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RESEARCH ARTICLE

Consortial effect of bio-inoculants for the management of powdery mildew of bhendi incited by *Golovinomyces cichoracearum* (DC.) V.P. Heluta

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Abstract

Powdery mildew of bhendi, incited by *Golovinomyces cichoracearum* (DC.) V.P. Heluta, results in substantial yield losses. To address this, an eco-friendly disease management strategy was developed using *Ampelomyces quisqualis*, *Trichoderma harzianum* and plant growth-promoting rhizobacteria (*Bacillus subtilis*). Strains AUAQ05 (*A. quisqualis*), AUTH02 (*T. harzianum*) and AUBS01 (*B. subtilis*) were evaluated for their efficacy against the pathogen under *in vitro*, pot culture and field conditions. In the *in vitro* spore germination assay, individual applications of AUAQ05 (84.64 %), AUTH02 (67.85 %) and AUBS01 (61.53 %) at 0.5 % concentration significantly inhibited conidial germination. Prior to combined application, the compatibility among the strains was assessed and confirmed. Under greenhouse (77.26 %) and field (71.69 %) conditions, the consortium of AUAQ05, AUTH02 and AUBS01 markedly reduced disease incidence. Additionally, field trials showed a 64.74 % increase in yield over the untreated control, surpassing the effect of individual treatments. These findings demonstrate that a compatible biocontrol consortium can serve as an effective and eco-friendly strategy for managing powdery mildew in bhendi.

Keywords: bhendi; bio-inoculants; consortia, field conditions; greenhouse; powdery mildew

Introduction

Bhendi (*Abelmoschus esculentus*), commonly known as ladies' finger or okra, is a vital vegetable crop grown worldwide, especially in tropical and subtropical regions and is highly valued for its tender green pods, which are consumed in various culinary forms and used for canning. Belonging to the family Malvaceae, it is rich in vitamins, minerals and dietary fibre, contributing to overall health by supporting immune function, gut health and reducing fasting glucose levels in pre-diabetics and diabetics (1). According to the Food and Agriculture Organization, global bhendi production in 2021 was approximately 9.8 million metric tons, with India contributing about 60 % of the world's production (2). India is the largest producer, with significant cultivation areas in states like West Bengal, Bihar, Andhra Pradesh and Tamil Nadu, where it occupies around 18000 hectares (3, 4). Despite its economic importance, bhendi yields are affected by various biotic and abiotic factors, with powdery mildew caused by *Erysiphe cichoracearum* being one of the most destructive diseases, impacting numerous crops, including bhendi (5).

Powdery mildew, caused by *E. cichoracearum*, is prevalent in bhendi-growing regions of Tamil Nadu, leading to significant yield losses (17.0 to 86.6 %) due to leaf defoliation (6). While chemical fungicides are commonly used for its control, their excessive use can lead to harmful residues, pathogen resistance and environmental pollution (7). To reduce pesticide reliance, alternative biocontrol methods involving microbial agents have been explored (8).

Powdery mildew of Bhendi causal organism, previously known as *E. cichoracearum* De Candolle, has recently been reclassified as *Golovinomyces cichoracearum* (DC.) VP Heluta. The fungus exhibits typical fungal morphology, with septate, colourless, thin-walled and branched hyphae. During asexual reproduction, *G. cichoracearum* produces conidiophores erect, hyaline structures that may or may not be branched which bear chains of single-celled and oval-shaped conidia. The primary infective propagules spread through air currents. The fungus also reproduces sexually by forming chasmothecia, fruiting bodies that produce ascospores. Ascospores are produce during unfavourable conditions, crucial for the overwintering and survival (9).

Ampelomyces quisqualis invades the hyphae and conidia of its fungal host through a combination of mechanical pressure and enzymatic degradation, ultimately resulting in the collapse and death of host cells (10). The commercial formulation AQ10, developed as water-dispersible granules, effectively targets the overwintering cleistothecia of powdery mildew pathogens (11). As a highly specific mycoparasite, *A. quisqualis* holds significant promise in biocontrol due to its non-toxic nature, specificity to powdery mildew fungi and ease of large-scale *in vitro* production. *Trichoderma* species are also widely known as effective biocontrol agents against diverse plant pathogens, including powdery mildew, owing to their proven antagonistic activity (12).

Bacillus species serve as effective biological control agents, largely attributed to their ability to form resilient endospores that can endure extreme environmental conditions, such as high temperature, pH fluctuations and nutrient scarcity (13). The suppression of powdery mildew by *Bacillus* involves direct mechanisms-such as the production of antifungal metabolites-and indirect mechanisms including competition for nutrients and space, siderophore production and the induction of systemic resistance in plants through modulation of phytohormones and molecular signalling pathways (14). The potential of eco-sensitive disease management in grapevines using a liquid formulation comprising *A. quisqualis* and *T. asperellum*, wherein a 2.0 % concentration of *A. quisqualis* followed by *T. asperellum* application significantly reduced disease severity (15). The application of *A. quisqualis* at 20 mL per litre, applied three times, was found to be particularly effective.

Despite extensive studies on *A. quisqualis*, *T. harzianum* and *B. subtilis* as microbial antagonists against powdery mildew, limited research has explored their combined efficacy against *G. cichoracearum* on bhendi. Existing studies primarily focus on individual agents, neglecting potential synergies and their formulation compatibility. Additionally, while molecular characterization of these agents has been reported, their regional diversity, pathogenic interactions and practical application in bioformulation development remain underexplored. Therefore, this study aims to isolate and characterize *Ampelomyces*, *Trichoderma* and *Bacillus* from bhendi-growing regions, assess their morphological, physiological and molecular diversity and evaluate their antagonistic potential through *in vitro* assays such as slide spore germination, leaf disc assay and detached leaf assay. Furthermore, it seeks to determine their compatibility for combined bioformulation and validate its efficacy under greenhouse conditions by comparing disease suppression, plant growth promotion and yield improvement against chemical control measures.

Materials and Methods

Source of seed material

The seed variety 'Sakthi,' used in this research, was obtained from the Seed Sales Counter at Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu.

Collection and maintenance of *G. cichoracearum* inoculum

Powdery mildew-infected leaves of the bhendi variety 'Sakthi' were collected from farmers' fields for conidial isolation. The method was followed with slight modifications (16). To obtain the conidial suspension, the infected leaf tissues were gently washed thrice with sterile double distilled water to dislodge the conidia. The washings then filtered using bilayer muslin to remove debris. The filtrate was centrifuged twice at 4000 rpm for 30 min to concentrate the spores. Finally, the conidial density was adjusted to 5×10^6 conidia per mL using sterile distilled water.

Sample collection and processing *A. quisqualis*

Field samples exhibiting powdery mildew symptoms were obtained from primary bhendi-producing districts of Tamil Nadu, namely Cuddalore, Dindigul, Krishnagiri, Salem and Dharmapuri. In total, 100 symptomatic samples were collected from bhendi cultivation areas and kept under refrigeration for subsequent laboratory evaluation. Additionally, five separate locations with visibly infected weed flora were surveyed to assess the occurrence of *Ampelomyces* spp., considering its known association with diseased weed hosts. Sampling in both crop fields and weed-infested zones was carried out in a structured manner to ensure adequate diversity in fungal isolates. At each site, four groups of 25 leaves were randomly chosen, specifically targeting the fifth leaf node along the shoot. Visual diagnosis was carried out to assess disease intensity by estimating the proportion of the leaf surface covered with characteristic powdery fungal growth on the adaxial surface. The number of infected leaves per replicate was recorded and disease severity was measured as the percentage of affected leaf area, while disease incidence was calculated as the proportion of affected leaves out of the total collected.

Ampelomyces spp. isolation using the pycnidia-picking method

Ampelomyces pycnidia were picked up using a sterilized needle while being examined under a stereo binocular microscope (Euromex, Netherlands, IS 3153 PLFi/3) at 10X magnification, equipped with a DC 20000i microscope camera (17). The collected pycnidia were then transferred onto potato dextrose agar for further analysis (18). To minimize microbial contamination, the culture medium was supplemented with either 0.3 % streptomycin sulfate or 2 % chloramphenicol. The inoculated plates were then placed in a BOD incubator (Sub-Zero Lab Instruments, Model SZ-044B, Serial No: 2001198) and maintained at a temperature of 23 ± 1 °C. Fungal growth was routinely observed and morphological traits of the emerging isolates were systematically documented.

Isolation of *Trichoderma* spp. from rhizospheric soil

Soil around the root zone were meticulously collected by uprooting bhendi plants and retrieving the soil adhering to their roots. The collected soil samples were then air-dried at ambient laboratory conditions (28 ± 2 °C). For microbial isolation, 1 g of the dried soil was suspended in 10 mL of sterile distilled water (SDW) and agitated for 15 min before performing a serial dilution. From the 10^4 dilution, 1 mL was inoculated onto *Trichoderma* selective medium (TSM) plates, which were incubated at 25 ± 1 °C. Fungal colonies showing typical *Trichoderma* morphology were observed under a microscope for confirmation. To obtain pure cultures, the hyphal tip method was employed by transferring the advancing edges of the mycelium onto PDA-enriched plates. A total of fifteen isolates were preserved on PDA slants and stored at 4 °C, as per the protocol (19).

Isolation of *Bacillus* spp. from bhendi rhizosphere

Soil around the bhendi root surfaces were meticulously gathered from the bhendi plants across 15 different bhendi-growing regions in Tamil Nadu. Loosely adhering soil was carefully removed from freshly excised roots and root segments (1 g) were suspended in 10

mL of sterile distilled water to obtain a 10^{-1} dilution. Serial dilutions were subsequently prepared up to 10^{-7}. From the 10^{-6} and 10^{-7} dilutions, 1 mL aliquots were transferred into sterile Petri plates containing nutrient agar medium, specific for *Bacillus* spp. The plates were gently rotated in both clockwise and counter-clockwise directions to ensure uniform distribution of the inoculum. Inoculated plates were incubated at room temperature (28 ± 2 °C) for 48 to 72 hr. Colonies exhibiting typical morphological characteristics of *Bacillus* spp. were isolated and purified using the streak plate method on nutrient agar. The purified cultures were maintained on nutrient agar slants and stored at 4 °C for further use.

In vitro evaluation against *G. cichoracearum*

Spore germination technique

The efficacy of 10 *Ampelomyces* isolates (AUAQ-01 to AUAQ-10), 15 *Trichoderma* isolates (AUTH-01 to AUTH-15) and 15 *Bacillus* isolates (AUBS-01 to AUBS-15) was evaluated against *G. cichoracearum* using the spore germination assay at three concentrations (0.1 %, 0.3 % and 0.5 %). Culture filtrates were prepared by growing *Ampelomyces* and *Trichoderma* isolates in potato dextrose broth (PDB) and *Bacillus* isolates in nutrient broth (NB). After incubation, the cultures were filtered and centrifuged and the resulting supernatant was sterilized using a 0.22 µm membrane filter. Desired concentrations were obtained by diluting the filtrates with sterile distilled water. Powdery mildew conidia were suspended in the respective diluted culture filtrates and two drops of each treatment were placed on clean cavity slides. A coverslip was gently placed over each cavity and the margins were sealed with Vaseline to prevent contamination and moisture loss. Each concentration was tested in triplicate cavities and a control treatment was included using sterile distilled water. The slides were incubated at room temperature (27 ± 1 °C) in Petri plates lined with moist blotting paper for 24 hr. Post-incubation, spore germination was observed under a microscope at 10X magnification. In each microscopic field, the total number of spores, as well as the germinated and non-germinated spores, were counted. The average spore germination percentage from the three replicates was calculated and the percentage inhibition of conidial germination by each biocontrol agent was computed using the formula (20).

$$I = \frac{C - T}{C} X100$$

Where,

I = percent inhibition

C = germination of conidia in control

T = germination of conidia in treatment

Detached leaf assay

Healthy, mature bhendi leaves of the *Sakthi* variety were collected from the fields of Annamalai University, sterilised with 0.1 % $MgCl_2$ and rinsed with sterile distilled water. The leaves were then soaked in suspensions of biocontrol agents (*Ampelomyces* spp., *Trichoderma* spp. and *Bacillus* spp.) at concentrations of 0.1 %, 0.3 % and 0.5 % for 30 min with gentle agitation. After treatment, the leaves were either placed on moist blotters in Petri plates with petioles wrapped in Hoagland solution-soaked cotton or inserted into Comstock vials containing the same nutrient solution and dried in a laminar flow chamber. The leaves were then inoculated by brushing conidia of *G. cichoracearum* on the adaxial surface under aseptic conditions inside a laminar airflow chamber and incubated for two weeks at 20-22 °C with 70-80 % relative humidity and a 15hr photoperiod. Disease coverage was assessed after the incubation period of 14 days using a four-point scale: +++ for over 25 % leaf area covers, ++ for 11-25 % leaf area covers, + for 1-10 % leaf area covers and - for no visible disease (20).

