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RESEARCH ARTICLE

Enhancing defense enzymes by fungal and bacterial biocontrol agents in cabbage against head rot disease caused by *Sclerotinia sclerotiorum*

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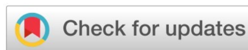

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Abstract

The pathogen *Sclerotinia sclerotiorum* is the source of cabbage head rot disease, which causes significant economic loss in the Nilgiris district, Tamil Nadu. To manage the disease, biocontrol agents were isolated from the rhizosphere region of cabbage and the antagonistic properties were assessed for their capacity to control cabbage head rot under *in vitro* conditions. *Trichoderma viride* (Tv3) recorded 86% inhibition and *Pseudomonas fluorescens* (PfC5) inhibited 80% reduction of mycelial growth of *Sclerotinia sclerotiorum.* During storage, the effectiveness of different carrier materials in maintaining the population of these biocontrol agents was evaluated. In glasshouse studies, the use of biocontrol agents in combination (Seed treatment + Soil application with (PfC5 + Tv3) + Foliar spray with PfC5) significantly recorded maximum (72.50) per cent disease reduction. Upregulation of defense genes triggered the enzymes viz., polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia lyase (PAL) and phenol were accumulated in cabbage treated with fungal and bacterial biocontrol agents and reduced the head rot disease incidence in cabbage and increase the yield.

Keywords

biocontrol agents; cabbage; defense related enzymes; head rot; *Sclerotinia sclerotiorum*

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is a green leafy vegetable crop cultivated worldwide, viz., Russia, Indonesia, India, China, Japan, Germany and Poland. In India, several states, including Tamil Nadu, Uttar Pradesh, Karnataka, Orissa, Bihar, Assam, Maharashtra and West Bengal. Notably, India ranks as the third-largest cabbage producer globally and its production was 2.17 MT between 2022 and 2023 (1). Diseases such as head rot, damping off, club root, black rot, Alternaria leaf blight and downy mildew are the main obstacles to cabbage production. In 1978, cabbage head rot disease was first reported in Tamil Nadu. (2). The initial symptom is water-soaked patches on the upper or lower leaves, which become larger

and soften the affected tissue before the outer leaves wilt. Additionally, the cabbage's head developed a white, cottony growth; later, the fungus produced the hardy, black resting structures (Fig. 1). The disease is caused by *Sclerotinia sclerotiorum* which is widely distributed to all cabbage growing regions in the world (3).

Chemical fungicides are used extensively to combat plant diseases, but the pollutants and phytotoxic residues they leave behind have increased the risks to human health. Plant diseases can be controlled biologically by utilizing bacteriophages, rhizobacteria, avirulent pathogen strains and bacterial metabolites as hostile organisms. Plant growth-promoting rhizobacteria (PGPR) are the most important antagonists for treating plant diseases. Biological control is a good alternative to chemical control, eliminating a wide range of micro and macroorganisms without affecting the environment (4). In addition to directly increasing plant development, plant growth-promoting rhizobacteria (PGPR) are used to manage diseases and create host resistance (5).

The high reproduction rate and fast pathogen expansion make it difficult to manage soil-borne diseases. Biocontrol agents are safe for people, non-polluting, biodegradable, selective in their mode of action, do not affect other beneficial microorganisms and usually improve the physical state of soil and agricultural sustainability, managing the diseases efficiently (6). *Pseudomonas fluorescens* and *Bacillus* strains are examples of rhizobacteria that have the potential to significantly reduce disease and improve plant growth and productivity. Antagonistic bacteria's quick growth, ease of handling and aggressive rhizosphere colonization make them excellent biological control agents (7). *Trichoderma* sp. can withstand other soil-dwelling organisms' antagonistic actions, allowing for incredibly fast growth and extensive spore, enzyme and antibiotic synthesis.

