

## Screening of *Beauveria bassiana* (Balsamo) Vuillemin Isolates Against Maize Stem Borer, *Chilo partellus* (Lepidoptera: Pyralidae) and the Effect of Solid Substrates on Conidial Production and Virulence

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In the present study, eighty seven isolates of *Beauveria bassiana* obtained from NBAIR culture collection were screened against maize stem borer, *Chilo partellus* in laboratory bioassay. Six *B. bassiana* isolates (Bb-5a, 7, 19, 45, 14 and 23) showed higher mortality (77.25-100%) and mycosis (71.11-97.78%). These isolates were further subjected to dose and time mortality tests. Among the six isolates tested, Bb-45 showed the least LC<sub>50</sub> (4.4x10<sup>5</sup> conidia/ml) and LT<sub>50</sub> (136 hrs) on second instar of *C. partellus*. Among the different substrates tested, rice supported higher conidial production of promising isolates of *B. bassiana* (1.66-2.83g conidia/100g of substrate) and also induced production of virulent conidia for all the isolates.

**Key words:** *Beauveria bassiana*, Biocontrol, *Chilo partellus*, LC<sub>50</sub>, LT<sub>50</sub>, *Zea mays* L.

Maize (*Zea mays* L.) is one of the most important cereal crops grown in India. More than 140 insect pests cause damage in maize (Jalali and Singh, 2003). Among them, lepidopteron pest, *Chilo partellus* (Swinhoe) is the one of most important borer pests of maize. It also infests other crops like, sorghum (*Sorghum bicolor* Moench) and Napier grass (*Pennisetum purpureum* Schumach) (Charles Midega *et al.*, 2011). It causes damage in leaf, stem, tassel and ear leading to significant reduction in yield of maize (Prem Nidhi Sharma and Purushottam Gautam, 2010), about 90-95 per cent of the damage observed in *kharif* season. Several insecticides like, cypermethrin, deltamethrin, endosulfan, triazophos and carbofuran are used for controlling the borer pest (Ganguli *et al.*, 1997). It is difficult to control the pest by using insecticides, because of multiple generations,

prolonged emergence pattern and cryptic life cycle of the pest. Using chemical insecticides to control of stem borers by small-scale farmers is uneconomical and chemical insecticides may cause ecological problems. Alternate methods of management using biocontrol agents (parasites, predators and pathogens) were also attempted in recent times.

One such alternative for stem borer management is the entomopathogenic fungus (EPF) *Beauveria bassiana* (Balsamo) Vuillemin, a deuteromycete fungus which has major advantages for insect biological control, the reasons for interest in this fungus is its mode of infection, reproduction in target insects and safety to non-target organisms (Jianzhong Sun *et al.*, 2003). It also has the ability to colonize endophytically in maize (Wagner and Lewis, 2000) and it is not proved as a plant pathogen. For the past 60 years, it has been used as a biocontrol agent (Bing and Lewis, 1993). The present study was carried out to identify the promising isolates

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of *B. bassiana* against *C. partellus* through laboratory bioassay studies and to identify the suitable solid substrates for virulent conidial production.

## MATERIALS AND METHODS

### Insect

Field collected *C. partellus* larvae were reared in the laboratory according to the protocol Chandish R Ballal *et al.*, 1995. Larvae were transferred into an artificial diet and were pupated on the diet and the pupae were transferred into a plastic container. Newly emerged male and female adults were transferred into another plastic container and fed on cotton pads soaked with 50% honey for stimulation of oviposition. Eggs were incubated at 25°C and then newly hatched larvae were transferred to an artificial diet. Second instar larvae were used for laboratory bioassay studies.

### Fungal cultures

Eighty seven isolates of *B. bassiana* collected from different insect hosts and soil samples from various geographical areas of India maintained at National Bureau of Agricultural Insect Resources (ICAR-NBAIR) culture repository, Bangalore were used in the laboratory bioassay studies. These fungal cultures were grown on Sabouraud's Dextrose Yeast Agar medium (SDYA) slants and stored at -20°C until further use.

