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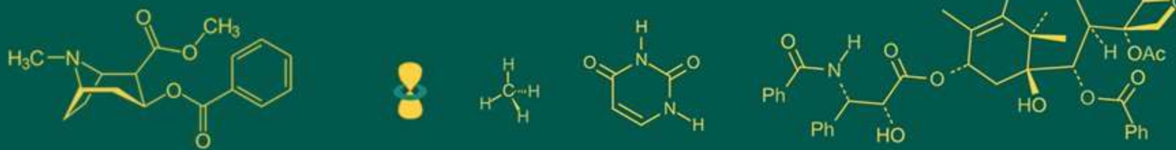

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Suppression of anthracnose (*Colletotrichum coccodes*) of tomato (*Lycopersicon esculentum* L.) by bio fortified vermicompost

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Abstract
Tomato (*Solanum lycopersicum*) stands as a widely cultivated and adaptable fruit, yet its production confronts numerous challenges, including both biotic and abiotic stresses. Among these, anthracnose disease, caused by the soil-borne pathogen *Colletotrichum coccodes*, stands out as a devastating ailment with global implications, leading to substantial economic losses in tomato cultivation. In this study, the potential utilization of bio fortified Vermicompost for anthracnose management was investigated. Organic control agents, namely *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*, were incorporated into the Vermicompost. The study assessed various antioxidants, plant growth markers, and disease incidence across different treatments at various intervals. The results revealed significant differences: tomato plants treated with bio fortified Vermicompost exhibited notable reductions in disease occurrence, improved growth, increased yields, and enhanced antioxidant activity. This highlights the promising role of bio fortified Vermicompost in managing anthracnose disease and enhancing tomato production. Among these treatments, the most noteworthy results were seen in plants treated with *Trichoderma harzianum* -enhanced Vermicompost, displaying the highest values recorded across the examined parameters. The data indicated that Tomato plants treated with Vermicompost enriched with *Trichoderma* exhibited the longest root length after 15 days of planting, followed by T_2 and T_3. The greatest dry weight was observed in plants treated with *Trichoderma*-enhanced Vermicompost. The highest PAL, PO, and PPO activity were noted in leaves from plants grown in Vermicompost fortified with T. harzianum (T_1) at 48 hours, followed by T2, T_3, and T_4. The findings demonstrated that tomato plants treated with bio fortified Vermicompost displayed significant reductions in disease incidence, enhanced growth, increased yields, and elevated levels of defensive enzyme activity compared to the control group.

Keywords: *Colletotrichum coccodes*, *Lycopersicon esculentum* L., bio fortified vermicompost

Introduction
Tomato (*Solanum lycopersicum*) is one of the most widely grown vegetable all over the world. Tomato belongs to the diverse family Solanaceae which includes about more than 3500 species, found in a wide variety of habitats [1]. Tomato is native to tropical Central and South America particularly in Peru, Ecuador, Bolivia and Andes. Tomato plant is adapted to wide variety of climate and cultivated in almost every country of the world and consumed fresh or processed. Tomato (*Solanum lycopersicum*) is considered as the most sensitive species of vegetable to excessive soil moisture. Over the last century, tomato becomes an important vegetable crop has achieved a tremendous popularity. Tomato is the seventh most important crop species after maize, rice, wheat, potatoes, soybeans and cassava and 2nd vegetable crops in the world after potato.

Tomato is a major vegetable crop and stands second in total production of vegetables worldwide. Tomato is the one of the most important vegetable crops cultivated for its fleshy. The tomato plant is very versatile crop and can be utilized as fresh and processed tomatoes [2]. In both the cases, world production and consumption has grown quite rapidly over the past 25 years. With their culinary role in the daily diet, tomatoes also represent a low energy food with very unique constituents that have positive effects on human health. The common phytochemicals present in raw tomato are the carotenoids including lycopene (60-40%), phytoene (10-12%), neurosporene (7-9%) and carotenes (10-15%).

Colletotrichum species pose a significant threat to tomato production, leading to substantial economic losses in fields and warehouses alike. While *C. coccodes* is the most prevalent species affecting tomato fruit, others like *C. truncatum*, *C. gloeosporioides*, *C. acutatum*, *C. dematium*, *C. fioriniael*, and *C. nymphaeae* also contribute to fruit infections [3]. The tomato industry faces challenges from both biotic and abiotic stresses, resulting in reduced yields. Pathogenic fungi, bacteria, viruses, and nematodes are among the primary constraints to tomato cultivation.

A comprehensive approach to disease management, integrating resistant cultivars, cultural practices, biological control, and soil management techniques, represents the most efficient and sustainable strategy for addressing anthracnose disease. Given its enduring presence and ability to persist in soil over long periods, a holistic strategy that combines multiple tactics tailored to the specific conditions of each farming system is essential for effectively managing this detrimental disease in tomato crops. Plant diseases present significant obstacles to global agriculture, impacting crop yield and food security. Biological management utilizing plant growth-promoting rhizobacteria (PGPR), particularly the antagonistic *Pseudomonas* species, has emerged as a robust strategy for controlling various soil-borne plant pathogens. Biocontrol agents such as *Pseudomonas fluorescens* and *Bacillus subtilis* are deployed as commercial bio pesticides [4-6].

Conventional methods of disease control often rely on synthetic chemicals, which can have harmful impacts on both the environment and human health. Recently, Vermicompost has emerged as a promising eco-friendly alternative for managing plant diseases. Vermicompost, an organic fertilizer rich in nutrients produced through the decomposition of organic matter by earthworms, has gained recognition as an effective tool in plant disease management. It contains beneficial microorganisms and compounds that exhibit inhibitory effects on various plant pathogens. These microorganisms can compete with or antagonize harmful pathogens, aiding in disease prevention and management [3, 8]. The use of Vermicompost offers several advantages that contribute to the overall health and resilience of plants. Its application has been linked to the activation of the plant's innate defense mechanisms. In this study, the effectiveness of bio fortified Vermicompost was investigated against the fungal plant pathogen anthracnose of tomato caused by *Colletotrichum coccodes*.