One of the biological control mechanisms known as induced systemic resistance (ISR) protects plants on a systemic level by enhancing their defense enzymes against various diseases. Once produced, ISR causes plants to activate multiple defense mechanisms, such as the improved activity of PPOs, PODs, phytoalexins, PAL and phenol. One enzyme that responds quickly to plant pathogens is POD, which is engaged explicitly in wound healing, hydroxyproline-rich glycoprotein polymerization, lignification, suberification, regulation of cell wall elongation and plant resistance to infections. Plants have an enzyme called PPO that controls growth and protects plants from biotic and abiotic stressors (8). Biocontrol agents enhance plant growth and reduce disease incidence in an environmentally friendly manner. The combined use of multiple biocontrol agents, each with a different mode of action, has proven highly effective. The present study investigates how biocontrol agents induce defense enzyme activity in cabbage plants and reduces the incidence of disease. An eco-friendly approach to disease management is always preferable, as it provides maximum disease suppression without causing harm to the ecosystem. The following research was designed to develop a sustainable system for managing cabbage head rot disease.

Materials and Methods

Pathogen isolation

The cabbage plants exhibiting head rot symptoms were gathered from several areas of Nilgiris, Tamil Nadu, India. To isolate the pathogen, the tissue segment approach was used on the infected cabbage tissues (9). After being cut into small pieces with a sterile scalpel, the infected cabbage was surface sterilized by 0.1% mercuric chloride for 1 min, rinsed in 3 rounds of sterile distilled water and then put in a petri dish with Potato Dextrose Agar (PDA) medium that had been poured and solidified before hand. The development of the fungus was monitored over the 5 days when these plates were incubated at (28 °C). The fungal hyphal tips were moved to PDA slants to preserve the culture for future research.

Isolation of bacterial and fungal biocontrol agents

Antagonistic bacteria and fungi were identified from the cabbage plants' rhizosphere soil, which was taken from Nilgiris, Tamil Nadu. Plants were carefully uprooted with intact roots and gently removed excess soil. In a 250 mL Erlenmeyer flask with 100 mL of sterile distilled water, 10 g of rhizosphere soil were added. The suspension was thoroughly shaken and antagonists were isolated using the serial dilution plate method. Sterilized petri plates containing *Trichoderma* special medium (TSM) and King's

Fig. 1. Symptoms of cabbage head rot disease and sclerotia.

B medium were filled with 1 mL of each of the final dilutions (10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) (10). The plates were incubated for 24 h at room temperature (28 ± 2 °C) after being rotated to ensure even distribution. The streak plate method on King's B medium was used to isolate and purify colonies that looked like *Pseudomonas* sp. (11). After being separated from TSM plates, *Trichoderma* sp. was purified on a PDA medium. At 4 °C, pure bacteria and fungus cultures were kept on corresponding agar slants.

Evaluation of antagonistic bacterial and fungal isolates against Sclerotinia sclerotiorum

Four isolates of *Trichoderma viride* (Tv1, Tv2, Tv3 and Tv4) and 6 strains of *Pseudomonas fluorescens* (PfC1, PfC2, PfC3, PfC4, PfC5 and PfC6) were tested for antifungal activity against *Sclerotinia sclerotiorum* using the dual culture method (12). Near the Petri plate's border, a mycelial disc measuring 9 mL *Trichoderma viride* or *Pseudomonas fluorescens* and *Sclerotinia sclerotiorum* were positioned opposite one another and kept at room temperature for incubation (28 ± 2 °C 3/7/2025). After 4 days incubation, the inhibition zone and the pathogen's mycelial growth were assessed in both treatment-imposed and control plates. Seven days after incubation, the antagonists' overgrowth over the pathogen was measured and the percentage inhibition (PI) of mycelial growth was computed (13). The zone of inhibition and overgrowth were measured and reported in millimeters and centimeters respectively.

