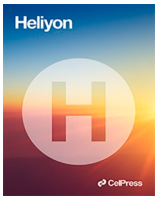
Heliyon 10 (2024) e23030

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



# Biochemical changes, antioxidative profile, and efficacy of the bio-stimulant in plant defense response against *Sclerotinia sclerotiorum* in common bean (*Phasaeolus vulgaris* L.)

Sunil Kumar [a,b,*], Vaishali Shukla [a], Yashoda Nandan Tripathi [a], Mohd Aamir [a,c], Kumari Divyanshu [a], Mukesh Yadav [a], Ram Sanmukh Upadhyay [a]

[a] *Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India*
[b] *Central Ayurveda Research Institute, Bhubaneswar, 751029, Odisha, India*
[c] *Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, 110012, India*

ARTICLE INFO

*Keywords:*
Sclerotinia Sclerotiorum
Phaseolus vulgaris L.
Biocontrol agents: Trichoderma erinaceum
Trichoderma viride
Plant defense
Antioxidant enzymes
ROS induced cellular toxicity

ABSTRACT

*Sclerotinia sclerotiorum,* is a highly destructive pathogen with widespread impact on common bean (*Phasaeolus vulgaris* L.) worldwide. In this work, we investigated the efficacy of microbial consortia in bolstering host defense against sclerotinia rot. Specifically, we evaluated the performance of a microbial consortia comprising of *Trichoderma erinaceum* (T51) and *Trichoderma viride* (T52) (referred to as the T4 treatment) in terms of biochemical parameters, alleviation of the ROS induced cellular toxicity, membrane integrity (measured as MDA content), nutrient profiling, and the host defense-related antioxidative enzyme activities. Our findings demonstrate a notable enhancement in thiamine content, exhibiting 1.887 and 1.513-fold higher in the T4 compared to the un-inoculated control and the T1 treatment (only *S. sclerotiorum* treated). Similarly, the total proline content exhibited 3.46 and 1.24-fold higher and the total phenol content was 4.083 and 2.625-fold higher in the T4 compared to the un-inoculated control and the T1 treatment, respectively. Likewise, a general trend was found for other antioxidative and non-oxidative enzyme activities. However, results found were approximately similar in T2 treatment (bioprimed with T51) or T3 treatments (bioprimed with T52). Further, host defense attribute (survival rate) under the pathogen challenged condition was maximum in the T4 (15.55 % disease incidence) compared to others. Therefore, bio priming with consortia could be useful in reducing the economic losses incited by *S. sclerotiorum* in common beans.

## 1. Introduction

The common bean (*Phaseolus vulgaris* L.) is an important grain legume crop and is mainly consumed in the form of its pods and edible seeds throughout the world [1]. FAO reports that the global common bean production in 2019 came to 28.9 million tons and the crop area was 33.1 million ha. There were five top common bean-producing countries in the world from 2000 to 2019: Myanmar, India, Brazil, China, and America (FAO, 2020). Over the past decade, Sclerotinia stem rot has transitioned from being a minor concern to becoming a major global threat to common bean crops, primarily due to shifts in climatic conditions. This disease is now responsible

* Corresponding author. Centre of Advanced Study in Botany (CAS), Institute of Science, Banaras Hindu University, Varanasi, 221005, India.
*E-mail address:* sunil.kumar2@bhu.ac.in (S. Kumar).

https://doi.org/10.1016/j.heliyon.2023.e23030
Received 19 December 2021; Received in revised form 17 November 2023; Accepted 24 November 2023
Available online 29 November 2023

for inflicting substantial damage, resulting in yield losses ranging from 50 % to 100 % [2]. *Sclerotinia sclerotiorum* (Lib.) deBary is responsible for causing sclerotinia wilt and rot. This necrotrophic fungal pathogen has a global distribution and can infect over 500 plant species, including common beans [3,4]. The fungus causes numerous soft rots in horticultural and agricultural crops. In India, the soft rot caused by *Sclerotinia* spp. is one of the significant concerns and affects agricultural productivity to a great extent. Since the resistant varieties against the white mold disease have not been developed so far, hence, the disease is managed through other conventional or other traditional approaches like deep plugging, increased row spacing, crop rotation with other non-host crops, use of the upright cultivars, and most importantly through the use of the systemic fungicides, particularly benomyl and procymidone [5]. However, the uses of systemic fungicides for disease control are non-sustainable and have several loopholes like higher economic cost, environmental issues, and sometimes their non-targeted effects. Furthermore, extensive use and prolonged exposure of these fungicides in the environment may leave harmful residues in soil, water, and atmosphere leading to environmental pollution, and deterioration of the soil quality, fertility, and productivity. In addition, these fungicides also influence the soil's beneficial microbial diversity disturbing their ecological functions and may lead to developing resistance in phytopathogens [6]. Hence, there is a strong need for the creation of effective and eco-friendly technologies aimed at minimizing or eliminating the use of synthetic fungicides in agriculture [7].

*Trichoderma* spp. is a fungal genus widely employed as one of the most important and commercially used microbial biocontrol agents (MBAs) with great potential to alleviate the use of agrochemicals in agriculture. The mechanism of action of *Trichoderma* against plant pathogens includes competition with pathogens for nutrients and space, antibiosis through secretion of potent secondary metabolites, and direct interaction with plant pathogens through mycoparasitism [8]. It is well-documented that the interaction between *Trichoderma* and plant pathogens is intricately regulated through a series of metabolic events, often involving diverse mechanisms. These signaling cascades encompass signaling compounds, enzymes, and various interfering metabolites that are generated in situ, typically at low concentrations during the interaction [9]. Some *Trichoderma* spp. have the potent ability to interact with host plant root and shoot by establishing a molecular dialogue that has positive effects in plants [10]. Several studies have reported the endophytic nature of *Trichoderma* that establishes communication with several host plants like tomato, *Arabidopsis*, cucumber, maize, and cotton [11–15]. The induced systemic resistance (ISR), in the presence of *Trichoderma asperellum* was found to be associated with increased antioxidative enzymatic activities, including enhanced secretion of chitinase and peroxidases along with modulation of plant defense transcriptome that is accompanied by an expression of genes involved in JA/ET signaling pathways [16]. Furthermore, *Trichoderma* colonization of plant tissues also activates the SAR pathways even when the ISR pathway is activated. They can enhance plant resistance to phytopathogens like S. sclerotiorum, with *Trichoderma* strains demonstrating their effectiveness through the induction of cell-wall degrading enzymes in the plant [13,17]. Several studies have reported the efficacy of *Trichoderma* spp. in controlling plant pathogens. Besides inducing ISR and SAR pathways, *Trichoderma* colonization improves nutrient uptake, induces phytohormonal signaling and overall improves plant growth and development.

Creating microbial consortia involving two distinct microorganisms, each with its unique function, represents a contemporary approach that has been adopted for the formulation of microbial products aimed at enhancing plant growth, bolstering disease resistance, and optimizing performance in challenging environmental conditions. Furthermore, the current biocontrol strategy employs blending various BCAs of different microbial species having plant growth-promoting attributes to achieve desired outcomes [18]. In pepper, *T. theobromicola, T. stilbohypoxyli, and T. caribbaeum* influenced the genetic expression related to the hypersensitive response and the biosynthesis of sesquiterpene phytoalexins [19]. The use of *Trichoderma* as MBCA against several plant pathogens is well-reported, and several studies have been done so far in this field. For example, *T. harzianum* (SQR-T307) and *T. asperellum* (T-34) are potent bio-control agents against the *Fusarium oxysporum* [20]. The antagonistic activity of the *T. gamsii* 6085 against *F. subtilis* and *F. graminearum* in rice is well studied [21]. Recently, biocontrol attributes of the *T. harzianum* and *T. erinaceum* against *F. oxysporum* in tomato is well documented [22–24]. In our previous report Kumar et al. (2021), reported that the *T. viride, T. erinaceum* and their consortium induced defense response in common bean against sclerotinia infection [25]. Likewise, bio-priming with the *T. erinaceum*, *T. brevicompactum* and *T. harzianum* against *S. rolfsii* increased antioxidative enzymatic activities, particularly, chitinases [26,27].

In plants, the most common resistance response following the pathogenic invaders includes accumulation of the phenolics, callose deposition, formation of the tyloses (outgrowths of the xylem contact cells), and gels in the infected vessels [28]. Additionally, plants biosynthesize secondary metabolites, defense-related proteins, activation of the ROS machinery, along with enhanced expression of defense-related antioxidants as part of their defense arsenal [25,29,30]. Secondary metabolites, including phenolic acids and proline, are integral to fortifying the defense system. They contribute to enhancing mechanical strength and preserving cell wall rigidity, thereby acting as a barrier against pathogen intrusion [31]. Phenylalanine ammonia-lyase (PAL) is the most important enzyme which is expressed during stress and acts as a regulatory enzyme in the biosynthesis of phenolic compounds [32]. Proline (an amino acid) is an excellent osmolyte and acts as a non-enzymatic antioxidant defense molecule during different abiotic and biotic stresses. Thus, proline plays three major roles in stressed plants, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule [33,34]. The peroxidation of the unsaturated lipid is the most observable symptom of oxidative stress. Lipid peroxidation is the most inimical process in the Plant, which disturbs the permeability of the cell membrane, degrades membrane protein, disturbs ion transport, and finally triggers the cell death process [35]. Malondialdehyde (MDA) is the most abundant secondary product of lipid peroxidation [36,37]. Fortunately, plants have evolved various effective mechanisms for the removal of ROS in order to protect themselves against various stresses. Different antioxidant enzymes such as the ascorbate peroxidase (APx), guaiacol peroxidase (GPx), glutathione reductase (GR), and the phenylalanine ammonia-lyase (PAL) involved in the ROS metabolism during pathogen infection [23].

However, limited information is available regarding the management of sclerotial wilt in beans through microbial consortia. This study seeks to fill this void by assessing the biocontrol effectiveness of *T. erinaceum* (NAIMCC-F-02171), *T. viride* (NAIMCC-F-02500),

and their combinations in common bean when challenged by *Sclerotinia*. The evaluation will encompass an assessment of disease severity, enzymatic activities (APx, GPx, GR, and PAL), and biochemical changes in total phenol, proline, lipid peroxidation, and thiamine content. Through this investigation, we seek to elucidate the influence of these biological microbes, individually and in combination, on both the growth and defense attributes of the host plant in response to *S. sclerotiorum* infection. This research endeavors to shed light on the potential of microbial consortia as an effective strategy for managing sclerotial wilt in common beans.

