

Elevation of Systemic Defense in Potato Against *Alternaria Solani* by a Consortium of Compatible *Trichoderma* Spp.

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Abstract

The crop loss due to phytopathogens is a serious problem affecting the entire world. To avoid economic losses due to phytopathogens synthetic chemicals have been used for years generating serious concerns about the human health and environment. Today the use of beneficial microorganisms to treat phytopathogens is gaining attention. In this way, *Trichoderma* spp. has been used for combating plant diseases and inducing defense response in plants. With this idea in mind, in this study we evaluate the effectiveness of *Trichoderma viride* and *T. harzianum* as single as well as in combination for elevating the defense response and growth promotion activities in potato challenged with *Alternaria solani*. The mycelial inhibition of *A. solani* by *T. viride* and *T. harzianum* was recorded and compared with control. Scanning electron microscope (SEM) observation revealed the collapsed hyphae and sunken conidia of *A. solani* due to antagonistic activity of *T. viride* and *T. harzianum*. Induction of defense enzymes including TPC, PAL, SOD and total protein content was increased in *Trichoderma* spp. treated plants as compared with pathogen inoculated plants. HPLC analysis demonstrated higher production in phenolic compounds during combined application of *Trichoderma* spp. treated potato plants in the response of *A. solani* infection. Moreover, treatment with *Trichoderma* spp. consortium showed significant growth promotion in potato plants comparing with the control.

Introduction

The crop loss due to numerous pests and pathogens threatens is a current serious problem worldwide. However, plants can have a complicated defense network in response to phytopathogens. The most important forms of induced resistance in plants fall into two categories such as systemic acquired resistance (SAR) and induced systemic resistance (ISR). The first resistance, called SAR, is triggered when pathogens invade plants where the potential signal molecule salicylic acid (SA) is required and it is noted and related with PR protein accumulation for its activation (Moghaddam et al., 2019). The second defense response of induced resistance through plant growth promoting rhizobacteria, called ISR, is produced by translocatable signals and indirectly related to SA, but it is directly regulated by jasmonic acid and ethylene (Conrath, 2011). When plants are attacked by phytopathogens, they can activate the defense genes and induce several reactive oxygen species (ROS), biosynthesize various PR proteins and firmly produce antimicrobial compounds. Current studies revealed that the beneficial microbes can induce defense response in crop plants, such as, induced systemic resistance (ISR) (Jain et al., 2012). Plants stimulate defense response via a wide array of defense enzymes including phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), β -1,3-glucanase, chitinase accumulation and proline that have been noted to be expressed with ISR, preventing infection caused by phytopathogens (Jetiyanon 2007; Ngadze et al., 2012; Awan and Shoaib, 2019).

Among the most important agricultural crops for human consumption is potato (*S. tuberosum* L.) which has been an important mankind's food crops and constituent of cuisines. Since it is an important source of vitamin B, vitamin C and carbohydrate, it has been the third most prominent cultivated crops worldwide. Early blight caused by *A. solani* is a major constraint in potato production, since generates severe reduction in this plant production (Shuman and Christ, 2005) hampering crop production globally. Therefore *A. solani* phytopathogen has been associated with remarkable losses in terms of quantity and quality during storage (Al-Mughrabi, 2005). Among the characteristics disease symptoms firstly there are irregular dark brown to black spots in concentric rings visible on older leaves because of unequal growth of pathogen in damaged tissue (Fairchild et al., 2013). The lesions look like "target-spot" and "bull's eyes" and prominently affect the tubers and diminish the germination ability of seed potatoes.

During the years the disease has been managed by a regular application of synthetic chemicals on the seed treatment as well as foliar spray, but these are not long-term solutions since the pathogen population has developed resistance against these chemicals (Edin et al., 2019; Odilbekov et al., 2019). Therefore, a green and environmentally safe solution is needed to increase defense response in potato plants. The use of beneficial microorganisms as biological control agents (BCAs) including *T. viride*, *T. harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces griseoviridis*, and *Paenibacillus acerans* for inducing defense response in potato plants against *A. solani* has been applied for some years ago (Murmuet et al., 2015; Zhang et al., 2020).

Trichoderma spp. is a soil borne genus, which has a well-established antagonistic properties, forms a green spores has wide diversity in ecosystem and can potentially colonize plant root and shoot promoting plant growth by producing beneficial metabolites (Contreras-Cornejo et al., 2014; Manganiello et al., 2018). *Trichoderma* spp., use their enzymatic and chemical weapons for combating the disease and induce defense response in plants. Defense signal transduction pathways have been employed to trigger the defense genes regulating the antagonistic activity. These pathways include mitogen-activated protein kinase (MAPK) cascades, heterotrimeric G-protein signaling and cAMP pathway (Zeilinger and Omann, 2007). *Trichoderma* spp. can induce ISR defense by the stimulation of chitinase and peroxidase activity, and induce genes that are involved in jasmonic acid or ethylene signaling pathways playing a pivotal role in to confer resistance to plant against several phytopathogens (Shoresh et al., 2005; Salas-Marina et al., 2011; Lopes et al., 2012).

In this study we evaluate the effectiveness of *T. viride* and *T. harzianum* as single as well as in combination for elevating the defense response and growth promotion activities in potato challenged with *A. solani*.

Materials And Methods

Microbial strains

The culture of *A. solani* pathogen strain was provided from the Indian Type Culture Collection (ITCC), New Delhi, India with the ITCC No. 3640. The fungus was cultured on Potato Dextrose Agar (PDA) medium. Moreover, the antagonistic cultures of *T. viride* (ITCC No. 7057) and *T. harzianum* (ITCC No.6908) were provided from ITCC, New Delhi. The biocontrol agents were cultured on *Trichoderma* Selective Medium (TSM) and stored at 4°C till further use.

Efficacy of *Trichoderma* spp. against the pathogen using the *in vitro* dual culture assay

Antagonistic potential of *Trichoderma* spp. was evaluated on PDA Petri plates by dual culture assay (Fokkema, 1978). The mycelial discs of 6 mm diameter were taken from the *Trichoderma* spp. five days old cultures and were placed at one end of the PDA plates keeping 1 cm distance from the edge. Mycelial discs (6 mm) of test pathogen were also placed opposite to the mycelial discs of *Trichoderma* spp. Control plates were maintained for both *Trichoderma* spp. and pathogen. These plates were incubated at 28°C and percent growth inhibition (PGI) was calculated on seventh day by using the following formula:

$$\text{PGI} = [(C - T) / C \times 100]$$

Where,

PGI = Growth inhibition of pathogen (%)

C = Radial growth of the pathogen (control)

T = Radial growth of the pathogen (treated).

Study of *A. solani* conidial germination inhibition by *Trichoderma* spp. using microscopy

A. solani was tested by microscopic slide technique to determine the conidial germination (Zivkovic et al, 2010). Edges of parasitized *A. solani* hyphae by *Trichoderma* spp. were taken and placed on clean slides. The cultures of *Trichoderma* spp. and *A. solani* grown on PDA medium were incubated in BOD at 28°C. Spores were harvested in Tween 20 solution (v/v 0.01%), and concentration of spore suspensions determined by hemocytometer. 50µl of *Trichoderma* spp. and *A. solani* suspensions were mixed separately, and then poured into cavity slides. In the control only conidial suspension of *A. solani* with sterile distilled water was taken and each slide was incubated for 24h in moist chambers. Three replications were conducted for each treatment. Inhibition of conidial germination was observed under a light microscope.

Scanning electron microscope (SEM) study of post-interaction events

1 cm bits of agar from the interaction region were taken from each plate and cultured on fresh sterile plate. The sample was vacuum dried with help of vapor diffusion dehydration assembly and fixed at 4°C for 2 h in 4 % (v/v) glutaraldehyde in 0.05% sodium cacodylate buffer solution (pH 7.2), after repeated washing with same buffer the sample was dehydrated with 70-100% acetone and post fixation was done with 1% (w/v) osmium tetroxide. After fixation, the sample was placed in sterile separate aluminum disc cup and critical points dried in CO₂, samples were mounted on double layer tape affixed to SEM specimen and sputter-coated with gold (Lopez-Llorca and Duncan 1988). These samples were examined at 15 kV in ZEISS electron microscope (Japan).

Preparation of Inoculum for Treatment of Potato tubers with *Trichoderma* spp.

Both *T. viride* and *T. harzianum* were selected and seven-day old cultures were washed properly with sterile distilled water and filtered through double layer muslin cloth and used in spore suspension preparation. The spore's quantity measured as 2×10⁷ spore per ml was maintained by using a hemocytometer.

Culture of *A. solani* (10 days old) was used for making spore suspension of test pathogen. Culture of *A. solani* was flooded with Tween 20, scraped with the help of sterile rubber spatula. The test pathogen spore suspension was filtered through double layers of cheese cloth. Final spore concentration in suspension was measured by hemocytometer and adjusted to 1.5-2.0×10⁵ conidia per ml to use for inoculation in potato plants.

Effect of treatments on plant growth activities *in vivo*

The experiments were conducted in the green house the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi. The details of the treatments used to determine the plant growth promotion activity are given below:

T1: *T. viride*

T2: *T. harzianum*

T3: *T. viride* + *T. harzianum*

T4: Control

The seed tubers of potato variety 'Kufri Bahar' were sown in 30 cm size pots, which were already filled with sterile soil mixture containing, sandy loam soil: farmyard manure: vermi-compost in the ratio of 2:1:1. Seed tubers were treated separately with talc-based formulation of *T. viride*, *T. harzianum* (2×10⁷ spore per ml) and their mixture (1:1). The treated potato tubers were further incubated for 24 h under room temperature and ready for sowing in pot. In control pot, tubers were treated with only formulation material without *Trichoderma* spp. There were three replications which were conducted for one treatment with each pot containing five seed tubers sown and maintained regularly with water. The whole experiment was repeated twice, and greenhouse conditions were maintained at 30°C and 80 % relative humidity (RH).

After 45 days of sowing, three plants were selected randomly from each treatment and growth parameters including shoot length, root length, fresh weight of shoot and root, dry weight of shoot and root, number of leaves and tuber yields were recorded.

Greenhouse experiments for estimating the defense related enzymes

The 45 days old potato plants were used for evaluating the effect of two *Trichoderma* strains separately or in combination on the induction of defense activities in *A. solani* challenged potato plants.

Biochemical analysis of defense related enzymes

The fresh leaves were collected at 0 to 72 h after pathogen inoculation. Take plant samples from each treatment and then gently washed through running tap water and use it for estimation of changes in the action of enzymes in potato plants. The samples that were collected were stored at -80°C for further used until the completion of experiments.

Total phenolic content (TPC) assay

The determination of total phenolic content was done following the method of Ragazzi and Veronese (1973). Briefly we took 0.1 g fresh leaf tissue was crushed in 10 ml of 95 % ethanol and left the sample for 1 h and centrifuged at 13,000 g for 10 min, collecting the supernatants. Enzyme solution 1 ml was taken in a separate test tube, 5 ml of distilled water and 0.5 ml of 50 % Folin-Ciocalteu's reagent (FCR) were added and gently mixed. After 10 min, 1.0 ml of sodium carbonate (5 %) was added, and the reaction mixture was vortexed and left for 1 h and to measure the absorbance at 725 nm with ethanol as black. The absorbance values were expressed in μg gallic acid (GA) equivalent g^{-1} fresh weight (FW).

