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RESEARCH ARTICLE

Identification of disease suppressive potential of *Trichoderma virens* and Jasmonic acid against fusarium wilt and damping-off in "Seed Primed" tomato plants

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Abstract

Disease causing phytopathogens are responsible for an approximately 15% reduction in worldwide food production. Therefore, for efficient management of plant diseases, a systematic understanding of the harmful impacts of pathogens on economic crops is essential. The practice of sustainable agriculture aims at the development of a system that supports the growth of plants but simultaneously induces adverse effects on the existence of pathogens. Therefore, the current research was designed to monitor the seed priming effects of *Trichoderma virens* (as Biocontrol Agent, BCA) and Jasmonic acid (a chemical inducer) in tomato plants infected with two devastating soil-borne pathogens viz., *Fusarium oxysporum lycopersici* (*Fol*) and *Rhizoctonia solani*. Application of these agents in infected plants alone or together leads to the establishment of various disease-suppressive mechanisms in the host plants as observed in the form of enhanced seedling vigour index, percentage germination, morphological growth, and a substantial decrease in the percentage of disease incidence. Furthermore, pathogen inoculation in diseased plants enhances the content of two compatible osmolytes i.e., proline and glycine betaine which themselves serve as defensive molecules by acting as osmoprotectants and signalling molecules in the induction of various defence-related pathways in the stressed plants. Our study provides important insights into the effectiveness of *T. virens* and JA in the amelioration of pathogen-induced damage in the host plants. The inferences obtained from this research highlight the better efficiency of combined applications of *T. virens* and JA against these two soil-borne pathogens.

Keywords

biotic stress; seed priming; biocontrol; disease incidence; plant defence

Introduction

Tomato (*Solanum lycopersicum* L.) is a valuable, widely grown, horticultural rabi crop. The growth and yield of this important horticultural crop are limited by several biotic and abiotic stresses. Abiotic stresses involve salinity, extremes in temperature, radiation, flood, drought, heavy metal toxicity, etc.; while biotic stress comprises pathogenic attacks by fungi, bacteria, nematodes, and herbivores. Above all, diseases are the most common limiting factors in tomato production. It is susceptible to being infected by differ-

ent kinds of pathogens viz., viral, bacterial, nematodes, fungal, etc. In comparison to other pathogens, fungal pathogens are responsible to a greater extent for lowering the quantity as well as the market value of this commercial crop (1). In the present investigation, we have studied the effects of two diseases i.e., fusarium wilt and damping off on infected tomato plants in terms of growth characteristics as well as some biochemical alterations.

Infection of tomato plants with *Fusarium oxysporum* f.s. *lycopersici* (*Fol*) causes severe water loss in diseased plants due to the clogging of xylem vessels. Furthermore, wilt-like symptoms such as defoliation, vein clearing, and epinasty of leaves were observed on infected plants (2). Similarly, *R. solani* mainly infects the crops during the seedling stage, but can also damage the roots, pods, leaves, and stem of affected host plants. Most peculiar symptoms of plant diseases appear in the form of brown-coloured lesions near the collar region, browning of the root system, and chlorosis on the upper parts (3).

To adapt themselves to stressful environments, plants produce and accumulate compatible solutes commonly designated as osmolytes. Numerous low molecular-weight substances or metabolites, including sugars, polyamines, secondary metabolites, amino acids, and polyols, are referred to as osmolytes (4). Numerous stress signalling pathways such as hormones, mitogen-activated protein (MAP) kinase, and calcium-signalling cause excessive formation of osmolytes. Among the several amino acids in plants, proline, glycine betaine (GB), and Gamma-Aminobutyric Acid (GABA) function as stress-responsive amino acids and play a key role in oxidative and osmotic adjustments in plants (4–7).

Even though presently a broad variety of chemical pesticides are accessible to handle plant diseases, these fungicides cannot be considered long-term remedies against pathogenic fungi because of health and environmental concerns (8). Furthermore, regular application of these chemicals not only establishes tolerance in target organisms through mutation, but their continuous application introduces several adverse effects in the numerous beneficial organisms also present in the rhizosphere. In recent times, agriculturists and business-related sectors have expressed intense concern regarding the development of some sustainable and cost-effectual approaches for plant disease management (9). Natural control procedures are intended as substantial measures for disease management in comparison to other approaches since the fungicides negatively affect other beneficial organisms too (10).

In recent years, the essential need for safe and chemical-free food has increased the demand and market for biopesticides. Moreover, in the pursuit of sustainable agriculture, biopesticides serve as an eco-friendly approach by minimising the application of harmful chemicals (11). Subsequently, the expression of many defence-responsive genes has been upregulated following the treatment with biotic and abiotic resistance inducers (12). *Trichoderma* spp. are plant symbiotic fungi that assist the plant in having better growth and metabolism in pathogen-contaminated soil by inhibiting the growth of pathogens through several mechanisms of antagonism like mycoparasitism, competition, and antibiosis (13). In our country, around 250 *Trichoderma*-derived products are utilized as bio-fungicides, but our farmers still rely on synthetic chemical fungicides to a larger magnitude as compared to biological control agents (14). During exposure to external stimuli, plants respond to them through the operation of a brilliant communication network of chemical messengers known as the hormonal signal transduction pathway. Additionally, following pathogen attack and successful recognition by specific Pattern Recognition Receptors (PRR), JA acid biosynthesis is initiated through the oxylipin biosynthesis pathway (15). Under the conditions of pathogen contamination, JA and its derivatives can instantly come into effect, which consequently induces nearly all chief secondary metabolites and protein expressions implicated in defence reaction (16). The present investigation aims to examine the ameliorative potential of *T. virens* and JA against *Fol* and *R. solani*-induced damage in the host tomato plants. We have studied the seed-priming effects of *T. virens* and JA individually and in combination to decrease the severity of tomato fusarium wilt and damping off caused by these two soils borne pathogens under *in vitro* and *in vivo* conditions as well as the extent of the disease's suppression abilities of these two stimulants.

Materials and Methods

Evaluation of antagonistic activities of T. virens and T. viride against F. oxysporum f. sp. lycopersici and R. solani

Dual culture assay

In dual plate assay, *T. virens* (ITCC No. 4177) and *T. viride* (ITCC 8315) were screened for assessment of antagonistic activity against test pathogens i.e., *Fusarium oxysporum lycopersici* (ITCC no. 8111) and *Rhizoctonia solani* (ITCC No. 1142) by inserting a 5 mm mycelial plug in the Petri plates (17). A 5 mm mycelial disc of a 5-day-old pathogen culture was kept at a 6 cm distance on the opposite side of the Petri dish, perpendicular to the *Trichoderma* fungal disc. The same disc of test fungus was placed in another PDA containing a Petri plate which served as untreated control. After this, these plates were incubated in a BOD incubator at 28 ± 2°C and the growth of pathogen mycelium towards the colonies of *Trichoderma* spp. was monitored and inhibition percentage was measured after 7 days of incubation. The experiment was carried out in triplicate and repeated twice.

Calculations

To calculate the percent inhibition in the radial colony growth, the following formula has been applied (18):

$$\text{Inhibition } \% = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Control-Radial growth (cm) in the control set, Treated-Radial growth in the treated set.

Screening of Trichoderma spp. for the synthesis of extracellular enzyme i.e., chitinase assay

The chitinase detection medium (19) consisted of a basal medium comprising 4.5 g of colloidal chitin, 0.30 g of $MgSO_4.7H_2O$, 3.00 g of NH_4SO_4, 2.00 g of KH_2PO_4, 1.00 g of Citric acid monohydrate, 15.00 g of Agar, 0.15 g of Bromo cresol purple, and 0.20 mL of Tween-80 per litre. The pH of this medium was adjusted to 4.7 and then autoclaved at 121°C for 15 min. Colloidal chitin so prepared can be stored at 4°C until further use. After cooling, the medium was poured into Petri plates and allowed to solidify. The actively growing *T. virens* and *T. viride* culture plugs of the isolates to be tested for chitinase activity were inoculated into the medium containing Petri plates and incubated at 26 ± 2°C for 3-5 days, and observed for the coloured zone formation. Chitinase activity was identified by the formation of a purple-coloured zone. The Colour intensity and diameter of the purple-coloured zone were taken as the criteria to determine the chitinase activity.

Preparation of Colloidal chitin

In the chitinase assay, the detection medium is supplemented with chitin as the sole carbon precursor, and it was prepared according to the method of Roberts and Selitrennikoff *et al*., 1988 (20) with a small modification. For this, 5 g of chitin was acid hydrolysed in 40 mL of conc. Hydrochloric acid (HCl) by continuous stirring on a magnetic stirrer for 24 hours at 4°C. This step was followed by adding 200 mL of chilled ethanol (95%) and then kept at 26°C for 24 hours. Subsequently, it was centrifuged for 20 min at 3000 rpm and 4°C. After that, the pellet so obtained was eroded with sterile distilled water by centrifugation at 3000 rpm and 4°C for 5 min until the complete removal of the smell of alcohol was achieved. The procured colloidal chitin had a pasty and soft consistency with wetness up to 90-95 %. It was preserved at 4°C till further use.

Preparation of inoculum

Fusarium oxysporum lycopersici

Two mycelial plugs (0.5 cm in diameter) from the 7-day-old culture of *Fol* were inoculated in 250 mL PDB (Potato Dextrose Broth) in an Erlenmeyer flask. This flask was incubated at 28 ± 2°C, at 100 rpm for 10 days. The mycelial mat was filtered through a double layer of sterilized cheesecloth to obtain conidial stock. The density of conidial stock was counted using a hemocytometer and the desired density i.e., 1×10^6 spores/mL was obtained by diluting the stock solution.

Rhizoctonia solani

Two mycelial plugs (0.5 cm in diameter) from a 7-day-old culture of *R. solani* were transferred to a 250 mL PDB in an Erlenmeyer flask. This flask was incubated at 28 ± 2°C, at 100 rpm for 10 days. The mycelial mat was filtered out with a double layer of sterilized cheesecloth. A known amount of mycelial mat was blended in sterilized distilled water (2 % W/V) in a blender for 30-60 sec.

Seed source

Tomato seeds, cultivar *Punjab Ratta* were obtained from the Department of Vegetable Science, Punjab Agriculture University (PAU), Ludhiana, Punjab, India.

Seed Treatment and Germination

Tomato seeds were surface sterilized with 0.01% mercuric chloride followed by frequent washing with double-distilled water. Afterwards, standardization of effective concentrations of *Trichoderma virens* and Jasmonic acid for seed treatment was carried out.