### Preparation of conidial suspension

Conidial suspension of each isolate was prepared by suspending one gram of 15 days old conidiated rice in sterile distilled water with 0.1% Tween 80. Suspension was filtrated through three layers of muslin cloth to get hyphal-free conidial suspension. The conidial concentration in the suspension was adjusted to  $1 \times 10^7$  conidia/ml using Neubauer's improved haemocytometer.

### Bioassays

#### Single concentration bioassay

The experiment was carried out according to the protocol of Safavi *et al.*, (2010). Conidial suspensions ( $1 \times 10^7$  conidia/ml) of eighty seven isolates were prepared for bioassay studies. Fifteen numbers of second instar larvae of *C. partellus* larvae were dipped in one ml of conidial suspension of each isolate for 30 seconds. Control larvae were treated with distilled water containing 0.1% Tween 80. All treatments were replicated three times.

Treated larvae were transferred into a sterile plastic container and fed on maize leaf bits. Larval mortality was recorded up to 10 days at 24h intervals. The dead larvae were transferred to sterile moist paper in sterile petri dishes to facilitate mycosis. The per cent mortality of *C. partellus* was calculated after deducting the control mortality using Abbott's formula (Abbott, 1925). Based on this study, promising isolates were identified for further studies.

### Dose and time mortality studies

The experiment was conducted to estimate the dose and time to kill 50 per cent of the population ( $LC_{50}$  &  $LT_{50}$ ) of 2<sup>nd</sup> instar larvae of *C. partellus* by six isolates of *B. bassiana* (Bb-5a, 7, 14, 19, 23 and 45). Conidial concentrations of  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ , and  $10^8$  conidia  $ml^{-1}$  of each isolate were prepared and bioassays were carried out on second instar of larvae of *C. partellus* as described above in single concentration bioassay procedure. The dose and time to kill 50 per cent of the population ( $LC_{50}$  &  $LT_{50}$ ) was determined by probit analysis (Finney, 1971).

### Conidial production of *B. bassiana* isolates on different solid substrates

Rice, wheat, sorghum and finger millet (*Eleusine coracana*) were selected for substrate evaluation for conidial production of the six isolates (Bb-5a, 7, 14, 19, 23 and 45).

### Preparation of inocula of *B. bassiana* for inoculation on solid substrates

Each of *B. bassiana* isolate was grown 25 ml of Sabouraud's Dextrose Yeast Broth (SDYB) in 100 ml conical flask at 25°C temperature in a rotary shaker at 150 rpm for five days. The hyphal cum mycelial suspension from the flask was used as inoculum.

### Preparation of solid substrates

Approximately 100g of each solid substrate (three replications / substrate) was washed thoroughly and soaked overnight in distilled water in polypropylene bags. After soaking, excess water was drained and bags were sterilized in an autoclave at 121°C for 20 minutes. After sterilization, substrates were cooled and then inoculated with 10ml of the respective inoculum. The bags were sealed and incubated at 25°C for 15 days (Nirmala *et al.*, 2005).

### Conidial harvest and spore yield estimation

After 15 days of incubation, the

conidiated substrates were dried for 48h at room temperature (25-30°C) under aseptic conditions. The dried conidia were harvested from the substrate by passing through a sieve (300  $\mu$ m) (Nirmala *et al.*, 2005). The quantity of harvested conidia from 100g of each of the substrates was recorded. The conidial load per gram substrate was estimated using Neubauer's improved haemocytometer.

### Virulence of the conidia produced on different substrates

Virulence of the conidia harvested from different solid substrates was tested against second instar *C. partellus* larvae as described earlier in single concentration bioassay procedure

### Statistical analysis

The data of mortality, mycosis and spore count data were subjected to the statistical analysis using SPSS windows version 16.0.

## RESULTS

### Single concentration bioassay

The larval mortality (%) and mycosis (%) observed after 10 days of treatment are presented in Table 1 & 2. Significant differences in the larval mortality (%) and mycosis (%) were observed among the different isolates screened. Isolates of Bb-7, 14, 19, 23 and 45 showed significantly higher mortality (86.40-100%) and the rest of the isolates showed 2.92-77.42% mortality (Table 1). With regard to mycosis, Bb-7, 14, 23 and 45 showed significantly higher mycosis (84.44-97.78%) and the rest of the isolates showed lower mycosis in the range of 2.22-71.11% (Table 1). Isolates of Bb-7, 14, 19, 23, 45 and 5a were identified for further detailed studies based on higher per cent mortality (>75%) and mycosis (>70%) (Table 2).