Leaf disc technique

Healthy young bhendi leaves of the *Sakthi* variety were harvested from the fields of Annamalai University. The leaves were sterilized with 0.1 % $MgCl_2$ for 30 sec and rinsed with sterile distilled water. 11 mm discs were cut and immersed in biocontrol agent suspensions (*Ampelomyces* spp., *Trichoderma* spp. and *Bacillus* spp.) at 0.1 %, 0.3 % and 0.5 % concentrations, prepared as described earlier, for 30 min with gentle agitation. After soaking, the discs were air-dried for 5-10 min, then placed on filter paper in Petri plates and transferred to 9 cm plates with 10 mL of 1.5 % water agar. Conidia of the pathogen were applied to the upper surface of the discs. The inoculated discs were incubated in a BOD incubator at 22 °C under 1000 lux light intensity, which was measured using a SIGMA 101 lux light meter, for two weeks. Disease coverage was assessed after the incubation period of 14 days using a four-point scale: +++ for over 25 % leaf area covered, ++ for 11-25 % leaf area covered, + for 1-10 % leaf area covered and (-) for no visible disease (20).

Evaluation of plant growth-promoting traits using *Bacillus* spp.

Bacillus isolates were cultured in 100 mL of nutrient broth (NB) using a rotary shaker (Labline Orbital Shaker, Model: OS124C, Serial No: OL134) operated at 150 rpm and maintained at 28 ± 2 °C for 48 hr. Post-incubation, cultures were centrifuged at 6000 rpm for 15 min and the harvested cells were suspended in 0.01 M phosphate buffer (pH 7.0). The suspension was adjusted to a concentration of approximately 10^8 CFU/mL (OD_{595} = 0.3) using a Labman LMSP-UV1200 UV-VIS Spectrophotometer (21).

To assess plant growth-promotion, bhendi seeds were surface-treated with 10 mL of the bacterial suspension for 2 hr. After drying, they were placed between moist paper towels and incubated in a growth chamber (MLR-32H-PE, PHC Corporation, Japan) at 25 ± 1 °C with 80 % relative humidity under a 14/10-hr light/dark cycle (22). Seeds were treated with sterile double distilled water served as the untreated control. After 21 days, seedling performance was measured by recording root and shoot lengths and germination percentage was calculated accordingly.

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total Number of seeds own}} \text{ x100}$$

The vigour index was calculated using the following formula (23),

Vigour index = percent germination × seedling length (shoot length + root length)

After completing the *in vitro* assays, the virulent strain of the biocontrol agents was identified by morphological analysis and subjected to molecular characterization for confirmation.

Compatibility among biocontrol agents

The dual culture technique was employed to evaluate the compatibility between *B. subtilis* and *T. harzianum* (AUTH02 + AUBS01) on NA and PDA media. Fungal cultures grown on PDA plates for four days and *B. subtilis* grown in nutrient broth for 48 hr at 28 ± 2 °C were used in this study. Forty-eight-hour-old bacterial cultures were streaked at one end of the NA plates, while mycelial discs of *T. harzianum*, excised from the periphery of an actively

growing colony, were placed at the opposite corner of the assay plates, maintaining a 1 cm distance between the disc and the periphery of the plate. A control plate was prepared without bacterial inoculation. All plates were incubated at 28 ± 2 °C. Once the hyphal growth of *T. harzianum* in the control plates reached the periphery, the fungal growth in the dual inoculation plates (*T. harzianum* + *B. subtilis*) was assessed. The absence of an inhibition zone indicated compatibility with the bacterial strain, whereas the presence of an inhibition zone signified incompatibility (24). The same protocol was followed for the compatibility tests between *A. quisqualis* (AUAQ05) and *B. subtilis* (AUBS01), as well as between *A. quisqualis* (AUAQ05) and *T. harzianum* (AUTH02), in a PDA medium.

Formulation of talc-based bioinoculants

Talc-based formulations of *A. quisqualis* (AUAQ05), *T. harzianum* (AUTH02) and *B. subtilis* (AUBS01) were developed as a consortium by culturing each biocontrol agent individually and subsequently combining the broths for formulation. *A. quisqualis* and *T. harzianum* were cultured in molasses-yeast broth (composed of 30 g molasses and 5 g yeast per 1000 mL of distilled water), which was sterilised at 121 °C for 30 min and inoculated with a 9 mm mycelial disc. The cultures were incubated for 15 days, yielding a biomass concentration of approximately 3×10^8 cfu/mL.

B. subtilis was cultured in nutrient broth and incubated on a rotary shaker at 150 rpm for 48 hr at 28 ± 2 °C. Equal volumes (150 mL each) of the three microbial broths were then mixed with sterilised talc powder (pre-heated at 105 °C for 12 hr). To enhance shelf-life and maintain spore viability, the pH of the talc was adjusted to 7.0 using 1 % calcium carbonate ($CaCO_3$). A total of 50 mL of microbial suspension was added per 100 g of talc, along with 5 g of carboxymethyl cellulose (CMC) as an adhesive (25). The resulting mixture was air-dried in the shade for 12 hr and stored in polythene bags. The microbial population in the formulations was periodically monitored using the serial dilution technique to ensure adequate levels of spores and mycelial fragments.

Assessing the efficacy of combined talc-based biocontrol formulations for managing powdery mildew

Management practices were employed to control *G. cichoracearum* in the bhendi variety *Sakthi*. Talc-based formulations of *A. quisqualis*, *T. harzianum* and *B. subtilis* were prepared and applied at the recommended dosages. These treatments were sprayed onto the aerial parts of the plants using a hand-held sprayer. The first application was carried out preventively at 40 days after sowing (DAS), before the onset of disease symptoms. Pot culture and field experiments were conducted with nine treatments, including an untreated control. Standard cultivation practices were followed consistently throughout the study.

Disease incidence in green house

A pot culture study was carried out to assess the effectiveness of talc-based formulations containing *A. quisqualis*, *T. harzianum* and *B. subtilis*, applied both singly and in various combinations. Cement pots with dimensions of 1.5 × 1 × 1 feet were used, each filled with a mixture of red soil, sand and farmyard manure (FYM) in a 2:1:1 proportion. Five seeds of the bhendi cultivar *Sakthi* were sown in each pot. The experiment included nine different treatment regimes, with each treatment replicated three times (Table 1).

Powdery mildew pathogen was inoculated into the plants 30 DAS. A spray on each treatment was administered immediately upon symptom appearance. The percent disease index (PDI) was recorded ten days after spraying, as per the method described by (26). The efficacy of various bioagents was compared with the fungicide hexaconazole.

Table 1. Treatment details

T1	Foliar application with *A. quisqualis* at 0.5 %
T2	Foliar application with *T. harzianum* at 0.5 %
T3	Seed treatment with *B. subtilis* at 10 g/kg + FA at 0.5 %
T4	Foliar application with *A. quisqualis* at 0.5 % + Foliar application with *T. harzianum* at 0.5 %
T5	Foliar application with *A. quisqualis* at 0.5 % + seed treatment with *B. subtilis* at 10 g/kg and FA at 0.5 %
T6	Foliar application with *A. quisqualis* at 0.5 % + foliar application with *T. harzianum* at 0.5 %+ Seed treatment with *B. subtilis* at 10 g/kg and foliar application at 0.5 %
T7	Foliar application of hexaconazole at 0.1 %
T8	Pathogen inoculated control
T9	Uninoculated control (healthy)

Growth and yield parameters in greenhouse

Plant height was measured 90 DAS, from the base to the tip and expressed in centimeters. Fruit length and girth were recorded from five harvested fruits and their averages were calculated. Fruit yield per plant was determined by weighing fruits after each harvest. Shoot height and root length were measured from the base to the tip of the longest shoot and root, respectively and the average values were recorded for each treatment. Dry matter accumulation was determined by oven-drying the harvested plants at 70 °C until a constant weight was attained, using a hot air oven (Matri Scientific Instruments, Model: PX80, Serial No: 10398, Pondicherry, India).

Disease incidence at field level

A field experiment was carried out on farmers' fields in Parathur North village, Chidambaram taluk, Tamil Nadu, India, during the period from December 2023 to April 2024. The trial was conducted in randomized block design (RBD) comprising eight treatments triplicated. Each experimental plot measured 5 × 4 meters and the crop was maintained at a spacing of 45 × 30 cm. Foliar sprays were applied immediately following the initial appearance of powdery mildew symptoms (Table 1).

The trial was carried out in accordance with the recommended agronomic practices. Observations on the PDI were recorded ten days after the spray application. Disease severity was assessed on 20 randomly selected plants per plot and the PDI was calculated using a standard disease rating scale as previously described.

Growth and yield parameters at field level

The growth and yield parameters of bhendi included fruit yield per plant, which was calculated as the total weight of fruits harvested from each plant, expressed in grams, with the mean value determined. The fruit yield per hectare, plant height, fruit length and girth of the fruit were recorded by periodically. Harvesting bhendi fruits and measuring the yield in tonnes per hectare. Additionally, the percentage increase in yield over the control was calculated using the formula:

$$\text{Percent Increase in Yield Over Control} = \frac{\text{Yield in Treated Plot - Yield in Control Plot}}{\text{Yield in Control Plot}} \times 100$$

Statistical analysis

Experimental data were statistically evaluated through analysis of variance (ANOVA) and treatment means were compared using Duncan's multiple range test (DMRT) at the 5 % significance level. To ensure data normalization, disease severity values expressed in percentages were transformed using the arcsine method before analysis (27).

Results and Discussion

Isolation of *Ampelomyces* sp. from Bhendi and Weed Hosts Across Different Districts

Ampelomyces sp. isolates were obtained from various bhendi-growing districts, namely Dindigul, Cuddalore, Salem and Krishnagiri, from both bhendi plants and weed hosts such as *Euphorbia geniculata* and *Xanthium indicum* as well as shown in the Table 2 and Fig. 1. In total, ten isolates (AUAQ01 to AUAQ10) were obtained. This aligns with a study that isolated *Ampelomyces* spp. hyperparasite isolates from powdery mildew-infected grapevine leaves in Coimbatore district, confirming their presence in regional vineyards (28). Likewise, 20 *Ampelomyces* sp. isolates from various plant hosts and powdery mildew pathogens (*Erysiphe*, *Sphaerotheca*, *Oidium* and *Leveillula*) across Tamil Nadu, recording up to 81 % mycoparasitization in *Erysiphe* spp. (29). Similarly, *Ampelomyces* spp. Isolated from bhendi in Coimbatore (30).

Variation in morphological traits and growth patterns across diverse isolates of *Ampelomyces* spp.

Ampelomyces spp. isolates exhibited variations in colony morphology, growth rate, pycnidial shape and pigmentation. Most isolates showed radial growth, except AUAQ01, AUAQ06 and AUAQ08, which were flat. Colony colour ranged from brownish black (AUAQ01, AUAQ08) to brown (AUAQ03, AUAQ05, AUAQ06, AUAQ07, AUAQ09) and brownish white (AUAQ04, AUAQ10), with AUAQ02 being white. Zonation was present in six isolates and margins varied between wavy, irregular and smooth. Growth was mostly slow, except for AUAQ05, AUAQ06, AUAQ09 and AUAQ10 (moderate). Pycnidia were diverse in shape, with unicellular, hyaline spores. AUAQ02 had the highest a total of 10 pycnidi, while AUAQ05 and AUAQ09 had the lowest only 3 as given in Table 3. These differences indicate potential functional variability among isolates (Fig. 2-4).

Our results align with those reporting radial, fluffy growth in *A. quisqualis*, with pycnidia ranging from ovoid to globose (31). *Ampelomyces* as a biocontrol agent against *E. cichoracearum* in bhendi, noting that pycnidia and pycnidiospores varied in size and shape. The mycelium transitioned from hyaline to greyish-white or brownish-black, with isolate AQB7 exhibiting the highest virulence (30). The powdery mildew fungus with hyaline, septate hyphae and sub globose to pyriform pycnidia, with unicellular, guttulate conidia (32).

