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DN Kambrekar
Department of Agricultural Entomology University of Agricultural Sciences, Dharwad, Karnataka, India

J Aruna
Department of Agricultural Entomology University of Agricultural Sciences, Dharwad, Karnataka, India

Correspondence
DN Kambrekar
Department of Agricultural Entomology University of Agricultural Sciences, Dharwad, Karnataka, India

Isolation of *Beauveria bassiana* from different host plants and its pathogenicity against sorghum stem borer, *Chilo partellus* swinhoe

DN Kambrekar and J Aruna

Abstract
The present experiment was conducted for isolation, identification of endophytic fungi, *Beauveria bassiana* (Balsamo) Vuillemin from several field crops and their pathogenicity against sorghum stem borer, *Chilo partellus* Swinhoe during June 2014 to March 2016 at Regional Agricultural Research Station (RARS), Vijayapur, Karnataka, India. The experimental results revealed that, the endophytic *B. bassiana* is known to present in different parts of the different plants i.e. maize stem, leaves of chickpea, chilli, green gram, citrus, pomegranate fruit, bean pod, cauliflower head and banana pseudo stem. The pathogenicity studies revealed that the endophytic *B. bassiana* isolated from leaves and stem of the sorghum and maize was found to be pathogenic to sorghum stem borer, *Chilo partellus* and army worm, *Mythimna separata*, from cabbage and cauliflower was pathogenic to diamond back moth, *Plutella xylostella*, and endophytic *Beauveria* from leaves and fruit of tomato and chilli leaves was pathogenic to chickpea pod borer, *Helicoverpa armigera*. Further, endophytic *B. bassiana* was evaluated for the pathogenicity against sorghum stem borer at different doses ($1x10^8$ conidia ml^{-1}) (0.1, 0.2, 0.4, 0.8, 1 and 2 ml per litre). Among all the treatments least population of sorghum stem borer larvae were observed in *B. bassiana* ($1x10^8$ conidia ml^{-1}) @ 2 ml at 10 days after treatment (4.00 larvae) followed by 7 days after treatment (4.67 larvae). While, highest number of larvae were observed in *B. bassiana* ($1x10^8$ conidia ml^{-1}) @ 0.1 ml (8.33 larvae) @ 10 DAT which was on par with untreated control (10 larvae). Further, Endophytic *B. bassiana* could cause larval mortality up to 60.00 per cent at 10 days after treatment. Hence, endophytic *B. bassiana* present in different parts of the different plants can be largely exploited for the controlling different insect pest in eco-friendly manner.

Keywords: *Beauveria bassiana*, endophytes, sorghum stem borer, *Chilo partellus*, pest management

Introduction
Sorghum (jowar) is an important nutrition cereal constituting staple diet in the country [6]. India contributes about 16 % of the world's sorghum production. It is the fourth most important cereal crop in the country. Sorghum grain yields on farmer's fields in Asia and Africa are generally low (500-800 kg/ha) mainly due to insects, diseases, weeds and drought [1]. Nearly 150 insect species have been reported as pests on sorghum [2, 3]. Sorghum is vulnerable to over 150 insect species from sowing to the final crop harvest [4] which includes, Shoot fly (*Atherigona soccata* Rondani), stem borer (*Chilo partellus* Swinhoe), army worm (*Mythimna separat*a (Walker)), shoot bug (*Peregrinus maidis* Ashmead), aphids *(Rhopalosiphum maidis* (Fitch.) and *Melanaphis sacchari* Zehntner), spider mites (*Oligonychus* spp.), grasshoppers and locusts (*Heiroglyphus* sp., *Oedaleus* sp., *Aiolopus* sp., *Schistocerca* sp. and *Locusta* sp.), sorghum midge (*Stenodiplosis sorghicola* Coquillett), mirid bug (*Calocoris angustatus* Lethiery) and panicle-feeding caterpillars (*Helicoverpa armigera* Hubner, *Eublemma* sp., *Cryptoblabes* sp., *Pyroderces* sp., and *Nola* sp.) are the major pests of sorghum worldwide [4].