Shelf life of Pseudomonas fluorescens (PfC5) and Trichoderma viride (Tv3) in various carrier materials

The best strains of *T. viride* (Tv3) and *P. fluorescens* (PfC5) were utilized for formulation development. Gypsum, vermiculite, talc, farmyard manure, lignite and peat soil were the 6 carriers used to assess the shelf life of bacterial and fungal cultures. The serial dilution technique was used to examine the survivability of biocontrol agents in various carrier materials kept at 28 $\pm$ 2 °C 3/7/2025 (14). For 10 days at room temperature (28 °C), strain PfC5 was cultured in King's B broth (KMB) for 48 h at 150 rpm in a shaking culture. 400 mL of 72-h-old *P. fluorescens* (PfC5) culture in their respective medium (1 kg) with a population of 9 x10^7 cfu/mL were mixed with 15 g of calcium carbonate and 10 g of carboxy methyl cellulose and packed in polythene bags. The contents were drained after growing *T. viride* (Tv3) in yeast molasses medium for 15 days. Subsequently, the fungal biomass was removed and combined with the appropriate carrier materials (1 kg) and 5 g of carboxyl methyl cellulose. The mixture was then sealed in polythene bags and incubated at room temperature. Following a 20 days storage period, samples were taken at 10 days intervals and dilution plate techniques were used to evaluate the *T. viride* (Tv3) population.

Efficacy of biocontrol agents against head rot disease of cabbage in glass house condition

An experiment investigated the effectiveness of *P. fluorescens* (PfC5) and *T. viride* (Tv3) against cabbage head rot disease. The *Sclerotinia sclerotiorum* were multiplied on sand-maize medium (15), including maize powder and sand (19:1), soaked in 400 mL of water per kilogram and then sealed in polypropylene bags. Two 9 mm culture discs of vigorously developing Potato Dextrose Agar (PDA) *S. sclerotiorum* were inoculated into each bag after the bags were sterilized for 2 days continuously at 120 °C and 15 psi for 20 min. For 15 days, they were kept at room temperature (28 °C) and utilized as a source of inoculum. 5 kg of soil were filled in each earthen pot (30 cm in diameter by 60 cm in height) and cabbage seedlings were planted in pots. Up to harvest, the disease incidence percentage was measured every 15 days. Before enzyme extraction for ISR investigations, the leaves were repeatedly cleaned with sterile distilled water after being removed from the pots at 0, 3, 5, 7 and 9 days following the challenge inoculation with *S. sclerotiorum*. At 15 day intervals till harvest, the percentage of disease incidence was noted.

Treatment details

Six treatments were formulated to study the effect of fungal biocontrol agents against *S. sclerotiorum*, as illustrated in Table 1.

Induced Systemic Resistance related enzymes

Estimation of peroxidase activity: 0.1 M sodium phosphate buffer (pH 6.5) was used in 2 mL to homogenize 1 g of cabbage leaf tissue. The supernatant, which acted as an enzyme source, was then extracted from the leaf after it had been centrifuged at 15000 × g for 15 min at 4 °C. Next, 1.5 mL of 0.05 M pyrogallol, 0.5 mL of 1% H_2O_2 and 0.1 mL of enzyme extract were mixed. For 3 min, at room temperature (28 °C), the change in absorbance at 420 nm was measured in a spectrophotometer at 30 sec intervals. The change in absorbance min^{-1} g^{-1} of the leaf was used to represent the enzyme activity of the reaction mixture (16).

Estimation of polyphenol oxidase activity: 1 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 1 g of freshly crushed leaf material were used for the experiment. The supernatant was used as the source of enzymes after the homogenate was centrifuged for 15 min at 15000 × g at 4 °C. To initiate the reaction, 0.2 mL of catechol (0.01 M) was added after 0.2 mL of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) had been combined. The activity was measured with a spectrophotometer (Cary 60, Agilent Technologies) and expressed as a change in absorbance at 495 nm. Per gram of leaf tissue, the change in absorbance per minute was used to express the enzyme activity (17).

