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Effect of entomopathogenic fungi against banana pseudostem weevil *Odoiporus longicollis* (Olivier) and elucidation of infection process

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Abstract

Background: The banana stem weevil, *Odoiporus longicollis* (Olivier), is a serious threat to banana cultivation world over. Since banana is a food crop, the use of naturally infecting biological control agents could be an effective alternative to manage the insect pest instead of harmful chemicals. Also, the efficacy of entomopathogenic fungi against *O. longicollis* was used in bioassay.

Results: Among the *Beauveria bassiana* isolates tested the median lethal concentration (LC_{50}) 10.468×10^5 conidia ml^{-1} when treated with *B. bassiana* (NRCBEFPMP1), two other isolates of *B. bassiana*, namely NRCBEPF22 and NRCBEPF2, were also effective against *O. longicollis* and recorded LC_{50} of 12.617×10^5 and 12.891×10^5 conidia ml^{-1}, respectively. The results of bioassay with different *Metarhizium* spp. showed variations in efficacy, where the most virulent isolate was *M. quizhouense* (NRCBEPF11) with LC_{50} 8.050×10^5 conidia ml^{-1}. Scanning electron microscopic analysis showed that *B. bassiana* and *M. quizhouense* caused infection by cuticle penetration and completed the infection process in 15 days. The composition of volatile organic compounds released by *B. bassiana* and *M. anisopliae* during pathogenesis showed that a significantly high number of known insect volatiles were present in infected insects. Consequently, these volatiles were emission in Insect attractant, Odorant receptor agonist, Plant hormone Plant, and Microbial Metabolites, through the biological activity, such as Methyl salicylate, Benzaldehyde, alpha-Terpineol, Limonene, Benzene, 1,2-dimethoxy, Phthalic acid, 1-Octadecene, Phenylacetaldehyde, 3-Octanone, Octanal, Methylheptenone and 2-Ethyl-1-hexyl alcohol.

Conclusion: Overall, the results show that EPF could significantly reduce damage by *O. longicollis* and produce a wide profile of secondary metabolites. Further, analysis was used for principal components to determine whether separated classes of fungi can be distinguished from one another based on their metabolite profiles.

Keywords: *Odoiporus longicollis*, *Beauveria* spp., *Metarhizium* spp., Bioassay, Insect–microbial interaction, Volatile organic compounds

Background

Banana (*Musa* sp.) is the second most important fruit crop in India next to mango. Over nineteen species of insects have been reported to infest banana cultivars (Padmanaban et al. 2001). One important pest

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is the banana pseudostem borer, *Odoiporus longicollis* (Olivier) (Coleoptera: Curculionidae). Also known as banana stem weevil (BSW). It can cause substantial damage in terms of production and productivity of banana (David 2008). Recent reports suggest that *O. longicollis* is distributed over diverse geographical locations in India (Isahaque 1978) and serious crop loss due to BSW (Jayanthi and Varghese 1999).

In India, application of synthetic chemical insecticides is the most common option to control BSW (Thippaiah et al. 2011). Since banana is a food crop, safer control methods of BSW are needed. One important safe alternative is use of biological control for entomopathogenic fungi (EPF), as they can grow naturally in soils and are capable of infecting various insects (Sharmila and Mohan 2015). Researchers have tried to use *Metarhizium anisopliae,* and *Beauveria bassiana* and their related species to control *O. longicollis* (Velavan et al. 2021; Sripriya et al. 2000). In addition, foliar application of EPF formulation against BSW has also been attempted in the fields (Azam et al. 2010). Studies have also shown certain endophytic isolates of *B. bassiana* can be highly effective on *O. longicollis,* if allowed to colonize in early stages of crop (Alagesan et al. 2019). Padmanaban et al. (2019) could identify endophytic EPF within the Musa germplasm cultivars that induced natural protection against banana pests. While EPF are identified based on their morphological and molecular characteristics, species identification among *Metarhizium* spp. is difficult because of its structural simplicity and the lack of distinctive features (Velavan et al. 2021). Recently the diversity of *Beauveria* spp. occurring in India was studied through phylogenetic analysis (Kisaakye et al. 2021) and taxonomic status of *Beauveria* spp. occurring worldwide was attempted using both morphological and molecular methods (Rehner et al. 2011).

At National Research Centre on Banana in India, six EPF, namely *B. bassiana*, *B. brongniartii, M. anisopliae, M. robertsii*, *M. quizhouense* and *M. pinghaense* native isolates, have been tested against leaf and fruit scarring banana pest *Basilepta subcostata.* Thus, *B. brongniartii, M. anisopliae* and *M. pinghaense* strains were found effectively infecting *B. subcostata* (Viswakethu et al. 2021). Although a number of EPF have been evaluated to control insect pests of banana, very limited data is available on use of EPF against *O. longicollis*. The present study was undertaken to know the biological control ability of EPF strains, isolated from naturally infected pests of banana. Consequently, electron microscopy analyses of the infection process and identification of insect specific volatiles released during pathogenesis were also studied.

Methods

Entomopathogenic fungi

Twenty-seven native strains of EPF belonging to *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae M. robertsii*, *M. quizhouense* and *M. pinghaense* were obtained from the culture collection of ICAR-National Research Centre on Banana (ICAR-NRCB), India. Naturally isolated at *Odoiporus longicollis, Cosmopolites sordidus, Basilepta subcostata* and *Galleria mellonella* identity was established by morphological and molecular studies (Table 1).

Rearing of Odoiporus longicollis

Specimens of banana stem weevils were collected from banana-growing regions of Thanjavur, Tiruchirappalli, and Theni in Tamil Nadu during 2017–2018. The weevils were maintained at Entomology Laboratory, Division of Crop Protection ICAR-NRCB, Tiruchirappalli. Banana pseudostem pieces (4–8 cm) were kept in plastic containers (10 kg volume) and weevils were released into the containers for feeding, mating and oviposition (Padmanaban et al. 2001). Pseudostems were changed once in 15 days. After hatching, for further development, the first instar grubs (larvae) were transferred on to fresh pieces of pseudostem. Larvae were reared in various stages viz. egg, 1st, 2nd, 3rd larval instars and adults. After adult emergence, they were placed in jars and supplied with cotton wicks saturated with 8–10% honey. Adults were fed with banana leaf sheath at 28 ± 1 °C, 75 ± 5% RH.