Phenylalanine ammonia-lyase (PAL) assay

Phenylalanine ammonia-lyase analysis was assayed following the method of Brueske (1980). Fresh leaf sample (0.1 g) was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) which consisted on 1.4 mM β mercaptoethanol in a pre-chilled mortar and pestle, and the solution was centrifuged at 16,000 g for 15 min at 4°C. The reaction mixture, comprising 0.2 ml of enzyme extract, was incubated with 0.2 M phosphate buffer (0.5 ml; pH 8.7) followed by the addition of distilled water 1.3 ml. The reaction started by the adding of 0.1 M phenylalanine and incubated for 30 min at 32°C. The reaction was stopped by addition of 1M trichloroacetic acid 0.5 ml and absorbance was recorded at 290 nm. The amount of *trans*-cinnamic acid was defined as the μmol TCA mg^{-1} fresh weight.

Total Protein Content

The method developed by Lowry et al. (1951) was used to determine the total protein content in potato leaves. Fresh leaf sample 1 g from each treatment was homogenized in extraction buffer in pre-chilled mortar and pestle. Enzyme extract was centrifuged at 10,000 g for 30 min at 4°C collecting the supernatants. 7.5 ml of supernatant were transferred in a separate tube, gently mixed with sample buffer 2.5 ml and used for protein estimation. The working standard solution was pipette out and 0.2, 0.4, 0.6, 0.8 and 1.0 ml were put into series of test tubes affording to 1 ml final volume with distilled water. A separate tube with 1 ml of water was performed as a blank. 5 ml of solution C were added in each tube and incubated at room temperature for 15 min and then 0.5 ml FCR were added and gently mixed and incubated 30 min in dark condition. Absorbance was recorded at 660 nm against the blank and amount of total protein was expressed as μg g^{-1} fresh weight.

Superoxide Dismutase (SOD) assay

Superoxide Dismutase (SOD) activity was performed following the Fridovich method (1974). 0.5 g fresh leaf sample were taken from each treatment and homogenized in 2 ml of 0.1M phosphate buffer containing 0.5 mM EDTA at pH 7.5. The homogenate was centrifuged at 15,000 g in 4°C for 20 min. The reaction mixture comprised methionine 200 mM, phosphate buffer 100 mM (7.8 pH), nitroblue tetrazolium chloride 2.25 mM, EDTA 3.0 mM, sodium carbonate 1.5 M and enzyme extract, making up 3 ml of reaction mixture final volume. The reaction was begun by adding 2 mM riboflavin (0.4 ml), and placing each tube under 15 W fluorescent lamps for 15 to 20 min. The reaction was stopped by switching off the lights and keeping the tubes in dark condition for 15 min and absorbance was observed at 560 nm. Reaction mixture without enzyme was performed as a control. SOD activity was defined as the amount of the enzyme reducing the absorbance to 50 % in comparison with control.

β -1, 3 glucanase assay

The β -1, 3 glucanase enzyme was determined following the method of Pan and co-workers (1991). Fresh leaf samples 0.1 g was homogenized in 0.05 M sodium acetate buffer 2.0 ml (pH 5.0) and the sample was centrifuged at 13,000 g at 4°C for 15 min. The reaction was started by mixing supernatant 0.25 ml, 1 M sodium acetate buffer 0.3 ml (pH 5.3) and laminarin 0.5 ml and after solution was incubated at 40°C for 60 min. The reaction is stopped by adding 3, 5-dinitrosalicylic acid 0.375 ml. The final-colored solution was diluted with distilled water and absorbance was measured at 500 nm. The β -1, 3 glucanase enzyme activity was defined as μg glucose released min^{-1} g^{-1} fresh weight.

Histochemical assay for visualization of O_2^- (superoxide radicals) and H_2O_2

Potato leaves were sampled randomly at 15 days after pathogen inoculation for each treatment and used for localization of O_2^- radicals. The superoxide radical detection level was measured by nitro blue tetrazolium (NBT; Hi Medea, India) solution with slight modification (Kumar et al., 2014) and its presence was detected by developing blue color on the leaves. For the H_2O_2 detection was further examined to visualize the localization pattern that was done by DAB staining with slight modification (Thordal-Christensen et al., 1997). The H_2O_2 reacts with DAB and developed reddish-brown color was visualized under a light microscope (Nikon DS-fi1, Japan).

High performance liquid chromatography (HPLC) analysis of phenylpropanoid derivatives

Take 1 g of fresh leaf sample harvested 0 h, 24 h, 48 h, and 72 h after inoculation of the pathogen and was homogenized with 10 ml of 50% methanol and centrifuged for 15 min at 13,000 g. After centrifugation solvent was removed under reduced pressure on rotary evaporator (Eyela N-N series, Japan). The residue was dissolved with HPLC grade methanol. The compounds separation was done using the protocol of Singh et al., (2009). The solvent flow rate was 1.0 mL min⁻¹ and phenolics were detected using UV detector SPD-10A. Their identification was done by comparing the retention times with those from authentic standards at 254 nm.

Measurement of Disease Severity

The disease severity observation was recorded after 15 days of pathogen inoculation and compared with control plants. Three leaves were randomly selected from each treatment for disease severity measurement using 0-9 scale as described by Mayee and Datar, 1986.

Statistical analysis

The analysis was done by using IBM SPSS, Version 20. The presented values from different experiments were the mean of three replications and \pm show standard deviation (SD) for each treatment. The present data was statistically analyzed by using variance one-way analysis (ANOVA) and treatment mean values were compared with Duncan's multiple range tests at the $p \leq 0.05$ significance level.

Results

Growth inhibition of pathogen by *Trichoderma* spp. in dual culture assay

The dual culture assay results revealed that both *T. viride* and *T. harzianum* significantly inhibited the growth of *A. solani* under *in vitro* condition (Figure 1). As shown in figure 1, maximum growth inhibition was recorded by *T. viride* against *A. solani* (91.88%) on seven day of inoculation, while for *T. harzianum*, the lowest growth inhibition against *A. solani* (80.11%) as compared to control.

Study of *A. solani* conidial germination inhibition by *Trichoderma* spp. using microscopy

A study of *A. solani* conidial germination inhibition assay by *T. viride* and *T. harzianum* was realized using microscopic slide technique. In general, both *Trichoderma* spp. significantly reduced *A. solani* conidial germination when compared to the control. The initial spore's concentration in the control suspension of *A. solani* was found to be 4.5×10^4 spore per ml against *Trichoderma* spp. The highest reduction of *A. solani* conidial germination was found when they were tested against *T. viride*, being the spore concentration of *A. solani* reduced up to 1.9×10^2 spore per ml. Whereas, when the pathogen was tested against *T. harzianum* the spore concentration of *A. solani* was slightly reduced to 3.31×10^2 spore per ml.

Ultrastructure defects on *A. solani* hyphae and spores under Scanning electron microscope

Scanning electron microscopy was used to identify the morphological changes in *A. solani* hyphae and spores due to antagonistic activity of *T. viride* and *T. harzianum* (Figure 2). The SEM results revealed that both *T. viride* and *T. harzianum* damaged mycelium and conidia of *A. solani*. As shown in figure 2A is observed that the hyphae (green arrow) and spores (yellow arrow) of *A. solani* were damaged by *T. viride* since the conidia and mycelia are collapsed and appeared broken and shrieked. In figure 2B can be observed some *T. harzianum* hyphae coiled (red arrows) around *A. solani* hyphae penetrating *A. solani* hyphae.

Effect of *Trichoderma* spp. on plant growth promotion and disease response *in vivo*

It was studied the *in vivo* effect of *Trichoderma* spp. on plant growth promotion response as shown in figure 3. Treatment with *T. harzianum* T2 recorded maximum shoot and root length (30.56 and 13.06 cm), respectively comparing to *T. viride* and control, while, when plants were treated with *T. viride* showed maximum number of leaves (54 per plant), shoot and root length, 32.74 and 2.07 cm respectively and their respective fresh and dry weight, 1.37 and 0.24 gm as compared to *T. harzianum* and control. Potato plants treated with the combination of both bioagents exhibited maximum growth parameters on all plant growth promotion activities compared to plants treated with individual *Trichoderma* spp. The control plants showed minimum plant growth promotion parameters (Table 1). The yield of potato tubers was recorded significantly higher in *Trichoderma* consortium treated plants as compared to control plants (Table 1). Plant treated with *T. viride* + *T. harzianum* consortium showing higher yield 621.45 gm per pot, corresponding to control 377.34 gm per pot was recorded. In the case of individual *T. viride* and *T. harzianum* treated plants was recorded 459.59 gm per pot and 425.49 gm per pot respectively.

The disease response results revealed that maximum early blight infection was observed in the control plants (*A. solani*) wherein the concentric rings were formed and covering the leaves. The response of disease development under various treatments is shown in figure 4 (A-D). Potato plants treated with *T. viride* were recorded to have 65.63 % of disease inhibition over control plants. On the other hand, minimum disease inhibition 34.37 % was recorded in *T. harzianum* treated plants. The maximum disease inhibition was recorded in plants treated with both *Trichoderma* spp. compared with control plants, that is, 85.94 % (Figure 5).

Table 1: Efficacy of *Trichoderma* spp. on growth parameters of potato plant

Treatment	Shoot height (cm)	Root height (cm)	No. of leave per plant	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)	Yield (gm/pot)
<i>T. viride</i>	27.4±2.06 ^b	10.76±1.59 ^c	54±3.60 ^b	32.74±3.08 ^b	2.07±0.21 ^b	1.37±0.23 ^b	0.24±0.08 ^b	459.59±115.72 ^{ab}
<i>T. harzianum</i>	30.56±2.30 ^{ab}	13.06±2.23 ^b	45±6.0 ^c	25.67±3.55 ^{ab}	1.46±0.15 ^{ab}	1.33±0.30 ^c	0.21±0.03 ^c	425.49±111.99 ^b
<i>T. viride</i> + <i>T. harzianum</i>	33.46±3.07 ^a	17.26±2.11 ^a	81.33±2.51 ^a	35.18±6.0 ^a	3.73±1.09 ^a	1.62±0.28 ^a	0.33±0.11 ^a	621.45±49.32 ^a
Control	18.8±3.41 ^c	7.06±0.85 ^d	27±2.0 ^d	13.88±3.14 ^c	1.34±0.51 ^c	1.22±0.22 ^d	0.14±0.05 ^d	377.34±100.06 ^c
CD (0.05%)	5.52	2.24	8.78	8.51	2.13	1.92	1.05	173.67

The results are expressed as means of three replicates, ± indicate standard deviations of means. Different letters indicate significant differences between the treatments according to DMRT at $p < 0.05$.

Effect of *Trichoderma* spp. on synthesis and accumulation of superoxide radicals (O₂⁻) and hydrogen peroxide (H₂O₂)

Microscopic visualization of superoxide radicals in potato leaves revealed that minimum reactive oxygen species (ROS) accumulation was found in control as compared to other treatments. However, higher production of ROS was found in the plant treated with the consortium of *Trichoderma* spp. Moreover, when plants were treated with a single *Trichoderma* spp. they have less production of ROS as compared to combinations of both *Trichoderma* spp. (Figure 6). The visualization of H₂O₂ accumulation was observed as brown polymerization reacting with DAB staining. The significantly less accumulation of H₂O₂ was observed in the potato leaves that were treated with consortium of both *Trichoderma* spp. strains as compared with individual inoculation of *Trichoderma* spp. strain. However, the least amount of brown pigmentation (H₂O₂) was observed in control plants (Figure 7).