Materials and Methods

Experimental site

The *in vitro* experiments were conducted in the plant pathology Laboratory, Department of Plant Pathology, Institute of Agricultural Sciences, Rama University Kanpur, India. The in vivo experiments were carried out in the polyhouse and agricultural field of the same department, where tomato crop was raised in pots (15 x 10 cm) and field and all physical precautions were kept in view in order to protect the crop from the external damage. The site of the experiment was unaltered during experimentation period. The University is located in Rama city, Mandhana, Kanpur, Uttar Pradesh-209217 which is at 25.5697°N latitude, 80.2169°E longitude and at an altitude of 3 18 m above mean sea level.

Collection of diseased samples from different districts of Uttar Pradesh

Survey for incidence of *Colletotrichum coccodes* from different zones of Eastern and Bundelkhand region of Uttar Pradesh and collection of diseased samples Random roving method of survey was carried out to record the severity of anthracnose disease in tomato. The survey was conducted in some districts of Eastern and Bundelkhand region of Uttar Pradesh. The observations on stage of crop and disease severity were recorded on the rating scale of (1-9). Tomato plants showing typical anthracnose symptoms were collected separately in paper bags and brought to the laboratory for isolation of associated pathogen and further investigations.

Sample Collection and Fungal Isolation

Tomato fruit samples displaying typical anthracnose symptoms were gathered from commercial tomato plants and transported to the university laboratory for fungal isolation. To begin the isolation process, the diseased tissues were cut into smaller pieces approximately 1 cm square. These tissue pieces were then subjected to surface sterilization by immersion in 70% ethanol for 3 minutes, followed by a 3-minute immersion in 1% sodium hypochlorite solution. Subsequently, the samples were rinsed three times in sterile distilled water for 1 minute each. Following sterilization, the treated samples were placed onto sterile potato dextrose agar (PDA) medium and incubated at room temperature (25±2 °C) for one week to allow for the growth of fungal mycelia. After incubation, the resulting fungal mycelia were subcultured onto fresh PDA plates to obtain pure cultures of fungal isolates. This process of obtaining pure cultures was conducted using the single conidium isolation method previously described by Zhang *et al*. 2016.

Morphological Characteristics

Fungal isolates obtained were cultured onto PDA plates and incubated at 25±2 °C for 7 days. The macroscopic characteristics example as the appearance and pigmentation of colony and growth rate of mycelium were recorded. For microscopic characteristics, the arrangement, shape, and size of acervuli, conidia, conidiogenous cells, appressoria, and setae were examined.

Maintenance and storing of the pathogen

The pure culture of the pathogen *Colletotricum coccodes* was maintained on PDA slants throughout the period of investigation by periodic sub culturing on fresh media and stored in a refrigerator at 4 0C.

Pathogenicity test

The pathogenicity of the pathogen was assessed using the tomato cultivar "Arka Rakshak." To prepare the seeds for experimentation, they were surface sterilized with 1% sodium hypochlorite for 30 seconds, followed by two rinses with sterilized distilled water and subsequent air-drying. A soil mixture comprising sandy loam soil, Vermicompost, and farmyard manure in a ratio of 2:1:1 was autoclaved at 15 lbs pressure for 30 minutes over three consecutive days to ensure sterility. Half of this soil mixture was then blended with crushed mycelial powder of *Colletotrichum coccodes*. The sterilized seeds were sown in 15 × 10 cm^2 pots under greenhouse conditions. Positive and negative controls were

established by sowing untreated seeds in soil infected with the pathogen and uninfected soil, respectively.

Preparation of Vermicompost

Temple, farmyard and kitchen wastes were used as feedstock in the present study. The temple wastes mainly consisted of *Hibiscus rosasinensis* flowers, Aegle marmelos leaves and *Tagetes erecta*. The offerings were collected from different temples in the city but the bulk from two temples namely ''JK temple'' and ''Sankatmochan temple'' which receive most of the devotees. The kitchen wastes were collected from the cafeteria of hostels in the Rama University campus which had major share of fruit and vegetable peels (60–75%) and the remainder consisted of used tea leaves, pulses extract, vegetables extract, peel of potato etc. Flesh, bones, fat, egg shells, etc. Were not included in the kitchen waste as they are not easily degradable and can be toxic to earthworms. The wastes of yard mainly contents of drie leaves (a mixture of *Mangifera indica*, Sarakaasoka, *Syzygium cumini*, *Tamarindus indica* and various grasses). Nine non-transparent, rectangular plastic containers measuring 340 cm × 160 cm × 60 cm were utilized for Vermicomposting. To ensure proper gas exchange, 45 holes, each with a diameter of 0.65 cm, were drilled at the base of each container. The experiment followed a randomized complete block design with three replications for each feedstock type. Before being placed in the plastic containers, each feedstock was kept for 2–3 days and thoroughly mixed to prevent clumping and compaction of the substrates after water addition. Mature cow dung was added at a ratio of approximately 1:7 to provide an immediate food source for the earthworms. The containers were then covered with a layer of soil for decomposition. *Eisenia fetida* adult clitellate worms, ranging from 4 to 8 cm in length, were introduced at a rate of 1.5 kg/m2 through developed cracks after 15 days of partial waste decomposition to protect the worms from the thermophilic reaction during composting. The moisture content of the feedstock was adjusted to 70±10% at the beginning of Vermicomposting and maintained throughout the process by periodically sprinkling water. Watering ceased when the Vermicompost reached readiness, indicated by a uniformly dark brown to black granular structure. Three days later, the compost, along with the worms, was harvested, and the worms were separated by sieving (< 2 mm). (Singh *et al.* 2013) [9].

Microbial fortification of Vermicompost

The three Biological Control Agents (BCAs)-*T. harzianum*, *P. fluorescens*, and *B. subtilis*-utilized in this study were selected based on their compatibility and proven efficacy in reducing soil borne diseases across various crops. Each of these BCAs was individually employed to fortify the Vermicompost. For the fortification process, 1 liter of 2-dayold bacterial cultures grown in Nutrient Broth (NB) with a colony-forming unit (CFU) count of approximately 2×10^7 was thoroughly mixed with 25 kg of freshly prepared Vermicompost in separate trays. Similarly, 1 liter of 5-day-old T. harzianum culture grown in Potato Dextrose Broth (PDB) with a CFU count of approximately 3.5×10^6 was used to fortify another set of Vermicompost trays, each weighing 25 kg.

Biological management of Anthracnose of tomato using bio fortified Vermicompost Source of BCAs used and viability test

The biological control agents used in this study *viz. Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the culture repository of Plant Health Laboratory of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Rama University, Uttar Pradesh, Kanpur India.

