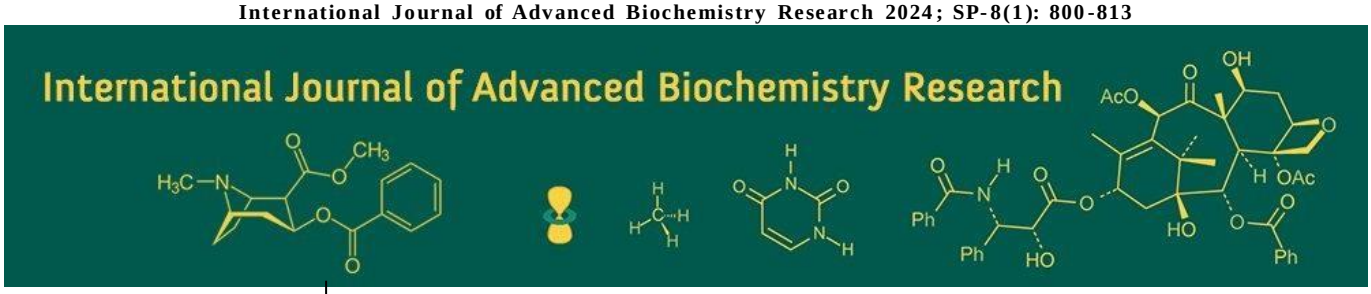

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Phylloplane microorganisms and induction of systemic resistance against pathogen *Alternaria solani* in tomato (*Solanum lycopersicum* L.)

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Abstract
Early blight disease caused by *Alternaria solani* is a widely distributed and notorious disease responsible for severe loses in crop yield in most parts of the world. In sustainable agriculture, use of biological agents is an imperative module approach against drastic plant hackers as they facilitate the fight off against phytopathogens by the production of phenolic compounds and defensive enzymes peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL). The phylloplane isolates viz, *Trichoderma* spp. (PF2 and PRF1), *Bacillus subtilis* (MNB2), *Bacillus mojavensis* (TB1) and *Ochrobactrum* sp. (EB1) were evaluated for their antagonistic effect against *Alternaria solani* in pot culture experiment. Increased expression of PO, PPO and PAL was observed in all the treated plants which lead to induced systemic resistance (ISR). Among the antagonists treatments, *Trichoderma* sp. (PF2) followed by *Bacillus subtilis* (MNB2) gave the highest activity of oxidative enzymes with good attributes of growth and yield parameters compared to the control. Foliar application of antagonists resulted in disease reduction and improved plant growth in tomato plant. The tested phylloplane isolates may consider as an ideal bioagents that play a pivotal role in management of early blight of tomato plants through induction of induced systemic resistance.

Keywords: *Alternaria solani*, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, *in vivo* study

Introduction
Tomato (*Solanum lycopersicum* L.) is one of the most significant widespread and common vegetables in most parts of world. It is placed second position after potato in economic importance among many countries (Prajapati *et al*., 2014) [35]. Generally, the fungicides use for the plant disease control is considered to be the most effective against pathogens, but the extensive use of these chemicals especially in agriculture lead to serious concerns resulting environmental pollution and health hazards. Moreover, continuous use of these fungicide chemicals can establish to resistant races and toxic residues (Chaerani *et al*., 2007 and Ramkissoon, *et al*., 2016) [9, 39].

Owing to aforementioned conditions, it is imperative to expand plant-derived plant-based pesticides or microbial pesticides, which is biological, biodegradable and environmentally friendly to control as effective against pathogens. Biotic and abiotic inducers are known to have great potential to manage and control plant diseases in agriculture (Anand *et al*., 2009 and Simonetti *et al*., 2012) [5, 44]. At present, use of biological control for antimicrobials, resistance promoters as well as growth promoters provides an outstanding alternative both practically and economically in controlling plant pathogens (Chandrashekara *et al*., 2012 and EL-Tanany *et al*., 2018) [10, 16].

Plant resistance induction by the application of various microorganisms or other organic materials offers an effective strategy in plant disease management (Rais *et al*., 2017) [37]. Biotic inducers are well-known recognized to elicit activities which emerge several defense reactions in plant host after being response by microbial infection, including defense related enzymes and production of phenolic compounds as well as specific flavonoids (Abd El-Rahman *et al*., 2012; Hussein *et al*., 2018 and Sarhan *et al*., 2018) [1, 23, 41].

