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Evaluation of bio-efficacy of *Metarhizium anisopliae* against the pink bollworm *Pectinophora gossypiella* (Saunders), with insights into its colonization potential and insecticide compatibility

THAVKAR Snehal[1], THUBE Shivaji[2,5*], PANCHBHAI Pramod[1], LAVHE Nandkishor[1], PILLAI Tinni[1], SHAH Vivek[2], DESHMUKH Vrushali[2], PANDIAN Thavaprakasa R.[3], FAND Babasaheb[2], TENGURI Prabhulinga[2], NIKOSHE Akash[2], PRIYANK Mhatre[4], BEHERE Gajanan[2] and PRASAD Yenumula[2]

Abstract

Background The pink bollworm (*Pectinophora gossypiella*, PBW) is a major cotton pest, causing economic losses by damaging seeds and fiber. Cotton growers typically use systemic and broad-spectrum insecticides for its management, which pose risks to human health and the environment. Consequently, there is a need for eco-friendly alternatives. This study evaluates the bio-efficacy of the entomopathogenic fungus *Metarhizium anisopliae* strain TMBMA1 against pink bollworm and assesses its compatibility with major insecticides. Additionally, to comprehend the dynamics of colonization and the infection processes of entomopathogenic fungi (EPF), scanning electron microscopy (SEM) of infected larvae was carried out.

Result We challenged the second instar PBW larvae to eight different concentrations (1×10^3 to 1×10^{10} conidia mL^{-1}) of an *M. anisopliae* strain TMBMA1. The highest mortality (100%) occurred at the higher concentrations i.e., 1×10^9 and 1×10^{10} spores mL^{-1}, while the lowest mortality rate (46.6%) was observed at 1×10^3 spores mL^{-1} concentration compared to control (3.33%). TMBMA1's biocontrol efficacy was validated by Probit analysis, exhibiting an exceptionally low median lethal concentration (LC_{50}) value of 7.1×10^5. The comparative evaluation revealed that the *M. anisopliae* strain TMBMA1 performed excellently with insecticide [Cypermethrin 10% (volume fraction) emulsifiable concentrate (EC) at 1 $mL{\cdot}L^{-1}$ water] giving 100% mortality, both being superior to a commercial product of *M. anisopliae* (60%). According to SEM analysis, the EPF strain was profusely colonized on both the internal and external surfaces of PBW larvae. Compatibility studies with insecticides revealed > 98% and > 96% reduction in the sporulation of *M. anisopliae* due to the treatment of Emamectin Benzoate 1.5% (mass fraction) + Profenofos 35% (mass fraction) water dispersible granules (WDG) and Profenofos 50% EC, respectively. In contrast, Cypermethrin 10% EC, Emamectin Benzoate 5% (mass fraction) Soluble Granules and Neem Seed Kernel Extract (NSKE) 0.15% (volume fraction) treatments reported lower reduction (11.45%, 13.79% and 21.21% respectively) in spore production.

*Correspondence:
Thube Shivaji
shivajithube@gmail.com; Shivaji.Thube@icar.gov.in
Full list of author information is available at the end of the article

Conclusion According to the current investigations, the *M. anisopliae* strain TMBMA1 exhibits high virulence against PBW and offers a promising eco-friendly solution for managing this pest. It shows significant potential to proliferate on both external and internal surfaces of PBW. This strain can be integrated into PBW management programs with chemical insecticides, improving pest control and lessening environmental impact.

Keywords Bio-efficacy, Compatibility, Entomopathogenic fungi, *Metarhizium anisopliae*, Pink bollworm, SEM

Background

Cotton is one of the most important fiber crops globally, grown in the tropical and subtropical regions of more than 70 nations including India (Shaheen et al. 2012; Chakravarthy et al. 2014). It is the major fiber crop grown in India and plays a dominant role in the agricultural and industrial sectors. It contributes 70% of total fiber consumption in the textile sector and 38% of India's exports, fetching over Rupees (₹) 42 000 crores (Mageshwaran et al. 2019). In India, cotton is cultivated on 12.47 million hectares, with a production of 32.3 million bales (170 kg per bale) and a lint productivity of 441 kg·hm^{-2}, compared to the world average of 712 kg·hm^{-2} (The Cotton Corporation of India 2022; ICAR-AICRP 2023). While India leads in total area covered with cotton cultivation, its productivity remains the lowest among major global cotton planting countries/regions. This low productivity is influenced by various biotic and abiotic factors. In particular, the infestation caused by insect pests is highlighted as a crucial constraint affecting cotton production (Nagrare et al. 2022).

The pink bollworm (PBW), *Pectinophora gossypiella*, is a major threat among various insect pests, this pest species is capable of causing yield reductions of up to 90% before the widespread use of broad-spectrum insecticides and the introduction of transgenic cotton (Patil 2003). The introduction of synthetic pyrethroids in India during the 1980s marked a significant advancement in managing this challenging cotton pest. However, the uncontrolled application of these chemical insecticides resulted in widespread ecological disruptions, exacerbating PBW problems and contributing to issues with other secondary pests in the cotton ecosystem (Kranthi et al. 2002; Kranthi et al. 2009).

Following the introduction of genetically engineered transgenic cotton (Bt cotton), which incorporates delta-endotoxin proteins (Cry1Ac and Cry2Ab) from *Bacillus thuringiensis*, there was a significant advancement in the management of PBW in cotton (Choudhary et al. 2010). In its initial phase, Bt cotton effectively controlled the bollworm complex. However, subsequent reports indicated resistance in PBW populations to Cry1Ac, allowing their survival on Bollgard I cotton (Dhurua and Gujaret al. 2011). While Bollgard II effectively managed PBW until 2013, widespread infestations of PBW have been recorded from 2014 onwards. The development of resistance, particularly against transgenic cotton (Bollgard I and Bollgard II), is the primary reason for the extensive infestation of PBW in Indian cotton (Fand et al. 2019). Significant PBW infestations have recently been reported in India, with incidences ranging from 8 to 92%. These infestation levels are predicted to have reduced yields by 10% to 30% (Nagrare et al. 2023).

To combat PBW infestation in cotton, farmers frequently resort to indiscriminate use of broad-spectrum insecticides. Apart from affecting human health, these chemicals contribute to environmental pollution in delicate agricultural ecosystems, causing significant harm to natural resources (Thube et al. 2022, 2023). Pesticide poisoning has claimed many lives among agricultural workers in cotton fields in India; within three months a total of 21 deaths were reported in 2017 due to the unintentional inhalation of pesticides during its application in the cotton field (The Hindu 2017). Acute pesticide poisoning affects both male and female cotton growers across India (Mancini et al. 2005). Additionally, insecticides not only harm beneficial soil microflora and natural pest enemies but also pose risks to pollinators; residues of neonicotinoids have been found in cotton flowers in China (Wu et al. 2022) and residues of various pesticides also persist in cotton seeds, a vital source of edible oil (Blossom et al. 2004).

Employing natural pest management techniques such as biological control agents (BCAs) like predators, parasitoids, and entomopathogenic fungi offers safe alternatives to synthetic pesticides (Colmenarez et al. 2020). Various species of entomopathogenic fungi (EPF) have been investigated as effective and environmentally sustainable methods for controlling a diverse array of agricultural pests (Kisaakye et al. 2021; Nawaz et al. 2022). Among BCAs, EPF has gained considerable attention due to their potential for biocontrol, self-sustaining behavior, environmentally friendly characteristics, ease of mass production, and specific action against pests (Thube et al. 2022). These fungi induce lethal infections in their hosts, serving as natural regulators of various pests, including soil-dwelling insects through epizootics (Goettel et al. 2005). They have gained prominence in biological control programs across diverse ecosystems (Lacey et al. 2015). *Metarhizium anisopliae* and *Beauveria bassiana*

are among the most extensively studied species of EPF, particularly in terms of commercial production (Goettel et al. 2005; Meyling et al. 2007). Numerous microbial pesticides have been developed worldwide using different EPF species (Fang et al. 2014). Within the genus *Metarhizium*, there are both generalist strains, such as *M. anisopliae*, *M. brunneum*, and *M. robertsii*, which infect a wide range of insect species across different orders, and specialist strains like *M. acridum*, which target specific insect species within the Orthoptera order (Wang et al. 2016), both these groups share similar mode of action (San Aw et al. 2017). Besides their efficacy against lepidopteran pests, *M. anisopliae* has demonstrated effectiveness against various sucking pests such as leafhoppers (Naik et al. 2018), *Thrips tabaci* (Gillespie 1986), and *Frankliniella occidentalis* in chrysanthemum (Lopes et al. 2000).

