Entomopathogenic fungi as a promising biological control agent against banana fruit scarring beetle, *Basilepta subcostata* (Jac.) (Chrysomelidae: Coleoptera)

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**Abstract**

**Background:** Banana fruit scarring beetle (BFSB), *Basilepta subcostata* (Jac.) (Chrysomelidae: Coleoptera), is an important insect pest feeds on leaf and fingers, which affects the cosmetic value of the fruit. The pest is distributed in Assam, Bihar, West Bengal, Chhattisgarh, and North-eastern Hill regions of India.

**Results:** The pest is currently managed by foliar spray with insecticides. In order to identify eco-friendly control of the pest, attempts were made to isolate microbial agent and evaluate their potential to control the pest. A total of 27 entomopathogenic fungal isolates were obtained from *Odoiporus longicollis* (Oliver), *Cosmopolites sordidus* (Germar), *Basilepta subcostata* (Jac), and other insect *Galleria mellonella* (Fabr). Based on colony morphology, the collected fungal isolates were identified as *Metarhizium* spp. (17) and *Beauveria* spp. (10). Through ITS sequencing, the fungal isolates were further characterized at species level as *B. bassiana* (8), *B. brongniartii* (2), *M. anisopliae* (8), *M. robertsii* (6), *M. guizhouense* (2), and *M. pinghaense* (1). Their sequences were submitted in GenBank and obtained accession numbers. Among 27 isolates tested against *B. subcostata* under laboratory conditions, 3 isolates (*M. anisopliae* NRCBEPF-36, *M. pinghaense* NRCBEPF-7 and *B. brongniartii* NRCBEPF-27) recorded 100% beetle mortality, followed by 11 isolates with 95–99% and 13 isolates with 88–93% within 8 days of treatment.

**Conclusion:** This study highlights the two native North East India isolates *B. brongniartii* NRCBEPF-27 (MT151781) and *M. anisopliae* NRCBEPF-36 (MT140308) showed the significance to use as potential biocontrol agents against banana fruit scarring beetle *B. subcostata*. Further experiments under field conditions are required to evaluate their biological control efficacy against the pest.

**Keywords:** *Basilepta subcostata*, *Metarhizium anisopliae*, *Beauveria bassiana*, ITS region, Bioassay

**Background**

Banana is the most important fruit crop and cultivated across 130 countries in the tropical and subtropical region. More than 200 species of insect and non-insect pests have been reported (Simmonds, 1966). The banana leaf and fruit scarring beetle *Basilepta subcostata* (Jacoby) (Chrysomelidae: Coleoptera) is considered as one of the most economically important pests in North-Eastern India (Mishra et al., 2015 and Bhagabati and Deka, 2016). Recently, taxonomic descriptions with illustrations of the genitalia of banana fruit and leaf scarring beetle led to correct identification of *B. subcostata* (Prathapan et al., 2019).

Entomopathogenic fungal (EPF) strains have been commercialized as a biocontrol agent, and the majority of them have been developed from Ascomycota,
Beauveria spp. (Bals-Criv.) Vuill. (Hypocreales: Clavicipitaceae) and Metarhizium spp. (Metschn.) Sorok. (Hypocreales: Clavicipitaceae) (Rehner et al., 2011 and Velavan et al., 2017). The efficacy of Beauveria spp. has been well established as a potential biocontrol agent of Cosmopolites sordidus (Akello et al., 2008 and Lopes et al., 2013). EPF have been isolated and evaluated against banana stem weevil, Odoiporus longicollis and showed promising results (Padmanaban et al., 2019 and Alagesan et al., 2019). Additionally, the combined use of myco-insecticides and the full or reduced concentration of botanical/chemical insecticides is a promising pest-control option for minimizing adverse chemical effects (Samui et al., 2004 and Choudhary et al., 2010). In this study, an attempt was made to collect, isolate and identify promising EPF as a biocontrol agent for managing scarring beetle of banana.

