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Pathogenicity of native strains of *Bacillus thuringiensis, Beauveria bassiana* and *Metarhizium rileyi* as entomopathogens against the polyphagous borer, *Conogethes punctiferalis* (Guenée) (Crambidae: Lepidoptera)

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Abstract

Background The shoot and fruit borer, *Conogethes punctiferalis* (Guenée) (Crambidae: Lepidoptera), is a significant pest causing substantial economic losses across various crops. The need for alternative control methods has prompted the exploration of biological control using entomopathogenic fungi and bacteria. In this study, the pathogenicity of *Beauveria bassiana, Metarhizium (Nomuraea) rileyi* and *Bacillus thuringiensis* (*Bt*) against *C. punctiferalis* larvae and pupae was assessed through laboratory bioassays.

Results Various concentrations of *B. bassiana* and *M. rileyi* spores, i.e. 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml, were tested alongside controls. Additionally, five strains of *Bt* (IIOR Bt-145, Bt-154, Bt-171, Bt-172 and Bt-127) were evaluated at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml. The LC_{50} values for *B. bassiana* and *M. rileyi* were 7.9×10^5 spores/ml and 8.7×10^4 spores/ml, respectively, after 4 and 6 days of post-treatment using the spray method. In the larval dip method, the LC_{50} values were 4.8×10^3 spores/ml for *B. bassiana* and 2.0×10^4 spores/ml for *M. rileyi* after 5 and 6 days of treatment, respectively. For pupae, the LC_{50} values were 1.2×10^6 spores/ml for *B. bassiana* and 4.3×10^4 spores/ml for *M. rileyi* after 4 and 7 days of treatment, respectively. Similarly, the five strains of *Bt* were effective against *C. punctiferalis*. However, *Bt*-154 demonstrated the highest efficacy, with LC_{50} values of 0.66 mg/ml in the spray method and 0.79 mg/ml in the larval dip method after 5 days of post-treatment.

Conclusion The potential of entomopathogenic isolates as biocontrol agents against *C. punctiferalis* provided a promising alternative to synthetic insecticides in pest management. The efficacy of *B. bassiana, M. rileyi* and *Bt* strains suggests their suitability for integrated pest management strategies, potentially reducing reliance on chemical pesticides and minimizing the environmental impacts. Further field studies are warranted to validate the efficacy and practicality of these biocontrol agents in real-world agricultural settings.

Keywords *Conogethes punctiferalis*, Entomopathogenic fungi, *Bacillus thuringiensis*, Biological control, Larval dip method

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Background

Conogethes punctiferalis Guenée (Crambidae: Lepidoptera), commonly known as the capsule borer or yellow peach moth, is a significant polyphagous pest with widespread distribution across tropical Asia, spanning

from India through Southeastern Asia to Australia (Pena et al. 2002). Additionally, it has been introduced to areas beyond its native range, including Britain and Europe, where it is recognized as a detrimental pest. This species poses a substantial threat to agricultural and forest ecosystems, as it has the potential to cause severe damage to over 40 species of fruits, field crops and forest trees. Notable hosts include peach, apple, plum, chestnut, durian, citrus, mango, papaya, maize, sorghum, sunflower, castor and various pine species. In India alone, *C. punctiferalis* infests 36 crop plants from 23 families. Its impact extends globally, being identified as the most serious insect pest of papaya in Australia (Chay-Prove et al. 2000), *Durio zibethinus* in Thailand, fruit crops and maize in China (CPCI 2005), as well as over 20 fruit crops including *Dimocarpus longan* and *Averrhoa carambola* in Korea and *Helianthus annuus* and *Macadamia ternifolia* in New Zealand (CPCI 2005). Furthermore, *C. punctiferalis* poses a significant threat to high-value spice crops such as castor (*Ricinus communis* L.), turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale* Rosc.) in India (Duraimurugan and Lakshminarayana 2016).

The larval stage of this species represents the damaging phase, as it bore into stems, shoots, buds, fruits and seeds of various plants. Its cryptic behaviour during the initial stages of infestation poses a significant challenge for effective control measures. Boring activity can predispose fruits to secondary pathogens. Common symptoms include yellowing or browning of terminal shoots, fruit damage and the presence of bore holes (Molet 2015).

Microbial biopesticides, particularly entomopathogenic fungi (EPF) and entomopathogenic bacteria (EPB), hold considerable promise as alternatives to chemical pesticides due to their unique mode of action and ability to infect a wide range of sucking and chewing insect pests. They are deemed environmentally safe and represent novel tools in pest management (Vimala Devi et al. 2021), offering advantages such as low cost, high efficiency, safety for beneficial organisms and reduced residues in the environment (Lacey et al. 2001). The objective of the present study was to assess the potential efficacy of different EPF, namely *B. bassiana* and *M. rileyi*, and certain isolates of EPB (*B. thuringiensis*) against *C. punctiferalis* infesting castor under laboratory conditions.

Methods

Insect rearing

The insects utilized in these studies were sourced from a laboratory colony of *C. punctiferalis*, initially established from larvae gathered in October 2020 from castor fields at the Department of Entomology, ICAR-Indian Institute of Oilseeds Research. The culture of *C. punctiferalis* was maintained by collecting larvae at various stages from stray castor crop. Infested castor capsules were placed in glass jars containing additional castor capsules. The adults emerging from these larvae were released into an oviposition chamber for mating and oviposition. Newly hatched larvae were reared on fresh capsules within glass jars measuring 15 cm in diameter and 21 cm in height, covered with muslin cloth. Fresh capsules were provided as needed when the old ones dried out or were consumed by the larvae. Upon pupation, pupae were individually transferred to specimen tubes measuring 10.2 cm in length and 2.5 cm in diameter for adult emergence. Five pairs (5 males and 10 females) of freshly emerged adults were introduced into the oviposition chamber. This chamber was equipped with castor inflorescences and immature capsules held in a vial containing water. Similarly, castor inflorescences and immature capsules were replenished every other day and eggs were collected accordingly. The resulting homogenous larval populations in subsequent generations were utilized for conducting further experiments (Shivakumar et al. 2020).