### Dose and time mortality studies

The  $LC_{50}$  values of Bb-7, 14, 19, 23, 45 and 5a isolates ranged from  $4.4 \times 10^5$ - $4.3 \times 10^7$  conidia/ml (Table 3). Among these, Bb45 showed the lowest  $LC_{50}$  value ( $4.4 \times 10^5$  conidia/ml). The time taken for each of the six isolates to kill 50% of the larvae is given in Table 3. The results indicated that Bb45 isolate showed the lowest  $LT_{50}$  values (136 hrs) and Bb19 isolate had the highest  $LT_{50}$  value (522 hrs). The  $LT_{50}$  values of rest of the isolates were in the range of 169-248hrs.

Table 1. Effect of different isolates of *B. bassiana* on the larval mortality and mycosis of *C. partellus*.

Isolates	% Mortality	Isolates	% Mycosis
Bb-7,14,23,45	86.41-100 <sup>a</sup>	Bb-7,14,23,45	84.44-97.78 <sup>a</sup>
Bb-19	86.40 <sup>a</sup>	Bb-19	71.11 <sup>b</sup>
Bb-5a,8,42	59.58-77.42 <sup>b</sup>	Bb-5a,8,42	55.56-71.11 <sup>b</sup>
Bb-30	57.15 <sup>b</sup>	Bb-30	46.67 <sup>c</sup>
Bb-5b,5c,8a,10,11,12,13,18,22,24,26,28,37,43,65,77	36.73-54.79 <sup>c</sup>	Bb-5b,5c,8a,10,11,12,13,18,22,24,26,28,37,43,65,77	26.67-48.89 <sup>c</sup>
Bb-9,16,27,34,41a,62,71,72	34.58-38.98 <sup>c</sup>	Bb-9,16,27,34,41a,62,71,72	28.89-36.67 <sup>d</sup>
Bb-1,4,5,6,15,20,29,31,32,33,39,41b,59,69,73,74,76,78,80	14.30-32.34 <sup>d</sup>	Bb-1,4,5,6,15,20,29,31,32,33,39,41b,59,69,73,74,76,78,80	11.11-26.67 <sup>d</sup>
Bb-58,60,67	14.34-21.17 <sup>d</sup>	Bb-58,60,67	6.67-17.78 <sup>e</sup>
Bb-54,57	12.01-14.32 <sup>e</sup>	Bb-54,57	11.11-13.33 <sup>d</sup>
Bb-2,3,17,25,34a,35,38,40,47,48,51,52,53,55,56,64,68,75,81,82	2.92-14.32 <sup>e</sup>	Bb-2,3,17,25,34a,35,38,40,47,48,51,52,53,55,56,64,68,75,81,82	2.22-8.89 <sup>e</sup>
Bb-44,46,49	0.00	Bb-44,46,49	0.00

\*Values in columns followed by the different letter are significantly different with each other according to LSD (P < 0.01)

### Conidial production on different solid substrates

Among the different substrates tested, rice supported higher conidial production for Bb-7, 14, 23, 45 and 5a isolates (1.66-2.83 g conidia / 100g of substrate), where as for Bb19 isolate, wheat and sorghum supported higher conidial production (1.04 and 1.0g conidia/100g of substrate respectively) (Table 4). Significantly higher conidial load per gram was observed on rice for Bb7, 14, 19, 5a and 23 isolates ( $4.85-18.95 \times 10^8$  conidia/g of substrate), where as sorghum gave higher conidial load ( $5 \times 10^8$  conidia/g of substrate) for Bb45 isolate. Rice has been identified as promising substrate for conidial production of Bb-5a, 7, 14 and 23 isolates based on quantity of conidia harvested from 100g of substrate and conidial load per gram substrate. Sorghum and wheat substrates were identified as ideal substrates for conidial production of Bb19 isolate, where as for Bb45 isolate sorghum has been identified as promising substrate for conidial production.

