**Bio-enriched vermicompost mediated suppression of fusarium wilt of chickpea**

**Abstract**

The present study evaluated the efficacy of bio-enriched vermicompost fortified with *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* in enhancing growth and inducing systemic resistance in chickpea (Cicer arietinum) against *Fusarium oxysporum* f. sp. *ciceris.* Vermicompost fortified with microbial biocontrol agents (BCAs) significantly improved morpho-physiological traits, including root and shoot length and dry weight. Plants treated with the fortified vermicompost exhibited markedly higher activities of defense-related enzymes phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), and superoxide dismutase (SOD) as well as increased total phenolic content (TPC), compared to controls. Peak enzyme activities and phenolic accumulation were recorded 48–72 hours after pathogen challenge, particularly in treatments involving *T. harzianum*. Disease incidence was significantly reduced in BCA-treated plants, correlating with enhanced biochemical defense responses. The results suggest that biofortified vermicompost is an effective, eco-friendly strategy for managing chickpea wilt and enhancing plant resilience through induced systemic resistance mechanisms.

Keywords: Bio-enriched vermicompost, Fusarium oxysporum f. sp. ciceris, biocontrol agents

**Introduction**

“Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops grown globally, particularly in the semi-arid regions of South Asia, the Middle East, and parts of Africa. It serves as a rich source of dietary protein, carbohydrates, vitamins, and minerals for millions of people, while also playing a vital role in improving soil fertility through atmospheric nitrogen fixation” (Kumar et al., 2025). “Despite its significance, chickpea cultivation is often hindered by various biotic stresses, among which Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Ciceris* is considered one of the most destructive and widespread soil-borne diseases. Under favorable environmental conditions, the pathogen can lead to complete crop failure, resulting in substantial economic losses and posing a serious threat to food and nutritional security” (Kumari et al., 2024).

Traditional methods for managing Fusarium wilt have primarily relied on the use of resistant cultivars and chemical fungicides. However, the effectiveness of these strategies is increasingly compromised by the emergence of new pathogenic races, limited genetic diversity in resistance sources, and growing concerns regarding the environmental and health impacts of chemical inputs. Moreover, the prolonged and indiscriminate use of fungicides can disrupt soil microbial diversity, cause the accumulation of chemical residues, and promote the development of fungicide-resistant pathogen strains. In light of these challenges, there is an urgent need for sustainable, eco-friendly, and cost-effective alternatives for disease management in chickpea cultivation (Chohan et al., 2024).

Organic amendments such as vermicompost have attracted increasing attention in recent years due to their potential to improve soil health, stimulate plant growth, and suppress soil-borne pathogens. Vermicompost, a stabilized organic product derived from composting via earthworm activity, is rich in plant nutrients, growth hormones, and beneficial microbial communities (Bashi et al., 2025). When further enriched with antagonistic biocontrol agents such as species of Trichoderma, Pseudomonas, and Bacillus vermicompost can be transformed into a biologically active formulation with enhanced disease-suppressive properties (Bisen et al., 2023; Bisen et al., 2019; Jyoti Singh et al., 2017). This process, known as biofortification, not only enhances microbial diversity and activity in the rhizosphere but also promotes the effective colonization of beneficial microbes, thereby creating an environment unfavorable for pathogenic organisms (Datta et al., 2022; Wonglom et al., 2025).

The present study focuses on the biological management of Fusarium wilt in chickpea through the application of biofortified vermicompost, which combines the nutritional and microbial benefits of organic matter with the antagonistic potential of selected biocontrol agents. This integrated approach is hypothesized to improve plant health, reduce disease incidence, and contribute to sustainable chickpea production. The objectives of this research are to evaluate the efficacy of biofortified vermicompost in suppressing *Fusarium oxysporum* f. sp. *ciceris* under both controlled and field conditions.

**Materials and Methods**

**Experimental site**

The *in vitro* experiments were conducted in the Department Plant Pathology, Faculty of Agricultural Sciences and Allied Industries, Rama University, Kanpur, India. The *in vivo* experiments were carried out in the polyhouse and agricultural field of the same department.