Entomopathogens

B. thuringiensis (Bt) isolates *Bt*-127, *Bt*-145, *Bt*-154, *Bt*-171 and *Bt*-172 and the fungal isolates *B. bassiana Bb*-4513 and *M. rileyi* IIOR-SlMr maintained at Crop Protection Section, ICAR-IIOR were used.

Maintenance of *B. thuringiensis* culture

The five *Bt* isolates, namely IIOR *Bt*-127, *Bt*-145, *Bt*-154, *Bt*-171 and *Bt*-172, were cultured on nutrient agar slants. Following a 72-h incubation period, the inoculum from the pure *Bt* culture was introduced into sterilized Luria broth medium and placed in an incubating shaker set at 180 rpm and 30 °C for 17 h. Subsequently, *Bt* multiplication was achieved through solid-state fermentation, wherein the inoculum was transferred to sterilized solid wheat bran medium supplemented with molasses and allowed to incubate for 2–3 days.

Maintenance of *B. bassiana* and *M. rileyi* culture

The fungal isolates *B. bassiana Bb*-4513 and *M. rileyi* IIOR-SlMr were cultured on potato dextrose agar- and Sabouraud maltose yeast extract agar-specific medium, respectively. They were then incubated at 25 °C for 15 days.

Multiple concentration preparation of *B. thuringiensis* for bioassay

The fully grown *Bt* on wheat bran medium was filtered using distilled water, and the filtrate was then centrifuged at 10,000 rpm for 20 min. The supernatant was discarded, and the pellet was dried and powdered. This technical powder was utilized for larval bioassays at various

concentrations of *Bt* technical powder, ranging from 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml, dissolved in water and 0.1% Tween 20 (Fite et al. 2019).

Multiple concentrations of *B. bassiana* and *M. rileyi* prepared for bioassay

For the fully grown fungal isolates, distilled water along with 0.1% Tween 20 was added and the mixture was filtered through muslin cloth. Pure conidial suspensions of *B. bassiana* and *M. rileyi* with concentrations of 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml were prepared using a hemocytometer (Fite et al. 2019).

Bioassay of *B. thuringiensis* isolates against *C. punctiferalis* larvae using larval dip method

Larvae were immersed in suspensions of *Bt* isolates IIOR *Bt*-145, Bt-154, Bt-127, Bt-171 and Bt-172 technical powder for 30 s, following which they were placed on filter paper and transferred to sterile insect rearing Petri dishes. Castor capsules or stem bits were provided daily as food. Various concentrations of *Bt* technical powder suspensions, specifically 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml, were employed for the insect bioassay. The control group was sprayed with distilled water and 0.1% Tween 20 (Senthil Kumar et al. 2016).

Bioassay of *B. thuringiensis* isolates against *C. punctiferalis* larvae using spray method

Larvae were placed on filter paper in a sterile insect rearing Petri dishes after spraying the filter paper with a volume of 3 ml suspension at multiple concentrations of IIOR *Bt*-145, *Bt*-154, *Bt*-171, *Bt*-172 and *Bt*-127 isolates technical powder, i.e. 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml diluted in distilled water and 0.1% Tween 20. The filter paper after inoculation was air dried before larvae were placed. Castor capsules or stem bits were provided daily as food. The filter paper of the control group was sprayed with 3 ml of distilled water and 0.1% Tween 20 (Chergui et al. 2020).

Bioassay of EPFs against *C. punctiferalis* larvae using larval dip method

Larvae were placed on filter paper after being immersed in the spore suspension for 30 s, following which they were transferred to sterile insect rearing Petri dishes. Castor capsules or stem bits were added daily as food. Conidial suspension at various concentrations of 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml was utilized for the insect bioassay. The control group was sprayed with distilled water and 0.1% Tween 20 (Senthil Kumar et al. 2016).

Bioassay of EPFs against *C. punctiferalis* larvae using spray method

Larvae were positioned on filter paper within a sterile Petri dish subsequent to the application of 3 ml of conidial suspension at various concentrations of 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml. The control group received a spray of 3 ml of distilled water and 0.1% Tween 20 (Chergui et al. 2020).

Bioassay of EPFs against *C. punctiferalis* pupae using the spray method

Pupae were placed onto filter paper within a Petri dish subsequent to spraying the filter paper with a volume of 3 ml of conidial suspension at various concentrations of 1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml. The filter paper for the control group was sprayed with 3 ml of distilled water and 0.1% Tween 20 (Chergui et al. 2020).

Data analysis

Observations on the number of living and dead larvae in the treatments were recorded for all experiments to calculate the mortality percentage. Three replications per treatment, each containing 30 larvae, were utilized for the bioassay of both larvae and pupae. The data were subjected to one-way analysis of variance (ANOVA) using the statistical software SPSS Windows. Probit analysis for LC_{50} calculation on specific days after treatment was also performed using the SPSS Windows software (Tesari et al. 2024).