### Methods

**Survey and collection of fungal infected insects**

Survey was conducted to collect healthy and infected insects in Tamil Nadu, Bihar, and North-Eastern region (Assam) in India (Table 1). Naturally infested banana stem weevils (Odoiporus longicollis Oliver), rhizome weevils (Cosmopolites sordidus Germar), several insect cadavers of leaf scarring beetle (Basilepta subcostata Jac), and other insect pests of the greater wax moth (Galleria mellonella L.) were collected (Table 1) and surface-sterilized with 1% sodium hypochlorite (NaOCl) for 30 s, followed by 3 washes with sterile distilled water to prevent external saprophytic contaminations. Dead insects were kept in Petri plate lined with a single layer of wet filter paper until signs of muscardine were observed. After that, they were dissected and placed into Petri plates containing potato dextrose agar yeast extract (PDA) medium.

### Table 1

| S.No | Isolates’ name | Species                          | Isolation sources | Geographic location | Place/state | GenBank accession |
|------|----------------|----------------------------------|-------------------|---------------------|-------------|------------------|
| 1    | NRCB EPF2      | Beauveria bassiana               | Odoiporus longicollis | 10.78° N 78.58° E   | Trichy TN   | MT645318        |
| 2    | NRCB EPF27     | B. brongniartii                  | Basilepta subcostata | 26.72° N, 94.19° E | Jorhat Assam | MT151781        |
| 3    | NRCB EPF28     | B. brongniartii                  | B. subcostata      | 26.72° N, 94.19° E  | Jorhat Assam | MT151784        |
| 4    | NRCB EPF29     | B. bassiana                      | B. subcostata      | 26.31° N, 94.11° E  | Jorhat Assam | MT151783        |
| 5    | NRCB EPF30     | B. bassiana                      | B. subcostata      | 26.31° N, 94.11° E  | Jorhat Assam | MT151786        |
| 6    | NRCB EPF32     | B. bassiana                      | B. subcostata      | 26.31° N, 94.11° E  | Jorhat Assam | MT140307        |
| 7    | NRCB EPFMP1    | B. bassiana                      | Cosmopolites sordidus | 09.80° N, 77.36° E | Theni TN    | MK899434        |
| 8    | NRCB EPF22     | B. bassiana                      | B. subcostata      | 25.85° N, 85.78° E  | Katihar Bihar | MK834817        |
| 9    | NRCB EPF8      | B. bassiana                      | B. subcostata      | 25.09° N 85.31° E   | Katihar Bihar | MT645316        |
| 10   | NRCB EPF14     | B. bassiana                      | B. subcostata      | 25.09° N 85.31° E   | Katihar Bihar | MT645319        |
| 11   | NRCB EPF16     | Metarhizium anisopliae           | Galleria mellonella | 10.78° N 78.58° E   | Trichy TN   | MK834813        |
| 12   | NRCB EPF17     | M. anisopliae                    | G. mellonella      | 10.78° N 78.58° E   | Trichy TN   | MN888761        |
| 13   | NRCB EPF18     | M. anisopliae                    | G. mellonella      | 10.78° N 78.58° E   | Trichy TN   | MN888763        |
| 14   | NRCB EPF19     | M. robertsii                     | G. mellonella      | 10.78° N 78.58° E   | Trichy TN   | MN889408        |
| 15   | NRCB EPF6      | M. anisopliae                    | O. longicollis     | 10.78° N 78.58° E   | Trichy TN   | MN892391        |
| 16   | NRCB EPF9      | M. anisopliae                    | O. longicollis     | 10.84° N 78.95° E   | Trichy TN   | MK834805        |
| 17   | NRCB EPF7      | M. pinghaense                    | O. longicollis     | 10.78° N 78.58° E   | Trichy TN   | MN892390        |
| 18   | NRCB EPF10     | M. robertsii                     | O. longicollis     | 10.84° N 78.95° E   | Trichy TN   | MN892393        |
| 19   | NRCB EPF11     | M. quizhouense                   | O. longicollis     | 10.84° N 78.95° E   | Trichy TN   | MN892392        |
| 20   | NRCB EPF12     | M. anisopliae                    | O. longicollis     | 10.78° N 78.58° E   | Trichy TN   | MN892390        |
| 21   | NRCB EPF13     | M. robertsii                     | O. longicollis     | 10.78° N 78.58° E   | Trichy TN   | MN892304        |
| 22   | NRCB EPF23     | M. robertsii                     | O. longicollis     | 10.78° N 78.58° E   | Trichy TN   | MN893382        |
| 23   | NRCB EPF24     | M. robertsii                     | B. subcostata      | 25.99° N, 85.59° E  | Samastipur  | MK836090        |
| 24   | NRCB EPF33     | M. robertsii                     | B. subcostata      | 26.48° N, 94.11° E  | Jorhat Assam | MN893380        |
| 25   | NRCB EPF34     | M. quizhouense                   | B. subcostata      | 26.30° N, 94.11° E  | Jorhat Assam | MN893383        |
| 26   | NRCB EPF35     | M. anisopliae                    | B. subcostata      | 26.43° N, 94.35° E  | Jorhat Assam | MT140304        |
| 27   | NRCB EPF36     | M. anisopliae                    | B. subcostata      | 26.43° N, 94.35° E  | Jorhat Assam | MT140308        |
amended with antibiotic (1% yeast extract 0.6 g, 100 μg/ml chloramphenicol, 50 μg/ml streptomycin, 2 mg crystal violet), and the plates were incubated at 28±1 °C and 90% RH to facilitate growth and sporulation of the fungus. The cultures were purified using Veen’s medium containing Dodine (Veen and Ferron, 1966). Morphologically distinct colonies Beauveria and Metarhizium were picked individually and inoculated on PDAY medium and incubated at 27 °C for 15 days. After that, the fungal isolates were transferred to PDAY slants and used for further study. Also, slides were prepared (Riddell, 1950) from 5 days old culture for the identification and the cultures were stained with permanent stain (Cotton blue-Lacto phenol) and mounted using dibutylphthalata in xylene (DPX). Identification was done on the basis of morphotaxonomic characters through microscopic inspection of conidia and conidiogenous structure (Bischoff et al., 2009 and Kepler et al., 2014). Also, for a long-term old culture and mixed in 10% glycerol and stored at −30 °C until use.