**Isolation of the pathogen**

The pathogen was isolated from infected chickpea roots using Potato Dextrose Agar (PDA) medium. Small tissue segments (3–5 mm) comprising both diseased and adjacent healthy areas were excised and surface-sterilized with 0.1% mercuric chloride (HgCl₂) for 30 seconds. The tissues were then rinsed thoroughly three times with sterile distilled water. Under aseptic conditions, the sterilized segments were placed onto sterile Petri dishes containing PDA medium and incubated at 20–25 °C for 5 to 6 days. Upon appearance of fungal growth, a portion of the actively growing colony was excised using a cork borer and transferred to fresh PDA plates to obtain a pure culture. Additionally, a small portion of the mycelium was transferred to PDA slants for long-term storage. The pure culture of *Fusarium oxysporum* was maintained on PDA slants through periodic sub-culturing onto fresh medium and stored at 4 °C for preservation throughout the study.

**Plant Material**

Seeds of chickpea variety “Pusa Chickpea 10216” were obtained from ICAR-Indian Institute of Pulse Research, Kanpur, India. Seeds were surface sterilized with 0.1% HgCl2 for 30sec, washed thrice with sterile distilled water and sown as per experimental design.

**Pathogenecity test**

The chickpea variety “Pusa Chickpea 10216” was selected to assess the pathogenicity of Fusarium oxysporum. Seeds were surface-sterilized using 1% sodium hypochlorite for 30 seconds, followed by two rinses with sterile distilled water, and then allowed to air dry. A sterilized soil mixture comprising sandy loam, vermicompost, and farmyard manure in a 2:1:1 ratio was prepared by autoclaving at 15 lbs pressure for 30 minutes over three consecutive days. Half of this sterilized soil was amended with crushed mycelial powder of F. oxysporum. The treated seeds were sown in 15 × 10 cm² pots and maintained under greenhouse conditions. As controls, untreated seeds were planted in both pathogen-infested and pathogen-free soil to serve as positive and negative controls, respectively.

**Vermicompost**

Vermicompost was obtained from the vermicompost production unit atFaculty of Agricultural Sciences and Allied Industries, Rama University, Kanpur, India

**Source of BCAs used**

The biological control agents used in this study *viz*. *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the culture repository of Department Plant Pathology, Faculty of Agricultural Sciences and Allied Industries, Rama University, Kanpur, India.

**Microbial fortification of vermicompost**

Three biological control agents (BCAs) Trichoderma harzianum, Pseudomonas fluorescens, and Bacillus subtilis were selected for this study due to their proven compatibility and established effectiveness in suppressing soilborne diseases in various crops. Each of these BCAs was used separately to enrich freshly prepared vermicompost. For bacterial treatments, 1 liter of 2-day-old cultures grown in nutrient broth (NB), with an approximate cell density of 2 × 10⁸ CFU/ml, was thoroughly mixed with 25 kg of vermicompost in individual trays. Similarly, for T. harzianum, 1 liter of a 5-day-old culture grown in potato dextrose broth (PDB), with a CFU count of around 4 × 10⁷, was blended into a separate tray containing 25 kg of vermicompost. All trays were placed in shaded conditions and covered with dark polyethylene sheets for 10 days to allow the BCAs to acclimate and colonize the substrate.

**Table 1** Combination of treatmentsused for conducting experiment

|  |  |
| --- | --- |
| **Treatment No.** | **Treatment** |
| **T1** | Vermicompost +*Trichoderma harzianum*+ Pathogen |
| **T2** | Vermicompost *+ Bacillus subtilis +* Pathogen |
| **T3** | Vermicompost *+ Pseudomonas flourescens +* Pathogen |
| **T4** | Vermicompost + Pathogen |
| **T5** | Control (Only vermicompost) |

**Pot experiments**

Plastic pots (15 × 10 cm) were used to evaluate the plant growth-promoting and antagonistic effects of vermicompost fortified with F. oxysporum antagonists. Sterilized soil was mixed with microbially fortified vermicompost in a 1:1 (w/w) ratio (1.5 kg per pot). Treatments included vermicompost enriched individually with T. harzianum, B. subtilis, and P. fluorescens. Positive control pots contained only vermicompost, while negative controls had only sterile soil.

**Pathogen inoculation**

Spore suspension was prepared by adding 20 ml of sterile distilled water to 5–7-day-old fungal cultures, followed by gentle scraping with a spore harvester. The conidial concentration was adjusted to 2–3 × 10⁷ conidia/ml using a haemocytometer. For all five treatments, 5 ml of this suspension was applied to each seedling via soil drenching, wherein the fungal suspension was pipetted around the root zone. Prior to inoculation, seedling roots were slightly wounded approximately 1 cm from the stem using a sterile needle to facilitate pathogen entry. Wilt symptoms were monitored for up to five weeks post-inoculation.