***In vitro* Efficacy of BCAs against pathogen**

The antagonistic ability of selected BCAs against the pathogen was studied *in vitro* following a dual culture assay described by Sharma *et al.* [13]. A nine mm disc (plug) of 15 days old cultures of *Colletotrichum coccodes* were cut with a sharp cork borer from the growing edge of the culture plate. The cut block was placed on PDA medium 1 cm away from the edge of the plate. 9 mm disc of biocontrol agent namely *T. harzianum* isolate was placed at opposite end of the Petri plate. PDA plates inoculated with the pathogen alone served as the control and incubated at 25±2 °C. Similarly, the *in vitro* antagonistic ability of the bacterial isolates was studied using a dual culture assay. A 9 mm plug of the *Colletotrichum coccodes* was placed at the centre of a Petri plate containing PDA, then the test bacterial isolate was streaked 3 cm away from the fungal plug at both the sides towards edge of the plate by a loop loaded with 48 h old bacterial culture. The plates were incubated at 28± 2 °C for 7 days and the inhibition zone was measured from the edge of mycelium to the bacterial streaks, when the control plates showed full growth. Percent inhibition over control was calculated as per the following formulae given by Whipps (1997):

$$PI = C\text{-}T/C \times 100$$

Where,
PI = percent inhibition over control
C = Growth of test pathogen with absence of antagonist (mm).
T = Growth of test pathogen with antagonist (mm)

Experimental details

Tomato seeds were surface sterilized by using 1% sodium hypochlorite for 30 sec and was rinsed twice with sterilized distilled water and then air-dried.

Table 1: Combination of treatments used for conducting experiment

No. Treatment
T_1: Vermicompost +*Trichoderma harzianum*+ Pathogen
T_2: Vermicompost + *Bacillus subtilis* + Pathogen
T_3: Vermicompost + *Pseudomonas flourescens* + Pathogen
T_4: Vermicompost + Pathogen
T_5: Control (Only vermicompost)

Pot experiments

Plastic pots of 15×10 cm were used to conduct the plant growth promotion and antagonistic potentials of fortified Vermicompost against *Colletotrichum coccodes*. Soil was autoclaved for 30 min at 15 psi for three consecutive days. Pots were filled with soil mixture containing sterile soil and microbially fortified Vermicompost in the ratio of 1:1 (w/w) (1.5 kg pot^{-1}). In the first three treatments, Vermicompost

was fortified individually with *T. harzianum*, *B. subtilis*, and *P. fluorescens* cultures as described above. Fourth treatment contained only Vermicompost (positive control), while the fifth treatment contained only soil (Negative control) [8, 15].

Pathogen inoculation

The spore suspension for inoculation was prepared by adding 20 ml of sterile distilled water to each culture plate containing 5-7 days old fungal mycelium. The mycelium was gently scraped using a spore harvester to dislodge the spores. The concentration of conidia was then adjusted to 2-3×10^7 conidia ml^-1 using a hemocytometer. For inoculation, 5 ml of the prepared spore suspension was used to treat each seedling in all five treatments using the soil drenching method as outlined by Patil *et al.* [16]. In this method, 5 ml of the fungal suspension (i.e., water containing the pathogen's conidia) was applied to each seedling by drenching the soil around the root zone using a pipette. Before inoculation, the roots were slightly wounded by inserting a needle 1 cm away from the stem. This wounding of the roots was performed to facilitate pathogen penetration through the roots. Observations on anthracnose symptoms were recorded for up to 5 weeks following inoculation.

Observations recorded

Random sampling technique was adopted for recording the observations of various morph physiological characters after 30, 60 and 90 days after sowing (DAS). Three plants of each treatment from each replication were selected at random at the time of recording the data on various characters. Data of the plants were averaged replication wise and mean data was used for statistical analysis. Recommended package of practices were applied to raise a healthy crop.

Morphological parameters

Shoot length (cm)

It was measured in centimeter from the ground level (base of the plant) to the tip of the main axis of the plant after stretching the main shoot of plant at the time span of 30 days after sowing with meter scale.

Length of roots (cm)

It was measured in centimeter from the ground level (base of the plant) to the tip of the main root of the plant at the time span of 30 days after sowing with the help of meter scale.

Dry weight (gm)

After washing the plants in the tap water and softly wiped with using blotting paper, fresh weight was determined by using an electronic balance (Sartorius BT-224S) and the values were expressed in grams. After taking fresh weight, the plants were placed to 100 °C pre-heated hot air oven for one hour. Then they were placed in an oven, maintained at 60±2 °C for drying purpose. The weight was measured regularly and expressed in grams.

Biochemical Analysis

Analysis of biochemical compounds to identify various antioxidants and reactive oxygen species (ROS), specifically hydrogen peroxide (H_2O_2), in tomato plant leaves post-pathogen inoculation was conducted using the protocol outlined by Singh *et al*. (2013). Enzyme assays including phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), and total phenol content (TPC) were carried out at 0, 24, 48, 72, and 96 hours following pathogen inoculation as described by Jain *et al*. (2011) [17, 18].

Phenylalanine ammonia-lyase (PAL) assay

Each leaf sample weighing 0.1 g underwent homogenization in 2 ml of 0.1 mol L-1 sodium borate buffer (pH 7.0; 4 °C), supplemented with 1.4 mmol L-1 ß-mercaptoethanol, followed by centrifugation at 16000 rpm at 4 °C for 15 min. The resulting supernatant served as the enzyme source. Subsequently, 0.2 ml of enzyme extract was combined with 0.5 ml of 0.2 mol L-1 borate buffer (pH 8.7) and 1.3 ml of water in the reaction mixture. The reaction commenced upon the addition of 1 ml of 0.1 mol L-1 phenylalanine (pH 8.7) and incubation for 30 min at 32 °C. Termination of the reaction was achieved by adding 0.5 ml of 1 M trichloroacetic acid (TCA). PAL (EC 4.1.3.5) activity was determined by monitoring trans-cinnamic acid formation at 290 nm, following the method outlined by Brueske, and was quantified in terms of µmol L-1 TCA per g fresh weight (FW).

