5408	40.00 (39.20)	22.50 (28.19)	20.00 (26.33)	17.50 (24.44)	0.00 (0.00)	17.50 (21.79)
6	Bb 5412	41.25 (39.80)	22.50 (28.27)	18.75 (25.52)	16.25 (23.49)	0.00 (0.00)	18.00 (22.17)
7	Bb7130	37.50 (37.63)	27.50 (31.59)	13.75 (21.55)	11.25 (19.52)	0.00 (0.00)	20.00 (23.74)
8	Bb7126	36.25 (36.89)	26.25 (30.55)	13.75 (21.69)	11.25 (19.22)	0.00 (0.00)	21.00 (24.32)
	Overall mean	46.56 (43.02)	30.16 (32.99)	22.66 (27.83)	19.06 (25.35)	0.00 (0.00)	
	CD (p = 0.05)	Isolates (A) = (3.01), Concentration (B) = (2.38) and A × B = (6.74)					

*Mean of 4 replications (each replication consisted of 20 larvae of *C. partellus*)
Figures in parentheses are mean of arc sine √percentage transformed values

Table 2. Virulence of selected isolates of *Beauveria bassiana* against *Chilo partellus* in terms of time

Sr. No.	Treatments	Concentration of spore suspension of different isolates			
		1×10^{10} spores/ml	1×10^{8} spores/ml	1×10^{7} spores/ml	1×10^{6} spores/ml
		Per cent mortality* ± S.E.	Per cent mortality* ± S.E.	Per cent mortality* ± S.E.	Per cent mortality* ± S.E.
1.	PAU Bb	68.75 ± 5.54	50.00 ± 4.56	42.50 ± 4.33	33.75 ± 3.15
2.	Bb4668	56.25 ± 5.54	42.50 ± 4.33	33.75 ± 3.75	25.00 ± 1.25
3.	Bb4644	48.75 ± 3.75	25.00 ± 2.04	20.00 ± 2.04	18.75 ± 2.39
4.	Commercial isolate	43.75 ± 5.54	25.00 ± 2.04	18.75 ± 2.39	17.50 ± 2.50
5	Bb5408	40.00 ± 2.04	22.50 ± 2.50	20.00 ± 3.54	17.50 ± 1.44
6.	Bb 5412	41.25 ± 3.75	22.50 ± 1.44	18.75 ± 2.39	16.25 ± 3.15
7.	Bb7130	37.50 ± 4.33	27.50 ± 1.44	13.75 ± 2.39	11.25 ± 1.25
8.	Bb7126	36.25± 3.23	26.25 ± 4.27	13.75 ±1.25	11.25 ± 2.39

*Mean of 4 replications (each replication consisted of 20 larvae of *C. partellus*)

S. E.: Standard error

Table 3. Concentration-mortality response of *Beauveria bassiana* against *Chilo partellus*

Sr. No.	Treatments	LC_{50}	Fiducial range (95%)	Slope	χ^2	Virulence ratio
1.	PAU Bb	5.93×10^{5}	$1.43 \times 10^{5} - 2.20 \times 10^{6}$	0.22 ± 0.02	3.48	56.49
2.	Bb4668	4.67×10^{6}	$1.20 \times 10^{6} - 1.77 \times 10^{7}$	0.21 ± 0.02	2.21	7.17
3.	Commercial isolate	3.35×10^{7}	$7.71 \times 10^{6} - 1.57 \times 10^{8}$	0.19 ± 0.02	0.95	1

LC_{50}: Lethal concentration-50

Table 4. Efficacy of *Beauveria bassiana* against maize stem borer, *Chilo partellus* under artificial infestation in screen house conditions during 2015

Sr. No.	Treatments	Mean * incidence of *C. partellus*						
		Days after artificial infestation of *C. partellus*						
		7		14		21		25
		Leaf injury** (%)	Deadhearts** (%)	Leaf injury** (%)	Deadhearts** (%)	Leaf injury** (%)	Deadhearts ** (%)	Mean LIR***
1.	*Beauveria bassiana* (PAU Bb isolate)	68.00 (55.81)	0.00 (0.00)	24.00 (29.09)	24.00 (29.09)	20.00 (26.55)	28.00 (31.62)	5.96 (0.49)
2.	*Beauveria bassiana* (Bb 4668)	72.00 (58.34)	0.00 (0.00)	36.00 (36.88)	32.00 (34.15)	24.00 (29.09)	36.00 (36.68)	6.64 (0.89)
3.	Isolate from commercial formulation	88.00 (74.03)	0.00 (0.00)	36.00 (36.88)	36.00 (36.68)	24.00 (29.09)	40.00 (39.22)	6.82 (0.91)
4.	Decis 2.8 EC (deltamethrin)	40.00 (39.22)	0.00 (0.00)	16.00 (23.30)	8.00 (10.62)	12.00 (15.93)	8.00 (10.62)	4.20 (0.09)
5.	Infested control	92.00 (79.34)	0.00 (0.00)	12.00 (20.05)	76.00 (60.88)	4.00 (5.31)	84.00 (68.72)	8.48 (1.41)
6.	Uninfested control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.00 (−1.49)
	CD (p = 0.05)	(12.14)	(NS)	(11.29)	(10.05)	(10.87)	(11.09)	(0.39)

* Mean of 5 replications

** Figures in parentheses are mean of arc sine √percentage transformed values

*** Figures in parentheses are mean of interpolated transformed values (ITV) according to Fisher and Yates (1976)

respectively) and Bb 4668 (24.00 and 36.00%, respectively) isolates of *B. bassiana.* However, LIR of infested plants was significantly lower on the plants sprayed with spore suspension of PAU Bb isolate (5.96) than the plants sprayed with the spore suspension of Bb 4668 and commercial isolate of *B. bassiana* (6.64–6.82) which was due to the lower deadhearts incidence in the earlier treatment. The plants in the infested control treatment exhibited minimum leaf injury (4.00%) but maximum deadhearts (84.00%) incidence and maximum LIR (8.48) of *C. partellus*. The lower LIR values in the *B. bassiana* isolates as compared to infested control treatment might be due to reduction in feeding of *C. partellus* larvae infected with *B. bassiana*, which has already been reported by different workers (Tefera and Pringle, 2004b).

The results of the experiment under screen house conditions clearly showed that the efficacy of *B. bassiana* isolates was lower than Decis which may be due to the quick knock down action against artificially infested larvae. The lower efficacy of *B. bassiana* isolates as compared with the tested insecticides had also been reported by Rameash *et al*. (2012). However, the higher efficacy of *B. bassiana* isolates as compared to tested insecticides against a target pest has also been reported (Maniania, 1993; Godonou *et al.,* 2009) which may be due to the favourable environmental conditions prevailing there, as the efficacy of entomopathogenic fungi depends on proper environmental conditions. Out of all the tested pathogenic isolates of *B. bassiana,* the PAU Bb isolate was most virulent against *C. partellus*. However, the efficacy of this isolate needs improvement which may be possible by using formulation that can improve pre and post application stability, persistence, efficacy and facilitate its easy delivery to the target pest.

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