Individual and consortium of *Trichoderma* spp. effects on specific phenolics compounds

Quantitative analysis of three potential phenolic compounds, such as, syringic acid, shikimic acid, and gallic acid was realized in potato leaves at different time of intervals after pathogen inoculation under the four treatments before mentioned. As shown in table 2 the level of the phenolic compounds is increased both under individual and consortium treated potato plants: however, the values are significantly higher in *Trichoderma* consortium treated plants when compared with pathogen treated control potato plants. The significant induction was found in all three compounds at 48 h after pathogen inoculation in singly or combined application of *Trichoderma* spp. The increase in syringic acid, shikimic acid and gallic acid was 3.4 times, 2.9 times and 3 times, respectively higher at 48 h after pathogen inoculation with the treatment of *T. viride* + *T. harzianum* + pathogen inoculated potato plants when compared with pathogen inoculated control plants. At 72 h after pathogen inoculation phenolic compounds were recorded to decline sharply in all treatments (Table 2).

Phenolic compounds	0 h after pathogen inoculation (hapi)				24 h after pathogen inoculation (hapi)				48 h after pathogen inoculation (hapi)				72 h after pathogen inoculation (hapi)			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Syringic acid	5.6	8.1	11.3	3.3	6.2	9.3	10.2	2.8	12.3	14.5	18.6	5.4	11.1	13.2	14.4	4.6
Shikimic acid	232.2	214.5	321.2	105.5	289.9	285.6	465.8	190.3	325.1	415.5	778.8	260.2	296.6	369.6	479.7	210.
Gallic acid	22.2	14.4	18.6	8.9	20.1	15.2	20.2	9.2	26.5	24.4	37.5	12.1	23.2	21.3	29.9	10.2

T1: *T. viride*, T2: *T. harzianum*, T3: *T. viride* + *T. harzianum*, T4: Control. The results show a mean of three replicates expressed in µg phenolic acid/g fresh weight (FW).

Effect of *Trichoderma* spp. on TPC and PAL activity

The total phenol content (TPC) was analyzed in *Trichoderma* spp. inoculated plants, either singly or consortium compared with control plants inoculated with pathogens (Figure 8). The results showed that the maximum amount of TPC in potato leaves was recorded at 48 h after pathogen inoculation and increasing trend continued till 48 h after which a decrease was recorded. It can be appreciated that potato plants treated with *T. viride* + *T. harzianum* and challenged with pathogen had the maximum amount of TPC, that is, 3.19 times higher than the corresponding control treatments at 48 h of pathogen inoculation. Individual application of *T. viride* and *T. harzianum* treated plants challenged with test fungus also showed significantly higher phenol content, 2.72 and 2.19 times higher at 48 h after pathogen inoculation compared with the pathogen treated control. In the same line, phenylalanine ammonia-lyase (PAL) activity was measured. Similarly, the consortium of *T. viride* and *T. harzianum* treated plants strongly stimulated the PAL activity with the maximum value at 48 h after pathogen inoculation compared to the control plants (Figure 9). The PAL activity level was significantly higher in consortium of *Trichoderma* spp. strain, approximately 3.72-fold, being 2.92-fold in *T. viride* and 3.07-fold in *T. harzianum* when compared with control pathogen inoculated plants at 48 h after pathogen inoculation. A decrease level of PAL activity was recorded at 72 h after pathogen inoculation in all treatments.

Effect of *Trichoderma* spp. on protein and SOD activity

The total protein content was measured in all treatments and the results showed the plants treated with *Trichoderma* spp. Consortium triggered the total protein content in the leaves of potato increasing trend 0 to 48 hpi (Figure 10). Maximum protein content was noted to be nearly 2.50 times higher in treatments with *Trichoderma* spp. consortium and 1.82 times and 1.86 times higher in treatments with *T. viride* and *T. harzianum* respectively at 48 h after inoculation of the pathogen compared to control. At 72 hpi total protein content declined sharply in all treatments (Figure 10). Similarly, the superoxide dismutase (SOD) activity was measured and the results showed an elevated level of SOD activity in plants treated with *Trichoderma* spp. consortia, challenged with *A. solani* exhibiting a significantly higher content of SOD than pathogen inoculated control plants (Figure 11). Individual application of *T. viride* and *T. harzianum* remarkably increased level of SOD observed. After 48 hpi the level of SOD was declining gradually.

Effect of *Trichoderma* spp. on activity of β -1, 3- glucanase

Pathogenesis related protein (PR-2) also is correlated with β -1,3glucanase enzyme activity providing first line defense against pathogen infection. Therefore, the β -1, 3 glucanase enzyme activity was measured and the results showed that this activity is significantly higher in the consortium of *T. viride* + *T. harzianum* treated plants as well as individually treated with *Trichoderma* spp. compared to the control plant (Figure 12) being the maximum β -1, 3- glucanase level at 72 h after pathogen inoculation, that is, 35.51 μg glucose released $\text{min}^{-1} \text{g}^{-1}$ fresh weight, it means 2.21- fold higher than control. The plants treated with singly *T. viride* and *T. harzianum* was recorded 25.15 μg glucose released $\text{min}^{-1} \text{g}^{-1}$ fresh weight and 23.71 μg glucose released $\text{min}^{-1} \text{g}^{-1}$ fresh weight respectively that were, 1.56 and 1.47-fold higher than control which contains the lowest value of β -1,3- glucanase, that is, 16.04 μg glucose released $\text{min}^{-1} \text{g}^{-1}$ fresh weight (Figure 12).

Discussion

Plants are armed with an array of cellular mechanisms to defend themselves against diseases causing phytopathogens. Biocontrol agents may regulate these cellular mechanisms and promote the expression of defense genes (Viterbo et al., 2005; Segarra et al., 2007), for the induction of plant metabolic process, to be expressed when plants challenged with biotic and abiotic stresses (Van der Ent et al., 2009) specially after plants inoculated with pathogens (Conrath et al., 2006). These defense mechanisms include the production of reactive oxygen species (ROS) and upregulation of disease defense related genes (Ahn et al., 2007). From the results of this study, we assess the effect of an individual and consortium of *T. viride* and *T. harzianum* on *in vitro* *A. solani* mycelial growth and conidial germination inhibition, and on *in vivo* suppression of early blight, studying at the same time the plant growth parameters in the presence of *Trichoderma* spp. All results indicated that both *Trichoderma* spp. strains, especially when they are applied together, are beneficial for the potato plant defense against *A. solani* and promote the growth response in the plant, being eco-friendly and an environmentally safe control strategy for combating the early blight caused by this pathogen. This is congruent with an earlier report of Yedidia et al., (1999) who showed the activation of induced systemic resistance in plants treated with *Trichoderma* spp. against infection of phytopathogens.

The inhibition of mycelial growth of pathogens by antagonistic organisms in dual culture is characterized by production of inhibitory compounds (Tapwal et al., 2015). Significant inhibition of radial growth of phytopathogens by various spp. of *Trichoderma*, *Bacillus* and *Pseudomonas* under *in vitro* condition was reported by many scientist (Buragohain et al., 2000; Shaikh and Sahera, 2013; Enespa and Dwivedi, 2014; Sansinenea et al., 2016). In the present study, results of dual culture assay with microscopic observation of the individual interaction between the *Trichoderma* spp. *A. solani* pathogen, revealed that the maximum inhibition of *A. solani* by *T. viride* was 91.88 % and by *T. harzianum* was 80.11 % compared with control. The similar findings were reported by Mokhtar and Aid (2013). Moreover, Prasad and Kumar (2011) revealed that *Trichoderma* spp. is a potent biocontrol agent under *in vitro* condition for reducing the mycelial growth of several pathogens including *F. oxysporum*, *A. alternata* and *R. solani*. *Trichoderma* spp., especially *T. viride* and *T. harzianum*, produces a wide array of volatile and non-volatile metabolites that significantly affect the growth of many microorganisms, including *Alternaria* spp., *R. solani* and *F. oxysporum* and reduce the mycelial growth of early blight pathogen (Amin et al., 2010; Tapwal and Pandey, 2016).

Plants have capability to produce many phenolic substances that are known to be antimicrobial (Abo-Elyousr et al., 2009) and play a pivotal role as a precursor to the synthesis and accumulation of lignin, that's provides strong barrier against pathogens. The phenolic substances regulate the signal molecules that are directly associated with defense related genes (Dakora, 1996; Keswani et al., 2017). In this work we report that activity of total phenolic content in potato plants was significantly increased by the treatment of the *T. viride* and *T. harzianum* consortium. The synthesis and accumulation of phenol is increased in plants treated with jasmonic acid alone or in combination with antagonistic fungus, inducing a plant defense response and changing plant metabolic processes (Gadzouska et al., 2007; Harman et al., 2004). In the present work the suppression of early blight of potato caused by *A. solani* could be related with the higher production of TPC.

Trichoderma spp. treated plants enhancing the plant growth promotion response has been widely studied in vitally important crop plants affected by various disease-causing agents (Singh et al., 2013). In the present study, potato plants that were treated with *Trichoderma* spp. strains showed significant increase in the shoot and root length, and plant dry weight. Interestingly, plants treated with individually *T. viride* enhanced the number of leaves along with fresh and dry weight compared with *T. harzianum* treated plants and control. These findings are correlated with the results of Srivastava and co-workers (2010) who reported that the use of *Trichoderma* spp. isolates as consortium in tomato plants showed the highest shoot length and combated Fusarium wilt. Contreras-Cornejo and co-workers (2014) demonstrated the systemic effect of *Trichoderma* spp. which was able to enhance plant growth by uptake of more plant beneficial micronutrients along with development of primary and secondary roots through augmentations of phytohormones.

Plants that are treated with bioagents can augment their effector molecules activity of the flux by phenylpropanoid pathway. PAL is the first line defense enzyme that is required for the crucial phenylpropanoid biosynthesis pathway, leading to synthesis of potential phytoalexins, which sharp capability in plants for fighting against infamous pathogens (Nicholson and Hammerschmidt, 1992). There is an increase level of mRNA that encodes the chalcone synthase and PAL in the early stage of plant-antagonistic microbes' interaction (Zodor and Anderson, 1992). Recently, Gallou and co-workers (2009) reported that potato plants treated with *T. harzianum* and challenged with *R. solani* can induce the expression of PAL being the highest PAL level in potato plants treated with the

Trichoderma spp. consortium. The results of our study showed that potato plants treated with *Trichoderma* spp. consortium have an increase of PAL level which is correlated with an enhanced defense against early blight infection.

Phenolic compounds are found naturally in living plants that provide support from invasion of pathogenic fungi and play crucial a role in biosynthesis of cinnamic acid, phenylalanine and phenylpropanoids. Singh and co-workers (2011) identified some potential phenolic compounds such as, ferulic acid, *t*-chlorogenic acid and protocatechuic acid which provide fortified defense against invaders,. The results of the present work showed that phenolic compounds, such as gallic acid, shikimic acid and syringic acid, were increase by the treatment of *Trichoderma* spp. consortium. Gallic acid is the important source of antifungal and converted into gallotannins, which showed antimicrobial properties (Salisbury and Ross, 1986). Moreover, the shikimic acid pathway may regulate the biosynthesis and accumulation of potential plant phenolic compounds, which are good precursors of lignin and tannins flavonoids (Shoresh et al., 2005).