Morphological characteristics of *Trichoderma* isolates from bhendi-growing regions in Tamil Nadu

Trichoderma spp. isolates from Tamil Nadu exhibited diverse colony characteristics, mycelial growth and conidia sizes. Mycelial growth ranged from 42.35 mm to 90.00 mm, with colonies varying from white to dark green and sporulation levels from minimal to very high (Fig. 5). Conidia sizes ranged from 2.03 to 3.65 µm, 1.83 to 5.23 µm in length and breadth respectively, with larger conidia observed in isolates displaying cottony, fluffy sporulation, emphasizing the potential diversity of these strains for biocontrol applications as indicated in Table 4. The findings align with the work, which detailed the microscopic features of *Trichoderma* isolates, with conidia ranging from 2.92 to 5.10 µm in size, having a globose to sub-globose shape and green coloration (33). Similarly, *T. asperellum* isolates exhibited dark green to green sporulation, consistent with the findings of the current research (34).

Table 2. List of *Ampelomyces* spp. isolates collected during the survey

S. no.	Isolate no	District	Place of collection	Plant source
1.	AUAQ01	Dindugal	Neikarapatti	*Xanthium indicum*
2.	AUAQ02	Dindugal	Oddanchatram	*A. esculentus*
3.	AUAQ03	Dindugal	Kollapatti	*A. esculentus*
4.	AUAQ05	Cuddalore	Paalur	*A. esculentus*
5.	AUAQ05	Cuddalore	Parathur North	*A. esculentus*
6.	AUAQ06	Cuddalore	Kottimalai	*A. esculentus*
7.	AUAQ07	Salem	Veeraganur	*A. esculentus*
8.	AUAQ08	Salem	Gengaveli	*X. indicum*
9.	AUAQ09	Krishnagiri	Ammaiyampatti	*Euphorbia geniculate*
10.	AUAQ10	Krishnagiri	Barur	*A. esculentus*

Fig. 1. Association of *Ampelomyces* sp. on different host species. **A:** *Xanthium indicum*, **B:** *Euphorbia geniculate* and **C:** *Abelmoschus esculentus* (bhendi).

Table 3. Morphological characters of different isolates of *Ampelomyces* spp.

S. no.	*Ampelomyces* spp. isolate	Mycelium	Topography	Colour of mature colonies	Zonation	Margin	Colony growth	Pycnidia shape	Pycnidiospore	No. of pycnidia
1	AUAQ01	Hyaline, septate	Flat	Brownish black	Absent	Wavy	Very slow	Pyriform	Unicellular, hyaline, round	6
2	AUAQ02	Hyaline, septate	Radial	White	Present	Irregular	Slow	Globose	Unicellular, hyaline, round	10
3	AUAQ03	Hyaline, septate	Radial	Brown	Absent	Smooth	Slow	Globose	Unicellular, hyaline, oval	4
4	AUAQ04	Hyaline, septate	Radial	Brownish white	Present	Wavy	Slow	Spherical	Unicellular, hyaline, round	5
5	AUAQ05	Hyaline, septate	Radial	Brown	Present	Wavy	Moderate	Ovoid	Unicellular, hyaline, round	3
6	AUAQ06	Hyaline, septate	Flat	Brown	Present	Irregular	Moderate	Spherical	Unicellular, hyaline, oval	8
7	AUAQ07	Hyaline, septate	Radial	Brown	Absent	Smooth	Slow	Round	Unicellular, hyaline, oval	7
8	AUAQ08	Hyaline, septate	Flat	Brownish black	Present	Irregular	Slow	Globose	Unicellular, hyaline, round	6
9	AUAQ09	Hyaline, septate	Radial	Brown	Present	Wavy	Moderate	Spherical	Unicellular, hyaline, oval	3
10	AUAQ10	Hyaline, septate	Radial	Brownish white	Absent	Irregular	Moderate	Oval	Unicellular, hyaline, oval	4

Fig. 2. Maintenance of isolates of *Ampelomyces* (on PDA slant).

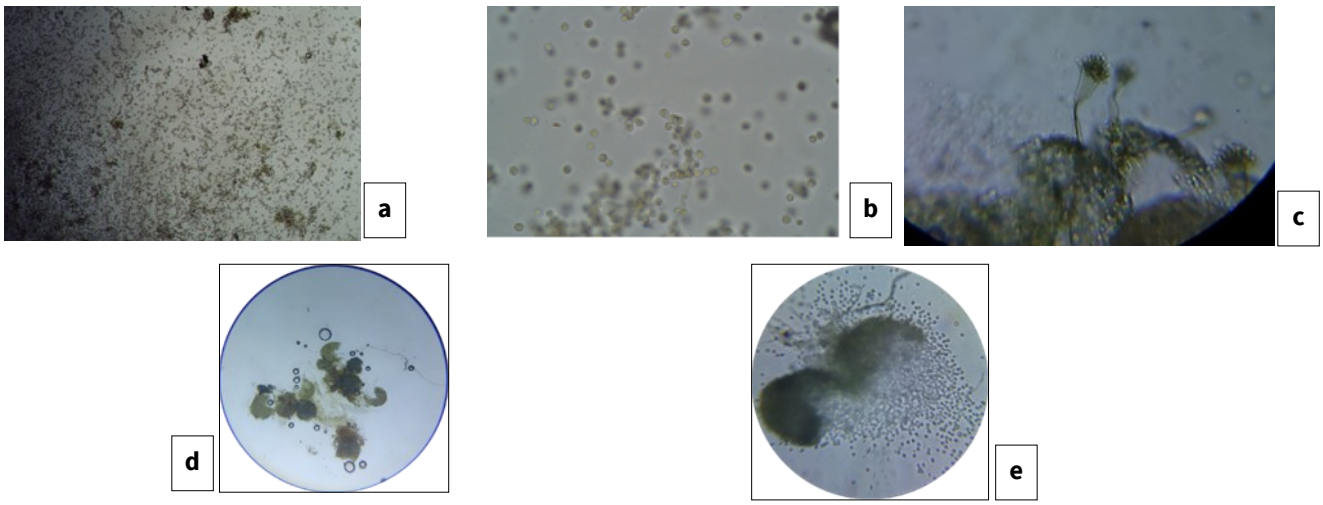

Fig. 3. Microscopic observation of *A. quisqualis* (AUAQ05). **A:** under 10 x, **B:** under 40 x, **C:** pycnidiophore (40x), **D:** pycnidia (10x) and **E:** Pycnidia (40x).

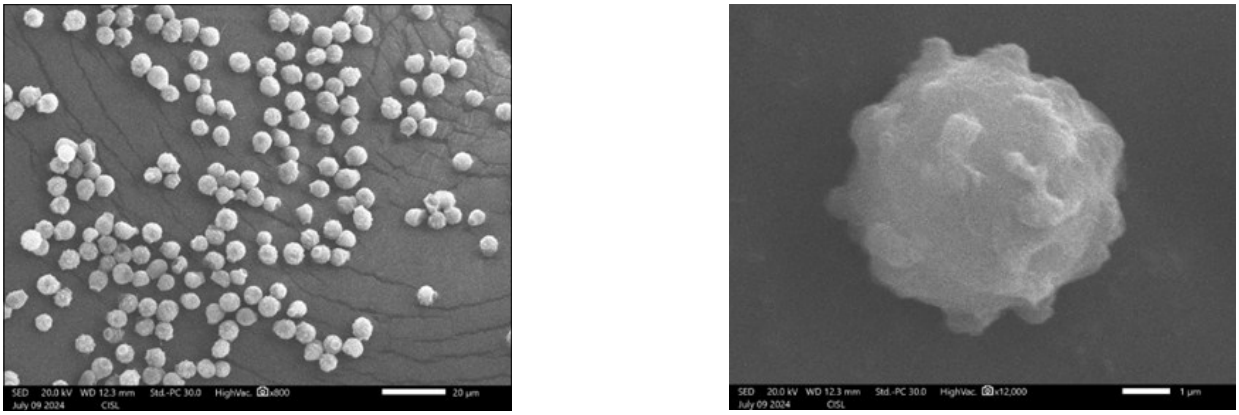

Fig. 4. SEM observation of *A. quisqualis* (Pycnidiospores) (AUAQ05).

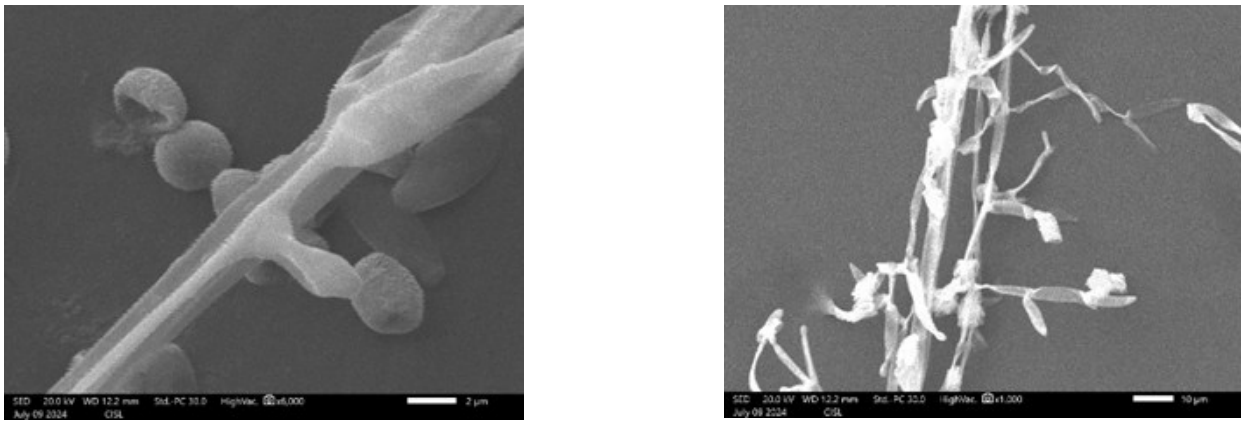

Fig. 5. SEM observation of *Trichoderma harzianum* (AUTH02).

Table 4. Collection details and morphological characteristics of *Trichoderma* isolates from bhendi-growing regions in Tamil Nadu

S. No.	*Trichoderma* spp. isolates	Location	Soil type	Colony colour	Mycelial growth* (mm)	Conidia Size		Sporulation
						Length (µm)	Breadth (µm)	
1.	AUTH01	Neikarapatti	Red loamy	Dark green	86.2^{ab} (68.42)	2.03-3.17	2.20-4.05	++
2.	AUTH02	Kannivadi	Red loamy	Green	90.00^{a} (71.65)	2.13-3.25	2.06-4.09	++++
3.	AUTH03	Kollapatti	Red loamy	Green	81.65^{bc} (64.64)	2.28-3.27	2.17-4.50	+
4.	AUTH04	Paalur	Loamy and clayey	Green	74.25^{de} (59.55)	2.83-3.60	2.53-5.18	++
5.	AUTH05	Parathur North	Loamy and sandy	Light green	70.36^{ef} (57.02)	2.06-3.10	2.26-4.18	**++++**
6.	AUTH06	Kottimalai	Loamy and clayey	Pale green	67.45^{fg} (55.22)	2.87-3.65	2.58-5.23	**++++**
7.	AUTH07	Sathapadi	Loamy and clayey	White	63.80^{gh} (52.88)	2.27-2.73	1.83-3.53	**+++**
8.	AUTH08	Veeraganur	Loamy and clayey	Greyish green	50.35^{jk} (45.20)	2.32-3.32	2.21-4.55	**+++**
9.	AUTH09	Gengaveli	Loamy upland	White	54.60^{j} (47.03)	2.32-2.78	2.37-3.73	**+++**
10.	AUTH10	Ikundham	Non-Calcareous	White	60.65^{h}(51.15)	2.35-2.83	2.41-3.78	**+++**
11.	AUTH11	Ammaiyampatti	Sandy loam.	Dull green	42.35^{l} (42.90)	2.53-3.28	2.43-4.58	**+**
12.	AUTH12	Barur	Black cotton	Faded green	84.85^{b} (67.20)	2.22-3.03	2.33-3.87	**++**
13.	AUTH13	Baderahalli	Sandy loam.	Soft green	76.65^{cd} (61.11)	2.18-2.98	2.29-3.78	**++++**
14.	AUTH14	Vediyur	Clay loam.	Whitish green	46.85^{kl} (44.34)	2.25-3.06	2.38-4.10	**+++**
15.	AUTH15	Eriyur	Calcareous Black	White	57.55^{i} (49.37)	2.16-3.15	2.08-4.21	**+**