Bioassay

The EPF cultures were grown on potato dextrose yeast agar (PDAY) medium for 10–15 days at 25 ± 0.5 °C. Mycelial mats containing spores were harvested in 100 ml sterile distilled water in tubes with continuous stirring. The harvested contents were filtered through a single layer of muslin cloth to remove debris and mycelia. Conidial concentration was estimated using a hemocytometer under a light microscope (Olympus model BX100). Subsequently conidial suspensions (1×10^7, 1×10^6, 1×10^5, 1×10^4, and 1×10^3 conidia ml^{-1}) were prepared with 0.1% TritonX-100, 0.2% Tween 80 and 0.1% glycerol. Adults of stem weevils were transferred aseptically to fresh plastic boxes (10 cm diameter and 30 cm height). The conidial suspensions of EPF isolates were swabbed on leaf sheaths (8–10 cm length) individually. For comparison, two commercial isolates, namely *M. anisopliae* (M1, M2) and *B. bassiana* (BCRL, TARI) were included in the experiment. Five replications were maintained for each treatment and each replication had 15 test adults. Leaf sheaths treated with water containing 0.1% Triton X-100, 0.2% Tween 80 and 0.1% glycerol served as control. Mortality was recorded every day up to

Table 1 Entomopathogenic fungi used in the study, their source and identity

Isolates name	Source of the isolate	Geographic location	GenBank accession
Beauveria bassiana (NRCB EPF2)	*Odoiporus longicollis**	10.78° N 78.58° E	MT645318
B. brongniartii (NRCB EPF27)	*Basilepta subcostata**	26.72° N, 94.19°E	MT151781
B. brongniartii (NRCB EPF28)	*B. subcostata**	26.72° N, 94.19°E	MT151784
B. bassiana (NRCB EPF29)	*B. subcostata**	26.31°N, 94.11°E	MT151783
B. bassiana (NRCB EPF30)	*B. subcostata**	26.31°N, 94.11°E	MT151786
B. bassiana (NRCB EPF32)	*B. subcostata**	26.31°N, 94.11°E	MT140307
B. bassiana (NRCBEFPMP1)	*Cosmopolites sordidus**	09.80° N, 77.36° E	MK899434
B. bassiana (NRCB EPF22)	*B. subcostata**	25.85° N, 85.78°E	MK834817
B. bassiana (NRCB EPF8)	*B. subcostata**	25.09° N 85.31° E	MT645316
B. bassiana (NRCB EPF14)	*B. subcostata**	25.09° N 85.31° E	MT645319
Metarhizium anisopliae (NRCB EPF16)	**	10.78° N 78.58° E	MK834813
M. anisopliae (NRCB EPF17)	**	10.78° N 78.58° E	MN888761
M. anisopliae (NRCB EPF18)	**	10.78° N 78.58° E	MN888763
M. robertsii (NRCB EPF19)	**	10.78° N 78.58° E	MN889408
M. anisopliae (NRCB EPF6)	*O.longicollis**	10.78° N 78.58° E	MN892391
M. anisopliae (NRCB EPF9)	*O.longicollis**	10.84° N 78.95° E	MK834805
M. pinghaense (NRCB EPF7)	*O.longicollis**	10.78° N 78.58° E	MN892389
M. robertsii (NRCB EPF10)	*O.longicollis**	10.84° N 78.95° E	MN892393
M. quizhouense (NRCB EPF11)	*O.longicollis**	10.84° N 78.95° E	MN892392
M. anisopliae NRCB EPF12	*O.longicollis**	10.78° N 78.58° E	MN892390
M. robertsii (NRCB EPF13)	*O.longicollis**	10.78° N 78.58° E	MN892394
M. robertsii (NRCB EPF23)	*O.longicollis**	10.78° N 78.58°E	MN893382
M. robertsii (NRCB EPF24)	*B. subcostata**	25.99° N, 85.59° E	MK836090
M. robertsii (NRCB EPF33)	*B. subcostata**	26.48°N, 94.11°E	MN893380
M. quizhouense (NRCB EPF34)	*B. subcostata**	26.30°N, 94.11°E	MN893383
M. anisopliae (NRCB EPF35)	*B. subcostata**	26.43°N, 94.35°E	MT140304
M. anisopliae (NRCB EPF36)	*B. subcostata**	26.43°N, 94.35°E	MT140308

*Naturally infected cadaver

**Isolated from *Galleria mellonella* when used as bait

15 days after inoculation. Infected insect cadavers were transferred to the wet chamber and the pathogenic fungus was re-isolated and confirmed based on culture characteristics and spore morphology.

Volatile organic compound detection from insect–fungus interaction

Virulent EPF isolates, namely *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, M. robertsii, M. quizhouense* and *M. pinghaense*, were selected to identify secretion of volatile organic compounds (VOC) or organic compound emission of secondary metabolites during insect–fungus interaction. Infected dead insects were carefully returned to individual flasks and maintained without feeding under rearing conditions as previously described (Crespo et al. 2006). VOC were sampled from adults previously treated with either 1×10^8 conidia ml^{-1} or sterile distilled water containing 0.01% Tween-80 (controls). For each condition, one adult of known sex was gently placed in a 20-ml glass vial sealed with a Teflon cover having a rubber septum. The vial was vortexed for 10 s to elicit the release of volatiles; the released volatiles were immediately sampled from the headspace corresponding to the gaseous phase in contact with the insect sample (Arthur and Pawliszyn 1990). Three independent replicates (each replicate from a different individual) were performed for each condition (fungus-treated and untreated males and females). A vial containing no insect was used as controls. Volatile collections from EPF uninfected and infected adults were performed with a (polydimethylsiloxane PDMS) fiber with a 50-µm film thickness (Supelco, Inc., Bellefonte, PA, U.S.A.). The fiber had been previously conditioned according to the manufacturer's instructions and was systematically reconditioned before each analysis.

