



REVIEW ARTICLE

# Synergistic use of biochar, *Trichoderma harzianum* and *Pseudomonas fluorescens* to combat biotic stress by *Phomopsis vexans* in brinjal

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## Abstract

The present study focuses on induced systemic resistance (ISR), where secondary metabolites and pathogenesis-related (PR) proteins are activated after the application of resistance inducers following the salicylic acid pathway. Biochar was used as an inducer to activate phenolic compounds and pathogenesis-related (PR) proteins, helping to combat *Phomopsis vexans*, which causes Phomopsis blight in brinjal. Physical and biochemical analysis showed biochar has significantly improved the yield of traits of the plant and reduced the amount of infection by increasing levels of total proteins, total phenols and sugar content. Increased levels of proteins and phenolic compounds with decreased levels of disease severity as shown by regression analysis, indicating a negative correlation (*r*) between soil pH, soil electrical conductivity (EC), plant height, number of branches, fruit yield, peroxidase activity, total protein content, total phenols and disease severity. The treatment T<sub>3</sub> with 3 % biochar, *Trichoderma harzianum* and *Pseudomonas fluorescens* was found to be the best treatment that exhibited positive effects on yield attributing traits and managed disease severity (22.2 %), followed by biochar concentrations of 3.5 % (T<sub>6</sub>) and 3 % (T<sub>5</sub>) alone with disease severities of 24.42 % and 26.6 %, respectively. Mixed application of biochar, *T. harzianum* and *P. fluorescens* has shown a synergistic effect on yield attributing traits and contributed to improving final yield and reducing disease severity. The data indicated a negative correlation between disease severity and yield attributing parameters, underscoring the effectiveness of this integrated approach in promoting plant resilience and productivity.

**Keywords:** biochar; biocontrol agents; brinjal; induced systemic resistance; phomopsis blight; synergism

**Abbreviations:** BC: Biochar; CRD: Completely randomised design; EC: Electric conductivity; JA: Jasmonic acid; PDI: % disease index; PGPB: Plant growth-promoting bacteria; PGPF: Plant growth-promoting fungi; PGPR: Plant growth-promoting rhizobacteria; SA: Salicylic acid; TPC: Total protein content; TP: Total phenol.

## Introduction

*Solanum melongena* is the most widely grown vegetable throughout the year in an array of agroclimatic conditions, especially in tropical and subtropical regions (1-3). Even though brinjal is rich in phenolic flavonoid phytochemicals known as anthocyanins, brinjal is prone to various biotic and abiotic stresses at various stages of growth and development (4). Phomopsis blight, also referred as fruit rot, tip over, fruit rot, stem blight or canker and damping off, impacts fruit germination, seedling mortality, plant death, fruit rotting and production at last, leading to yield losses ranging from 30 to 50 % because of fruit rot and flower drop, make this disease devastating (5-7).

Little circular spots develop on diseased leaves that have a light-colored centre, changing from grey to brown as the disease gets older. Lesions are also formed on the petiole and stem, resulting in blighted portions and the blighted area grows larger over time to form concentric circles. Critical symptoms are observed on the fruits

as water-soaked rings with brown margins and gray centers that grow decay fruits to become mummified if not dropped, A hot, humid climate with a temperature ranging 27-35 °C is necessary for spore germination, pycnidia development and fruit rotting; a temperature greater than 40 °C doesn't promote the growth of the disease (8, 9). *Phomopsis vexans* produces two conidia types, alpha and beta, of which alpha is known to cause infection. The alpha conidium is sub-cylindrical, measuring 5.5-9.05 mm long × 1.9-2.3 mm wide and the beta conidium is filiform, measuring 19.9-28.2 mm long × 0.95-1.32 mm wide (10).

Compared to many other methods of plant disease management, such as cultural, biological and chemical, integrated plant disease management poses less or no risk to the ecosystem (9-16). A plain or combined application of the above-mentioned approaches induces resistance into a plant system with a defined protocol, which is considered one of the best techniques for the management of various plant diseases (17). Secondary metabolites,

i.e., phenols and PR proteins, get activated in the respective plant to combat the metabolic phenomenon of the causal biotic agent. Secondary metabolite production is controlled and activated by inducers, which create signals to plant cells and hints for production when encountering the causal biotic agent. Inducers may include living organisms, organic compounds and inorganic compounds. Plant growth-promoting rhizobacteria (PGPR), Mycorrhizae, *Trichoderma* spp., fungicides, salicylic acid, biochar, inorganic salts, etc., are common inducers that have been used for the past few years and have imparted a satisfactory management of plant diseases by reducing disease infection and improving ecosystems of soil, water and humans (18-21).

**Materials and Methods**

The present study, conducted at the Department of Plant Pathology, Lovely Professional University, Phagwara (Punjab), India, during the kharif season of 2024, aimed to evaluate the impact of plant resistance inducers against *P. vexans*. The experiment was laid out in a completely randomised design (CRD) with 14 treatments in triplicate.

**Treatment details**

**Isolation, pure culture and identification of *P. vexans* through morphological characteristics**

Diseased specimens showing the conspicuous symptoms were supposed to be of Phomopsis blight and to confirm the causal organism, isolation was done using the tissue isolation method from the infected fruit samples (10). Diseased samples underwent careful microscopic examination, which confirms the presence of pycnidia in diseased lesions used for isolation. The diseased section was cut and sliced into smaller sections carrying healthy and infected portions, surface sterilised with sodium hypochlorite (NaClO) at 1 %, followed by triple rinsing with

distilled water (13). Blotted and dried sections were then transferred onto potato dextrose agar (PDA) poured Petri plates to incubate at 25 ± 2 °C for 10 days. Purification was done using the hyphal tip method (22). *Phomopsis vexans* produces hyaline, septate and branched mycelium measuring 2.9 to 3.7 µm in diameter. Alpha conidia are hyaline, one-celled, subcylindrical, bi-guttulate and 5.5-9.05 µm long × 1.9-2.3 µm wide in size (Fig. 1). Research has found a similar observation as the previous finding (Table 1) (10).

**Pot culture experiment**

Twenty-day-old seedlings were transplanted into earthen pots filled with a mixture of sandy loam soil added with various concentrations of biochar. On the 7th day after transplanting biocontrol agents (BCAs), *T. harzianum* and *P. fluorescens* were applied at the rate of 5 g/5 L with (1 × 10<sup>9</sup>CFU/g) of spores and bacterial cells, respectively.

**Preparation of biochar**

Biochar is a highly stable, carbon-rich residue formed by the pyrolysis method (Table 2). It is generally known as charred organic matter purposely applied to soils to improve soil physical, biological and chemical properties. The biochar was crushed into fine powder with the help of a pestle and mortar and kept in a tightly sealed container. The biochar chunks (>7 mm) were crushed into 4 mm pieces and again crushed into <2 mm size for perfect texture (Fig. 2). After crushing, the biochar was passed through a sieve (2 mm) to remove any large pieces of biochar.

**Table 2.** Properties of biochar

Parameters	Remarks
Feedstock material	Teak wood
Category of biochar	Hardwood Biochar
Biochar limiting potential	2.5 %
Biochar pH	8.52
Biochar EC	1.806 mhos/cm

**Table 1.** Treatment details

Treatment No.	Treatment Name	Concentration (% W/V)	Composition (% W/W) [Soil + BC]
T <sub>1</sub>	Sterilized Soil	(-)	5.5 kg + (-)
T <sub>2</sub>	Sterilised Soil + BC	3 %	5.5 kg + 165 g
T <sub>3</sub>	Biochar	2 %	5.5 kg + 110 g
T <sub>4</sub>	Biochar	2.5 %	5.5 kg + 137.5 g
T <sub>5</sub>	Biochar	3 %	5.5 kg + 165 g
T <sub>6</sub>	Biochar	3.5 %	5.5 kg + 192.5 g
T <sub>7</sub>	Biochar	4 %	5.5 kg + 220 g
T <sub>8</sub>	<i>Trichoderma harzianum</i>	5 g/5 L	5.5 kg + (-)
T <sub>9</sub>	<i>Pseudomonas fluorescens</i>	5 g/5 L	5.5 kg + (-)
T <sub>10</sub>	<i>T. harzianum</i> + <i>P. fluorescens</i>	5 g/5 L + 5 g/5 L	5.5 kg + (-)
T <sub>11</sub>	BC-(3 %) + <i>T. harzianum</i>	3 % + 5 g/5 L	5.5 kg + 165 g
T <sub>12</sub>	BC-(3 %) + <i>P. fluorescens</i>	3 % + 5 g/5 L	5.5 kg + 165 g
T <sub>13</sub>	BC-(3 %) <i>T. harzianum</i> + <i>P. fluorescens</i>	3 % + 5 g/5 L + 5 g/5 L	5.5 kg + 165 g
T <sub>14</sub>	Control	-	5.5 kg (Only soil)