Overall, this study addresses a critical agricultural challenge - the management of sclerotial wilt in common beans caused by *Sclerotinia sclerotiorum*. The research focuses on harnessing the biocontrol potential of *Trichoderma* spp., either individually or as a consortium, to combat this devastating disease. The investigation encompasses a comprehensive evaluation of disease severity, enzymatic activities, and biochemical changes in the host plant. By elucidating the intricate interplay between *Trichoderma* and *S. sclerotiorum*, this research aims to contribute to the development of sustainable and environmentally friendly strategies for disease management in agriculture.

## 2. Material and methods

### 2.1. Fungal culture and growth conditions

*S. sclerotiorum* (GenBank accession number MW485172) was isolated from infected common bean plants from Sonbhadra district of Uttar Pradesh (India). The pathogen was isolated, sub-cultured, and maintained on potato dextrose agar (PDA) medium incubated at 25–27 °C for 5–7 days. The biocontrol microbes *T. erinaceum* (T51) (NAIMCC-F-02171) and the *T. viride* (T52) (NAIMCC-F-02500) were procured from National Agriculturally Important Microbial Culture Collection (NAIMCC), Kushmaur, Mau, and Uttar Pradesh, India. The BCAs and pathogen culture were cultured on PDA slants prepared with glycerol stock and were kept in incubator at 4 °C.

### 2.2. Pathogenicity test

The pathogenicity test was performed using the Cut-Petiole method [38]. In this method, the stem of test plant was injured with a sterile needle at the second node or 2 cm above from the soil surface. In the experiment, a mycelial plug (5 mm) or sclerotial body from *S. sclerotiorum* was introduced into the damaged section of the bean stem and covered with moist cotton. Following the inoculation, the pots were placed inside the greenhouse, maintaining a temperature of 23 ± 2 °C, and they were consistently watered as required. One week after the initial inoculation, the fungus was retrieved from all the inoculated bean plants, and Koch's postulate was affirmed by re-inoculating the re-isolated pathogen. This process was replicated three times.

### 2.3. Preparation of Trichoderma spp. spore suspension and seed bio-priming

The Trichoderma isolates *T. erinaceum* (T51) and *T. viride* (T52) were cultivated in a potato dextrose broth (PDB) medium at a temperature of 27 ± 2 °C for a period of 7 days. Following this incubation, the spores were collected by rinsing them with sterile double-distilled water and then filtered through a sterile muslin cloth. The spores were harvested and final concentration was maintained to $1 \times 10^5$ CFU/ml which was count by using the hemocytometer. The spore suspension was centrifuged at 10,000 rpm for 10 min then, the pellet was re-suspended in the 10 ml of 1.5 % CMC (Carboxy methyl cellulose) solution separately to make slurry [39]. In the case of consortia, equal amount of T51 and T52 suspension (v/v) was mixed. The bean seeds (Anupama variety) susceptible (83.67 %) to the *S. sclerotiorum* pathogen were used in this experiment [40]. For seed bio-priming, fresh and healthy seeds were surface sterilized with 0.01 % sodium hypochlorite aqueous solution for 25–30 s, followed by washing with double-distilled water and further dried in sterile condition [39]. The sterilized and dried seeds were soaked in the spore suspensions with $1 \times 10^5$ CFU/ml concentration (100 seeds/100 ml of spore suspension) of *T. erinaceum, T. viride* and their consortium for overnight in dark condition. The seeds were dried for overnight under aseptic condition (Under Laminar flow) to drained off the excess suspension [41]. The control seeds were treated solely with CMC, serving as an adhesive compound that facilitated the adherence of *Trichoderma* spores to the seed surface.

### 2.4. Seed sowing and experimental design

Four bioprimed bean seeds of each treatment were sown in pots (9 × 9 × 10 cm) containing autoclaved soil under controlled environmental conditions. Pots with untreated bean seeds were used as controls. All pots were kept in a greenhouse at 25 ± 2 °C with alternate day watering.

For inoculums preparation, we grew the pathogen *S. sclerotiorum* on fresh PDA plates for 5 days. The 24 days-old bean plant (4–6 leaves) was inoculated with a mycelium plug (5 mm diameter) of *S. sclerotiorum* on the shoot-root collar region. One set of control plants was left unchallenged. A cycle of 12 h dark / 12 h light and temperature of 25 ± 2 °C was maintained in the greenhouse. The plants were categorized into 5 groups and treated as the following: *viz*. the **CNT** (control; without any treatment), **T1-**Control plants exposed to the *S. sclerotiorum* pathogen challenge, **T2**-Plants treated with bio-control agent T51 and exposed to the *S. sclerotiorum* pathogen challenge, **T3**-Plants treated with bio-control agent T52 and exposed to the *S. sclerotiorum* pathogen challenge, **T4**-Plants treated with a consortium of both T51 and T52 bio-control agents, followed by exposure to the *S. sclerotiorum* pathogen challenge.

### 2.5. Measurement of the disease incidence

The disease incidence was assessed three days following pathogen infection using a scale adapted from Song et al. (2004). The scale ranged from 0 to 4, where a score of zero indicated no infection, and a score of four denoted the entire plant leaves turning yellow, signifying complete infection [42]. Scores of 1, 2, and 3 corresponded to varying degrees of stem rot in different color regions, reflecting the extent of disease severity. Two independent experiments were conducted, each with five replicates for every treatment. Disease incidence, as described by Song et al. (2004), encompasses both disease percentage and disease severity [42].

$$\text{Disease incidence } (\%) = \frac{(\Sigma \text{ scale } \times \text{ number of plants infected})}{(\text{Highest scale } \times \text{ total number of Plant})} \times 100$$

### 2.6. Plant harvest and analysis

The plant leaves were harvested from treated and control plants at five different time points: 0, 24, 48, 72, and 96 h after inoculation with the *S. sclerotiorum* pathogen. These samples were collected to evaluate various parameters, including total phenol content, proline content, lipid peroxidation, thiamine content, and the activity of several enzymes such as phenylalanine lyase (PAL), guaiacol peroxidase (GPx), glutathione reductase (GR), and ascorbate peroxidase (APx). Each of the biochemical assays was conducted with three replicates, and each replicate consisted of four seedlings.

### 2.7. Scanning electron microscopy (SEM) to study the colonization of T. erinaceum and T. viride with P. vulgaris

Plant of common bean (4 week-old) was inoculated by drenching with conidial suspension of *T. erinaceum* (T51) and *T. viride* (T52) (final concentration of $1 \times 10^5$ CFU/ml). The inoculum was freshly prepared from the 10-day culture of T51 and T52. To collect conidia, each plate was with 5 ml sterile water, and scraped the culture to collect the conidia. The collecting conidial suspension was filtered through muslin cloth and adjusted to the required conidial concentration after counting with a haemocytometer. Three plants were used for each treatment. For every treatment, 100 ml conidial suspension of *Trichoderma* spp. was used, and 100 ml of sterile water was used for control plants. After 6-days of inoculation, the root of each treatment was washed with distilled water was, followed by drying at 37 °C. Then it was fixed with 2 % glutaraldehyde in 0.1 M phosphate buffer for 12 h, and thereafter the samples were washed with the same buffer 2–3 times. The specimens were treated for dehydration using a sequence of ethanol concentrations (10 %, 20 %, 30 %, 50 %, 70 %, and 90 %) for 10–15 min each. For critical dryness, the samples were mounted on the metal stubs and coated with the fine platinum layer using a metallic covering apparatus. The platinum-coated root specimens were analyzed in the SEM (JEOL 1600, Japan) with a high-vacuum mode.

### 2.8. Thiamine estimation (vit. B1)

The determination of thiamine (Vitamin B1) content in the leaves of common bean plants involved an adapted method based on the approach outlined by Jordan (2000). Leaf samples weighing 0.1 g from various treatments were homogenized in 50 ml of ethanolic sodium hydroxide [43]. A 10 ml filtrate extracted from this mixture was combined with potassium dichromate, and the optical density was measured at 360 nm once color development occurred.

### 2.9. Lipid peroxidation (LPO)

The determination of the LPO range was conducted in accordance with the approach recommended by Ref. [44]. Assessing the overall Malondialdehyde (MDA) content, generated as a secondary byproduct of peroxidation of polyunsaturated fatty acids, was a crucial factor in gauging the level of LPO content. Leaf samples from all treatment groups were first homogenized in a buffer solution, followed by centrifugation. Subsequently, the supernatant was employed as an extract for the subsequent assay. For the reaction, a mixture was prepared comprising 0.1 ml of the extract, 0.2 ml of 8.1 % sodium dodecyl sulfate (SDS), 1.5 ml of 20 % acetic acid, and 1.5 ml of a 0.8 % aqueous TBA solution. The volume of this mixture was adjusted to 4.0 ml with distilled water and subsequently incubated at 95 °C for 1 h in a water bath. After cooling, 1.0 ml of distilled water and 5.0 ml of a n-butanol and pyridine mixture (15:1 by volume) were added, followed by centrifugation at 10,000 g for 15 min. The absorbance at 532 nm was measured, and the MDA content was determined by applying an extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$, and it was expressed in nmol MDA per gram of fresh weight (FW).

### 2.10. Proline content

The Proline content was quantified following the method outlined by Bates et al. (1973) with minor adaptations [45]. For this analysis, 0.1 g of plant samples were homogenized with 5 mL of 3 % sulphosalicylic acid in a pre-chilled mortar and pestle. The resulting mixture was then centrifuged at 10,000 g for 10 min. Next, 2 mL of enzyme extract was combined with 2 mL of glacial acetic acid and 2 mL of the Ninhydrin reagent. This mixture was incubated in a water bath held at 100 °C for 30 min. After incubation, the sample was rapidly cooled in an ice bath to stabilize the purple color of the extract and maintained at room temperature. Subsequently, 4 mL of toluene was added to each tube, and the content was vortexed for 15–20 s. The spectrophotometric absorbance of the upper

purple aqueous layer was measured at 520 nm. The proline concentration was then estimated using a standard curve generated with known concentrations of L-proline.