The crucial superoxide dismutase (SOD) enzyme directly is involved in the activation of ascorbate-glutathione pathway to enhance the scavenging of free radicals (Hernandez et al., 2011). Under biotic and abiotic stress conditions, plants produce huge amounts of ROS that can lead the scavenging capacity and damaged the plant cellular system through lipid peroxidation (Mittler, 2002). SOD worked as a denature enzyme and catalyzed the huge production of O₂⁻ and H₂O₂ from superoxide radicals. Proteomic analysis revealed that the *Trichoderma* spp. treated roots can enhance the augmentation of SOD and other detoxifying enzymes that protect from collar rot pathogen infection (Smirnoff, 1993). In this work we show that potato plants treated with *Trichoderma* spp. consortium have a high production of SOD enhancing ROS scavengers and therefore, protecting the potato plants against *A. solani*.

Plant β-1, 3-glucanase enzymes are PR proteins belonging to PR-2 family and important parts of plant defense against pathogen infection (Karthikeyan et al., 2006). Moreover, maize seedlings treated with *T. harzianum* T22 augmented the level of PR-2 proteins when plants were inoculated with *Pythium ultimum* (Harman et al., 2004). In the same line, β-1, 3-glucanase was induced when cucumber seedlings were treated with *Trichoderma* spp. inducing systemic disease resistance to pathogens (Shoresh et al., 2005). In the present work we showed a significant increase of β-1, 3-glucanase levels when potato plants were treated with *Trichoderma* spp. consortium.

In summary the results obtained in this study suggest that *T. viride* and *T. harzianum*, especially when both are applied together, are effective for elevating the defense response and growth promotion activities in potato against the phytopathogen *A. solani*. Therefore, these *Trichoderma* strains can be good candidates as biological control agents to use in important agricultural crops such as potato.

Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest/ competing interests whatsoever.

Availability of data and material (data transparency)

The data would be made available on demand basis.

Code availability (software application or custom code)

Not applicable

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

SK, CK, RC and ES were involved in the idea generation. SK, LB, CK conducted the experiments and analyzed the data. SK, CK, ES were involved in manuscript preparation and editing. RC supervised the work.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable

Ethics approval (include appropriate approvals or waivers)

Not applicable

Consent to participate (include appropriate statements)

All authors have significantly contribute to this manuscript and agreed to submit this work in the [World Journal of Microbiology and Biotechnology](#).

Consent for publication (include appropriate statements)

All authors have approved the final draft of the manuscript and provided their consent for publication of the same in the [World Journal of Microbiology and Biotechnology](#).

References

1. Abo-Elyousr, K.A.M., Hashem, M., Ali, E.H. (2009) Integrated control of cotton root rot disease by mixing fungal biocontrol agents and resistance inducers. *Crop Prot* 28, 295–301.
2. Ahn, I.P., Lee, S.W., Suh, S.C. (2007) Rhizobacteria induced priming in *Arabidopsis* is dependent on ethylene, jasmonic acid, and NPR1. *Mol Plant Microbe Interact* 20, 759–768.
3. Al-Mughrabi, K.I. (2005) Efficacy of oxidate for control of early blight (*Alternaria solani*) in potato storages. *Plant Pathol. J.* 4, 1–4.
4. Amin, F., Razdan, V.K., Mohiddin, F.A., Bhat, K.A., Sheikh, P.A. (2010) Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens in vitro. *J Phytol.* 2,34–37.
5. Awan, Z.A., Shoaib, A. (2019) Combating early blight infection by employing *Bacillus subtilis* combination with plant fertilizers, *Current Plant Biology*, 20, 100125.
6. Brueske, C.H. (1980) Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root knot nematode *Meloidogyne incognita*. *Physiol Plant Pathol.* 16,409–414.
7. Buragohain, A.M., Das, B.C., Islam, M. (2000) In-vitro studies of *Trichoderma* species against *Sclerotium rolfsii* *J Agric Sci.* 13, 99–100.
8. Conrath, U., Beckers, G.J., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., Newman, M.A., Pieterse, C.M.J. et al. (2006) Priming: getting ready for battle. *Mol Plant Microbe Interact* 19, 1062–1071.
9. Conrath, U. (2011) Molecular Aspects of Defence Priming. *Trends in Plant Science*, 16, 524-531.
10. Contreras-Cornejo, H.A., Macías-Rodríguez, L.I., Alfaro Cuevas, R., López-Bucio, J. (2014) *Trichoderma* improves growth of *Arabidopsis* seedlings under salt stress through enhanced root development, osmolite production and Na⁺ elimination through root exudates. *Mol Plant Microbe Interact* 27,503–514.
11. Edin, E.; Liljeroth, E.; Andersson, B. (2019) Long term field sampling in Sweden reveals a shift in occurrence of cytochrome b genotype and amino acid substitution F129L in *Alternaria solani*, together with a high incidence of the G143A substitution in *Alternaria alternata*. *Eur. J. Plant Pathol.* 155, 1–15.
12. Enespa, Dwivedi, S.K. (2014) Effectiveness of some antagonistic fungi and botanicals against *Fusarium solani* and *Fusarium oxysporum* sp. *lycopersici* infecting brinjal and tomato plants. *Asian J Plant Pathol.* 8,18–25.
13. Fairchild, K.L., Miles, T.D., Wharton, P.S. (2013) Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. *Crop Prot* 49, 31–39.
14. Fridovich, I. (1974) Superoxide dismutases. *Adv Enzymol Relat Areas Mol Biol.* 41,35–97.
15. Fokkema, N.J. (1978) Fungal antagonism in the phyllosphere. *Ann Appl Biol* 89, 115-117.
16. Gadzouska, S., Delaunay, A., Spasenoski, M., Maury, S., Joseph, C., Hagege, D. (2007) Jasmonic acid elicitation of *Hypericum perforatum* Cell suspensions and effects on the production of phenylpropanoids and naphthodiantrones. *Plant Cell Tissue Organ Cult.* 89, 1–13.
17. Gallou, A., Cranenbrouck, S., Declerck, S. (2009) *Trichoderma harzianum* elicits defence response genes in roots of potato plantlets challenged by *Rhizoctonia solani*. *Eur J Plant Pathol.* 124, 219–230.
18. Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, I.M. (2004) *Trichoderma* species-Opportunistic, avirulent plant symbionts. *Nature Rev* 2, 43–56.
19. Hernandez, J.A., Ferrer, M.A., Jimenez, A., Barcelo, A.R., Sevilla, F. (2001) Antioxidant systems and O₂ /H₂O₂ production in the apoplast of pea leaves. Its relation with salt induced necrotic lesions in minor veins. *Plant Physiol* 127, 827–831.
20. Jain, A., Singh, S., Sarma, B.K., Singh, H.B. (2012) Microbial consortium mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. *J Appl Microbiol* 112, 537–550.
21. Jetiyanon, K. (2007) Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strains (IN937a and IN937b) against different pathogens. *Biol Control* 42, 178–185.
22. Karthikeyan, M., Radhika, K., Mathiyazhagan, S., Bhaskaran, R., Samiyappan, R., Velazhahan, R. (2006) Induction of phenolics and defense-related enzymes in coconut (*Cocos nucifera*) roots treated with biocontrol agents. *Braz J Plant Physiol.* 18, 367–377.
23. Keswani, C., Bisen, K., Chitara, M.K., Sarma, B.K., Singh, H.B. (2017) Exploring the role of secondary metabolites of *Trichoderma* in tripartite interaction with plant and pathogens. In: Singh JS, Seneviratne G, editors. *Agro-environmental sustainability*. 1st ed. Cham: Springer; p. 63–79.
24. Kumar, D., Yusuf, M., Singh, P., Sardar, M., Sarin, N. (2014) Histochemical Detection of Superoxide and H₂O₂ Accumulation in *Brassica juncea* Bio-Protocol. 2014, 4.
25. Lopes, F. A., Steindorff, A. S., Geraldine, A. M., Brandao, R. S., Monteiro, V. N., Lobo, M. Jr., et al. (2012). Biochemical and metabolic profiles of *Trichoderma* strains isolated from common bean crops in the Brazilian Cerrado, and potential antagonism against *Sclerotinia sclerotiorum*. *Fungal Biol.* 116, 815–824.
26. Lopez-Llorca, L.V., Duncan, G.H. (1988) A study of fungal endoparasitism of the cereal cyst nematode *Heterodera avenae* by scanning electron microscopy. *Can J Microbiol* 34, 613-619.
27. Lowry, O.H., Nira, J., Rosenburgh, A., Lewis, F., Rose, J.R. (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275.
28. Manganiello, G., Sacco, A., Ercolano, M. R., Vinale, F., Lanzuise, S., Pascale, A., Napolitano, M., Lombardi N, Lorito M, Woo SL (2018). Modulation of tomato response to *Rhizoctonia solani* by *Trichoderma harzianum* and its secondary metabolite harzianic acid. *Microbiol.* 9, 1966.
29. Mayee, C.D., Datar, V.V. (1986) Phytopathometry. *Tech. Bull-1 Marathwada Agric. Univ. Parbhani* PP:66.
30. Mittler R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7, 405–410.
31. Moghaddam GA, Rezayatmand Z, Nasr-Esfahani M, Khozaei M. (2019) Genetic defense analysis of tomatoes in response to early blight disease, *Alternaria alternata*, *Plant Physiol Biochem*, 142, 500-509.