One group of biological control agents that provide a source for novel pest control is the mutualistic microbial symbionts, which are termed as "Endophytes". Entomopathogenic endophytes play vital role in reducing pest load by imparting host defense mechanisms against various crop insect pests. In recent years, entomopathogenic endophytes have been explored for pest management [5]. Some endophytes belong to genera that include fungal entomopathogens such as *Beauveria* has been reported as an endophyte in maize [6, 7], potato, in sorghum, chilli, sunflower and beans [7]. In addition, cocoa [8] and coffee seedlings [9] have been successfully inoculated with *Beauveria bassiana* (Bals.-Criv.) by depositing a spore

suspension on the radicle shortly after germination. Referring to *B. bassiana*, Steinhaus (1949) wrote, ''It also grows on corn and certain other plants but not so well as on insects [10].'' The entomopathogenic fungus *B. bassiana* contains a diverse assemblage of genotypes and probably comprises species complexes. Therefore, it is conceivable to have individual isolates or pathotypes which exhibit a substantially restricted host range. Unique endophytes could be used directly to treat seeds or transplants. The capability of colonizing internal host tissues has made endophytes valuable for agriculture as a tool to improve crop protection. In the recent years, biological control of insect pests using endophytic entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin has been receiving research attention. In view of this, the present experiment was conducted with a view of isolation of endophytic entomopathogen, *B. bassiana* and to examine its pathogenicity against sorghum stem borer, *Chilo partellus* Swinhoe.

2. Material and methods

The present experiment was conducted at Regional Agricultural Research Station, College of Agriculture, Vijayapur during 2014-16. The plant samples were collected from different location of different zones at particular season and brought to the laboratory for further isolation and pathogenicity studies. The materials used and methodologies followed are as given below.

2.1 Isolation of Microbial Endophyte, *B. bassiana* isolated from different plant/plant parts

2.1.1 Collection of different plant/ plant parts

Different plants/plant parts were collected from the surrounding villages during the year 2014 for isolation of microbial endophytes i.e. *Beauveria bassiana* which includes cotton leaves were collected from Dharwad (Zone 8) region during the month of August, cauliflower heads were collected from Ranebennur region during November, chilli leaves were collected from Haveri and Ranebennur region during November, fruits and leaves from pomegranate were collected from Vijaypur region during the month of June, green gram leaves were collected from Vijaypur during the month of August, bean pods were collected from Ranebennur region during month of November, leaves of *rabi* sun flower, mango leaves were collected from Bagalkot region during the month of July, chick pea leaves were collected from Annigeri region during the month of December and brinjal leaves were collected from Haveri and Ranebennur region during month of November and December.

2.1.2 Sterilization

All the glassware were sterilized in an autoclave at 121 °C 15 lbs pressure for 15 min and then kept in hot air oven at 55 °C for one hour. The plants parts like leaves, fruits, head and stems collected from the different locations were brought to the laboratory and were excised with sterilized knife in laminar air flow chamber. Each explant of leaves and stems were treated with double distilled water for 2-3 minutes. It was then surface sterilized with 0.1% sodium hypochlorite for 5 minutes. Again the explants were treated with double distilled water for 2-3 minutes. Later, were surface sterilized with 0.01 % Bavistin. The explants were kept in distilled water for 5 minutes. For further sterilization the explants were exposed to 0.05 % streptomycin to prevent the growth of bacterial mould followed by treatment with double distilled water for 5 minutes. Then the explants were exposed to 70 % ethanol and again kept in double distilled water for 5 minutes and were then air dried in laminar flow.

2.1.3 Preparation of PDA media

Potato infusion can be made by boiling 200 grams of sliced (washed but unpeeled) potatoes in ~ 1 litre distilled water for 30 minutes and then decanting or straining the broth through cheese cloth. Distilled water is added such that the total volume of the suspension is 1 litre. 20 grams dextrose and 20 grams agar powder is then added and the medium is sterilized by autoclaving at 121 °C 15 lbs for 15 minutes.

2.1.4 Isolation of pure culture

Sterilized Petri plates were taken and PDA media was poured uniformly in the Petri - plates. The plant parts were cut into small bits and were surface sterilized with 1:1000 Mercuric chloride solutions for 30 seconds and washed three times in sterile distilled water before transferring them to Potato Dextrose Agar (PDA). Then the piece of plant sample was kept in the centre of Petri plates. The plates were incubated at room temperature (28 ± 2 °C) and observed periodically for fungal growth. The colonies which developed from the tissue bits were transferred to PDA slants. The purity and sporulation of the culture by examined under microscope.