Polyphenol oxidase (PPO) assay

Leaf samples weighing 0.1 g each were homogenized with 2 ml of ice-cold phosphate buffer (0.1 mol L-1, pH 6.5). Following centrifugation at 16000 rpm for 30 min at 4 °C, the resulting supernatant was directly utilized in the enzyme assay. The reaction mixture comprised 0.4 ml of catechol (1 mmol L-1) in 3 ml of sodium phosphate buffer (0.05 mol L-1; pH 6.5) and 0.4 ml of enzyme extract. A control reaction mixture containing only the substrate was included. Catechol served as the substrate for PPO (EC 1.14.18.1), and the increase in absorbance was monitored at 405 nm (Gauillard *et al*., 1993). PPO enzyme activity was quantified based on the linear portion of the activity curve, expressed as the change in optical density (O.D.) per minute per gram fresh weight (FW).

Peroxidase (PO) assay

PO (EC 1.11.1.7) activity was determined following the method outlined by Hammerschmidt *et al*. (1982), with slight modifications. Leaf samples weighing 0.1 g were individually homogenized in 2 ml of 0.1 mol L-1 phosphate buffer (pH 7.0) at 4 °C, followed by centrifugation at 16000 x g at 4 °C for 15 min, and the resulting supernatant was utilized as the enzyme source. The reaction mixture comprised 1.5 ml of pyrogallol (0.05 mol L-1), 0.05 ml of enzyme extract, and 0.5 ml of H_2O_2 (1% v/v). A control reaction mixture without the enzyme was also prepared. Changes in absorbance at 420 nm were monitored at 30-second intervals for 3 min. Enzyme activity was expressed as the change in units (U) per minute per gram fresh weight (FW).

Results

Isolation, purification and maintenance of *Colletotrichum coccodes* isolates

The isolates purified and identified as *Colletotrichum coccodes* based on morphological and cultural characters using the descriptions given by C.M.I (1970). The isolates were designated serially from Col 1 to Col 10. The purified isolates were maintained in PDA slants and stored at 4 degrees C for further use.

Following the isolation and purification of *Colletotrichum coccodes* from the collected samples, pathogenicity tests were conducted using a susceptible genotype of the tomato cultivar 'ArkaRakshak' via the soil inoculation method. Among the 10 isolates of *Colletotrichum coccodes* tested for pathogenicity, 5 isolates exhibited typical anthracnose symptoms. Symptoms observed on the plants included yellowing of lower leaves, occasional formation of adventitious roots, burning of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and eventual death of the plant. Consequently, 5 isolates demonstrated positive results for Koch's postulate, while the remaining isolates failed to fulfill Koch's postulate, indicating their non-pathogenicity to tomato. The 5 isolates that showed positive results for Koch's postulate were designated as Col 1, Col 2, Col 4, Col 8, and Col 10 (as listed in Table 2).

Further analysis included the study of colony characteristics of these selected isolates, along with an assessment of the percent disease incidence (PDI) through the 'soil inoculation' method. The results of the pathogenicity tests are summarized in Table 2.

Study of the effect of selected isolates of *C. coccodes* on tomato in pots: The investigation of percent disease incidence (PDI) of selected isolates of *Colletotrichum coccodes* was conducted using soil inoculation methods in pots under greenhouse conditions. Data were collected from 30 DAI to 120 DAI (as presented in Table 2). Up to 30 DAI, none of the isolates exhibited any PDI. However, by 60 DAI, three isolates, specifically Col 2, Col 8, and Col 10, showed PDIs of 18.72%, 17.21%, and 16.5%, respectively. At 90 DAI, all five isolates demonstrated varying levels of PDI. The highest PDI, 34.65%, was observed in plants treated with Col 2, followed by a PDI of 31.22% for Col 8. Subsequently, PDI values decreased for the other isolates. Col 2 consistently displayed the highest PDI among all isolates from 60 to 120 DAI, while Col 8 held the second-highest position from 90 to 120 DAI. Fourisolates, namely Col 1, Col 2, Col 8, and Col 10, recorded PDIs of more than 70.0%, whereas the remaining isolate, Col 4, recorded a PDI of less than 70.0%. Based on the observations from Table 2, Col 2, isolated from Maudaha, exhibited the highest aggressiveness among the five isolates of *Colletotrichum coccodes*. Therefore, it was selected as the test pathogen for conducting further experiments.

Table 2: Test of pathogenicity of *Colletotrichum coccodes* isolates

	Percent disease incidence (%)				
	Days after inoculation				
	30	60	90	105	120
Control	0±0	0±0	0±0	0±0	0±0
Col1	0 ±0	0±0	22.54±6.10	48.55±7.21	72.25±12.45
Col2	0±0	18.7±5.10	34.65±6.73	52.45±8.50	90.70±14.33
Col4	0±0	0±0	23.20±6.78	41.83±8.75	65.85±14.33
Col8	0±0	17.21±6.00	31.2±5.25	48.55±9.22	80.85±10.75
Col10	0±0	16.50±5.98	28.2±6.25	42.86±13.11	72.58±12.55

Biological Management of Anthracnose of Tomato using Bio fortified Vermicompost

***In vitro* Efficacy of Bio Control Agents against Pathogen**

The described biocontrol agents (BCAs) were assessed for their antagonistic activities against *Colletotrichum coccodes* after 4 days in dual culture assay. Table 3 demonstrates that the bio agents notably decreased the radial growth of *Colletotrichum coccodes*. T. harzianum exhibited greater antagonistic activity compared to B. subtilis and P. fluorescens against the radial growth of *Colletotrichum coccodes*.

Table 3: Microbial dynamics after fortification of Vermicompost.