Validating the colonization potential of virulent EPF strains through Scanning Electron Microscopy (SEM) is crucial for investigating the mechanisms by which EPF interact with their insect hosts, offering detailed insights into colonization dynamics and infection processes (Sun et al. 2016). This technique enables researchers to visualize and analyze critical aspects such as spore attachment, hyphal growth, and penetration into insect cuticles, essential for assessing the efficacy and biocontrol potential of EPF. SEM's high-resolution imaging capabilities not only aid in understanding the mode of action of EPF but also facilitate comparative studies between different strains or treatments, supporting informed decisions in EPF-based pest management strategies.

Additionally, if any EPF proves to be successful, its compatibility for integration into other pest management approaches has to be investigated to incorporate it into integrated pest management (IPM) programs. Compatibility studies between EPFs and chemical insecticides are crucial for enhancing the effectiveness of pest control, preserving beneficial microbes, minimizing resistance development, reducing chemical residues, and promoting sustainable agricultural practices. EPFs, being slow-acting, benefit from integration with faster-acting insecticides, achieving quicker pest mortality and more sustained control. However, many insecticides are lethal to beneficial microbes, including EPFs, necessitating the avoidance of such insecticides post-EPF application to protect EPF spores for self-perpetuation. This combined approach leverages the strengths of both control methods, supporting IPM strategies that reduce chemical dependency, enhance ecological balance, and ensure long-term pest control. Hence, the present study aimed to evaluate the bio-efficacy of an indigenous insect pathogenic isolate of *M. anisopliae* against PBW, validate its virulence through scanning electron microscopy, and study the compatibility of this isolate with recommended insecticides for designing better management strategies for PBW in cotton.

Methods

Culture of Metarhizium anisopliae TMBMA1

The pure culture of the EPF *M. anisopliae* strain TMBMA1 was obtained from the Fungal Culture Collection of ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal Karnataka. This strain was isolated from the tea mosquito bug (*Helopeltis theivora*, TMB) infesting cocoa and was found to be the most virulent native strain infecting TMB (Thube et al. 2022).

Mass culturing of EPF

A 5 mm size mycelial disc of 10 days pure EPF (TMBMA1) culture was placed into 50 mL of potato dextrose broth (PDB) and flasks were incubated at (28 ± 2) °C for 5–7 days under shaking conditions (150 r·min^{-1}). The spore suspension was harvested through two layers of muslin cloth to remove the media remnants. The resulting spore suspensions were adjusted to the concentrations of 1×10^{3} to 1×10^{10} conidia mL^{-1} using a Neuberger chamber/ hemocytometer under a compound microscope.

Rearing of pink bollworm

The nucleus culture of PBW larvae was collected from the cotton variety Suraj (*Gossypium hirsutum*) from the research farm of the Indian Council of Agricultural Research-Central Institute for Cotton Research (ICAR-CICR), Nagpur during 2022–2023. The population was maintained on a chickpea-based artificial diet under controlled conditions in the insectary of ICAR-CICR, Nagpur at a temperature of 27 °C ± 1 °C, relative humidity 65% ± 5% with 14 h : 10 h photoperiod (Shah et al. 2021). Moths were allowed to pair and lay their eggs on cotton twigs; a cotton plug dipped in 10% (volume fraction) honey solution was provided as a food, moisture and energy source. Egg-laden twigs were transferred to plastic jars with a tissue paper lining placed at the bottom for clear visibility of neonate larvae. The newly hatched larvae were further reared on an artificial diet.

In-vitro bio-efficacy evaluation

The efficacy of the EPF isolate TMBMA1 was evaluated against 2nd instar larvae of PBW in an *in-vitro* condition. The 2nd instar larvae of PBW were chosen for the study based on preliminary investigations, which indicated that 1st instar larvae were more susceptible to handling during experiments due to their delicate nature, resulting in higher mortality rates even in the control. We hypothesized that the virulence response of 2nd instar larvae to

EPF would similarly apply to 1st instar larvae. Initially, a broad-range bioassay was conducted to finalize the various conidial concentrations used in the final bioassay. In addition to the six desired conidial concentrations of TMBMA1, a commercial formulation of *M. anisopliae* (IPL Biological) and cypermethrin 10% emulsifiable concentrate (EC) (1 mL·L^{-1}) were evaluated against 2nd instar larvae of PBW. The laboratory experiment was conducted under a completely randomized design with ten treatments, including an untreated control. Uniform-size cotton squares/bolls were collected from a cotton field (Cultivar: Suraj Non-BT) and surface sterilized with 1% (volume fraction) sodium hypochlorite solution, followed by washing with sterile distilled water. Air-dried surface sterilized cotton squares/bolls were used to evaluate the efficacy of various treatments against PBW. About 50 mL of each desired conidial suspension was prepared in a 100 mL beaker. The sterilized cotton bolls were dipped into the suspension of various treatments. After air drying, the petiole of each boll was immersed in a 2 mL Eppendorf tube containing 10% (volume fraction) sucrose solution to maintain the turgidity of the detached bolls. Each single boll was then transferred to a separate insect breeding dish (diameter 90 mm, height 40 mm). A single 2nd instar larva was introduced into each dish. Ten such inoculated insect breeding dishes were treated as one replication, and three replications were maintained for each treatment (total insects, $n=30$ per treatment). Inoculated larvae were maintained separately, and insect mortality was recorded 96 h after inoculation by destructive sampling. Dead larvae were incubated in a humidity chamber containing filter paper moistened with sterile distilled water to observe mycosis. Once mycelium growth developed over the insect body, the fungus was isolated on PDA under laminar airflow.

Another assay was performed to determine the median lethal dose (LD_{50}) of TMBMA1 with 10 treatments, including nine conidial suspensions (ranging from 1×10^1 to 1×10^9) and one control. The bioassay was conducted as previously described. Ten 2nd instar larvae exposed to each conidial suspension were treated as one replication, with three replications maintained for each treatment (total insects, $n=30$ per treatment). Mortality data collected during the experiment were subjected to probit analysis for calculation of median lethal concentration (LC_{50}) using PoloPlus software. The progression of growth and development of TMBMA1 on infected larval cadavers was documented using a stereoscopic microscope (Model: Leica M205A) equipped with an image analysis tool.

Scanning electron microscopy (SEM) analysis

Conidial suspension of TMBMA1 at 10^8 conidia mL^{-1} was sprayed on PBW larvae and SEM images were taken after 24, 48, 72, 96, and 120 h of incubation. Three samples were taken out at each interval and processed for SEM studies by using the method of Talaei-hassanloui et al. (2007) with some modifications. For sample preparation to study the colonization of TMBMA1 on PBW larvae using SEM, the larvae were initially subjected to primary fixation in Karnovsky's fluid, which consists of 2.5% (volume fraction) glutaraldehyde and 4% (volume fraction) paraformaldehyde in 0.1 mol·L^{-1} phosphate buffer (pH 7.4) for 24 h at 4 °C. Following this, the samples were washed three times with 0.1 mol·L^{-1} phosphate buffer. Secondary fixation was carried out by immersing the samples in a 1% (volume fraction) osmium tetroxide (OsO_4) solution in 0.1 mol·L^{-1} phosphate buffer (1 : 1 ratio) for 2–4 h. After secondary fixation, the samples were subjected to a series of three washes with 0.1 mol·L^{-1} phosphate buffer. The dehydration process involved immersing the samples in a graded series of acetone solutions, starting from 30% and gradually increasing to 95%. The samples were then cleaned with toluene. Subsequently, the processed larvae were mounted onto stubs and sputter-coated using the JEOL JFC-1600 Auto Fine Coater. Finally, the samples were observed in secondary electron imaging mode using the JEOL JSM-6380A (Tokyo, Japan) SEM to study the activity of the fungal isolate on the infected larvae.