Preparation of spore suspension of Trichoderma virens

Trichoderma virens with ITCC no. 4177 was grown on PDA for 7 days at 28 ± 2°C in a BOD incubator. Two mycelial plugs (0.5 cm in diameter) from a 7-d old culture of *T. virens* were transferred to 250 mL PDB in Erlenmeyer flask. This flask was incubated at 28 ± 2°C, at 100 rpm for 10 days. The mycelial mat was filtered through a double layer of sterilized cheesecloth to get the spore suspension. This suspension was further diluted, and the number of spores was counted through a hemocytometer under the microscope. To this spore suspension, 1% carboxy methyl cellulose (CMC) was added which serves as an adherent during seed treatment. The stock solution was further diluted to 1×10^6, 1×10^7, and 1×10^8 spores/mL to get the optimum concentration for seed treatment. Sterilized tomato seeds were treated with different concentrations of *T. virens* for 4 hrs (50 seeds in 20 mL) and allowed to germinate in Petri plates at 25 ± 2 °C and 80 % humidity in a seed germinator.

Jasmonic acid

A JA stock solution of concentration 10 mM was prepared. This stock was further diluted to attain concentrations of 0.1, 1.0, and 10 µM. Sterilized tomato seeds were treated with different concentrations of JA (50 seeds in 20 mL) for 4 hrs and allowed to germinate in Petri plates at 25 ± 2 °C and 80% humidity in a seed germinator. Each treatment was maintained with three replicates. Seed germination percentage and root-shoot length were monitored after 10 days of germination.

In vitro seedling germination and vigour index under pathogens infection

Sterilized tomato seeds of uniform size were selected and imbibed with 0.1, 1, 10 µM of JA and 1×10^6, 1×10^7, and 1×10^8 spores/mL of *T. virens* for four hours to get the optimum concentration for seed treatment. Then, these imbibed seeds were infected with the desired pathogens inoculum, as described previously, by soaking them in their respective inoculum for 30 minutes. After that, the seeds were washed with distilled water (DW) and then 20 seeds were transferred to each Petri plate for germination. Petri plates were lined with three layers of filter paper made wet with 7 mL of DW. After 10 days, seedlings' growth was measured in terms of root length, shoot length and percentage germination.

The seedling vigour index was measured through the formula described by (21):

Seed germination percentage was calculated according to

$$\text{Vigour Index} = (\text{Mean Root Length} + \text{Mean Shoot Length}) \times \text{Germination (\%)}$$

the following equation

$$\text{Seed germination percentage} = \frac{\text{No.of seeds germinated}}{\text{Total no.of seeds}} \times 100$$

Seed Priming and germination

Tomato seeds were sterilized with 0.01% mercuric chloride followed by frequent washing with double-distilled water and after that, these were dried in laminar air flow on autoclaved blotting paper (22). Thereafter, these dried and surface sterilized seeds were treated by immersing them for 4 hours in 1×10^7 spores/mL in 1% CMC of *T. virens* and 1μM of JA. Seed treatment in combination (*T. virens* + JA) comprised an equal volume of individually prepared *T. virens* and JA. The control seeds were treated with distilled water alone. Further, all these seeds were placed in the seed germinator at 80% relative humidity, 25–27°C and kept for 24 h (23). These treated seeds were then grown in seedling trays containing sterilized soil and vermicompost in the ratio of 3:1 in the plant growth chamber at a temperature between 25-27°C, 16 hours (h) light and 8 h dark cycle, and relative humidity of 70% for thirty days. The thirty days old tomato seedlings were then used for determining sensitivity against pathogens and for further experimentations.

Pathogen Inoculation

Fusarium oxysporum lycopersici

Thirty days old primed as well as unprimed tomato seedlings were treated with a conidial suspension (10^6 spores/mL) of *Fol* for 30 minutes by the standard root dip method (24).

Rhizoctonia solani

The soil was inoculated with 2% w/v suspension of a 10-day-old mycelial mat on PDB of *Rhizoctonia solani.* After inoculation, 10 days incubation period was given for the proliferation of pathogens in the soil. After that, the primed and unprimed 30 days old tomato seedlings were transplanted into pathogen-infested soil (25).

Experimental Design and treatments

The experiment of this study was conducted under open field conditions at the experimental farm, at Lovely Professional University with latitude 31°24.500' NS and longitude 75°69.507' EW. The design of the experiment was Random Block Design (RBD) and a total of twelve treatments with three replications each were distributed in a 20×20 m experimental field separated by a 60×90 cm distance from each other. The following treatments were examined: 1) Control, 2) *T. virens* (Tv), 3) Jasmonic acid (JA), 4) Tv+JA, 5) *F. oxysporum lycoprsici* (Fol), 6) Fol+JA, 7) Fol+Tv, 8) Fol+JA+Tv, 9) *R. solani* (Rs), 10) Rs+JA, 11) Rs+Tv, 12) Rs+JA+Tv. Sampling was done randomly for recording various physiochemical parameters after 30 days of pathogen inoculation.

Growth Characteristics

In sixty days old tomato plants, the effect of tomato seeds primed with *T. virens* (Tv) and Jasmonic acid (JA) on different morphological parameters were studied with and without pathogenic stress. The root and shoot lengths were measured in cm from the base of the stem to the tip of the root and shoot, respectively. Fresh weight and number of leaves were taken immediately at the time of harvesting of the plant samples. After the estimation of fresh weight, the plant samples with different treatments were placed in a pre-heated hot-air oven and dried at 60°C for 6 hours and the dry weight thus obtained was recorded in g/plant.

Disease Incidence

After thirty days of pathogen inoculation, the percentage of disease incidence (PDI) was determined by using the following formula:

$$\text{PDI} = \frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \times 100$$

Free Proline content

Proline content was determined according to Bates *et al.*, 1973 (26). For proline estimation, 250 mg of plant tissue was homogenized with 5 mL of 3% Sulfosalicylic acid followed by centrifugation at 10,000 rpm for 10 minutes. 1 mL of obtained supernatant was reacted with 1 mL each of freshly prepared ninhydrin and Glacial Acetic Acid and then kept in a boiling water bath for 1 h. The reaction was instantly terminated in an ice bath and then brought to room temperature. Subsequently, the reaction mixture was extracted with 2 mL of toluene and then vortexed for 15-20 sec. The chromophore containing toluene was separated from the aqueous phase and absorbance was read at 520 nm. The free proline content was quantified from the calibration curve.

Glycine-Betaine (GB) Content

GB content was quantified by following the method of Grieve & Grattan, 1983 (27). 1 g of dried plant sample was crushed in 10 mL of double DW and then filtered through Whatman filter paper (No. 1). 1 mL of this filtrate was mixed with 1 mL of 2 M HCl. To 0.5 mL of this mixture, 0.2 mL of Lugol's reagent was added. The constituents were mixed properly followed by cooling for 90 min. in an ice bath with constant shaking. After this, 2.0 mL of ice-cooled DW and 20 mL of 1,2-dichloromethane were added to it. The two layers produced in the mixture were combined properly by giving a continuous flow of air to it. The upper aqueous layer was removed and the absorbance of the lower organic layer was recorded at 365 nm. Glycine betaine content present in the given sample was quantified against the value of the standard curve.

Statistical Analysis

All data were exposed to a one-way analysis of variance (ANOVA) and presented as mean ± standard error of three replicates. The *Tukey post hoc test* at the significance level of $p \le 0.05$ was carried out by using the SPSS (IBM Statistics version 24) software package for expressing the statistical significance.

Results

Evaluation of antagonistic activities of T. virens and T. viride against F. oxysporum f.sp. lycopersici and R. solani by dual culture assay

Two Trichoderma spp., namely T. virens and T. viride, were screened for their antagonistic activity against Fol and *R. solani* through a dual culture assay technique (Fig. 1.1 and 1.2). In comparison to the control, *T. virens* and *T. viride* caused a significant reduction in mycelial growth, with a magnitude of 76.3% and 39% for Fol, and 78.9% and 73.6% for *R. solani*, respectively (Fig. 1.3)

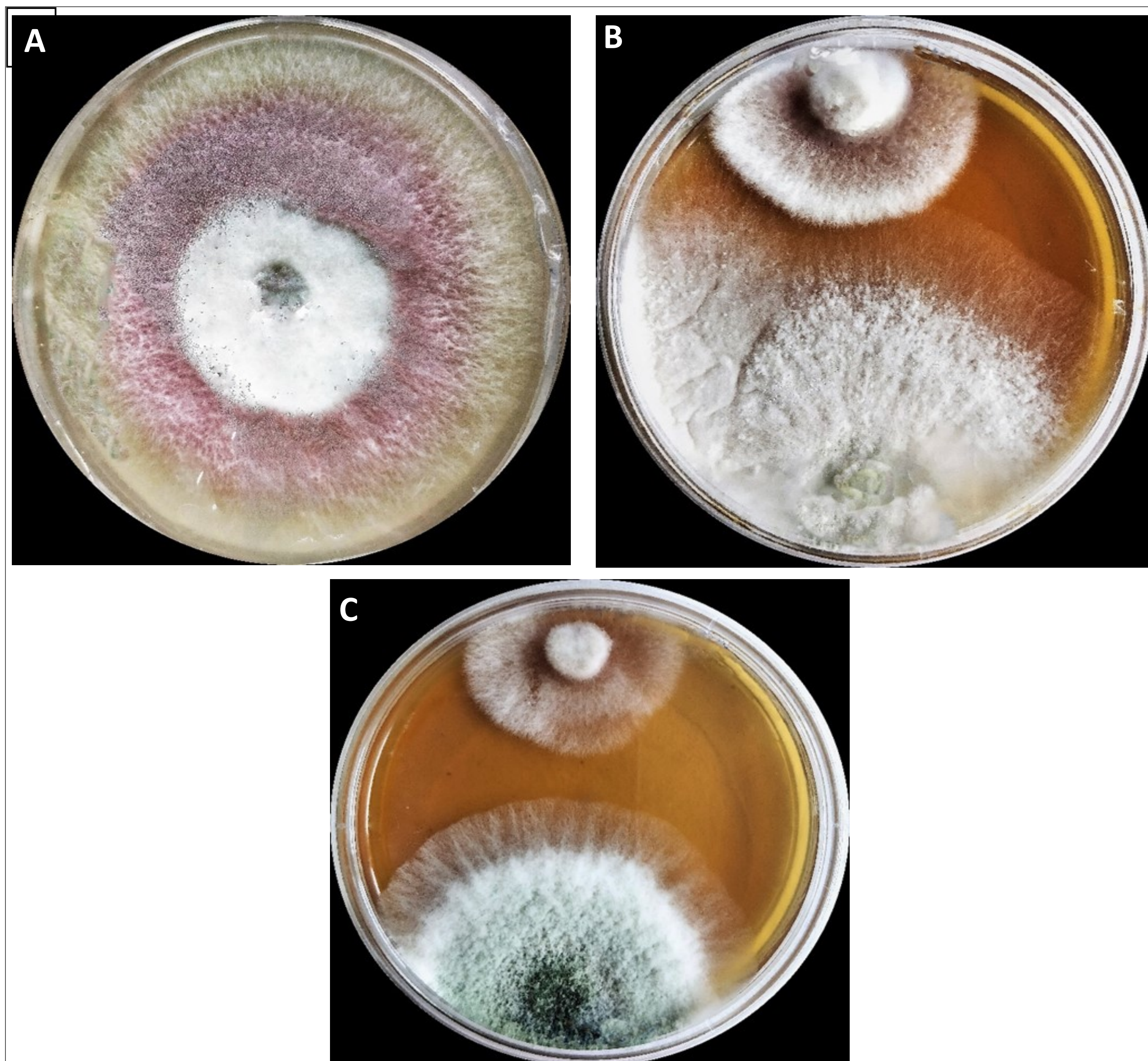


Fig. 1.1 Antagonistic activity of *T. virens* and *T. viride* against *Fusarium oxysporum* f. sp. *lycopersici* in dual culture assay (**A**) Control, (**B**) *T. virens* and *Fusarium oxysporum* f. sp. *lycopersici* (**C**) *T. viride* and *Fusarium oxysporum* f. sp. *lycopersici*