BCAs	0 day after fortification ($CFUg^{-1}$)	10 days after fortification (CFU^{-1})
B. subtilis	2×10^7±0.55	7×10^8±0.5
P. fluorescens	1.5×10^8±0.62	4.8×10^7±0.8
Trichoderma sp.	3.5×10^6±0.18	5.8×10^6±0.06

Table 4: Effect of bio agents on the growth of *Colletotrichum coccodes*

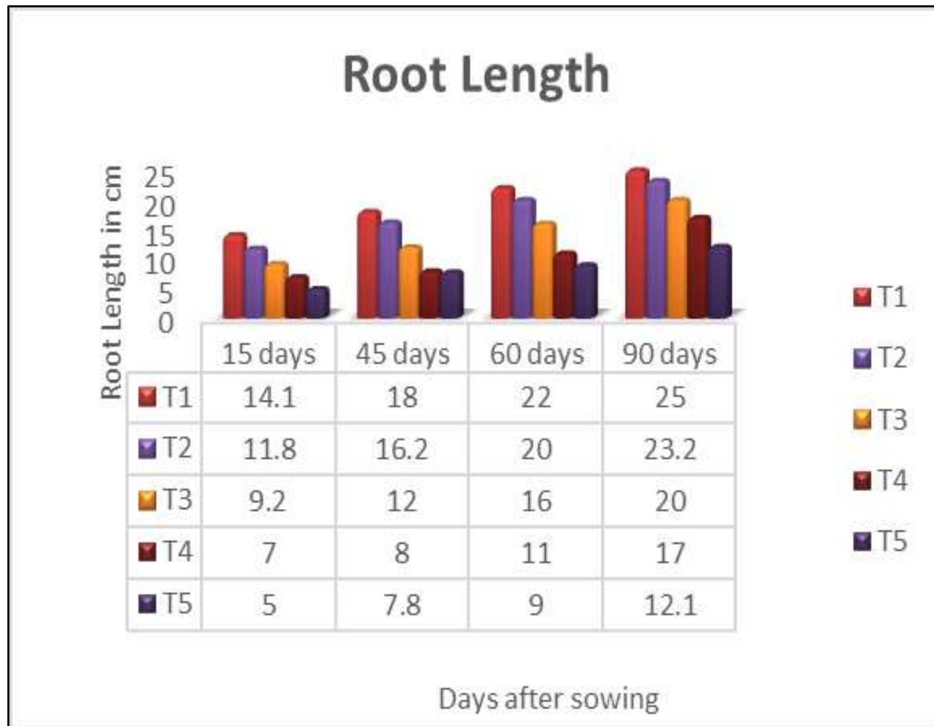
Microbial strain	Radial growth (cm.)	Inhibition Percentage (%)
T. harzianum	0.45±0.25	97.10±1.2
B. subtilis	1.1±0.2	87.89±1.65
P. fluorescens	1.25±0.12	82.75±0.1
Control	8.0±01	0.0±0

The dynamics of microbial population observed in bio fortified Vermicompost 10 days after fortification indicated that there was a high adaptability of the selected BCAs.

Effect of various treatments on growth parameters of tomato crop Root length

The influence of various microbes used to fortify Vermicompost on growth characteristics became apparent 15 days after transplantation. All treated plants demonstrated a significant improvement in root length compared to the control. Tomato plants treated with Vermicompost fortified with *Trichoderma* exhibited the longest roots (14.1 cm) after 15 days of sowing, followed by T_2 (11.8 cm) and T_3 (9.2 cm) than T_4 and T_5. After 45 days of sowing, *Trichoderma*-treated plants displayed the longest roots (18 cm), followed by T_2 (16.2 cm), T_3 (12 cm) and T_4

treated plant displayed the root length of (8 cm) and T_5 (7.8 cm). Similar trends were observed after 60 and 90 days post-sowing. After 60 DAS *Trichoderma harzianum* treated plant showed max root length (22 cm) followed by T_2 and T_3 (refer to Figure 1).

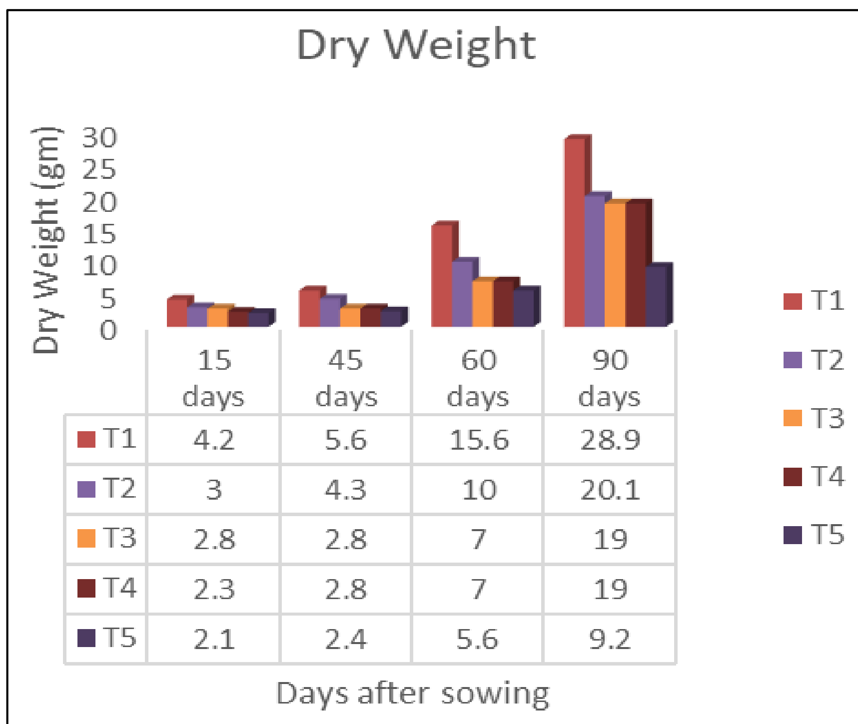


	15 days	45 days	60 days	90 days
T1	14.1	18	22	25
T2	11.8	16.2	20	23.2
T3	9.2	12	16	20
T4	7	8	11	17
T5	5	7.8	9	12.1