32. Mokhtar, H., Aid, D. (2013) Contribution in isolation and identification of some pathogenic fungi from wheat seeds, and evaluation of antagonistic capability of *Trichoderma harzianum* against those isolated fungi in vitro. *Agri Biol J North Am.* 4, 145–154.
33. Murmu, S., Dey S., Chakraborty A. (2015) Management of early blight of potato using bio control agents and plant extracts, *J. Appl Nat Sci.* 7, 860- 864.
34. Ngadze, E., Icishahayo, D., Coutinho, T. A., van der Waals, J. E. (2012) Role of Polyphenol Oxidase, Peroxidase, Phenylalanine Ammonia Lyase, Chlorogenic Acid, and Total Soluble Phenols in Resistance of Potatoes to Soft Rot. *Plant Disease*, 96, 186-192.
35. Nicholson, R.L., Hammerschmidt, R. (1992) Phenolic compounds and their role in disease resistance. *Annu Rev Phytopathol* 30, 369–389.
36. Odilbekov, F., Edin, E., Mostafanezhad, H., Coolman, H., Grenville-Briggs, L.J., Liljeroth, E. (2019) Within-season changes in *Alternaria solani* populations in potato in response to fungicide application strategies. *Eur. J. Plant Pathol.* 155, 953–965.
37. Pan, S.Q., Ye, X.S., Kuc, J. (1991) Association of b-1,3 glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. *Physiol Mol Plant Pathol.* 39(1):25–39.
38. Prasad, B.N., Kumar, M.R. (2011) Comparative efficacy of different isolates of *Trichoderma* spp. against *Rhizoctonia solani*, incitant of sheath blight of rice. *Ind J Fund Appl Life Sci.* 1, 107–111.
39. Ragazzi, E., Veronese, G. (1973) Quantitative analysis of phenolic compounds after thin layer chromatographic separation. *J Chromatogr.* 77, 369–375.
40. Rahman, M.A., Razvy, M.A., Alam, M.F. (2013) Antagonistic activities of *Trichoderma* strains against chili anthracnose pathogen. *Int J Microbiol Mycol.* 1, 7–22.
41. Salas-Marina, M.A., Silva-Flores, M. A., Uresti-Rivera, E. E., Castro-Longoria, E., Herrera-Estrella, A., Casas-Flores, S. (2011). Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur. J. Plant Pathol.* 131, 15–26.
42. Salisbury FB, Ross CW. 1986. *Plant physiology*. Delhi: CBS Publishers Distributors.
43. Sansinenea, E., Almaraz, M., Ramírez, M.D., Ortiz, A. (2016) Cellular damage of plant pathogenic fungi by antifungal compound produced by *Bacillus* isolates, *Chem Ecol*, 32, 722-732
44. Segarra, G., Casanova, E., Bellido, D., Odena, M.A., Oliveira, E., Trillas, I. (2007) Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. *Proteomics.* 7(21):3943–3952.
45. Shaikh, F.T., Sahera, N. (2013) In vitro assessment of antagonistic activity of *Trichoderma harzianum* against pathogen fungi. *Int J Appl Res.* 3, 57–59.
46. Shores, M., Yedidia, I., Chet, I. (2005) Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* Phytopathology. 95, 76–84.
47. Shuman, J.L.; Christ, B.J. (2005) Integrating a host-resistance factor into the fast system to forecast early blight of potato. *Am. J. Potato Res.* 82, 9–19.
48. Singh, A., Sarma, B.K., Upadhyay, R.S., Singh, H.B. (2013) Compatible rhizosphere microbes mediated alleviation of biotic stress in chickpea through enhanced antioxidant and phenylpropanoid activities. *Microbiol Res* 168, 33–40.
49. Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Singh, D.P., Sarma, B.K., Upadhyay, G., Singh, H.B. (2009) Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology*, 47, 1161–1167.
50. Singh, B.N., Singh, A., Singh, S.P., Singh, H.B. (2011) *Trichoderma harzianum* mediated reprogramming of oxidative stress response in root apoplast of sunflower enhances defence against *Rhizoctonia solani*. *Eur J Plant Pathol.* 131, 121–134.
51. Smirnov, N. (1993) Tansley Review No. 52. The role of active oxygen in the response of plants to water deficit and desiccation. *Plant Phytol.* 125.
52. Srivastava, R., Khalid, A., Singh, U.S., Sharma, A.K. (2010). Evaluation of arbuscular mycorrhizal fungus and *Trichoderma harzianum* formulation against *Alternaria solani* for the management of early blight of potato. *Biological control.* 53, 24-31.
53. Tapwal, A., Pandey, H. (2016) In vitro evaluation of *Trichoderma* species for virulence efficacy on *Botryodiplodia* *Curr Life Sci.* 2, 86–91.
54. Tapwal, A., Thakur, G., Chandra, S., Tyagi, A. (2015) In-vitro evaluation of *Trichoderma* species against seed borne pathogens. *Int J Biol Chem Sci.* 1, 2349–2724.
55. Thordal-Christensen, H., Zhang, Z., Wei, Y., Collinge, D.B. (1997) Subcellular localization of H₂O₂ in plants, H₂O₂ accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant J.*, 11, 1187–1194.
56. Van der Ent, S., Van Wees, S.C., Pieterse, C.M. (2009) Jasmonate signaling in plant interactions with resistance inducing beneficial microbes. *Phytochemistry* 70, 1581– 1588.
57. Viterbo, A., Harel, M., Horwitz, B.A., Chet, I., Mukherjee, P.K. (2005) *Trichoderma* mitogen activated protein kinase signaling is involved in induction of plant systemic resistance. *Appl Environ Microbiol.* 71, 6241–6246.
58. Yedidia, I., Benhamou, N., Chet, I. (1999) Induction of defense responses in cucumber plants (*Cucumis sativus*) by the biocontrol agent *Trichoderma harzianum*. *Appl Environ Microbiol.* 65, 1061–1070.
59. Zhang, D.; Yu, S., Yang, Y., Zhang, J., Zhao, D., Pan, Y., Fan, S., Yang, Z., Zhu J. (2020) Antifungal effects of volatiles produced by *Bacillus subtilis* against *Alternaria solani* in Potato, *Frontiers Microbiol.*, 11, 1196.
60. Zeilinger, S., Omann, M. (2007) *Trichoderma* biocontrol: signal transduction pathways involved in host sensing and mycoparasitism. *Gene Regul Syst Biol* 1, 227–234
61. Zodor, R.E., Anderson, A.J. (1992) Influence of root colonization bacteria on the defense responses in bean. *Plant Soil.* 140, 99–107.
62. Zivkovic, S., Stojanovic, S., Ivanovic, Z., Gavrilovic, V., Popovic, T., Balaz, J. (2010) Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Arch Biol Sci* 62, 611–623.

Figures



Figure 1

Antagonistic activity of *Trichoderma* spp. (A) *T. viride*, (B) *T. harzianum*, and (C) Control plate of *A. solani*.

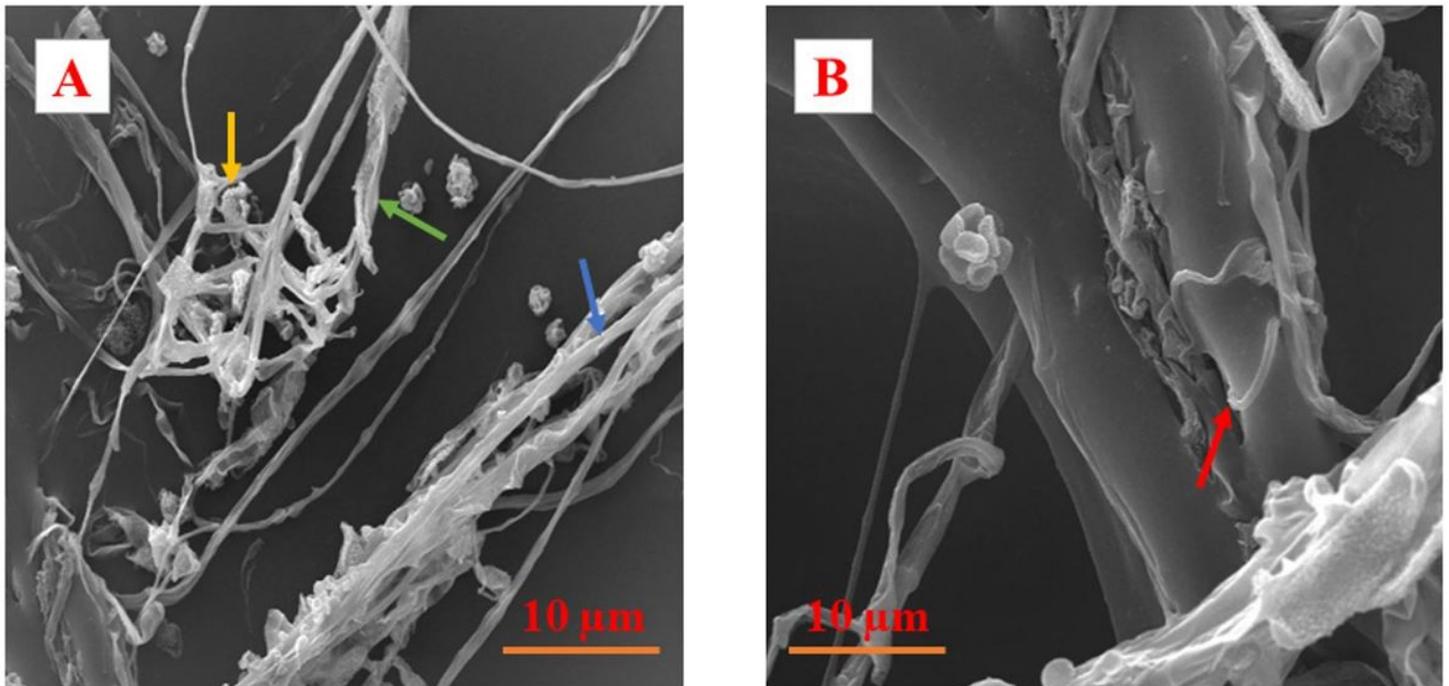


Figure 2

Scanning electron microscope (SEM) Images show morphological alteration and damage on hyphae and spores of *A. solani* due to the antagonistic of *T. viride* and *T. harzianum*. (A) Showing deformation of *A. solani* caused by *T. viride*, damage hyphae (green arrow) and damage spores of *A. solani* (yellow arrow). Normal hyphae of *A. solani* (blue arrow). (B) *T. harzianum* hyphae coiled on the *A. solani* hyphae (red arrow).

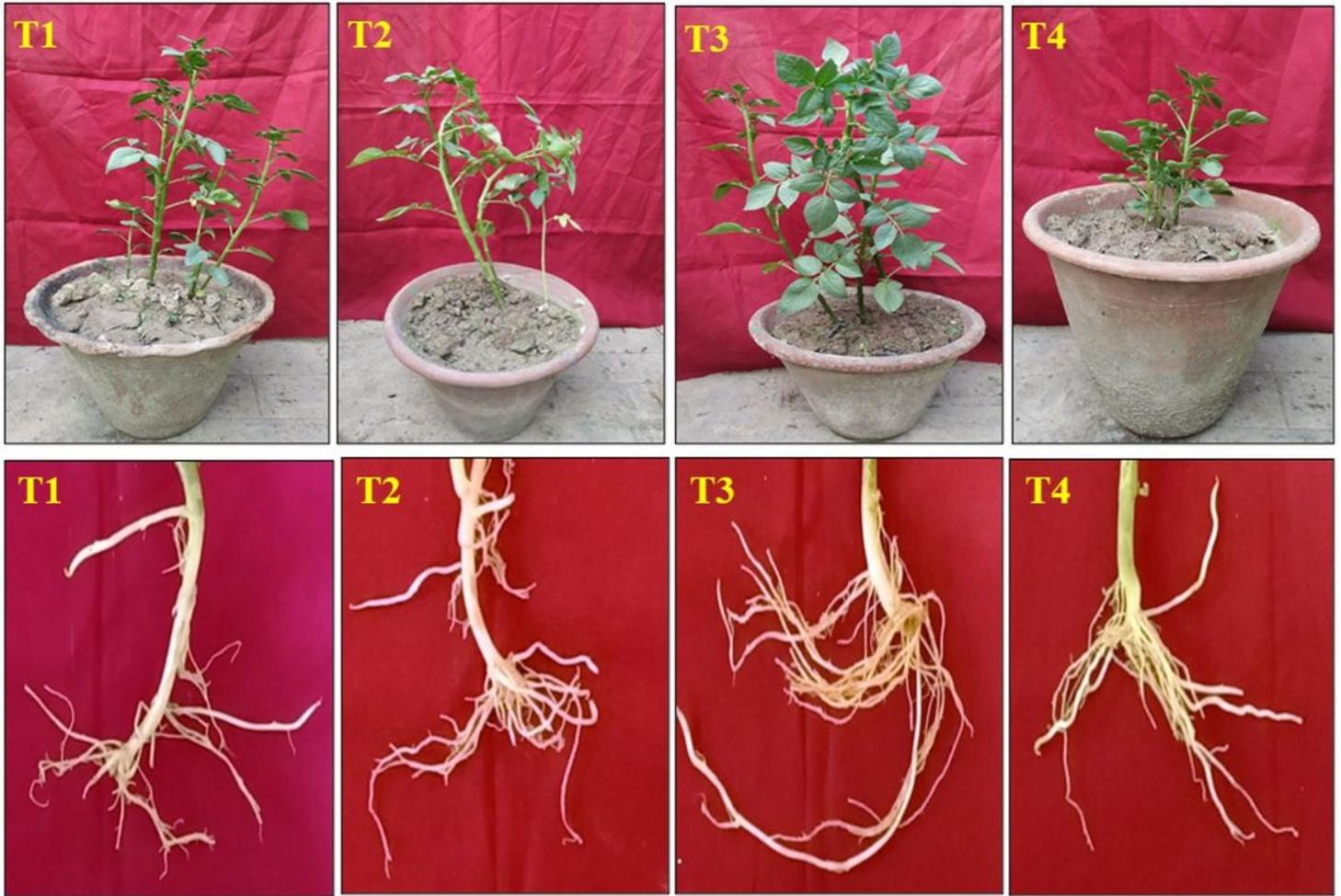


Figure 3
Effect of different treatments on the growth and root development of potato plants (T1- *T. viride*, T2- *T. harzianum*, T3- *T. viride* + *T. harzianum*, T4- control).