Compatibility studies on Metarhizium anisopliae TMBMA1 and insecticides

We used the poison food technique to assess the lethal effect of different test insecticides on the EPF, *M. anisopliae* TMBMA1. Six insecticides, recommended by the Central Insecticide Board and Registration Committee (CIB) of the Government of India for the management of PBW, were included in the study (Table 1). Potato dextrose agar (PDA) was prepared by mixing 200 g sliced potatoes, 20 g dextrose, and 20 g agar in 1 L of water. Then 150 mL of PDA was poured into 250 mL conical flasks and autoclaved at 121 °C and 103 421 Pa. Once cooled, the recommended concentration of each insecticide was added to the PDA media using a pipette (Table 1). The medium containing the insecticides was then poured into 9-cm petri dishes and allowed to solidify under laminar airflow. PDA without insecticides served as a control. A 5 mm mycelial plug from a seven-day-old pure culture of TMBMA1 was inoculated at the center of each PDA plate containing insecticides. Plates were sealed with parafilm and incubated at (28 ± 2) °C. A total of seven treatments were evaluated, including the control. Each treatment was replicated in seven plates. The colony morphology and mycelium growth were measured at 24-h intervals until full growth was achieved

Table 1 Details of insecticides used in compatibility assay

Insecticides	Manufacturer	Recommended dose
Profenofos 50% EC	Spectron Ethers Ltd	1.0 mL·L^{-1}
Cypermethrin 10% EC	Syngenta India Pvt. Ltd	3.0 mL·L^{-1}
Emamectin benzoate 5% SG	Spectrum Ethers Ltd	0.5 g·L^{-1}
Indoxacarb 14.5% SC	Gharda Chemicals Ltd	0.5 mL·L^{-1}
Emamectin benzoate 1.5% + Profenofos 35% WDG	Parijat Industries Pvt. Ltd	1.5 mL·L^{-1}
Neem seed kernal extract 0.15% EC	P.J. Margo Pvt. Ltd	5.0 mL·L^{-1}

in the control plate. The mean colony inhibition percentage was calculated using the following formula:

$$\text{Mean colony inhibition (\%)} = \frac{C - T}{C} \times 100$$

where, C and T are the radial growth of *M. anisopliae* in cm in the control plate and test plate respectively.

The effect of insecticides on spore production of *M. anisopliae* TMBMA1 was assessed by quantifying conidia production across all treatments. Spores produced on each plate were counted using a haemocytometer. A 50 mL spore suspension was prepared by scraping the EPF from a PDA plate into 50 mL distilled water containing 0.05% Tween 80. The suspension was homogenized in a centrifuge for 10 min (Abidin et al. 2017).

A 100 µLaliquot of the conidial suspension was placed on the haemocytometer and allowed to settle for 1–2 min. A cover glass was placed over the grid to prevent bubble formation. The mean number of conidia produced in each PDA plate was recorded as described by Thube et al. (2018). The data obtained were compared with the control data. Conidial production (spores mL^{-1}) was calculated using the following formula:

$$\text{Spores per mL} = \text{Number of spores in all square of Haemocytometer} \times 5 \times 10000$$

The effect of the test insecticides on the sporulation of *M. anisopliae* was further studied by calculating the percent reduction of spore in various treatments by the following formula:

$$\text{Spore reduction (\%)} = \frac{X - Y}{X} \times 100$$

where, X is the number of spores mL^{-1} in the control plate and Y is the number of spore mL^{-1} in the treated plate.

Statistical analysis

Experiment data, recorded on larval mortality, were subjected to probit analysis (Finney 1971) using Polo Plus software (Version 2.0, LeOra) to calculate LC_{50} value. The fiducial limit was taken at a 95% confidence interval. The data on the mortality of PBW under laboratory bioassay, and efficacy of *M. anisopliae* were subjected to one-way analysis of variance (ANOVA) using SPSS Software. Before conducting ANOVA, Levene's test for homogeneity of variance was performed to ensure the assumption of equal variances. When ANOVA was significant, a comparison of relevant means was made using Tukey's significance test at the 5% level of significance.

Results

Larval susceptibility of *P. gossypiella* to *Metarhizium anisopliae* TMBMA1

The inoculation of conidial suspensions of TMBMA1 on 2nd instar PBW larvae led to significantly high insect mortality within four days of treatment (Table 2). Levene's test for homogeneity of variance indicated no significant differences in the variances of percent mortality among the treatments ($p = 0.13$), confirming that the assumption of homogeneity was met. The study revealed a significant difference ($F_{8,\ 18} = 37.77$; $P < 0.001$) in insect mortality across the various concentrations tested. The data obtained showed that the maximum mortality (100%) was observed at the higher concentrations (1×10^9 and 1×10^{10} spores mL^{-1}), while the minimum mortality (46.6%) was noted at the lowest concentration (1×10^3 spores mL^{-1}), however only 3.33% mortality was observed in control larvae (Fig. 1).

The higher conidial suspensions (1×10^{10} and 1×10^9 spores mL^{-1}) were notably more virulent, resulting in 100% insect mortality within four days of inoculation. The total percentage of mortality increased progressively with the increasing concentrations. The LC_{50} of TMBMA1 against the 2nd larval instar of the PBW was found to be 718 518.504 (7.1×10^5) spores mL^{-1}, falling within a lower fiducial limit of 95 375.715 spores mL^{-1} and an upper fiducial limit of 3 189 259.437 spores mL^{-1} over 4 days (Table 2).

Table 2 Dose–mortality response (expressed as lethal concentration) of larvae of *Pectinophora gossypiella* to *Metarhizium anisopliae* (TMBMA1)

Entomopathogenic fungi	*df*[a]	Slope ± SE[b]	χ^{2c}	LC_{50}[d] (FL)[e] in spores mL^{-1}
Metarhizium anisopliae (TMBMA1)	8	1.68 ± 0.344	0.387	718 518.504 (95 375.715 – 3 189 259.437)

[a] *df* (degree of freedom); [b]slope ± SE (standard error); [c]χ^2 indicate a good fit to the line to the data; [d]LC_{50} lethal dose are expressed in conidial per larvae; [e](FL) (fiducial limits)

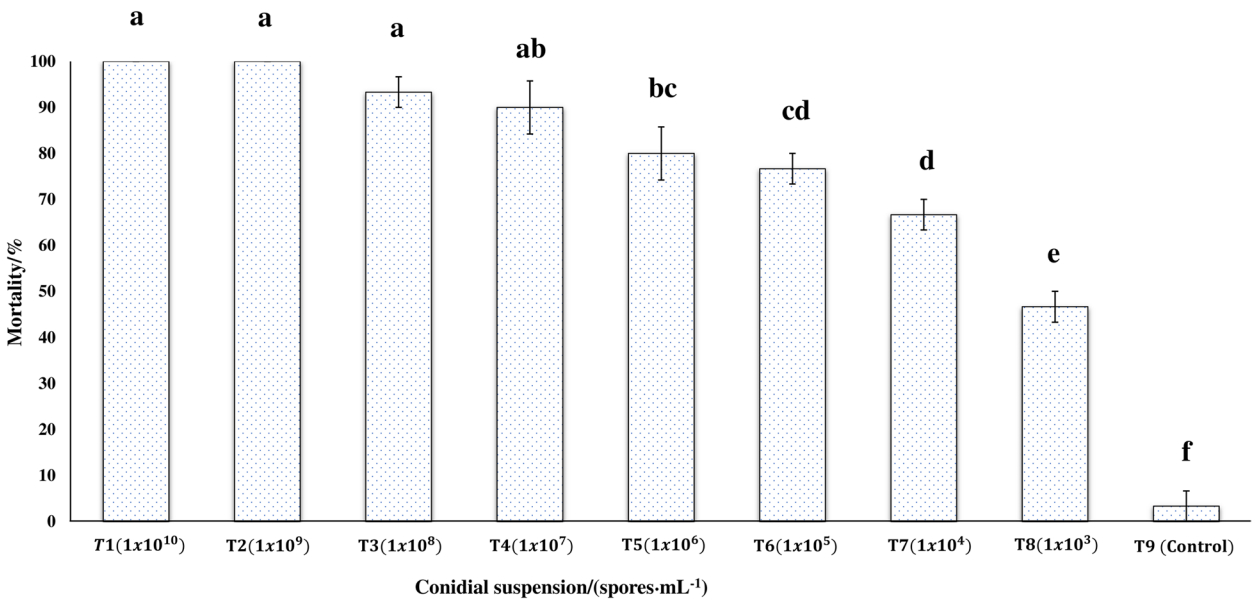

Fig. 1 Susceptibility of PBW larvae to *Metarhizium anisopliae* TMBMA expressed in percent mortality (Different letters above the bars indicating the significant difference among the various treatments)