Fig 1: Effect of different treatments on root length of tomato

Shoot length

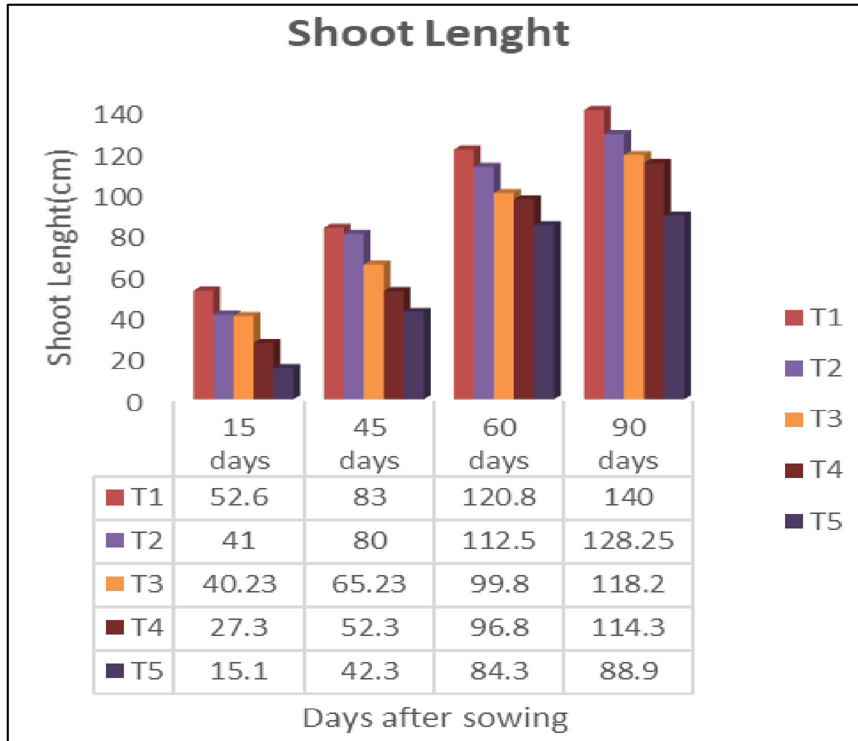
Shoot length was measured at 15, 45, 60, and 90 days after sowing. Throughout each interval, tomato plants treated with *Trichoderma*-fortified Vermicompost displayed the longest shoot lengths. At 15 days after sowing (DAS), the maximum shoot length of 52.6 cm was observed in T_1, followed by T_2 (41.0 cm) and T_3 (40.23 cm) than T_4 and T_5. This pattern persisted at 45, 60, and 90 DAS. After the 60 days of sowing treatment T_1 showed maximum shoot length (120.8 cm) followed by T_2 (112.5 cm). Remarkably, at 90 DAS, T_1 exhibited the maximum shoot length of 140.0 cm. A significant difference between treated and control plants was evident after 90 DAS, as illustrated in Figure 2.



	15 days	45 days	60 days	90 days
T1	4.2	5.6	15.6	28.9
T2	3	4.3	10	20.1
T3	2.8	2.8	7	19
T4	2.3	2.8	7	19
T5	2.1	2.4	5.6	9.2

Fig 2: Effect of different treatments on shoot length of tomato

Dry weight
Dry weight was recorded at 15, 45, 60, and 90 days after sowing. The highest dry weight was observed in plants treated with Vermicompost fortified with *Trichoderma*. After 15 DAS, (4.2 gm) of dry weight was recorded in T_1, followed by T_2 (3 gm) and T_3 (2.8 gm). This pattern persisted at 45, 60, and 90 days after sowing. At 60 DAS maximum dry weight observed (15.6 gm) in plants treated with Vermicompost fortified with *Trichoderma* followed by T_2 (10 gm) and T_3 (7 gm). The max. dry weight observed after 90 days of sowing with the treatment of *Trichoderma* which treatment-1 showed (28.9 gm) of dry weight followed by T_2 (20.1 gm) and T_3 (19 gm) (refer to Figure 3).



	15 days	45 days	60 days	90 days
T1	52.6	83	120.8	140
T2	41	80	112.5	128.25
T3	40.23	65.23	99.8	118.2
T4	27.3	52.3	96.8	114.3
T5	15.1	42.3	84.3	88.9

Fig 3: Effect of different on treatments on dry weigth of tomato

The impact of bio fortified Vermicompost on defense-related enzymes in tomato plants challenged with *Colletotrichum coccodes*

Phenylalanine Ammonia Lyase (PAL)
The PAL levels demonstrated a notable increase across all treatments up to 48 hours, followed by a decline in activity. The highest PAL activity was recorded in leaves from plants grown in Vermicompost fortified with *T. harzianum* (T_1) at 48 hours, followed by T_2, T_3, and T_4. At 48 hours, T_1 exhibited a 2.3-fold increase in PAL activity compared to the control. Similarly, T_2 and T_3 showed 2.0-fold and 1.5-fold increases in PAL accumulation, respectively, compared to the control. Plants from non-fortified Vermicompost also displayed higher PAL accumulation