Screening of Trichoderma spp. for the synthesis of chitinase, an extracellular enzyme assay

Chitinolytic activity was assessed by determining the release of reducing saccharides from colloidal chitin. Two *Trichoderma* spp. viz., *T. viride* and *T. virens* were grown on basal chitinase detection medium with colloidal chitin as a sole source of carbon. Out of the two observed spp. of *Trichoderma, T. virens* exhibited approximately 16 percent more chitinase activity than *T. viride* (Fig. 2.1 and 2.2).Ten days old pure culture of *T. virens* (A) and *T. viride* (B) was shown in (Fig. 3).

Effect of different concentrations of JA and T. virens on in vitro seed germination and seedling vigour of tomato seeds under pathogen infection

In comparison to unprimed tomato seeds, pre-treatment with *T. virens* and JA had significant improvement in mean root-shoot length, percentage germination and vigour index. Under *Fol*-challenged tomato seedlings, the highest improvement in growth parameters viz., mean root length, mean shoot length, percent germination and vigour index are achieved through priming with 1μM JA and 1×10^{7} spores/mL *T. virens*. A similar trend of improvement has been repeated by *R. solani*-infected tomato seedlings with the same concentrations of *T. virens* and JA as observed in terms of different growth parameters in the case of *Fol* (Table 1).

Fig. 1.2. Antagonistic activity of *T. virens* and *T. viride* against *Rhizoctonia solani* in dual culture assay (**A**) Control, (**B**) *T. virens* and *Rhizoctonia solani* (**C**) *T. viride* and *Rhizoctonia solani*

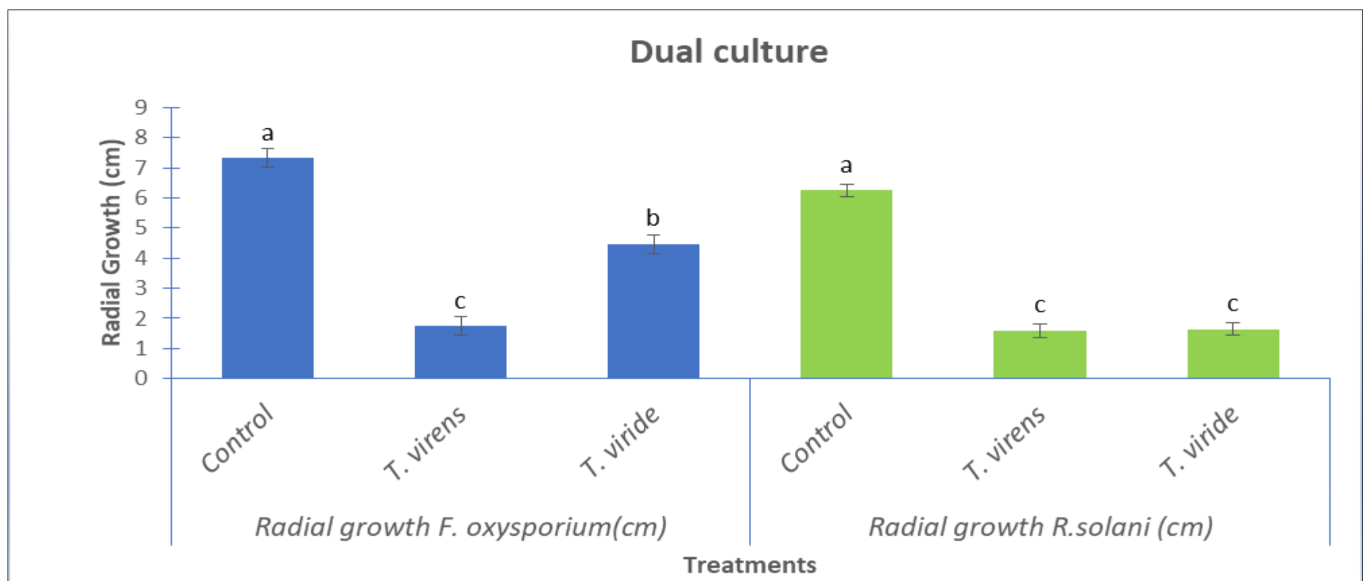


Fig. 1.3 Screening of *Trichoderma* spp. against pathogens in dual culture technique. Bars represent standard error of the mean [n=3]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).

Fig. 2.1. Chitinase activity of *Trichoderma* spp. (**A**) Control and (**B**) *T. virens* (**C**)*T. viride*

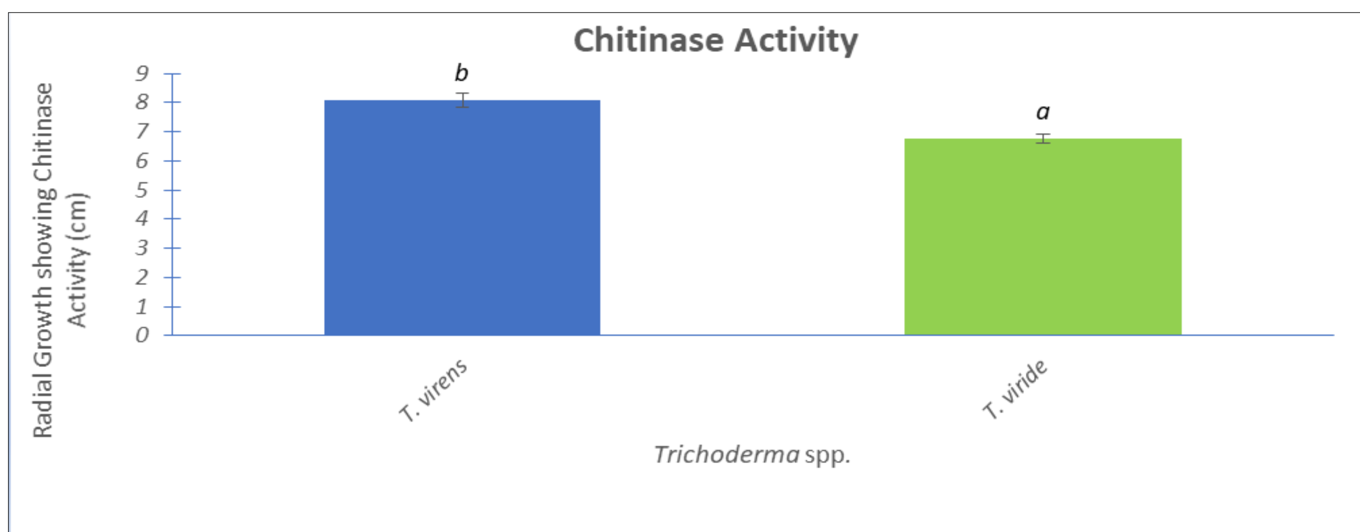


Fig. 2.2 Screening of *Trichoderma* spp. for chitinase activity on medium supplemented with colloidal chitin. Bars represent standard error of the mean [n=6]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).

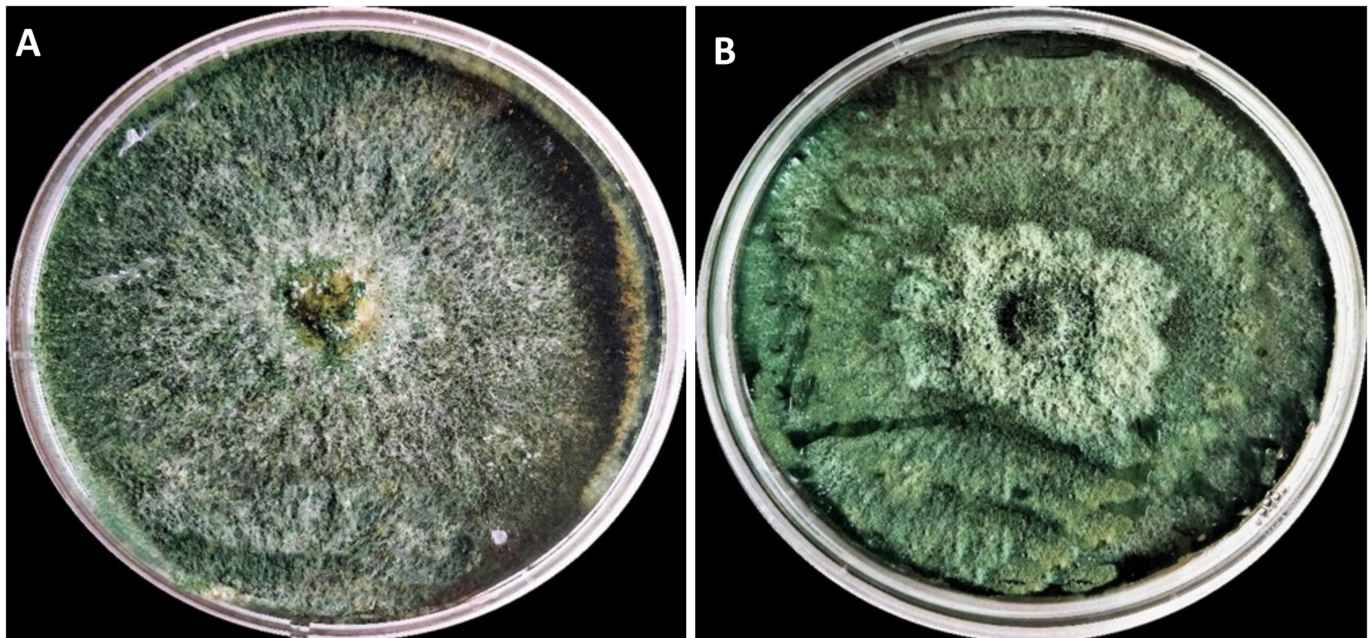


Fig. 3 Pure culture of *Trichoderma* spp. (**A**) *T. virens* (**B**) *T. viride*

Table 1. Effect of different concentrations of JA and *T. virens* on *in vitro* seed germination and seedling vigour of tomato seeds under pathogen infection.