The comparative effects of TMBMA1 (1×10^9 spores mL^{-1}), a commercial market product of *M. anisopliae* (1×10^9 spores mL^{-1}), and an insecticide on PBW mortality revealed notable differences in mortality across the various treatments. The mortality induced by both TMBMA1 and the insecticide was significantly higher (100% in both treatments) compared to the commercial product IPL Biological (< 60%) and control (3.33%) and interestingly, they were comparable in their effectiveness in causing mortality (Fig. 2).

Freshly treated larvae (Fig. 3a) with TMBMA1 took about 72–96 h to exhibit mortality. After infection with TMBMA1, freshly dead larvae took about 24–48 h to initiate the whitish mycelial growth in a humidity chamber (Fig. 3b). Gradually, white mycelia spread over the entire larval body (Fig. 3c). Following this initial growth phase, the infected larvae begin to develop greenish spores after 48 h, eventually forming a dark greenish conidial mat over their entire bodies (Fig. 3d).

SEM results of infected larvae

Upon examining the larvae under scanning electron microscopy after 24 h of inoculation, a multitude of conidia were discovered adhered to the larval body (Fig. 4a). Within 48–72 h, the development of germ tubes and emergence of active mycelium penetrating directly into the epidermis could be observed (Fig. 4b). By 72 h, nearly all conidia had germinated, and mycelium had formed on the host surface (Fig. 4b). After 96 h, these mycelia became tangled, and new conidia emerged from the mycelium (Fig. 4c). The conidia attached to the pores and bristle sockets exhibited the highest rates of germination, with profuse production of the next generation of conidia (Fig. 4d). Sporulation

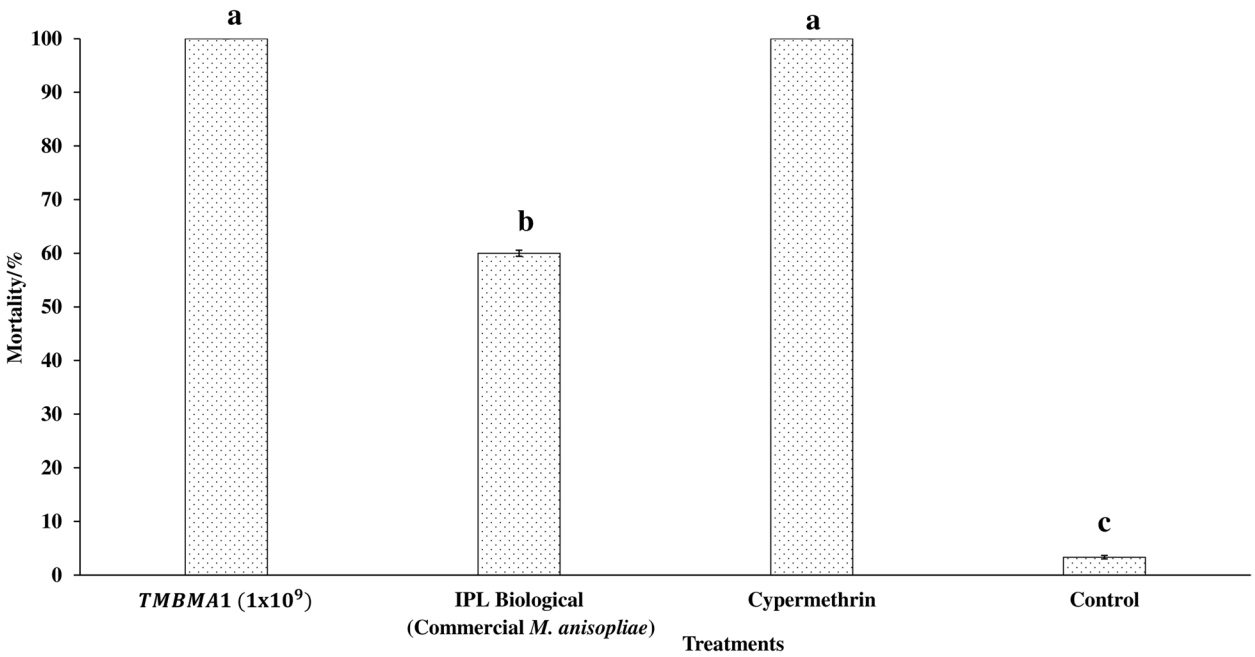

Fig. 2 Comparative virulence of *Metarhizium anisopliae* TMBMA1 with a commercial market product and a chemical insecticide against PBW (Different letters above the bars indicate the significant difference among the various treatments)

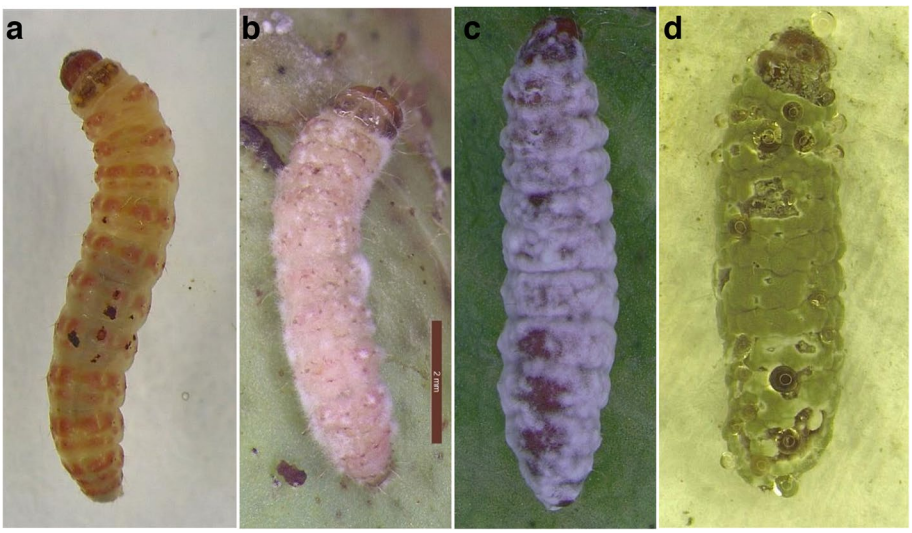

Fig. 3 Infection of *Metarhizium anisopliae* TMBMA1 to *P. gossypiella* (**a** Freshly inoculated larvae; **b** Initial growth of mycelium after 48 h in humidity chamber; **c** Whitish mycelial growth over entire larval cadaver after 72 h in humidity chamber; **d** Greenish conidial mat on larval cadaver during advance stage of infection)

data of TMBMA1 indicated that conidial germination correlated with the adherence of inoculated conidia to the body wall. Conidia in close contact with the insect cuticle had greater and faster sporulation rates compared to those on the sclerotized portions of the head and anal end (Fig. 4e), where sporulation was less pronounced. Interestingly, there was profuse conidial production within the hemocoel of infected larvae (Fig. 4f).