**Observations recorded**

Observations on various morpho-physiological traits were recorded at 30, 60, and 90 days after sowing (DAS) using a random sampling method. Three plants per treatment per replication were randomly selected for data collection. The values were averaged for each replication, and the means were used for statistical analysis. Standard agronomic practices were followed to ensure healthy crop growth.

**Biochemical Analysis**

Biochemical analysis for determination of different antioxidants and ROS (H2O2) in the leaves of chickpea plants at different time intervals after pathogen inoculation was performed according to the method of Singh *et al*. (2013). The enzymatic assays namely phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD) and total phenol content (TPC) was performed after 0, 24, 48, 72 and 96 h pathogen inoculation as described by Jain *et al*. (2011).

***Superoxide dismutase (SOD) assay***

SOD (EC 1.15.1.1) activity was assayed following the method of Fridovich (1974) by measuring the ability of enzyme extract from samples to inhibit photochemical reduction of nitroblue tetrazolium (NBT) chloride. Fresh leaves (0.1 g) from each treatment group were ground in 2.0 ml of extraction buffer (0.1 M phosphate buffer with 0.5 mM EDTA, pH 7.5) using a chilled mortar and pestle. The resulting homogenate was centrifuged at 15,000 × g for 20 minutes at 4 °C. For the assay, the reaction mixture was prepared with 200 mM methionine, 2.25 mM NBT, 3 mM EDTA, 100 mM phosphate buffer (pH 7.8), 1.5 M sodium carbonate, and the enzyme extract, adjusting the total volume to 3 ml. The reaction was initiated by adding 0.4 ml of 2 μM riboflavin, and the tubes were exposed to light from two 15 W fluorescent lamps for 15 minutes. Tubes without enzyme extract were used as controls. The reaction was stopped by turning off the light and placing the tubes in the dark until absorbance was measured at 560 nm. One unit of superoxide dismutase (SOD) activity was defined as the amount of enzyme required to inhibit the absorbance by 50% compared to the control lacking the enzyme.

***Phenylalanine ammonia-lyase (PAL) assay***

A 0.1 g leaf sample from each treatment was ground in 2 ml of 0.1 M sodium borate buffer (pH 7.0, 4 °C) containing 1.4 mM β-mercaptoethanol, using chilled equipment. The homogenate was centrifuged at 16,000 rpm for 15 minutes at 4 °C, and the supernatant was collected as the enzyme extract. For the enzyme assay, 0.2 ml of the extract was mixed with 0.5 ml of 0.2 M borate buffer (pH 8.7) and 1.3 ml of distilled water. The reaction was started by adding 1 ml of 0.1 M phenylalanine (pH 8.7) and incubated at 32 °C for 30 minutes. To stop the reaction, 0.5 ml of 1 M trichloroacetic acid (TCA) was added. Phenylalanine ammonia-lyase (PAL; EC 4.1.3.5) activity was quantified by measuring the formation of trans-cinnamic acid at 290 nm, following the method described by Brueske (1980). The results were expressed as µmol of trans-cinnamic acid per gram of fresh weight (FW).

***Total phenolic content (TPC) assay***

Total phenolic content (TPC) was estimated using the method described by Zheng and Shetty (2000). Leaf samples (0.1 g) were soaked in 5 ml of 95% ethanol and stored at 0 °C for 48 hours. The samples were then homogenized and centrifuged at 13,000 rpm for 10 minutes. From the resulting supernatant, 1 ml was taken and mixed with 1 ml of 95% ethanol, 5 ml of sterile distilled water, and 0.5 ml of 50% Folin–Ciocalteu reagent. The mixture was thoroughly mixed and, after 5 minutes, 1 ml of 5% sodium carbonate solution was added. The reaction mixture was left to stand for 1 hour, after which the absorbance was measured at 725 nm. A standard curve was generated using different concentrations of gallic acid (GA; Sigma-Aldrich-27645) in 95% ethanol, and TPC was expressed as mg gallic acid equivalents (GAE) per gram of fresh weight (FW).