Pathogen	Priming agent	Mean Root Length (cm)	Mean Shoot Length (cm)	Percentage Germination	Vigour Index
Fusarium oxysporum lycopersici	Jasmonic Acid (μ M)				
	0	$2.12^{a} \pm 0.15$	$4.17^{a} \pm 0.10$	$63.33^{a} \pm 4.41$	398.35
	0.1	$2.34^{a} \pm 0.07$	$5.10^{b} \pm 0.09$	$76.67^{ab} \pm 4.41$	570.43
	1	$3.01^{b} \pm 0.13$	$6.03^{c} \pm 0.12$	$90.00^{b} \pm 2.89$	813.6
	10	$2.91^{b} \pm 0.14$	$5.49^{b} \pm 0.14$	$85.00^{b} \pm 2.89$	714
	T. virens (Spores/ mL)				
	0	$2.12^{a} \pm 0.15$	$4.17^{a} \pm 0.10$	$63.33^{a} \pm 4.41$	398.35
	10^{6}	$2.55^{a} \pm 0.06$	$5.22^{b} \pm 0.11$	$81.67^{b} \pm 1.67$	634.58
	10^{7}	$3.30^{b} \pm 0.11$	$6.19^{c} \pm 0.12$	$90.00^{b} \pm 2.89$	854.1
	10^{8}	$3.14^{b} \pm 0.14$	$5.90^{c} \pm 0.11$	$85.00^{b} \pm 2.89$	768.4
Rhizoctonia solani	Jasmonic Acid (μ M)				
	0	$1.89^{a} \pm 0.13$	$3.99^{a} \pm 0.10$	$61.67^{a} \pm 6.67$	362.62
	0.1	$2.21^{a} \pm 0.09$	$5.01^{b} \pm 0.09$	$80.00^{ab} \pm 2.89$	577.6
	1	$2.95^{b} \pm 0.13$	$5.79^{c} \pm 0.15$	$85.00^{b} \pm 2.89$	742.9
	10	$2.71^{b} \pm 0.15$	$5.52^{c} \pm 0.14$	$85.00^{b} \pm 2.89$	700.0
	T. virens (Spores/ mL)				
	0	$1.89^{a} \pm 0.13$	$3.99^{a} \pm 0.10$	$61.67^{a} \pm 6.67$	362.62
	10^{6}	$2.45^{b} \pm 0.10$	$5.19^{b} \pm 0.09$	$83.33^{b} \pm 4.41$	636.64
	10^{7}	$3.04^{c} \pm 0.12$	$5.95^{c} \pm 0.14$	$88.33^{b} \pm 1.67$	794.09
	10^{8}	$3.07^{c} \pm 0.12$	$5.76^{c} \pm 0.10$	$85.00^{b} \pm 2.89$	750.55

Morphological Aspects

After thirty days of pathogen inoculation, morphological parameters viz. root-shoot length, fresh-dry weight and the number of leaves were recorded in sixty days old tomato plants under different treatments.

Root-Shoot Length

Significant variations were observed among root and shoot length in sixty days old treated/untreated tomato plants. In contrast to non-primed control plants, seed-primed tomato plants without any pathogenic stress express a significant enhancement in root length of 3.12, 9.9 and 16.4% under the treatments JA, Tv and Tv+JA, respectively. Furthermore, when compared with the control, a remarkable decrease in root length was detected in tomato plants infected with *Fol* (45.2%) and *R. solani* (42.3%). Pre-treatment of tomato seeds with *T. virens* and JA, either individually or together, brought significant improvement

in root length in infected plants as observed in the case of *Fol*-treated plants. Enhancements of 29, 37 and 53% were observed in the case of treatments with Fol+JA, Fol+Tv and Fol+Tv+JA, respectively. A similar trend of increase has also been perceived in the case of *R. solani* inoculated tomato plants with the treatments Rs+JA (38.7%), Rs+Tv (44.6%) and Rs+Tv+JA (55.9%). The most significant increase in the root length in the pathogens-infected tomato plants has been measured in the treatments where seed-priming with both the ameliorative agents has been performed prior to the exposure of each pathogen (Fig. 4 A). As observed in the case of root length, a similar trend of increase in shoot length has also been depicted by inoculated as well as non-inoculated tomato plants. In both cases, the most remarkable improvement has been exhibited by tomato plants where tomato seeds were primed with the combination of *T. virens* and JA. In continuation to root length observations, in comparison to control, shoot length was also observed to decrease in *Fol* (21.6%) and *R. solani* (24%) challenged tomato plants. Although under pathogenic challenges, priming of tomato seeds individually with *T. virens* (except in the treatment Rs+Tv) and JA causes a significant increase in the shoot length when compared with non-primed plants; but the highest improvement in the shoot length was observed in the treatments Fol+Tv+JA (17.6%) and RS+TV+JA (16.7%) in the case of *Fol* and *R. solani* diseased plants, respectively (Fig. 4 B).

Fresh-Dry Weight

Like root-shoot length, tomato plants' fresh and dry weights were also affected adversely due to pathogen-induced damage to them. A significant decrease in fresh (70.8 and 73.7%) and dry weight (73.6 and 76.5%) was monitored in the case of tomato plants challenged by *Fol* and *R. solani*, respectively. Further, priming of tomato

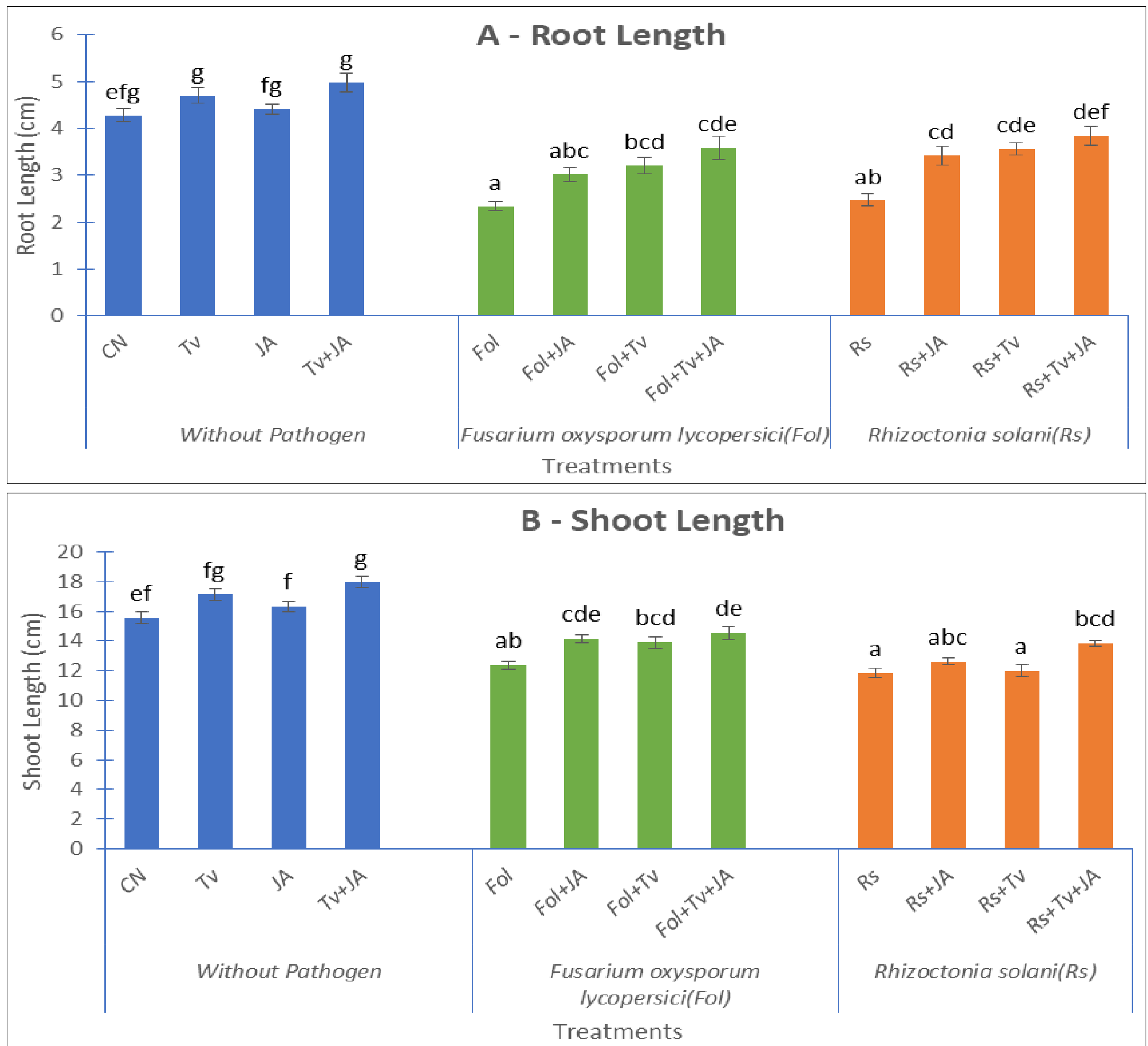


Fig. 4. Effect of *Trichoderma virens* (Tv) and Jasmonic acid (JA) on (**A**) Root length and (**B**) Shoot length in sixty days old tomato plants with and without pathogenic stress. Bars represent the standard error of the mean [n=9]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).

seeds with these two ameliorative agents either individually or in combination did not bring any significant improvement in the fresh-dry weight in the case of plants without any pathogenic infection. In comparison to pathogens-stressed plants, individual treatments with *T. virens* and JA also contribute towards an increase in the fresh weight but the maximum enhancement in fresh weight of 2.3- and 2.5-fold was observed in the treatments with Fol+Tv+JA and Rs+Tv+JA, respectively (Fig. 5A). A similar pattern of a significant reduction in dry weight was also observed in pathogen-infected tomato plants. However, after the application of bio stimulators, a similar trend of enhancement in dry weight has also been repeated in *T. virens* and JA-primed tomato plants under different treatments Fol+Tv+JA (123%), Rs+JA (83.9%), Rs+Tv (139.7%) and Rs+Tv+JA (137.6%) (Fig. 5B).