Compatibility of *Metarhizium anisopliae* TMBMA1 with insecticides

The effect of recommended insecticides on the colony growth and sporulation of TMBMA1 was also studied to

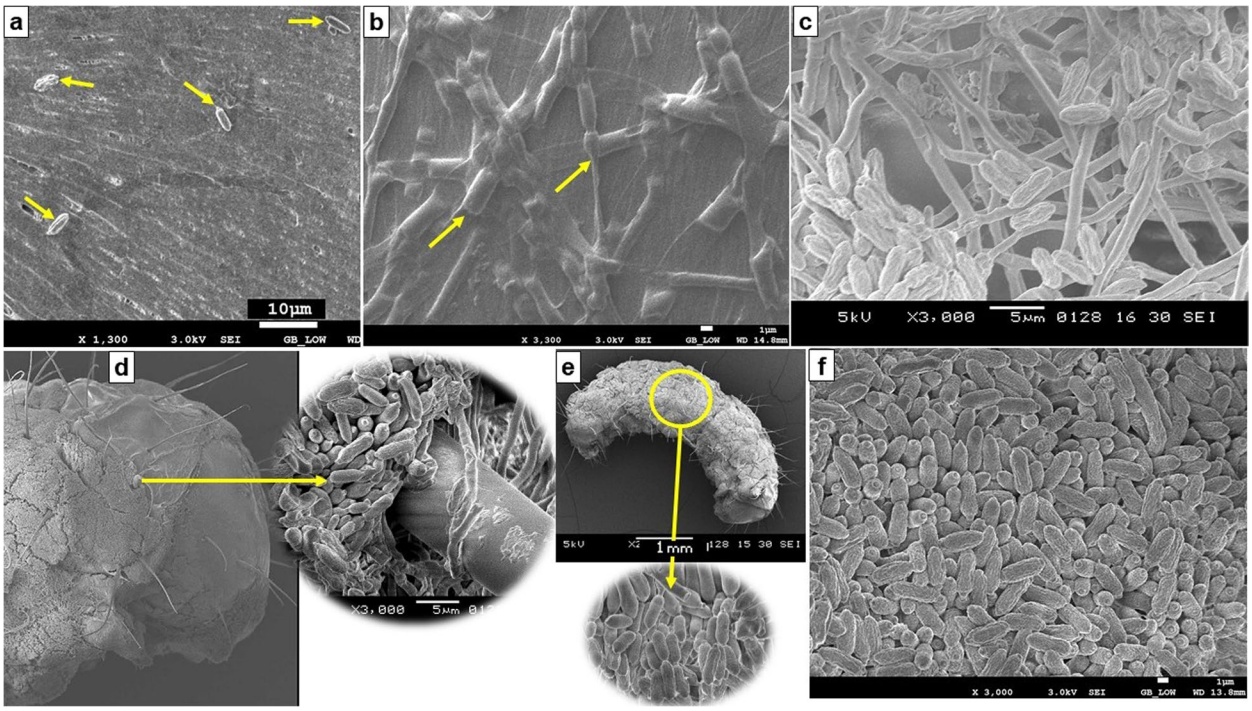

Fig. 4 The SEM analysis of *Metarhizium anisopliae* TMBMA1 infected larvae of *P. gossypiella* [**a** Cuticular attachment of conidia on freshly inoculated larvae (1 h post inoculation); **b** Development of germ tubes, and penetration of active mycelium through cuticle (72 h post inoculation); **c** Tangled mycelia, and newly produced conidia (96 h post inoculation); **d** Profuse sporulation in cuticular pores and bristle sockets (120 h post inoculation); **e** Significant sporulation on entire larval cuticle except head and anal end in advance infection (120 h post inoculation); **f** conidial density within hemocoel (120 h post inoculation)]

ascertain the compatibility of EPF and chemical insecticides. The results (Fig. 5) showed significant variation in the colony growth of TMBMA1 across insecticide treatments ($F_{6,42}=276.9$; $p<0.001$). The percent reduction in colony growth varied from 28.00% ± 0.99% to 68.73% ± 1.04%, with the least inhibition observed in Emamectin benzoate 5% SG and the maximum in Profenofos 50% EC (Fig. 6). Treatments with neem seed kernel extract [0.15% (volume fraction) EC], indoxacarb 14.5% SC, and cypermethrin 10% EC caused moderate reductions in colony growth, with reductions of 30.88% ± 1.07%, 31.99% ± 1.09%, and 37.21% ± 2.76%, respectively.

The impact of various insecticides on the sporulation of *Metarhizium anisopliae* TMBMA1 was assessed, showing significant differences in percent reduction of spore production compared to the control ($F_{6,42}=124.9$; $P<0.001$). Profenofos 50% EC resulted in a 96.66% reduction, and Emamectin Benzoate 1.5% + Profenofos 35% WDG caused a 98.55% reduction, indicating these treatments have higher inhibitory effects. Indoxacarb 14.5% SC showed a moderate inhibitory effect with a 58.12% reduction in sporulation. Neem oil led to a 21.21% reduction, suggesting a mild inhibitory effect. In contrast, Cypermethrin 10% EC and Emamectin benzoate 5% SG exhibited lower impact on spore production, with reductions of 11.45% and 13.79%, respectively. These findings indicate that Profenofos-based treatments are highly inhibitory to the sporulation of *M. anisopliae* TMBMA1 however it can be combined with Cypermethrin 10% EC and Emamectin benzoate 5% SG.

Discussion

Insecticides are vital for boosting crop yields and enhancing economic outcomes through effective pest management. However, their extensive and often indiscriminate application has led to issues such as pest resistance, the resurgence of secondary pests, and the disruption of natural enemy complexes. These challenges diminish the effectiveness of natural pest control processes and harm ecosystem health (Mantzoukas et al. 2020). Furthermore, worries about the safety of humans and the environment call for the development of more dependable, affordable, and ecologically friendly pest control techniques. The PBW has become a significant threat to cotton production due to the development of resistance to Bt cotton. Recently, India has experienced severe infestations of pink bollworm, with incidence rates ranging from 8%

Fig. 5 The colony growth of *Metarhizium anisopliae* TMBMA1 on insecticide amended media (**a** Profenofos 50% EC; **b** Emamectin Benzoate 5% SG; **c** Cypermethrin 10% EC; **d** Indoxacarb 14.5% SC; **e** NSKE 0.15% EC; **f** Emamectin benzoate 1.5% + Profenofos 35% WDG; **g** Control)

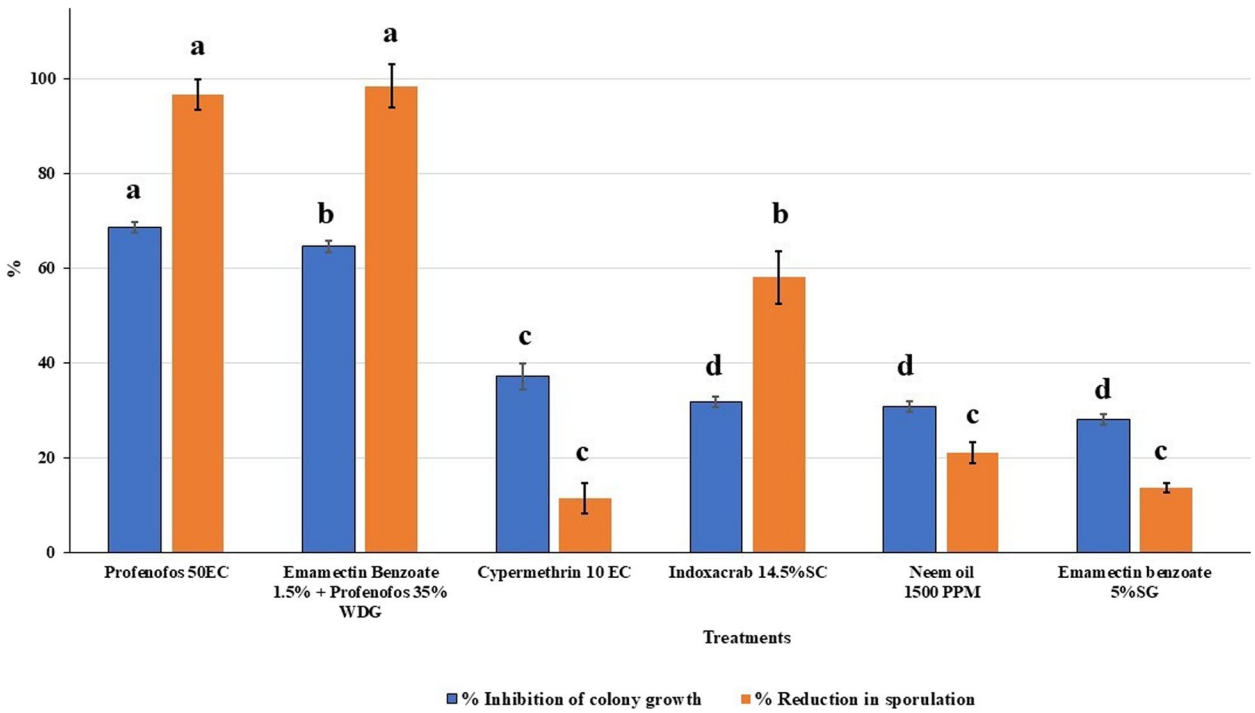

Fig. 6 The effect of insecticides on colony growth and sporulation of *Metarhizium anisopliae* TMBMA1 (Different letters above the bars indicating the significant difference among the various treatments)