Molecular characterization through sequencing of ITS region of entomopathogenic fungi (EPF) isolates
DNA was extracted from 50 to 100 g of lyophilized mycelium of the fungus from 10–15-day-old cultures grown in potato dextrose broth (PDB) following cetyltrimethylammonium bromide (CTAB) methods (Rogers and Bendich, 1994). Extracted DNA was suspended in EB buffer (10 mM Tris-Hcl, pH 8.5) and mounted using dibutylphthalata in xylene (DPX). Identification was done on the basis of morphotaxonomic characters through microscopic inspection of conidia and conidiogenous structure (Bischoff et al., 2009 and Kepler et al., 2014). Also, for a long-term old culture and mixed in 10% glycerol and stored at −30 °C until use.

Bioassay of EPF on Basilepta subcostata
The banana fruit scarring beetles (B. subcostata) were collected from the infested banana field and reared in entomology laboratory of the institute using perforated plastic containers at room temperature. The leaf midribs of cv. Jahaji (a susceptible host) were utilized for feeding the beetles. The entire test beetles were kept in these containers for at least 1 month before use in the experiments for acclimatization. Whereby beetles were observed using a hand lens and males were differentiated from females on the basis of pulsations on their rostrums spreading beyond the point of antennae insertion. These adult beetles were utilized for the present study.

The 27 indigenous EPF isolates were used for bioassay against B. subcostata. The cultures were grown on PDAY medium for 10 to 15 days at 25 ± 0.5 °C. The mycelia mat containing spores were harvested in tube using sterile distilled water 100 ml with continuous stirring the contents in a tube, filtered through a single layer of muslin cloth to remove debris and mycelia. Conidial suspension (CS) was prepared as per the procedure described by Lopes et al. (2013) and Velavan et al. (2017). The conidial concentration was estimated using hemocytometer under light microscope. Subsequently, the conidial suspension was diluted to make a final suspension of 1×10^5 spores/ml with 0.1% Triton X-100, 0.2% Tween 80 and 0.1% glycerol. B. subcostata was transferred aseptically to fresh plastic boxes (10-cm diameter and 30-cm height). The conidial suspension of EPF isolates (27) were swabbed on leaf sheaths (8–10-cm lengths) individually. For comparison, 2 commercial isolates; each M. anisopliae and B. bassiana was also included in the experiment. Each treatment had 5 replications, and each replication consisted of 15 beetles. In control, similar numbers of beetles were introduced on to the leaf sheaths treated with water, 0.1% Triton X-100, 0.2% Tween 80, and 0.1% glycerol alone. Observations were taken on morality of insects at 3, 5, and 8 days after inoculations. Infected insect cadavers were transferred to
wet chamber and the fungus was re-isolated and confirmed based on culture spore morphology.