### 2.11. Total phenol content (TPC)

To determine the Total Phenolic Content (TPC), 1 g of leaf tissue was immersed in 5 mL of 95 % ethanol and kept at 0 °C for 48 h. Afterward, the samples were macerated in a phosphate buffer and subjected to centrifugation at 16,000 g for 15 min. A 1 mL aliquot of the enzyme extract was combined with 1 mL of 95 % ethanol, 5 mL of water, and 0.5 mL of a 50 % Folin–Ciocalteau reagent, thoroughly mixed, and allowed to rest for 5 min. Following this, 1 mL of 5 % sodium carbonate was added, and the mixture was left to stand for 1 h. The color developed in the solution was assessed at a wavelength of 725 nm [46]. Gallic acid was employed as the reference standard, and the phenolic content was quantified as mmol $L^{-1}$ GAE (Gallic acid equivalent) per gram of fresh weight (FW).

### 2.12. Phenylalanine ammonia-lyase (PAL) activity assay

PAL (EC 4.1.3.5) activity was assessed following the method outlined by Brueske (1980) with minor adaptations [47]. Leaf samples weighing 0.1 g were homogenized in 2 mL of sodium borate buffer (0.1 M, pH 7.0) supplemented with β-mercaptoethanol (1.4 mM). The samples were homogenized and subsequently centrifuged at 16,000 g for 10 min at 4 °C to obtain the enzyme extract. The reaction mixture was prepared with 0.2 mL of enzyme extract, 0.5 mL of borate buffer (0.2 M, pH 8.7), and 1.3 mL of water. The enzymatic reaction commenced with the addition of 1 mL of L-phenylalanine solution (0.1 M, pH 8.7) and was then allowed to incubate for 25 min at a temperature of 37 °C. To halt the reaction, 0.5 mL of trichloroacetic acid (TCA, 1 M) was added. Enzyme activity was assessed by measuring the absorbance at 290 nm, indicating the production of *trans*-cinnamic acid, and expressed as micromoles of TCA per gram of fresh weight (FW).

### 2.13. Ascorbate peroxidase (APx) activity

The APx (E.C. 1.11.1.11) activity was determined following the Nakano and Asada (1981) method with slight modifications, where the oxidation of ascorbic acid was measured at 290 nm [48]. In this procedure, 0.1 g of leaf tissue was homogenized in 2 ml of an extraction buffer (comprising 90 mM-$Na_2HPO_4$ buffer at pH 7.8, 8 % glycerol, 1 mM-EDTA, and 5 mM ascorbate). PVP (0.3 g/g tissue) was introduced, and the homogenate was subsequently subjected to centrifugation at 15,000 g for 10 min at 4 °C. The resulting supernatants served as the enzyme extract. For the assay, 200 μl of the enzyme extract was introduced into a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 0.1 mM-EDTA, 0.25 mM ascorbic acid, and 1.0 mM $H_2O_2$. The reduction in absorbance was monitored 30 s after the enzyme extract was added. APx activity was determined using an extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$, and the enzymatic activity was quantified as nmol of ascorbate oxidized per minute per milligram of protein.

### 2.14. Guaiacol peroxidase (GPx) activity

GPx activity (EC 1.11.1.7) was assessed using a spectrophotometer following the methodology recommended by Zheng and Van Huystee in 1992 [49]. The assay involved monitoring the increase in absorbance at 470 nm as guaiacol was oxidized to tetra-guaiacol. The reaction mixture comprised 10 mM sodium phosphate buffer (pH 6.0), 0.3 % (v/v) $H_2O_2$, 1 % (v/v) tetra-guaiacol, and 0.3 ml of the enzyme extract. The reaction was initiated by adding $H_2O_2$. The enzyme activity was determined through spectrophotometric measurements, following the method proposed by Zheng and Van Huystee in 1992. The enzyme activity was expressed as units per milligram of protein, where one unit represents the oxidation of 1 μmol of guaiacol per minute per milligram of protein. Protein concentration was determined following the method described by Lowry (1951) [50].

### 2.15. Glutathione reductase (GR) activity

GR (EC 1.8.1.7) activity was determined following the methodology outlined by Schaedle and Bassham (1977), employing a spectrophotometer [51]. Leaf samples weighing 0.1 g were homogenized in 5 ml of 50 mM Tris–HCl buffer at pH 7.6. After centrifugation of the homogenized samples at 15,000 rpm for 30 min at 4 °C, the resulting supernatant was utilized as the enzyme extract. The conversion of reducing glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH) was gauged by observing the reduction in absorbance of NADPH at 340 nm, considering an extinction coefficient of 6.22 $mM^{-1}$ $cm^{-1}$. In a 2 ml reaction mixture comprising 50 mM Tris-HCl buffer at pH 6.0, NADPH (0.15 mM), 100 μl of oxidized glutathione (1 mM GSSG), 3 mM $MgCl_2$, and 0.3 ml of enzyme extract. The enzyme activity is expressed as μmol NADPH oxidized $min^{-1}$ $mg^{-1}$ protein.

### 2.16. Statistical analysis

The data for physiological and biochemical analyses were analyzed using one-way analysis of variance (ANOVA) in the Statistical Package for the Social Sciences (SPSS) version 16, USA. To determine significant differences among the means, Duncan's multiple range tests were conducted at a significance level of $\leq 0.05$. The results were presented as means ± standard error (SE) based on three replicates.

### 2.17. Sampling Mechanism

In this work, a combination of random and stratified sampling was used to achieve representative sample. Plants were chosen at random within each treatment group (T1, T2, T3, and T4) to eliminate the experimental bias. Furthermore, to adjust for any confounding factors, plants were stratified depending on the key parameters such as age and growth stage. The randomization procedure was detailed so that other researchers may verify and replicate it.

## 3. Results

### 3.1. Measurement of disease incidence

Three days after the challenge with *S. sclerotiorum*, soft brown lesions had appeared on the control plant's collar region. The disease incidence was reduced to a maximum when the consortium of both *T. erinacuem* and *T viride* microbes were used (15.55 % disease incidence) compared with only pathogen-challenged plants (80 % disease incidence). The minimum disease incidence was observed in T4 treatment (15.55 % disease incidence) followed by T2 (17.78 % disease incidence) and the T3 (33.33 % disease incidence) treatments (Fig. 1).

### 3.2. Scanning electron microscopic analysis

Plant-root colonization with *T. erinaceum* (T51) and *T. viride* (T52) was monitored by scanning electron microscope after 6 days of inoculation. The colonization degree of *T. erinaceum* (Fig. 2b) and *T viride* (Fig. 2c) with plant roots was examined in the image, which depicted the extent of Trichoderma spp. colonization on the surface of bean roots. The image revealed that abundant numbers of *Trichoderma* spores formed dense colonies on the root surface showing the best colonization abilities. The *Trichoderma* hyphae and spores were observed to have closer intimacy with the root surface. In contrast, in uninoculated control plants (seedling not treated with *Trichoderma* inoculants), the root surface was observed to be smooth and undamaged (Fig. 2a).

### 3.3. Estimation of thiamine content

Thiamine accumulations in plants treated with microbial agents were significantly higher than untreated plants challenged or unchallenged with *S. sclerotiorum*. The beans seed treated with a consortium of both *Trichoderma* strains (T51 and T52) shown the maximum thiamine content, which was 1.887 and 1.513 fold higher than controls (CNT) and pathogen-challenged Plant (T1), respectively. In addition, the T2 and T3 treatment also contained higher level of thiamine than T1 and CNT (Fig. 3a**)**.

### 3.4. Lipid peroxidation assesment

The lipid peroxidation activity was estimated by Malondialdehyde (MDA) content. The MDA content exhibited a significant increase in pathogen-challenged plants but reduce in plants treated with T51, T52, and their consortium. The maximum reduction in

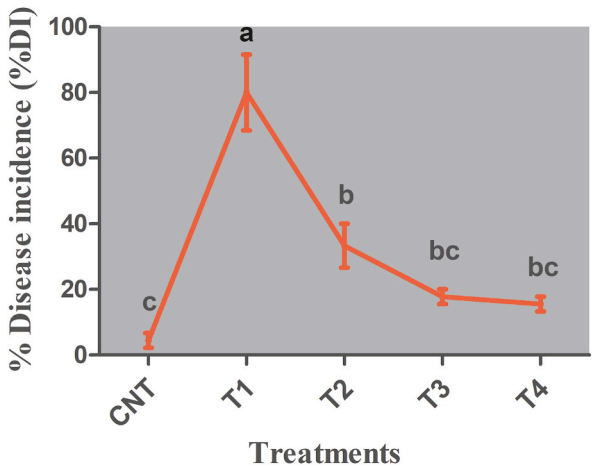



**Fig. 1.** Disease severity rate in common bean plants infected with *Sclerotinia sclerotiorum* and pretreated with *Trichoderma erinaceum*, *Trichoderma viride,* and its consortium. The control plant without any treatment (CNT), Plant infected with pathogen only (T1), Plant treated with *T. erinaceum* + *S. sclerotiorum* (T2), Plant treated with *T. viride* + *S. sclerotiorum* (T3), and Plant treated with *T. erinaceum* + *T. viride* + *S. sclerotiorum* (T4). Results are expressed as the mean of three replicates, and vertical bars indicate the SE of the mean. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

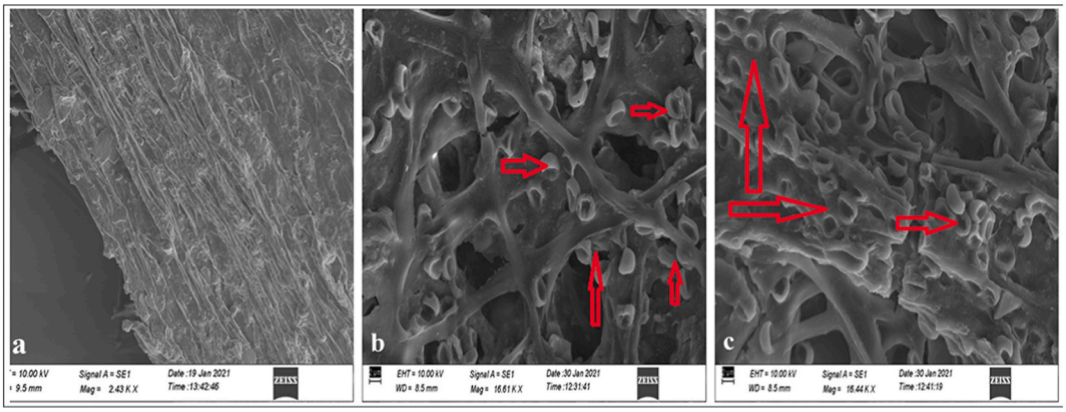



**Fig. 2.** Scanning electronic microscopy images (SEM) showing bean root colonization with. *erinaceum* and *T. viride*. **a**, Uncolonize root of bean. **b**, Root surface colonization by *T. erinaceum*. **c**, Root surface colonization by *T. viride*. Conidia germination and adhered to the plant root cuticle. Scale bars are indicated in the figure.