However, many researchers have identified to stimulate the induced systemic resistance (ISR) in host plants with some chemical inducers such as chitosan and salicylic acid, as these enhance the same physiological and biochemical changes in plants as do systemic active biological resistance (Ramkissoon *et al*., 2016 and Moustafa *et al*., 2018) [39, 32]. Also, the enzymes peroxidase (PO), polyphenoloxidase (PPO) and phenylalanine ammonia-lyase (PAL) were described as elicitors of the ISR in disease control of host plants (Yasmin, *et al*., 2016) [48]. These enzymes are well-known as active elicitors of phenylpropanoid pathway, which creates biosynthesis of a wide array of plant metabolites such as phenolic compounds, flavonoids, lignin and tannins. These products have a capacity to provide resistance against pathogenic attack in host plants (Hahlbrock and Scheel, 1989) [21]. In addition, the enzymes synergistically achieve in partial degradation of fungal cell walls. Furthermore, uniform increase of these enzyme activities is considerably optimal in plant defense (Saikia, *et al*., 2005) [40]. Many research workers has experienced that high accumulation of phenolics obtained due to increase activities of these oxidative enzymes which are responsible mainly for the protection of pathogenic microbes against many plant diseases (Abd-El-Rahman *et al*., 2012 and Hussein *et al*., 2018) [1, 23].

The current study was carried out to investigate *in vivo* bio control efficacy against early blight disease of tomato. This research was mainly focus to evaluate the effect of selected biotic agents for the management of early blight of tomatoes under rain shelter conditions, as well as to estimate the biochemical response among the treatments, for the induction of tomato plant resistance. The activity of PO, PPO and PAL defense enzymes were explored with selected isolates.

Materials and Methods

Isolation of *Alternaria solani* in pure culture

The early blight pathogen causing *Alternaria solani* was isolated from symptomatic tomato leaves following the protocol of (Shoaib *et al*., 2019) [43]. For the *in vivo* study, the conidial suspension of 10^6 spores ml^{-1} was prepared in sterile water by scraping the fungal mycelium from the Petri dishes followed by inoculation.

Isolation and selection of potential phylloplane antagonistic microbes of tomato

Leaf washings by serial dilution on suitable different media were followed suggested by Elad and Krishner, (1993) to estimate leaf surface microorganisms. One ml aliquots of microbial suspension were surface plated in triplicate and incubated at 25±1 ºC. By dual culture technique, *Trichoderma* sp. (PF2), *Trichoderma* sp. (PRF1), *Bacillus subtilis* (MNB2), *Bacillus mojavensis* (TB1) and *Ocrobactrum* sp. (EB1) were selected based on the best per cent inhibition of pathogen and used for their ability to suppress *Alternaria solani*.

Experimental design

The experiments were conducted in rain shelter conditions (200 m^2) constructed with gable type roof and UV stabilized sheets facing North-South direction in the Department of Plant Pathology, College of Agriclture, Vellanikkara, Kerala Agricultural University, Thrissur, with tomato variety Anagha in Completely Randomized Design (CRD) to test the effectiveness of selected potential antagonists against early blight disease in three replications each along with Control. The contact fungicide Propineb (0.2%) was used and the standard recommended practices were followed. In addition, two reference biocontrol agents, *Pseudomonas fluorescens* (KAU) and *Trichoderma* sp. (KAU) were also used procured from Department of Plant Pathology, KAU, Thrissur. The seeds were sown in Pro tray containing soil less medium of coir pith, perlite and vermiculate. The seedlings were then transferred in pot filled with soil and manure maintaining at a spacing 45 x 30 cm.