2.1.5 Identification of pure culture

The isolated pure cultures were identified at Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad.

2.1 Pathogenicity of endophyte, *Beauveria bassiana* against target insect pests:

2.2.1 Test Insects

The larvae of sorghum stem borer, *Chilo partellus* (Swinhoe) were maintained in the laboratory at 28 ± 2 °C. The larvae were reared on their respective host plant kept in cages.

2.2.2 Susceptibility of larvae to the endophytes

Fourth instar healthy larvae were used for bio assay studies. Fungal suspensions were prepared by flooding the spores from culture plates with sterile distilled water containing 0.05 % Tween-80. The clumping of the fungal spores was removed by gently scrubbing the concentrated suspension with a teflon hand homogenizer adapted for 1.5 ml Eppendorf tubes. The spores were counted with the help of an improved Neubauer Haemocytometer. The spore concentrations were then adjusted for 1x 10^{8} conidia/ ml in sterile distilled water.

Larvae were exposed to the fungus by the dipping technique. Seven treatments of *B. bassiana* ($1x10^{8}$ conidia ml^{-1}) @ 0.1, 0.2, 0.4, 0.8, 1 and 2 ml along with untreated control were maintained. Each treatment contains 10 larvae and it was replicated thrice. Sterile-distilled water was used instead of fungal suspension for control experiments. After drying for one minute, larvae were placed individually in 24 multi-well tissue culture plates to avoid cross contamination. Larvae were offered appropriate and equal pieces of sterile food as diet. Treated and for control insects were incubated at 28 ± 2 °C. Larval mortalities were recorded 3, 5, 7 and 10 days after post-exposure mortality.

2.3 Statistical analysis

The observation recorded on reduction in number larval population was converted to $\sqrt{x} + 0.5$ transformation and

finally data were subjected to ANOVA by using completely randomized design (CRD) for analysis. Per cent reduction of larval population over untreated control was calculated by using the following formulae.

$$\text{Per cent reduction (\%)} = \frac{\text{Number of larvae in treatment}}{\text{Total number of larvae in untreated control}} \times 100$$

3. Results and Discussion

3.1 Isolation of Microbial Endophyte, *B. bassiana* isolated from different plant/plant parts

As a part of the investigation, the presence of entomopathogenic fungal endophytes and their identity has been confirmed in different agricultural and horticultural crops. As an endophyte, *B. bassiana* was present in different parts of rabi sunflower, maize, chickpea, cauliflower, greengram, banana, citrus, tomato, brinjal, chilli, beans and pomegranate (Table 1). Similar results were observed by many authors where, *Beauveria* has been reported as an endophyte in maize [6, 7], potato, in sorghum, chilli, sunflower and beans [7], tomato [11], in bananas [12] and in coffee [13]. In addition, cocoa [8] and coffee seedlings [9] have been successfully inoculated with *B. bassiana* (Bals.-Criv.) by depositing a spore suspension on the radicle shortly after germination.

Table 1: Isolation of Microbial Endophyte, *B. bassiana* from different plant/plant parts

S. No.	Name of the crop	Plant/ plant part	Place of collection
1	Maize	Stem	Belagavi and Dharwad
2	Chickpea	Leaves	Annigeri
3	Greengram	Leaves	Vijaypur
4	Rabi sunflower	Leaves	Vijaypur and Dharwad
5	Banana	Pseudostem	Vijaypur
6	Pomegranate	Fruit	Vijaypur
7	Citrus	Leaves	Vijaypur
8	Tomato	Fruits	Haveri and Ranebennur
9	Chilli	Leaves	Haveri and Ranebennur
10	Brinjal	Leaves	Haveri and Ranebennur
11	Cauliflower	Head	Ranebennur
12	Beans	Pods	Ranebennur

3.2 Status of pathogenicity of endophytic *B. bassiana* against target insect pests

The preliminary studies on status of pathogenicity of endophytic *Beauveria* revealed that the endophytic *B. bassiana* isolated from leaves and stem of the sorghum and maize was found to be pathogenic to sorghum stem borer, *Chilo partellus* and army worm, *Mythimna separata*, from cabbage and cauliflower was pathogenic to diamond back moth, *Plutella xylostella* and endophytic Further, *Beauveria* from leaves of tomato and chilli and fruit of tomato was pathogenic to chickpea pod borer, *Helicoverpa armigera* (Table 2).