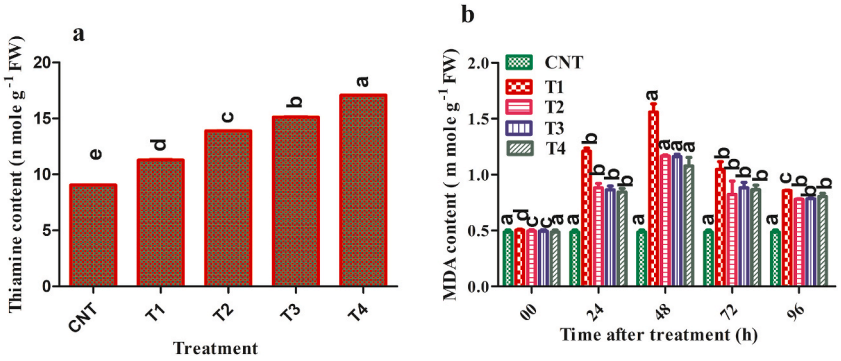



**Fig. 3a.** Thiamine content in bean raised from seeds pretreated with *Trichoderma erinaceum*, *Trichoderma viride* and its consortium and challenged with *Sclerotinia sclerotiorum*. **3b**, The range of lipid peroxidation (LPO) was determined by the MDA content at different time intervals in bean raised from seeds pretreated with *Trichoderma erinaceum*, *Trichoderma viride,* and its consortium and challenged with *Sclerotinia sclerotiorum*. Results are expressed as the mean of three replicates, and vertical bars indicate the SE of the mean. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

MDA content was observed in T4 treatment (T51; *T. erinaceum* and T52; *T. viride*), which was 1.448 fold lower than in plants that were only challenged by pathogen (T1). The MDA reduction was also showed by T2 (*T. erinaceum*), T3 (*T. viride*) treatment in comparison to T2 (pathogen treatment only) (Fig. 3b).

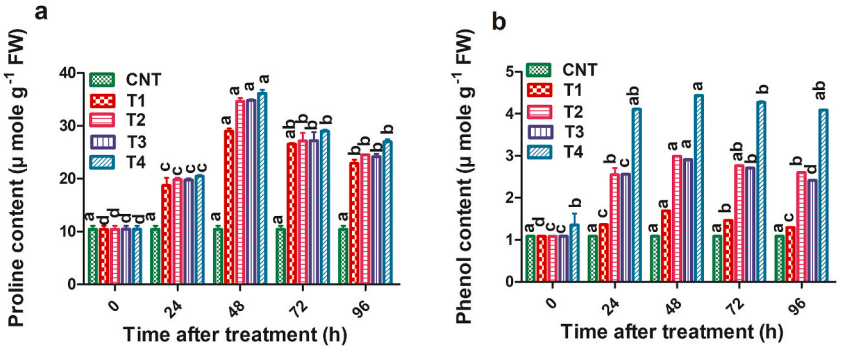



**Fig. 4a.** Change in the concentration of proline. **4b**, Estimating total phenol content in leaves of bean plants at different time interval, infected with *Sclerotinia sclerotiorum* and pretreated with *Trichoderma erinaceum*, *Trichoderma viride,* and its consortium. Results are expressed as the mean of three replicates, and vertical bars indicate the SE of the mean. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

### 3.5. Measurement of the proline content

Proline content exhibited significant enhance in all treatments up to 48 h, compared with the control plant. Among all the treatments, the maximum proline content was found in T4 treatment, while the minimum was in T1 treatment. No significant difference was found in the amount of proline content in T2 and T3 treatments, but both showed significantly higher in comparison to T1 treatment. Specifically, at the 48-h mark, proline content was 3.46 times higher in the T4 treatment compared to the control (CNT) and 1.24 times higher than in pathogen-challenged plants (T1) (Fig. 4a). The control plant exhibited the lowest proline content, which remained relatively consistent throughout the experiment.

### 3.6. Total phenol content (TPC)

Phenol accumulations in plants treated with microbial agents, *T. erinaceum* (T51), and *T. viride* (T52) and their consortium were significantly increased in comparison to control (CNT) or challenged with *S. sclerotiorum* (Fig. 4b). Seeds treated with consortium of *T. erinaceum* (T51), and *T. viride* (T52) exhibited higher level of phenols in their leaves, measuring 4.083 and 2.625 fold higher as compared to control and pathogen-challenged Plant, respectively (Fig. 4b). The maximum TPC content was recorded in T4 treatment, followed by T2 and T3. The assays also detected a small amount of phenols in unchallenged healthy control plants.

### 3.7. Phenyl ammonia lyase (PAL) activity measurement

PAL (Phenylalanine Ammonia Lyase) activity increased significantly in all treatments up to 48 h after treatment and after which, the activity declined slowly. Seeds were treated with a consortium of *T. erinaceum* (T51), and *T. viride* (T52) shown higher level of PAL activity in leaves, measuring 3.129 and 1.116 fold higher as compared to control and pathogen-challenged Plant, respectively (Fig. 5). The maximum PAL activity was recorded in T4 treatment, followed by T2 and T3. A small amount of PAL activity was also detected in unchallenged healthy control in all the assays.

### 3.8. Antioxidant enzyme activities

In general, the pathogen contamination led to be increase in antioxidant enzyme activity in comparison to the control plant throughout the experiments. The maximum activity was observed in consortia of *T. erinaceum* and *T. viride*, followed by a single *Trichoderma* treated plants. In control plants, the activities of APx (Ascorbate peroxidase, GPx (Guaiacol peroxiadse), and GR (Glutathione reductase) increased after pathogen treatment, reaching their maximum at 48 h, and there after showed slightly decreased in all treatments (Fig. 6a and b, 7). The maximum APx activity was recorded in T4 treatment, which was 2.939 and 1.217 fold higher than the control and pathogen-challenged Plant, respectively (Fig. 6a). The APx activity was significantly higher in the T4-treated plants when compared with other treatments at every interval of experiments. The APx activity in T2 and T3 treatments were not significantly difference but higher than the control and pathogen-challenged plants. In control plants quantified, a minimum APx activity than the all treated plants, and this remained relatively constant throughout the entire experiment. The maximum GPx activity was recorded in T4 treatment at 48 h after pathogen infection, which was 11.969 and 1.477 fold higher than the control and pathogen-challenged plants, respectively (Fig. 6b). T2 and T3 were not significantly difference, but higher than the control and pathogen-challenged plants. GR activity was enhanced in all treatments when compared with unchallenged plants. At 48 h after pathogen

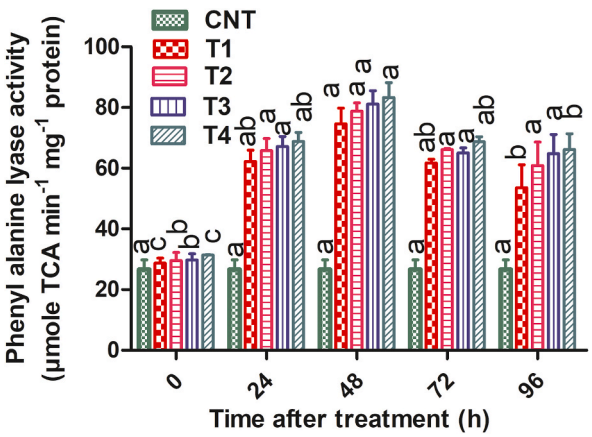



**Fig. 5.** Change in the phenylalanine lyase activity in leaves of bean plants at different time intervals infected with *Sclerotinia sclerotiorum* and pretreated with *Trichoderma erinaceum*, *Trichoderma viride,* and its consortium. Results are expressed as the mean of three replicates, and vertical bars indicate the SE of the mean. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

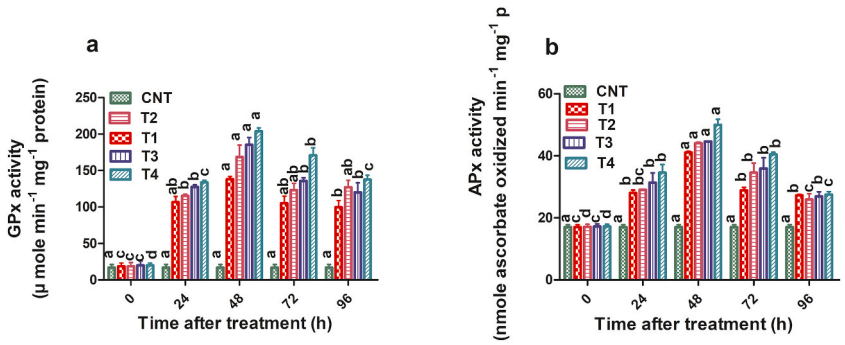



**Fig. 6a.** Change in the Guaiacol peroxidise activity. **6b**, Change in Ascorbic peroxidise activity in leaves of bean plants at different time intervals, infected with *Sclerotinia sclerotiorum* and pretreated with *Trichoderma erinaceum*, *Trichoderma viride* and its consortium. Results are expressed as the mean of three replicates, and vertical bars indicate the SE of the mean. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

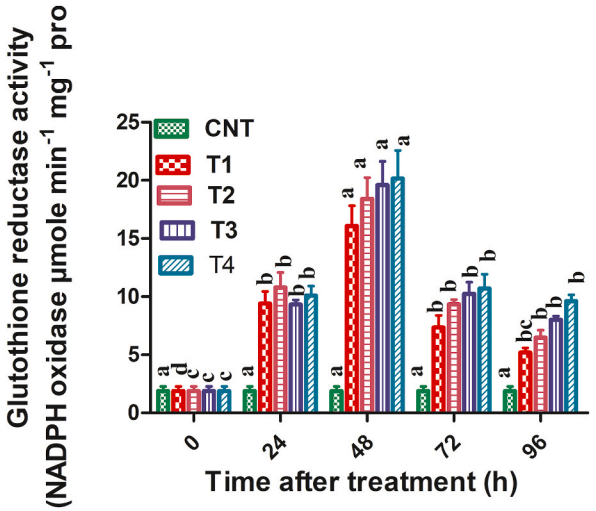



**Fig. 7.** Change in Glutathione reductase activity in leaves of bean plants at different time interval, infected with *Sclerotinia sclerotiorum* and pretreated with *Trichoderma erinaceum*, *Trichoderma viride* and its consortium. Results are expressed as the mean of three replicates, and vertical bars indicate the SE of the mean. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

treatment, recorded maximum GR activity in all treatments. The maximum GR activity was recorded in the T4 treatment, which was 10.673 and 1.253 fold higher in comparison with unchallenged control and pathogen-challenged (T1) plants (Fig. 7). The minimum GR activity was recorded in control plants; compared to all treated plants, and this remained relatively constant during the entire experiment. In T2 and T3 treatments, no significant differences were observed during the entire experiments, but they were higher than the unchallenged control and pathogen treatment.