Table 1. Treatment details adopted in the study

T1	Foliar spray with *P. fluorescens* (PfC 5) 5 g/L
T2	Seed treatment (ST) 10 g/kg of seeds + Soil application (SA) with *P. fluorescens* at 2.5 kg/ha
T3	Seed treatment (ST) 4 g/kg of seeds + Soil application (SA) with *T. viride* (Tv3) at 2.5 kg/ha
T4	ST+ SA with (PfC5 + Tv3) + Foliar spray with PfC5
T5	Carbendazim (0.1%)
T6	Untreated control

Estimation of phenyl alanine lyase activity: 1 g of leaf was homogenized in 5 mL of cold 25 mM borate HCl buffer (pH 8.8) that contained 5 mL of mercaptoethanol (0.4 mL/L) to estimate the activity of PAL. The supernatant was used as an enzyme source following a 15 min centrifugation of the homogenate at 15000 x g. The assay mixture comprises 0.2 mL of enzyme extract, 1.3 mL of water and 0.5 mL of borate buffer. After adding 1 mL of 12 mM L-phenylalanine to initiate the reaction, it was incubated at 32 ˚C for 1 hr. After adding 0.5 mL of 2 N HCl, the process ceased. The absorbance was measured with a spectrophotometer (Cary 60, Agilent Technologies) at 290 nm. A value of m mol of cinnamic acid/min/g of leaf tissue was used to express the enzyme activity (18).

Estimation of phenols activity: In a pestle and mortar, 1 g of the leaf sample was ground in 10 mL of 80% methanol. For 20 min, the homogenate was centrifuged at 10000 rpm. 5 mL of distilled water was used to dissolve the residue after the supernatant was dried off by evaporation. From this, 0.2 mL was extracted, distilled water was added to bring the volume up to 3 mL and then the Folin-Ciocalteau reagent (1 N) was added in 0.25 mL. After 3 min, 1 mL of 20% sodium carbonate was added and thoroughly mixed. The tubes were submerged in boiling water for 1 min before being cooled. The absorbance at 725 nm was measured using a reagent blank. The phenol activity was expressed as mg of catechol/g of leaf tissue (19).

Statistical Analysis

The statistical analysis of the experiment data was carried out by adopting a standard method (20).

Results and Discussion

Fungal and bacterial cultures were isolated from the soil collected from the rhizospheric region of cabbage growing in different Nilgiris, Tamil Nadu. *Sclerotinia sclerotiorum*, the pathogen that causes head rot, was studied *in vitro* for antagonistic action by biocontrol agents. Of the tested isolates, *T. viride* (Tv3) had the highest (86%) inhibition on the pathogen's mycelial growth with an inhibition zone of 3.15 mm. *Pseudomonas fluorescens* (PfC5) had the second highest (80%) inhibition on mycelial growth of *S. sclerotiorum* with an inhibition zone of 2.56 mm (Table 2). In the pot culture experiment, the efficacy of biocontrol agents against head rot disease of cabbage was tested. Among the 6 treatments, T4 (ST + SA with (PfC5 + Tv3) + FA with PfC5) treatment significantly recorded maximum (72.50%) disease reduction of head rot disease followed by T3 and T2 treatments were recorded 67 and 66% reduction of the disease respectively (Fig. 2, Table 3).

As a novel defense mechanism against pathogen attack, fluorescent pseudomonads have been shown to induce defense responses against various diseases. Two types of resistance mechanisms were induced in plants: SAR (Systemic Acquired Resistance) was produced by other than the biocontrol agents and ISR (Induced Systemic Resistance) was induced by biotic agents (21). Examples of active or induced defense responses include the hypersensitive reaction, the production of PR proteins and phytoalexins, ion fluxes across the plasma membrane, the production of reactive oxygen species (ROS) and reactive nitrogen species (oxidative bursts), lignification, the cross-linking of structural proteins of the cell wall and the deposition of callose.

Fig. 2. Efficiency of biocontrol agents against cabbage head rot disease in pot culture under glasshouse condition.