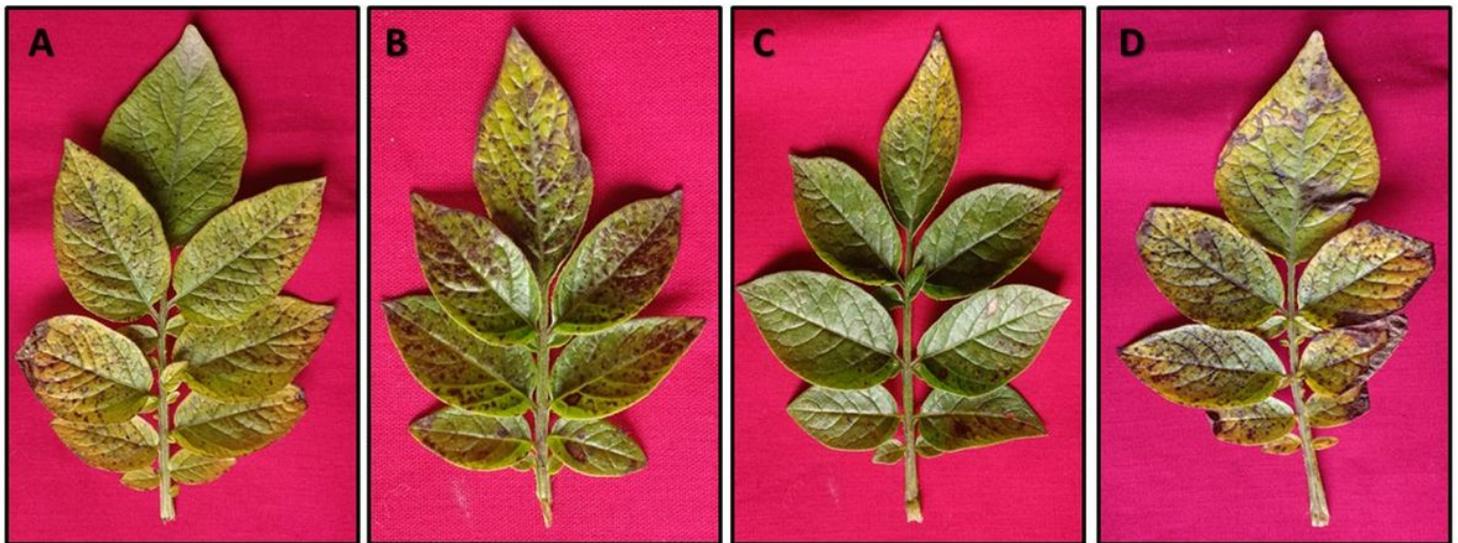


Figure 4
Efficacy of different treatments in disease development on leaves on potato plants after *A. solani* challenge (A- *T. viride*, B- *T. harzianum*, C- *T. viride* + *T. harzianum*, D- control).

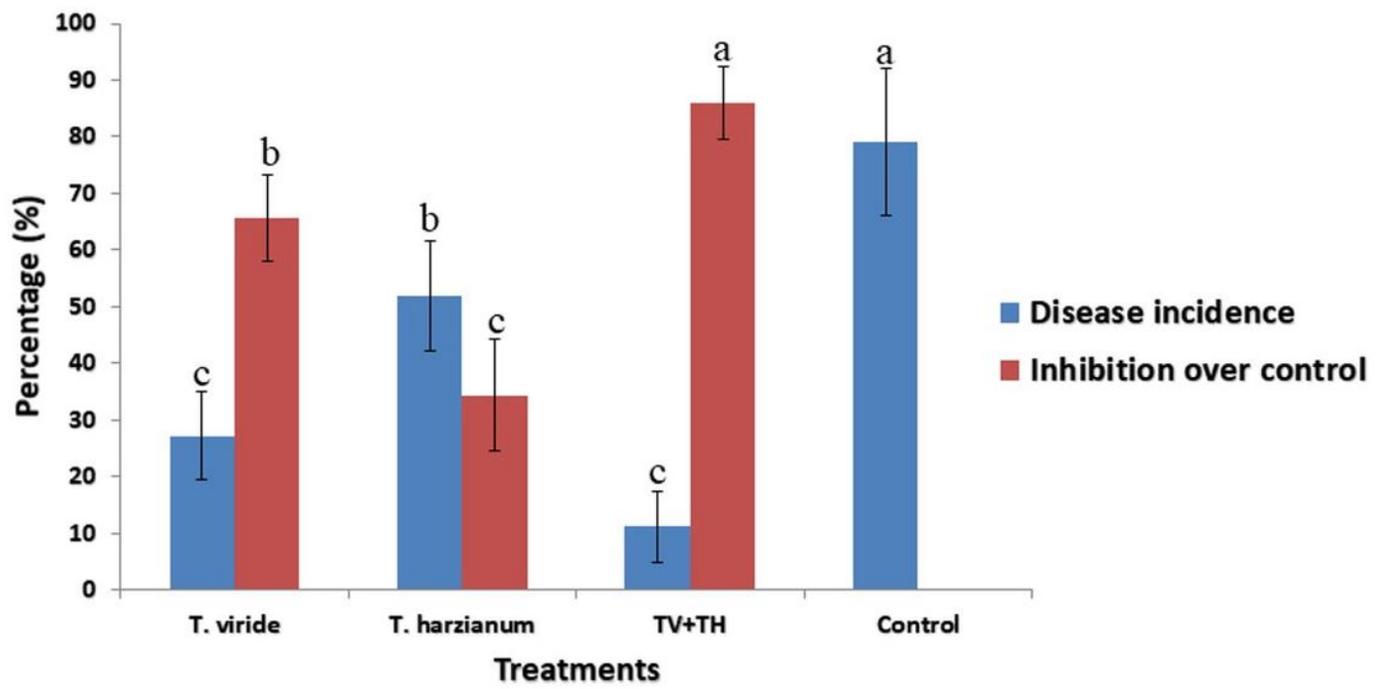


Figure 5

Effect of *Trichoderma* spp. on reduction of early blight caused by *A. solani*. Results are indicated as means of three replicates, and vertical bars showed standard deviations of means.

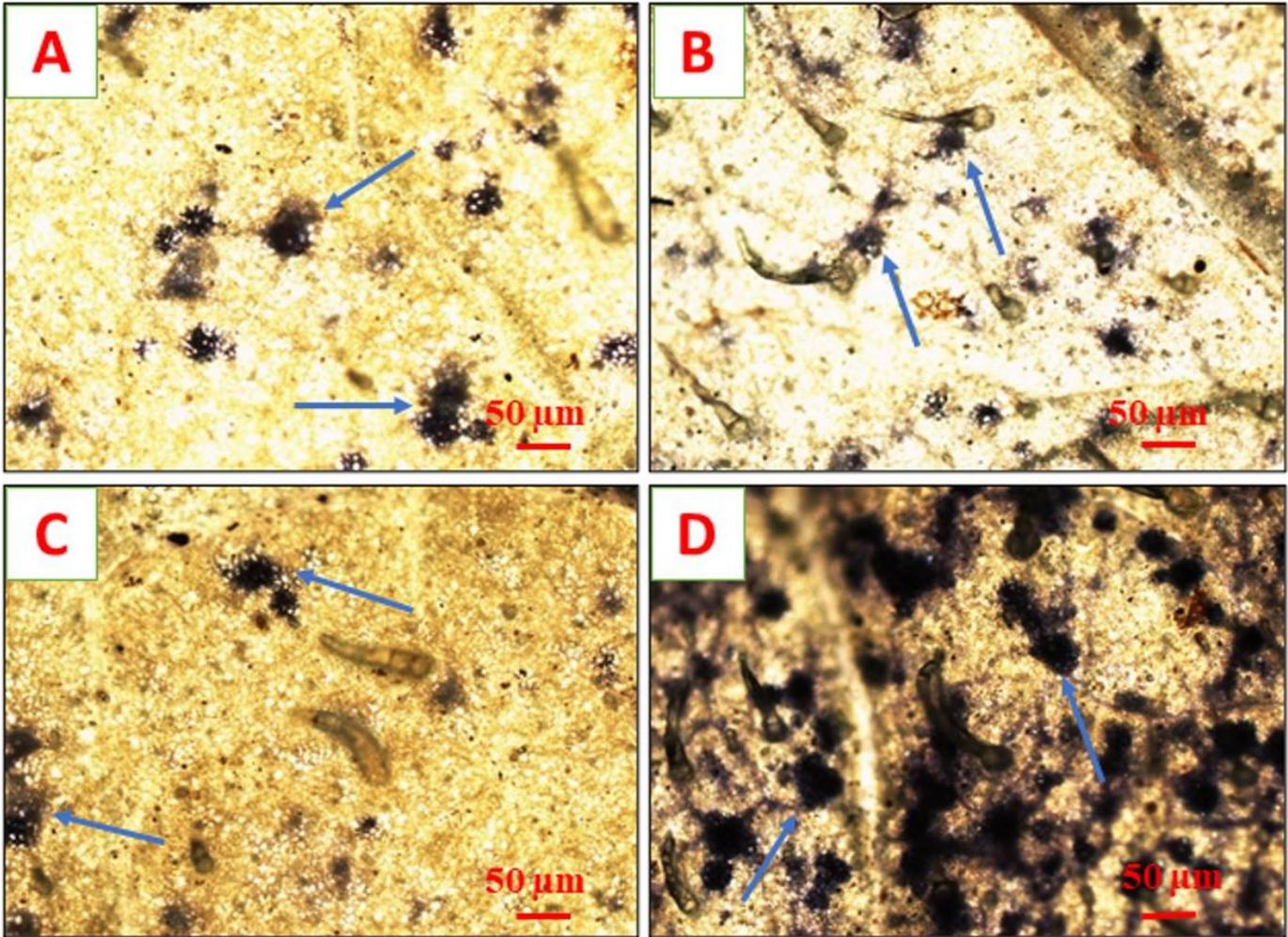


Figure 6
Microscopic detection of superoxide radicles (arrow). Effect of *Trichoderma* spp. in potato leaves inoculated with *A. solani* (A- *T. viride*, B- *T. harzianum*, C- *T. viride* + *T. harzianum*, D- control).