Number of Leaves

As observed in the previous growth parameters, a significant increase in the number of leaves was observed in JA+Tv seed-primed tomato plants with and without pathogenic stress. However, owing to fungal infections, a highly significant decrease in the number of leaves was observed. In pathogen-challenged plants, a percentage reduction of approximately 43 and 46% was observed in the case of *Fol* and *R. solani* diseased plants, respectively. However, due to seed priming, a slight improvement of 25% was observed in the treatments with Fol+Tv and Fol+Tv+JA while a remarkable improvement of 37% was achieved in the treatment with Rs+Tv+JA in the case of *R. solani* diseased tomato plants (Fig. 6).

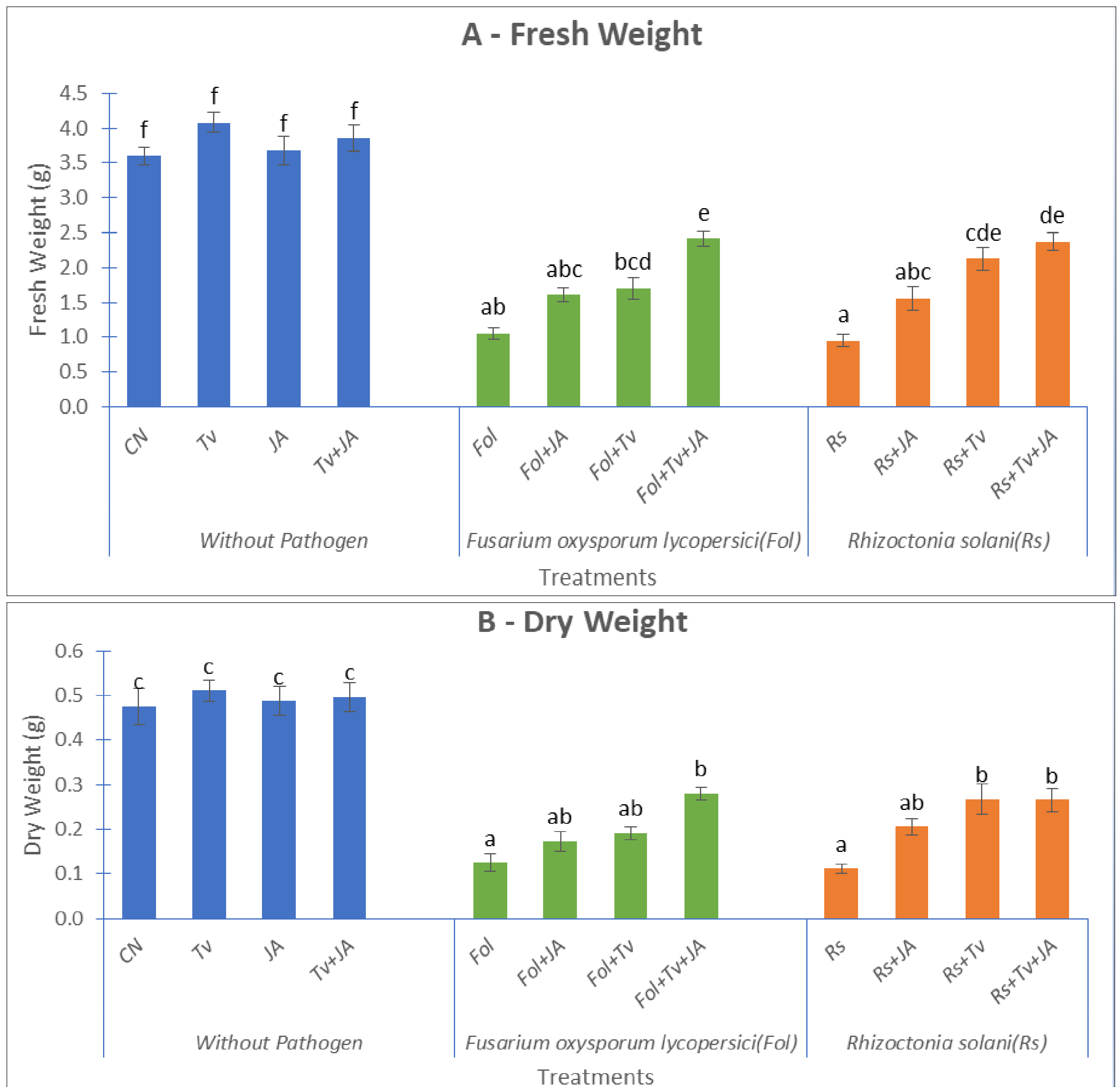


Fig. 5. Effect of *Trichoderma virens* (Tv) and Jasmonic acid (JA) on (**A**) Fresh and (**B**) Dry weight in sixty days old tomato plants with and without pathogenic stress. Bars represent the standard error of the mean [n=9]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).

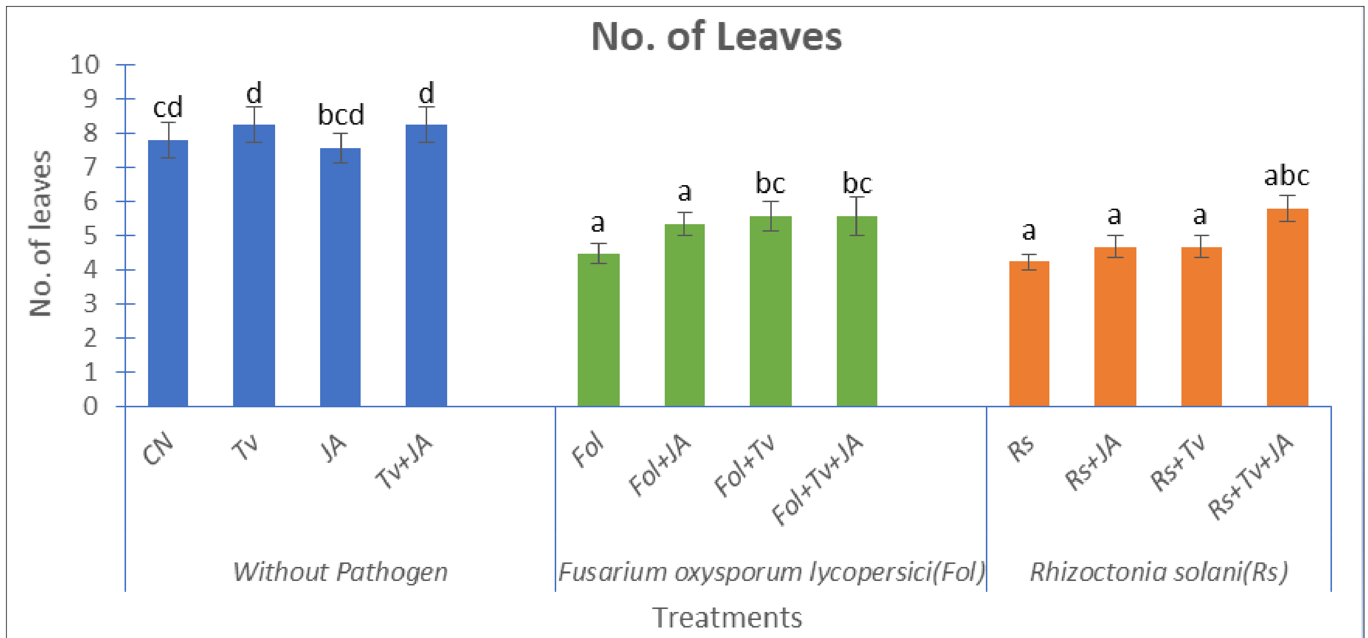


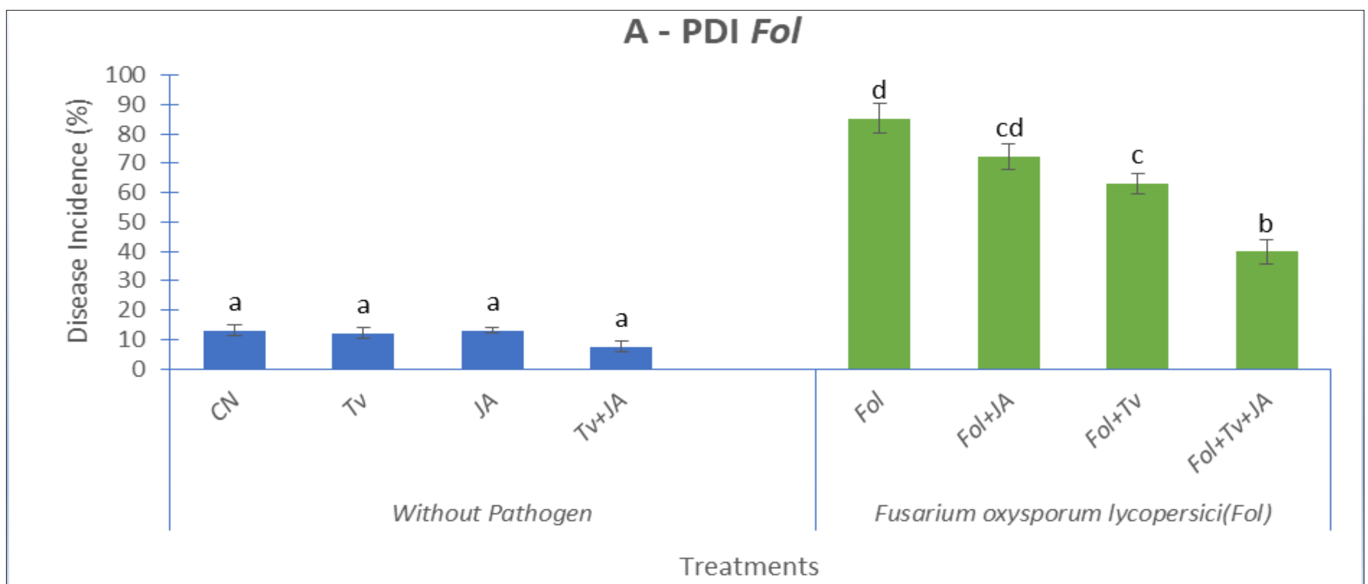
Fig. 6. Effect of *Trichoderma virens* (Tv) and Jasmonic acid (JA) on number of leaves in sixty days old tomato plants with and without pathogenic stress. Bars represent the standard error of the mean [n=9]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).

Disease Incidence

Priming the tomato seeds with *T. virens* and JA, either alone or in combination, did not insert any marked influence on PDI in the case of plants which were not inoculated with either of the pathogens. However, the extent of disease suppression in tomato plants through pre-treatment with *T. virens* and JA was revealed by the reduction of wilt and damping-off incidence in tomato plants infected with *F. oxysporum lycopersici* and *R. solani,* respectively. Our results revealed that the highest PDI of 85% was detected in pathogen-challenged plants. It has been further observed that priming the tomato seeds with *T. virens* and JA, alone or in combination, significantly reduces the PDI when compared to pathogen-inoculated plants. However, in the case of *Fol*, the lowest PDI (40%) was observed in the case of treatment with Fol+Tv+JA (Fig. 7A). A similar trend of reduction has also been followed by the treatment with Rs+Tv+JA, with the PDI falling to 42% (Fig. 7B).