### Virulence of the conidia produced on different substrates

Conidia of the Bb-5a, 7, 19, 14 and 23 isolates produced on rice showed higher mortality (77.25-100%) and mycosis (71.11-97.78%) of *C. partellus* compared the virulence of the conidia produced on other substrates (18.14-93.14% mortality and 20.0-88.89% mycosis) (Table 5). Conidia of Bb45 isolate produced on sorghum showed higher mortality (97.74%) than the conidia produced on rice (90.88%), however the % mycosis observed with conidia produced on rice and

sorghum were on par with each other (88.89%). The conidia produced on wheat and finger millet showed lower mortality (18.14-47.71% and 22.74-56.83% respectively) and mycosis (20.0-46.67% and 22.22-57.78% respectively) for all isolates (Table 5).

## DISCUSSION

There was significant variation in the virulence of the eighty seven isolates on *C. partellus* (Table 1). Among the eighty seven isolates, Bb5a, 7, 14, 19, 23 and 45 showed significantly higher mortality and mycosis of *C. partellus*. These isolates were originally isolated from insect cadaver and soil samples from different geographic areas (Table 2). According to Goettel *et al.*, (1990) isolates showed higher virulence towards the same or related host species from which they derived. But our studies indicated that higher virulence was observed even in the isolates that have been derived from the soil and non-related host species. Uma Devi *et al.*, (2001) reported that *B. bassiana* isolates which derived from different Coleopteran hosts showed less virulence against *C. partellus*.

In the present study, conidial production of six isolates of *B. bassiana* on four cereal substrates (rice, wheat, sorghum and finger millet) was evaluated. Significant differences in the conidial production of different isolates on different substrates were observed. The conidial yield may be affected by innate characteristics of the isolates,

**Table 2.** Promising isolates of *B. bassiana* identified against *C. partellus*

S. No	Isolate Code	Genbank Accession number	Source	% Mortality $\pm$ SE	% Mycosis $\pm$ SE
1	NBAII-Bb23	JF837082	<i>Maruca testulalis</i> (Legume pod borer)	100.00 $\pm$ 0.00 <sup>a</sup>	97.78 $\pm$ 9.56 <sup>a</sup>
2	NBAII-Bb14	JF837092	Unknown insect	93.10 $\pm$ 2.68 <sup>a</sup>	84.44 $\pm$ 2.30 <sup>a</sup>
3	NBAII-Bb45	JF837094	Soil	90.88 $\pm$ 1.62 <sup>a</sup>	88.89 $\pm$ 2.30 <sup>a</sup>
4	NBAII-Bb19	KC121555	Soil	86.40 $\pm$ 1.70 <sup>a</sup>	71.11 $\pm$ 1.12 <sup>b</sup>
5	NBAII-Bb7	JF837097	<i>Plocaederus ferrugineus</i> (Cashew root & stem borer)	86.36 $\pm$ 3.69 <sup>a</sup>	80.00 $\pm$ 3.12 <sup>b</sup>
6	NBAII-Bb5a	JF837134	<i>Hypothenemus hampei</i> (Coffee berry borer)	77.25 $\pm$ 3.25 <sup>b</sup>	71.11 $\pm$ 3.48 <sup>b</sup>

\*Values in columns followed by the different letter are significantly different with each other according to LSD (P < 0.01).

Table 3. Dose and time mortality response of *C. partellus* to six isolates of *B. bassiana*