*Values represent the mean of three replicates. Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

Morphological characteristics of *Bacillus* isolates from bhendi-growing regions in Tamil Nadu

The *Bacillus* isolates demonstrated considerable variability in terms of soil origin, colony morphology and motility characteristics. All isolates were rod-shaped with serrated edges; however, colony elevation differed-some were flat (AUBs-2, AUBs-6, AUBs-12, AUBs-14), others raised (AUBs-1, AUBs-4, AUBs-7, AUBs-8, AUBs-10, AUBs -11, AUBs-13) and a few convex (AUBs-3, AUBs-5, AUBs-9, AUBs-15) (Fig. 6). Colony pigmentation varied between white (e.g., AUBs-1, AUBs-3, AUBs-5, AUBs-6, AUBs-10, AUBs-11, AUBs-14) and creamy yellow (e.g., AUBs-2, AUBs-4, AUBs-7, AUBs-8, AUBs-9, AUBs-12, AUBs-13, AUBs-15). Colony texture also differed, being smooth (AUBs-2, AUBs-3, AUBs-4, AUBs-5, AUBs-11), mucoid (AUBs-1, AUBs -7, AUBs-8, AUBs-10, AUBs-13, AUBs-14), or dry (AUBs-6, AUBs-9, AUBs-12, AUBs-15). Motility assays revealed that eight isolates (AUBs-1, AUBs-2, AUBs-3, AUBs-10, AUBs-11, AUBs-12, AUBs-13) were motile, while the remaining seven lacked motility, as detailed in Table 5. Morphological analysis of sixteen *B. subtilis* isolates. All were Gram-positive, rod-shaped and capable of forming endospores. Colony colour ranged from white to cream, with most forming circular colonies featuring irregular margins. On selective media, green pigmentation was common among isolates, with four showing irregular colony shapes and the rest maintaining circular forms (35). Similar research involving *Bacillus* strains from rhizosphere soils in southern India also noted their antifungal properties against various plant pathogens (36).

In-vitro efficaciousness of different microbial antagonists against *G. cichoracearum*

Slide spore germination method

In this study, the efficacy of with 10 isolates of *Ampelomyces* spp., 15 isolates each of *Trichoderma* spp. and *Bacillus* spp., was evaluated against G. *cichoracearum* using the slide spore germination method as shown in Table 6. *Ampelomyces* spp. (AUAQ05), *Trichoderma* spp. (AUTH02) and *Bacillus* spp. (AUBS01) showed significant conidial germination inhibition, with the highest inhibition rates at 0.5 % concentration (Fig. 7-9) *Ampelomyces* sp. (AUAQ05) was the most effective (Fig. 10), achieving 73.64 % inhibition, compared to high germination rates (63.25 %) in the control. These findings align with reports that neem leaf and garlic extract as highly effective in inhibiting spore germination, achieving up to 82.23 % inhibition as depicted in Table 7 (37). Additionally, the efficacy of ten *Ampelomyces* isolates against *E. cichoracearum*, with the highest parasitism observed at 10^8 conidia/mL, highlighting the potential of *Ampelomyces* in biocontrol applications (30).

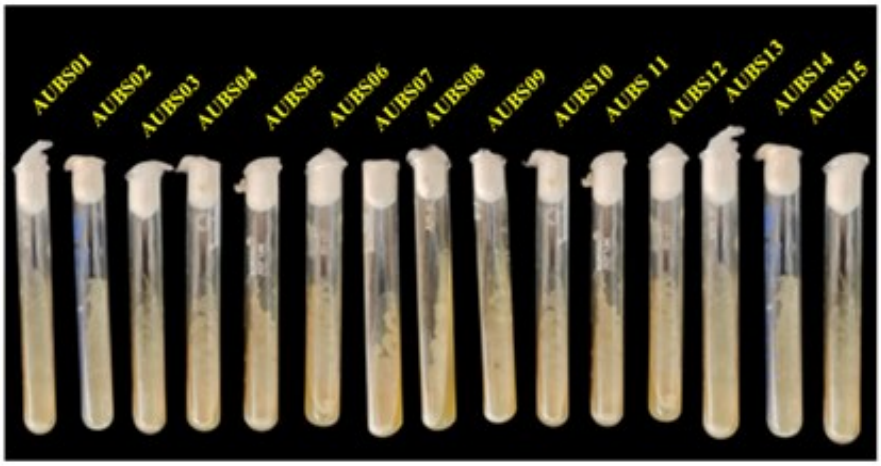

Fig. 6. Different isolates of *Bacillus* spp. (on NA slant).

Table 5. Morphological Characteristics of *Bacillus* spp. isolates from bhendi-growing regions in Tamil Nadu

S. no.	Bacillus spp. isolate	Location	Soil type	Shape	Margin	Elevation	Colour	Texture	Motility
1	AUBs-1	Neikarapatti	Red loamy	Rod	Serrated	Raised	White	Mucoid	+
2	AUBs-2	Kannivadi	Red loamy	Rod	Serrated	Flat	Creamy yellow	Smooth	+
3	AUBs-3	Kollapatti	Red loamy	Rod	Serrated	Convex	White	Smooth	+
4	AUBs-4	Paalur	Loamy and clayey	Rod	Serrated	Raised	Creamy yellow	Smooth	-
5	AUBs-5	Parathur North	Loamy and sandy	Rod	Serrated	Convex	White	Smooth	-
6	AUBs-6	Kottimalai	Loamy and clayey	Rod	Serrated	Flat	White	Dry	-
7	AUBs-7	Sathapadi	Loamy and clayey	Rod	Serrated	Raised	Creamy yellow	Mucoid	-
8	AUBs-8	Veeraganur	Loamy and clayey	Rod	Serrated	Raised	Creamy yellow	Mucoid	-
9	AUBs-9	Gengaveli	Loamy upland	Rod	Serrated	Convex	Creamy yellow	Dry	-
10	AUBs-10	Ikundham	Non-Calcareous	Rod	Serrated	Raised	White	Mucoid	+
11	AUBs-11	Ammaiyampatti	Sandy loam.	Rod	Serrated	Raised	White	Smooth	+
12	AUBs-12	Barur	Black cotton soil	Rod	Serrated	Flat	Creamy yellow	Dry	+
13	AUBs-13	Baderahalli	Sandy loam.	Rod	Serrated	Raised	Creamy yellow	Mucoid	+
14	AUBs-14	Vediyur	Clay loam.	Rod	Serrated	Flat	White	Mucoid	-
15	AUBs-15	Eriyur	Calcareous Black	Rod	Serrated	Convex	Creamy yellow	Dry	-

Table 6. *In vitro* evaluation of different bio-control agents on the spore germination of *G. cichoracearum*

***Ampelomyces* isolates**	**Reduction of spore germination (%)**			***Trichoderma* isolates**	**Reduction of spore germination (%)**			***Bacillus* isolates**	**Reduction of spore germination (%)**		
	Culture filtrate concentration (%)				**Culture filtrate concentration (%)**				**Culture filtrate concentration (%)**		
	0.1	**0.3**	**0.5**		**0.1**	**0.3**	**0.5**		**0.1**	**0.3**	**0.5**
AUAQ01	44.80	64.40	80.50	AUTH01	0	0	0	**AUBS01**	**41.77**	**57.77**	**61.53**
AUAQ02	46.90	66.00	81.50	**AUTH02**	**46.24**	**62.05**	**67.85**	AUBS02	35.60	52.20	59.00
AUAQ03	39.00	59.20	73.50	AUTH03	10.15	14.85	19.72	AUBS03	0	0	0
AUAQ04	32.40	56.90	70.20	AUTH04	0	0	0	AUBS04	0	0	0
AUAQ05	**48.65**	**68.17**	**84.64**	AUTH05	20.48	30.76	40.29	AUBS05	28.44	47.85	55.21
AUAQ06	35.40	58.60	75.40	AUTH06	0	0	0	AUBS06	0	0	0
AUAQ07	45.90	65.40	80.90	AUTH07	25.35	34.89	44.62	AUBS07	0	0	0
AUAQ08	37.20	56.80	72.70	AUTH08	5.10	7.55	10.20	AUBS08	20.00	32.00	45.50
AUAQ09	45.10	63.90	80.10	AUTH09	15.22	24.68	34.75	AUBS09	15.33	25.90	40.89
AUAQ10	47.30	66.60	82.60	AUTH10	0	0	0	AUBS10	0	0	0
Control	0	0	0	AUTH11	38.00	43.65	51.35	AUBS11	15.33	25.90	40.89
-	-	-	-	AUTH12	30.13	40.56	50.94	AUBS12	22.50	34.45	48.35
-	-	-	-	AUTH13	0	0	0	AUBS13	0	0	0
-	-	-	-	AUTH14	40.89	47.22	50.94	AUBS14	32.56	45.62	52.15
-	-	-	-	AUTH15	12.37	18.45	23.91	AUBS15	0	0	0

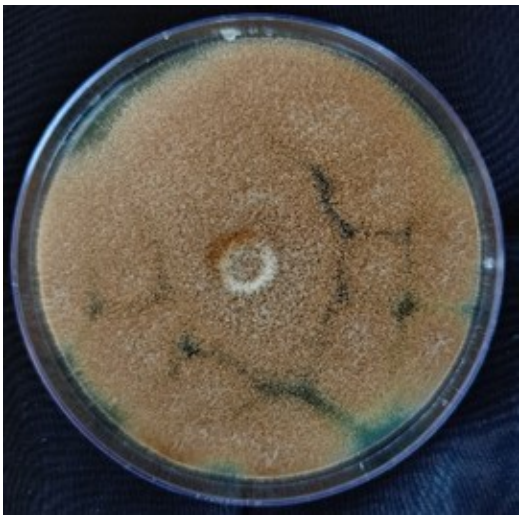

Fig. 7. Axenic culture of *A. quisqualis* (Pycnidiospores) (AUAQ05).

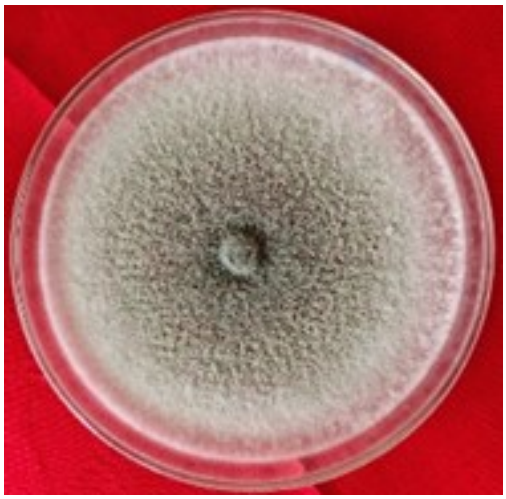

Fig. 8. Axenic culture of *T. harzianum (*AUTH02).

Fig. 9. Axenic culture *Bacillus subtills* (AUBS01).

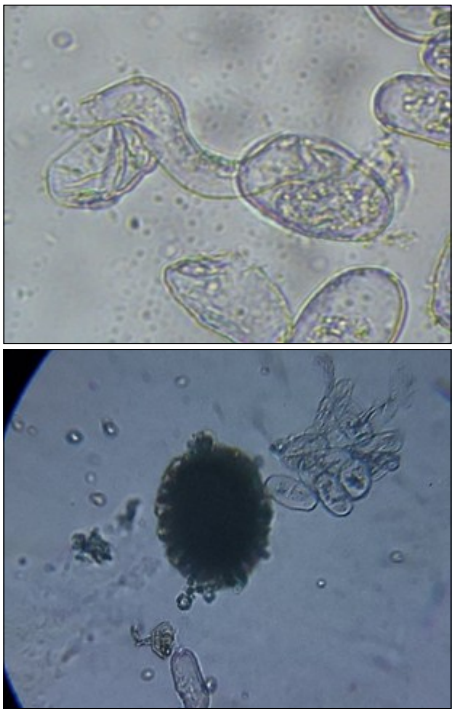

Fig. 10. *In vitro* spore germination assay. **A:** germination of conidia and **B:** Ampelomyces association with conidia.