**Statistical analyses**
Obtained data on beetle mortality rates were Arc Sin transformed and analyzed in Completely Randomized Design using Web Agri Stat Package 2.0 (WASP) https://ccari.res.in/wasp2.0/.

**Results**
**Isolation and characterization of EPF isolates**
During 2017–2018 in Tamil Nadu, Bihar, and Assam fungal-infected insects such as corn weevil, pseudostem weevil, and scarring beetle besides *Galleria* were collected, reared, and identified. In total, 27 fungal isolates were isolated and purified from the dead beetles and *Galleria* larvae Table 1. Based on colony color and spore morphology, the isolates were broadly classified as *Metarhizium* (17 isolates) and *Beauveria* (10 isolates) as they produced green and white colonies, respectively. Among the fungal isolates, 13 isolates consisting of 8 *Beauveria* and 5 *Metarhizium* were obtained from dead adult beetles of *B. subcostata*, while 8 isolates of *Metarhizium* and one isolate of *Beauveria* were isolated from *Odoiporus longicollis*. Also, one isolate of *Beauveria* and 4 isolates of *Metarhizium* were obtained from adult of *Cosmopolites sordidus* and larvae of

![Fig. 1 Colony and conidial morphology of entomopathogenic fungi](image)
Galleria, respectively. Observations on morphological characteristic indicated that Metarhizium isolates produced 4 different colonies and conidial morphologies, while Beauveria showed 2 morphological differences (Fig. 1).

Molecular characterization of EPF isolates

Molecular characterization of EPF cultures through sequencing of ITS regions and BLAST analysis with data base sequences revealed that they belonged to B. bassiana (8 isolates), B. brongniartii (2 isolates), M. anisopliae (12 isolates), M. robertsii (3 isolates), and M. pinghaense (1 isolate).

Table 2: In vitro bio-efficacy of Metarhizium and Beauveria isolates against Basilepta subcostata