Table 2. Antagonistic activity of biocontrol agents against the pathogen *Sclerotinia sclerotiorum in vitro* condition

Sl. No.	Isolates	Mycelial growth (cm)*	Percent Reduction Over Control (%)	Inhibition Zone (mm)
1	Tv1	2.65	70.42	1.87
2	Tv2	1.92	78.57	2.14
3	Tv3	1.28	85.71	3.15
4	Tv4	2.95	67.07	1.24
5	PfC 1	2.23	75.11	1.94
6	PfC 2	4.70	47.54	0.65
7	PfC 3	2.97	66.85	1.18
8	PfC 4	5.71	36.27	0.47
9	PfC 5	1.87	79.12	2.56
10	PfC 6	3.42	61.18	0.82
11	Control	8.94	-	
	CD (P=0.05)	**0.36**		

* Mean of 3 replications

Table 3. Efficiency of biocontrol agents against Head rot disease of cabbage under glass house condition

Sl. No.	Treatments	Percent Disease Incidence *	Percent reduction over control
1	T1- Foliar spray with *P. fluorescens* (PfC5)	22.46	61.48
2	T2- ST + SA with *P. fluorescens* (PfC5)	19.95	65.80
3	T3- ST + SA with *T. viride* (Tv3)	19.13	67.19
4	T4- (ST + SA with (Pf + Tv) + Foliar spray with PfC5)	16.05	72.47
5	T5- Carbendazim (0.1%)	15.42	73.55
6	T6- Untreated control	58.32	-
	CD (P=0.05)	**1.58**	-

Twenty days following storage, the *T. viride* (Tv3) population was evaluated. The carrier materials showed that the population decreased from the initial level. The population was higher in talc powder (33.80 × 10^4 cfu/g) at the end of 90 days, followed by gypsum (26.06 × 10^4 cfu/g). The minimum population was observed in lignite (18.75 × 10^4 cfu/g) after 90 days. Therefore, it is determined that the talc-based formulation is an appropriate carrier to transport *T. viride*. (Table 4). *P. fluorescens* (PfC5) survival was assessed in different carrier materials. Talc recorded the maximum population (35.96 × 10^7 cfu/g) at the end of 90 days, followed by lignite (22.45 × 10^7 cfu/g). The minimum population was observed in vermiculite (13.57 × 10^7 cfu/g) at the end of 90 days. Therefore, the talc-based formulation is a highly suitable carrier material to deliver *P. fluorescens* (PfC5) (Table 5). Several formulations of biocontrol agents are being used to manage plant diseases. Rice blast, rice sheath blight, chickpea wilt and pigeon pea wilt were all successfully combatted by the talc-based powder formulation that included the antagonistic bacteria *P. fluorescens* (22). Using a variety of materials, including rice bran, peat soil, wheat bran, rice straw and farmyard manure (FYM), it was found that the optimum substrates for the bulk proliferation of *Trichoderma* spp. were FYM and wheat bran (23).

Proteins and lignin or suberin precursors are polymerized into plant cell walls by POD enzymes, creating a physical barrier that may prevent pathogens from penetrating cell walls or moving through vessels (24). Peroxidase activity was stimulated in cabbage plants treated with biocontrol agents and challenged inoculated with the pathogen *Sclerotinia sclerotiorum.* Five days following challenge inoculation with *Sclerotinia sclerotiorum*, the results showed that cabbage pretreated with consortia formulation T4 (ST + SA with (PfC5 + Tv3) + FA with PfC5) had considerably increased PO enzyme activity (1.348 changes in absorbance/min/g of leaf tissue). Throughout the monitoring period, there was no discernible change in the PO activity in the untreated plants (Fig. 3).