to 92%, which have led to yield reductions estimated between 10% and 30% (Nagrare et al. 2023). The heavy reliance on insecticides for managing the pink bollworm has resulted in pesticide poisoning, the extinction of natural enemies and beneficial microbes, the development of resistance, and ecosystem contamination. Therefore, the outcomes of this study offer a promising opportunity for cotton growers by providing a more cost-effective, eco-friendly, and host-specific approach for the sustainable management of the pink bollworm, as compared to traditional chemical insecticides. Furthermore, for more efficient PBW management, the current work offers a way to combine EPF with chemical insecticides that are generally compatible. A significant limitation of employing EPF for pest control is the extended duration needed to eliminate the host. Therefore, before field deployment, it is imperative to evaluate the pathogenicity of EPF strains in vitro. For use in the field, only extremely virulent strains with low LC_{50} and median lethal time (LT_{50}) values ought to be encouraged.

The strain TMBMA1, evaluated in the present investigation, was originally isolated from the TMB. It caused 100% mortality at a concentration of 1×10^8 spores mL^{-1} in its original host. However, to achieve 100% mortality of PBW larvae, a higher concentration of 1×10^9 spores mL^{-1} was required. This difference in virulence could be attributed to the distinct taxonomic orders of the two insect hosts, leading to variations in cuticular chitin composition and other biochemical properties of their cuticles. In the present investigation, the EPF strain demonstrated high virulence against 2nd instar larvae of PBW with a significantly low LC_{50} value of 7.1×10^5 spores mL^{-1}. This is a substantial improvement compared to the *M. anisopliae* strain isolated from whitefly, which showed LC_{50} values of 6.03×10^9 spores mL^{-1} for neonate larvae and 1.29×10^{10} spores mL^{-1} for early 4th instar larvae of PBW (Omar et al. 2021). Our strain is over 850 to 1 800 times more effective than those reported by Omar et al. (2021). Additionally, *M. anisopliae* was found to be virulent against 3rd instar PBW larvae with an LC_{50} value of 1.59×10^5 spores mL^{-1} (Sahab et al. 2011). When four strains of *M. anisopliae* were injected with spore suspensions ranging from 1×10^6 to 1×10^8 spores mL^{-1}, the mortality of PBW larvae varied between 53% and 86% (Awad et al. 2022). Our findings are in line with this study, where mortality rates varied from 46% to 100% with spore suspensions of 1×10^3 to 1×10^{10} spores mL^{-1}.

The virulence of *M. anisopliae* against insect pests is primarily governed by the fungal strain, with certain strains exhibiting higher virulence due to enhanced spore adhesion, toxin production, and efficient host cuticle penetration. The production of secondary metabolites like destruxins and enzymes such as proteases and chitinases further contribute to the breakdown of host defenses and tissues, enhancing virulence. The susceptibility of the host insect, determined by factors like immune response, cuticle composition, and behavior, also plays a critical role. Collectively, these factors interact to determine the overall virulence of *M. anisopliae* (Inglis et al. 2001; Butt et al. 2016; Meyling et al. 2007). Accordingly, the higher virulence of TMBMA1 compared to other existing strains may be attributed to the presence of elevated levels of cuticle-degrading enzymes such as chitinases, proteases, and lipases, as well as higher titers of lethal toxins like destruxins.

The ability of an EPF to successfully infect and complete its life cycle on a host depends on the effective parasitism of the host epidermis (Duan et al. 2017). This process involves both mechanical and passive factors, relying not only on suitable environmental conditions and temperature but also on nutritional factors and the absence of inhibitory substances. These factors are essential for the successful germination of conidia (Maketon et al. 2007). SEM analysis of the Colorado potato beetle inoculated with *Beauveria bassiana* showed that conidia primarily attached and germinated within intersegmental folds, pores, and terminal setae (Maketon et al. 2007). In the present study, we also observed profuse sporulation of TMBMA1 in pores and cuticular setae sockets. Although infection initiated in the intersegmental region, the fungus eventually spread across the entire cuticle except the head and anal end. The reduced infection or sporulation in these areas could be due to higher levels of sclerotin and lower levels of chitin. Our observations of abundant sporulation in the hemocoel during advanced stages of infection align with the findings of Maketon et al. (2007). Similarly, the infection of the Asian long-horned beetle (*Anoplophora glabripennis*) was primarily through germ tubes, with some appressorial reinvasion observed, which is consistent with our results (Deng et al. 2012).

Studying the compatibility of insecticides with EPF is crucial for developing IPM strategies that are both effective and environmentally sustainable (Kopparthi 2020). EPFs, such as *B. bassiana* and *M. anisopliae*, play a significant role in biological control by infecting and killing a wide range of insect pests. However, the simultaneous use of chemical insecticides and these biocontrol agents can lead to potential interactions that may affect the efficacy of either or both. Compatibility studies are essential for determining if certain insecticides inhibit the growth, spore germination, or infectivity of entomopathogenic fungi. This ensures that their combined use does not compromise pest control efficacy. Additionally, understanding these interactions aids in minimizing negative environmental impacts and promoting the longevity of both chemical and biological control methods

by preventing the overuse of any single approach, which can lead to resistance development in pest populations (Zimmermann 2007). Therefore, these studies are vital for optimizing IPM programs that harness the strengths of both chemical and biological controls in a complementary manner.

Hence, in addition to the bio-efficacy and SEM studies of TMBMA1, its compatibility with insecticides recommended for PBW management in cotton was evaluated. The compatibility study confirmed that Profenofos 50% EC is toxic and non-compatible with TMBMA1. This finding is consistent with previous research by Joshi et al. (2018), Amutha et al. (2012), and Banu et al. (2014), which reported the toxicity of this insecticide towards the mycelial growth and spore production of *M. anisopliae*. This non-compatibility suggests that profenofos negatively impacts the efficacy of *M. anisopliae* irrespective of strain as a biological control agent by inhibiting crucial growth and reproductive processes. Though Indoxacarb 14.5% SC caused about a 30.0% reduction in colony growth and significantly reduced spore production by 50% in TMBMA1, these findings align with Akbar et al. (2012), who also reported the moderate compatibility of indoxacarb with *M. anisopliae*. In contrast, Khun et al. (2021) found indoxacarb to be compatible with several Australian strains of *M. anisopliae*, suggesting strain-specific differences in response. Our study showed that TMBMA1 is moderately compatible with both Emamectin benzoate 5% SG and Indoxacarb 14.5% SC, supporting the findings of Akbar et al. (2012) and indicating these insecticides can be integrated into pest management programs with *M. anisopliae*, although their partial inhibitory effects on fungal growth and reproduction should be considered to optimize biocontrol efficacy. Although Cypermethrin 10% EC significantly affected the colony growth of TMBMA1 with a 37.21% reduction, it only minimally impacted spore production, showing an 11.45% reduction, the lowest among all treatments, making it comparatively less toxic to the fungus. These results align with Akbar et al. (2012), who also found cypermethrin to be less detrimental to *M. anisopliae*, particularly in terms of spore production. However, our findings contrast with da Silva et al. (2013), who reported a 100% reduction in sporulation. The slight reduction (21.21%) in spore production of TMBMA1 observed with the Neem oil treatment differs from Bhalchim et al. (2023), who reported a significant reduction (46%) in germination of *M. anisopliae*. Similarly, Gomes et al. (2015) also reported minimal impact on the growth and sporulation of *M. anisopliae*. These contrasting findings underscore the importance of strain-specific responses to NSKE, likely due to genetic variations in resistance to azadirachtin, the active ingredient in Neem oil. The minimal impact of Neem oil, Cypermethrin and Emamectin benzoate on spore production suggests that these insecticides can be integrated into IPM programs utilizing *M. anisopliae* TMBMA1, with careful management of its application to balance effective pest control and the preservation of EPF efficacy. These results highlight the need for careful selection of pesticides in IPM to avoid compromising the effectiveness of beneficial microorganisms, advocating for the use of alternatives that maintain the ecological balance and sustainability of agricultural practices.