Table 7. Effect of biocontrol agents on conidial germination, germination inhibition and germ tube length at different concentrations (%) through slide spore germination method

BCA(s)	Conidial germination (%) inhibition at different concentrations (%)			Mean	Germ tube length at different concentrations (%)			Mean
	0.1	0.3	0.5		0.1	0.3	0.5	
***Trichoderma* spp. (AUTH-02)**	46.24	62.05	67.85	58.71	75.67	59.33	45.67	60.22
***Bacillus* spp. (AUBS-01)**	41.77	57.77	61.53	53.69	80.33	62.33	48.67	63.11
***Ampelomyces* spp. (AUAQ-05)**	48.65	68.17	84.64	63.48	72.00	56.67	42.67	57.11
Control	-	-	-		108.56	108.56	108.56	108.56
Mean	45.55	62.66	67.67		83.39	71.72	61.39	

*Values represent the mean of three replicates. Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

Leaf disc assay and detached leaf assay

In this study, 15 isolates each of *Trichoderma* sp. and *Bacillus* sp., along with 10 isolates of *Ampelomyces* sp., were evaluated against Bhendi powdery mildew using leaf disc and detached leaf assays (Fig. 11, 12). Among the biocontrol agents tested, *Trichoderma* spp. (AUTH02), *Bacillus* spp. (AUBS01) and *Ampelomyces* spp. (AUAQ05) exhibited varying degrees of efficacy. At 0.1 % concentration, more than 25 % of the leaf disc area was covered by powdery mildew for both *Trichoderma* spp. (AUTH02) and *Bacillus* spp. (AUBS01), whereas *Ampelomyces* spp. (AUAQ05) showed reduced disease severity with only 1-10% coverage. Increasing the concentration to 0.3 % resulted in a decline in disease severity, with *Trichoderma* spp. and *Bacillus* spp. reducing infection to 11-25 %, while *Ampelomyces* spp. maintained minimal infection at 1-10 %. At 0.5 % concentration, *Trichoderma* spp. and *Bacillus* spp. limited infection to just 1-10 %, while *Ampelomyces* spp. completely suppressed powdery mildew. These findings highlight the superior disease control efficacy of *Ampelomyces* spp. (AUAQ05) at higher concentrations, demonstrating its potential as an effective biocontrol agent (Table 8). These findings are consistent with the report confirming the efficacy of *A. quisqualis* in controlling powdery mildew through a detached leaf assay, showing a mean conidiophore collapse of 81 % across clones, the highest in AZA17 (88 %) and the highest pycnidia density per cm^2 in AC4 (63 %) (38).

Growth-promoting activity of *Bacillus* isolates on bhendi

The plant growth-promoting activity of *B. subtilis* isolates was assessed using the roll towel method, with AUBS01 showing the highest performance, achieving a 95 % germination rate, mean shoot and root lengths of 12.5 cm and 9.6 cm, respectively and the highest vigour index of 2097.5 (Fig. 13). AUBS14 also performed well, with a 93.5 % germination rate and a vigour index of 1831.0. Even the lowest-performing isolate, AUBS07, outperformed the control in shoot and root growth as evidenced by Table 9. These findings are consistent with earlier studies indicating that bioagent treatments, such as *Bacillus* and *Pseudomonas* strains, enhance okra growth by facilitating nutrient uptake (nitrogen, phosphorus and essential minerals) or regulating plant hormone levels (39).

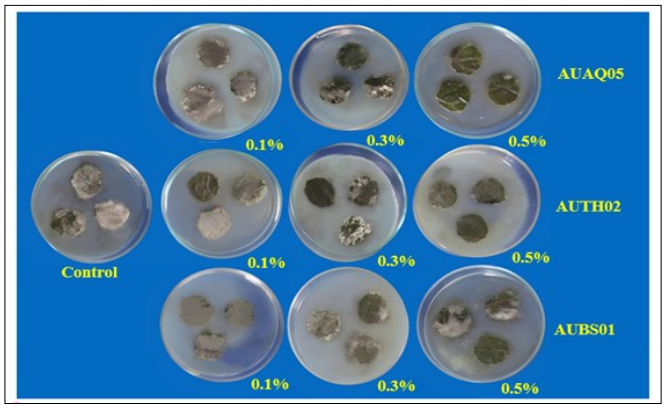

Fig. 11. Leaf disc assay.

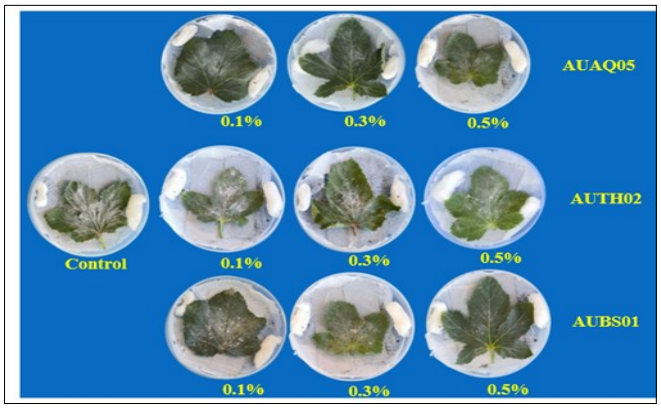

Fig. 12. Detached leaf assay.

Table 8. Efficacy of biocontrol agents against bhendi powdery mildew through leaf disc assay and detached leaf assay

S. no.	Culture filtrate concentration (%)	Powdery mildew growth					
		Biocontrol agents					
		Leaf disc assay			Detached leaf assay		
		AUTH02	AUBS01	AUAQ05	AUTH02	AUBS01	AUAQ05
1.	0.1	+++	+++	+	+++	+++	+
2.	0.3	++	++	+	++	++	+
3.	0.5	+	+	-	+	+	-
4.	Control		+++			+++	

AUTH02- *Trichoderma* spp, AUBS01– *Bacillus* spp, AUAQ05 – Ampelomyces spp.

+++ = > 25 % leaf disc covered by powdery mildew

++ = > 11-25 % leaf disc covered by powdery mildew

+ = > 1-10 % leaf disc covered by powdery mildew

- = > No powdery mildew

Fig. 13. *In vitro* PGPR effect on the growth of bhendi seeds (roll towel methods).

Table 9. Plant growth-promoting activity of *Bacillus* spp. isolates through roll towel method

Isolates	Germination (%)	Mean shoot length (cm)	Mean root length (cm)	Vigour index
AUBs-1	95.00a	12.5^{a}	9.6^{a}	2097.5^{a}
AUBs-2	94.00^{ab}	11.8^{b}	9.2^{b}	1926.0^{b}
AUBs-5	91.50^{cd}	10.5^{d}	8.3^{e}	1684.2^{e}
AUBs-8	89.00^{de}	10.7^{cd}	8.5^{d}	1703.8^{d}
AUBs-9	90.50^{d}	10.3^{d}	8.4^{de}	1654.5^{f}
AUBs-11	93.00^{bc}	11.2^{bc}	8.9^{bc}	1836^{bc}
AUBs-12	92.00^{c}	11.0^{c}	8.7^{cd}	1781.4^{cd}
AUBs-14	93.50^{b}	11.0^{c}	8.8^{c}	1831.0^{c}
Control	87.00^{e}	10.2^{de}	8.2^{e}	1608.0^{g}

*Values represent the mean of three replicates. Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

Molecular characterization of biocontrol agents

In vitro observations indicated that, the most effective biocontrol agents against bhendi powdery mildew-*Ampelomyces* spp. (AUAQ05), *Trichoderma* spp. (AUTH02) and *Bacillus* spp. (AUBS01)-have been selected for further molecular characterization. The isolate AUAQ05 from Parathur North corresponds to *A. quisqualis*, indexed under PQ066229. Additionally, isolate AUTH02 from Kannivadi was identified as *T. harzianum* with accession number PP425902 and isolate AUBS01 from Neikarapatti was confirmed as *B. subtilis* with accession number PP384198 as presented in Table 10. These molecular identifications and their GenBank deposits validate the accuracy of pathogen identification in the study.

Compatibility among biocontrol agents

The compatibility of the antagonistic microbial agents *T. harzianum*, *B. subtilis* and *A. quisqualis* is shown in Table 11. The results indicated

Table 10. Molecular characterization of biocontrol agents

S. no.	Location	Isolate no.	Accession no.	Biocontrol agent
02.	Parathur North	AUAQ05	PQ066229	*A. quisqualis*
03.	Kannivadi	AUTH02	PP425902	*T. harzianum*
04.	Neikarapatti	AUBS01	PP384198	*B. subtilis*

that *T. harzianum* demonstrated high compatibility with both *A. quisqualis* and *B. subtilis*, with the highest compatibility observed with *A. quisqualis*. Similarly, *B. subtilis* exhibited high compatibility with *T. harzianum* and moderate compatibility with *A. quisqualis*. *A. quisqualis* was highly compatible with *T. harzianum* and moderately compatible with *B. subtilis*. There was no inhibition zone between these biocontrol agents, suggesting that they can potentially be used in combination for effective management of powdery mildew. *A. quisqualis* showing strong compatibility with both *T. harzianum* and *B. subtilis* (Fig. 14). The dual-culture experiment confirmed the compatibility of *A. quisqualis* with

Table 11. Testing the compatibility among biocontrol agents

S. no.	Antagonist	Compatibility level		
		A. quisqualis	***T. harzianum***	***B. subtilis***
1.	*A. quisqualis*	NT	+++	++
2.	*T. harzianum*	+++	NT	+++
3.	*B. subtilis*	++	+++	NT

NT - not tested

++ - Compatible

+++ - Highly Compatible

T. asperellum, with microscopic and SEM analyses revealing hyperparasitism, where *Trichoderma* conidiophores carried *Ampelomyces* conidia and *Trichoderma* spores encircled and bound to *Ampelomyces* conidiophores (15). Compatibility studies showed that *Bacillus* isolates B03, B570, B571 and B854, together with *Trichoderma* isolates SF45, SF32 and SF311, exhibited effective inhibition of *Sclerotium cepivorum* mycelial growth and were mutually compatible (40).

Efficacy of combined talc-based biocontrol formulations for managing powdery mildew

Disease incidence at greenhouse condition

Under greenhouse conditions, individual talc-based biocontrol agents at 0.5 % concentration-*A. quisqualis*, *T. harzianum* and *B. subtilis*-ontrolled powdery mildew by 58.31 %, 69.07 % and 63.25 %, respectively (Table 12, Fig. 15). The combination of *A. quisqualis* + *T. harzianum* at 0.5 % and *A. quisqualis* + *B. subtilis* at 0.5 % further enhanced disease control to 74.83 % and 72.09 %, respectively. A significant improvement was observed with the three-agent combination (*A. quisqualis* + *T. harzianum* + *B. subtilis* at 0.5 %), along with seed treatment of *B. subtilis* at 10 g/kg of bhendi seeds, achieving 77.26 % disease control. This was comparable to the 87.71 % disease control provided by the check fungicide hexaconazole at 0.1 % (Table 12). *A. quisqualis* and

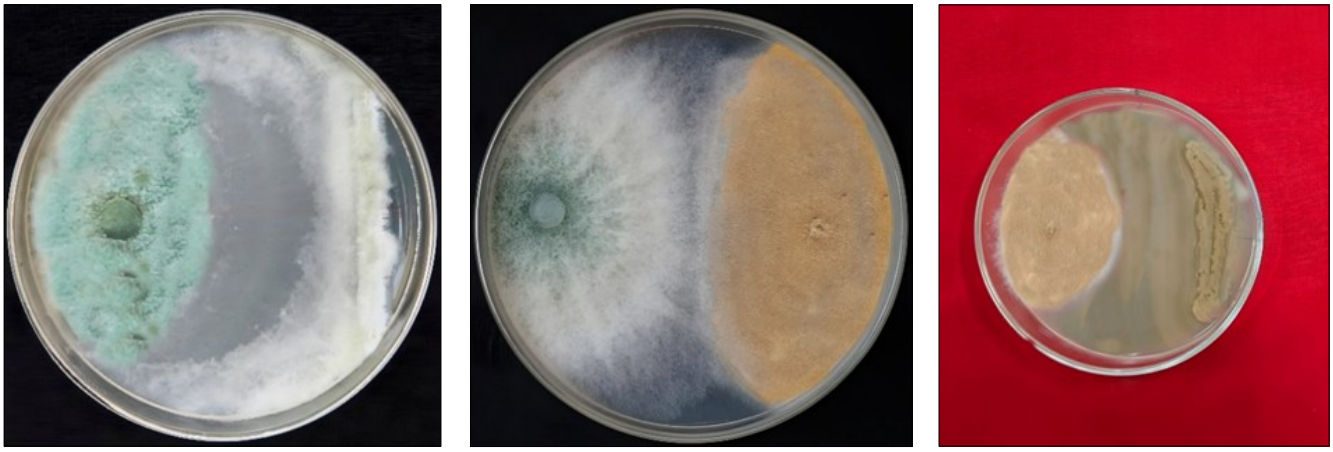

Fig. 14. *In vitro* compatibility among biocontrol agents. **A: c**ompatibility between *T. harzianum* and *B. subtilis,* **B: c**ompatibility between *T. harzianum* and *A. quisqualis* and **C: c**ompatibility between *A. quisqualis* and *B. subtilis.*