Comprehending PPO function and control using biochemical methods is challenging since the enzyme is cross-linked and covalently modified by the quinonoid reaction products. PPO activation was elevated in cucumber leaves around lesions brought on by specific foliar pathogens (25). After being treated with efficient biocontrol formulations and challenged with the pathogen *Sclerotinia sclerotiorum*, the PPO activity peaked 5 days later. Compared to the uninoculated control, the induction of PPO was doubled with these treatments. In comparison to the other treatments in cabbage, the treatment T4 (ST + SA with (PfC5 + Tv3) + FA with PfC5) recorded the highest degree of PPO activity (0.998 changes in absorbance /min/g of leaf tissue) (Fig. 4).

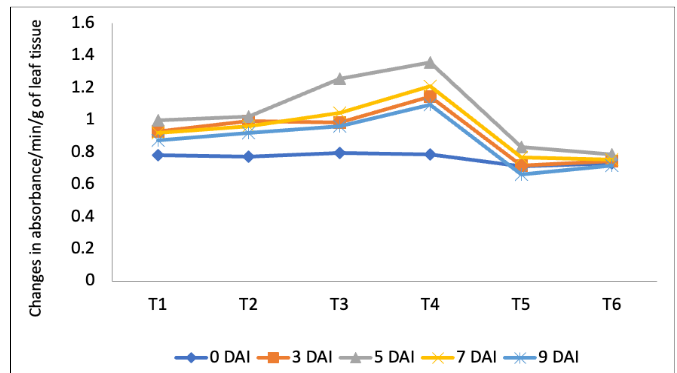

Fig. 3. Induction of peroxidase activity in cabbage plants treated with biocontrol agents.

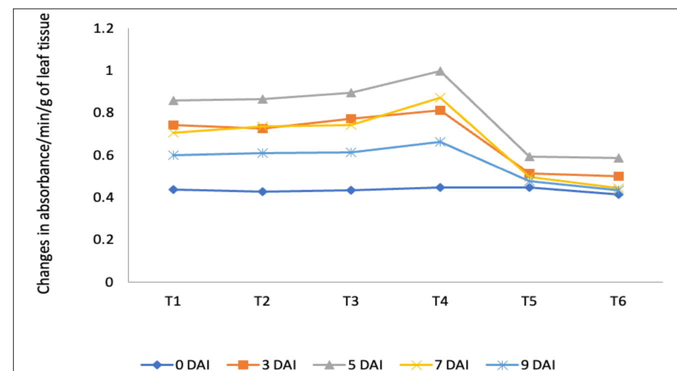

Fig. 4. Induction of polyphenol oxidase activity in cabbage plants treated with biocontrol agents.

Table 4. Shelf life of *Trichoderma viride* (Tv3) in six different carrier material

Sl. No.	Carrier materials	Population of *Trichoderma viride* ×10^4 CFU/g*								
		Days after inoculation (DAI)								
		0	20	30	40	50	60	70	80	90
1	Talc	46.60	46.23	43.82	41.62	40.55	38.91	36.47	35.21	33.80
2	Lignite	45.34	42.81	38.69	33.82	30.52	26.50	24.10	20.37	18.75
3	Peat soil	45.34	43.45	40.01	37.91	35.92	32.71	27.01	23.88	20.21
4	FYM	45.22	42.76	36.87	34.22	30.62	28.75	26.81	23.53	21.37
5	Vermiculate	45.43	43.12	40.95	38.15	35.62	33.82	31.84	29.91	25.17
6	Gypsum	46.32	43.29	42.12	40.61	37.61	34.92	31.41	29.90	26.06

*Mean of 3 replications

CD (P=0.05) Treatments = 0.93 Days = 0.99 Treatments × Days = 2.81

Table 5. Shelf life of *Pseudomonas fluorescens* (PfC5) in six different carrier material