\* (-) means no Biochar; BC- Biochar



**Fig. 1.** Morphological characteristics of *P. vexans*.

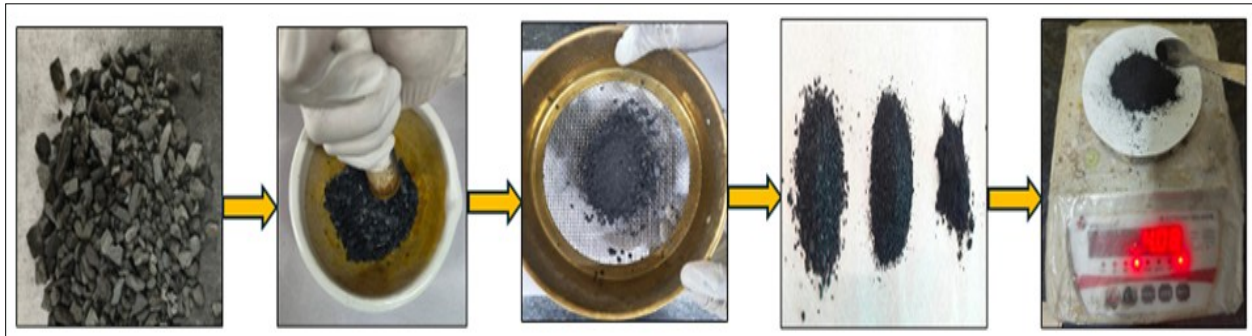


Fig. 2. Preparation of biochar.

#### Preparation and field inoculation of *P. vexans* for artificial infection

A conidial suspension with a concentration of  $1 \times 10^6$  conidia/mL was prepared as per the standard method and inoculated into 30-day-old plants of the 'Long Green' variety of brinjal (23). Subsequently, a series of serial dilutions was performed by transferring 1 mL of the initial suspension into a sterile tube containing 9 mL of sterile distilled water, resulting in a 1:10 dilution ( $1 \times 10^5$  conidia/mL). This process was repeated, transferring 1 mL from the first dilution into another sterile tube with 9 mL of thinner to create a second dilution ( $1 \times 10^4$  conidia/mL) and continued to create further dilutions ( $1 \times 10^3$ ,  $1 \times 10^2$ , etc.), ensuring thorough mixing after each transfer to achieve the desired range of conidial concentrations for experimental use. After inoculation, plants were covered with polythene bags for 48 h to provide adequate humidity for the initiation of disease.

#### Pathogenicity test

Kochs' postulates are followed to prove the pathogenicity of *P. vexans* (1882). Diseased specimens showing the conspicuous symptoms were supposed to be of Phomopsis blight and to confirm the causal organism, isolation was done using the tissue isolation method from the infected fruit samples (26). Once the pathogen was confirmed as *P. vexans* upon microscopic studies, again, inoculation of healthy seedlings was done to recreate the disease. Artificially inoculated plants could show the symptoms of Phomopsis blight and were again re-isolated, which confirmed the pathogenicity test using Kochs' postulates (Fig. 3).

#### Data recording for yield attributing traits and disease severity

Data recording of the shoot length of plants in cm, branches per plant in number, time to first flowering in days and fruits per plant in number was done using a measuring scale and manual reading by visualisation to distinguish the effects of different concentrations on plant growth patterns. Disease severity was recorded from the onset of infection through the cropping season at regular intervals. The severity of fruit rot disease was assessed by categorising all the infected samples into different grades of infection using the grading scale 0–5 (Table 3) (24).

#### Soil analysis on pH and EC

For the analysis of soil pH, soil samples were weighed at 5g for each treatment, air dried and added to Borosilicate conical flasks of 250 mL (Ishan Enterprises, Jalandhar). 25 mL of 0.01M  $\text{CaCl}_2$  (HiMedia) was added and was put on the shaker for 1 hour, then the pH was evaluated using a digital pH meter (Labtronics).

For the analysis of EC, soil samples were weighed and 20 g of each treatment was added to conical flasks (250 mL). After adding 200 ml of double-distilled water, shaking is done for 1 h. The samples were filtered with Whatman filter paper and kept at room temperature for 3 min and EC was evaluated using a digital EC meter (Labtronics).

Table 3. Rating % disease incidence

S.no	Grading	Description
1.	0 PDI	Free from the disease
2.	0.1–5.0 PDI	Poorly affected
3.	5.1–20.0 PDI	Moderately affected
4.	20.1–50.0 PDI	Severely affected
5.	>50.1 PDI	Very severely affected by the disease

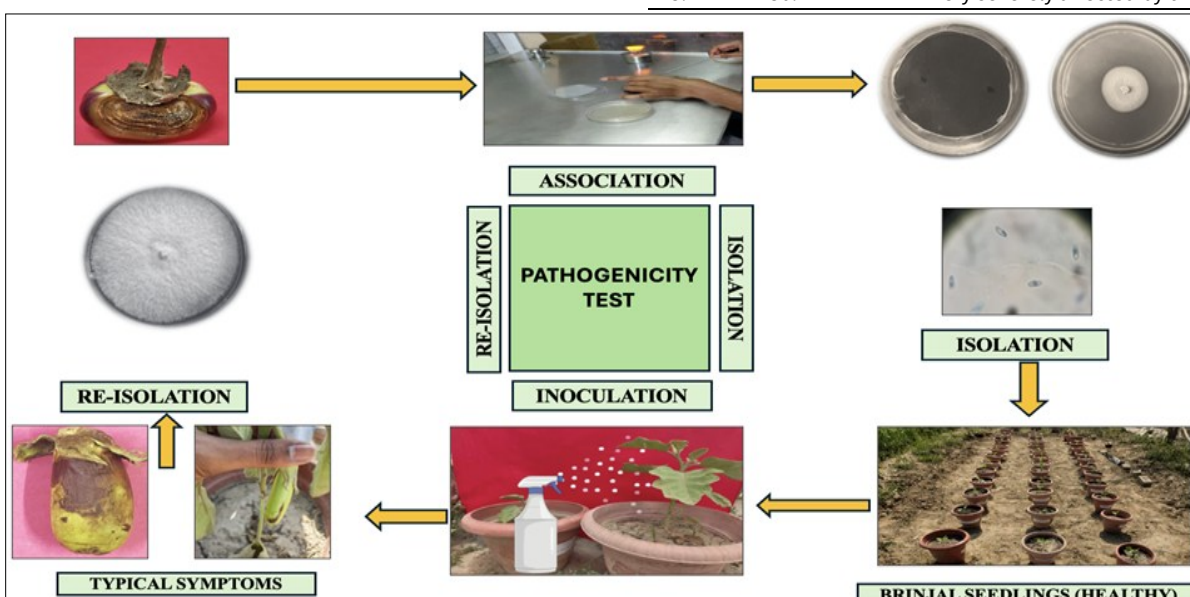


Fig. 3. Pathogenicity test to prove Kochs' postulates of *P. vexans* on brinjal.

**Biochemical analysis of plants for total soluble proteins and phenols**

The efficacy of treatments against the pathogen was assessed through various biochemical analyses involving estimation of total soluble proteins, total phenols, peroxidase activity estimation, total chlorophyll content and sugar content, which were analysed before and after pathogen inoculation (25–29).

**Statistical analysis**

The recorded data were subjected to statistical analysis at a 5 % probability level of significance ( $p < 0.05$ ) using Fishers’ LSD under CRD in OPSTAT (Beta version developed by Chaudhary Charan Singh Haryana Agriculture University, Haryana). To establish correlation among total soluble proteins, total phenols, peroxidase activity, reducing sugar, total chlorophyll content and disease severity, simple regression equations ( $Y = a + bx$ ) were developed.