Table 2: Status of pathogen city of endophytic *B. bassiana* against target insect pests

Plant /plant part	Target pest	Pathogen city status
Sorghum stem	*Chilo partellus*	Pathogenic
Sorghum leaf	*Chilo partellus*	Pathogenic
Sorghum leaf	*Mythimna separate*	Pathogenic
Cabbage	*Plutella xylostella*	Pathogenic
Cauliflower	*Plutella xylostella*	Pathogenic
Maize stem	*Chilo partellus*	Pathogenic
Maize leaf	*Chilo partellus*	Pathogenic
Tomato fruit	*Helicoverpa armigera*	Pathogenic
Chilli leaf	*Helicoverpa armigera*	Pathogenic
Chick pea leaves	*Helicoverpa armigera*	Pathogenic

Evaluation of endophytic *B. bassiana* for the pathogenicity against sorghum stem borer, *Chilo partellus* Swinhoe

Evaluation of endophytic *B. bassiana* for the pathogenicity against sorghum stem borer, *Chilo partellus* Swinhoe is presented in Table 3. Each treatment was contained 10 larvae before treatment. At three days after treatment, there was no significant difference between the treatments since there was no growth of fungal hyphae on the treated larvae. After 5th day significant difference was observed between the treatments compared to untreated control. Fungal hyphae were developed on the treated larvae. *B. bassiana* @ 2 ml has showed least number of larvae (5.00) followed by *B. bassiana* @ 1 ml (7.67) than compared to untreated control (10.00) whereas, same number of larvae was observed in *B. bassiana* @ 0.1 ml and 0.2 ml which were statistically at par with each other (9.67). Highest per cent reduction of larval population over untreated control was noticed in *B. bassiana* @ 2 ml (50.00 %) and least was noticed in *B. bassiana* @ 0.1 ml (3.30 %).

At 7 days after treatment, least number of larvae was observed in treatment, *B. bassiana* @ 2 ml (4.67) which is statistically on par with the treatment *B. bassiana* @ 1 ml (7.33). Highest larvae were observed in the treatment *B. bassiana* @ 0.1 ml (9.00) which is on par with untreated control (10.00). Similar trend was noticed in 10 day after treatment, wherein least number of larvae were noticed in *B. bassiana* @ 2 ml (4.00) followed by *B. bassiana* @ 1 ml (6.33). Highest number of larvae was noticed in *B. bassiana* @ 0.2 ml (8.00) and *B. bassiana* @ 0.1 ml (8.33). Per cent reduction of larval population over untreated control was registered high in *B. bassiana* @ 2 ml (53.30 %) and lowest was in *B. bassiana* @ 0.1 ml (10.00 %).

Similar trend was noticed in 10 days after treatment, wherein least number of larvae were noticed in *B. bassiana* @ 2 ml (4.00) followed by *B. bassiana* @ 1 ml (6.33). Highest number of larvae was noticed in *B. bassiana* @ 0.2 ml (8.00) and *B. bassiana* @ 0.1 ml (8.33). Highest per cent reduction of larval population over untreated control was registered in *B. bassiana* @ 2 ml up to 60.00 per cent (Table 3).

Table 3: Evaluation of endophytic *Beauveria bassiana* for the pathogen city against sorghum stem borer, *Chilo partellus* Swinhoe.