Sl. No.	Carrier materials	Population of PfC5×10^7CFU/g*								
		Days after inoculation (DAI)								
		0	20	30	40	50	60	70	80	90
1	Talc	47.98	47.25	46.82	45.02	43.85	40.82	39.77	37.21	35.96
2	Lignite	47.63	44.60	42.52	39.71	37.64	33.64	30.81	26.43	22.45
3	Peat soil	47.34	42.54	40.11	36.90	32.92	29.71	26.01	23.48	15.21
4	FYM	46.72	45.78	39.87	35.24	31.67	29.15	26.81	24.53	21.07
5	Vermiculate	46.43	42.25	39.95	34.15	30.62	28.82	21.84	17.91	13.57
6	Gypsum	46.62	43.72	39.15	36.61	33.53	32.81	29.48	26.90	20.16

*Mean of 3 replications .CD (P=0.05) Treatments =0.96 Days= 1.01 Treatments × Days = 2.56

The PAL enzyme's activity reached 5 days after inoculation, remained elevated for 7 days and gradually decreased in all treatments. Consortial formulation of biocontrol agents T4 (ST + SA with (Pf C5 + Tv3) + FA with PfC5) recorded higher activity of PAL enzyme in leaves (Fig. 5). By transforming L-phenylalanine into trans-cinnamic acid, the enzyme PAL starts the metabolism of phenyl propanoid, which supplies the building blocks for lignins, phytoalexins and flavonoid pigments (26). A key player in the manufacture of phenolic phytoalexins was PAL. Transcinnamic acid, a consequence of PAL activity, served as the direct precursor for the synthesis of salicylic acid, a signal molecule in systemic acquired resistance (SAR) (27). Environmental events can trigger the phenylpropanoid pathway in plants, one of the most common metabolic stress responses. PAL catalyzes the production of natural plant products based on the phenylpropane skeleton, such as phytoalexins and the conversion of L-phenylalanine to transcinnamic acid (28).

Phenol accumulation began to rise on the third day following treatment, peaked 5 days after inoculation and gradually decreased. In pretreated cabbage with the consortial formulation of T4 (ST + SA with (PfC5 + Tv3) + FA with PfC5), maximum total phenol content on fifth DAI (664.97 µg of catechol/g leaf tissue) was recorded followed by T3 which accounted 623.21 µg of catechol/g leaf tissue (Fig. 6). *T. harzianum* enhanced the yield of sucrose, green foliage and roots per ha in sugar beetroot and considerably decreased the occurrence of Sclerotium root rot (29). *Trichoderma* species use coiling, hooks, or appressorium-like structures to adhere to the host hyphae. They then use hydrolytic enzymes, like a basic proteinase, to break through the host cell walls (30).

Biological control is an economical and environmentally responsible alternative method of managing diseases. Biological control takes on particular importance. Rhizobacteria, like strains of *Bacillus* and *P. fluorescens*, have the potential to significantly improve plant growth and grain yield while also offering high levels of disease suppression. Due to their quick growth, ease of handling and aggressive rhizosphere colonization, antagonistic bacteria are regarded as the best biological control agents (31). The growth of *Macrophomina phaseolina* was successfully inhibited by the antibiotic 2,4 DAPG and phenazine extracted from the cell cultures of *Pseudomonas* isolates Pf32, Pf93 and B49 (32).

P. fluorescens, representing about 20% of the bacterial community, are expected to offer improved defense against infections transmitted through soil. Their strong affinity for amino acid exudates likely contributes to their notable competence in the rhizosphere. Higher levels of defense-related proteins, such as PAL, POD, β-1,3-glucanase, phenols, PPO and chitinase were observed in treatments with a bioformulation containing *P. fluorescens* (Pf1) targeting fungal pathogens and root-knot nematodes in cauliflower and cabbage (33). Using biocontrol agents like *P. fluorescens* and *Trichoderma* species induced coleus plants to generate higher levels of total phenols, PAL, PPO and POD (34). Additionally, delivering *P. chlororaphis* and *B. subtilis* through seed treatment, seedling dips and soil application effectively controlled damping-off in hot pepper by inducing the expression of genes linked with defense, including PPO, POD, chitinase, PAL and glucanase (35). Combined application of fungicide Nativo and *Bacillus amyloliqufacious* (B15) effectively control the cabbage head rot pathogen (36).