**Results**

**% inhibition in radial growth of fungal mycelium**

The results of the radial growth were compared with the % inhibition of mycelium and it was found that 3 % of biochar added with *T. harzianum* ( $T_9$ ) inhibited the maximum growth of mycelium *in vitro*. It was recorded as inhibiting 98.71 % of mycelium growth, which is followed by *T. harzianum* + *P. fluorescens* ( $T_8$ ) and BC-3 % + *T. harzianum* + *P. fluorescens* ( $T_{13}$ ), inhibiting 90.38 % and 89.74 % of the mycelial growth, respectively. Furthermore, it was recorded that in the treatments BC-3 % ( $T_3$ ) and BC-3 % + *P. fluorescens* ( $T_{10}$ ) inhibited mycelial growth by 83.97 and 83.74 %, respectively and were the next best treatments. The straight applications of biocontrol agents *T. harzianum* ( $T_6$ ) and *P. fluorescens* ( $T_7$ ) and

biochar ( $T_1$ ,  $T_2$ ,  $T_4$  and  $T_5$ ) were recorded as less than that of their combined application with biochar and biochar alone at 3 % (Fig. 4-6).

**Impact of plant resistance inducers on soil pH and soil EC**

The results of *in vivo* studies presented in Table 4 demonstrated various treatments and their respective pH, out of which the treatments  $T_{13}$  (BC-3 % + *T. harzianum* + *P. fluorescens*),  $T_4$  (BC-2.5 %),  $T_1$  (Sterilized soil) and  $T_9$  (*P. fluorescens*) were found to be the best treatments out of all other treatments, respectively, with noted pH variations ranging from 7.79–7.91. Whereas, coming to the electrical conductivity (EC) of the soil, i.e. results from Table 3 demonstrate that the treatment  $T_7$  (BC-4 %) showed the highest EC value 0.75 ds/m, followed by the treatments  $T_6$ (BC-3.5 %) and  $T_5$  (BC-3 %) with 0.66 and 0.62 ds/m, respectively. Also, we noticed a negative correlation between PDI and soil EC ( $R = -0.383$ ), especially in the treatments  $T_6$  (BC-3.5 %),  $T_{11}$  (BC-3 % + *T. harzianum*),  $T_4$  (BC-2.5 %) and  $T_8$  (*T. harzianum*), where we found that the soil EC and PDI are indirectly proportional to each other, stating that PDI had decreased with increased soil EC in the respective treatments.

**Impact of plant resistance inducers on plant growth attributes with respect to PDI**

**Plant height**

The results of *in vivo* studies presented in Table 4 demonstrated that maximum shoot length was observed in the treatments  $T_6$  (BC-3.5 %) with 59.4 cm,  $T_{13}$  (BC-3 % + *T. harzianum* + *P. fluorescens*) with 58.83 cm,  $T_5$  (BC-3 %) with 58 cm and  $T_{11}$  (BC-3 % + *T. harzianum*) with 57.4 cm, respectively. A negative correlation between PDI and plant height ( $R = -0.453$ ), was observed in the treatments  $T_8$  (*T. harzianum*),  $T_4$  (BC-2.5 %) and  $T_{11}$  (BC-3 % + *T. harzianum*), where we

**Table 4.** Evaluation of growth parameters, soil pH and soil EC of brinjal plants under different biochar treatments

Treatments	Plant Height	No. of branches per plant	Days to first flowering	No of fruits per plant	Yield per treatment	Soil pH	Soil EC	PDI
Sterilised soil ( $T_1$ )	25.300 ± 1.55 <sup>h</sup>	3.667 ± 0.333 <sup>e</sup>	42.667 ± 1.453 <sup>ab</sup>	4.333 ± 0.333 <sup>e</sup>	98.000 <sup>j</sup>	7.787 ± 0.015 <sup>e</sup>	0.412 ± 0.006 <sup>h</sup>	42.200 ± 2.200 <sup>def</sup>
Sterilized soil + BC-(3 %) ( $T_2$ )	51.300 ± 0.608 <sup>e</sup>	4.000 ± 0.577 <sup>de</sup>	38.000 ± 0.577 <sup>e</sup>	6.000 ± 0.577 <sup>ef</sup>	128.000 <sup>hi</sup>	8.530 ± 0.017	0.526 ± 0.058 <sup>de</sup>	37.767 ± 2.233 <sup>fg</sup>
BC- 2 % ( $T_3$ )	48.433 ± 0.410 <sup>f</sup>	4.667 ± 0.333 <sup>cde</sup>	39.000 ± 0.577 <sup>de</sup>	7.000 ± 0.577 <sup>cde</sup>	134.667 <sup>gh</sup>	8.173 ± 0.018 <sup>abc</sup>	0.477 ± 0.003 <sup>fg</sup>	51.067 ± 2.233 <sup>bcd</sup>
BC- 2.5 % ( $T_4$ )	51.867 ± 0.273 <sup>de</sup>	4.667 ± 0.333 <sup>cde</sup>	40.333 ± 1.453 <sup>bcd</sup>	6.333 ± 0.333 <sup>def</sup>	141.333 <sup>g</sup>	7.897 ± 0.047 <sup>de</sup>	0.546 ± 0.008 <sup>cd</sup>	39.967 ± 10.202 <sup>ef</sup>
BC- 3 % ( $T_5$ )	58.033 ± 0.448 <sup>ab</sup>	6.333 ± 0.333 <sup>a</sup>	39.000 ± 1.528 <sup>de</sup>	7.333 ± 0.667 <sup>cd</sup>	200.000 <sup>e</sup>	8.317 ± 0.102 <sup>a</sup>	0.616 ± 0.003 <sup>b</sup>	26.600 ± 0.00 <sup>gh</sup>
BC- 3.5 % ( $T_6$ )	59.467 ± 0.467 <sup>a</sup>	6.667 ± 0.333 <sup>a</sup>	40.667 ± 0.882 <sup>bcd</sup>	9.333 ± 0.333 <sup>a</sup>	232.000 <sup>b</sup>	8.257 ± 0.042 <sup>ab</sup>	0.670 ± 0.009 <sup>a</sup>	24.420 ± 2.210 <sup>h</sup>
BC- 4 % ( $T_7$ )	53.567 ± 0.876 <sup>cd</sup>	5.000 ± 0.577 <sup>bcd</sup>	44.000 ± 0.577 <sup>a</sup>	6.333 ± 0.333 <sup>def</sup>	179.000 <sup>f</sup>	8.147 ± 0.024 <sup>bc</sup>	0.753 ± 0.002	53.300 ± 3.868 <sup>bcd</sup>
<i>T. harzianum</i> ( $T_8$ )	48.400 ± 0.608 <sup>f</sup>	4.333 ± 0.333 <sup>de</sup>	38.000 ± 0.577 <sup>e</sup>	6.000 ± 0.577 <sup>ef</sup>	202.667 <sup>de</sup>	8.223 ± 0.071 <sup>ab</sup>	0.540 ± 0.001 <sup>cd</sup>	46.643 ± 3.848 <sup>cdef</sup>
<i>P. fluorescens</i> ( $T_9$ )	48.167 ± 0.376 <sup>f</sup>	4.000 ± 0.577 <sup>de</sup>	38.000 ± 0.577 <sup>e</sup>	5.333 ± 0.333 <sup>fg</sup>	185.333 <sup>f</sup>	7.907 ± 0.058 <sup>de</sup>	0.492 ± 0.003 <sup>ef</sup>	62.200 ± 2.200 <sup>ab</sup>
<i>T. harzianum</i> + <i>P. fluorescens</i> ( $T_{10}$ )	54.200 ± 0.265 <sup>c</sup>	5.000 ± 0.577 <sup>bcd</sup>	40.000 ± 0.577 <sup>cde</sup>	6.667 ± 0.333 <sup>cde</sup>	217.333 <sup>c</sup>	8.193 ± 0.047 <sup>abc</sup>	0.549 ± 0.005 <sup>cd</sup>	53.300 ± 0.00 <sup>bcd</sup>
BC- 3 % + <i>T. harzianum</i> ( $T_{11}$ )	57.400 ± 0.635 <sup>b</sup>	6.000 ± 0.577 <sup>ab</sup>	38.000 ± 0.577 <sup>e</sup>	7.667 ± 0.333 <sup>bc</sup>	249.667 <sup>a</sup>	8.150 ± 0.015 <sup>bc</sup>	0.612 ± 0.003 <sup>b</sup>	37.743 ± 4.428 <sup>fg</sup>
BC- 3 % + <i>P. fluorescens</i> ( $T_{12}$ )	55.133 ± 1.167 <sup>c</sup>	5.667 ± 0.333 <sup>abc</sup>	42.000 ± 0.577 <sup>abc</sup>	5.333 ± 0.333 <sup>fg</sup>	210.333 <sup>cd</sup>	8.233 ± 0.018 <sup>ab</sup>	0.555 ± 0.004 <sup>cd</sup>	55.533 ± 5.871 <sup>bc</sup>
BC- 3 % + <i>P. fluorescens</i> + <i>T. harzianum</i> ( $T_{13}$ )	58.833 ± 0.696 <sup>ab</sup>	6.667 ± 0.333 <sup>a</sup>	40.000 ± 0.577 <sup>cde</sup>	8.667 ± 0.333 <sup>ab</sup>	266.333	7.800 ± 0.058 <sup>e</sup>	0.579 ± 0.006 <sup>bc</sup>	22.200 ± 2.200 <sup>h</sup>
Control ( $T_{14}$ )	37.333 ± 0.970 <sup>g</sup>	4.333 ± 0.333 <sup>de</sup>	47.000 ± 0.577	6.000 ± 0.577 <sup>ef</sup>	125.333 <sup>i</sup>	8.043 ± 0.106 <sup>cd</sup>	0.436 ± 0.003 <sup>gh</sup>	71.067 ± 2.233 <sup>a</sup>
CD at (5 %)	2.034	1.264	2.529	1.290	8.973	0.158	0.047	11.543