Assessment of induced systemic resistance (ISR) in tomato by phylloplane antagonists

The selected phylloplane isolates were evaluated under *in vivo* for management of early blight disease on tomato plants. For bacterial isolates, a loopful of bacterium was inoculated into nutrient broth followed by incubation on a rotary shaker at 150 rpm for 48 hours at room temperature (28 ± 2 ºC). After which, the broth containing bacterial cells was then centrifuged at 10,000 rpm. To this, the supernatant was discarded and harvested bacterial cells were re-suspended in 100 ml sterile water used as foliar spray against the pathogen *Alternaria solani*. However, selected potential *Trichoderma* spp. (PF2 and PRF1) grown on potato dextrose (PD) broth for 3 to 5 days. The mycelia growth were then scraped by sterilized scraper and crushed in 100 ml of sterile water, and resulting debris was filtered through 3 layers of Whatman filter paper to remove fragmented mycelium from spores. They are then used as foliar spray in tomato plant after the challenge inoculation of pathogen. The treatment that sprayed with only water served as Absolute control. In contrast, diseased control plants were sprayed with pathogen *Alternaria solani* alone. The following antagonists were applied as foliar spray from the first day after the pathogen inoculation. Whereas, the treatments *viz* T_1-T_{10} were further evaluated separately under pot culture conditions so as to evaluate an integrated module for disease management.

Assessment of disease incidence and disease severity of early blight of tomato

The severity of disease on treated plants was assessed to measure the progression of early blight disease in terms of disease intensity. The per cent disease severity (PDS) for early blight on foliage was assessed by using disease score chart from 0-5 rating scale proposed by (Pandey *et al*., 2003) [27].

$$PDS = \frac{\text{Sum of all ratings}}{\text{Total no. of leaf samples assessed x Maximum disease grade}} \times 100$$

Early blight disease incidence was recorded one week after the challenge inoculation of the pathogen. Spore suspension spray was used for the artificial inoculation and calculated proposed by (Wheeler 1969) [49].

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

The plants were harvested two months after transplanting to record the total fresh and dry biomass of shoot and root. The dry biomass was determined in oven at 60°C for 48 hours.

Assessment of defensive enzymes

Different biochemical changes in plants were assessed after pathogen inoculation as followed:

Tomato leaf extract was prepared by grinding one gram leaf tissues in chilled 0.1 M sodium phosphate buffer and centrifuged. The leaf extract as supernatant (crude extract) was collected in microcentrifuge tubes and used to assess the activities of defensive antioxidants *viz*., peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL). The activity of defense-related enzymes for PO, PPO and PAL were estimated in the leaves at one day interval upto ninth day after pathogen inoculation. The tomato plants were given treatment with a common fungicide Propineb (0.2%) and biocontrol agents *Trichoderma* sp. (KAU) and *Pseudomonas fluorescens* (KAU). In addition, five promising selected phylloplane antagonists were also used. About five leaflets of the same height were randomly collected from the plants per replicates at each specific sampling time.

Estimation of relative activity of peroxidase (PO)

Leaves of each replicate were homogenized and protein content of one gram sample was extracted in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4ºC. The collected supernatant was used for PO enzyme activity. The reaction mixture was 1.5 ml of pyrogallol (0.05 M), 0.5 ml of enzyme, and 0.5 ml of H_2O_2 (1%). They are incubated at 28 ± 2ºC. The absorbance of the mixture was recorded every 20 second for 3 minutes at 420 nm. The PO activity was expressed as change in the absorbance of the reaction mixture $min^{-1}g^{-1}$ fresh matter (FM) (Hammerschmidt *et al*., 1982) [22].

Estimation of relative activity of polyphenol oxidase (PPO): Leaf samples of one gram was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) using a pre-chilled mortar and pestle. The homogenate PPO activity was measured by mixing 1.5 ml of the phosphate buffer, 200 µl of the crude enzyme, and 200 µl of catechol (0.01 M). The reaction mixture was incubated at room temperature for 2 minutes and absorbance was recorded at 495 nm. The changes in absorbance were recorded every 30 second interval for 2 minutes. The enzyme activity was expressed as change in absorbance min^{-1} g^{-1} FM (Mayer *et al*., 1965) [29].