The accumulation of phenolic compounds and their oxidized derivatives is linked to tissue browning, with these substances demonstrating inhibitory effects on the growth and development of microorganisms (37). Plant phenolics are recognized for their antifungal, antibacterial and antiviral properties. They possess fungitoxic characteristics and enhance host cell walls' physical and mechanical strength. These rapid defense responses at the site of fungal invasion can delay the infection process, allowing the host ample time to initiate additional defense mechanisms to curb pathogen proliferation. Highly toxic to invading fungi, plant phenolics and their oxidation products, such as quinones, play a crucial role in this defense (38). Increased activity of defense-related enzymes, including POD, PPO and PAL, has been observed after treatment with *T. harzianum*, showing significant enhancements compared to the control (39). It is natural for defense genes to be abundant in plants; however, these genes require specific signals to be activated. Reports indicate that biocontrol agents can trigger dormant defense systems when pathogens infect plants.

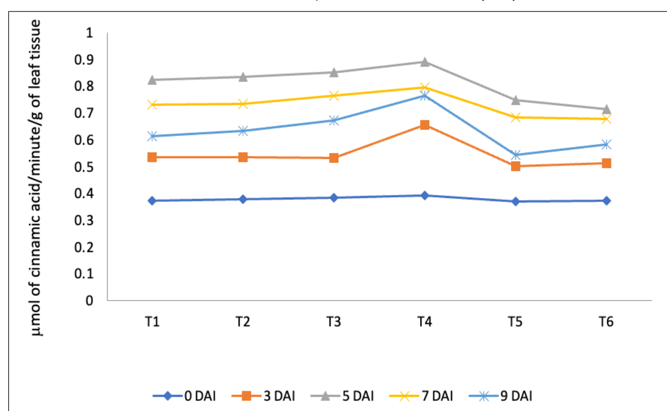

Fig. 5. Induction of phenylalanine ammonia lyase activity in cabbage plants treated with biocontrol agents.

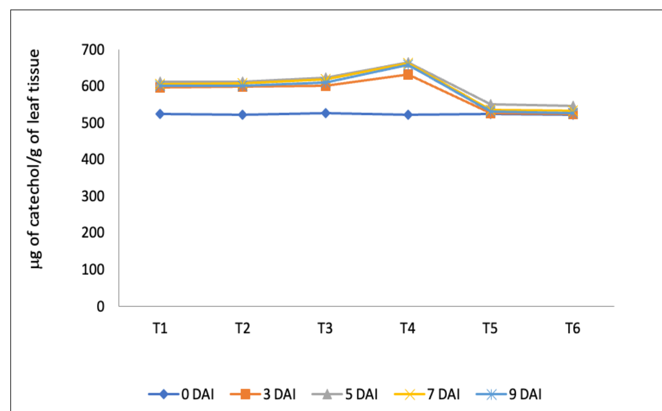

Fig. 6. Induction of phenol activity in cabbage plants treated with biocontrol agents.

Conclusion

Applying microbial biocontrol agents is one of the approaches to plant disease management, with the ultimate goal of protecting the environment and human health by lowering the use of pesticides and producing harvested products free of pollution. Upregulation of these enzymes produces defense-related metabolites such as phenols, phytoalexins, lignin, flavonoids and antibacterial qualities in plants, as well as signaling molecules like SA and JA. The current study clearly states that biocontrol agents effectively control cabbage head rot disease. Future research will focus on mass multiplication, commercialization and widespread application of biocontrol agents for the eco-friendly management of diseases and to create awareness among farmers for the utilization of biocontrol agents for sustainable crop cultivation.

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Authors' contributions

SM has executed the conceptualization, methodology and supervision. SM, VKS, MD and PA conducted the formal analysis and investigation. VKS, PA and ST finalized the preliminary manuscript preparation. SM, MD, PA and ST performed the last round of editing and review. All authors reviewed and approved the final 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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