isolates	LC <sub>50</sub> (Conidia/ml)	95% fiducial limit	Slope±SE	X <sup>2</sup>	P value	Isolates	LT <sub>50</sub> (hrs)	95% fiducial limit	slope±SE	X <sup>2</sup>	P value
Bb45	4.4X10 <sup>5</sup>	8.6X10 <sup>4</sup> -2.7X10 <sup>6</sup>	4.602±0.494	7.94	0.047	Bb45	136.25	98.04-556.31	0.0155±0.0060	6.60	0.0102
Bb14	1.1X10 <sup>6</sup>	2.7X10 <sup>5</sup> -5.4X10 <sup>6</sup>	5.673±0.570	7.64	0.054	Bb7	169.28	124.24-596.94	0.0125±0.0047	7.14	0.0075
Bb7	1.1X10 <sup>6</sup>	3.3X10 <sup>5</sup> -3.8X10 <sup>6</sup>	5.047±0.509	1.43	0.698	Bb23	186.75	135.02-616.79	0.0094±0.0034	7.82	0.0052
Bb23	1.3X10 <sup>6</sup>	2.7X10 <sup>5</sup> -7.97X10 <sup>6</sup>	5.232±0.533	8.16	0.043	Bb14	199.01	134.79-5373.49	0.0079±0.0033	5.54	0.0186
Bb5a	1.7X10 <sup>6</sup>	1.06X10 <sup>6</sup> -3.42X10 <sup>6</sup>	4.740±0.499	2.50	0.475	Bb5a	247.60	201.72-921.34	0.0066±0.0032	4.42	0.0355
Bb19	4.3X10 <sup>7</sup>	2.31X10 <sup>7</sup> -1.02X10 <sup>8</sup>	6.425±0.860	3.52	0.317	Bb19	522.39	489.20-1034.51	0.0035±0.0026	1.77	0.1836

\*All the mean values were angular transformed (Log<sub>10</sub>) before subjected to analysis.

Table 4. Conidial production on different solid substrates

Substrate	Bb5a		Bb7		Bb14		Bb19		Bb23		Bb45	
	1	2	1	2	1	2	1	2	1	2	1	2
Rice	2.04 <sup>a</sup>	9.85 <sup>a</sup>	1.66 <sup>a</sup>	18.95 <sup>a</sup>	2.83 <sup>a</sup>	11.95 <sup>a</sup>	0.61 <sup>b</sup>	9.95 <sup>a</sup>	1.85 <sup>a</sup>	4.85 <sup>a</sup>	2.27 <sup>a</sup>	4.00 <sup>b</sup>
Sorghum	0.66 <sup>b</sup>	0.70 <sup>b</sup>	1.25 <sup>b</sup>	0.65 <sup>c</sup>	0.91 <sup>b</sup>	0.85 <sup>b</sup>	1.00 <sup>a</sup>	0.15 <sup>c</sup>	0.49 <sup>c</sup>	0.20 <sup>d</sup>	1.32 <sup>b</sup>	5.00 <sup>a</sup>
Wheat	0.41 <sup>b</sup>	0.60 <sup>c</sup>	1.15 <sup>b</sup>	0.15 <sup>d</sup>	1.20 <sup>b</sup>	0.20 <sup>c</sup>	1.04 <sup>a</sup>	5.70 <sup>b</sup>	0.94 <sup>b</sup>	3.00 <sup>b</sup>	1.00 <sup>b</sup>	3.60 <sup>c</sup>
Finger millet	0.55 <sup>b</sup>	0.80 <sup>b</sup>	0.40 <sup>c</sup>	5.50 <sup>b</sup>	0.86 <sup>b</sup>	0.35 <sup>c</sup>	0.15 <sup>c</sup>	0.15 <sup>c</sup>	0.82 <sup>b</sup>	0.90 <sup>c</sup>	0.86 <sup>c</sup>	1.0 <sup>d</sup>

\*Values in columns followed by the different letter are significantly different with each other according to LSD (P <0.01).  
1-Quantity of harvested spore (g) from 100g of substrate (g/100g)  
2-Spore/g of substrate (10<sup>8</sup>/g)

production phase (solid/liquid) and incubation period (Taylor *et al.*, 2013). Nelson *et al.*, (1996) reported higher conidial production on rice for *Beauveria brongniartii*, but percentage viable conidia were less. In contrast, we found that, rice supported higher viable conidial production of *B. bassiana* isolates. Similar observation was reported by Thet Thet Mar *et al.*, (2012), Masoud Latifian *et al.*, (2013) and Sivakalai and Ramanathan, (2015).