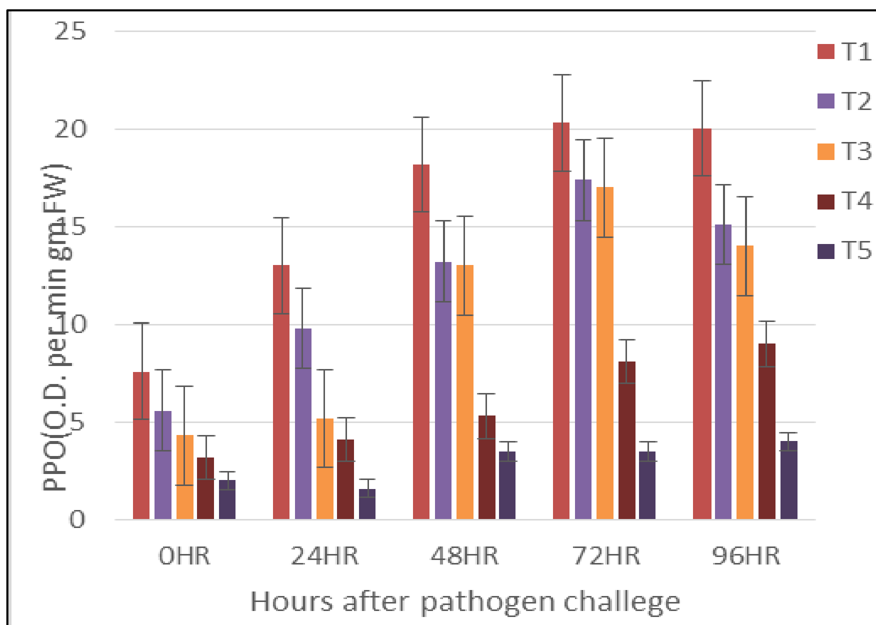
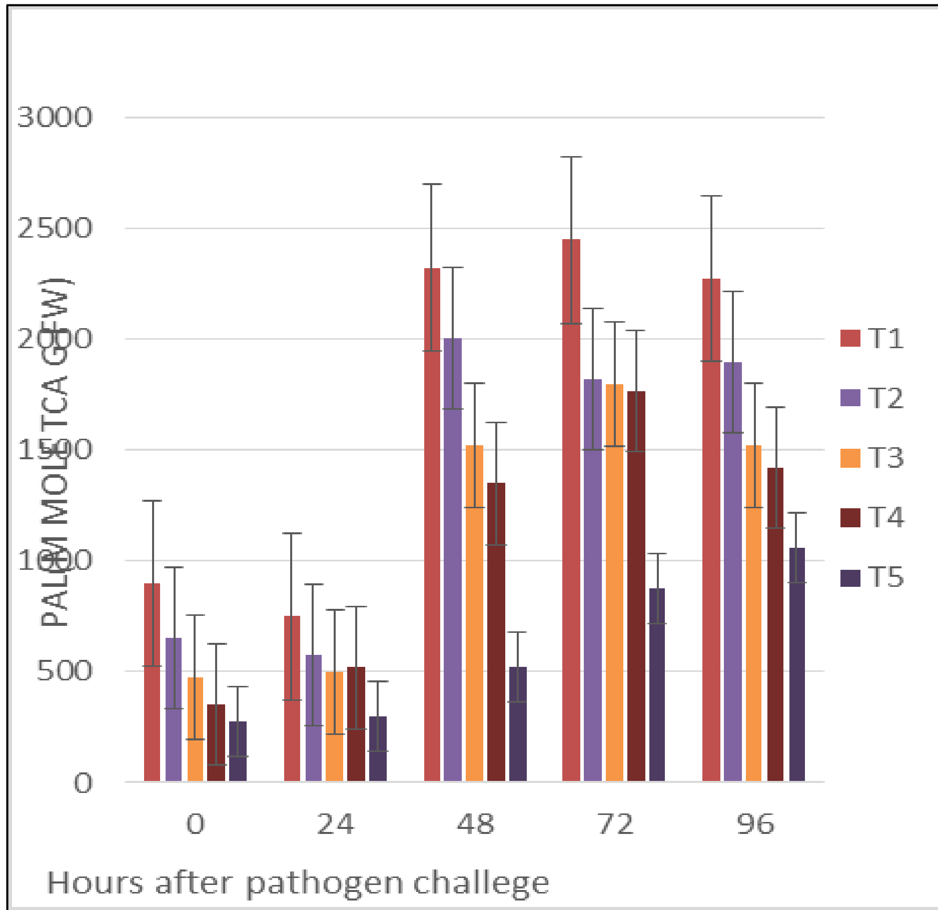


Fig 4: PAL activity at different time intervals in tomato raised from seeds

Polyphenol oxydase (PPO)

The levels of PPO increased significantly across all treatments up to 72 hours, after which its activity declined. The highest PPO activity was observed in leaves from plants grown in Vermicompost fortified with T. harzianum (T_1) at 72 hours, followed by T_2, T_3, and T_4. At 72 hours, T_1 exhibited a 4.8-fold increase in PPO accumulation compared to the control. Additionally, Figure 4 The impact of microbial fortified Vermicompost on PPO activity. Different letters signify significant differences among treatment results taken at the same time interval, according to Duncan's multiple range test at $P \leq 0.05$.



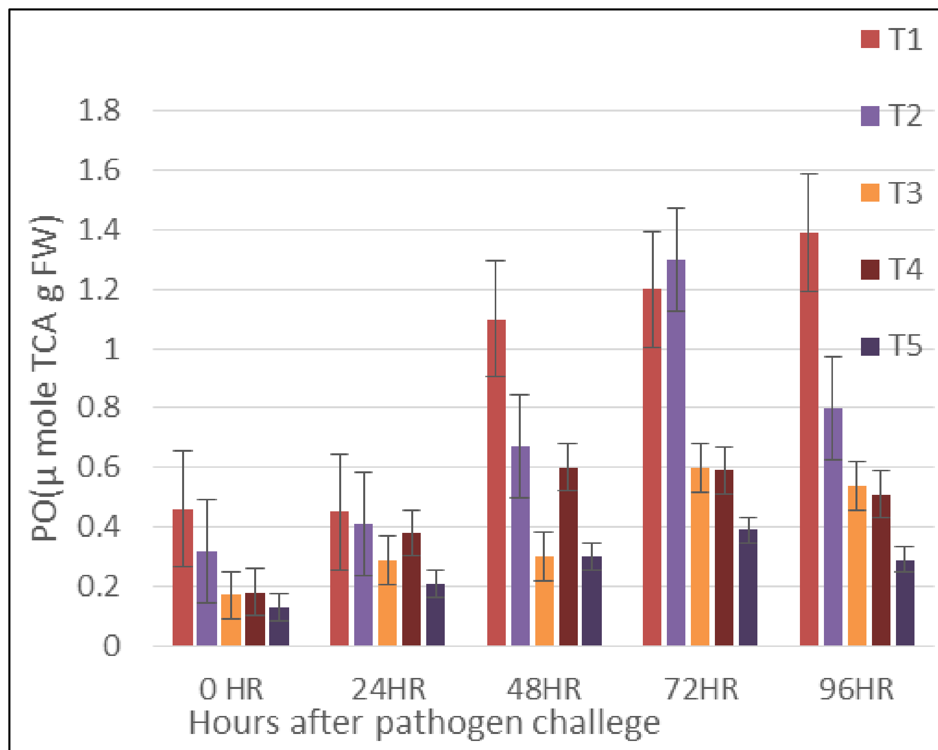


Fig 5: Figure Effect of microbial fortified Vermicompost on PO activity

Peroxidase (PO)

The levels of PO exhibited a notable increase across all treatments up to 72 hours, followed by a decline in their activity. The highest PO activity was observed in leaves from plants cultivated in Vermicompost enriched with T. harzianum (T_1) at 72 hours, followed by T_2, T_3, and T_4. At 72 hours, T_1 displayed a 7.2-fold increase in PO accumulation compared to the control. Similarly, T_2 and T_3 exhibited 6.5-fold and 3.5-fold increases in PO activity, respectively, compared to the control. Plants cultivated solely in Vermicompost also displayed higher PO accumulation compared to the control. At 48 hours and 72 hours, plants cultivated solely in Vermicompost (T_4) exhibited a 3.04-fold and 2.3-fold increase in PO accumulation, respectively, compared to the control (refer to Figure 5.