Table 12. Assessing the efficacy of combined talc-based biocontrol formulations for managing powdery mildew in greenhouse conditions

S. no.	Treatments	Disease index (%)	Disease reduction over control (%)
T1	FA with *A. quisqualis* at 0.5 %	17.82^{e} (24.29)	17.82^{e} (24.29)
T2	FA with *T. harzianum* at 0.5 %	13.65d^{f}	69.07
T3	ST with *B. subtilis* at 10 g/kg + FA at 0.5 %	15.71^{g} (23.34)	63.25
T4	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %	10.76^{c} (19.14)	74.83
T5	FA with *A. quisqualis* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	11.93^{d} (20.20)	72.09
T6	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %+ ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	09.72^{b}	77.26
T7	FA of hexaconazole at 0.1 %	8.25^{a} (13.24)	87.71
T8	Inoculated control	42.75^{h}	-

*ST- seed treatment *FA- foliar application.

*Values represent the mean of three replicates.

Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

Fig. 15. Overview of experiment (under pot culture condition).

T. asperellum effectively control powdery mildew in grapevines, with *A. quisqualis* showing high potential for biological control and compatibility with other biocontrol agents (15). Similarly, the early application of *A. quisqualis* significantly decreased the symptoms of cucurbit powdery mildew and hindered the development of *S. fusca* on melon leaves under controlled conditions, highlighting the potential of these mycoparasites as effective biocontrol agents in melon greenhouses (41).

The talc-based formulation of *P. fluorescens* strain Pf1, applied at 2 % under glasshouse conditions, significantly reduced powdery mildew in grapevine by inducing defense enzymes, including peroxidase, polyphenol oxidase and chitinase (42). The effectiveness of *Bacillus* culture filtrates against powdery mildew (12). The management of powdery mildew and grey mold in cucumber using *T. harzianum* T39 and *A. quisqualis* AQ10 (43). The combined use of biocontrol agents such as *T. harzianum*, *T. viride*, *B. subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae*, along with chemical resistance inducers like calcium chloride, potassium monohydrogen phosphate, potassium bicarbonate, saccharin, ascorbic acid, chitosan, humic acid and folic acid, has been suggested as an easy, safe and cost-effective control method (44).

Growth and Yield Parameters

The greenhouse assessment of talc-based biocontrol formulations demonstrated that the treatment T6, along with seed treatment T3, significantly enhanced bhendi growth and yield, achieving the highest plant height (138.99 cm), fruit length (19.72 cm) and yield per plant (364.72 g). Dual combinations also improved efficacy, with the treatment T5 recording a plant height of 130.87 cm and a yield of 338.34 g, while T4 showed moderate improvements. Among individual treatments, treatment T3 performed best

Table 12. Assessing the efficacy of combined talc-based biocontrol formulations for managing powdery mildew in greenhouse conditions

S. no.	Treatments	Disease index (%)	Disease reduction over control (%)
T1	FA with *A. quisqualis* at 0.5 %	17.82^{e} (24.29)	58.31
T2	FA with *T. harzianum* at 0.5 %	13.65d^{f} (21.68)	69.07
T3	ST with *B. subtilis* at 10 g/kg + FA at 0.5 %	15.71^{g} (23.34)	63.25
T4	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %	10.76^{c} (19.14)	74.83
T5	FA with *A. quisqualis* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	11.93^{d} (20.20)	72.09
T6	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %+ ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	09.72^{b} (18.16)	77.26
T7	FA of hexaconazole at 0.1 %	8.25^{a} (13.24)	87.71
T8	Inoculated control	42.75^{h} (40.82)	-

*ST- seed treatment *FA- foliar application.

***Values represent the mean of three replicates.**

Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

(125.19 cm, 318.99 g), whereas T1 and T2 exhibited lower but significant effects. The chemical check treatment T7, provided moderate disease control, while the T8 recorded the lowest growth and yield (Table 13). These results highlight the superior efficacy of biocontrol consortia over individual applications in managing bhendi powdery mildew. These findings align with the study, which demonstrated that all treatments significantly enhanced okra growth, nutrient content and yield while also increasing microbial biomass in the soil (45). This improvement is attributed to their ability to solubilize mineral nutrients for crop uptake and release phytohormones that promote plant growth. *Bacillus* species, in particular, produce various compounds that enhance plant growth and contribute to disease suppression. Additionally, they regulate phytohormones and activate defense -related genes under biotic stress conditions (46).

Disease incidence at field level

Field evaluation of talc-based biocontrol agents showed that individual treatments T1, T2 and T3 resulted in disease reductions of 64.77 %, 61.74 % and 57.44 %, respectively (Fig. 16-18). However, their combinations were more effective. Dual treatments, such as treatment T4 and T5, provided 68.40 % and 66.33 % disease control. The combination of all three agents' treatment T6, along with seed treatment of *B. subtilis*, achieved the highest disease control at 71.69 %, followed by chemical control T7, which showed 78.30 % disease reduction. The untreated control (T8) exhibited the highest disease incidence at 43.52 %. These results emphasize the superior efficacy of combined biocontrol agents over individual treatments in managing bhendi powdery mildew under field conditions.

Table 13. Growth and yield parameters of bhendi: assessing the efficacy of combined talc-based biocontrol formulations under greenhouse conditions

S. no.	Treatment	Plant height (cm)	Fruit length (cm)	Girth of the fruit (cm)	Yield/ plant (gm)	Shoot length (cm)	Root length (cm)	Plant biomass (g)
T1	FA with *A. quisqualis* at 0.5 %	109.13^{ef}	15.35^{d}	4.25^{ef}	261.77^{ef}	85.5^{ef}	23.6^{cd}	56.43^{e}
T2	FA with *T. harzianum* at 0.5 %	105.21^{f}	14.98^{de}	4.15^{fg}	258.36^{f}	82.3^{f}	22.9^{d}	54.76^{ef}
T3	ST with *B. subtilis* at 10 g/kg + FA at 0.5 %	125.19^{c}	18.52^{b}	4.91^{c}	318.99^{c}	95.8^{c}	29.4^{b}	62.56^{c}
T4	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %	110.04^{e}	16.02^{cd}	4.42^{e}	272.22^{e}	92.1^{d}	27.8^{bc}	59.55^{d}
T5	FA with *A. quisqualis* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	130.87^{b}	19.06^{ab}	5.54^{b}	338.34^{b}	101.2^{b}	29.7^{ab}	65.98^{b}
T6	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %+ ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	138.99^{a}	19.72^{a}	5.79^{a}	364.72^{a}	108.3^{a}	30.7^{a}	68.58^{a}
T7	FA of hexaconazole at 0.1 %	119.95^{d}	16.66^{c}	4.60^{d}	294.43^{d}	86.4^{e}	24.6^{c}	60.53^{cd}
T8	Inoculated control	96.88^{h}	14.36^{f}	3.80^{h}	221.03^{g}	74.3^{g}	22.5^{e}	48.52^{f}
T9	Uninoculated control (healthy)	99.90^{g}	14.61^{e}	4.03^{g}	243.40^{f}	76.5^{fg}	23.4^{de}	46.20^{g}

*ST- seed treatment *FA- foliar application.

*Values represent the mean of three replicates.

Means followed by the same letter are not significantly different at the 5% level as determined by DMRT.

Fig. 16. Overview of experiment (under field condition).

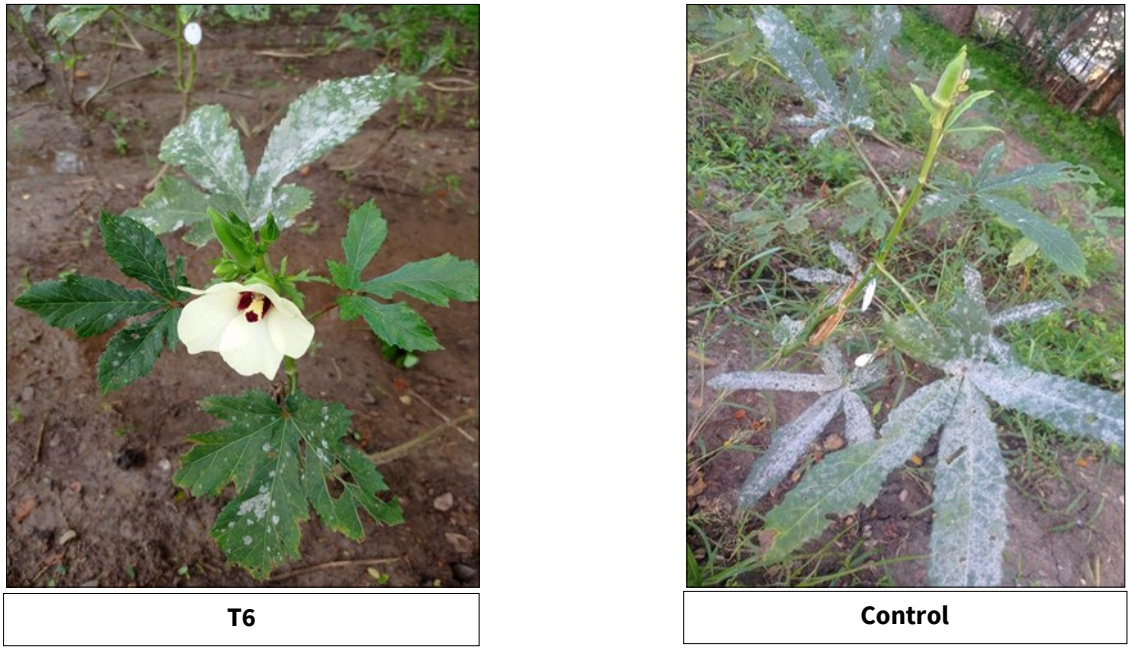

Fig. 17. Powdery mildew disease incidence (under field condition).

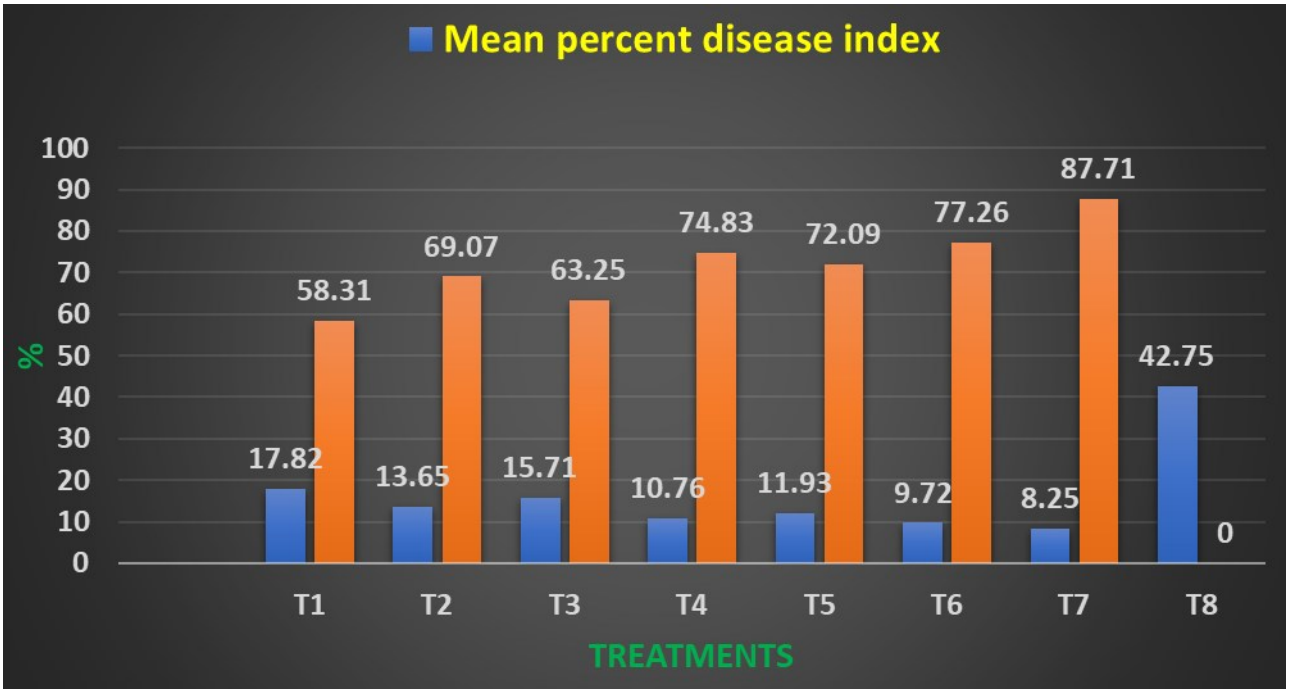

Fig. 18. Assessing the efficacy of combined talc-based biocontrol formulations for managing powdery mildew in greenhouse conditions.