Statistical analysis

The corrected mortality rate was calculated using the Abbotts formula. The lethal concentration LC_{50} was analyzed by using Probit analysis software in SPSS® version 25. The concentration responses of each replicate were observed for estimation of median lethal concentration (LC_{50}) to kill 50% of exposed adults only. Principal components analysis (PCA), to illustrate the differences in the profiles of volatile compounds released during Insect–Fungal interaction, was also subjected to statistical analysis (Origin vs. OriginPro).

Results

Bioassay *against Odoiporus longicollis*

Twelve *B. bassiana* isolates that included two commercial (BCRL & TARI) and two *B. brongniartii* strains were tested against adults of *O. longicollis*. The adults of *O. longicollis* were susceptible to all the tested *Beauveria* spp. used in a concentration-dependent manner in five concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 conidia ml^{-1}). High mortality was observed at low conidial concentrations that varied significantly (Table 2). It was observed that median lethal concentration (LC_{50}) value for weevils was 10.468×10^5 conidia ml^{-1} in *B. bassiana* (NRCBEFPMP1) indicating that this isolate was the most virulent (Table 1). Two other isolates, namely (NRCBEPF22 and NRCBEPF2), were also virulent and showed LC_{50} of 12.617×10^5 and 12.891×10^5 conidia ml^{-1} (Table 2). The highest concentration requirement was exhibited by the two commercial strains BCRL and TARI and they exhibited the same LC_{50} value of 29.962 conidia ml^{-1}. The results established that *B. bassiana* strains (NRCBEFPMP1, NRCBEPF22 and NRCBEPF2) were virulent and had potential to be deployed in the field.

Bioassay of 19 *Metarhizium* spp., to determine concentration dependent virulence against *O. longicollis* showed significant differences in mortality (Table 3). Probit analysis showed a variation in virulence among the different *Metarhizium* isolates. Based on LC_{50} values the most virulent isolate was *M. quizhouense* (NRCBEPF11) that exhibited the ability to kill 50% of the tested adults with 8.050×10^5 conidia ml^{-1}. Other virulent isolates were *M. pinghaense* (NRCBEPF7), *M. robertsii* (NRCBEPF23) and *M. robertsii (*NRCBEPF24) with median lethal concentration (LC_{50}) of 10.155, 10.497 and 12.443×10^5 conidia ml^{-1}, respectively (Table 3). Hence, these isolates could be further tested in the field. The least virulent was *M.*

Table 2 Probit analysis of concentration-mortality response at 15 days post inoculation of *Beauveria* spp. against *Odoiporus longicollis*

Organism	LC_{50} (*10^5 conidia/ml)	95% Confidence limits		Slope (SE)	χ^2 (*df* = 3) (p value)
		Lower	Upper		
Beauveria bassiana NRCBEPF2	12.891	2.414	21.702	3.40 (±0.345)	1.721 (0.01)
B. bassiana NRCBEPF8	16.675	5.007	26.553	2.23 (±0.479)	0.636 (0.25)
B. bassiana NRCBEPF14	18.751	10.599	26.645	2.65 (±0.594)	2.323 (0.08)
B. bassiana NRCBEFPMP1	10.468	5.182	10.714	2.65 (±0.594)	2.323 (0.08)
B. bassiana NRCBEPF22	12.617	3.367	20.355	2.65 (±0.594)	2.323 (0.08)
B. bassiana NRCBEPF29	21.413	6.425	30.447	2.39 (±0.474)	1.154 (0.16)
B. bassiana NRCBEPF30	21.774	8.129	31.625	3.74 (±0.489)	3.052 (0.01)
B. bassiana NRCBEPF32	21.933	7.471	32.169	3.49 (±0.503)	2.774 (0.01)
B. brongniartii NRCBEPF27	25.289	6.037	38.180	2.97 (±0.478)	0.714 (0.03)
B. brongniartii NRCBEPF28	25.575	11.183	35.840	3.73 (±0.489)	3.052 (0.01)
B. bassiana BCRL	29.962	15.181	40.873	3.73 (±0.489)	3.052 (0.01)
B. bassiana TARI	29.962	15.181	40.873	3.73 (±0.489)	3.052 (0.01)

LC_{50} estimated concentration that causes 50% mortality (SPSS version 25); χ^2 = Chi-square value, *df* = degrees of freedom; slope ± standard error of the slope (covariates X are transformed using the base 10.000 logarithm)

Table 3 Probit analysis of concentration-mortality response at 15 days post inoculation of *Metarhizium* spp. against *Odoiporus longicollis*

Organism	LC_{50} (*10^5 conidia/ml)	95% confidence limits		Slope (SE)	χ^2 ($df = 3$) (p-value)
		Lower	Upper		
Metarhizium anisopliae NRCB EPF6	14.746	3.142	23.796	3.40 (±0.553)	1.153 (0.01)
M. anisopliae NRCB EPF9	18.857	12.03	26.362	2.54 (±0.557)	0.902 (0.11)
M. anisopliae NRCB EPF12	20.752	4.368	31.012	2.54 (±0.557)	0.902 (0.11)
M. anisopliae NRCB EPF16	15.171	3.570	24.057	3.45 (±0.560)	3.231 (0.01)
M. anisopliae NRCB EPF17	20.931	5.781	31.659	3.31 (±0.498)	1.068 (0.01)
M. anisopliae NRCB PF18	15.010	7.527	20.165	2.65 (±0.499)	1.255 (0.08)
M. anisopliae NRCB EPF35	14.710	2.395	24.412	3.21 (±.0538)	1.825 (0.01)
M. anisopliae NRCB EPF36	21.261	5.573	32.277	3.25 (±0.495)	1.365 (0.01)
M. robertsii NRCB EPF10	21.261	5.573	32.277	3.25 (±0.495)	1.365 (0.01)
M. robertsii NRCB EPF13	15.126	4.856	22.817	3.74 (±0.616)	3.695 (0.01)
M. robertsii NRCB EPF19	16.042	5.827	23.649	3.88 (±0.607)	2.136 (0.01)
M. robertsii NRCB EPF23	10.497	1.028	19.413	3.22 (±0.538)	1.825 (0.01)
M.robertsii NRCB EPF24	12.443	1.576	21.764	3.22 (±0.538)	1.825 (0.01)
M. robertsii NRCB EPF33	17.389	3.627	27.476	3.23 (±0.538)	1.825 (0.01)
M.quizhouense NRCB PF34	14.710	2.395	24.412	3.21 (±0.538)	1.825 (0.01)
M.quizhouense NRCB PF11	8.050	4.043	13.763	2.59 (±0.601)	1.869 (0.10)
M. pinghaense NRCB EPF7	10.155	2.540	17.339	2.92 (±0.598)	1.684 (0.03)
M. robertsii M1(ArMz6W)	25.448	8.534	37.098	3.24 (±0.495)	1.365 (0.01)
M. majus M2 (VjMz1W)	20.613	5.499	31.148	3.22 (±0.538)	1.825 (0.01)