| Isolates' name   | Species         | Per cent mortality (in days after treatment) |
|------------------|-----------------|---------------------------------------------|
|                  |                 | Day 3            | Day 5            | Day 8            |
| NRCB EPF2        | B. bassiana     | 32.7 (34.0)      | 61.7 (51.8)      | 91.7 (73.4)      |
| NRCB EPF8        | B. bassiana     | 33.3 (35.3)      | 68.3 (55.9)      | 93.3 (77.5)      |
| NRCB EPF14       | B. bassiana     | 28.3 (31.6)      | 56.7 (48.8)      | 93.3 (77.9)      |
| NRCB EPF22       | B. bassiana     | 41.3 (40.2)      | 71.7 (57.9)      | 93.3 (80.7)      |
| NRCB EPFMP1      | B. bassiana     | 38.3 (38.2)      | 80.0 (63.5)      | 91.7 (73.4)      |
| NRCB EPF27       | B. brongniartii | 45.0 (42.1)      | 95.0 (79.3)      | 100.0 (89.4)     |
| NRCB EPF28       | B. brongniartii | 30.0 (32.9)      | 70.3 (56.9)      | 93.3 (77.5)      |
| NRCB EPF29       | B. bassiana     | 38.3 (38.2)      | 78.3 (62.8)      | 91.7 (76.0)      |
| NRCB EPF30       | B. bassiana     | 30.0 (33.2)      | 60.0 (50.9)      | 93.3 (77.5)      |
| NRCB EPF32       | B. bassiana     | 40.0 (39.2)      | 80.0 (63.4)      | 98.3 (85.3)      |
| NRCB EPF16       | M. anisopliae   | 41.7 (40.2)      | 73.3 (60.0)      | 91.7 (73.4)      |
| NRCB EPF17       | M. anisopliae   | 33.3 (35.1)      | 68.3 (56.3)      | 95.0 (79.3)      |
| NRCB EPF18       | M. anisopliae   | 38.3 (38.2)      | 78.3 (60.1)      | 96.7 (81.2)      |
| NRCB EPF19       | M. anisopliae   | 40.0 (39.2)      | 70.0 (57.3)      | 88.3 (73.6)      |
| NRCB EPF6        | M. anisopliae   | 45.0 (36.2)      | 76.7 (61.1)      | 91.7 (76.0)      |
| NRCB EPF7        | M. pinghaense   | 35.0 (42.1)      | 75.0 (62.5)      | 100.0 (89.4)     |
| NRCB EPF9        | M. anisopliae   | 31.7 (34.2)      | 66.7 (54.8)      | 91.7 (73.4)      |
| NRCB EPF10       | M. robertsii    | 30.0 (32.9)      | 71.7 (58.3)      | 95.0 (82.0)      |
| NRCB EPF11       | M. quizhouense  | 35.0 (36.2)      | 73.3 (59.0)      | 93.3 (75.2)      |
| NRCB EPF12       | M. anisopliae   | 40.0 (39.2)      | 75.0 (60.1)      | 96.7 (83.4)      |
| NRCB EPF13       | M. anisopliae   | 36.7 (37.2)      | 78.3 (61.1)      | 95.0 (79.3)      |
| NRCB EPF23       | M. robertsii    | 36.7 (37.1)      | 78.3 (62.5)      | 98.3 (85.3)      |
| NRCB EPF24       | M. robertsii    | 36.7 (37.3)      | 78.3 (60.0)      | 95.0 (79.3)      |
| NRCB EPF33       | M. robertsii    | 36.7 (37.1)      | 78.3 (62.5)      | 98.3 (85.3)      |
| NRCB EPF34       | M. robertsii    | 36.7 (37.1)      | 78.3 (62.5)      | 98.3 (85.3)      |
| NRCB EPF35       | M. anisopliae   | 30.0 (42.1)      | 85.0 (79.3)      | 98.3 (85.3)      |
| NRCB EPF36       | M. anisopliae   | 35.0 (36.2)      | 85.0 (67.4)      | 100.0 (89.4)     |
| Commercial       | M. anisopliae   | 36.7 (36.2)      | 78.3 (59.0)      | 98.3 (75.2)      |
| Commercial       | M. anisopliae   | 36.7 (37.1)      | 78.3 (62.5)      | 98.3 (85.3)      |
| BCRL formulation | B. bassiana     | 36.7 (37.1)      | 75.0 (62.5)      | 95.0 (85.3)      |
| TARI formulation | B. bassiana     | 30.0 (37.3)      | 71.7 (60.0)      | 95.0 (79.3)      |
| Control          | Water+Glycerol  | (0.6)            | (0.6)            | 0.864            |

*p < 0.001 (highly significant) and p < 0.05 (significant)

Two-way factorial analysis of variance (ANOVA) at α = 0.05, CV% coefficient of variation

Values represent means of three replicates

Values in parentheses represent arcsine transformations
anisopliae (8 isolates), M. robertsii (6 isolates), M. guizhouense (2 isolates), and M. pinghaense (1 isolate) as they had 100, 94, 99, 100, and 100% homology with NCBI data base sequences of MT635020, AB027381, FJ545286, MF681599, HM055445, and HM055446, respectively, and all the isolates details with GenBank accessions were depicted in Table 1.

Bioassay of EPF isolates on Basilepta subcostata

All 27 EPF isolates of Beauveria spp. and Metarhizium spp. along with standard commercial isolates were tested against B. subcostata in vitro (Table 2, Fig. 2). Invariably, all the isolated strains showed significant mortality rates of the beetle in 3 days after treatment and the effects were on par with 4 commercial isolates. In 5 days after treatment, M. anisopliae (NRCBEPF35 and NRCBEPF36) and B. brongniartii (NRCBEPF27) isolated from B. subcastata from Jorhat, Assam recorded 85–95% mortality rate of the beetle. The effect of the isolate (NRCBEPF27) was significantly better than all commercial isolates and other isolated strains. However, other isolated strains of M. anisopliae (NRCBEPF36), M. pinghaense (NRCBEPF7), and B. brongniartii (NRCBEPF27) are recorded 100% mortality on the 8th day of treatment (Table 2).