Results

Bioassay of *B. thuringiensis* isolates against *C. punctiferalis* larvae

Following the bioassay with five isolates of *Bt* against *C. punctiferalis* larvae using different concentrations of technical powder (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml diluted in distilled water and 0.1% Tween 20), it became evident that *Bt*-154 exhibited significantly greater potential than the other tested *Bt* isolates. *Bt*-154 recorded lower LC_{50} values of 0.79 mg/ml with the larval dip method and 0.66 mg/ml with the spray method of bioassay compared to the other isolates (Table 1).

Larval dip method

Using larval dip method, at a concentration of 0.5 mg/ml, larval mortality ranged from 20.00 to 40.00%. This mortality increased to 20.00–60.00% at 1.0 mg/ml, 40.00–60.00% at 1.5 and 2.0 mg/ml and reached 80.00–100.00% at 2.5 mg/ml of technical powder of various *Bt* isolates after 5 days post-treatment, as detailed in Table 2. The results indicated that with an increase in concentration,

Table 1 LC_{50} values of *Bacillus thuringiensis* isolates tested against *Conogethes punctiferalis* larvae using larval dip and spray methods after 5 days of treatment

EPB	Larval dip method					Spray method				
	LC_{50}	Lower limit	Upper limit	Regression equation	Chi-square	LC_{50}	Lower limit	Upper limit	Regression equation	Chi-square
Bt-145	1.00	0.53	1.42	Y = 0.001 + 1.389X	3.42	0.87	0.36	1.25	Y = 0.078 + 1.315X	2.75
Bt-154	0.79	0.21	1.18	Y = 0.124 + 1.202X	2.30	0.66	0.09	1.03	Y = 0.204 + 1.134X	1.72
Bt-171	1.40	1.09	1.82	Y = 0.309 + 2.116X	3.57	1.68	1.30	2.43	Y = 0.428 + 1.909X	8.39
Bt-172	1.80	1.42	2.59	Y = 0.528 + 2.067X	9.67	1.68	1.30	2.43	Y = 0.428 + 1.909X	8.39
Bt-127	1.66	1.22	2.71	Y = 0.349 + 1.579X	1.11	1.29	0.97	1.67	Y = 0.218 + 1.991X	2.95

– *EPB* Entomopathogenic bacteria, *Bt Bacillus thuringiensis*, LC_{50}, median lethal concentration

Table 2 Mortality percentages of *Conogethes punctiferalis* 5 days after treating with 5 concentrations of *Bacillus thuringiensis* isolates using larval dip and spray bioassay methods

Concentrations (mg/ml)	Mortality (%) for larval dip method					Mortality (%) for spray method				
	***Bt*-145**	***Bt*-154**	***Bt*-171**	***Bt*-172**	***Bt*-127**	***Bt*-145**	***Bt*-154**	***Bt*-171**	***Bt*-172**	***Bt*-127**
0.5	40.00 (39.23)^{bc}	40.00 (39.23)^{c}	20.00 (26.57)^{d}	20.00 (26.57)^{c}	20.00 (26.57)^{d}	43.33 (41.14)^{c}	43.33 (41.14)^{c}	23.33 (28.86)^{c}	23.33 (28.86)^{c}	23.33 (28.86)^{d}
1	40.00 (39.23)^{bc}	60.00 (50.77)^{b}	40.00 (39.23)^{c}	20.00 (39.23)^{c}	40.00 (39.23)^{c}	43.33 (41.14)^{c}	63.33 (52.71)^{b}	23.33 (28.86)^{c}	23.33 (28.86)^{c}	43.33 (41.14)^{c}
1.5	60.00 (50.77)^{ab}	60.00 (50.77)^{b}	40.00 (39.23)^{c}	40.00 (39.23)^{b}	40.00 (39.23)^{c}	63.33 (52.71)^{b}	63.33 (52.71)^{b}	43.33 (41.14)^{b}	43.33 (41.14)^{b}	43.33 (41.14)^{c}
2	60.00 (50.77)^{ab}	60.00 (50.77)^{b}	60.00 (50.77)^{b}	40.00 (39.23)^{b}	60.00 (50.77)^{b}	63.33 (52.71)^{b}	63.33 (52.71)^{b}	43.33 (41.14)^{b}	43.33 (41.14)^{b}	63.33 (52.71)^{b}
2.5	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}
Control	20.00 (26.57)^{c}	20.00 (26.57)^{d}	20.00 (26.57)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{c}	13.33 (21.38)^{d}	13.33 (21.38)^{d}	0.00 (0.00)^{d}	6.60 (0.00)^{d}	6.60 (0.00)^{e}
CD (0.01)	4.815	5.087	4.344	3.744	4.281	4.494	4.726	4.307	4.058	4.062
CV (1%)	3.861	3.824	4.019	4.503	4.291	3.968	4.002	3.643	3.683	4.090

Values between parentheses are mortality percentages

CD @ $P=0.01$ significant at (0.01) per cent level of significance

Percentages followed by same letters in a column not significantly different by DMRT

the mortality percentage also increased. Larval mortality of 20.00% was observed in the control group of *Bt*-145, *Bt*-154 and *Bt*-171.

Spray method

Using spray method, at a concentration of 0.5 mg/ml, larval mortality ranged from 23.33 to 43.33%. This mortality increased to 23.33–63.33% at 1.0 mg/ml and reached 43.33–63.33% at 1.5 mg/ml and further increased to 43.33–63.33% at 2.0 mg/ml and to 80.00% at 2.5 mg/ml of technical powder of various *Bt* isolates after 5 days post-treatment, as detailed in Table 2. The results indicated that with an increase in concentration, the mortality percentage also increased. Larval mortality of 6.60% was observed in the control group of *Bt*-172 and *Bt*-127 and 13.33% was observed in the control group of *Bt*-145 and *Bt*-154. Larval death induced by *Bt* was confirmed by the appearance of black discoloration over the entire body of the larvae, as depicted in Fig. 1.