LC_{50}estimated concentration that causes 50% mortality (SPSS version 25); χ^2 = Chi-Square value, df = degrees of freedom; slope ± standard error of the slope (covariates X are transformed using the base 10.000 logarithm)

anisopliae M1 (25.448×10^5 conidia ml^{-1}). There was no evidence of mycosis in any control cadavers, whereas mycosis was confirmed on all dead *O. longicollis* treated with *Beauveria* or *Metarhizium* spp.

Scanning electron microscope (SEM) studies

SEM analysis was conducted by the model number TM3030 Plus (Hitachi) at Indian Institute of Horticultural Research, Bengaluru, India. SEM images of adults treated with strains of *B. bassiana* (NRCBEFPMP1) and *M. quizhouense* (NRCBEPF11) revealed that after adhesion of conidia onto the cuticle of *O. longicollis*, some of the conidia germinated and mycelia appeared after 10 days. The SEM studies revealed detailed information about the attachment and infection process of *B. bassiana* and *M. quizhouense.* In the sporulated cadavers, hyphae were detected in cuticle, inter-segments of legs, antenna and abdomen. Conidiogenesis was observed on the cuticle (Figs. 1 and 2). The images showed that the virulent EPF infected approximately 15 days to complete its infection process (Fig. 3).

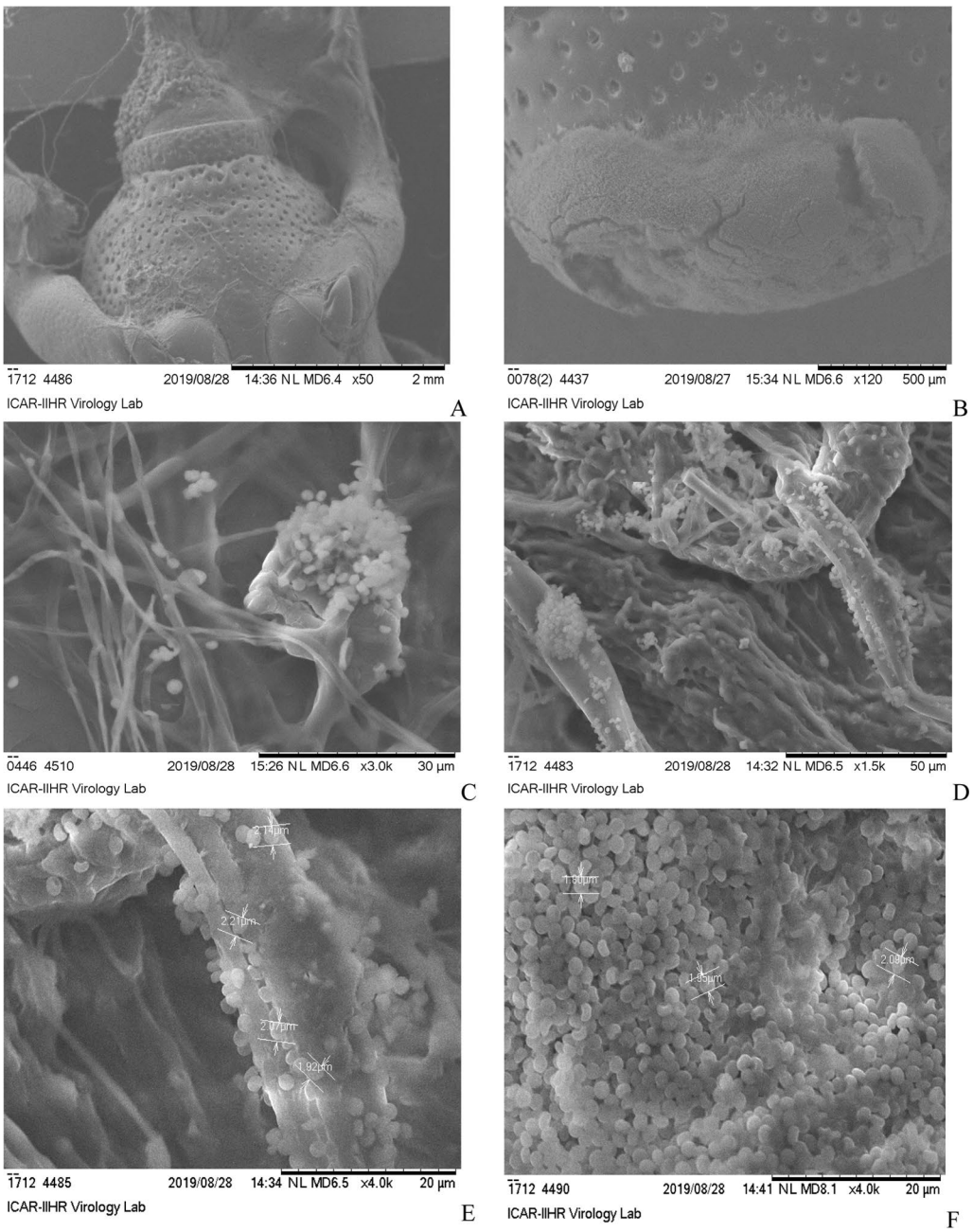

Fig. 1 SEM image showing the process of *Beauveria bassiana* infection in *Odoiporus longicollis*. **A**, **B** Conidia adhered to the surface of adults insect (2 mm & 500 µm), **C**, **D** the cuticle surface of the insect of hyphae (scale par 50 µm), **E**, **F** large amount formed in mycelia of *B. bassiana* (scale par 20 µm & 20 µm)