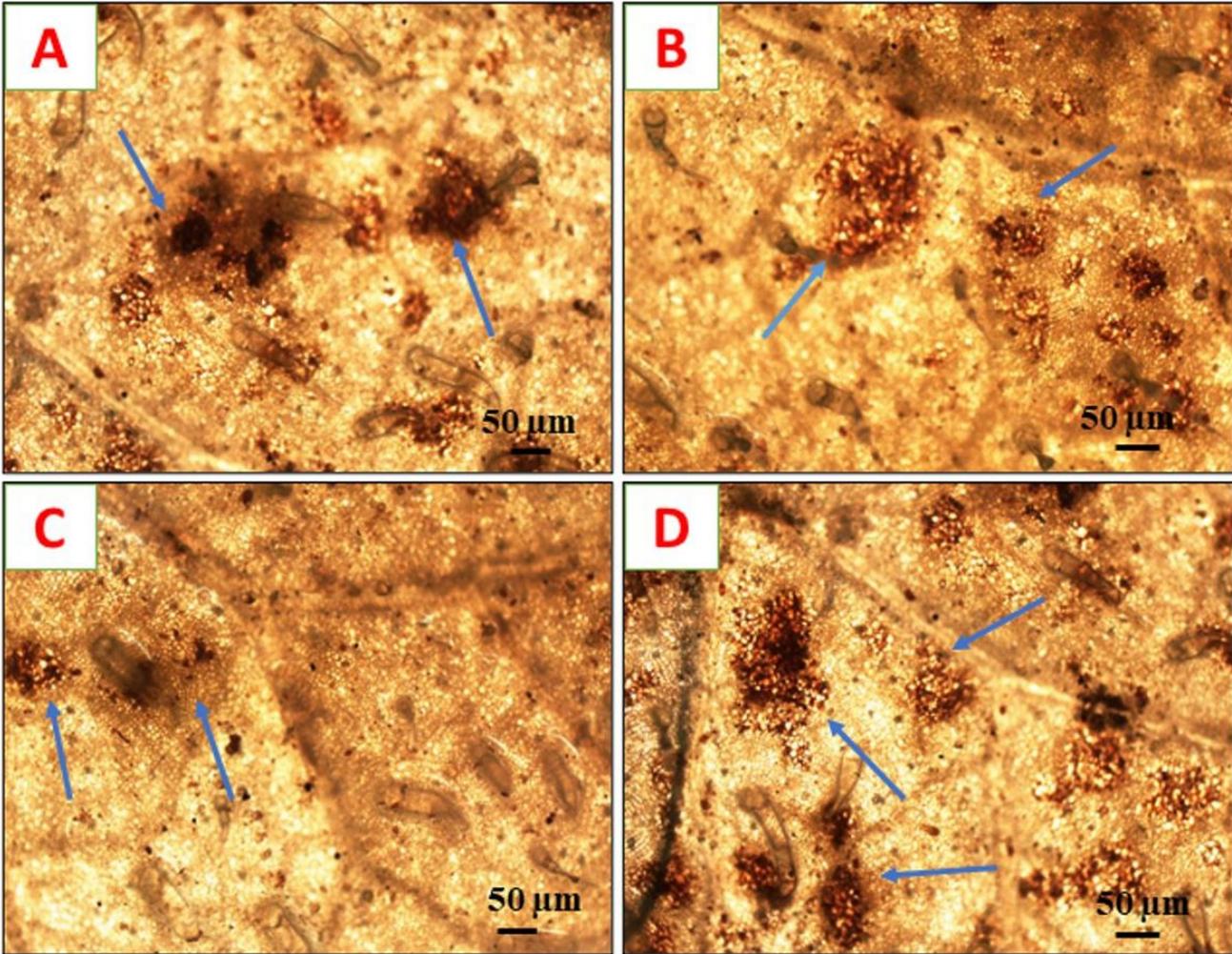


Figure 7
Microscopic detection of Hydrogen peroxide (arrow). Effects of *Trichoderma* spp. in potato leaves inoculated with *A. solani* (A- *T. viride*, B- *T. harzianum*, C- *T. viride* + *T. harzianum*, D- control).

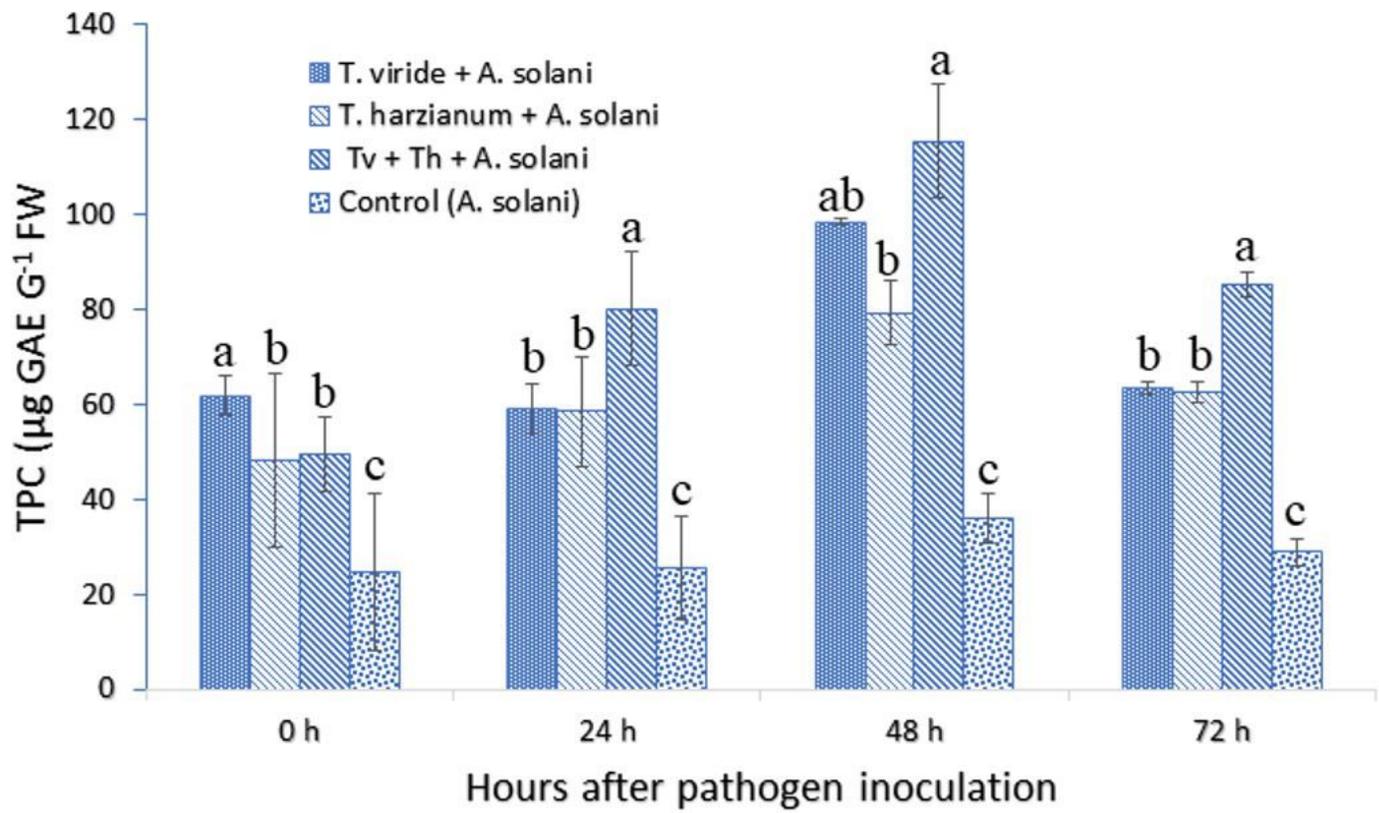


Figure 8

Accumulation of total phenolic content (TPC) at different time intervals in potato plants against *A. solani* challenged and plants treated with individual and consortium of *Trichoderma* strain.

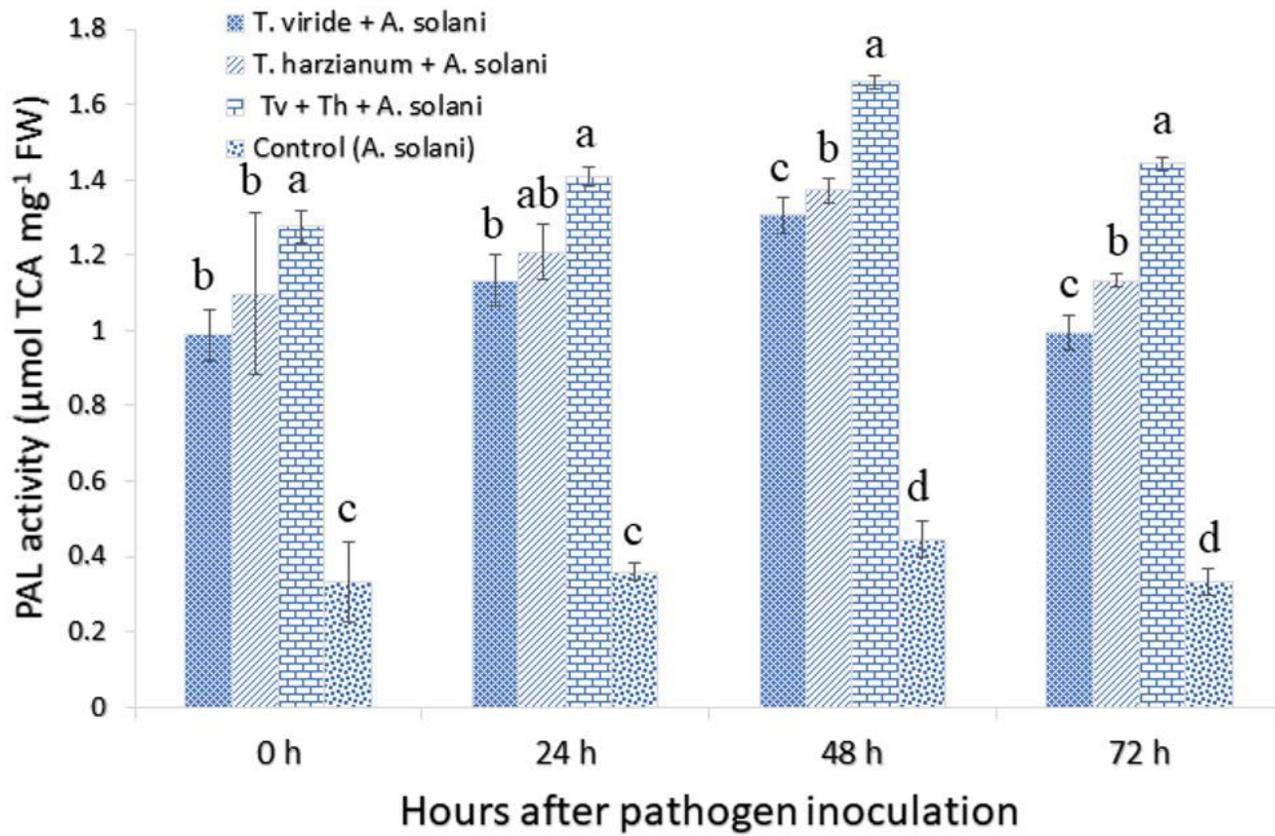


Figure 9

Effect of Trichoderma spp. on PAL activity in potato leaves challenged with pathogen at different time intervals.