### *3.9. Principal component analysis (PCA)*

A multivariate Principal component analysis (PCA) was conducted to understanding of the relationships, similarities, and dissimilarities between the results for biochemical and physiological traits. The multivariate PCA allows many variables to be lessened to only a few, which largely account for the variance in the experimental results. PCA analysis revealed positive scores for proline, phenol, PAL, GR, GPx, and LPO. In contrast, the APx activity was associated with a negative score (Fig. 8).

## 4. Discussion

The fungus *Sclerotinia sclerotiorum* is a notorious fungal pathogen responsible for white mold disease in economically important crops, such as common bean, soybean, and cotton [52]. This pathogen persists in the soil for many years, germinating during favorable conditions to produce ascospores, which subsequently infect host plants [53]. Upon infection, S. sclerotiorum colonizes various plant

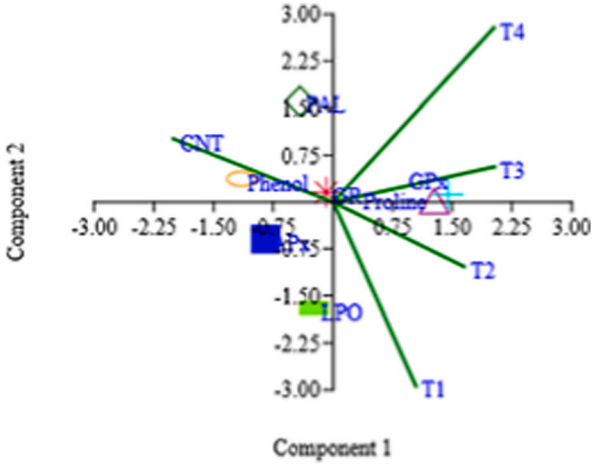



**Fig. 8.** Principal component analysis (PCA) of beans treated with BCA under pathogen-challenged, untreated, and unchallenged as a negative control, and untreated and pathogen-challenged condition as positive.

tissues extensively through the secretion of phytotoxic metabolites and cell wall-degrading enzymes [54]. Notably, it employs oxalic acid secretion to suppress host defense mechanisms initially, eventually leading to cell death [55]. The ascospores are released and function as a source of primary inoculums for infection and progression of the disease. It has been reported that the pathogen can infect all above-ground foliage. The lesions produced from infection are characterized by a white cotton-like mass of mycelium growing on the surface of stem, leaf, or pod tissue. Infected tissue becomes soft and slimy with a water-soaked appearance. Microbial biocontrol agents (MBAs) are generally deployed to control the biotic effects of plant pathogens [9,22,23,56–58] and other abiotic environmental stresses like soil salinity, heat stress, drought, metal toxicity [59–62]. These MBAs are supplied to host crops for biological control against several biotic and abiotic stresses. The biocontrol mechanism incited by these MBCAs against the plant pathogens might induce systemic resistance or priming without direct interaction [24]. An integrated approach to Trichoderma-mediated disease suppression includes mycoparasitism, antibiosis, induced defense response, and competitive exclusion [9,63], while others inhibit the plant pathogens by imparting competition for both nutrients and space [64]. However, under their natural environment, the microbial diversity associated with plant hosts is confined to a specific region and associated with other interacting microbes of the ecosystem while associated with its original host and constitutes the so-called "Plant microbiome." The plant microbiome provides an indigenous system associated with host crops to impart different functions and influence each other's biological activity and functional dynamics in a multi-dimensional manner [25,27]. The microbial diversity residing in soil or that remains part of an active plant microbiome may comprise several beneficial bacteria like *Pseudomonas* spp. or actinomycetes [65]. The microbial biocontrol agents (MBCAs), including biocontrol fungi like *Clonostachys* and *Trichoderma* spp. or other bacterial or fungal endophytes with biocontrol factors that may alleviate the harmful effects of both abiotic and biotic stresses [66].

Bio priming results in an aggravated defense response upon encountering biotic challenges of plant pathogens or against several environmental stresses [25]. Pathogenic elicitors generally activate the defense response in plants, which can be stimulated by the co-inoculation of bio-control microbes. Plants treated with BCAs became primed to respond faster by activating cellular defense responses and up-regulation of defense genes [67,68]. *Trichoderma*-based products and synthetic fungicides may result in the combined interaction for Sclerotinia stem rot disease management [69]. In this study, we have reported that induced systemic resistance (ISR) play a crucial role during the interaction of pathogenic elicitors with host plants, rendering spatially distant plant parts resistant to the pathogens. Plants perceive the ISR elicitors to show an exaggerated immune response against the pathogen challenges [70,71]. The rise in levels of defense enzymes, proline, and other defense-related compounds could help provide resistance against pathogen attack. The ISR pathway activates the expression of various defense enzymes, including PAL, peroxidases, and hydroperoxide lyases, through jasmonate/ethylene (JA/ET) signaling cascades [72]. The systematic acquired resistance (SAR) mediated the expression of PR proteins, particularly chitinase and β-1,3-glucanases that lysis the pathogen's cell and infected host cells [73,74]. In the present study, two potential biocontrol agents *T. viride* (T51) and *T. erinaceum* (T52) have been used to assess of their biocontrol attributes against the *S. sclerotiorum*. Our results have shown that both *T. viride* and *T. erinaceum* have significant potential to counteract the pathogen infection in host tissues. The previous reports show inhibitory activities of the *T. viride* and *T. erinaceum* against the pathogen *S. sclerotiorum* [25,75]. *Trichoderma* spp. promotes plant growth through various indirect and direct mechanisms and induces systemic resistance against subsequent pathogen attacks [76–78]. However, *Trichoderma*-induced systemic resistance (TISR) has been reported to involve multiple signaling routes, cross-communicating hormonal pathways, and networks that constitute a complex web of signaling cascades [79]. The previous report suggested that TISR might involve both JA and SA signaling pathways. However, the interaction of *T. harzianum* Rifai T39 in the *Arabidopsis-Botrytis cinerea* pathosystem revealed that TISR-mediated resistance is well regulated by JA, ET, and ABA signaling rather than direct activation of SA-related pathways [80]. In one report, about an 80 % reduction in the Fusarium wilt was observed by *Trichoderma asperellum* UDEAGIEM-H01 in *Stevia rebaudiana*. Similarly, the Fusarium wilt of banana is managed using only *Trichoderma* spp. in the integrated management approach [81,82]. *Trichoderma* interaction with

plants or symbiosis could also lead to the expression of plant defense-related genes, which immune the plants against the pathogen challenge, and, therefore, assist in growth and development [83–87]. Aamir et al. (2019) reported that the bio-priming of tomatoes seeds with *T. erinaceum* helps in the transcriptional reprogramming of the defense-related genes in tomato against the Fusarium wilt [24]. Another study reported that *Trichoderma hamatum* 382 induced systemic resistance against *Botrytis cinerea* in *Arabidopsis* [88], *Rhizoctonia solani* and *S. sclerotiorum* in lettuce [89]. In many studies, *Trichoderma* spp. Mediated priming against phytopathogens and its effect on crucial defense players have been well demonstrated [90]. SaravanaKumar et al. (2017) reported that the T. harzianum strain is a potential BCA against Fusarium graminearum and induces systemic resistance in maize [91]. Sanchez-Montesinos et al. (2019) reported a reduction of the incidence of the disease caused by *Pythium ultimum* by different *Trichoderma* species [92]. However, studies on the application of microbial consortium of *T. erinaceum and T. viride* against *S. scletiorum* in the common bean are not reported yet.

We have estimated the biochemical attributes, including the amount of thiamine, level of ant-oxidative defense enzymes, and proline content under the pathogen-challenged condition. The same were when bio-primed plants were challenged with biocontrol agents alone or in microbial consortia. In the present study, we have found the maximum thiamine content in T4 treatment compared to all treatments. This result suggested that high concentrations of thiamine in the chloroplast and other cellular organelles play a significant role in defense mechanisms during pathogen infection. Pyrophosphate (TPP) is the active form of thiamine that plays the role of a cofactor in many metabolic reactions such as glycolysis, pentose phosphate pathway, and tricarboxylic acid (TCA) cycle, and also associated with the induction of systemic acquired resistance (SAR) in all living plants. The phenyl propanoid pathway play an essential role in host defense through several fundamental mechanisms, including cellular-reinforcement antimicrobial activity and biosynthesis of signaling compounds such as SA [93,94]. PAL is a key enzyme that catalyzes the deamination reaction of phenylalanine to *trans*-cinnamate. It is a secondary metabolites synthesis pathway that synthesizes phenolic compounds such as flavonoids, isoflavonoids, anthocyanins, hormones, phytoalexins, and lignin. The phenolic compounds play a significant role in plant defense mechanisms against biotic challenges [95,96]. In this experiment, the maximum PAL activity was recorded in the T4 treatment, followed by the T2, T3, and T1 treatments, indicating that the T4 treatment provides the maximum defense response against the *S. sclerotiorum*. An increase in phenolic content is positively related to the increasing plant resistance against pathogens as an antimicrobial defense weapon of plants [97,98]. Besides, phenols induction is linked with PAL activity, which catalyzes the initiation step of synthesizing phenols. We found a significant increase in phenolic content in the T4 treatment, similar to PAL activity recorded following the pathogen-challenged condition. The higher phenolic content and PAL activity can be correlated with enhanced defense response against *S. sclerotiorum* by a synergistic effect of biocontrol agents. Amino acids are considered precursors and components of protein synthesis and play a significant role in plant development and metabolism [33].