Discussion

Effect of microbial fortified Vermicompost on growth parameters of tomato crop

The utilization of composts and Vermicomposts as amendments for soil is widely acknowledged for its ability to enhance soil nutrient levels, foster soil health, and improve various attributes of crop plants when compared to synthetic fertilizers. The introduction of biofortified Vermicompost has resulted in significant improvements in yield and disease resistance. The outcomes of this research indicate a discernible disparity in the growth promotion of tomato plants treated with microbially fortified Vermicompost or Vermicompost alone. Noticeable disparities were observed in root length, shoot length, and dry weight across the different treatments. These findings align with the study by Wang *et al.* [24], which highlighted the growth-promoting effects of Vermicompost combined with other bioinoculants in tomatoes. Wang *et al.* demonstrated that the most significant growth in tomato plants was achieved through a combination of Vermicompost, *Bacillus pumilus*, *Trichoderma*, and the mycorrhizal fungus Glomus mosseae. Similarly, Bachman and Metzger [25] reported positive impacts on productivity enhancement and nematode control in brinjal through the use of Vermicompost and bio-pesticides. These results corroborate previous experimental findings that indicate the beneficial role of Vermicompost, either alone or in combination with *Pseudomonas fluorescens*-based biopesticides, in promoting tomato growth [26].

Effect of Fortified Vermicompost on Activity of Defense Related Enzymes in Tomato

Plants deploy various mechanisms to fend off invading phytopathogens. Application of biofortified Vermicompost may enhance cellular defense responses, which are typically activated only when plants are under pathogenic attack. Findings from this study revealed that tomato plants treated with biofortified Vermicompost displayed elevated levels of defense-related enzyme activity and phenol accumulation in leaves when faced with *C. coccodes* challenge. Induced systemic resistance (ISR) occurs throughout the plant in response to colonization of roots by beneficial microorganisms present in Vermicompost and selected biocontrol agents (BCAs) [27]. These cellular responses involve an early oxidative burst and intensified upregulation of defense genes [14]. The induction of defense proteins and enzymes in this study signifies a defense mechanism triggered against pathogen invasion in tomatoes. The results demonstrate that treatment with biofortified Vermicompost significantly increased the activities of defense-related enzymes such as PAL, PO, PPO, and SOD, indicating their role in disease resistance. The highest enzyme activities were observed in plants treated with Vermicompost fortified with *T. harzianum*. Phenols play diverse roles in plant defense, including strengthening cell walls, exhibiting antimicrobial activity, and serving as precursors for signaling compounds like salicylic acid [28]. The highest PAL activity was observed in leaves of plants grown in Vermicompost fortified with

T. harzianum (T_1) at 48 hours. Our findings align with research on the suppression of damping off diseases in psyllium using *Bacillus subtilis* and Vermicompost. Amooaghaie *et al.* [29] noted that Vermicompost and B. subtilis induce systemic resistance via nitric oxide (NO) signaling, leading to the accumulation of defense-related enzymes such as β-1,3-glucanase, PAL, and PPO, while also reducing lipid peroxidation in psyllium leaves. The increased PAL activity correlates with enhanced antimicrobial activity, contributing to greater resistance of the host plant against pathogens. Enzymes like SOD and PO collaborate with other components of the ascorbate-glutathione cycle to scavenge free radicals [14]. SOD is crucial in mitigating oxidative stress by catalyzing the dismutation of O2- to H_2O_2 and O2 [30, 31]. PO enzyme facilitates the reduction of H_2O_2 by transferring electrons to various donor molecules, linking to a wide range of physiological processes such as lignification, auxin metabolism, cell wall protein cross-linking, and defense against phytopathogens (Sarma *et al*., 2015). In our study, analysis post-pathogen infection revealed a significant increase in PO levels across all treatments up to 72 hours, followed by a decrease in activity. The highest PO activity was observed in leaves from plants grown in Vermicompost fortified with *T. harzianum* (T_1) at 72 hours. Similar findings were reported in a previous study where Vermicompost biofertilizer induced maximum PO and SOD activity in greenhouse cucumber after 72 hours of *Pythium aphanidermatum* challenge [30]. Following treatment with bio fortified Vermicompost, the increased PO activity in treated plants may lead to the accumulation of lignin, an important physical barrier against pathogen invasion. Enhanced PPO activities in plant tissues against phytopathogens and insect pests have been documented in various beneficial plant-microbe interactions [14]. The increased activity of PAL and PPO is directly related to heightened antimicrobial activity and reduced accumulation of toxic oxidation products, thereby enhancing resistance to pathogens. Maximum PO activity was recorded in leaves from plants grown in Vermicompost fortified with T. harzianum at 72 hours. Increased PPO activity in all plants treated with fortified Vermicompost indicates enhanced pathogen tolerance. Elevated levels of defense-related enzymes PAL, PO, and PPO were observed in plants treated with B. subtilis and Vermicompost under stress induced by C. coccodes in Plantago psyllium [28]. Our results align with those of Bosco *et al*. (2017), where increased PAL, PO, and PPO activity were recorded in tomatoes treated with bio fortified Vermicompost against anthracnose. The heightened activity of defense-related enzymes in plants treated with bio fortified Vermicompost may contribute to the host cell's response to pathogens, aiding in controlling fungal

development without causing further damage to surrounding tissues and potentially delaying symptom development.

Conclusion

The three biocontrol agents (BCAs), namely *T. harzianum*, *P. fluorescens*, and *B. subtilis*, were selected based on their compatibility and proven ability to mitigate soil borne diseases in various crops. Each BCA was used to fortify the Vermicompost individually, and the effect of bio fortified Vermicompost on plant growth and disease suppression was assessed. All treated plants exhibited significant improvements in root length compared to the control group. Biochemical analysis of plants from each treatment revealed a significant increase in PAL, PO, and PPO levels up to 48 hours, followed by a decline in activity. The highest activity of PAL, PO, and PPO was recorded in leaves from plants grown in Vermicompost fortified with *T. harzianum* (T_1). The utilization of Vermicompost enriched with specific biocontrol agents proved highly effective in biologically managing anthracnose in tomatoes caused by *Colletotrichum coccodes*. Tomatoes grown in soil blended with fortified Vermicompost exhibited notable increases in defense-related enzymes. Moreover, the fortified Vermicompost significantly influenced plant morphology and various growth parameters. This observed growth enhancement and biocontrol potential in Vermicompost could be attributed to its hosting of beneficial microorganisms for plants.

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