**Note:** The means with different Letters as superscripts are significant ( $P < 0.05$ ). The means with the same letters or having common letter(s) are not significantly different.

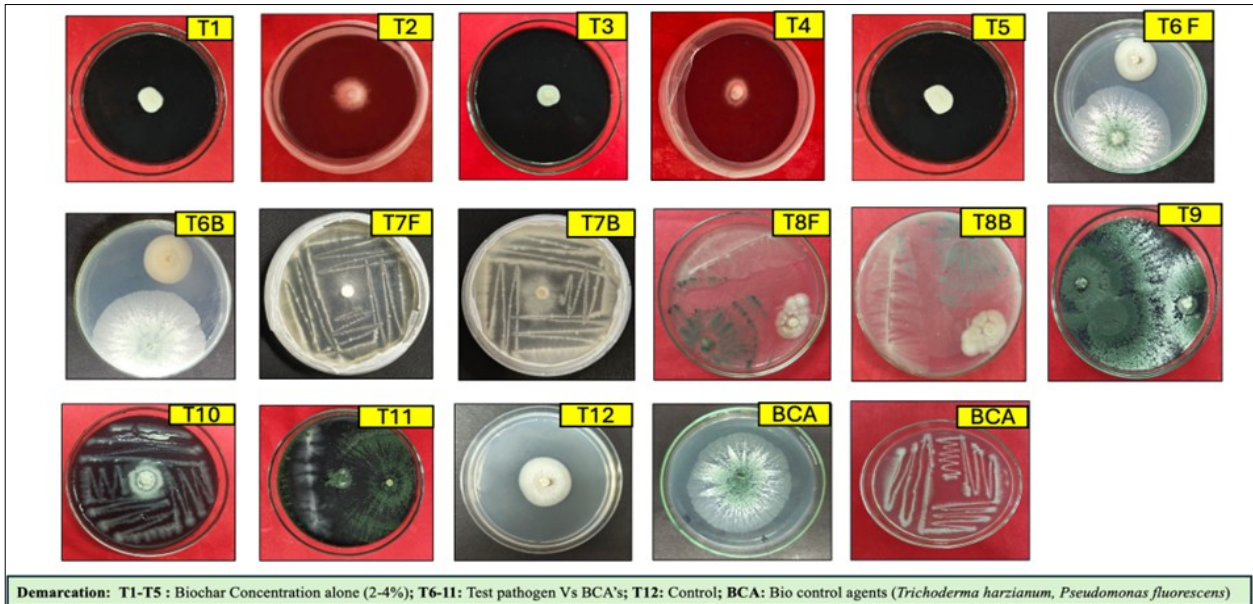


Fig. 4. Cultural characteristics of *P. vexans*.

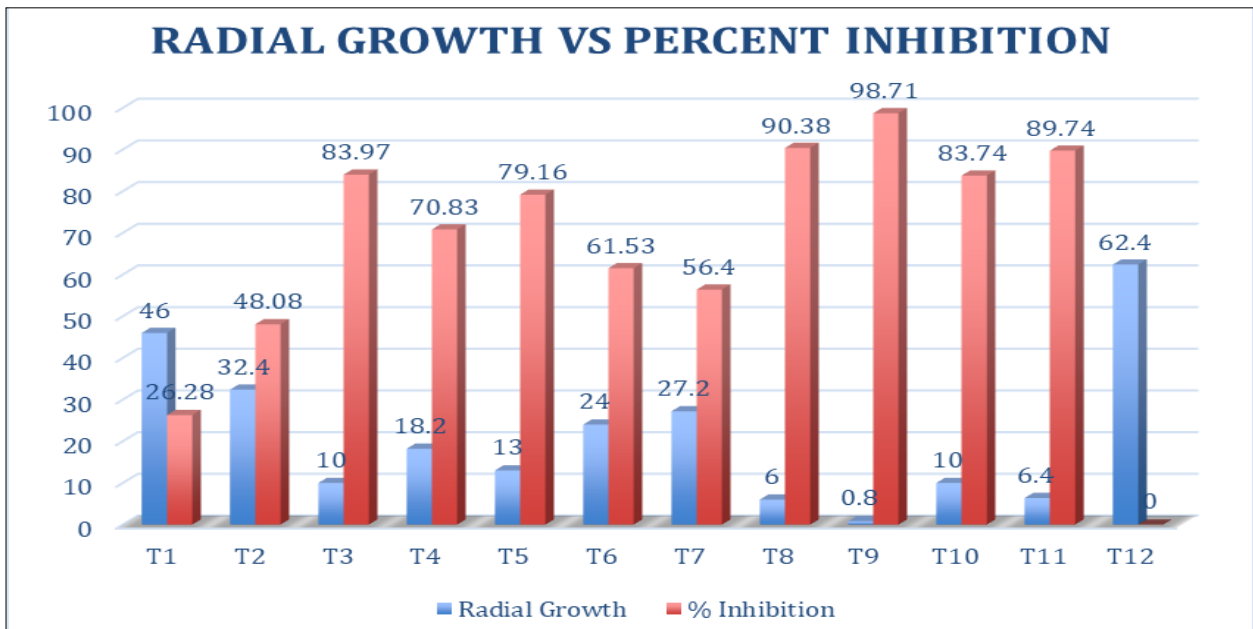


Fig. 5. Effect of different treatments on radial growth of mycelium.