***Polyphenol oxidase (PPO) assay***

Leaf tissue (0.1 g) was homogenized in 2 ml of ice-cold 0.1 M phosphate buffer (pH 6.5). The homogenate was then centrifuged at 16,000 rpm for 30 minutes at 4 °C, and the resulting supernatant was used directly for the enzyme assay. The reaction mixture consisted of 0.4 ml of 1 mM catechol and 0.4 ml of the enzyme extract, both added to 3 ml of 0.05 M sodium phosphate buffer (pH 6.5). A control reaction containing only the substrate was also prepared. Polyphenol oxidase (PPO; EC 1.14.18.1) activity was assessed using catechol as the substrate, and the increase in absorbance was measured at 405 nm as described by Gauillard et al. (1993). PPO activity was expressed as the rate of change in optical density per minute per gram of fresh weight (ΔOD min⁻¹ g⁻¹ FW) based on the linear portion of the absorbance curve.

***Peroxidase (PO) assay***

Peroxidase (PO; EC 1.11.1.7) activity was measured following the method of Hammerschmidt *et al*. (1982) with minor modifications. Individual leaf samples (0.1 g) were homogenized in 2 ml of 0.1 M phosphate buffer (pH 7.0) at 4 °C. The homogenates were centrifuged at 16,000 × g for 15 minutes at 4 °C, and the resulting supernatants were used as the enzyme source. The reaction mixture contained 1.5 ml of 0.05 M pyrogallol, 0.05 ml of the enzyme extract, and 0.5 ml of 1% (v/v) hydrogen peroxide. A control without the enzyme extract was also prepared. Absorbance was recorded at 420 nm at 30-second intervals over a period of 3 minutes. Peroxidase activity was expressed as units of change in absorbance per minute per gram of fresh weight (U min⁻¹ g⁻¹ FW).

**Determination of Disease Incidence**

Disease incidence was assessed using a 0–4 scale based on the severity of wilt symptoms, as described by Song *et al*. (2004). A score of 0 indicated a completely healthy plant with no visible symptoms, while a score of 4 represented a fully wilted plant. Intermediate scores reflected varying levels of disease severity:

* **Scale 1**: 1–20% leaf yellowing and wilting
* **Scale 2**: 21–40% leaf yellowing and wilting
* **Scale 3**: 41–60% leaf yellowing and wilting
* **Scale 4**: Entire plant exhibited yellowing and complete wilting, indicating full infection

**Results**

**Isolation, purification and maintenance of *F. oxysporum* isolates**

The isolates purified and identified as *F. oxysporum*based on morphological and cultural characters using the descriptions given by C.M.I (1970). The isolates were designated serially from Foc 1 to Foc 7. The purified isolates were maintained in PDA slants and stored at 4 ͦ C for further use.

**Test of pathogenicity of *F. oxysporum* isolates**

` After isolation and purification of *F. oxysporum*from the collected samples, they were subjected to the pathogenicity tests on susceptible genotype of chickpea cultivar “Pusa Chickpea 10216” through soil inoculation method. Out of 7 isolates of *F. oxysporum*tested for pathogenicity, 5 isolates showed typical wilt symptoms like drooping and wilting of lower leaves. Plants showed yellowing of the lower leaves, occasional formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of the remaining leaves, and finally death of the plant. Thus, 7 isolates showed positive result for Koch’s postulate while the remaining 2 isolates failed to prove Koch’s postulate indicating their non-pathogenicity to chickpea. Those 5 isolates which showed positive results for Koch’s postulate were selected for further study. The selected isolates were named as Foc 1, Foc 2, Foc 3 Foc 4, Foc 5 (Table 1). The colony characteristics of these isolates were studied .These selected isolates were further studied for per cent disease incidence (PDI) through ‘soil inoculation’ method. The results of pathogenicity are presented in Table 2.

**Table 2** Test of pathogenicity of *F. oxysporum*isolates collected from different districts of Eastern Uttar Pradesh

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Isolate name** | **Root rot symptoms** | **Koch postulates** |
| 1 | Foc1 | +ve | +ve |
| 2 | Foc 2 | +ve | +ve |
| 3 | Foc 3 | +ve | +ve |
| 4 | Foc 4 | +ve | +ve |
| 5 | Foc 5 | +ve | +ve |
| 6 | Foc 6 | -ve | -ve |
| 7 | Foc 7 | -ve | -ve |

**Study of the effect of selected isolates of *F. oxysporum* on chickpea in pots**

The percent disease incidence (PDI) of ten selected Fusarium oxysporum isolates was evaluated under greenhouse conditions using a soil inoculation method. Observations were recorded from 30 to 120 days after inoculation (DAI) (Table 3). No PDI was observed for any isolate at 30 DAI. However, by 60 DAI, isolates Foc 2 and Foc 4 showed PDIs of 19.52% and 16.43%, respectively. By 90 DAI, all ten isolates exhibited varying levels of disease incidence. The highest PDI at this stage was recorded for Foc 2 (35.55%), followed by Fol 8 (30.12%), with other isolates showing comparatively lower values. Fol 2 consistently showed the highest PDI from 60 to 120 DAI, while Foc 4 ranked second in aggressiveness during 90–120 DAI. Based on these results (Table 3), Fol 2, isolated from Kanpur, was identified as the most virulent and was therefore selected as the test pathogen for further experimentation.