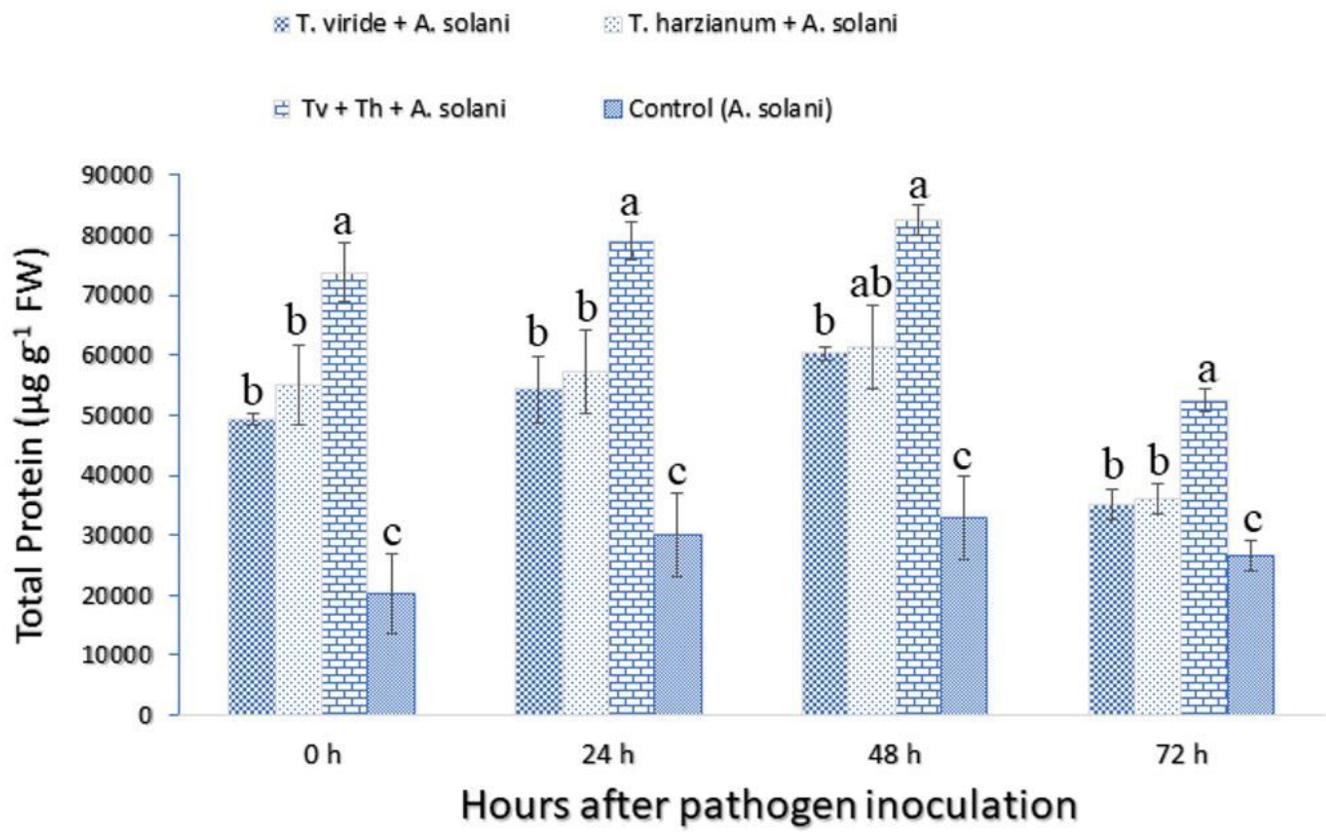


Figure 10

Total protein content at different time of intervals potato plants treated with *Trichoderma* spp. either individual or consortium and challenged with *A. solani*.

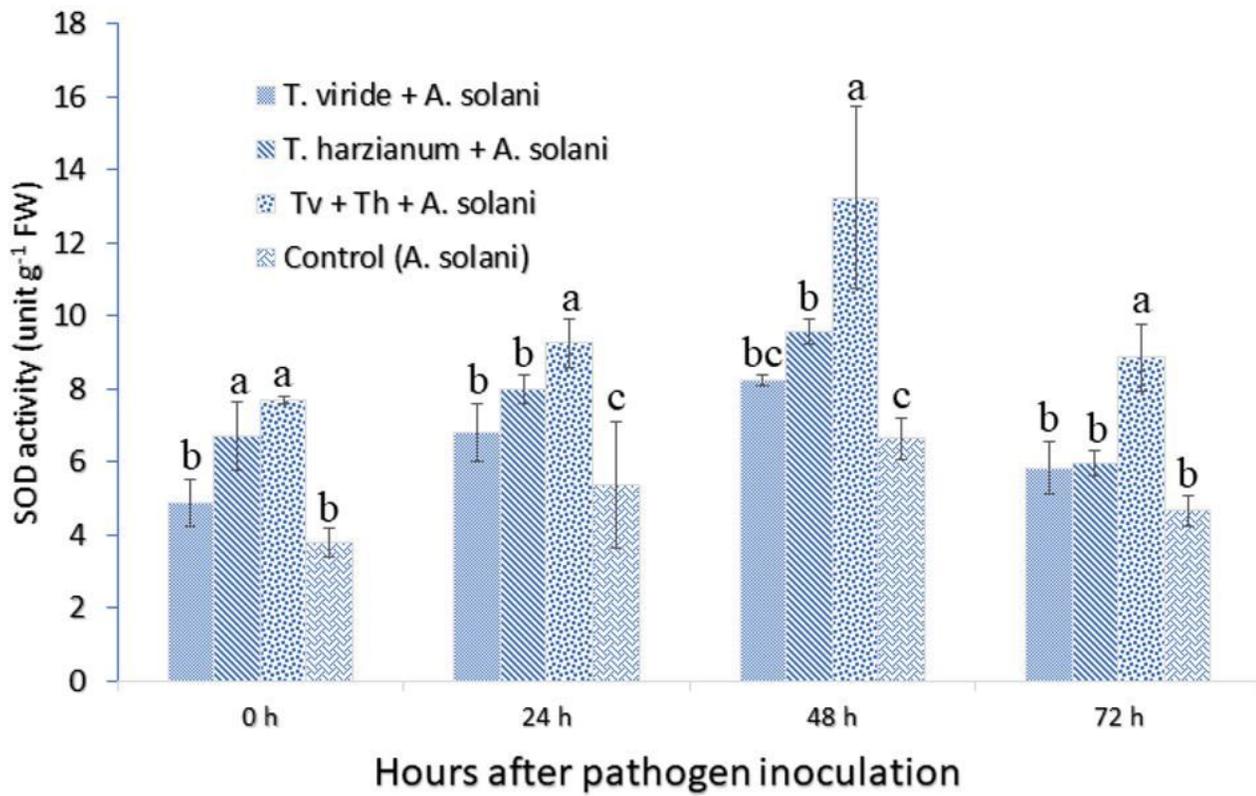


Figure 11

Effect of *Trichoderma* spp. and their consortium on SOD activity (unit g⁻¹ FW) in potato leaves against *A. solani* at different time of intervals challenged with pathogen.

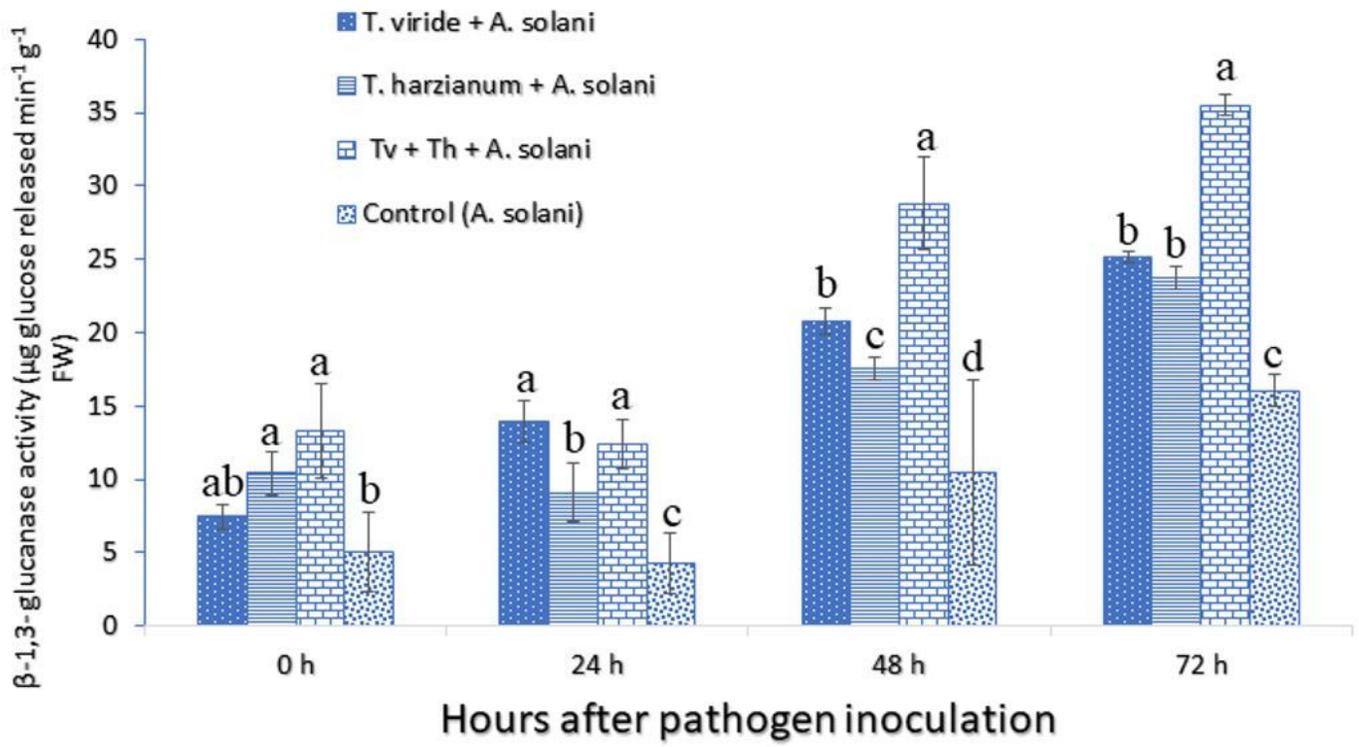


Figure 12

Effect of individual or consortium of *Trichoderma* spp. on β -1,3- glucanase activity (μg glucose released $\text{min}^{-1} \text{g}^{-1}$ fresh weight) in potato leaves at different time of intervals challenged with pathogen.