Estimation of relative activity of phenylalanine ammonia -lyase (PAL): One gram leaf samples of each replicate was homogenized and extracted with 3 ml ice-cold sodium borate (0.1 M) buffer at pH 7.0, which contains 2-Mercapto ethanol (1.4 mM), and insoluble polyvinylpyrrolidone (PVP) (0.1 g). The extract was centrifuged at 10,000 rpm for 15 minutes at 4ºC. The supernatant was used as enzyme source. About 0.4 ml of enzyme was incubated with 0.5 ml of borate buffer (0.1 M) at pH 8.8 and 0.5 ml of L-phenylalanine (12 mM) for 30 minutes at 30ºC. Enzyme activity was expressed as 1 µmol trans-cinnamic acid $min^{-1}g^{-1}$ FM (Dickerson *et al*., 1984) [13].

Statistical Analysis

Analysis of variance was performed on the data collected in various experiments using the statistical package GRAPES (General R-shiny based Analysis Platform Empowered by Statistics). Transformations of the resultant data were done if required.

Results

Effect of treatments on per cent disease incidence and per cent disease severity under rain shelter

The per cent disease incidence was determined by the formula as mentioned in methodology. As artificial inoculation was given at fifteen days interval, early blight incidence was noticed in most of the plants in all the treatments. Hence, significant difference in per cent disease incidence was found between different treatments under rain shelter conditions. While comparing early blight incidence at three different intervals between the treatments, highest per cent incidence was noticed in treatment inoculated with pathogen alone with respect to control (46.60, 68.20 and 90.60) respectively. Similarly, days taken for disease incidence took 7 to 9 days after inoculation (DAI) as compared to remaining treatments condition. However, the selected treatments showed lesser rate of disease progress compared to control and treatment inoculated with pathogen alone. Amongst the treatments, T_1 *Trichoderma* sp. (PF2) and T_3 *Bacillus subtilis* (MNB2) showed lower disease incidence occurrence recording the highest per cent disease reduction of 64.25 and 61.01 respectively. The effectiveness of selected bioagents and a fungicide propineb (0.2%) were tested against the early blight disease of tomato and observed to be superior as compared to control. Foliar application of treatments was given at the onset of early blight disease.

Early blight disease severity was recorded at the time of treatment applications and ten days after first, second and third spraying of treatments applications. Four plants per replication per treatments were selected randomly and observation on severity of the disease on the foliage was recorded and per cent disease severity was assessed. Results of the pot experiment for management of early blight disease of tomato caused by *Alternaria solani* in rain shelter are presented in Figure 1 and 2. It was observed from the graph that, all treatments were superior to control and significant difference was noticed among the treatments at all intervals of observations. Moreover, it was noticed that, rate of disease progress was very less in all treatments compared to control at different intervals of observation which indicates the positive effect of treatments on the spread of infection. Since artificial inoculation of pathogen was given uniformly, there was no significant difference in disease severity among treatments before foliar application. Among the treatments, spraying of T_1*Trichoderma* sp. (PF2) showed the lowest disease severity at all spray intervals recording 19.67, 20 and 22.60 per cent with 68.58, 69.51 and 68.30 per cent reduction over control respectively. This was statistically on par with spraying of T_3 *Bacillus subtilis* (MNB2) with 21.96, 24.30 and 31.64 per cent disease severity after first, second and third spray recording 64.92 per cent disease reduction over control after first spray. The plants treated with propineb (0.2%) fungicide showed low disease severity (25.60%) compared to T_9 Control and T_{10} Pathogen alone treated plants which recorded 59.11 per cent diseased reduction over control after first spray. Hence, it was evident that, fungicidal treatment T_6 Propineb (0.2%) was noticed efficient in the management of early

blight disease in rain shelter condition. All bioagents recorded more than 45-50 per cent reduction of early blight disease over control. Among them, foliar spray with T_1 *Trichoderma* sp. (PF2) gave best result in the reduction of early blight severity recording 22.60 per cent with 68.30 per cent disease reduction over control after third spray. This was followed by T_3 *Bacillus subtilis* (MNB2) where 31.64 per cent severity and 55.62 per cent disease reduction over control was recorded and it was statistically on par with T_8 *Pseudomonas fluorescens* (KAU) which accounted 32.45 per cent disease severity with 54.49 per cent disease reduction of early blight disease over control.

Effect of treatments on biometric characters of tomato plant

Biometric characters such as number of leaves and plant height, fresh and dry biomass of shoot-root, average number of fruits per plant, average fruit weight and yield per pot were recorded during pot culture experiment under rain shelter and presented in Figure 3, 4, 5, 6, 7 and 8.