Proline is a multifunctional amino acid that enhances the plants tolerance level against various types of abiotic and biotic stresses [99]. It acts as an osmoregulator and helps maintain plants' water potential to absorb water from the soil [100]. The hypersensitive response (HR) is a mechanism plants use to reduce the spread of infection by pathogens because of the rapid death of plant cells in the local regions surrounding an infection. The increased accumulation of the proline well promotes HR during a pathogen attack. In this experiment, the maximum proline accumulation was found in T4 followed by T2 and T3. Thus, this result suggested that the consortium of *T. erinaceum and T. viride* showed the maximum HR in the bean during *S. sclerotiorum* infection compared to a single treatment with BCA. In the present study, we have found that the consortium of T. erinaceum and T. viride increased the activities of the APx, GPx and GR after the inoculation with S. sclerotiorum. These results suggested that the consortium of the *T. erinaceum* and *T. viride* increased the resistance against the S. *sclerotiorum* in the common bean by increasing the antioxidative activities, thereby minimizing the ROS-induced cell death incited by pathogen-challenged conditions. ROS-induced cell death was further confirmed, and damage was minimized in microbial consortia compared to control and pathogen-challenged samples. Lipid peroxidation is a series of oxidative degradation reactions that affect lipids. It involves the extraction of electrons from lipids in cell membranes by free radicals, leading to cellular damage. This chain reaction is driven by the actions of free radicals. However, the rate of lipid peroxidation is estimated by the MDA content in the plant cells. In our study, the MDA content was found to gradually increase in infected leaf bean plants with an increase in infection duration. In the common bean plant, pretreated with *T. erinaceum* and *T. viride* and their consortium, the MDA content significantly decreased compared to pathogen-challenged plants. Among all the treatments, the pathogen-challenged common bean plant (T1) had maximum MDA content compared to all treatments, including the control. The increased catalytic activities of defense enzymes such as SOD, CAT, GPx, APx, PPO, PO, etc., could be correlated with the increased amount of lipid peroxidation. Bio-primed seeds with single and consortium of both Trichoderma had comparatively lesser MDA content than pathogen-challenged samples. A decrease in the MDA content in common bean plants might enhance the antioxidant enzyme activity, decrease the generation of free radicals and subsequently, damage membrane against pathogen *S. sclerotiorum*. So, we concluded that the consortium of *T. erinaceum* and *T. viride* has good potential to reduce the disease severity against *S. sclerotiorum,* and it may increase the plant growth promotion and defense mechanisms in common beans against *S. sclerotiorum*. The combined effect of *T. erinaceum* and *T. viride* may be enhanced the survival rate of common bean seedlings against *S. sclerotiorum* than individually. Overall, the findings in this study highlight the relevance of microbial consortia in controlling *Sclerotinia* infections in the common bean. Therefore, microbial consortia with BCAs like *T. erinaceum* and *T. viride* could be an alternative platform to chemical pesticides and provide a cost-effective, stable, and sustainable method for preventing the economic damages caused by this pathogen.

## 5. Conclusion

The present study demonstrated that the consortium of T51 (*T. erinaceum*) and T52 (*T. viride*) strains on seed coating and foliar spraying reduced the stem rot disease incidence and severity of common beans under the greenhouse study. The present biocontrol

approach involves combining different biological control agents (BCAs) from diverse microbial species with plant growth-promoting characteristics to attain specific objectives. A successful biocontrol strategy relies on the harmonious coexistence of all co-inoculated microorganisms in the rhizosphere without competitive interactions. The evaluation phase is undoubtedly a pivotal step in the development of a microbial consortium as it offers insights into its effectiveness in reducing disease severity and promoting plant growth. Efforts are underway to create a microbial consortium with the aim of mitigating diseases and enhancing plant growth. In a consortium, multiple microbial groups coexist symbiotically, resulting in highly effective, resilient, adaptable, and dependable systems [101]. The combined inoculation of beneficial microorganisms led to enhanced growth and yield characteristics, such as germination rate, nutrient uptake, plant height, branch count, nodulation, and increased overall biomass of the plants. The use of consortia boosts microbial efficacy, ensuring consistent and reliable performance across various soil conditions. The amalgamation of biocontrol agents within the consortium is anticipated to provide heightened protection and the potential to suppress multiple plant diseases.

## Funding information

No funding for this article.

## Data availability statement

Data will be made available upon request. The 18s rRNA gene sequence of *Sclerotinia sclerotiorum* SSIT1 has been deposited to GenBank in the NCBI database with accession number: MW485172.

## CRediT authorship contribution statement

**Sunil Kumar:** Software, Methodology, Formal analysis, Data curation, Conceptualization, Writing - original draft, Writing - review & editing. **Vaishali Shukla:** Formal analysis. **Yashoda Nandan Tripathi:** Writing – review & editing. **Mohd Aamir:** Writing – review & editing. **Kumari Divyanshu:** Formal analysis. **Mukesh Yadav:** Formal analysis. **Ram Sanmukh Upadhyay:** Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that there is no conflict of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We are highly thankful to the National Agriculturally Important Microbial culture collection (NAIMCC) for providing *Trichoderma erinaceum* and *Trichoderma viride*. SK would also like to acknowledge the Head of the Department of Botany, Banaras Hindu University, Central Instrument Laboratory, ISLS, Department of Botany, Centre of Advance Study (CAS) BHU, Varanasi for providing instrument facilities. SK is highly thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing financial support in the form of fellowship for research work and chemicals only (Grant/Award Number 09/013(0725)/2017-EMR-l)

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23030.

## References

[1] M.A. Nadeem, M.Z. Yeken, M.Q. Shahid, E. Habyarimana, H. Yılmaz, A. Alsaleh, R. Hatipoğlu, Y. Çilesiz, K.M. Khawar, N. Ludidi, S. Ercişli, Common bean as a potential crop for future food security: an overview of past, current and future contributions in genomics, transcriptomics, transgenics and proteomics, Biotechnol. Biotechnol. Equip. 35 (1) (2021) 759–787.
[2] J.R. Steadman, White mold-A serious yield-limiting disease of bean, Plant Dis. 63 (1983) 346–350.
[3] H.J. Willets, J.A.L. Wong, The biology of *Sclerotiniasclerotiorum, S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature, Bot. Rev. 46 (1980) 101–165.
[4] A. Elgorban, B.A. Al-Sum, M. Elsheshtawi, A.H. Bahkali, Factors affecting on *Sclerotinia sclerotiorum* isolated from beans growing in Ismailia, Egypt, Life Sci. J. 10 (2013) 1278–1283.
[5] L.A. Rothmann, N.W. McLaren, *Sclerotinia sclerotiorum* disease prediction: a review and potential applications in South Africa, South Afr. J. Sci. 114 (2018) 1–9.
[6] M.F. Villarreal-Delgado, E.D. Villa-Rodríguez, L.A. Cira-Chávez, M.I. Estrada-Alvarado, F.I. Parra-Cota, S.D.L. Santos-Villalobos, The genus *Bacillus* as a biological control agent and its implications in the agricultural biosecurity, Rev. Mex. Fitopatol. 36 (2018) 95–130.
[7] G. Santoyo, M.D.C. Orozco-Mosqueda, M. Govindappa, Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of Bacillus and Pseudomonas: a review, Biocontrol Sci. Technol. 22 (2012) 855–872.
[8] K. Mukherjee, D.M. Pandey, A.S. Vidyarthi, Molecular dynamics simulation of Rap 1 Myb-type domain in *Saccharomyces cerevisiae*, Bioinformatics 8 (2012) 881.