Conclusion

The present study has generated valuable insights into the efficacy of *M. anisopliae* strain TMBMA1 against PBW. The dose-dependent mortality of PBW was observed with EPF. Comparative analysis revealed that TMBMA1 and chemical insecticide achieved significantly higher mortality than a commercial *M. anisopliae* product. SEM studies documented profuse colonization of PBW by TMBMA1, both internally and externally. Compatibility tests indicated that Cypermethrin 10% EC, Emamectin Benzoate 5% SG, and NSKE (0.15% EC) are compatible with TMBMA1, while Profenofos 50% EC and Emamectin Benzoate 1.5% + Profenofos 35% WDG significantly reduced its sporulation, indicating incompatibility. This suggests that strain TMBMA1 can be effectively integrated into PBW management programs with selected insecticides, enhancing pest control and lessening environmental impact.

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Authors' contributions

Thube S, Panchbhai P, Fand B, Pandian RT, Prabhulinga T, Mhatre P, Behere G, and Prasad Y planned the work, designed the experimental setup, supervised the conduct of experiments, analyzed and interpreted the data; Thavakar S, Deshmukh V, Thube S, Prabhulinga T, Shah V, and Nikoshe A conducted laboratory experiments, recorded the data; Thube S, Thavakar S, Panchbhai P, Lavhe N, Pillai T drafted the manuscript; Behere G, Prasad Y, Shah V, Mhatre P, Pandian RT edited manuscript, and Prasad Y approved the work plan, provided the research facilities/ infrastructure and monitored the work progress. All authors have read and agreed to the published version of the manuscript.

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Data availability
All the data relevant to the present study are included in the article. Any further details related to the experiments conducted can be made available by requesting the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no conflict of interest related to the content of this article.

Author details
[1]College of Agriculture, Nagpur, Maharashtra 440001, India. [2]Indian Council of Agricultural Research (ICAR)-Central Institute for Cotton Research, Nagpur, Maharashtra 440010, India. [3]ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka 574243, India. [4]ICAR-Central Potato Research Institute Regional Station, Tamil Nadu, Udhagamandalam 643004, India. [5]Division of Crop Protection, ICAR-Central Institute for Cotton Research, Nagpur, 440010, India.

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References

Abidin AF, Ekowati N, Ratnaningtyas NI. Compatibility of insecticides with entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Scr Biol. 2017;4(4):273–9. https://doi.org/10.20884/1.sb.2017.4.4.695.

Akbar S, Freed S, Hameed A, et al. Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. Afr J Microbiol Res. 2012;6(17):3956–62.

Amutha M, Banu JG. Compatability of *Metarhizium anisopliae* and *Pochonia lecanii* with insecticides. Ann Plant Prot Sci. 2012;20(2):354–7.

Awad AS, Ali SS, El-Habal NA. Pathogenicity of entomopathogenic fungal isolates on immature stages of *Pectinophora gossypiella* (Saunders), (Lepidoptera: Gelechiidae). Egypt J Agric Res. 2022;100(4):475–82. https://doi.org/10.21608/EJAR.2022.120684.1205.

Banu GJ, Amutha M, Rajalakshmi S. Compatibility of insecticides with *Metarhizium anisopliae* (ARSEF-9613), a native entomopathogenic fungal isolate from mealybug. J Insect Sci. 2014;27(1):144–6.

Bhalchim NR, Tamboli ND, More SA, et al. Compatibility of *Metarhizium anisopliae* with botanicals under laboratory condition. Pharma Innov J. 2023;12(3):5747–51.

Blossom B, Singh G. Monitoring of insecticide residues in cotton seed in Punjab. India Bull Environ Contam Toxicol. 2004;73:707–12. https://doi.org/10.1007/s00128-004-0483-0.

Butt TM, Coates CJ, Dubovskiy IM, et al. Entomopathogenic fungi: new insights into host-pathogen interactions. Adv Genet. 2016;94:307–64. https://doi.org/10.1016/bs.adgen.2016.01.006.

Chakravarthy VS, Reddy TP, Reddy VD, et al. Current status of genetic engineering in cotton (*Gossypium hirsutum* L): an assessment. Criti Revie Biotech. 2014;34(2):144–60. https://doi.org/10.3109/07388551.2012.743502.

Choudhary B, Gaur K. Bt cotton in India: a country profile. ISAAA series of iotech crop profiles. Ithaca, NY: ISAAA; 2010. p. 1–24. http://re.indiaenvironmentportal.org.in/files/Bt_Cotton_in_India-A_Country_Profile.pdf .

Colmenarez CY, Corniani N, Mundstock J S, et al. Use of parasitoids as a biocontrol agent in the neotropical region: challenges and potential. In: Baimey HK, Hamamouch N, Kolombia YA, editors. Horticultural crops. 2nd ed. Longdon: Intech Open; 2020. p. 1–23. https://doi.org/10.5772/intechopen.80720.

Deng CP, Yan XZ, Liu HX, et al. Pathological observation of *Anoplophora glabripennis* larva infected by *Beauveria bassiana* by using SEM and light microscope. Sci Silvae Sin. 2012;3:105–9. https://doi.org/10.11707/j.1001-7488.20120317.

Dhurua S, Gujar GT. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag Sci. 2011;67(8):898–903. https://doi.org/10.1002/ps.2127.

Duan Y, Wu H, Ma Z, et al. Scanning electron microscopy and histopathological observations of *Beauveria bassiana* infection of Colorado potato beetle larvae. Microb Pathog. 2017;111:435–9. https://doi.org/10.1016/j.micpath.2017.09.025.

Fand BB, Nagrare VS, Gawande SP, et al. Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) on Bt cotton in Central India: a new threat and concerns for cotton production. Phytoparasitica. 2019;47:313–25. https://doi.org/10.1007/s12600-019-00738-x.

Fang WG, Lu HL, King GF, et al. Construction of a hypervirulent and specific mycoinsecticide for locust control. Sci Rep. 2014;4:1–13. https://doi.org/10.1038/srep07345.

Finney DJ. Probit analysis. 3rd ed. Cambridge, UK: Cambridge University; 1971.

Gillespie AT. The potential of entomogenous fungi as control agents for onion thrips, *Thrips tabaci*. Monograph, British Crop Protection Council. 1986;34:237–43.

Goettel MS, Eilenberg J, Glare T. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, Iatrou K, Gill SS, editors. Comprehensive molecular insect science. Amsterdam: Elsevier; 2005.

Gomes SA, Paula AR, Ribeiro A, et al. Neem oil increases the efficiency of the entomopathogenic fungus *Metarhizium anisopliae* for the control of *Aedes aegypti* (Diptera: Culicidae) larvae. Parasit Vectors. 2015;8:1–8. https://doi.org/10.1186/s13071-015-1280-9.

ICAR-AICRP. (Cotton) Annual report (2023–24). ICAR - All India coordinated research project on cotton. Nagpur: ICAR; 2024. (https://aiccip.cicr.org.in/pdf/annual_report/AICRP_AR_2023_24.pdf). Accessed 15 July 2024.

Inglis GD, Goettel MS, Butt TM, et al. Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N, editors. Fungi as biocontrol agents: progress, problems and potential. Rockville: CABI; 2001. https://doi.org/10.1079/9780851993560.002.