Effect of treatments on number of leaves and plant height

It was observed from the graph presented in Figure 3 and 4 that, significant difference was noticed among different treatments with respect to number of leaves and plant height. In rain shelter, the maximum number of leaves at two months after transplanting (MAT) was observed in T_1 *Trichoderma* sp. PF2 (22.63) which was on par with T_3 *Bacillus subtilis* (MNB2) (22). It was followed by T_8 *Pseudomonas fluorescens* (KAU) and T_5 *Bacillus mojavensis* (TB1) with 21 and 19.33 respectively, while T_5 *Bacillus mojavensis* (TB1) is on par with T_4 *Ochrobactrum* sp. (EB1) (19). Plants in control recorded (6 and 17.44) at first and second month after transplanting which was higher than the pathogen inoculated plants and which was statistically on par with T_2 *Trichoderma* sp. (PRF1) (18.66) and T_6 Propineb (0.2%) (18.33). Plant inoculated pathogen alone showed the lowest number of leaves (15.27) in (Figure 3).

The results from graph presented in (Figure 4) revealed that, significant difference was noticed among different treatments with respect to plant height. Plants treated with T_1 *Trichoderma* sp. (PF2) showed maximum plant height (69.18 and 211.82 cm) during first and second month after transplanting. This was followed by T_3 *Bacillus subtilis* (MNB2) (68.58 and 207.33 cm). Here, T_9 Control (65.30 and 195.27 cm) plants showed comparatively better response on plant height than fungicide propineb (0.2%). However, this was statistically on par with T_5 *Bacillus mojavensis* (TB1) (191.22 cm) at second month after transplanting. The minimum plant height of (56.73 and 149.10 cm) was recorded in T_{10} pathogen alone. Summing up the results, the effect of different treatments of bioagents on number of leaves and plant height showed almost same trend of the results in rain shelter conditions. Moreover, in bioagents treatments, plant growth rate was more compared to control. In these cases, plants treated with bioagents showed the higher number of leaves and plant height than fungicide treated plants and control. The plants applied with T_1 *Trichoderma* sp. (PF2) and T_3 *Bacillus subtilis* (MNB2) recorded maximum number of leaves and plant height, and minimum in T_{10} Pathogen alone inoculation plants. Similarly, among different bioagents, *Trichoderma* sp. (PF2) and *Bacillus subtilis* (MNB2) showed a better response with respect to number of leaves and plant height.

Effect of treatments on biomass of shoot and root

The graph in (Figure 5 and 6) revealed that, significant difference was noticed among different treatments with respect to fresh and dry weight of shoot and root. In fresh weight of tomato shoot and root, the maximum fresh weight of shoot and root was observed in T_1 *Trichoderma* sp. (PF2) (11.50 g and 11 g) respectively and it was followed by T_3 *Bacillus subtilis* (MNB2) (10.80 g and 10.37 g) respectively. It was then followed by T_8 *Pseudomonas fluorescens* (KAU)(10.67 g and 10.37 g). However, in fresh weight of shoot, T_5 *Bacillus mojavensis* (TB1) recorded 9.90 g, While in fresh weight of root, it was followed by T_4 *Ochrobactrum* sp. (EB1) weighing 9.97 g. Fresh weight of shoot and root in control recorded 8.36 g and 8.60 g which was higher than the pathogen inoculated plants and which was statistically onpar with T_6 Propineb (0.2%) (8.60 g), T_7 *Trichoderma* sp. (KAU) (8.67 g) and T_5 *Bacillus mojavensis* (TB1) (8.53 g) respectively. Plant inoculated pathogen alone showed the lowest number of biomass on both shoots (8.10 g) and root (8 g).