Identification of volatile organic compounds during pathogenesis

The composition of volatile organic compounds (VOC) released during pathogenesis by virulent EPF was investigated. Gas chromatography mass spectrometry (GC–MS) analyses showed that a significantly higher number of known insect volatile compounds were present among fungus-treated insects, when compared to untreated control. Insect toxic compounds were detected only in the volatiles of the fungus-exposed insects. These toxic compounds were identified as: Dihydrothiophenone, Benzaldehyde, Phenylacetaldehyde, Methylheptenone, 3-Octanone, 1-Hexanol, 2-ethyl-, Methyl salicylate, alpha-Terpineol, N-(3-Butenyl)-N-methylcyclohexanamine,

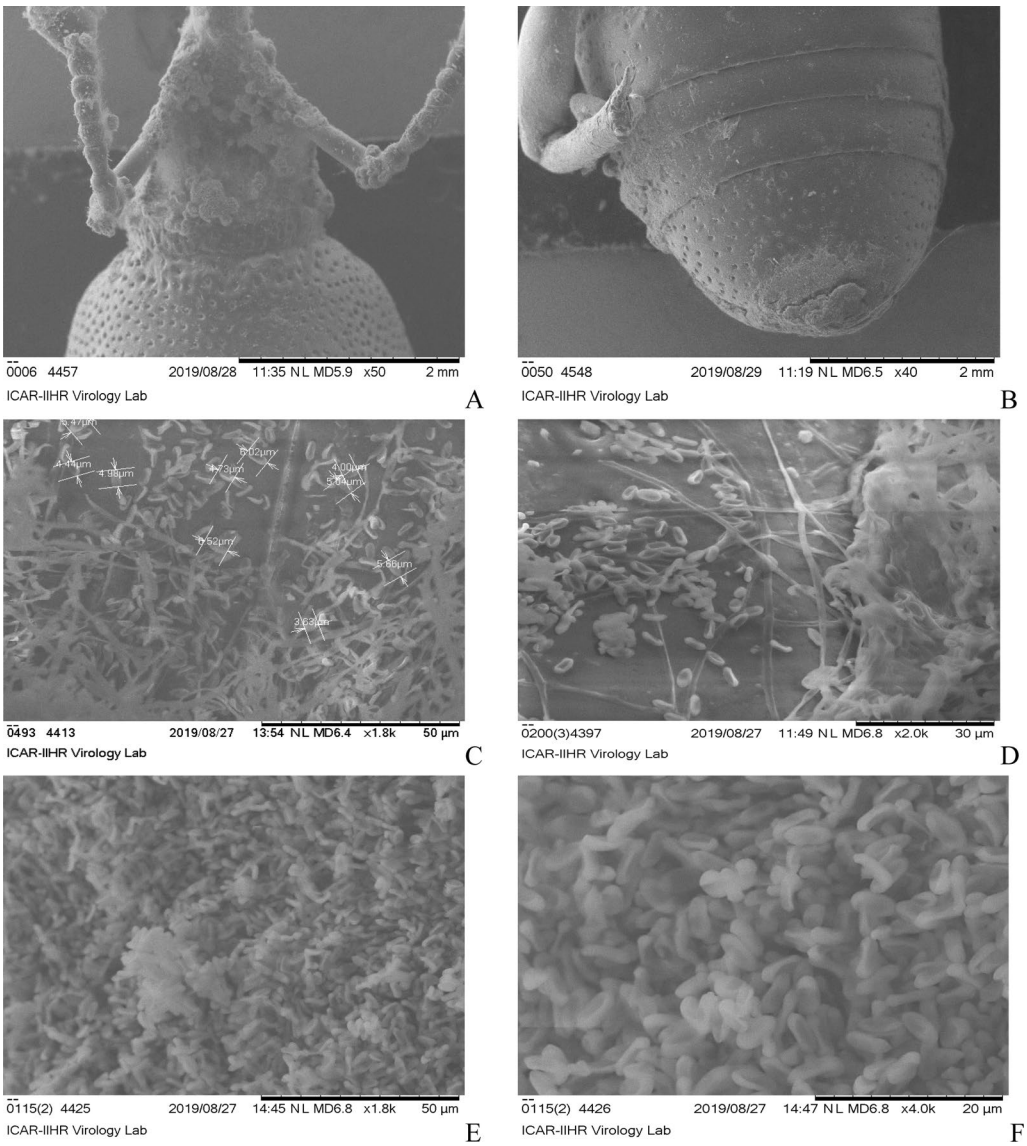

Fig. 2 SEM image showing the process of *Metarhizium quizhouense* infection in *Odoiporus longicollis*. **A**, **B** Conidia adhered to the surface of adults insect (2 mm), **C**, **D** the cuticle surface of the insect of hyphae (scale par 50 µm & 30 µm), **E**, **F** large amount formed in mycelia of *M. quizhouense* (scale par 50 µm & 20 µm)

Benzene, 1,2-dimethoxy-4-(methoxymethoxy), N,N'-Ethylenedi-beta-alanine, Palmitic acid, Caryophyllene oxide, 1-Octadecene, Hexadecanoic acid, Cyclohexane, 1,1'-dodecylidenebis [4-methyl] and these compounds were mainly categorized as insect repellents, attractants, plant metabolite, antimicrobial or antioxidants..

The PCA plot is presented in Fig. 4. PCA was applied to reduce the redundant information in data and to group the correlated responses into principal components (Hammoudaa et al. 2017). Each of the first principal components explains a variance of 88.53%. The relative peak areas of all identified volatile compounds were selected for the calculation of the main components. A multivariate analysis showed clear class separation of *Metarhizium quizhouense* species (Table 4). Slight differences were observed in the metabolite profiles of *B. bassiana*, *B. brongniartii, M. robertsii, M. pinghaense* and *M. anisopliae.*

Discussion

In the present study, 27 native strains of EPF belonging to *B. bassiana, B. brongniartii, M. anisopliae, M. robertsii, M. quizhouense* and *M. pinghaense* (Table 1)