Joshi M, Gaur N, Pandey R. Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with selective pesticides. J Entomol Zool Stud. 2018;6(4):867–72.

Khun KK, Ash GJ, Stevens MM, et al. Compatibility of *Metarhizium anisopliae* and *Beauveria bassiana* with insecticides and fungicides used in macadamia production in Australia. Pest Manag Sci. 2021;77(2):709–18. https://doi.org/10.1002/ps.6065.

Kisaakye J, Fourie H, Coyne D, et al. Evaluation of the entomopathogenic potential of *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* for management of cosmopolites sordidus Germar (Coleoptera: Curculionidae). Agriculture. 2021;11:1290. https://doi.org/10.3390/agriculture11121290.

Kopparthi AV. Compatibility of biopesticides with insecticides in IPM. Indian J Entomol. 2020;82(3):588–92. https://doi.org/10.5958/0974-8172.2020.00146.7.

Kranthi KR, Russell DA. Changing trends in cotton pest management. In: Gaugler R, Kaya HK, editors. Integrated pest management: innovation-development. Dordrecht: Springer; 2009. p. 499–541.

Kranthi KR, Jadhav DR, Kranthi S, et al. Insecticide resistance in five major insect pests of cotton in India. Crop Prot. 2002;21(6):449–60. https://doi.org/10.1016/S0261-2194(01)00131-4.

Lacey LA, Grzywacz D, Shapiro-Ilan DI, et al. Insect pathogens as biological control agents: back to the future. J Invertebr Pathol. 2015;1(132):1–41. https://doi.org/10.1016/j.jip.2015.07.009.

Lopes RB, Alves SB, Tamai MA. Control of *Frankliniella occidentalis* in hidroponic lettuce by *Metarhizium anisopliae*. Sci Agric. 2000;57:239–43. https://doi.org/10.1590/S0103-90162000000200007.

Mageshwaran V, Satankar V, Shukla SK, et al. Current status of organic cotton production. Indian Farming. 2019;69(2):9–14.

Maketon M, Sawangwan P, Sawatwarakul W. Laboratory study on the efficacy of *Metarhizium anisopliae* (Deuteromycota: Hyphomycetes) in controlling *Coptotermes gestroi* (Isoptera: Rhinotermitidae). Entomol Gen. 2007;30(241):203–18. https://doi.org/10.1127/entom.gen/30/2007/203.

Mancini F, van Bruggen AH, Jiggins JL, et al. Acute pesticide poisoning among female and male cotton growers in India. Int J Occup Environ Health. 2005;11(3):221–32. https://doi.org/10.1179/10773.52058.00246.064.

Mantzoukas S, Eliopoulos PA. Endophytic entomopathogenic fungi: a valuable biological control tool against plant pests. Appl Sci. 2020;10(1):360. https://doi.org/10.3390/app10010360.
Meyling NV, Eilenberg J. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol Control. 2007;43(2):145–55. https://doi.org/10.1016/j.biocontrol.2007.07.007.
Nagrare VS, Fand BB, Kumar R, et al. Arthropod pests and their natural enemies associated with cotton in India: a review. Indian J Entomol. 2022;84(3):713–25. https://doi.org/10.55446/IJE.2022.167.
Nagrare VS, Fand BB, Kumar R, et al. Pink bollworm, *Pectinophora gossypiella* (Saunders) management strategy, dissemination and impact assessment in India. Crop Prot. 2023;174:106424. https://doi.org/10.1016/j.cropro.2023.106424.
Naik VC, Kumbhare S, Kranthi S, et al. Field-evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (Bt) cotton expressing crystal 1Ac (Cry1Ac) and Cry2Ab in India. Pest Manag Sci. 2018;74(11):2544–54. https://doi.org/10.1002/ps.5038.
Nawaz A, Razzaq F, Razzaq A, et al. Compatibility and synergistic interactions of fungi, *Metarhizium anisopliae*, and insecticide combinations against the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). Sci Rep. 2022;12:4843. https://doi.org/10.1038/s41598-022-08841-6.
Omar G, Ibrahim A, Hamadah K, et al. Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* on different stages of the pink bollworm, *Pectinophora gossypiella* (Saunders)(Lepidoptera: Gelechiidae). Egypt J Biol Pest Control. 2021;31(1):1–7. https://doi.org/10.1186/s41938-021-00447-w.
Patil SB. Studies on management of cotton pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). Dharwad, India: University of Agricultural Sciences; 2003.
Sahab A, Sabbour MM. Virulence of four entomopathogenic fungi on some cotton pests with special reference to impact of some pesticides, nutritional and environmental factors on fungal growth. Egypt J Biol Pest Control. 2011;21(1):61–7.
San AW, Hue MK. Mode of infection of *Metarhizium* spp. fungus and their potential as biological control agents. J Fungi. 2017;3:30–50. https://doi.org/10.3390/jof3020030.
Shah V, Pande R, Fand BB, et al. Innovative on-plant bioassay method for selection of superior genotype/cultivar/event against pink bollworm, *Pectinophora gossypiella* (Saunders) in cotton in protected screen house condition. Int J Trop Insect Sci. 2021;41:3167–72. https://doi.org/10.1007/s42690-021-00512-9.
Shaheen HL, Shahbaz M, Ullah I, et al. Morpho-physiological responses of cotton (*Gossypium hirsutum*) to salt stress. Int J Agric Biol. 2012; 14:980–4.
Silva RAD, Quintela ED, Mascarin GM, et al. Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae*. Sci Agric. 2013;70:152–60. https://doi.org/10.1590/S0103-90162013000300003.
Sun X, Yan W, Zhang J, et al. Frozen section and electron microscopy studies of the infection of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) by the entomopathogenic fungus *Metarhizium anisopliae*. Springerplus. 2016;5:1748. https://doi.org/10.1186/s40064-016-3416-6.
Talaei-Hassanloui R, Kharazi-pakdel A, Goettel MS, et al. Germination polarity of *Beauveria bassiana* conidia and its possible correlation with virulence. J Invertebr Pathol. 2007;1:94(2):102–7. https://doi.org/10.1016/j.jip.2006.09.009.
The Cotton Corporation of India (CCI). Statistics. 2022. https://cotcorp.org.in/statistics.aspx?AspxAutoDetectCookieSupport=1. Accessed 15 July 2024.
The Hindu. Pesticide poisoning continues to claim farmers' lives in Maharashtra. The Hindu Ground Zero. https://www.thehindu.com/news/national/when-every-breath-kills/article61847648.ece#:~:text=In%20the%20cotton%20belt%20of,that%20continues%20to%20claim%20lives. Access 3 Dec 2021.
Thube SH, Pandian RT, Babu M, et al. Evaluation of a native isolate of *Metarhizium anisopliae* (Metschn.) Sorokin TMBMA1 against tea mosquito bug, *Helopeltis theivora* infesting cocoa (*Theobroma cacao* L.). Biol Control. 2022;170:104909. https://doi.org/10.1016/j.biocontrol.2022.104909.
Thube SH, Pandian RTP, Mahapatro GK, et al. Compatibility study of insecticides recommended for the management of tea mosquito bug *Helopeltis* spp. with bio-fungicide, *Trichoderma harzianum*. J Entomol Zool Stud. 2018;6(5):2034–9.
Thube S, Shinde S, Shah V, et al. Biocontrol potential of entomopathogenic nematode, *Heterorhabditis indica* against pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). J Cotton Res. 2023;6:23. https://doi.org/10.1186/s42397-023-00159-6.
Wang JB, St Leger RJ, Wang C. Advances in genomics of entomopathogenic fungi. Adv Genet. 2016;94:67–105. https://doi.org/10.1016/bs.adgen.2016.01.002.
Wu C, Pan S, Shan Y, et al. Residue status and risk assessment of neonicotinoids under real field conditions: based on a two-year survey of cotton fields throughout China. Environ Technol Innov. 2022;28:102689. https://doi.org/10.1016/j.eti.2022.102689.
Zimmermann G. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci Techn. 2007;17(9):879–920. https://doi.org/10.1080/09583150701593963.