Free Proline and Glycine Betaine Content

Compatible osmolytes play a tremendous role in plant defence by maintaining cellular turgor through decreasing the water potential as well as acting as signalling molecules to initiate a cascade of defensive responses in diseased plants. In the case of uninoculated plants, pre-treatment of tomato seeds with *T. virens* and JA non-significantly decreases the free proline accumulation in the tomato leaves. Furthermore, in comparison to uninoculated plants, a significant increase in the levels of free proline has been observed in the case of both wilting and damping off stressed tomato plants. Maximum enhancement in proline content was depicted in the *Fol* i.e., 2.64-fold as compared to control followed by *R. solani* treated plants (2.61-fold). However, pre-treatment of tomato seeds with JA and *T. virens,* either individually or in combination, significantly decreases the accumulation of free proline content in the case of both the studied pathogens (Fig. 8A). Like proline content, seed-priming of tomato seeds with biocontrol agent and chemical inducer either



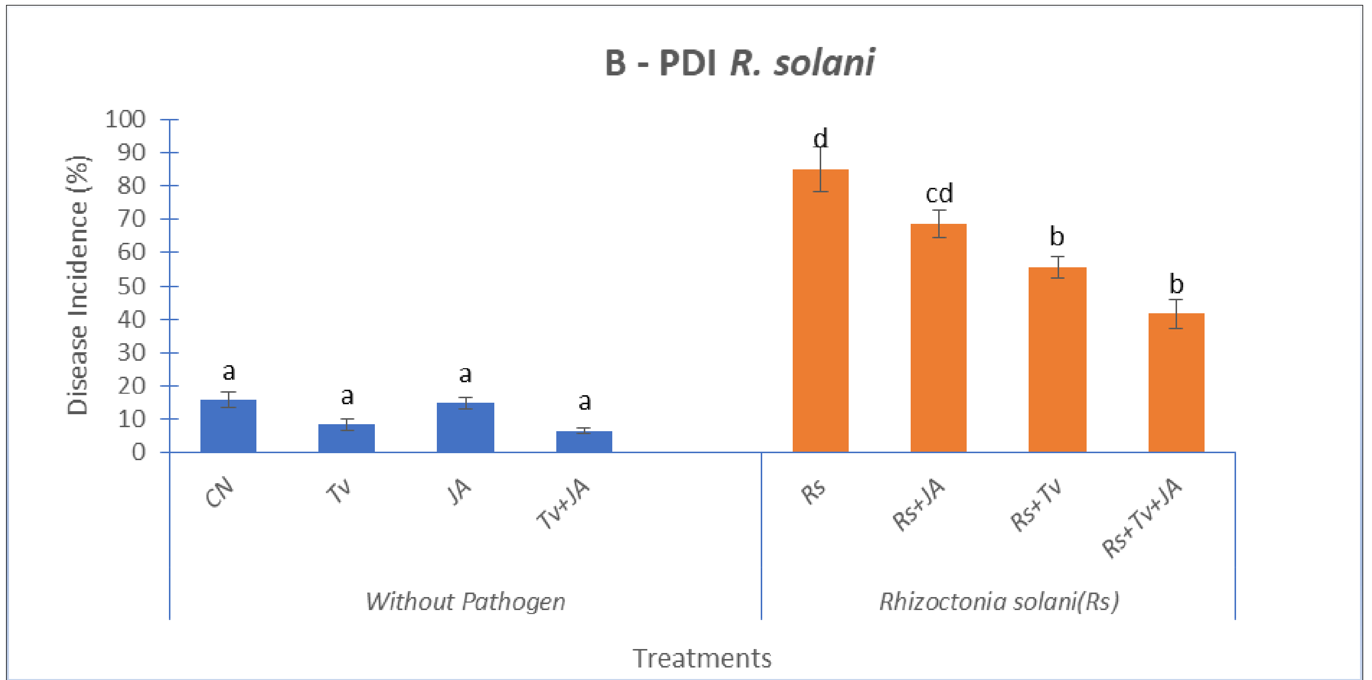
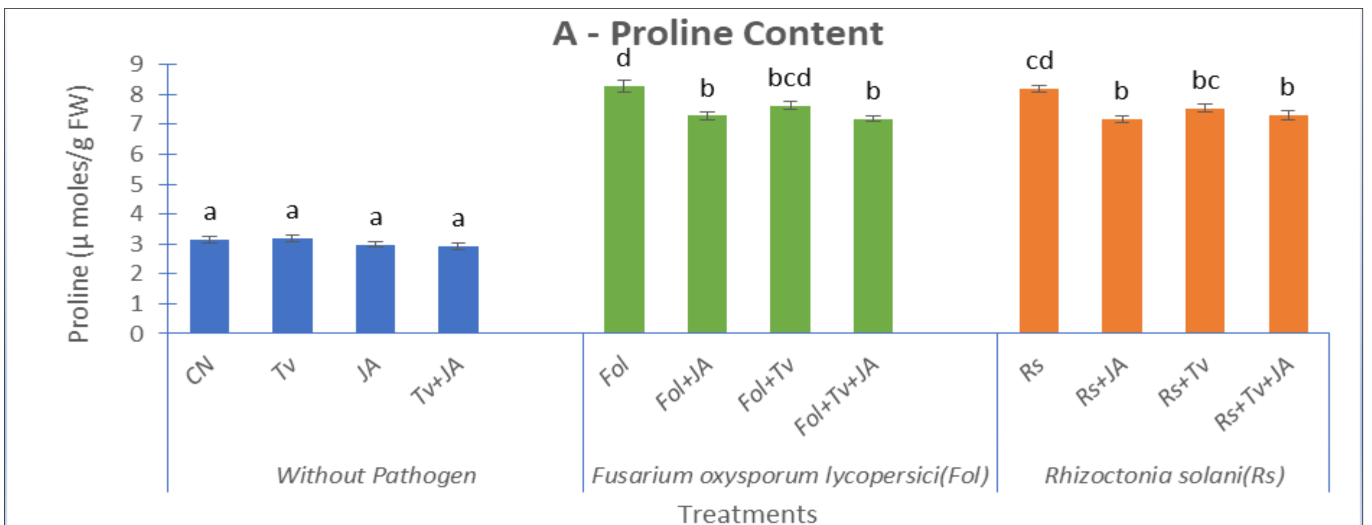


Fig. 7. Effect of *Trichoderma virens* (Tv) and Jasmonic acid (JA) on Percentage Disease Incidence (PDI) of (**A**) *F. oxysporum lycopersici* and (**B**) *R. solani* in sixty days old tomato plants with and without pathogenic stress. Bars represent the standard error of the mean [n=36]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).



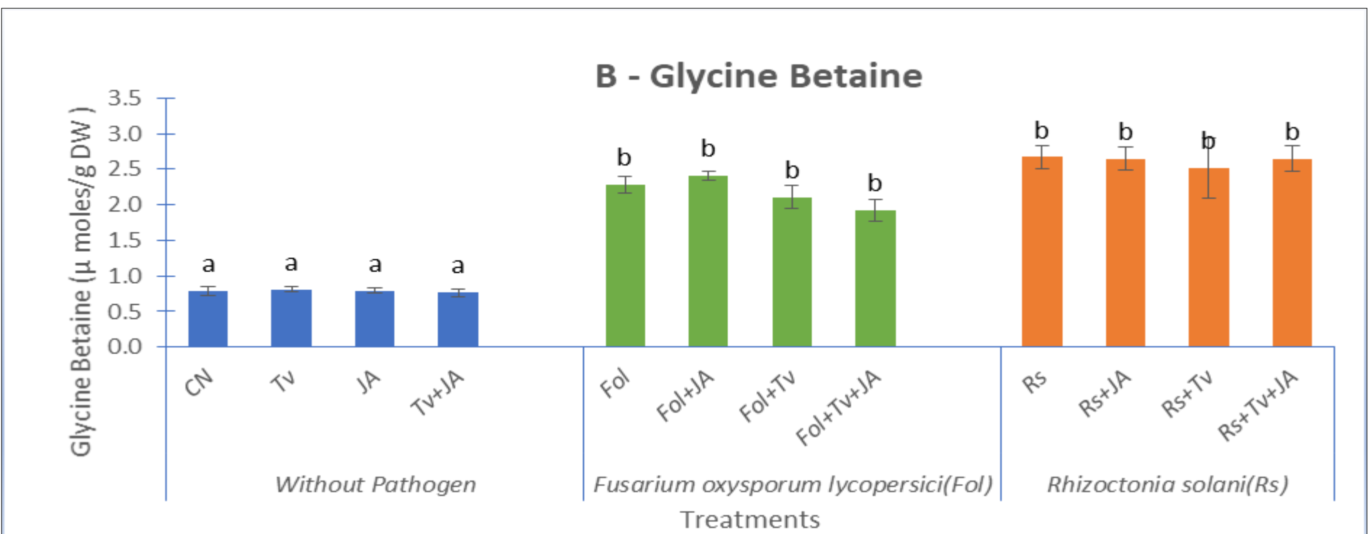


Fig. 8. Effect of *Trichoderma virens* (Tv) and Jasmonic acid (JA) on (**A**) Free Proline and (**B**)Glycine Betaine Content in sixty days old tomato plants with and without pathogenic stress. Bars represent the standard error of the mean [n=3]. Different letters within the column indicate statistically significant differences among the treatments, according to Tukey's multiple comparison test ($P < 0.05$).

individually or together did not alter the glycine betaine content to the significant level in the case of tomato plants without any pathogenic inoculation. However, as observed in the case of diseased plants, a marked increase of approximately 3- and 3.4-fold, as compared to control in glycine betaine content, has been observed in *Fol* and *R. solani* stressed tomato plants, respectively. Further, pretreatment of tomato seeds with *T. virens* and JA alone or together non-significantly decreases the accumulation of glycine betaine in diseased plants (Fig. 8B).