Discussion

Scarring beetle is a major problem in banana cultivation especially in North, East, and North East parts of India; Bangladesh; and South East Asia (Prathapan et al., 2019). It causes severe damage to banana, and it has been estimated up to 95% damage to the crop in different parts of India (Choudhary et al., 2010; Bhagabati and Deka 2016; Saikia et al., 2018 and Daizy et al., 2019). In India, bananas are consumed majorly as raw fruits and some extent as processed food. Therefore, application of chemical pesticides on banana is hugely discouraged. Alternatively, entomopathogens have been promising in management of pests in different crops. However, success of the biocontrol agent is mainly depending on efficacy of specific strains against target pest and performance of such agents in the given environment. Hence, the present study focused to collect EPF isolates from North, North East, and Southern parts of India and screen against B. subcostata.

In total, 27 EPF isolates were obtained and based on spore and colony morphologies, and they were characterized (Bischoff et al., 2009; Rehner et al., 2011; Ravindran et al., 2015; and Ramanujam et al., 2015) majorly as Metarhizium (17 isolates) and Beauveria (10 isolates). Molecular identification of fungal species is generally carried out through sequencing of ITS regions (Bischoff et al., 2009; Rehner et al., 2011; Lopes et al., 2013; Kepler et al., 2014; Ravindran et al., 2015; and Ramanujam et al., 2015) and BLAST analysis with data base sequences. Similarly, sequencing of ITS region of EPF cultures revealed that they belonged to B. bassiana, B. brongniartii, M. anisopliae, M. robertsii, M. guizhouense, and M. pinghaense. In the present study, majority of isolates were belonging to Metarhizium spp. followed by Beauveria spp. Over all, comparatively Metarhizium isolates out performed than Beauveria isolates against B. subcastata. Though management strategy for lowering infestation of fruit scaring beetle by biopesticide (B. bassiana) was profitable (Samui et al., 2004 and Choudhary et al., 2010), the present study results corroborated with Tuncer et al. (2019) study where Metarhizium isolate showed better results than Beauveria on controlling the coleopteran insect (Ambrosia beetle, Xylosandrus germanus).

**Fig. 2** Effect of EPF native isolates on Basilepta subcostata after 8 days of inoculation. a Insect cadaver fully covered with bright white mycelia of Beauveria brongniartii. b Insect cadaver fully covered with mycelium of Metarhizium pinghaense. c Insect cadaver covered with conidia of Metarhizium anisopliae
Conclusions

EPF isolates M. anisopliae NRCB EPF 35 and NRCB EPF36, and B. brongniartii NRCBEFPF27 showed the highest mortality rates of B. subcastata. They could be further exploited to extend a wide spread testing of the isolates against the pest in different ecological zone and to commercialize the strain for wider adoptability by end-users. It also gives the way for preparation of consortium, using the best strains such as NRCBEFPF36, NRCBEFPF7, and NRCBEFPF27. Such M. anisopliae and B. brongniartii strains with biocontrol properties can fit into the Integrated Pest Management (IPM) of the banana fruit scarring beetle, B. subcastata.

Abbreviations

PDAY: Potato dextrose agar yeast extract; TBE: (Tris/Borate/EDTA); EDTA: Ethylenediaminetetraacetic acid; NaOCl: Sodium hypochlorite; CTAB: Cetyltrimethylammonium bromide; NCBI: National Center for Biotechnology Information; BFSB: Banana fruit scarring beetle; NRCB: National Research Center for Banana; EPF: Entomopathogenic fungi; ITS: Internal transcribed spacer

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Authors’ contributions

VV, BP, ML, and US analyzed and interpreted the data; VV wrote the manuscript; BP contributed to the taxonomic identification of B. subcastata; BN performed the laboratory experimentation; and ML edited the manuscript. The authors have read and approved the manuscript.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high-quality and grade materials were used in this study.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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