[9] J. Köhle, R. Kolnaar, W.J. Ravensberg, Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy, Front. Plant Sci. 845 (2019).
[10] L. Macías-Rodríguez, H.A. Contreras-Cornejo, S.G. Adame-Garnica, E. Del-Val, J. Larsen, The interactions of *Trichoderma* at multiple trophic levels: inter-kingdom communication, Microbiol. Res. 240 (2020), 126552.
[11] H.A. Contreras-Cornejo, L. Macías-Rodríguez, C. Cortés-Penagos, J. López-Bucio, *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis, Plant Physiol. 149 (2009) 1579–1592.
[12] J. Vishnevetsky, T.L. White, A.J. Palmateer, M. Flaishman, Y. Cohen, Y. Elad, M. Velcheva, U. Hanania, N. Sahar, O. Dgani, A. Perl, Improved tolerance toward fungal diseases in transgenic Cavendish banana (Musa spp. AAA group) cv, Grand Nain. Transgenic Res. 20 (2011) 61–72.
[13] R.B. Lopes, M.A.V. Paiva e Brito, S.C.M. de Mello, O.J. Saggin, A. Wendland, Microbial collections at Embrapa: conservation and value added to biodiversity, Documento-Embrapa. Agroener. 15 (2013) 15–22.
[14] Z.H. Zhang, D.J. Jhaveri, V.M. Marshall, D.C. Bauer, J. Edson, R.K. Narayanan, G.J. Robinson, A.E. Lundberg, P.F. Bartlett, N.R. Wray, Q.Y. Zhao, A comparative study of techniques for differential expression analysis on RNA-Seqdata, PLoS One 9 (2014), e103207.
[15] N.L. Lamdan, S. Shalaby, T. Ziv, C.M. Kenerley, B.A. Horwitz, Secretome of *Trichoderma* interacting with maize roots: role in induced systemic resistance*[S], Mol. Cell. Proteomics 14 (2015) 1054–1063.
[16] M. Shoresh, I. Yedidia, I. Chet, Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203, Phytopathology 95 (2005) 76–84.
[17] M.A. Salas-Marina, M.A. Silva-Flores, M.G. Cervantes-Badillo, M.T. Rosales-Saavedra, M.A. Islas-Osuna, S. Casas-Flores, The plant growth-promoting fungus Aspergillus ustus promotes growth and induces resistance against different lifestyle pathogens in Arabidopsis thaliana, J. Microbiol. Biotechnol. 21 (2011) 686–696.
[18] R.M. Ram, A. Debnath, S. Negi, H.B. Singh, Use of microbial consortia for broad spectrum protection of plant pathogens: regulatory hurdles, present status and future prospects, in: Biopesticides, Woodhead Publishing, 2022, pp. 319–335.
[19] H. Bae, D.P. Roberts, H.S. Lim, M.D. Strem, S.C. Park, C.M. Ryu, R.L. Melnick, B.A. Bailey, Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms, Mol. Plant Microbe Interact. 24 (2011) 336–351.
[20] X. Yang, L. Chen, X. Yong, Q. Shen, Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against Fusarium wilt of cucumbers, Biol. Fertil. Soils 47 (2011) 239–248.
[21] F. Matarese, S. Sarrocco, S. Gruber, V. Seidl-Seiboth, G. Vannacci, Biocontrol of Fusarium head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*, Microbiology 158 (2012) 98–106.
[22] A. Zehra, M. Meena, M.K. Dubey, M. Aamir, R.S. Upadhyay, Activation of defense response in tomato against Fusarium wilt disease triggered by *Trichoderma harzianum* supplemented with exogenous chemical inducers (SA and MeJA), Braz. J. Bot. 40 (2017) 51–664.
[23] A. Zehra, M. Meena, M.K. Dubey, M. Aamir, R.S. Upadhyay, Synergistic effects of plant defense elicitors and *Trichoderma harzianum* on enhanced induction of antioxidant defense system in tomato against Fusarium wilt disease, Bot. Stud. 58 (2017) 1–14.
[24] M. Aamir, S.P. Kashyap, A. Zehra, M.K. Dubey, V.K. Singh, W.A. Ansari, R.S. Upadhyay, S. Singh, *Trichoderma erinaceum* bio-priming modulates the WRKYs defense programming in tomato against the *Fusarium oxysporum* f. sp. *lycopersici* (Fol) challenged condition, Front. Plant Sci. 10 (2019) 911.
[25] S. Kumar, V. Shukla, M.K. Dubey, R.S. Upadhyay, Activation of defense response in common bean against stem rot disease triggered by *Trichoderma erinaceum* and *Trichoderma viride*, J. Basic Microbiol. 61 (2021) 910–922.
[26] K. Kumar, N. Amaresan, S. Bhagat, K. Madhuri, R.C. Srivastava, Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens, Indian J. Microbial. 52 (2012) 137–144.
[27] V. Shukla, S. Kumar, Y.N. Tripathi, R.S. Upadhyay, *Bacillus subtilis*-And *Pseudomonas fluorescens*-mediated systemic resistance in tomato against *Sclerotium rolfsii* and study of physio-chemical alterations, Front. Fungal Biol. 3 (2022), 851002, https://doi.org/10.3389/ffunb.
[28] F. Takken, M. Rep, The arms race between tomato and *Fusarium oxysporum*, Mol. Plant Pathol. 11 (2010) 309–314.
[29] R. González-Lamothe, G. Mitchell, M. Gattuso, M. Diarra, F. Malouin, K. Bouarab, Plant antimicrobial agents and their effects on Plant and human pathogens, Int. J. Mol. Sci. 10 (2009) 3400–3419.
[30] S. Lehmann, M. Serrano, F. L'Haridon, S.E. Tjamos, J.P. Metraux, Reactive oxygen species and plant resistance to fungal pathogens, Phytochemistry 112 (2015) 54–62, https://doi.org/10.1016/j.phytochemistry.2014.08.027.
[31] S.M. El-Khallal, Induction and modulation of resistance in tomato plants against *Fusarium* Wilt disease by bioagent fungi (*Arbuscular mycorrhiza*) and/or hormonal elicitors (Jasmonic Acid & Salicylic Acid): 2—changes in the antioxidant enzymes, phenolic compounds and pathogen related-proteins, Aust. J. Basic. Appl. Sci. 1 (2007) 717–732.
[32] S. Caretto, V. Linsalata, G. Colella, G. Mita, V. Lattanzio, Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress, Int. J. Mol. Sci. 16 (2015) 26378–26394.
[33] S. Hayat, Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel, A. Ahmad, A role of proline under changing environments: a review, Plant Signal. Behav. 7 (2012) 1456–1466.
[34] S.S. Gill, N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, Plant Physiol. Biochem. 48 (2010) 909–930.
[35] Y. Yamamoto, Y. Kobayashi, H. Matsumoto, Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots, Plant Physiol. 125 (2001) 199–208.
[36] L. Ma, Y. Li, C. Yu, Y. Wang, X. Li, N. Li, Q. Chen, N. Bu, Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress, Protoplasma 249 (2012) 393–399.
[37] J.P. Awasthi, B. Saha, P. Regon, S. Sahoo, U. Chowra, A. Pradhan, A. Roy, S.K. Panda, Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India, PLoS One 12 (2017), e0176357.
[38] L.E. Del Rio, N.C. Kurtzweil, C.R. Grau, Petiole inoculation as a tool to screen soybean germplasm for resistance to *Sclerotinia sclerotiorum*, Phytopathology 91 (2001) 176.
[39] A. Jain, S. Singh, B.K. Sarma, H.B. Singh, Microbial consortium- mediated reprogramming of defense network in pea to enhance tolerance against *Sclerotinia sclerotiorum*, J. Appl. microbial. 112 (2012) 537–550.
[40] G.B. Chopada, S. Pushpendra, Cultural and morphological variability among *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato under south Gujarat, India, J. Pure Appl. Microbiol. 8 (2014) 1109–1114.
[41] A. Jain, A. Singh, S. Singh, H.B. Singh, Phenols enhancement effect of microbial consortium in pea plants restrains Sclerotinia sclerotiorum, Biol. Control 89 (2015) 23–32.
[42] W. Song, L. Zhou, C. Yang, X. Cao, L. Zhang, X. Liu, Tomato *Fusarium* Wilt its chemical control strategies in a hydroponic system, Crop Protect. 23 (2004) 243–247.
[43] L. Jordan, Cohen, The Official Compendia of Standards. The United States Pharmocopedia (USP 24 NF 19). The United States Pharmocopedial Convention Press 12601, Twinbrook Parkway Rockville M. D., 1975, 20852.
[44] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358.
[45] L. Bates, R.P. Waldren, I.D. Teare, Rapid determination of free proline for water-stress studies, Plant Soil 39 (1973) 205–207.
[46] Z. Zheng, K. Shetty, Solid-state bioconversion of phenolics from cranberry pomace and role of Lentinusedodes b-glucosidase, J. Agric. Food Chem. 48 (2000) 895–900.
[47] C.H. Brueske, Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode (*Meloidogyne incognita*), Physiol. Plant Pathol. 16 (1980) 409–414.
[48] Y. Nakano, K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, Plant Cell Physiol. 22 (1981) 867–880.
[49] X. Zheng, R.B. Van Huystee, Peroxidase-regulated elongation of segments from peanut hypocotyls, Plant Sci. 81 (1992) 47–56.
[50] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[51] M. Schaedle, J.A. Bassham, Chloroplast glutathione reductase, Plant Physiol. 59 (1977) 1011–1012.
[52] L.D.S. Botelho, W.L.A. Zancan, J.D.C. Machado, E.N. Barrocas, Performance of common bean seeds infected by the fungus *Sclerotinia sclerotiorum*, J. Seed Sci. 35 (2013) 153–160.
[53] M.D. Bolton, B.P.H.J. Thomma, B.D. Nelson, *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen, Mol. Plant Pathol. 7 (2006) 1–16.
[54] J.A. Van Kan, Methods for gene function analysis in Botrytis cinerea, Trends Plant Sci. 5 (2006) 247–253.
[55] B. Williams, M. Kabbage, H.J. Kim, R. Britt, M.B. Dickman, Tipping the balance: *Sclerotinia sclerotiorum* secreted oxalic acid suppresses host defenses by manipulating the host redox environment, PLoS Pathog. 7 (2011), e1002107.
[56] M. Meena, P. Swapnil, K. Divyanshu, S. Kumar, Y.N. Tripathi, A. Zehra, A. Marwal, R.S. Upadhyay, PGPR mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: current perspectives, J. Basic Microbiol. 60 (2020) 828–861.
[57] M. Aamir, V.K. Singh, M.K. Dubey, S.P. Kashyap, A. Zehra, R.S. Upadhyay, S. Singh, Structural and functional dissection of differentially expressed tomato WRKY transcripts in host defense response against the vascular wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*), PLoS One 13 (2018), e0193922.
[58] M. Legein, W. Smets, D. Vandenheuvel, T. Eilers, B. Muyshondt, E. Prinsen, R. Samson, S. Lebeer, Modes of action of microbial biocontrol in the phyllosphere, Front. Microbiol. 11 (2020) 1619.
[59] W.A. Ansari, N. Atri, J. Ahmad, M.I. Qureshi, B. Singh, R. Kumar, V. Rai, S. Pandey, Drought mediated physiological and molecular changes in muskmelon (*Cucumis melo* L.), PLoS One 14 (2019), e0222647.
[60] K.K. Rai, N. Rai, M. Aamir, D. Tripathi, S.P. Rai, Interactive role of salicylic acid and nitric oxide on transcriptional reprogramming for high temperature tolerance in lablab purpureus L.: structural and functional insights using computational approaches, J. Biotechnol. 309 (2020) 113–130.
[61] S.P. Kashyap, N. Kumari, P. Mishra, D.P. Moharana, M. Aamir, B. Singh, H.C. Prasanna, Transcriptional regulation-mediating ROS homeostasis and physio-biochemical changes in wild tomato (*Solanum chilense*) and cultivated tomato (*Solanum lycopersicum*) under high salinity, Saudi J. Biol. Sci. 27 (2020) 1999–2009.
[62] M.T. Zeyad, W.A. Ansari, M. Aamir, R. Krishna, S.K. Singh, N. Atri, A. Malik, Heavy metals toxicity to food crops and application of microorganisms in bioremediation, in: Microbe Mediated Remediation of Environmental Contaminants, Woodhead Publishing, 2021, pp. 421–434.
[63] U. Conrath, G.J. Beckers, C.J. Langenbach, M.R. Jaskiewicz, Priming for enhanced defense, Annu. Rev. Phytopathol. 53 (2015) 97–119.
[64] D. Spadaro, S. Droby, Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists, Trends Food Sci. Technol. 47 (2016) 39–49.
[65] M. Aamir, K.K. Rai, A. Zehra, M.K. Dubey, S. Samal, M. Yadav, R.S. Upadhyay, Endophytic actinomycetes in bioactive compounds production and plant defense system in bioactive compounds production and plant defense system, in: Microbial Endophyt, Woodhead Publishing, 2020, pp. 189–229.
[66] M. Aamir, K.K. Rai, A. Zehra, S. Kumar, M. Yadav, V. Shukla, R.S. Upadhyay, Fungal endophytes: classification, diversity, ecological role, and their relevance in sustainable agriculture, in: Microbial Endophyt, Woodhead Publishing, 2020, pp. 291–323.
[67] U. Conrath, G.J. Beckers, V. Flors, P. García-Agustín, G. Jakab, F. Mauch, M.A. Newman, C.M. Pieterse, B. Poinssot, M.J. Pozo, A. Pugin, Priming: getting ready for battle, Mol. Plant Microbe Interact. 19 (2006) 1062–1071.
[68] I.P. Ahn, S.W. Lee, S.C. Suh, *Rhizobacteria* induced priming in Arabidopsis is dependent on ethylene, jasmonic acid, and NPR1, Mol. Plant Microbe Interact. 20 (2007) 759–768.
[69] D. Yu, C. Li, Y. Huang, Z. Huang, Joint action of *Trichoderma hamatum* and difenoconazole on growth of a phytopathogen *Sclerotinia sclerotiorum* under laboratory conditions, Pakistan J. Zool. 50 (2019) 2249–2259.
[70] K. Jetiyanon, Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strains (IN937a and IN937b) against different pathogens, Biol. Control 42 (2007) 178–185.
[71] M. Magnin-Robert, P. Trotel-Aziz, D. Quantinet, S. Biagianti, A. Aziz, Biological control of *Botrytis cinerea* by selected grapevine-associated bacteria and stimulation of chitinase and b-1,3 glucanase activities under field conditions, Eur. J. Plant Pathol. 118 (2007) 43–57.
[72] P.K. Mukherjee, B.A. Horwitz, C.M. Kenerley, Secondary metabolism in Trichoderma–a genomic perspective, Microbiology 158 (1) (2012) 35–45.
[73] G.E. Harman, C.R. Howell, A. Viterbo, I. Chet, M. Lorito, *Trichoderma* species opportunistic, avirulent plant symbionts, Nat. Rev. Microbiol. 2 (2004) 43–56.
[74] A. Yadav, A. Radhakrishnan, G. Bhanot, H. Sinha, Differential regulation of antagonistic pleiotropy in synthetic and natural populations suggests its role in adaptation, G3: Genes, Genomes, Genetics 5 (2015) 699–709.
[75] M.T. Abdullah, N.Y. Ali, P. Suleman, Biological control of *Sclerotinia sclerotiorum* (lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*, Crop Protect. 27 (2008) 1354–1359.
[76] A. Ali, M.A. Zeshan, M. Mehtab, S. Khursheed, M. Mudasir, M. Abid, M. Mahdi, H.A. Rauf, S. Ameer, M. Younis, M.T. Altaf, A comprehensive note on *Trichoderma* as a potential biocontrol agent against soil borne fungal pathogens: a review, Plant Prot. 5 (3) (2021) 171–196.
[77] K. Saravanakumar, L. Fan, K. Fu, C. Yu, M. Wang, H. Xia, J. Sun, Y. Li, J. Chen, Cellulase from *Trichoderma harzianum* interacts with roots and triggers induced systemic resistance to foliar disease in maize, Sci. Rep. 6 (2016) 1–18.
[78] A. Abbas, D. Jiang, Y. Fu, *Trichoderma* spp. as antagonist of *Rhizoctonia solani*, J. Plant Pathol. Microbiol. 8 (2017) 1–9.
[79] A. Martínez-Medina, I. Fernández, M.J. Sánchez-Guzmán, S.C. Jung, J.A. Pascual, M.J. Pozo, Deciphering the hormonal signaling network behind the systemic resistance induced by *Trichoderma harzianum* in tomato, Front. Plant Sci. 4 (2013) 206.
[80] N. Korolev, D. Rav David, Y. Elad, The role of phytohormones in basal resistance and Trichoderma-induced systemic resistance to *Botrytis cinerea* in *Arabidopsis thaliana*, BioControl 53 (2008) 667–683.
[81] W. Raza, N. Ling, R. Zhang, Q. Huang, Y. Xu, Q. Shen, Success evaluation of the biological control of Fusarium wilts of cucumber, banana, and tomato since 2000 and future research strategies, Crit. Rev. Biotechnol. 37 (2017) 202–212.
[82] G. Bubici, M. Kaushal, M.I. Prigigallo, C. Gómez-Lama Cabanás, J. Mercado-Blanco, Corrigendum: biological control agents against Fusarium wilt of banana, Front. Microbiol. 10 (2019) 1290.
[83] R. Hermosa, A. Viterbo, I. Chet, E. Monte, Plant-beneficial effects of *Trichoderma* and of its genes, Microbiology 158 (2012) 17–25.
[84] Y. Brotman, U. Landau, A. Caudros-Inostroza, T. Tohge, A.R. Fernie, I. Chet, et al., *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance, PLos Pathol 9 (2013), e1003221.
[85] M. De Palma, M. Salzano, C. Villano, R. Aversano, M. Lorito, M. Ruocco, T. Docimo, A.L. Piccinelli, N. D'Agostino, M. Tucci, Transcriptome reprogramming, epigenetic modifications and alternative splicing orchestrate the tomato root response to the beneficial fungus, Trichoderma harzianum. Hortic. Res. 6 (2019).
[86] S. Galletti, R. Paris, S. Cianchetta, Selected isolates of *Trichoderma gamsii* induce different pathways of systemic resistance in maize upon *Fusarium verticillioides* challenge, Microbiol. Res. 233 (2020), 126406.
[87] M.F. Pimentel, E. Arnão, A.J. Warner, A. Subedi, L.F. Rocha, A. Srour, J.P. Bond, A.M. Fakhoury, *Trichoderma* isolates inhibit *Fusarium virguliforme* growth, reduce root rot, and induce defense-related genes on soybean seedlings, Plant Dis. 104 (2020) 1949–1959.
[88] J. Mathys, K. De Cremer, P. Timmermans, S. Van Kerckhove, B. Lievens, M. Vanhaecke, B.P. Cammue, B. De Coninck, Genome-wide characterization of ISR induced in Arabidopsis thaliana by *Trichoderma hamatum* T382 against Botrytis cinerea infection, Front. Plant Sci. 3 (2012) 108.
[89] L.S. Ryder, B.D. Harris, D.M. Soanes, M.J. Kershaw, N.J. Talbot, C.R. Thornton, Saprotrophic competitiveness and biocontrol fitness of a genetically modified strain of the plant-growth-promoting fungus *Trichoderma hamatum* GD12, Microbiology 158 (2012) 84–97.
[90] N.A. Boccardo, M.E. Segretin, I. Hernandez, F.G. Mirkin, O. Chacón, Y. Lopez, O. Borrás-Hidalgo, F.F. Bravo-Almonacid, Expression of pathogenesis-related proteins in transplastomic tobacco plants confers resistance to filamentous pathogens under field trials, Sci. Rep. 9 (2019) 1–13.
[91] K. Saravanakumar, Y. Li, C. Yu, Q.Q. Wang, M. Wang, J. Sun, J.X. Gao, J. Chen, Effect of *Trichoderma harzianum* on maize rhizosphere microbiome and biocontrol of Fusarium Stalk rot, Sci. Rep. 7 (2017) 1–13.
[92] B. Sánchez-Montesinos, F. Diánez, A. Moreno-Gavira, F.J. Gea, M. Santos, Plant growth promotion and biocontrol of *Pythium ultimum* by saline tolerant *Trichoderma* isolates under salinity stress, Int. J. Environ. Res. Publ. Health 16 (2019) 2053.
[93] R.L. Nicholson, R. Hammerschmidt, Phenolic compounds and their role in disease resistance, Annu. Rev. Phytopathol. 30 (1992) 369–380.