Bioassay of EPFs against *C. punctiferalis* larvae

Larval dip method

Following the larval dip method of bioassay with *B. bassiana* against *C. punctiferalis* larvae using different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml), the mortality percentage increased with an increase in spore concentration and with an increase in days after treatment. At concentration of 1×10^2 spores/ml, the mortality ranged from 16.70 to 70.00% and 3–7 days after treatment, respectively, but with 1×10^4 spores/ml, it ranged from 23.33 to 83.33% between the same period, respectively. At 1×10^6 spores/ml, the mortality was between 30.00 and 90.00% from three to 7 days, while at 1×10^7 spores/ml, it ranged from 46.70 to 96.70% at the same period, respectively, and it increased from 66.70 to 100.00% at the concentration of 1×10^8 spores/ml between three to seventh day after treatment. No mortality was observed in the control group (Table 3).

Following the larval dip method of bioassay with *M. rileyi* against *C. punctiferalis* larvae using different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml), the mortality percentage increased with an increase in spore concentration and with an increase in days after treatment. At concentration of 1×10^2 spores/ml, the mortality ranged from 3.33 to 43.33% and 3–7 days, respectively, and at 1×10^4 spores/ml, it ranged from 13.33 to 56.70% at the same period, respectively. After the period from 3 to 7 days post-treatment, the mortality ranged between 20.00 and 83.33% at concentration of 1×10^6 spores/ml, respectively, while it was 36.70–90.00% at 1×10^7 spores/ml, respectively, and it increased to 60.00–100.00%, respectively, at 1×10^8 spores/ml. No mortality was observed in the control group (Table 3).

Spray method

Following the spray method of bioassay with *B. bassiana* against *C. punctiferalis* larvae using different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml), the mortality percentage increased with an increase in spore concentration and with an increase in days after treatment. At concentration of 1×10^2 spores/ml, the mortality ranged from 13.33 to 50.00% and 3–7 days, respectively, and with 1×10^4 spores/ml, it ranged from 20.00 to 53.00% and 3–7 days, respectively. At 1×10^6 spores/ml, the mortality was between 30.00 and 70.00%, at the same mortality period, respectively, while at 1×10^7 spores/ml, it ranged from 36.70 to 97.00% and 3–7 days, respectively, and also, it increased from 43.33 to 100.00%, respectively, at concentration of 1×10^8 spores/ml. No mortality was observed in the control group (Table 4).

Following the spray method of bioassay with *M. rileyi* against *C. punctiferalis* larvae using different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml), the mortality percentage increased with an increase in spore concentration and with an increase in days after treatment. With 1×10^2 spores/ml, the mortality ranged from 3.33 to 30.00% after 3–7 days post-treatments, and with 1×10^4 spores/ml, it ranged from 16.70 to 50.00%. At 1×10^6 spores/ml, the mortality rate was between 26.70 and 63.00% after and 3–7 days post-treatments, respectively, while at 1×10^7 spores/ml, it ranged from 40.00 to 77.00% it increased to 63.30–97.00% and 3–7 days, respectively, with 1×10^8 spores/ml. No mortality was observed in the control group (Table 4).

Bioassay of EPFs against *C. punctiferalis* pupae using the spray method

Following the spray method of bioassay with *B. bassiana* against *C. punctiferalis* pupae using different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml), at concentration of 1×10^2 spores/ml, the mortality ranged from 10.00 to 63.33% and 3–7 days, respectively, and with 1×10^4 spores/ml, it ranged from 13.33 to 63.33% and 3–7 days, respectively. At 1×10^6 spores/ml, the mortality was between 30.00 and 80.00% from 3 to 7 days, respectively, while at 1×10^7 spores/ml, it ranged from 36.67 to 96.67% at the same period of treatment, respectively, and it increased to 53.33–100.00% with 1×10^8 spores/ml between 3–7 days, respectively. No mortality was observed in the control group (Table 5).

Following the spray method of bioassay with *M. rileyi* against *C. punctiferalis* pupae using different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^7 and 1×10^8 spores/ml), at concentration of 1×10^2 spores/ml, the mortality ranged from 0.00 to 13.33%, while at 1×10^4 spores/ml, it was between from 6.67 to 46.67% for 3–7 days, respectively. At 1×10^6 spores/ml, the mortality was between

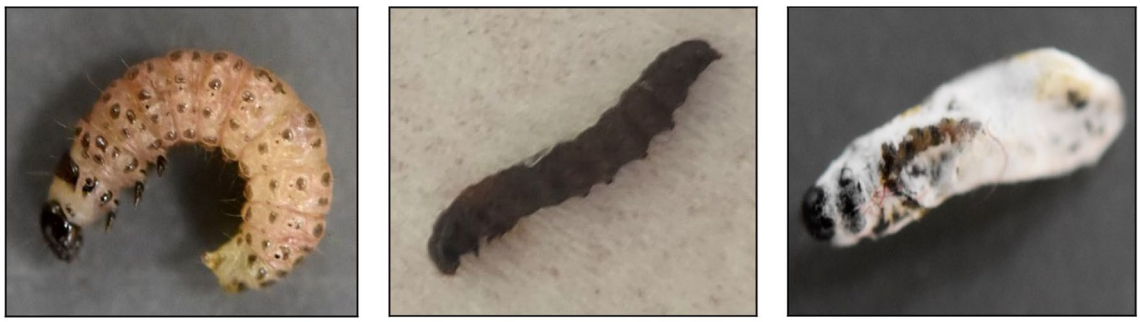

Fig. 1 Effectiveness of entomopathogens against larvae of *Conogethes punctiferalis*