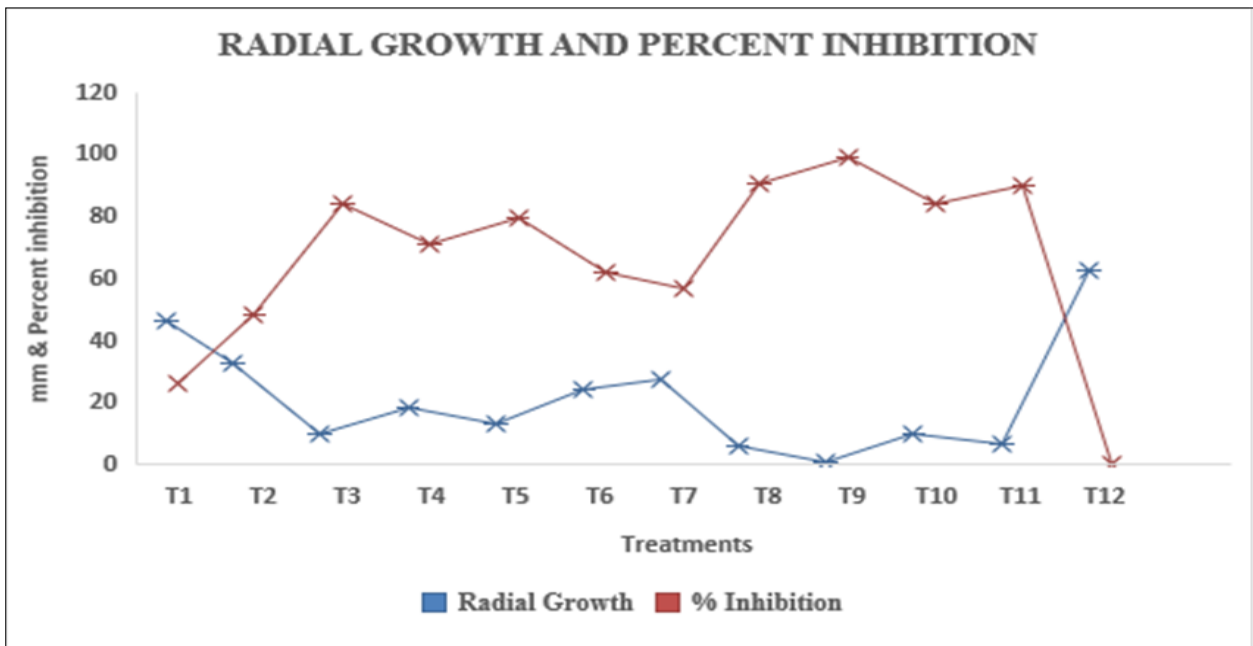


Fig. 6. *In vitro* efficacy of bioagents and biochar against *P. vexans*.

found that the plant height and PDI are indirectly proportional to each other, i.e., the treatment in which the PDI is lower showed the maximal shoot length. Research has demonstrated that several agronomic traits, such as plant height, fruit set and yield, were positively affected by the application of biochar as an organic supplement along with the recommended fertiliser dose (30).

#### Number of branches per plant

The results of *in vivo* studies presented in Table 4 demonstrated that the maximal number of branches is recorded in the treatments T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) with 6.66, T<sub>6</sub> (BC-3.5 %) with 6.66, T<sub>5</sub> (BC-3 %) with 6.33 and T<sub>11</sub> (BC-3 % + *T. harzianum*) with 6.0. A negative correlation between PDI and number of branches ( $R = -0.637$ ), especially in the treatments T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*), T<sub>5</sub> (BC-3 %) and T<sub>11</sub> (BC-3 % + *T. harzianum*), is to be found, stating that the number of branches per plant and PDI are indirectly proportional to each other, stating that the number of branches increased as compared with other treatments. *Trichoderma-Pseudomonas* have shown a synergistic effect on several plant attributes and contributed to increased yield alongside biotic stress management (21).

#### Days to first flowering

The results of *in vivo* studies presented in Table 4 demonstrated that treatments T<sub>11</sub> (BC-3 % + *T. harzianum*) with 38 days, T<sub>5</sub> (BC-3 %) with 39 days, T<sub>8</sub> (*T. harzianum*) with 38 days, T<sub>9</sub> (*P. fluorescens*) with 38 days and T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) with 40 days outperformed the other treatments along with T<sub>14</sub>, i.e., (the control) with 47 days.

#### Number of fruits per plant

The results of *in vivo* studies presented in Table 4 demonstrated that in these treatments, i.e., T<sub>6</sub> (BC-3.5 %), T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) and T<sub>11</sub> (BC-3 % + *T. harzianum*), the maximum number of fruits per plant was obtained. A negative correlation between PDI and yield per plant ( $R = -0.616$ ), especially in the treatments T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*), T<sub>11</sub> (BC-3 % + *T. harzianum*), T<sub>8</sub> (*T. harzianum*) and T<sub>9</sub> (*P. fluorescens*), where we found that the number of fruits per plant and PDI are indirectly proportional to each other, stating that the number of fruits increased as compared with other treatments where the PDI is low. Research has demonstrated that biochar application has influenced plant growth through the enhancement of certain morphological and chemical traits during vegetative and fruit stages (31).

#### Yield per treatment

The results of *in vivo* studies presented in Table 4 demonstrated that maximum yield was observed in the treatments T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) with 266.3 Kg, T<sub>11</sub> (BC-3 % + *T. harzianum*) with 249.6 kg, T<sub>6</sub> (BC-3.5 %) with 232 kg. A negative correlation between PDI and yield per plant ( $R = -0.421$ ), especially in the treatments T<sub>3</sub> (BC-2 %), T<sub>8</sub> (*T. harzianum*) and T<sub>11</sub> (BC-3 % + *T. harzianum*), where we found that the yield and PDI are indirectly proportional to each other, i.e., yield had drastically increased when the PDI had lowered.

### Impact of plant resistance inducers on biochemical parameters with respect to PDI

#### Total phenols

The results of *in vivo* studies presented in Fig. 7 depict the % increase over control in these treatments, i.e. T<sub>6</sub> (BC-3.5 %) with 40.44 %, T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) with 34.2 % and T<sub>11</sub> (BC-3 %

+ *T. harzianum*) with 33.77 %, which outperformed all the other respective treatments. However, there is a negative correlation between PDI and total phenol content ( $R = -0.864$ ). The results summarised that in the treatments T<sub>4</sub> (BC-2.5 %), T<sub>11</sub> (BC-3 % + *T. harzianum*) and T<sub>6</sub> (BC-3.5 %), the application of the above-mentioned treatments sensitised brinjal plants to increase elevated levels of total phenolic content and antioxidants, which resulted in induced resistance of brinjal plants against *Phomopsis vexans*. The results are in accordance with (1), where they have concluded that application of both fungal and bacterial biocontrol agents had significantly reduced the disease severity in the rice with respect to the increase in the total phenolic content in the rice leaves. Research has demonstrated a negative correlation between disease incidence and total phenolic content ( $r = -0.34$ ), peroxidase activity ( $r = -0.38$ ) and polyphenol oxidase activity ( $r = -0.32$ ) (32).

#### Total protein content

The results of *in vivo* studies presented in Fig. 8 depict the % increase over control in these treatments, i.e., T<sub>6</sub> (BC-3.5 %) with 43.94 %, T<sub>11</sub> (BC-3 % + *T. harzianum*) with 43.88 % and T<sub>13</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) with 43.56 % had outperformed the remaining treatments. A negative correlation between PDI and total protein content ( $R = -0.793$ ). The results are summarised as follows that in the treatments T<sub>14</sub> (Control), T<sub>9</sub> (*P. fluorescens*), T<sub>12</sub> (BC-3 % + *P. fluorescens*) and T<sub>8</sub> (*T. harzianum*), i.e., having a protein content range of 16.5 to 22.4, have shown a gradual decrease in the PDI, respectively. Thus, application of the above-mentioned treatments sensitised brinjal plants to increase elevated levels of total protein content and antioxidants, which resulted in induced resistance of brinjal plants against *Phomopsis vexans*. These findings were consistent with those of (33); they noticed elevated soluble protein levels in the treatments that were treated with different inducers and said it is indirectly associated with the defence response.

#### Peroxidase content

The results of *in vivo* studies presented in Table 5 and Fig. 9 demonstrate that these treatments, i.e., T<sub>5</sub> (BC-3 %) with 0.819, T<sub>4</sub> (BC-2.5 % + *T. harzianum*) with 0.432 and T<sub>10</sub> (BC-3 % + *T. harzianum* + *P. fluorescens*) with 0.352, had outperformed the remaining treatments. The results of *in vitro* studies presented in Table 3 demonstrate a negative correlation between PDI and peroxidase content ( $R = -0.363$ ). The results are summarised as follows that in the treatments T<sub>9</sub> (*P. fluorescens*), T<sub>7</sub> (BC-4 %), T<sub>8</sub> (*T. harzianum*) and T<sub>2</sub> (sterilised soil + BC-3 %), i.e., having a peroxidase content range of 0.24 to 0.32, there has been a gradual decrease in the PDI, respectively. Thus, application of the above-mentioned treatments sensitised brinjal plants to increase elevated levels of peroxidase content and antioxidants, which resulted in induced resistance of brinjal plants against *Phomopsis vexans*. Research has demonstrated that bion and salicylic acid (inducers) treated treatments that were pathogen inoculated had responded with higher peroxidase content and stated that an increase in the peroxidase activity is associated with systemic acquired resistance (34).