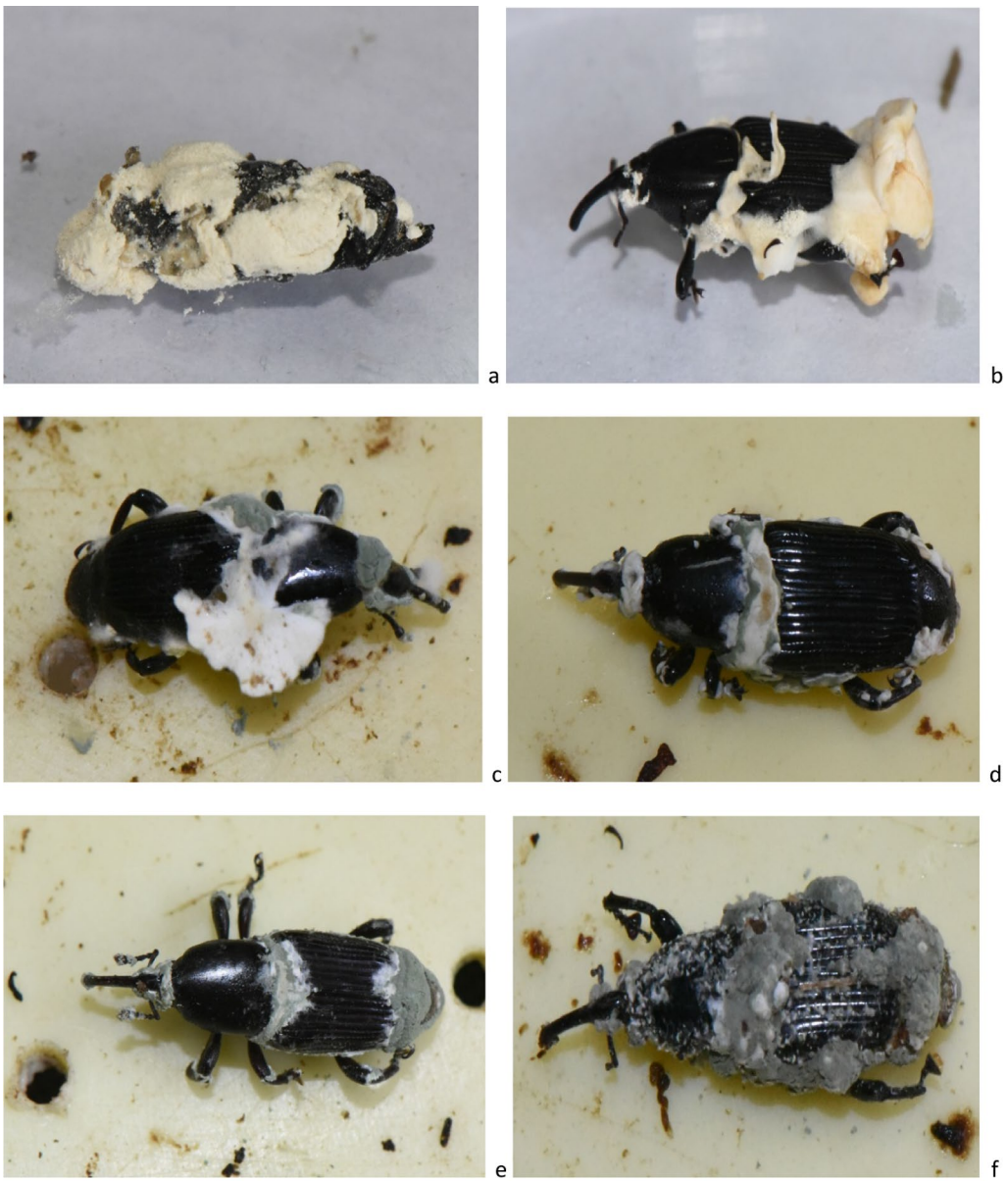

Fig. 3 Bioassays performed in entomopathogenic fungi native isolated were affected by mummification of insects after 15 days of infection; **a**, **b** *Beauveria brongniartii,* and *Beauveria bassiana* cadaver fully covered by bright white mycelia power; **c** *Metarhizium robertsii*; **d** *Metarhizium quizhouense*; **e** *Metarhizium pinghaense*; **f** *Metarhizium anisopliae* the conidia formatted and the insect cadaver green

against adults of banana stem weevil *O. longicollis* were tested under laboratory conditions. Among the tested organisms, *M. quizhouense* (NRCBPF11) was observed to be the most virulent needing 8.050×10^5 conidia/ml to kill 50% of the beetles. However, isolates such as *M. pinghaense*, *M. robertsii, B. brongniartii* and *B. bassiana* were also found to be virulent (LC_{50} of 10.468 to 12.617×10^5conidia ml^{-1}).

Bioassay studies showed that the dead adults were wholly covered by white or green conidia of the tested virulent fungi (Fig. 2). Fungal germination was also observed on dead insect, while the insect's color became white with spongy mycelia of dark green or yellowish-green. The strategy of the present study was to see if adults get infected through treated leaf sheath and the results establish this fact. Systemic fungicides worked well in controlling the larval stages of *O. longicollis* as they feed on the inside of the leaf sheath. Adults were found feeding on fallen leaves and spraying these leaves helped infecting them. Studies showed that *M. anisopliae*

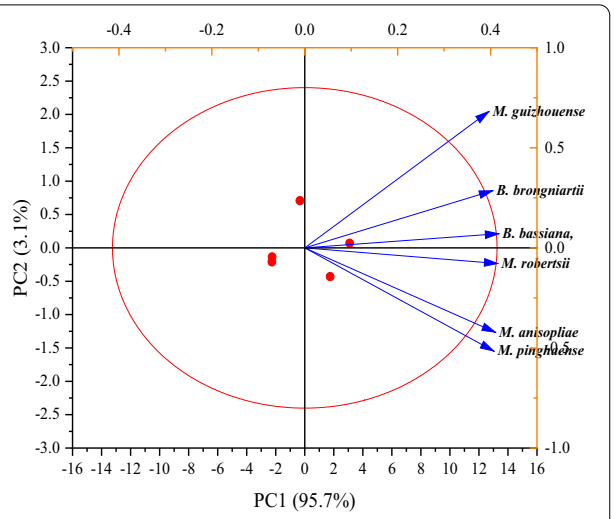

Fig. 4 Principal component analysis (PCA) obtained from the profiles of volatile compounds released by entomopathogenic fungi