The present study reported that the conidia of *B. bassiana* isolates produced on different solid substrates showed significant differences in their virulence against *C. partellus*. The differences in the virulence of the conidia of entomopathogenic fungi that were produced on different solid substrates may be due to varying C:N ratios in the substrates. According to Rodríguez-Gomez *et al.*, (2009), nutritional factors and environmental growth conditions had an impact on virulence of entomopathogenic fungi, the carbon and nitrogen sources of the substrates were one of the virulent parameters and *B. bassiana* conidia produced on rice showed higher virulence than conidia produced on insect cadaver. Francisco and Posada (2008) also reported that, *B. bassiana* conidia produced on rice substrate showed higher virulence against *Hypothenemus hampei*. Our results also indicate that the conidia of *B. bassiana* produced on rice substrate showed higher virulence towards *C. partellus*. According to Asghar Mohammadbeigi (2013), under starvation conditions, fungal virulence get enhanced because of the increased production of cuticle degrading enzyme protease pr1 a virulent determinant. The increased level of this enzyme leads to higher virulence, where as it gets depressed under nutrient rich condition which lowers the virulence. The nutrients in the rice may get depleted quickly leading to the starvation of the entomopathogenic fungus which may result in virulent conidial production. According to Shah *et al.*, (2005), virulent conidia had endogenous CN ratio less than 5.2:1 and elevated level of spore bound protease pr1. Tamarai chelvi *et al.*, 2010 reported higher virulence of *B. brongniartii* conidia produced on cow pea on *Holotrichia serrata*. According to Parisa Bena-Molaei *et al.*, (2011), conidia of *B. bassiana* harvested from wheat showed higher

**Table 5.** Virulence of the conidia of *B. bassiana* obtained from different substrates against *C. partellus*

Substrate	Bb-5a		Bb-7		Bb-14		Bb-19		Bb-23		Bb-45	
	1	2	1	2	1	2	1	2	1	2	1	2
Rice	77.25 <sup>a</sup>	71.11 <sup>a</sup>	86.36 <sup>a</sup>	80.00 <sup>a</sup>	93.10 <sup>a</sup>	84.44 <sup>a</sup>	86.40 <sup>a</sup>	71.11 <sup>a</sup>	100.00 <sup>a</sup>	97.78 <sup>a</sup>	90.88 <sup>b</sup>	88.89 <sup>a</sup>
Sorghum	61.35 <sup>b</sup>	57.78 <sup>b</sup>	81.80 <sup>a</sup>	75.56 <sup>a</sup>	81.84 <sup>b</sup>	75.56 <sup>b</sup>	61.27 <sup>b</sup>	37.78 <sup>b</sup>	93.14 <sup>b</sup>	88.89 <sup>b</sup>	97.74 <sup>a</sup>	88.89 <sup>a</sup>
Wheat	20.44 <sup>d</sup>	22.22 <sup>d</sup>	40.89 <sup>b</sup>	40.00 <sup>b</sup>	29.55 <sup>c</sup>	31.11 <sup>c</sup>	18.14 <sup>d</sup>	18.14 <sup>c</sup>	38.63 <sup>c</sup>	37.78 <sup>d</sup>	47.71 <sup>d</sup>	46.67 <sup>c</sup>
Finger millet	31.81 <sup>c</sup>	31.11 <sup>c</sup>	34.07 <sup>b</sup>	35.56 <sup>b</sup>	22.74 <sup>c</sup>	22.22 <sup>d</sup>	27.22 <sup>c</sup>	22.22 <sup>c</sup>	45.41 <sup>c</sup>	46.67 <sup>c</sup>	56.83 <sup>c</sup>	57.78 <sup>b</sup>

\*Values in columns followed by the different letter are significantly different with each other according to LSD ( $P < 0.01$ ).  
1: % Mortality, 2: % Mycosis



virulence to brown tail moth, *Euproctis chrysorrhoea*.

In the present study, we have identified the promising isolates of *B. bassiana* (Bb-5a, 7, 14, 19, 23 and 45) which can be further field tested for management of maize stem borer *C. partellus*. These isolates can be mass produced on rice as it supports virulent conidial production. Furthermore these *B. bassiana* isolates can be used for establishing as endophytes in maize for management of stem borer.

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