Table 3 Mortality percentages of *Conogethes punctiferalis* larvae treated with 5 concentrations of entomopathogenic fungi using larval dip method

Concentrations (spore/ml)	Mortality (%) after *Beauveria bassiana* treatments					Mortality (%) after *Metarhizium rileyi* treatments				
	3DAT	4DAT	5DAT	6DAT	7DAT	3DAT	4DAT	5DAT	6DAT	7DAT
1×10^2	16.70 (24.12)^{d}	26.67 (31.09)^{d}	36.67 (37.27)^{d}	53.33 (46.91)^{d}	70.00 (56.79)^{b}	3.33 (10.51)^{d}	10.00 (18.43)^{e}	16.67 (24.10)^{e}	30.00 (33.21)^{c}	43.33 (41.15)^{c}
1×10^4	23.33 (28.86)^{cd}	43.33 (41.17)^{c}	56.67 (48.83)^{c}	70.00 (56.79)^{c}	83.33 (65.88)^{ab}	13.33 (21.39)^{c}	23.33 (28.88)^{d}	30.00 (33.21)^{d}	40.00 (39.23)^{c}	56.70 (48.85)^{c}
1×10^6	30.00 (33.21)^{c}	50.00 (45.00)^{c}	60.00 (50.77)^{bc}	73.33 (58.91)^{bc}	90.00 (71.57)^{ab}	20.00 (26.57)^{c}	36.67 (37.27)^{c}	46.67 (43.09)^{c}	63.33 (52.73)^{b}	83.33 (65.88)^{b}
1×10^7	46.70 (43.11)^{b}	66.67 (54.74)^{b}	73.33 (58.91)^{b}	80.00 (63.43)^{b}	96.70 (79.53)^{a}	36.70 (37.29)^{b}	50.00 (45.00)^{b}	60.00 (50.77)^{b}	76.67 (61.12)^{b}	90.00 (71.57)^{ab}
1×10^8	66.70 (54.76)^{a}	93.33 (75.03)^{a}	96.67 (79.49)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	60.00 (50.77)^{a}	70.00 (56.79)^{a}	86.67 (68.59)^{a}	96.67 (79.49)^{a}	100.00 (90.00)^{a}
Control	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{c}	0.00 (0.00)^{d}	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{d}	0.00 (0.00)^{d}
CD (0.01)	3.289	4.427	4.909	5.631	6.422	2.723	3.356	3.954	4.743	5.631
CV (1%)	4.299	4.311	4.290	4.286	4.247	4.472	2.394	2.820	4.294	4.267

– *EPF* Entomopathogenic fungi, *DAT* Days after treatment

Values between parentheses are mortality percentages

CD @ $P=0.01$ significant at (0.01) per cent level of significance

Percentages followed by same letters in a column not significantly different by DMRT

16.67 and 60.00%, but at 1×10^7 spores/ml, it ranged from 36.67 to 80.00% from 3 to 7 days, respectively, and it increased to 56.67 to 100.00% for the same period of treatment, respectively, at 1×10^8 spores/ml. No mortality was observed in the control group (Table 5). After the death of larvae and pupae due to EPFs, the development of mycosis was observed, characterized by white sporulation due to *B. bassiana* infection and green sporulation due to *M. rileyi* infection, confirming that the death was caused by fungi only (Fig. 2).

In the present study, the bioassay results underscore the efficacy of EPFs against *C. punctiferalis* larvae and pupae, as mortality escalates with high spore concentrations.

Additionally, the development of mycosis, characterized by distinctive sporulation patterns, provided further evidence of fungal infection as the cause of larval mortality. These findings highlight the potential of EPFs as a promising biocontrol agent for the management of *C. punctiferalis*, offering a sustainable and environmentally friendly alternative to chemical pesticides.

Concentration–mortality relationship of EPFs against larvae and pupae of *C. punctiferalis*

The LC_{50} value of *B. bassiana* is 7.9×10^5 spores/ml and the LC_{50} value of *M. rileyi* was 8.7×10^4 spores/ml at 4 and 6 days post-treatment using the spray method

Table 4 Mortality percentages of *Conogethes punctiferalis* larvae treated with 5 concentrations of entomopathogenic fungi using the spray method