Table 14. Assessing the efficacy of combined talc-based biocontrol formulations for managing powdery mildew in field conditions

S. no.	Treatments	Disease index (%)	Disease reduction over control (%)
T1	FA with *A. quisqualis* at 0.5 %	15.33^{d} (23.13)	64.77
T2	FA with *T. harzianum* at 0.5 %	16.65^{c} (24.08)	61.74
T3	ST with *B. subtilis* at 10 g/kg + FA at 0.5 %	18.52^{b} (25.48)	57.44
T4	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %	13.75^{e} (21.76)	68.40
T5	FA with *A. quisqualis* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	14.65^{de} (22.50)	66.33
T6	FA with *A. quisqualis* at 0.5 %+ FA with *T. harzianum* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5%	12.32^{f} (20.54)	71.69
T7	FA of hexaconazole at 0.1 %	9.44^{g} (17.88)	78.30
T8	Control (untreated)	43.52^{a} (41.27)	-

*ST - seed treatment, **FS**- foliar spray.

*Values represent the mean of three replicates.

Means followed by the same letter are not significantly different at the 5% level as determined by DMRT.

This highlights the enhanced efficacy of combining biocontrol agents compared to chemical treatments. AQ 10 is formulated as water-dispersible granules that act on overwintering cleistothecia of powdery mildew (11). Similarly, AQ10 effectively halted new chasmothecia production and reduced mature ascocarp numbers in potted grapevine plants, owing to their mycoparasitic activity, ability to induce systemic resistance in plants and competition for nutrients and space (47, 48). *Trichoderma* spp. exhibit antibiosis against powdery mildew by producing antifungal compounds that diffuse into the substrate, leading to the complete plasmolysis of mildew cells within 24-48 hours (12). *Trichoderma* employs several mechanisms to attack powdery mildew, including mycoparasitism, space competition, induction of plant resistance and antibiosis. In the case of antibiosis, *Trichoderma* spp. produces antifungal compounds that diffuse into the substrate, inhibiting the growth of other species. This process can result in the complete plasmolysis of powdery mildew cells within 24 to 48 hours (49).

Growth and yield promotion at field level

Field evaluation of talc-based biocontrol agents demonstrated that the combination of *A. quisqualis* at 0.5 %, *T. harzianum* at 0.5 % and *B. subtilis* at 0.5 %, with seed treatment of *B. subtilis* at 10 g/kg, was the most effective, achieving a 64.74 % yield increase (13.46 t/ha) (Fig. 19, 20; Table 15). This was closely followed by hexaconazole at 0.1 % (13.95 t/ha, 70.74 % increase). Individual treatments showed moderate improvements: *A. quisqualis* (T1) achieved a 33.04 % yield increase, *T. harzianum* (T2) 23.13 % and *B. subtilis* + seed treatment (T3) 13.21 %. Dual treatments like *A. quisqualis* + *T. harzianum* (T4) and *A. quisqualis* + *B. subtilis* + seed treatment (T5) improved growth and yield by 52.99 % and 43.94 %, respectively. The untreated control (T8) had the lowest growth and yield. These results emphasize the effectiveness of combined biocontrol agents in managing bhendi growth and yield. The treatment containing the PGPR consortium with peat led to the maximum improvements (50). From these results, it is clear that the application of combined talc-based biocontrol formulations of *A. quisqualis*, *T. harzianum* and *B. subtilis* effectively improves control measures for bhendi powdery mildew.

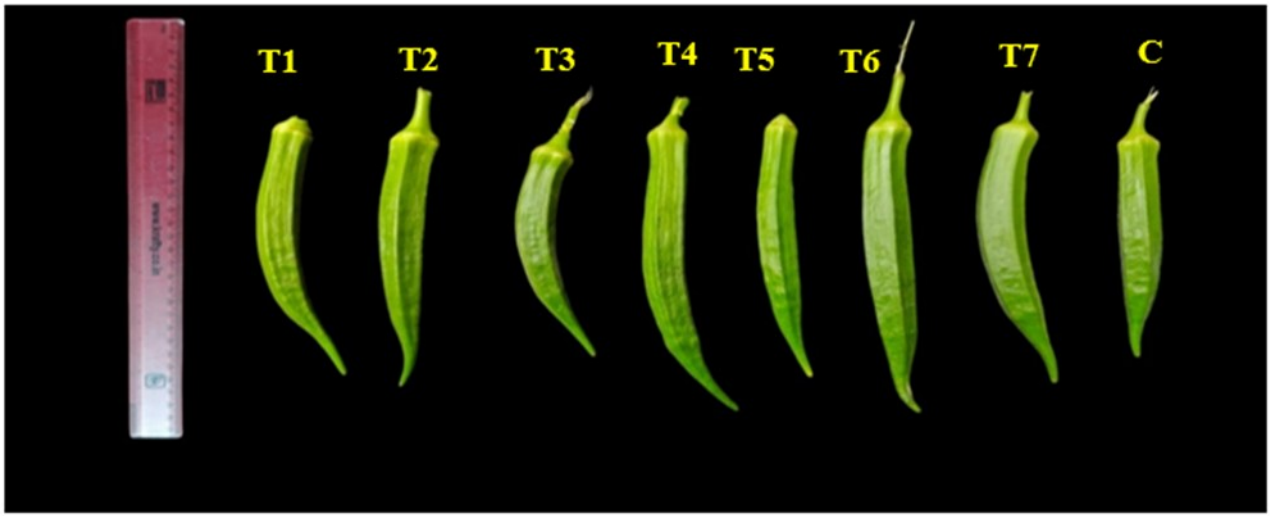

Fig. 19. Growth and yield parameters (field condition).

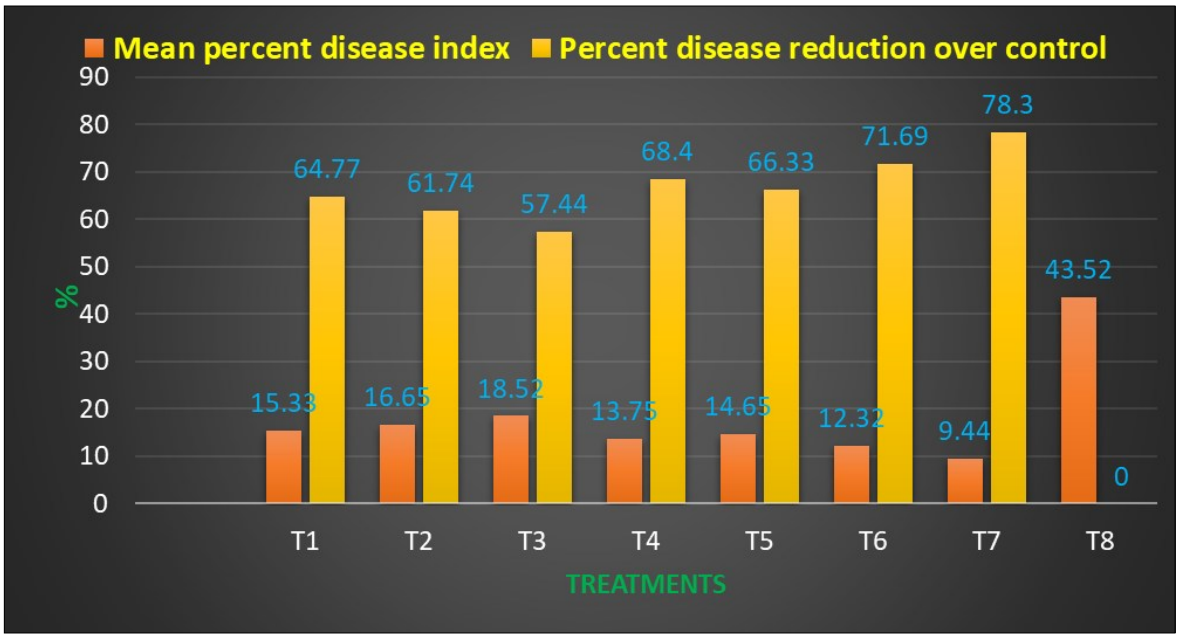

Fig. 20. Assessing the efficacy of combined talc-based biocontrol formulations for managing powdery mildew in field conditions.

Table 15. Growth and yield parameters of bhendi: evaluating the efficacy of combined talc-based biocontrol formulations under field conditions

Sl. No.	Treatment	Plant height (cm)	Fruit length (cm)	Girth of the fruit(cm)	Yield/ plant (gm)	Yield (t/ha)	Yield over control (%)
T1	FA with *A. quisqualis* at 0.5 %	110.04^{e}	16.02^{c}	4.60^{d}	272.22^{e}	10.87^{e}(19.25)	33.04
T2	FA with *T. harzianum* at 0.5 %	109.13^{ef}	14.61^{e}	4.25^{ef}	261.77^{f}	10.06^{f}(18.49)	23.13
T3	ST with *B. subtilis* at 10 g/kg + FA at 0.5 %	105.21^{f}	14.98^{de}	4.15^{fg}	258.36^{fg}	9.25^{g}(17.70)	13.21
T4	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 %	125.19^{c}	18.52^{b}	5.54	338.34^{b}	12.50^{c}(20.70)	520.99
T5	FA with *A. quisqualis* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5%	119.95^{d}	16.66^{c}	4.91^{c}	294.43^{d}	11.76^{d}(20.05)	43.94
T6	FA with *A. quisqualis* at 0.5 % + FA with *T. harzianum* at 0.5 % + ST with *B. subtilis* at 10 g/kg and FA at 0.5 %	138.99^{a}	19.72^{a}	5.79^{a}	364.72^{a}	13.46^{b}(21.52)	64.74
T7	FA of hexaconazole at 0.1 %	130.87^{b}	19.06^{ab}	4.42^{e}	318.99^{c}	13.95^{a}(21.93)	70.74
T8	Control (untreated)	99.90^{g}	15.35^{d}	4.03^{g}	221.03^{g}	8.17^{h}(16.60)	-

*Values represent the mean of three replicates.

Means followed by the same letter are not significantly different at the 5% level as determined by DMRT.

Conclusion

This study presents a sustainable and eco-friendly approach to managing powdery mildew in bhendi, providing farmers with an effective alternative to synthetic fungicides. Through the integration of biocontrol agents such as *A. quisqualis*, *T. harzianum* and *B. subtilis*, significant disease control was achieved, alongside enhanced plant health and productivity. The synergistic interactions among these agents were particularly noteworthy, leading to improved disease management and yield outcomes.

Authors' contributions

All authors read and approved the final manuscript. Authors declare that this research article is an original work and has not been previously published or submitted for publication. I confirm that all data presented in the article are accurate and have been obtained through ethical and legal means. Any data or materials obtained from other sources have been acknowledged and cited appropriately.

Compliance with ethical standards

Conflict of interest: Authors declare that they have no financial or non-financial interests that could potentially be perceived as a conflict of interest related to the research.