In dry weight biomass of shoot and root, plants treated with T_1 *Trichoderma* sp. (PF2) showed maximum weight (0.59 g and 2.51 g) respectively. This was followed by T_3 *Bacillus subtilis* (MNB2) (0.52 g and 2.36 g and this was statistically on par with T_8 *Pseudomonas fluorescens* (KAU) (0.52 and 2.10 g). However, T_6 Propineb (0.2%) on dry weight of shoot and root recorded 0.42 g and 1.36 g respectively. Here also control plants showed comparatively better response on dry biomass of shoot and root weight than pathogen alone treatment. However, the minimum was recorded in pathogen alone. Summing up the results, the effect of different treatments on each fresh and dry weight of shoot and root showed certainly almost similar with respect to biomass parameters. In both cases, plants treated with bioagents showed more in biomass of shoot and root than control. The results clearly indicates that the plants applied with T_1 *Trichoderma* sp. (PF2) and T_3 *Bacillus subtilis* (MNB2) recorded maximum weight of shoot and root and minimum in control plants. Similarly, a fungicide propineb (0.2%) showed a better response with respect to shoot and root biomass than control and pathogen inoculated treatment.

Effect of treatments on yield parameters

Significant difference was noticed among the treatments with respect to all the observed yield parameters such as average number of fruits per plant, average fruit weight and yield per pot under rain shelter conditions (Figure 7 and 8). Moreover, all treatments in pot culture were significantly superior to control in all the observed yield attributes. In case of bioagents treatments, average number of fruits per plant was more in plants sprayed with T_1 *Trichoderma* sp. (PF2) with 9 fruits per plant and this was statistically on par with T_3 *Bacillus subtilis* (MNB2) followed by T_8 *Pseudomonas fluorescens* (KAU) that obtained 8.68 and 8 fruits per plant. It was followed by T_6 Propineb (0.2%) produced 3.83 fruits per plant and this was on par with T_2 *Trichoderma* sp. (PRF1), T_5 *Bacillus mojavensis* (TB1), T_7 *Trichoderma* sp. (KAU) and T_9 Control. Least average number of fruits per plant was recorded in T_{10} Pathogen alone (1.67 per plant). While in case of average weight of

fruits, T_1 *Trichoderma* sp. (PF2) recorded maximum (48.03 g) and it was on par with T_3 *Bacillus subtilis* (47.17), T_8 *Pseudomonas fluorescens* (KAU) (46.60), and T_2 *Trichoderma* sp. (PRF1) (45.97) and remaining treatments were almost on par with each other. Regarding average yield per pot, all treatments were significantly superior to T_9 Control and T_{10} Pathogen inoculated alone and maximum was observed in T_1 (410 g) followed by T_3 *Bacillus subtilis* (MNB2) (389.33 g) and it was on par with T_8 *Pseudomonas fluorescens* (KAU) (376.67 g) during first harvest of tomato. While in second harvest, T_1 (300 g) showed maximum average yield and it was on par with T_3 *Bacillus subtilis* (MNB2) and T_8 *Pseudomonas fluorescens* (KAU) with 291 g and 273.33 g respectively against 102.67 g in control under rain shelter conditions. Hence, the bioagents applications showed better yield performance than pathogen alone and control treatments.

Induction of systemic resistance in tomato against *Alternaria solani* by phylloplane antagonists

The activity of defense-related enzymes for PO, PPO and PAL were estimated in the leaves using spectrophotometer at one day interval upto ninth day after pathogen inoculation. The results of the enzyme assays are described below.