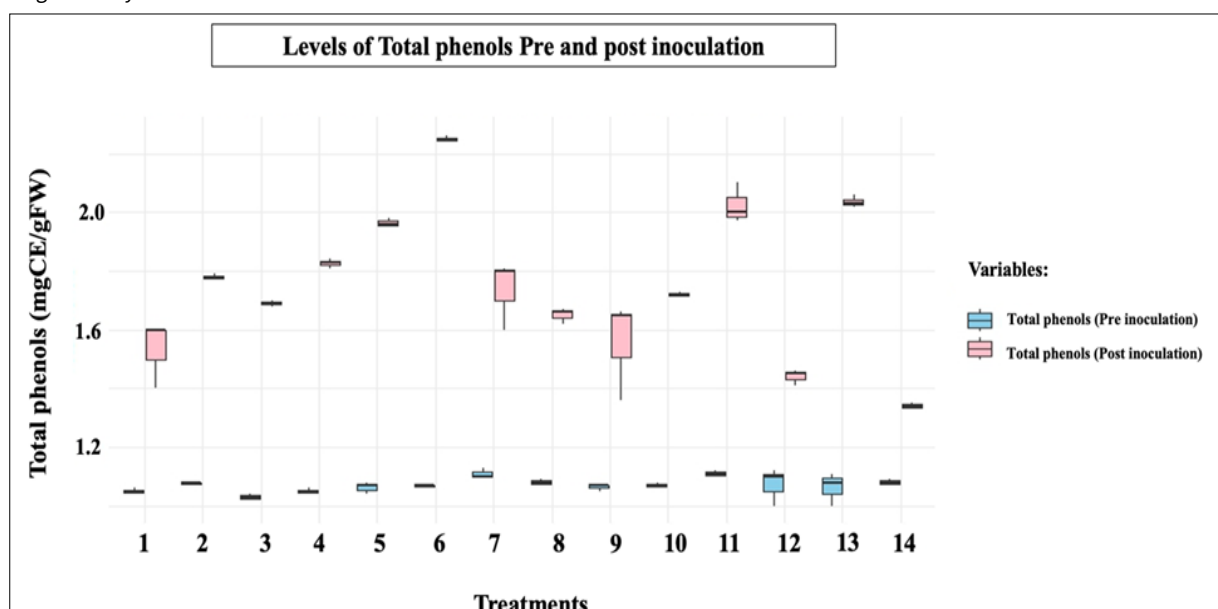
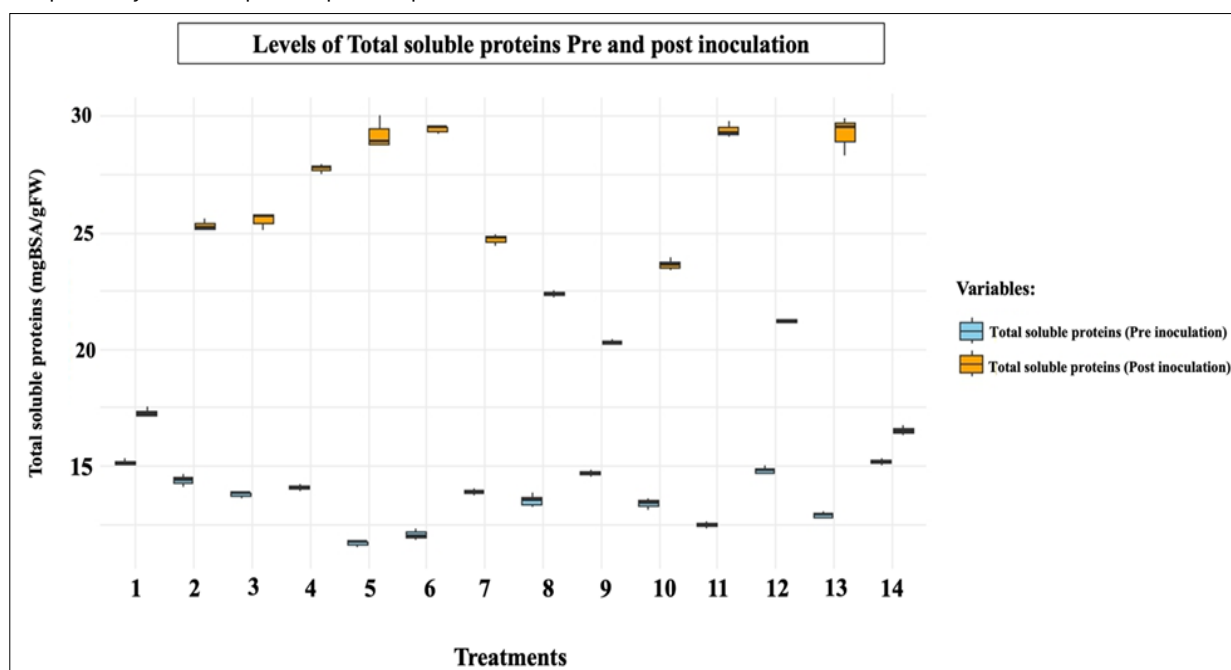
#### Total chlorophyll content

The results of *in vivo* studies presented in Table 5 and Fig. 10 demonstrated that these treatments, i.e., T<sub>7</sub> (BC-4 %), had shown the highest value in all three absorbances, i.e., 470 nm, 645 nm and 663 nm, with values of 2.990, 2.990 and 2.207, respectively. In the absorbance, we noticed that the treatments T<sub>3</sub> (BC-2 %) and T<sub>4</sub> (BC-2.5 %) showed values ranging near the treatment T<sub>7</sub> (BC-4 %), i.e., 2.934 and 2.827, whereas in the absorbances at 645 nm and 663 nm,