Discussion

In the present investigation, we have observed that priming of tomato seeds with *T. virens* and JA, individually as well as in combination, was successful in defending tomato plants infected with two soil-borne pathogens viz., *Fusarium oxysporum lycopersici* and *Rhizoctonia solani* under *in vitro* and *in vivo* conditions. Presently, the cultivated tomato possesses a low genetic diversity because of severe selection and intensive genetic bottlenecks which occurred over the course of evolution and domestication (28). Due to these reasons, tomato is highly prone to be influenced by more than 200 diseases produced by different kinds of pathogens worldwide (29). Among these, yield destruction of this important horticultural crop by soil-borne pathogens is the worst as these pathogens are more difficult to manage because of the wide range of hosts as well as the fact that they persist in the soil for a longer duration by developing different kinds of perennating structures. *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* are important soil-borne pathogens which were identified through different methods. Further, their disease-producing abilities were evaluated through the pathogenicity test by perceiving symptoms like vein clearing, reduced rate of photosynthesis and transpiration, disruption of leaf cell membrane permeability, wilting, leaf epinasty, necrosis, and cell death (30, 31). Adhikari et.al., have sequenced and analysed the existence of fifteen pathogenicity genes through PCR (Polymerase Chain Reaction) studies and confirmed that these genes can be used as a molecular marker to recognise and differentiate the different isolates of *Fol* (30). Similarly, Abdelghany et. al., have examined the pathogenicity of 17 isolates of *R. solani* towards *Arabidopsis thaliana* in Japan through the application of soil and leaf inoculations (31).

Pathogen exposure disturbs the plant by interfering with plant vegetative growth and numerous physiological processes. For example, root rot disease of tomatoes caused by *Rhizoctonia solani* infection results in a decline in total root length, a decrease in the number of root tips, as well as in the magnitude of root branching, which eventually makes plants incapable to access water from underground soil layers and consequently, lowers the shoot growth (32). Similar findings were also obtained with *Phytophthora parasitica* causing infection in tomato seedlings (33). Our study has also revealed the same results which were measured in terms of root length, shoot length, fresh and dry weight, and the number of leaves. Fusarium wilt diminishes plant growth by clogging and blocking the xylem vessels which are responsible for the translocation of water and essential minerals through the plants. In addition to this, chlorosis, stomatal closure, etc. are the reasons which might be responsible for the pathogen-induced decrease in the growth of infected tomato plants (34). Similarly, *R. solani*-infected tomato plants exhibit poor growth of lateral roots which ultimately leads to less vigorous plants with decreased water and nutrient uptake abilities. Consequently, these plants look chlorotic and stunted. Furthermore, with the subsequent decrease in access to soil water and minerals, plants eventually begin to wilt and die (35).

In the pursuit of sustainable agriculture, various spp. of the genus *Trichoderma* serve as economically important biostimulants in terms of plant growth promotion, induction of disease resistance and disease control. Several investigations have reported the antagonistic activities of *Trichoderma* spp. against several fungal pathogens of economically important crops through dual culture assay. In the present research work, screening of two strains of *Trichoderma* i.e., *T. virens* and *T. viride* against *Fol* and *R. solani* was assessed through this technique. Maximum reduction in the *in vitro* radial growth of both pathogens has been observed with *T. virens*. The development of an inhibition zone at the contiguous spot between pathogens and *Trichoderma* spp. in dual culture assay might be ascribed to the synthesis and accumulation of mycoparasitic volatile and non-volatile metabolites along with the assembly of various extracellular hydrolytic enzymes by these species. Antagonistic effects of *T. virens* in radial growth of *R. solani* in dual culture assay have been reported by Halifu et. al., 2020 (36). Furthermore, the inhibitory action of *T. virens* against fungal pathogens such as *Alternaria alternata*, *Geotrichum candidum*, *Fusarium oxysporum* f. sp. *spinaciae* and *Macrophomina phaseolina* has been confirmed in previous investigations. In addition to this, synthesis and release of cell wall degrading lytic enzymes like chitinase and β-1,3 glucanases along with antibiotics are extremely valuable weapons used by *Trichoderma* spp. to reduce the pathogenicity of fungal pathogens in the host plants, as the cell wall of fungal pathogens is composed of chitin and β-1,3 glucan. The present investigation also explores the biocontrol potential of *T. virens* and *T. virde* through chitinase activity in the chitinase detection media which could be participated in the degradation of the pathogen cell wall during antagonism. Further, these fragments of pathogenic cell walls serve as ligands which by binding with suitable receptors, initiate the downstream signalling cascades via induction of mitogen-activated protein kinase (MAPK) and G proteins (13).

Compatible osmolytes like proline and glycine betaine serve as significant metabolites by maintaining cellular homeostasis through various mechanisms like facilitation of driving gradient for water absorption, regulation of cell turgor through fine osmotic tuning, the establishment of cellular redox status by eliminating excess ROS and providing protection to cellular machinery from oxidative injury and osmotic stress. Numerous stress signalling pathways such as hormones, mitogen-activated protein (MAP)

kinase, and calcium-signalling cause excessive formation of osmolytes. In the present investigation, enhancement in the accumulation of free proline and glycine betaine against pathogen-induced injury to the host plant might support their osmoprotectant role under stressed circumstances. Similar findings were also reported by several previous investigations (37–39).

Seed priming is an evolving technique for sustainable agricultural practices, designed particularly to increase seed value, seedling strength, and productivity; and most importantly to induce resistance against several kinds of biotic and abiotic stresses by minimising the use of several harmful chemical compounds (40). The present study provides an important insight into the seed priming-induced ameliorative potential of *T. virens* and Jasmonic acid against two devastating diseases of tomato plants. As both our *in vitro* and *in vivo* findings demonstrated, seed priming with both these biocontrol agents leads to better growth of tomato seedlings and plants in pathogen-contaminated conditions. The presence of *Trichoderma* spp. in the plants' rhizosphere leads to many significant changes in plant metabolism, predominantly including the synthesis of phytohormones, compatible osmolytes, secondary metabolites and amino acids; and regulates important physiological processes like transpiration, photosynthesis and water potential in host plants (13). Moreover, *Trichoderma* spp. are known to enhance resistance in diseased plants by upregulating the synthesis of pathogenesis-related (PR) proteins like chitinase and glucanase as well as antioxidative defensive responses (41). As an important instance, as reported in the case of potato and tobacco plants, an increase in the expression of *T. harzianum*-derived chitinase in these plants made them highly resistant to many soil-borne pathogens such as *R. solani* and foliar pathogens like *Alternaria solani*, *A. alternata*, *Botrytis cineria* etc. (42). In addition to this, numerous previous reports highlight the mycoparasitic action of *Trichoderma* strains against several fungal phytopathogens like *Fusarium* spp., *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Pythium* spp., *Ustilago maydis, Rhizoctonia solani, Botrytis cinerea* through the mechanisms like competition, antibiosis etc. (43).

Being a significant chemical inducer, JA and its derivatives play a crucial role in plants' immune systems to defend them against pathogens (44). Previous studies revealed that the exogenous application of JA upregulates the expression of defence-related genes and eventually enhances disease resistance against numerous necrotrophic pathogens (45). As reported in transgenic rice plants, the constitutive expression of the Allene oxide synthase (AOS) gene involved in JA biosynthesis resulted in a higher resistance to fungal pathogens by positively regulating the expression of anti-pathogen-related genes (46). Similarly, as reported in the case of *Fusarium graminearum*-infected wheat plants, the treatment by JA inhibits the direct invasion by the pathogens through significant changes in the accumulation of secondary metabolites and defensive enzymes (47, 48). Furthermore, the application of JA assists in plant development and defence by diminishing the accumulation of ROS like MDA (Malondialdehyde) and H_2O_2 as reported in the case of *Fusarium culmorum*-infected wheat plants (49). Through this investigation, our findings demonstrated that tomato seed priming with biological (*T. virens*) and chemical inducer (JA), specifically when applied together, increases plant growth and resistance, and simultaneously decreases the disease incidence in the pathogenically infected tomato plants.

Conclusion

A considerable element that determines the superior morphological and reproductive growth of plants is 'defence'. A defended plant can well endure distinct kinds of stresses. The defensive attitude of plants is centred upon the quickness to identify a particular pathogen and mount a signalling network to begin the production and accumulation of defence molecules. In the present investigation, we have studied the deteriorating fluences of two soil-borne pathogens on the vegetative growth of tomato plants. Furthermore, our study also revealed that pre-treatment of tomato seedlings with *T. virens* and JA, before exposure to pathogens leads to a marked improvement not only in morphological parameters but also lowers the percentage disease incidence (PDI) through various mechanisms of disease resistance like inhibition of growth and proliferation of pathogens, induction of SAR (Systemic Acquired Resistance) and ISR (Induced Systemic Resistance) in the diseased plants through the synthesis and accumulation of secondary metabolites, PR (Pathogenesis Related) proteins and induction of antioxidative enzymatic and non-enzymatic defence system in the stressed plants. Our findings also demonstrated that pathogen-induced damage in the infected tomato plants leads to the accumulation of compatible osmolytes i.e., proline and glycine betaine which themselves assist in osmoprotection by lowering the water potential of wilted plants and alleviate the pathogen challenges by mitigating the ROS and initiating a signalling cascade. Therefore, from the above analysis, we can conclude that in the present study, higher induction of ameliorative compounds in the plants pre-treated with *T. virens* as BCA in combination with JA as chemical inducer can be correlated to an improved defence response activated against damping off and fusarium wilt infection in tomato plants. Therefore seed-priming with these agents, especially in combination, can be recommended as a cost-effective, environment- and farmer-friendly approach to raising the productivity of this important horticultural crop on a commercial scale.