Table 4 Percentage rate of total variability explained by principal components obtained in the PCA analysis

Principal component number	Eigen values	Percentage of variance (%)	Cumulative (%)
1	4.42677	88.53539	88.53539
2	0.43915	8.78295	97.31834
3	0.08125	1.62503	98.94337
4	0.0297	0.59396	99.53733
5	0.02313	0.46267	100

and *B. bassiana)* were found to be infective at 1×10^8 conidia ml^{-1} against adults *O. longicollis* (Alagesan et al. 2019). In other studies, a high mortality was obtained with "beauvericide" *B. bassiana*-based formulation when used @ 1×10^7 spores ml^{-1} (Awasthi et al. 2017). Formulations of *B. bassiana* and *M. anisopliae* exhibited LC_{50} value of 1.25×10^6 conidia ml^{-1} against *O. longicollis* at 15 days (Sharmila and Mohan 2015). The present studies showed that *Metarhizium* spp. needed less conidia to cause 50% mortality. Similar results were obtained against other banana pests like *Cosmopolites sordidus* (stem borer) with conidial suspension (1.1×10^7 conidia ml^{-1}) of *B. bassiana* (Lopes et al. 2011). Reports also suggested that with a high conidial concentration, a high mortality can be obtained (Akello et al. 2007). Further studies are needed to evaluate the virulent EPF so as to verify whether the mechanism observed in vitro occurs also in vivo.

Lozano-Soria et al. (2020) reported that in infected banana black weevil (BW), *Cosmopolites sordidus* and EPF can secrete (styrene, benzothiazole, camphor, borneol, 1,3-dimethoxy-benzene, 1-octen-3-ol and 3-cyclohepten-1-on, Alcohol (3-Octanone), and Phthalic acid) volatile compounds that can behave as attractants and repellents. This was also found in EPF infected banana fruit scarring beetle *Basilepta subcostata* Jac (Padamnaban et al. 2019). In another study Kavitha et al. (2020) could show that steroid in Stigmasterol-3-O-Glucoside was toxic to *O. longicollis*. Through, volatile was investigate in secondary metabolites, such as (Z)-9-octadecenamide, (Z,Z)-9,12-octadecadienoic acid (linoleic acid), (Z,Z,Z)-9,12,15-ocatadecadienoic acid (linolenic acid) and n-hexadecanoic acid (palmitic acid) for the identified and other invertebrates were evaluated as attractants or repellents (Rivero-Borja et al. 2018). VOCs mediate interactions between micro-organisms such as fungal insects that have been correlated with their pathogenic activity (Hummadi et al. 2021). Overall, EPF was emitted through the Volatile organic compounds (VOCs) can be used in chemotaxonomic profiling such as antifungal activity, phytotoxicity, symbiotic regulation, insect attractant, and repellent activities also other environmental parameters (Lobo et al. 2018).

In the present study, a total of 50 volatile compounds were identified from EPF infected banana stem weevil with major constituents having methyl group, methyl ester, aromatic aldehyde, carbon atoms, carboxyl groups, and alcohol. These compounds may alter the behavior of *O. longicollis* and hence some of the identified chemicals could interrupt and modify its behavior and in general its search ability for the host (banana) and could be served as a tool for management of *O. longicollis*. Recently researchers have reported the attraction of *O. longicollis* towards "male aggregation pheromone" such as "2-methyl-4-heptanol" but it was less effective but when it was used in combination with pseudostem extract it resulted in significant attraction (Palanichamy et al. 2019).

As the adults were targeted in this study, the infection process through SEM was targeted. During, infection process by both *B. bassiana* and *M. anisopliae* was typical with adhesion of conidia on surface, germination, penetration of the host cuticle and completion of life cycle by mycelial growth and sporulation on surface (Aw and Hue, 2017). Hydrophobins and cuticles were degrading enzymes such as chitinases, proteases and lipases are produced by EPF which help in penetration (Aw and Hue, 2017). The host was killed by the pathogen growth and release of fungal toxins in 15 days.

Conclusion

Biological control agents such as EPF could be used for management of banana pseudostem weevil. In the present study virulent isolates belonging to *Metarhizium* sp., and *Beauveria* sp., were identified as promising alternatives to chemical control. SEM studies showed that the infection process was completed in 15 days. The study had also identified volatiles produced during pathogenesis of virulent *Beauveria* sp. and *Metarhizium* sp. The development of possible new bio-control agents against the invasive banana pest is now possible with a better understanding of the potential use of indigenous EPF.

Abbreviations

VOC: Volatile organic compounds; SEM: Scanning electron microscopic; BSW: Banana stem weevil; LC_{50}: Lethal concentration; EPF: Entomopathogenic fungi; NRCB: National Research Centre on Banana; PDAY: Potato dextrose yeast agar; PCA: Principal components analysis; GC: Gas chromatography; MS: Mass spectrometry.

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Author contributions

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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. Data associated with this study has been deposited at NCBI GenBank: Database under the accession numbers (NCBI Acc. MT645316, 318, 309; MT140307; MT151783, 786; MK899434; MK834817), *M. anisopliae* (MK834813, 805; MN888761, 763; MN892390, 391; MT140304, 308); *B. brongniartii* (MT151781, 784), *M. robertsii* (MN889408; MN892393, 394; MN893382, 380; MK836090), *M. quizhouense* (MN892392, MN893383) and *M. pinghaense* (MN892389).

Code availability

(Graph was generated using Origin Lab Professional Version 2021b software; URL link:www.OriginLab.com/2021b, accessed on 29 September 2021) software was used in the paper for statistical analysis.