**Table 5.** Evaluation of biochemical parameters of brinjal plants under *P. vexans* stress at 30 DAT

Treatments	(TCC)-470	(TCC)-645	(TCC)-663	Total Phenols (TP) (30 Days)	Sugar content	Peroxidase detection	Total protein content (TPC) (30 Days)
Sterilised soil ( $T_1$ )	0.956 ± 0.011 <sup>g</sup>	0.773 ± 0.040 <sup>d</sup>	0.775 ± 0.005 <sup>bcd</sup>	1.050 ± 0.006 <sup>bc</sup>	0.626 ± 0.004 <sup>d</sup>	0.245 ± 0.005 <sup>ef</sup>	15.133 ± 0.088 <sup>a</sup>
Sterilised soil + BC-(3%) ( $T_2$ )	0.835 ± 0.003 <sup>h</sup>	0.527 ± 0.005 <sup>e</sup>	0.541 ± 0.009 <sup>cd</sup>	1.077 ± 0.003 <sup>ab</sup>	1.106 ± 0.009 <sup>a</sup>	0.320 ± 0.010 <sup>c</sup>	14.367 ± 0.145 <sup>cd</sup>
BC- 2% ( $T_3$ )	2.934 ± 0.007 <sup>a</sup>	1.062 ± 0.003 <sup>a</sup>	1.458 ± 0.111 <sup>a</sup>	1.030 ± 0.006 <sup>c</sup>	0.452 ± 0.006 <sup>f</sup>	0.171 ± 0.001 <sup>g</sup>	13.767 ± 0.088 <sup>ef</sup>
BC- 2.5% ( $T_4$ )	2.827 ± 0.024 <sup>b</sup>	0.953 ± 0.002 <sup>b</sup>	1.238 ± 0.004 <sup>ab</sup>	1.050 ± 0.006 <sup>bc</sup>	0.561 ± 0.005 <sup>e</sup>	0.432 ± 0.002 <sup>a</sup>	14.067 ± 0.088 <sup>de</sup>
BC- 3% ( $T_5$ )	1.527 ± 0.024 <sup>d</sup>	0.465 ± 0.018 <sup>f</sup>	0.750 ± 0.006 <sup>bcd</sup>	1.063 ± 0.012 <sup>bc</sup>	0.845 ± 0.013 <sup>b</sup>	0.819 ± 0.015	11.667 ± 0.088 <sup>k</sup>
BC- 3.5% ( $T_6$ )	1.589 ± 0.011 <sup>c</sup>	0.397 ± 0.003 <sup>h</sup>	0.623 ± 0.020 <sup>bcd</sup>	1.067 ± 0.003 <sup>bc</sup>	0.740 ± 0.004 <sup>c</sup>	0.281 ± 0.002 <sup>d</sup>	12.033 ± 0.145 <sup>j</sup>
BC- 4% ( $T_7$ )	2.990 ± 0.010	2.990 ± 0.006	2.207 ± 0.793	1.110 ± 0.010 <sup>a</sup>	0.823 ± 0.032 <sup>b</sup>	0.261 ± 0.003 <sup>de</sup>	13.867 ± 0.088 <sup>e</sup>
<i>T. harzianum</i> ( $T_8$ )	1.610 ± 0.019 <sup>c</sup>	0.411 ± 0.007 <sup>gh</sup>	0.803 ± 0.009 <sup>bcd</sup>	1.080 ± 0.006 <sup>ab</sup>	1.074 ± 0.010 <sup>a</sup>	0.317 ± 0.001 <sup>c</sup>	13.500 ± 0.173 <sup>fg</sup>
<i>P. fluorescens</i> ( $T_9$ )	0.718 ± 0.002 <sup>i</sup>	0.204 ± 0.005 <sup>j</sup>	0.219 ± 0.002 <sup>d</sup>	1.063 ± 0.007 <sup>bc</sup>	0.347 ± 0.025 <sup>g</sup>	0.243 ± 0.023 <sup>ef</sup>	14.667 ± 0.088 <sup>bc</sup>
<i>T. harzianum</i> + <i>P. Fluorescen</i> ( $T_{10}$ )	1.248 ± 0.007 <sup>f</sup>	0.258 ± 0.005 <sup>i</sup>	0.455 ± 0.004 <sup>cd</sup>	1.070 ± 0.006 <sup>abc</sup>	0.660 ± 0.033 <sup>d</sup>	0.352 ± 0.003 <sup>b</sup>	13.367 ± 0.145 <sup>g</sup>
BC- 3% + <i>T. harzianum</i> ( $T_{11}$ )	0.806 ± 0.002 <sup>h</sup>	0.435 ± 0.003 <sup>fg</sup>	0.484 ± 0.002 <sup>cd</sup>	1.110 ± 0.006 <sup>a</sup>	0.229 ± 0.031 <sup>h</sup>	0.284 ± 0.006 <sup>d</sup>	12.467 ± 0.088 <sup>i</sup>
BC- 3% + <i>P. fluorescens</i> ( $T_{12}$ )	1.615 ± 0.004 <sup>c</sup>	0.806 ± 0.006 <sup>cd</sup>	0.995 ± 0.058 <sup>abc</sup>	1.073 ± 0.037 <sup>ab</sup>	0.832 ± 0.013 <sup>b</sup>	0.173 ± 0.003 <sup>g</sup>	14.800 ± 0.115 <sup>b</sup>
BC- 3% + <i>P. fluorescens</i> + <i>T. harzianum</i> ( $T_{13}$ )	1.414 ± 0.026 <sup>e</sup>	0.816 ± 0.003 <sup>c</sup>	1.020 ± 0.015 <sup>abc</sup>	1.063 ± 0.033 <sup>bc</sup>	0.233 ± 0.010 <sup>h</sup>	0.237 ± 0.004 <sup>f</sup>	12.867 ± 0.088 <sup>h</sup>
Control ( $T_{14}$ )	1.385 ± 0.003 <sup>e</sup>	0.378 ± 0.001 <sup>h</sup>	0.702 ± 0.004 <sup>bcd</sup>	1.080 ± 0.006 <sup>ab</sup>	0.864 ± 0.012 <sup>b</sup>	0.313 ± 0.008 <sup>c</sup>	15.167 ± 0.088 <sup>a</sup>
CD at (5%)	0.040	0.036	0.622	0.042	0.052	0.025	0.325

**Note:** The means with different Letters as superscripts are significant ( $P < 0.05$ ). The means with the same letters or having common letter(s) are not significantly different.

**Fig. 7.** Boxplot analysis of total phenols pre- and post-inoculation.**Fig. 8.** Boxplot analysis of total soluble proteins pre- and post-inoculation.

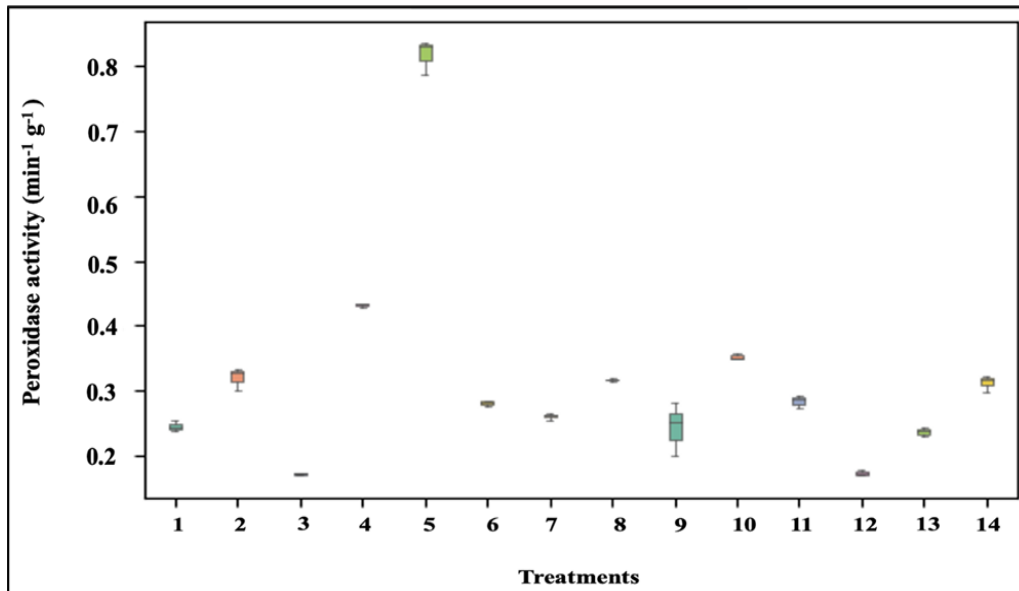


Fig. 9. Boxplot analysis of Peroxidase activity- pre-inoculation.

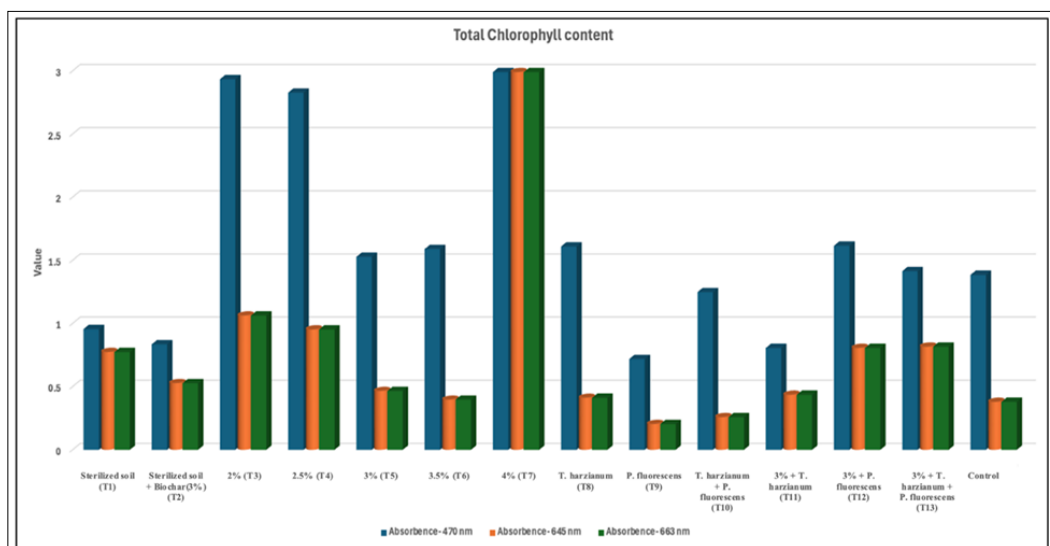


Fig. 10. Impact of plant resistance inducers on total chlorophyll content (Absorbances- 470 nm, 645 nm and 663 nm).

we noticed a drastic reduction in the values, which ranged between 0.204 and 2.990 overall.

**Sugar content**

The results of *in vivo* studies presented in Table 5 demonstrated that these treatments, i.e., T<sub>2</sub> (BC-3% + sterilised soil) with 1.106, followed by T<sub>8</sub> (*T. harzianum*) with 1.074 and T<sub>14</sub> (Control) with 0.864, showed higher sugar content in comparison with all other treatments.