**Table 3** Effect of selected *F. oxysporum* isolates on disease incidence of chickpea through soil inoculation method**.** Results are expressed as mean of triplicates ± S.D.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name of the isolates** | **Per cent disease incidence (%)** | | | | |
| **Days after inoculation** | | | | |
| **30** | **60** | **90** | **105** | **120** |
| Control | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 |
| Foc 1 | 0 ±0 | 0 ±0 | 23.63 ±5.25 | 47.95 ±8.22 | 70.25 ±13.41 |
| Foc 2 | 0 ±0 | 19.52 ±5.25 | 35.55 ±7.33 | 53.85 ±8.33 | 89.63 ±14.33 |
| Foc 3 | 0 ±0 | 0 ±0 | 22.75 ±6.38 | 42.32 ±9.75 | 65.85 ±14.33 |
| Foc 4 | 0 ±0 | 16.43 ±5.25 | 30.12 ±6.25 | 47.51 ±8.22 | 81.25 ±10.25 |
| Foc 5 | 0 ±0 | 0 ±0 | 21.40 ±8.45 | 31.42 ±9.25 | 62.70 ±12.33 |

**Effect of biofortified vermicompost on defense related enzymes in chickpea plants challenged with *F*. *oxysporum* f. sp. *ciceris***

***Phenylalanine Ammonia Lyase (PAL)***

PAL activity showed a significant increase across all treatments up to 48 hours, after which a decline was observed. The highest PAL activity at 48 hours was noted in plants treated with T. harzianum-fortified vermicompost (T1), followed by treatments T2, T3, and T4. Specifically, T1 exhibited a 3.6-fold increase in PAL activity compared to the control. Similarly, T2 and T3 recorded 3.5-fold and 2.5-fold increases, respectively. Even plants treated with non-fortified vermicompost (T4) demonstrated elevated PAL levels relative to the control, with a 2.3-fold increase observed at 48 hours (Figure 1).



**Figure 1** PAL activity at different time intervals in chickpea raised from seeds sown in soil amended with biofortified vermicompost challenged with *F. oxysporum*.

***Peroxidase (PO)***

Peroxidase (PO) activity increased significantly across all treatments up to 72 hours, followed by a subsequent decline. The highest PO activity at 72 hours was observed in plants treated with T. harzianum-enriched vermicompost (T1), followed by T2, T3, and T4. At this time point, T1 recorded an 8.2-fold increase in PO activity relative to the control. Treatments T2 and T3 also showed substantial increases of 7.5- and 4.5-fold, respectively. Even plants grown with non-fortified vermicompost (T4) exhibited higher PO levels compared to the control, with 3.09- and 2.3-fold increases at 48 and 72 hours, respectively (Figure 2).



**Figure 2** Effect of microbial fortified vermicompost on PO activity

***Polyphenol oxydase (PPO)***

Polyphenol oxidase (PPO) activity showed a notable increase in all treatments up to 72 hours, after which a decline was observed. The highest PPO activity at 72 hours was found in plants treated with T. harzianum-fortified vermicompost (T1), followed by treatments T2, T3, and T4. At this time point, T1 exhibited a 5.4-fold increase in PPO levels compared to the control. Plants receiving only vermicompost (T4) also demonstrated elevated PPO activity, showing a 2.3-fold increase over the control at 72 hours (Figure 3).



**Figure 3** Effect of microbial fortified vermicompost on PPO activity**.**

**Total phenol content (TPC)**

TPC followed a pattern similar to that of PAL, peaking at 48 hours in T1 before dropping sharply. Significant differences in TPC were observed among the treatments. The highest phenolic accumulation occurred in T1, showing a 6.5-fold increase over the control. T2, T3, and T4 also showed elevated levels at 48 hours, with 5.4-, 3.9-, and 3.8-fold increases, respectively, compared to the control (Figure 4).