Ethical issues: None

References

1. Abdel-Kader MM, El-Mougy NS, Aly MDE, Lashin SM. Integration of biological and fungicidal alternatives for controlling foliar diseases of vegetables under greenhouse conditions. Int J Agric & Forest. 2012;2(2):38–48.10.5923/j.ijaf.20120202.07
2. Abdul-Baki AA anderson JD. Vigour determination in soybean seed by multiple criteria.CropSci.1973;13(6):63033.
3. Ashwini R, Amaresh YS, Yenjerappa ST, Kulkarni S, Aswathanarayana DS. Studies on symptomatology, morphological and molecular characterization of *Erysiphe cichoracearum* causing powdery mildew of okra. Int J Environ Clim Change. 2023;13 (10):2921-28.10.9734/ijecc/2023/v13i102958

4. Asis A, Shahriar SA, Naher L, Saallah S, Fatihah HNN, Kumar V, et al. Identification patterns of *Trichoderma* strains using morphological characteristics, phylogenetic analyses and lignocellulolytic activities. Mol Biol Rep. 2021; 48:3285-3301. 10.1007/s11033-021-06321-0

5. Athira K, Ragupathi N, Raguchander T. Morphological characterization of *Ampelomyces* spp., a hyperparasite of Bhendi (*Abelmoschus esculentus* (L.) Moench) powdery mildew. J Appl Nat Sci. 2017;9(4):1954-57. 10.31018/jans.v9i4.1471

6. Ayaz M, Ali Q, Zhao W, Chi YK, Ali F, Rashid KA, et al. Exploring plant growth promoting traits and biocontrol potential of *Bacillus subtilis* BS-2301 strain in suppressing *Sclerotinia sclerotiorum* through various mechanisms. Front Plant Sci. 2024;15:1444328. https://doi.org/10.3389/fpls.2024.1444328

7. Ayyappan S, Sruthi K, Aravind US. Isolation and characterization of antagonistic *Bacillus* spp. against fungal phytopathogens from rhizosphere soil of different plants in southern India. J Microbiol Biotechnol Food Sci. 2023;13(1):30-42.

8. Bacha AA, Suhail M, Awwad FA, Ismail EA, Ahmad H. Role of dietary fiber and lifestyle modification in gut health and sleep quality. Front Nutr. 2024; 11:1324793. 10.3389/fnut.2024.1324793

9. Bachihal S. Investigations on powdery mildew of okra caused by *Erysiphe cichoracearum* DC. (Master's thesis, University of Agricultural Sciences, Raichur); 2012.

10. Banupriya M, Ushamalini C, Nakkeeran S, Raguchander T. Morphological characterization of *Ampelomyces* spp., a hyperparasite of grapevine [*Vitis vinifera* (L.)] powdery mildew. Int J Curr Microbiol App Sci. 2019;8(06):1725-31. https://doi.org/10.20546/ijcmas.2019.806.206

11. Bélanger RR, Labbé C. Control of powdery mildews without chemicals. In: Bélanger RR, Bushnell WR, Dik AJ, TLW C, editors. The powdery mildews: a comprehensive treatise. Am Phytopathol Soc; 2002. p. 256–67. https://www.cabidigitallibrary.org/doi/full/10.5555/20023170360

12. Benuzzi M, Baldoni G. AQ10- a New Bio fungicide Based on *Ampelomyces quisqualis* for Powdery Mildew Control on Grapes. Intrachem Bio Italia s.r.L., Cesena,Italy; 2000. https://www.cabidigitallibrary.org/doi/full/10.5555/20001007582

13. Braun U. A monograph of the Erysiphales (powdery mildews). Nova Hedwigia. 1987; 89:1–700. https://www.cabidigitallibrary.org/doi/full/10.5555/19871337454

14. Braun U, Cook RTA. Taxonomic Manual of the Erysiphales (Powdery Mildews). ResearchGate; 2012. https://cir.nii.ac.jp/crid/1573668924100070656

15. Chowdhury SP, Hartmann A, Gao X, Borriss R. Biocontrol mechanism by *Bacillus amyloliquefaciens* FZB42: a review. Front Microbiol. 2015; 6:780. https://doi.org/10.3389/fmicb.2015.00780

16. Department of Agriculture, Government of Tamil Nadu. Annual Report 2021-22; 2022. https://agriwelfare.gov.in/Documents/annual-report-2021-22.pdf

17. Elad Y, Chet I, Henis Y. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica. 1981;9(1):59–67. https://link.springer.com/article/10.1007/BF03158330

18. Elad Y, Kirshner B, Yehuda N, Sztejnberg A. Management of powdery mildew and gray mold of cucumber by *Trichoderma harzianum* T39. Biocontrol. 1998; 43:241-51. https://link.springer.com/article/10.1023/A:1009919417481

19. Fondevilla S, Rubiales D. Powdery mildew control in pea: a review. Agron Sustain Dev. 2012; 32:401-9. https://doi.org/10.1007/s13593-011-0033-1

20. Food and Agriculture Organization. Global production statistics for Bhendi; 2022. https://openknowledge.fao.org/server/api/core/bitstreams/0c372c04-8b29-4093-bba6-8674b1d237c7/content

21. García-Gutiérrez L, Zeriouh H, Romero D, Cubero J, Vicente A, Pérez-García A. The antagonistic strain *Bacillus subtilis* UMAF6639 also confers protection to melon plants against cucurbit powdery mildew by activation of jasmonate- and salicylic acid-dependent defence responses. Microb Biotechnol. 2013;6(3):264–74. https://doi.org/10.1111/1751-7915.12028

22. Gireesha D, Patil PV, Vishwas Gowda GR, Vijaykumar KN, Doggalli G. Morphological and biochemical characterization of *Bacillus subtilis* isolated from rhizosphere of sugarbeet. Biochem Cell Arch. 2024; 24:1077-82. https://doi.org/10.51470/bca.2024.24.1.1077

23. Goh TK. Single spore isolation using a hand-made glass needle. Fungal Divers. 1999; 2:47-63.

24. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. John Wiley & Sons, New York; 1984.

25. Grace S, Salman M, Prabha D, Chauhan JS, Gupta H. To study Effect of biocontrol agents on the growth of okra (*Abelmoschus esculentus* (L.) Moench). Int J Chem Stud. 2019; SP6:880-5.

26. Zeledón-Castro I, Blandón Díaz JU, Romero SD, Castillo-Arévalo T. Isolation and characterization of *Trichoderma* spp., for their potential use in peanut (*Arachis hypogaea*) crops. Sch J Agric Vet Sci. 2023;10(6):57-66.

27. ISTA (International Seed Testing Association). International rules for seed testing. Seed Sci Technol. 1993;21(Suppl):1–75. https://www.seedtest.org/en/publications/international-rules-seed-testing.html

28. Jadav AH, Kadvani DL. Efficacy of different phytoextracts against *Erysiphe cichoracearum* DC causing powdery mildew of okra. J Pharmacogn Phytochem. 2019;8(2):538–40.

29. Jena RK, Raja IY, Ramamoorthy V, Narayanan SL, Renuka R, Subbiah A, et al. Exploring eco-sensitive strategies for effective powdery mildew management in grapevines. J Environ Sci. 2023;57(4):301–10. DOI: 10.18311/jbc/2023/34206

30. Kanipriya R, Rajendran L, Raguchander T, Karthikeyan *G. Characterization* of *Ampelomyces* and its potentiality as an effective biocontrol agent against *Erysiphe cichoracearum* DC causing powdery mildew disease in bhendi (*Abelmoschus esculentus* (L.) Moench). Madras Agric J. 2019;106(4):325–30. doi:10.29321/MAJ 2019.000258

31. Keerthana S, Sendhilvel V, Raguchander T, Varanavasiappan S, Swarnapriya R. Diversity of powdery mildew mycoparasite *A. quisqualis* under natural ecosystem and its molecular characterization. Int J Plant Soil Sci. 2022;34(9):48-59. DOI: 10.9734/IJPSS/2022/v34i930913

32. Kokare NB, Saha S. Bioefficacy studies of *Trichoderma asperelloides* and *Ampelomyces quisqualis* in combination with sulphur for the management of powdery mildew of grapes. Grape Insight. 2023;144–51. https://doi.org/10.59904/gi.v2.i1.2024.25

33. Kusch S, Qian J, Loos A, Kümmel F, Spanu PD, Panstruga R. Long-term and rapid evolution in powdery mildew fungi. Mol Ecol. 2024;33(10). https://doi.org/10.1111/mec.16909

34. Legler SE, Caffi T, Benuzzi M, Ladurner E, Rossi V. New perspectives for the use of *Ampelomyces*-based biofungicides for effective control of powdery mildew on grapevine. In: Proceedings of the Fourth International Conference on Non-Chemical Crop Protection Methods, Lille, France; 2011. p. 546–51. https://www.cabidigitallibrary.org/doi/full/10.5555/20113378898

35. López CG, Ramírez HA, Castellanos LN. Control of Pepper Powdery mildew using Antagonistic Microorganisms. In: Biol Control. 2020. p. 385–420. https://doi.org/10.1007/978-3-030-51034-3_15

36. Manjunatha L, Singh S, Ravikumara BM, Reddy GN, Senthilkumar M. *Ampelomyces*. In: Beneficial Microbes in Agro-Ecology. Elsevier Inc; 2020. Chapter 44. https://doi.org/10.1016/B978-0-12-823414-3.00044-7

37. Menge D. Biological control of cashew powdery mildew using *A. quisqualis* Ces. J Biol Control. 2016;30. http://hdl.handle.net/123456789/4800

38. Ministry of Agriculture & Farmers Welfare. Horticultural statistics at a glance 2023; 2023.

39. Perveen R, Hussain A, Ditta A, Dar A, Aimen A, Ahmad M, et al. Growth and yield of okra exposed to a consortium of rhizobacteria with different organic carriers under controlled and natural field conditions. Horticulturae. 2022;9(1):8. https://doi.org/10.3390/horticulturae9010008

40. Piggot PJ, Hilbert DW. Sporulation of Bacillus subtilis. Curr Opin Microbiol. 2004;7(6):579–86. https://doi.org/10.1016/j.mib.2004.10.001

41. Ramakrishnan G, Jeyarajan R, Dinakaran D. Talc-based formulation of *Trichoderma viride* for biocontrol of *Macrophomina phaseolina*. J Biol Control. 1994;8(1):41–4.

42. Ravinder Reddy M. Evaluation of different methods of disease assessment and effect of some cultural practices on powdery mildew of mungbean. MSc (Ag.) Thesis andhra Pradesh Agricultural University, Hyderabad, India; 1982.

43. Sendhilvel V, Marimuthu T, Samiappan R. Talc-based formulation of *Pseudomonas fluorescens*-induced defense genes against powdery mildew of grapevine. Arch Phytopathol Plant Protect. 2007;40(2):81–9. https://doi.org/10.1080/03235400500321677

44. Sundaramoorthy S, Balabaskar P. Consortial effect of endophytic and plant growth promoting rhizobacteria for the management of early blight of tomato incited by *Alternaria solani*. J Plant Pathol Microbiol. 2012;3(7).

45. Sundheim L, Krekling T. Host-parasite relationships of the hyperparasite *Ampelomyces quisqualis* and its powdery mildew host *Sphaerotheca fuliginea*. I. Scanning electron microscopy. Phytopathol Z. 1982;104:202–10. https://www.cabidigitallibrary.org/doi/full/10.5555/19821387273

46. Thompson DC, Clarke BB, Kobayashi DY. Evaluation of bacterial antagonists for reduction of summer patch symptoms in Kentucky bluegrass. Plant Dis. 1996;80:856–62. https://www.cabidigitallibrary.org/doi/full/10.5555/19971000546

47. Mahalakshmi S, Vijayapriya M, Pandeeswari N. Studies on developing PGPR consortium with improved shelf life. J Pharmacogn Phytochem. 2019;8(2S):545-8.

48. Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J. *Trichoderma*: the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int J Mol Sci. 2022;23(4):2329. https://doi.org/10.3390/ijms23042329

49. Vimala R. Molecular and biochemical markers for characterization of *Erysiphe pisi* resistance in pea (*Pisum sativum* L.) [dissertation]. New Delhi: Indian Agricultural Research Institute; 2005.

50. Fuga CA, Lopes EA, Vieira BS, Cunha WV. Efficiency and compatibility of *Trichoderma* spp. and *Bacillus* spp. isolates on the inhibition of *Sclerotium cepivorum*. 2016; p.526-31.

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