Concentrations (spore/ml)	Mortality (%) after *Beauveria bassiana* treatments					Mortality (%) after *Metarhizium rileyi* treatments				
	3DAT	4DAT	5DAT	6DAT	7DAT	3DAT	4DAT	5DAT	6DAT	7DAT
1×10^2	13.33 (21.39)^{d}	23.33 (28.86)^{c}	30.00 (33.21)^{d}	40.00 (39.23)^{d}	50.00 (45.00)^{c}	3.33 (10.47)^{d}	10.00 (18.43)^{e}	13.00 (21.13)^{e}	23.00 (28.66)^{d}	30.00 (33.21)^{d}
1×10^4	20.00 (26.57)^{cd}	30.00 (33.21)^{c}	37.00 (37.46)^{cd}	43.00 (40.98)^{d}	53.00 (46.72)^{c}	16.70 (24.12)^{c}	23.33 (28.86)^{d}	30.00 (33.21)^{d}	40.00 (39.23)^{cd}	50.00 (45.00)^{c}
1×10^6	30.00 (33.21)^{bc}	40.00 (39.23)^{b}	53.00 (46.72)^{bc}	63.00 (52.54)^{c}	70.00 (56.79)^{b}	26.70 (31.11)^{bc}	36.70 (37.29)^{c}	47.00 (43.28)^{c}	53.00 (46.72)^{bc}	63.00 (52.54)^{bc}
1×10^7	36.70 (37.29)^{ab}	56.70 (48.85)^{a}	70.00 (56.79)^{b}	83.00 (65.65)^{b}	97.00 (80.03)^{a}	40.00 (39.23)^{b}	53.33 (46.89)^{b}	60.00 (50.77)^{b}	67.00 (54.94)^{b}	77.00 (61.34)^{b}
1×10^8	43.33 (41.15)^{a}	80.00 (63.43)^{a}	93.00 (74.66)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	63.30 (52.71)^{a}	70.00 (56.79)^{a}	77.00 (61.34)^{a}	90.00 (71.57)^{a}	97.00 (80.03)^{a}
Control	0.00 (0.00)^{e}	0.00 (0.00)^{d}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{e}	0.00 (0.00)^{e}
CD (0.01)	2.817	3.811	4.443	5.175	5.064	2.913	3.390	3.758	4.308	4.856
CV (1%)	4.246	4.293	4.295	4.317	4.284	4.445	2.418	4.310	4.299	3.464

– *EPF* Entomopathogenic fungi, *DAT* Days after treatment

Values between parentheses are mortality percentages

CD @ $P=0.01$ significant at (0.01) per cent level of significance

Percentages followed by same letters in a column not significantly different by DMRT

Table 5 Mortality percentage of *Conogethes punctiferalis* pupae with 5 concentrations of entomopathogenic fungi using the spray method

Concentrations (spore/ml)	Mortality (%) after *Beauveria bassiana* treatments					Mortality (%) after *Metarhizium rileyi* treatments				
	3DAT	4DAT	5DAT	6DAT	7DAT	3DAT	4DAT	5DAT	6DAT	7DAT
1×10^2	10.00 (18.43)^{c}	23.33 (28.86)^{c}	30.00 (33.21)^{d}	43.33 (41.17)^{c}	63.33 (52.73)^{c}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	13.33 (21.41)^{e}
1×10^4	13.33 (21.41)^{c}	26.70 (31.11)^{c}	40.00 (39.23)^{d}	53.33 (46.91)^{c}	63.33 (52.73)^{c}	6.67 (14.89)^{d}	13.33 (21.39)^{d}	20.00 (26.57)^{d}	30.00 (33.21)^{d}	46.67 (43.09)^{d}
1×10^6	30.00 (33.21)^{b}	43.33 (41.15)^{b}	56.67 (48.83)^{c}	70.00 (56.79)^{b}	80.00 (63.43)^{bc}	16.67 (24.04)^{c}	26.70 (31.11)^{c}	36.67 (37.23)^{c}	50.00 (45.00)^{c}	60.00 (50.77)^{c}
1×10^7	36.67 (37.27)^{ab}	56.70 (48.85)^{b}	76.67 (61.12)^{b}	86.67 (68.59)^{a}	96.67 (79.49)^{ab}	36.67 (37.23)^{b}	46.70 (43.11)^{b}	56.67 (48.79)^{b}	66.67 (54.70)^{b}	80.00 (63.43)^{b}
1×10^8	53.33 (46.91)^{a}	73.33 (58.89)^{a}	90.00 (79.49)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	56.67 (48.79)^{a}	70.00 (56.79)^{a}	90.00 (71.57)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
Control	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{e}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{e}	0.00 (0.00)^{f}
CD (0.01)	2.810	3.704	4.936	5.411	5.984	2.508	3.009	3.656	4.455	4.937
CV (1%)	4.299	2.642	4.360	4.290	4.254	4.830	4.751	2.608	4.808	4.420

– *EPF* Entomopathogenic fungi, *DAT* Days after treatment

Values between parentheses are mortality percentages

CD @ $P=0.01$ significant at (0.01) per cent level of significance

Percentages followed by same letters in a column not significantly different by DMRT

of bioassay. In the larval dip method, LC_{50} value of *B. bassiana* was 4.8×10^3 spores/ml, while it was 2.0×10^4 spores/ml for *M. rileyi* at 5 and 6 days post-treatment. For pupae, the LC_{50} values were 1.2×10^6 and 4.3×10^4 spores/ml for *B. bassiana* and *M. rileyi*, respectively, at 4 and 7 days post-treatments, respectively, as presented in Table 6.

Both bioassay methods were employed to assess the efficacy of *Bt, B. bassiana* and *M. rileyi* against *C. punctiferalis* larvae, while only the spray method was utilized

Fig. 2 Effectiveness of entomopathogenic fungi against pupae of *Conogethes punctiferalis*

Table 6 Effect of the tested EPF against *Conogethes punctiferalis* larval and pupal stages