Declarations

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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References

Akello J, Dubois T, Gold CS, Coyne D, Nakavuma J, Paparu P (2007) *Beauveria bassiana* (Balsamo) Vuillemin as an endophyte in tissue culture banana (Musa spp.). J Invertebr Pathol 96:34–42

Alagesan A, Tharani G, Padmanaban B, Manivannan S, Jawahar S (2019) An assessment of biological control of the banana pseudostem weevil *Odoiporus longicollis* (Olivier) by entomopathogenic fungi *Beauveria bassiana*. Biocatal Agric Biotechnol 20:101262

Arthur CL, Pawliszyn J (1990) Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem 62(19):2145–2148

Aw KMS, Hue SM (2017) Mode of infection of *Metarhizium* spp. fungus and their potential as biological control agents. J Fungi. https://doi.org/10.3390/jof3020030

Awasthi NS, Sridharan S, Mohankumar S (2017) In vitro evaluation of native isolate of *Metarhizium anisopliae* (Metchinkoff) sorokin and its oil in water formulations against *Odoiporus longicollis* Olivier. J Biol Control 31(4):248–252

Azam M, Tara JS, Ayri S, Feroz M, Ramamurthy VV (2010) Bionomics of *Odoiporus longicollis* Olivier (Coleoptera: Rhynchophoridae) on banana plant (Musa paradisica). Mun Ent Zool 5(2):627–635

Crespo R, Pedrini N, Juárez MP, Bello GMD (2006) Volatile organic compounds released by the entomopathogenic fungus *Beauveria bassiana*. Microbiol Res 163:148–151

David BV (2008) Biotechnological approaches in IPM and their impact on environment. J Biopesti 1:01–05

Hammoudaa IB, Freitasb F, Ammara S, Gomes Da Silva MDR, Bouaziz M (2017) Comparison and characterization of volatile compounds as markers of oils stability during frying by HS–SPME-GC/MS and Chemometric analysis. J Chromatogr B 1068(1069):322–334

Hummadi EH, Dearden A, Generalovic T, Clunie B, Harrott A, Cetin Y et al (2021) Volatile organic compounds of *Metarhizium brunneum* influence the efficacy of entomopathogenic nematodes in insect control. Biol Control 155:104527

Isahaque NMM (1978) Note on the incidence of *Odoiporus longicollis* Olivier on banana in Assam. Pesticides 12(6):22–24

Jayanthi PDK, Verghese A (1999) Report of the occurrence of banana weevils in Bangalore. Insect Environ 4(4):153

Kavitha KJ, Anil John J, Evans DA (2020) Stigmasterol-3-O-glucoside, an allelopathic molecule responsible for pest resistance of Thenkaali (AAB), a Musa cultivar against *Odoiporus longicollis* Olivier. Cur Sci 118(6):946–953

Kisaakye J, Fourie H, Coyne D, Cortada L, Masinde S, Subramanian S, Haukeland S (2021) Evaluation of the Entomopathogenic Potential of *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* for Management of *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). Agriculture 11:1290

Lobo LS, Girotti JR, Mijailovsky SJ, Fernandes ÉKK, Luz C, Pedrini N (2018) Synthesis and secretion of volatile short-chain fatty acids in *Triatoma infestans* infected with *Beauveria bassiana*. Med Vet Entomol. https://doi.org/10.1111/mve.12306

Lopes RB, Michereff-Filho M, Tigano MS, Oliveria JN, Lopez EL, Fancelli M, da Silva JP (2011) Virulence and horizontal transmission of selected Brazilian strains of *Beauveria bassiana* against *Cosmopolites sordidus* under laboratory conditions. B Insectol 64:201–208

Lozano-Soria A, Ugo Picciott U, Lopez-Moya F, Lopez-Cepero J, Porcelli F, Lopez-Llorca VV (2020) Volatile Organic Compounds from

Entomopathogenic and Nematophagous Fungi, repel banana black weevil (*Cosmopolites sordidus*). Insects 11:509
Padmanaban B, Sundararaju P, Sathiamoorthy S (2001) Incidence of banana pseudostem borer, *Odoiporus longicollis* (Oliv.) (Curculionidae: Coleoptera) in banana peduncle. Indian J Entomon 63:204–205
Padmanaban B, Kamala Jaynth, PD, Bakthavatsalam N, Sarvan kumar P, Baskar N, Velavan V, Karthikeyan C, Uma S (2019) Role of host plant volatiles in adult attraction and auto dissemination of entomopathogenic fungi: A case study with Banana fruit scarring beetle *Basilepta subcostata* (Jac.). In: International conference on plant protection in horticulture: advances and challenges, 24–27 July 2019 in ICAR-IIHR, Bengaluru, India
Palanichamy S, Padmanaban B, Vaganan MM, Uma S (2019) Electrophysiological and behavioural responses of banana pseudostem weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to aggregation pheromone, 2-methyl-4-heptanol and host plant kairomones. Cur Sci 116(10):1753–1757
Rehner SA, Minnis AM, Sung G, Jennifer J, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103(5):1055–1073
Rivero-Borja M, Guzmán-Franco AW, Rodríguez-Leyva E, Santillán-Ortega C, Pérez-Panduro A (2018) Interaction of *Beauveria bassiana* and *Metarhizium anisopliae* with chlorpyrifos ethyl and spinosad in *Spodoptera frugiperda* larvae. Pest Manag Sci 74(9):2047–2052
Sharmila Bharathi C, Mohan B (2015) Bioefficacy of liquid *Beauveria bassiana* for the management of pseudostem borer *Odoiporus longicollis* (Olivier) in hill banana of Kolli hills. Asian J Plant Sci Res 5:55–60
Sripriya C, Padmanaban BS, Uma S (2000) Evaluation of banana (Musa sp.) germplasm against insect pests. Indian J Entomon 62:382–390
Thippaiah M, Ashok Kumar CT, Shivaraju C, Sudhir KS, Naveena NL (2011) Study of biology of banana pseudostem weevil, *Odoiporus longicollis* Oliv. Internat J Entomol 2:1–5
Velavan V, Rangeshwaran R, Sivakumar G, Sasidharan TO, Sundararaj R, Kandan A (2021) Occurrence of *Metarhizium* spp. Isolated from forest samples in South India and their potential in biological control of banana stem weevil *Odoiporus longicollis* Oliver. Egypt J Biol Pest Control 31:131–142
Viswakethu V, Balakrishanan P, Murugan L, Narayanaswamy B, Subbaraya U (2021) Entomopathogenic fungi as a promising biological control agent against banana fruit scarring beetle, *Basilepta subcostata* (Jac.) (Chrysomelidae: Coleoptera). Egypt J Biol Pest Control 31:53–59

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