Treatments	No. of larvae before treatment	Number of larvae after treatment				Per cent reduction of larval population over untreated control			
		3 DAT	5 DAT	7 DAT	10 DAT	3 DAT	5 DAT	7 DAT	10 DAT
Beauveria bassiana ($1x10^8$ conidia ml^{-1}) @ 0.1 ml	10	10.00 (3.24)abc	9.67 (3.19)cde	9.00 (3.08)cde	8.33 (2.97)def	0.00	3.30	10.00	16.70
Beauveria bassiana ($1x10^8$ conidia ml^{-1}) @ 0.2 ml	10	10.00 (3. 24) abc	9.67 (3.19)cde	9.00 (3.08)cde	8.00 (2.92)cde	0.00	3.30	10.00	20.00
Beauveria bassiana ($1x10^8$ conidia ml^{-1}) @ 0.4 ml	10	10.00 (3.24) abc	9.33 (3.13)bcd	8.67 (3.03)bcd	7.67 (2.86)cd	0.00	6.70	13.30	23.30
Beauveria bassiana ($1x10^8$ conidia ml^{-1}) @ 0.8 ml	10	9.67 (3.19) ab	8.33 (2.97)bc	8.00 (2.91)bc	7.00 (2.74)bc	3.30	16.70	20.00	30.00
Beauveria bassiana ($1x10^8$ conidia ml^{-1}) @ 1 ml	10	9.33 (3.13)a	7.67 (2.86)b	7.33 (2.80)ab	6.33 (2.61)b	6.70	23.30	26.70	36.70
Beauveria bassiana ($1x10^8$ conidia ml^{-1}) @ 2 ml	10	9.33 (3.13)a	5.00 (2.34)a	4.67 (2.27)a	4.00 (2.11)a	6.70	50.00	53.3	60.00
Untreated control	10	10.00 (3.24)abc	10.00 (3.24)cdef	10.00 (3.24)def	9.67 (3.19)g				
CV		NS	3.81	3.33	4.23				
CD @ 1%			0.28	0.24	0.21				
S. Em			0.07	0.06	0.07				

*Figures in parentheses are $\sqrt{}$ X+0.5 transformed values.
Means followed by same letters do not differ significantly by DMRT (p = 0.05).
DAS: Days After Treatment

The present study is in accordance with the results of [14] who reported that lower percentage of dead hearts (2.2-11.1%) and lower stem tunneling (2.7-4.3cm/ plant) was observed significantly in *B. bassiana* treated plants as compared to the untreated control plants (28.86 % of dead hearts and 13.41cm/plant stem tunneling). Similar results were obtained by [15] who reported that *B. bassiana* treated and untreated (control) sorghum plants were artificially infested with stem borer (*Chilo partellus*) larvae 15 days post treatment and the extent of damage was compared. About 40 % of the control plants developed dead heart while no plant in the *B. bassiana* treated plot did. In the surviving control plants, stem tunneling by shoot borer was significantly higher compared to *B. bassiana* treated sorghum plants.

Earlier reports [16, 17] isolated *B. bassiana* from maize (*Zea mays*) and the fungus was used to control the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Noctuidae). Some of the *Beauveria* strains isolated from maize were used against the insect pest, *Spodoptera frugiperda* [18]. *Beauveria* can reduce pest damage [19] by inhibiting insect development and establishment [20, 11, 21, 22] observed long hyphal structures to follow the leaf apoplast in any direction from the point of penetration. A few hyphae were observed within xylem elements. Because, vascular bundles are interconnected throughout the corn plant, this may explain how *B. bassiana* travels within the plant and ultimately provides overall insecticidal protection. Virulency bioassays demonstrated that *B. bassiana* does not lose virulence towards the European corn borer once it colonizes corn. This endophytic relationship between an entomopathogenic fungus and a plant suggests possibilities for biological control, including the use of indigenous fungal inocula as insecticides.

4. Conclusion

The results of present experiment concluded that the endophytic *B. bassiana* is known to present in different parts of the different plants i.e. maize stem, leaves of chickpea, chilli, green gram, citrus, pomegranate fruit, bean pod, cauliflower head and banana pseudo stem. Among all the treatments least population of sorghum stem borer larvae were observed in endophytic *B. bassiana* ($1x10^8$ conidia ml^{-1}) @ 2 ml at 10 days after treatment (4.00 larvae) followed by 7 days after treatment (4.67 larvae). Further, Endophytic *B. bassiana* could cause larval mortality up to 60.00 per cent at 10 days after treatment. Hence, endophytic *B. bassiana* present in different parts of the different plants can be largely exploited for the controlling sorghum stem borer in eco-friendly manner.

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