**Figure 4** TPC activities at different time intervals in chickpea in different treatment.

**Discussion**

**Effect of microbial fortified vermicompost on growth parameters of chickpea crop**

It is widely accepted that the application of composts and vermicomposts as soil amendments can improve nutrient availability, enhance soil health, and positively influence various plant traits when compared to synthetic fertilizers (Singh *et al*., 2022; Oyege *et al*., 2023; Mulatu and Bayata, 2024). Vermicomposts, in particular, play a crucial role in boosting plant growth and yield due to their rich composition of micro- and macronutrients, enzymes, hormones, and vitamins. These amendments provide essential nutrients like nitrates, soluble potassium, exchangeable phosphorus, magnesium, and calcium in bioavailable forms. Additionally, vermicomposts offer a large surface area and numerous microsites, supporting prolonged nutrient retention and heightened microbial activity in the rhizosphere (Raza *et al*., 2022; Nieto-Cantero *et al*., 2025; Goldan *et al.*, 2023).

The use of biofortified vermicompost resulted in notable improvements in chickpea growth and a significant reduction in disease incidence. Marked differences were observed in root and shoot length, as well as dry biomass among the treatments. These findings align with previous studies. For example, Wang *et al*. (2017) reported enhanced chickpea growth when vermicompost was combined with microbial inoculants like Bacillus pumilus, Trichoderma, and Glomus mosseae. Similarly, Bachman and Metzger (2008) highlighted increased productivity and nematode suppression in brinjal using vermicompost and biopesticides. Shariati *et al*., 2013 also noted the growth-promoting effects of vermicompost, especially when used alongside Pseudomonas fluorescens-based biopesticides in chickpea.

**Effect of fortified vermicompost on activity of defense related enzymes in chickpea**

Plants have evolved various defense mechanisms to combat invading pathogens. The present study shows that application of biofortified vermicompost enhances these defense responses in chickpea, particularly when challenged with Fusarium oxysporum. Plants treated with vermicompost enriched with beneficial microbes like T. harzianum, Bacillus subtilis, and Pseudomonas fluorescens demonstrated higher levels of defense-related enzymes—PAL, PO, PPO, SOD—and total phenolic content. These responses are indicative of induced systemic resistance (ISR), which is known to be triggered by root-colonizing beneficial microbes (van der Ent *et al*., 2009).

Among the treatments, T. harzianum-fortified vermicompost (T1) consistently showed the highest enzyme activities, particularly PAL at 48 h and PO and PPO at 72 h. PAL plays a key role in phenylpropanoid metabolism and is directly associated with the production of antimicrobial compounds and lignin precursors (Bisen *et al*., 2019; Nawrocka *et al*., 2018; AbuQamar *et al*., 2017). Similar findings were reported by Amooaghaie *et al*. (2018), who observed increased resistance in psyllium with combined application of vermicompost and B. subtilis.

Enzymes such as SOD and PO are crucial for mitigating oxidative stress and enhancing cell wall fortification (Jain *et al*., 2012; Rao *et al*., 2015). Our findings of increased PO levels up to 72 h align with reports in cucumber treated with vermicompost under Pythium infection (Sabbagh and Valizadeh, 2016). PPO activity also rose significantly in all treated plants, supporting previous reports that link PPO induction with enhanced resistance (Jain et al., 2012).

Overall, the elevated activity of PAL, PO, and PPO in biofortified treatments—especially with T. harzianum—suggests an effective defense strategy through enzyme-mediated resistance. These results confirm earlier findings by Bosco et al. (2017) in chickpea and emphasize the role of fortified vermicompost as a sustainable approach to improve disease resistance against F. oxysporum.

**Conclusion**

The findings of this study suggest that the application of vermicompost, whether used alone or enriched with agriculturally beneficial microorganisms, offers a sustainable and environmentally responsible method for managing plant diseases. This approach holds strong potential to support sustainable agricultural practices. Specifically, biofortifying vermicompost with microbes such as Trichoderma harzianum, Pseudomonas fluorescens, and Bacillus subtilis was found to enhance plant growth and improve the nutritional quality of tomato crops. These results indicate that biofortified vermicompost could be further evaluated in large-scale field applications as part of an eco-friendly plant disease management strategy. Additionally, vermicomposting represents a valuable method for converting organic waste into nutrient-rich compost, thereby contributing to waste reduction and environmental cleanliness.

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