[94] P.F. Wen, J.Y. Chen, W.F. Kong, Q.H. Pan, S.B. Wan, W.D. Huang, Salicylic acid induced the expression of phenylalanine ammonia lyase gene in grape berry, Plant Sci. 169 (2005) 928–934.
[95] S. La Camera, G. Gouzerh, S. Dhondt, L. Hoffmann, B. Fritig, M. Legrand, T. Heitz, Metabolic reprogramming in Plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways, Immunol. Rev. 198 (2004) 267–284.
[96] M.J.D. MacDonald, G.B. Cunha, A modern view of phenylalanine ammonia lyase, Biochem. Cell. Biol. 85 (2007) 273–282.
[97] M. Shoresh, G.E. Harman, The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach, Plant Physiol. 147 (2008) 2147–2163.
[98] K.A. Abo-Elyousr, M. Hashem, E.H. Ali, Integrated control of cotton root rot disease by mixing fungal biocontrol agents and resistance inducers, Crop Protect. 28 (2009) 295–301.
[99] S. Lehmann, D. Funck, L. Szabados, D. Rentsch, Proline metabolism and transport in plant development, Amino Acids 39 (2010) 949–962.
[100] A.D. Hanson, C.E. Nelsen, A.R. Pedersen, E.H. Everson, Capacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance, Crop Sci. 19 (1997) 489–493.
[101] R.M. Ram, H.B. Singh, Biocontrol potential of drought tolerant rhizospheric bacteria isolated from stressed ecosystems against dry root rot of chickpea (*Cicer arietinum* L.) caused by *Rhizoctonia bataticola*, Int. J. Environ. Agric. Biotech. 10 (2017) 333.