	DAT	LC_{50}	Lower limit	Upper limit	Regression equation	Chi-square
Larval bioassay—spray method						
Beauveria bassiana	5	7.9×10^{5}	9.4×10^{4}	9.7×10^{6}	Y = 1.394 + 0.236X	4.00
Metarhizium rileyi	6	8.7×10^{4}	1.1×10^{4}	5.1×10^{5}	Y = 1.402 + 0.284X	3.18
Larval bioassay—larval dip method						
Beauveria bassiana	4	4.8×10^{3}	1.5×10^{2}	3.8×10^{4}	Y = 0.912 + 0.248X	5.90
Metarhizium rileyi	6	2.0×10^{4}	2.5×10^{3}	1.0×10^{5}	Y = 1.365 + 0.317X	4.37
Pupal bioassay						
Beauveria bassiana	4	1.2×10^{6}	1.3×10^{5}	2.1×10^{7}	Y = 1.361 + 0.224X	2.02
Metarhizium rileyi	7	4.3×10^{4}	9.6×10^{3}	1.5×10^{5}	Y = 1.960 + 0.423X	5.64

– *EPF* Entomopathogenic fungi, *DAT* Days after treatment, LC_{50}, median lethal concentration

for testing *B. bassiana* and *M. rileyi* against *C. punctiferalis* pupae. Following the bioassay with *Bt*, both methods demonstrated a maximum mortality of 80.00% with the highest concentration of 2.5 mg/ml after 5 days of treatment. However, among the five native isolates of *Bt* (IIOR *Bt*-127, *Bt*-145, *Bt*-154, *Bt*-171 and *Bt*-172), *Bt*-154 was found to be effective against *C. punctiferalis* larvae, recording lower LC_{50} values of 0.79 mg/ml and 0.66 mg/ml with the larval dip and spray method of bioassay, respectively, after 5 days of treatment, than other isolates. With the EPF evaluated against larvae and pupae of *C. punctiferalis,* both *B. bassiana* and *M. rileyi* were found to be the most effective against the two stages.

Discussion

Entomopathogenic microorganisms, such as certain strains of bacteria and fungi, offer a promising alternative for the control of *C. punctiferalis*. These biocontrol agents specifically target and infect the pest insect, exerting their lethal effects while posing minimal risk to non-target organisms and the surrounding ecosystem. By harnessing the natural enemies of *C. punctiferalis* especially entomopathogenic bacteria and fungi, eco-friendly management practices can be implemented to effectively reduce pest populations while preserving environmental integrity and promoting sustainable agricultural practices.

B. thuringiensis

Efforts to develop biopesticides for controlling *C. punctiferalis* emphasize the need to screen new, potentially more potent isolates. Integrated pest management strategies could benefit from the incorporation of these biopesticides. However, it is worth noting that *Bt* has been found to have adverse effects on various biological aspects of larval, pupal and adult stages of many pests.

In the present study, we evaluated the efficacy of *Bt* across a range of concentrations, observing significant mortality rates among the tested isolates. This range facilitated the accurate estimation of the median lethal

concentration (LC_{50}). Consistent with our findings, the previous research by Lalitha and Muralikrishna 2012 demonstrated high mortality rates in *S. litura* following *Bt* treatment.

The observed lower mortality of *C. punctiferalis* when exposed to *Bt*-171 and *Bt*-172 isolates in the present study suggested the potential development of resistance by *C. punctiferalis* larvae against *Bt* toxins. To achieve early mortality, high concentrations of *Bt* could be applied against *C. punctiferalis* larvae. These differences may stem from variations in insecticidal genes present or differences in the insect strain utilized, as noted by Lone et al. 2016. Additionally, Alsaedi et al. (2017) reported the susceptibility of *Tuta obsoluta* larval instars to *Bt* toxins.

The variation in LC_{50} values among larval hosts exposed to different concentrations of *Bt* powder provides evidence for the disparity in mortality observed in the present study. These findings can be contextualized with the research of Lone et al. (2016), who demonstrated that the *Bt* isolate JK12 exhibited high toxicity against *H. armigera*, requiring a median lethal concentration of 0.184 mg/ml to cause 50.00% larval mortality.

Considerable variability exists in the effectiveness of *Bt* isolates against target insects, as noted by Yaradoni (1999). The degree of pathogenicity varies with the concentration of bacterial isolate, as well as the duration of exposure and the stage of metamorphosis of the silkworm (Savitri and Murali Mohan 2003). Knowles (1994) suggested that the variations in efficacy against different lepidopteran species may stem from differences in the number of cry genes and the absence of specific binding sites.

The disparity in LC_{50} values among different *Bt* isolates suggested variations in their efficacy against the target pest species. Factors influencing this variation may include differences in the genetic makeup of the isolates, variations in the expression of insecticidal proteins and the specific mechanisms by which each isolate interacts with the target pest's biology. For instance, *Bt*-154 showed a low LC_{50} value indicated that this isolate's efficiency was higher at the lowest concentrations compared to other isolates, implying potentially greater potency or a more effective mode of action against *C. punctiferalis*.

B. bassiana and *M. rileyi*

Both spray and larval dip methods demonstrated efficacy in inducing mortality among the target insects. These findings are consistent with the previous research conducted by Gonzalez-Cabrera et al. (2011) in bioassays targeting the coffee berry borer using fungal entomopathogens, wherein various application methods were employed. Specifically, high mortality among coffee berry borers treated with *Bb* using the dipping method was documented the effectiveness of different bioassay methods in inducing larval mortality (Gonzalez-Cabrera et al. 2011).

The larval dipping method offers advantages by providing direct exposure of spores to the insects, facilitating mortality. Moreover, this method reduces labour requirements, particularly beneficial when screening multiple isolates and proves useful in assessing the potency of different strains. Notably, our control experiment with entomopathogens did not result in any observed mortality, consistent with findings reported by Gonzalez-Cabrera et al. (2011). Furthermore, the implementation of biocontrol agents, such as EPF strains, has been shown to decrease pest incidence, as observed by Yun et al. (2017). The mortality rate observed in such cases was influenced by various factors including spore concentration; time elapsed after fungal treatment and temperature levels.