Induction of peroxidase (PO) by phylloplane antagonists

Application of phylloplane antagonists resulted in an increase in the activity of peroxidase (PO). The activity of PO as expressed by the change in absorbance ranged from control to before inoculation. Spectrophotometer reading of peroxidase activity against *Alternaria solani* was carried out and results are presented in (Figure 9). After foliar inoculation of pathogen *Alternaria solani* resulted in PO activity over days and the treatment T_1 *Trichoderma* sp. (PF2) and T_2 *Trichoderma* sp. (PRF1) exhibit higher activity of PO with 0.37 $min^{-1}g^{-1}$ and 0.28 $min^{-1}g^{-1}$ respectively. The inoculation of T_8 *Pseudomonas fluorescens* (KAU) recorded highest PO activity of 0.572 $min^{-1}g^{-1}$ at three days after inoculation, followed by T_5 *Bacillus mojavensis* (TB1) and T_1 *Trichoderma* sp. (PF2) which showed 0.50 $min^{-1}g^{-1}$ and 0.46 $min^{-1}g^{-1}$ respectively. The lowest activity at 1st and 3rd day after inoculation (DAI) was observed in T_9 Absolute control, which recorded 0.14 $min^{-1}g^{-1}$ and 0.25 $min^{-1}g^{-1}$ respectively. At 5th DAI, highest activity was recorded by T_1 *Trichoderma* sp. (PF2) (1 $min^{-1}g^{-1}$) followed by the treatment T_8 *P. fluorescens* (KAU) (0.96 $min^{-1}g^{-1}$) and T_4 *Bacillus subtilis* (MNB2) (0.94 $min^{-1}g^{-1}$). The expression of PO activity elevated and showed highest peaked at five days after inoculation of pathogen, where highest activity was noticed in plants treated with T_1 *Trichoderma* sp. (PF2) and was followed by T_8 *P. fluorescens* (KAU) and T_4 *B. subtilis* (MNB2). The activity increased from 1st to 5th DAI in all the treatments. All the isolates showed subsequent increase in PO activity upto 5th DAI. However, the activity of PO gradually decreases after 5th DAI as the days of pathogen inoculation increases from 7th to 9th DAI. However, the higher activity of PO were noticed in T_1 *Trichoderma* sp. (PF2) (0.86 $min^{-1}g^{-1}$), T_8 *P. fluorescens* (KAU) (0.84 $min^{-1}g^{-1}$) followed by T_4 *Bacillus subtilis* (0.74 $min^{-1}g^{-1}$) at 7th DAI. While the high PO activity at 9th DAI were almost all similar in T_1 *Trichoderma* sp. (PF2) (0.69 $min^{-1}g^{-1}$), T_8 *P. fluorescens* (KAU) (0.68 $min^{-1}g^{-1}$) followed by T_4 *Bacillus subtilis* (MNB2) (0.65 $min^{-1}g^{-1}$). In this study, it was observed that the higher activity of PO enzyme was recorded in plants treated with *Trichoderma* sp. (PF2) followed by *P. fluorescens* (KAU) and *B. subtilis* (MNB2). The highest activity of PO was exhibited by T_1 *Trichoderma* sp. (PF2) with per cent increase over control of 169.54 at 5th day after inoculation.

Induction of polyphenol oxidase (PPO) by phylloplane antagonists

The foliar application of phylloplane isolates after challenge inoculation resulted in an increase in the activity of polyphenol oxidase (PPO) (Figure 10). Before challenge inoculation, the expression of PPO activity is almost certainly uniform and non-significant between treatments. After pathogen inoculation and bioagents were sprayed, the results exhibited at different range of PPO activity at different applied treatments. At 1st day after inoculation (DAI), the treatment sprayed with T_1 *Trichoderma* sp. (PF2) (0.37 $min^{-1}g^{-1}$) followed by T_2 *Trichoderma* sp. (PRF1) (0.28 $min^{-1}g^{-1}$) and T_4 *Bacillus subtilis* (MNB2) (0.30 $min^{-1}g^{-1}$) showed high polyphenol oxidase activity. However, gradual increase in PO activity from 3rd day after inoculation was recorded by T_8 *P. fluorescens* (KAU) (0.57 $min^{-1}g^{-1}$), followed by T_4 *Bacillus subtilis* (MNB2) (0.50 $min^{-1}g^{-1}$) and T_1 *Trichoderma* sp. (PF2) (0.46 $min^{-1}g^{-1}$). At 5th day after inoculation, *Trichoderma* sp. (PF2) T_1 showed rapid increase in PO activity (1.11 $min^{-1}g^{-1}$) followed by T_8 *P. fluorescens* (KAU) (1.07 $min^{-1}g^{-1}$) and T_4 (1.01 $min^{-1}g^{-1}$) and the same trend with the activity value of (0.85 $min^{-1}g^{-1}$, 0.81 $min^{-1}g^{-1}$ and 0.73 $min^{-1}g^{-1}$) respectively was followed at 7th DAI which gradually decreases and the lowest activity was recorded by T_9 Absolute control (0.33 $min^{-1}g^{-1}$) (Figure 10). However, there was subsequent increase in PPO activity by all the isolates from 3rd to 5th DAI. The phylloplane antagonistic isolate *Trichoderma* sp. (PF2) recorded highest peak activity at 5th DAI. Higher activity of polyphenol PPO enzyme was recorded in plants treated with isolates, T_1 *Trichoderma* sp. (PF2) (0.69 $min^{-1}g^{-1}$) followed by T_8 *P. fluorescens* (KAU) (0.67 $min^{-1}g^{-1}$) and T_4 *Bacillus subtilis* (MNB2) (0.64 $min^{-1}g^{-1}$). At 5th day after inoculation, the highest activity of PPO was exhibited by T_1 *Trichoderma* sp. (PF2) with per cent increase over control of 199.19.