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Authors contributions

MS and VK designed the research. MS performed the experimentation, data analysis and wrote the initial draft. SK reviewed and edited the manuscript. All authors reviewed and accepted the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Panno S, Davino S, Caruso AG, Bertacca S, Crnogorac A, Mandić A *et al*. A review of the most common and economically important diseases that undermine the cultivation of tomato crop in the Mediterranean basin. Agronomy 2021 Nov 1 ;11(11):2188. https://doi.org/10.3390/agronomy11112188
2. Joshi R. A review of *Fusarium oxysporum* on its plant interaction and industrial use. J Med Pl Studies. 2018;6(3):112-15. https://doi.org/10.22271/plants.2018.v6.i3b.07
3. Clocchiatti A, Hannula SE, Rizaludin MS, Hundscheid MPJ, Klein Gunnewiek PJA, Schilder MT *et al*. Impact of cellulose-rich organic soil amendments on growth dynamics and pathogenicity of *Rhizoctonia solani*. Microorganisms. 2021 Jun 1;9(6). https://doi.org/10.3390/microorganisms9061285
4. Ghosh UK, Islam MN, Siddiqui MN, Khan MAR. Understanding the roles of osmolytes for acclimatizing plants to changing environment: A review of potential mechanism. Plant Signal Behav Aug;16(8):1913306 https://doi.org/10.1080/15592324.2021.1913306
5. Giri J. Glycinebetaine and abiotic stress tolerance in plants. Plant Signal Behav 2011 Nov 6(11):1746-51
6. Huang J, Hirji R, Adam L, Rozwadowski KL, Hammerlindl JK, Keller WA *et al*. Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. Plant Physiol 1;122(3):747-56
7. Suprasanna P, Rai AN, Hima Kumari P, Kumar SA, Kavi Kishor PB. Modulation of proline: implications in plant stress tolerance and development. In: Anjum NA, Gill SS, Gill R, editors. Plant Adaptation to Environmental UK: CABI Publishers. 2014 ; p. 68-93.
8. Meena RS, Kumar S, Datta R, Lal R, Vijayakumar V, Brtnicky M *et al*. Impact of agrochemicals on soil microbiota and management: A review. 2020 Jan 23;9(2):34 https://doi.org/10.3390/land9020034
9. Panth M, Hassler SC, Baysal-Gurel F. Methods for management of soilborne diseases in crop production. Agriculture. 2020 Jan 11;10(1):16. https://doi.org/10.3390/agriculture10010016
10. Collinge DB, Jensen DF, Rabiey M, Sarrocco S, Shaw MW, Shaw RH. Biological control of plant diseases – What has been achieved and what is the direction?. Plant Pathol 2022 Jun 1. https://doi.org/10.1111/ppa.13555
11. Fenibo EO, Ijoma GN, Matambo T. Biopesticides in sustainable agriculture: A critical sustainable development driver governed by green chemistry principles. Front Sustain Food Syst. 2021 Jun 11;5:141. https://doi.org/10.3389/fsufs.2021.619058
12. Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, el Hamss H *et al*. Biological control of plant pathogens: A global perspective. Microorganisms. 2022 https://doi.org/10.3390/microorganisms10030596
13. Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M *et al*. Trichoderma: The “Secrets” of a multitalented biocontrol agent. Plants (Basel). 2020
14. Mishra J, Dutta V, Arora NK. Biopesticides in India: technology and sustainability linkages. 3 Biotech. 2020 May 1. https://doi.org/10.1007/s13205-020-02192-7
15. Zhang L, Zhang F, Melotto M, Yao J, He SY. Jasmonatesignaling and manipulation by pathogens and insects. J Exp Bot. 2017 Mar 3. https://doi.org/10.1093/jxb/erw478
16. Wang Y, Mostafa S, Zeng W, Jin B. Function and mechanism of jasmonicacid in plant responses to abiotic and biotic stresses. Int J Mol Sci. 2021 Aug. https://doi.orger/10.3390/ijms22168568
17. Morton DJ, Stroube WH. Antagonistic and stimulatory effects of soil microorganisms upon *Sclerotium rolfsii*. Phytopath. 1955 ; 45(8) 417-20
18. Garrett S. Biology of root-infecting fungi. Cambridge University Press. 1956; 294 p. https://doi.org/10.1097/00010694-195607000-00011
19. Agrawal T, Kotasthane AS. Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. Springerplus. 2012 https://doi.org/10.1186/2193-1801-1-73
20. Roberts WK, Selitrennikoff CP. Plant and bacterial chitinases differ in antifungal activity. Microbiology (N Y). 1988 Jan 1 https://doi.org/10.1099/00221287-134-1-169
21. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria1. Crop Sci. 1973 Nov 1 https://doi.org/10.2135/cropsci1973.0011183X001300060013x
22. Jain A, Singh S, Kumar Sarma B, Bahadur Singh H. Microbial consortium-mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. J Appl Microbiol]. 2012 Mar. https://doi.org/10.1111/j.1365-2672.2011.05220.x
23. Jensen B, Knudsen IMB, Madsen M, Jensen DF. Biopriming of infected carrot seed with an antagonist, *Clonostachys rosea*, selected for control of seedborne *Alternaria* spp. Phytopathology.2004. https://doi.org/10.1094/PHYTO.2004.94.6.551
24. Nirmaladevi D, Venkataramana M, Srivastava RK, Uppalapati SR, Gupta VK, Yli-Mattila T *et al*. Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. Scientific Reports. 2016 Feb 17 https://doi.org/10.1038/srep21367
25. Manganiello G, Sacco A, Ercolano MR, Vinale F, Lanzuise S, Pascale A *et al*. Modulation of tomato response to *Rhizoctonia solani* by *Trichoderma harzianum* and its secondary metabolite Harzianic acid. Front Microbiol. 2018 Aug 30;9(AUG):1966. https://doi.org/10.3389/fmicb.2018.01966
26. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973 Aug. https://doi.org/doi.org/10.1007/BF00018060
27. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil. 1983 Jun;70(2):303-07. https://doi.org/10.1007/BF02374789
28. Tamburino R, Sannino L, Cafasso D, Cantarella C, Orrù L, Cardi T *et al*. Cultivated tomato (*Solanum lycopersicum*L.) suffered a severe cytoplasmic bottleneck during domestication: Implications from chloroplast genomes. Plants. 2020 Oct https://doi.org/10.3390/plants9111443
29. Singh VK, Singh AK, Kumar A. Disease management of tomato through PGPB: current trends and future perspective. 3 Biotech. 2017 Jul 20. https://doi.org/10.1007/s13205-017-0896-1
30. Adhikari TB, Gao A, Ingram T, Louws FJ. Pathogenomicscharacterization of an emerging fungal pathogen, *Fusarium oxysporum* f. sp. *lycopersici*in greenhouse tomato production systems. Front Microbiol. 2020 Aug 27;11:1995. https://doi.org/10.3389/

fmicb.2020.01995

31. Abdelghany MMA, Kurikawa M, Watanabe M, Matsui H, Yamamoto M, Ichinose Y *et al*. Surveillance of pathogenicity of *Rhizoctonia solani* Japanese isolates with varied anastomosis groups and subgroups on *Arabidopsis thaliana*. Life. 2022 Jan 1. https://doi.org/10.3390/life12010076
32. Heflish AA, Abdelkhalek A, Al-Askar AA, Behiry SI. Protective and curative effects of *Trichoderma asperelloides* ta41 on tomato root rot caused by *Rhizoctonia solani* Rs33. Agronomy. 2021 Jun 1;11(6). https://doi.org/10.3390/agronomy11061162
33. Larousse M, Rancurel C, Syska C, Palero F, Etienne C, Industri B *et al*. Tomato root microbiota and *Phytophthora parasitica*-associated disease. Microbiome. 2017;5(1). https://doi.org/10.1186/s40168-017-0273-7
34. Chaturvedi H, Singh B, Jajoo A, Prakash A. Shielding of photosynthetic apparatus by consortia of bacterial endophytes in tomato plants suffering from *Fusarium* wilt. Frontiers in Agronomy. 2022 May 18;4:33. https://doi.org/10.3389/fagro.2022.831731
35. Ajayi-Oyetunde OO, Bradley CA. *Rhizoctonia solani*: taxonomy, population biology and management of *Rhizoctonia* seedling disease of soybean. Plant Pathol. 2018 Jan 1. https://doi.org/10.1111/ppa.12733
36. Halifu S, Deng X, Song X, Song R, Liang X. Inhibitory mechanism of *Trichoderma virens* ZT05 on *Rhizoctonia solani*. Plants. 2020 Jul 19 . https://doi.org/10.3390/plants9070912
37. Zehra A, Meena M, Dubey MK, Aamir M, Upadhyay RS. Activation of defense response in tomato against *Fusarium* wilt disease triggered by *Trichoderma harzianum* supplemented with exogenous chemical inducers (SA and MeJA). Revista Brasil Botanica. 2017 Sep 1;40(3):651-64. https://doi.org/10.1007/s40415-017-0382-3
38. Ayub MA, Rehman MZU, Umar W, Farooqi ZUR, Sarfraz M, Ahmad HR *et al*. Role of glycine betaine in stress management in plants. Emerging Plant Growth Regulators in Agriculture: Roles in Stress Tolerance. https://doi.org/10.1016/B978-0-323-91005-7.00005-9
39. Aydi-Ben-Abdallah R, Jabnoun-Khiareddine H, Daami-Remadi M. *Fusarium* wilt biocontrol and tomato growth stimulation, using endophytic bacteria naturally associated with *Solanum sodomaeum* and *S. bonariense* plants. Egypt J Biol Pest Control. 2020 Dec 1. https://doi.org/10.1186/s41938-020-00313-1
40. Sood M, Kumar V, Rawal R. Seed biopriming a novel method to control seed borne diseases of crops. Biocontrol Agents and Secondary Metabolites. 2021 Jan 1;181-223. https://doi.org/10.1016/B978-0-12-822919-4.00008-9
41. Abbas A, Mubeen M, Zheng H, Sohail MA, Shakeel Q, Solanki MK *et al*. *Trichoderma* spp. genes involved in the biocontrol activity against *Rhizoctonia solani*. Front Microbiol. 2022 May 25 . https://doi.org/10.3389/ fmicb.2022.884469
42. Howell CR. Mechanisms employed by *Trichoderma*species in the biological control of plant diseases: The history and evolution of current concepts.2007 Feb 23. https://doi.org/10.1094/PDIS.2003.87.1.4
43. Harwoko H, Daletos G, Stuhldreier F, Lee J, Wesselborg S, Feldbrügge M *et al*. Dithiodiketopiperazine derivatives from endophytic fungi *Trichoderma harzianum* and *Epicoccum nigrum*. Nat Prod Res. 2021. https://doi.org/10.1080/14786419.2019.1627348
44. Wang J, Song L, Gong X, Xu J, Li M. Functions of jasmonicacid in plant regulation and response to abiotic stress. Int J Mol Sci 2020. Feb 1. https://doi.org/10.3390/ijms21041446
45. Ameye M, Audenaert K, de Zutter N, Steppe K, van Meulebroek L, Vanhaecke L *et al*. Priming of wheat with the green leaf volatile Z-3-hexenyl acetate enhances defense against *Fusarium graminearum* but boosts deoxynivalenol production. Plant Physiol. 2015 Apr 1;167(4):1671-84. https://doi.org/10.1104/pp.15.00107
46. Mei C, Qi M, Sheng G, Yang Y. Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression and host resistance to fungal infection. Mol Plant Microbe Interact. 2006 Oct. https://doi.org/10.1094/MPMI-19-1127
47. Zhao S, Li Y. Current understanding of the interplays between host hormones and plant viral infections. PLoSPathog. 2021 Feb 25;17(2). https://doi.org/10.1371/journal.ppat.1009242
48. Moosa A, Sahi S, Khan S, Horticulturae AMF, 2019 undefined. Salicylic acid and jasmonic acid can suppress green and blue moulds of citrus fruit and induce the activity of polyphenol oxidase and peroxidase. researchgate.net. 2019 Jun 1. https://doi.org/10.2478/fhort-2019-0014
49. Motallebi P, Niknam V, Ebrahimzadeh H, Hashemi M, Enferadi ST. Exogenous methyl jasmonatetreatment induces defenseresponse against *Fusarium culmorum* in wheat seedlings. J Plant Growth Regul. 2017 Mar 1;36(1):71-82. https://doi.org/10.1007/s00344-016-9620-3