The present study elucidated that the screening of two EPF isolates revealed the virulence of *B. bassiana* and *M. rileyi* against larvae of *C. punctiferalis*. The majority of isolates induced larval mortality, indicating their capacity to effectively combat *C. punctiferalis* larvae. Specifically, *B. bassiana* and *M. rileyi* exhibited virulence under laboratory conditions. These findings are comparable to the research conducted by Douro et al. (2012), who identified isolates of *B. bassiana* and *M. anisopliae* that caused significant larval mortality in *Heliothis armigera* L.

Additionally, it was observed that the mycosis development of the EPF, *M. rileyi* was slower than *B. bassiana*. Furthermore, the mortality of larvae increased with higher spore concentrations, suggesting a concentration-dependent relationship in the efficacy of these EPF isolates against *C. punctiferalis* larvae. Similarly, Akmal et al. (2013) investigated the effectiveness of different entomopathogenic fungal strains against various aphid species under controlled laboratory conditions. They observed that the mortality of the aphids increased with higher spore concentrations.

In the present study, the efficacy of EPF in controlling insect pests has been well documented and added valuable insights into their potential application. The susceptibility of *C. punctiferalis* larvae to *B. bassiana,* determined through the spray method, aligns with previous findings by Swati et al. (2017), who reported significant pathogenicity of *B. bassiana* against *H. armigera*. Similarly, for *M. rileyi* indicate its substantial effectiveness, which is consistent with Senthil Kumar (2021) who demonstrated notable susceptibility of *C. punctiferalis* to *Metarhizium*.

In the larval dip method, *B. bassiana* and *M. rileyi* also showed significant pathogenicity. These results are corroborated by Fite et al. (2019) who reported high mortality rates in *H. armigera* larvae treated with these

entomopathogenic fungi. Additionally, Ramanujam et al. (2020) provided evidence of the substantial impact of *B. bassiana* on *S. frugiperda* larvae, further supporting our findings.

It has been established that a concentration of 1×10^8 spores/ml induces the highest mortality in *C. punctiferalis* larvae when inoculated with *M. rileyi* and *B. bassiana*. This result can be compared to the mortality observed in *H. armigera* larvae treated with *B. bassiana*-APPRC-9604 isolate at a concentration of 1×10^8 conidia/ml, which was identified as a more virulent strain under laboratory conditions, reducing larval infection (Fite et al. 2019). Ana et al. (2018) also noted the highest susceptibility of *S. frugiperda* larvae to *B. bassiana*, resulting in 100.00% larval mortality at a concentration of 1×10^8 conidia/ml. Kalvnadi et al. (2018) observed the virulence of *B. bassiana* and *M. anisopliae* isolates, respectively, causing a high larval mortality and affecting the biological parameters of *H. armigera*.

The present findings can be compared to those of Wraight et al. 2010, who conducted a study comparing the virulence of *B. bassiana* isolates against lepidopteran insect pests. Their research indicated that larvae of *Heliothis zea* and *Spodoptera exigua* were susceptible to fungal infection. Swathi et al. (2017) analysed the lowest LC_{50} and observed the highest larval mortality (100%) against *H. armigera* when treated with *B. bassiana.*

Mycosis observed on the larvae in the present results, along with the outgrowth of white sporulation due to *B. bassiana* and green sporulation due to *M. rileyi* infestations on larval cadavers of *C. punctiferalis*, provided evidence that the mortality of larvae was attributed to the treated fungal isolates. These results can be compared to the findings of Lacey et al. (2015), who studied the role of fungal conidia, variations in concentrations of spore suspensions, larval species and larval instars in causing infection by entering the host body through the cuticle. The increase in mortality of larvae with the rise in concentration of spore suspension was compared to the study of Rogge et al. (2017), who reported that high conidial application rates led to a significant increase in mycosis, consequently reducing the incidence of wireworm, *Agriotes obscurus* (L.).

Conclusion

In conclusion, both *Bt* and EPF demonstrated remarkable potential as biocontrol agents against *C. punctiferalis*. Particularly, among the *Bt* isolates, IIOR *Bt*-154 exhibited superior virulence compared to others, under laboratory conditions, effectively reducing larval infestations. Additionally, both *B. bassiana* and *M. rileyi* were highly effective against both larvae and pupae of C. *punctiferalis*, showcasing their potential as efficient entomopathogenic fungi for integrated pest management strategies. These findings highlight the promise of utilizing these agents as biopesticides, offering a viable alternative to synthetic insecticides. However, to fully harness their potential, further efforts are needed to develop formulations for these virulent isolates, enhancing their shelf life and efficacy as entomopathogens. Ultimately, the future of integrated pest management for *C. punctiferalis* could be significantly enhanced through the strategic incorporation of these biopesticides into existing pest control strategies.

Abbreviations

Bt	*Bacillus thuringiensis*
LC_{50}	Median lethal concentration
EPF	Entomopathogenic fungi
EPB	Entomopathogenic bacteria
SPSS	Statistical Package for the Social Sciences

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-024-00808-1.

Supplementary file 1.

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Author Contributions

P. Duraimurugan: Supervision, Resources, Project administration, Investigation. E. Bharathi: Methodology, Investigation, Formal analysis. Neethu Roy. D: Writing – original draft, review and editing. Hariharan Selvam: Review and editing.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

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Not applicable.

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No potential conflict of interest was reported by the author(s).

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