**Quantification of defence**

The results presented in Table 4 revealed that the disease severity had declined from 71.067 in T<sub>14</sub> (Control) to 22.20 in T<sub>13</sub> (BC-3% + *P.*

*fluorescens* + *T. harzianum*). This outcome is likely attributed to the effective suppression of *P. vexans* spore germination and reduction in the pathogenicity (35).

**Correlation coefficient (r) and regression equation**

The results listed in Table 6 show a negative correlation (r) between disease severity and various biochemical parameters, including soil pH, soil EC, plant height, number of branches, number of fruits, yield per treatment, total soluble proteins, total phenols and peroxidase activity.

Table 6. Correlation coefficient (r) of disease severity with various parameters

Variables	Correlation coefficient with disease severity	Correlation equation
Soil pH	-0.085137	y = 92.377 -5.8693x
Soil EC	-0.3828395	y = 79.153-61.949x
Plant height	-0.45305	y =80.387 -0.7057x
Number of branches	-0.6372044	y =90.344 -8.9821x
Number of fruits	-0.6169247	y = 84.658 -6.1433x
Yield per treatment	-0.421446	y = 66.734-0.1197x
Peroxidase activity	-0.3631934	y = 0.4967-0.004x
Total Protein content	-0.7935579	y = 101.58-2.3489x
Total phenols	-0.8644274	y =131.98 -49.757x

**Note:** The means with different Letters as superscripts are significant ( $P < 0.05$ ). The means with the same letters or having common letter(s) are not significantly different.

## Discussion

In the era of sustainable disease management, our study highlights the application of biochar along with fungal (*Trichoderma harzianum*) and bacterial (*Pseudomonas fluorescens*) biocontrol agents, which were investigated on various growth and biochemical parameters against the Phomopsis blight in brinjal using single and combined applications, i.e., 2 %, 2.5 %, 3 %, 3.5 % and 4 %. *In vitro* and *in vivo* conditions, the effectiveness of biocontrol agents was observed to be improved when coupled with biochar (36). Secondary metabolites, key components to biochemical defence, were synthesised in increased amounts and became an integral part of induced systemic resistance (11, 37). Biochar, which enriches the soil with high carbon and high porosity, helps enhance plant-microbe interactions in the rhizosphere and rhizoplane, supporting sustainable crop yield and disease management (38, 39). A study supported current research; they highlighted the effect of green waste biochar (GWB), wood biochar (WB), compost (Comp) and *Bacillus subtilis* (PGPR) on the production and protection of tomato from *Alternaria solani*, causing early blight. Green waste biochar significantly reduced disease severity compared to WB when combined with *B. subtilis*. Few studies on biochar in relation to plant disease management were conducted to manage certain soil and foliar diseases (40, 41). No research has been carried out concerning the sustainable management of Phomopsis blight using biochar and biocontrol agents. The results presented are the first to show the synergistic impact of biochar, *T. harzianum* and *P. fluorescens* on induced systemic resistance against the Phomopsis blight in brinjal. Research has demonstrated the importance of biochar application and supports the current study with respect to soil reclamation, an increase in beneficial microbes' population and yield-attributing traits (42, 43).

It is believed that whenever the biocontrol agents recognise the host plant, they trigger certain defensive mechanisms, which boost hormonal and enzymatic activities (15). Although the exact process and effects of this trigger are unknown, it appears likely that the production of certain antifungal compounds, physical barriers and cell wall development driven by these biochemical defence mechanisms could be potentially useful (44, 45). The biochar, PGPF (plant growth-promoting fungi), PGPB (plant growth-promoting bacteria) and PGPR (plant growth-promoting rhizobacteria) can refine and enrich the production of auxin, cytokinin, ACC deaminase, gibberellin, phosphorus solubilization and nitrogen fixation (46, 47). Research found similar observations from the previous study regarding the *Fusarium* wilt in tomato (48). Biochar as a soil amendment has resulted in improving plant vigour by increasing nutrient absorption. The addition of 0.5-5 % of biochar to soil resulted in enhancing the tomato plant resilience, with a 3 % concentration raising protein and phenols, which act as building blocks for disease resistance.

The ecosystem and its interactions are not always favorable to plants, as they are vulnerable to plethora of stresses (abiotic and biotic) from various habitats during their lifetime and its' so fascinating that how plants have been evolved during these times and able to cope its survival by utilizing their myriad defense mechanisms is key to be investigated, this is where the role of secondary metabolites comes to play and they are necessary when plants interaction with or within other habitats from different ecosystems, Solanaceous crops release endogenous phenolic substances in response to pathogen attack and other secondary

metabolites together notably are the reason for changes in cell wall rigidity and cell wall hydrophobicity, also specifically these phenolic compounds also been contributing for allelopathic effect and increasing the survivability of plants by disrupting the growth other plant around (49-51).

In 2024, their colleagues also reported similar findings during their study in evaluating the efficacy of the biocontrol agents against the bacterial leaf spot and speck disease on tomato, shedding light on bioagents and their ability to boost the physiological defences in tomato, which guides us nearer to sustainable management of the diseases (52). Plant defence machinery is triggered when there is proper recognition of stimuli or elicitors by specific receptors, resulting in ISR. *Trichoderma* spp. and *Pseudomonas* spp. have been proven effective as elicitors for the induction of ISR (53). Research indicates that the effects of biochar, chemical fungicides and *Trichoderma harzianum* against *Alternaria solani*, which causes early blight in tomato and reported that biochar at 3.25 % by weight has been most effective, as it has improved the plant immunity and provided a robust resistance against the disease (45).

Nevertheless, the assessment of the genes responsible, i.e., genes upregulated and genes downregulated in response to biochar-BCA against the disease, has to be further explored with the onset of a series of bilateral experiments, i.e., pot and large-scale field cultivation, to understand these unexplored concepts. Overall, our study will help understand the importance of biochar, as well as how it diverges as a growth enhancer and finally emphasises it as a key feasible element in this modern sustainable disease management era. Many genes related to jasmonic acid (JA) biosynthesis and signalling were found systemically upregulated in the presence of biochar (39). In contrast to this, nearly all the genes that were involved in the salicylic acid (SA) biosynthesis and signalling were downregulated, except the regulatory protein NPR1. Cellulose and xylan biosynthesis genes and phenylpropanoid, phenylalanine, flavonoid and lignin biosynthesis genes, known to make cell walls firm and stable and accelerate several plant growth hormone genes, were found systematically upregulated by biochar. The genes involved in the synthesis of a phyto-steroidal hormone (brassinosteroid), which plays a pivotal role in the growth and activation of defence response, are also systematically upregulated. Research has demonstrated that biochar application induced the expression of defence-associated genes, including jasmonic acid-related PI2, TomloxD, salicylic acid-related PR1a, PR2 and ethylene-responsive Pti4.

## Conclusion

Using biochar seems to be one of the best cost-effective waste management practices, which, being used as a soil amendment, improves soil quality and benefits the soil microbial consortia directly or indirectly by playing a vital role in tackling the plethora of pathogens and also by activating several defence mechanisms. During our study, we further tried to explore the possible interactions in relation to biochar-host-pathogen. It is found that optimal concentrations of biochar together with biocontrol agents have yielded a mutualistic interaction, which gave a synergistic outcome in managing the Phomopsis blight in both *in vitro* and *in vivo* conditions. We also noticed an increase in the amount of key phenolic compounds, which is directly linked with the activation of induced systemic resistance in the plant system. The key theme of

researchers has already started drifting towards sustainable disease management, which is directly linked to crop production, where biochar, now being referred to as the future of plant disease management in the field of Plant Pathology, is being applied increasingly with increased interest and trust. We, as researchers, feel that it is going to play an important role to have a multi-tiered investigation on the diversity in the feed stocks, pyrolysis temperatures and their mechanisms behind the activation of key biochemical parameters responsible for plant growth, disease management and inducing resistance into the host system.

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### Authors' contributions

All authors have made substantial contributions to this manuscript. MSM and AK conceptualised and designed the study, conducted the experiments and analysed the data. AM helped in the interpretation of results in the light of its industrial application. VK and SK provided expertise on the application of biocontrol agents and assisted in drafting the manuscript. All authors have reviewed and approved the final version of the manuscript.

### Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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