Induction of phenylalanine ammonia-lyase (PAL) by phylloplane antagonists

After challenge inoculation of pathogen *Alternaria solani*, applied phylloplane isolates enhance the activity of phenylalanine ammonia-lyase (PAL) in all the given treatments (Fig 11). No significant observation was obtained before inoculation. The bioagents treated treatments synthesized more PAL enzyme activity compared to T_9 Absolute control. At 3rd day after inoculation (DAI), the increase in PAL enzyme activity was shown by T_4 *Bacillus subtilis* (MNB2) (0.51 $min^{-1}g^{-1}$) followed by T_1 *Trichoderma* sp. (PF2) (0.49 $min^{-1}g^{-1}$) and T_8 *Pseudomonas fluorescens* (KAU) (0.45 $min^{-1}g^{-1}$) to the lowest activity by absolute control (0.27 $min^{-1}g^{-1}$). The rapid increase in enzyme activity was recorded by T_8 *P. fluorescens* (KAU) (1.10 $min^{-1}g^{-1}$), followed by T_1 *Trichoderma* sp. (PF2) (1.06 $min^{-1}g^{-1}$) and T_4 *Bacillus subtilis* (1.00 $min^{-1}g^{-1}$), while decreasing trend was observed in the pathogen inoculated alone and absolute control from 7th to 9th DAI. However, the enzyme activity gradually decreases in which the higher PAL activity was exhibited byT_1 *Trichoderma* sp. (PF2) isolate at both 7th and 9th day after inoculation with a value of (0.90 $min^{-1}g^{-1}$) and (0.70 $min^{-1}g^{-1}$) respectively (Figure 11). While the next

higher activity was exhibited by the T_8 *P. fluorescens* (KAU) (0.87 $min^{-1}g^{-1}$) and T_5 *Bacillus mojavensis* (TB1) (10.64 $min^{-1}g^{-1}$). The highest activity of PAL was exhibited by T_8 *P. fluorescens* (KAU), T_1 *Trichoderma* sp. (PF2) and T_4 *Bacillus subtilis* (MNB2) with percent increase over control of 209.86, 198.59 and 180.85 respectively at 5th day after inoculation.

Discussion

All the treatments considerably reduced the disease intensity as compared to control and inoculated pathogen alone. However, the degree of disease reduction intensity varied from treatment to treatment. The range of disease severity at 15 intervals from first to third spray in treatments varied from 19.67 to 22.60%. In first spray, minimum disease severity of 19.67% was observed in plants treated with *Trichoderma* sp. (PF2), followed by *Bacillus subtilis* (MNB2) (21.96%). The Propineb (0.2%) showed disease severity after first spray of 25.60%. However, besides pathogen alone (65.72%), control was least effective in disease incidence (62.6%). Thus, all the treatments observed superior and noticed significant disease incidence over control (90.60%) at 75 days after transplanting (DAT) (Fig 1).

Amongst the treatments used, *Trichoderma* sp. (PF2) was established as effective in reducing occurrence of disease blight incidence as minimum 16.66%, 31.50% and 80.83% with per cent reduction over control of 64.25, 53.81 and 10.78 at first, second and third spray respectively of 15 days interval under protected rain shelter conditions. Bio-inoculant *Trichoderma* sp. (PF2) was also observed greatly significant in disease severity reduction with the per cent reduction control of 68.58 at first spray (Fig 1 and 2). All other treatments revealed an effective outcome in early blight disease suppression as compared to control. Progression of disease incidence was reduced in all the treatments except in control and pathogen inoculated alone. Several strains of plant growth promoting microbes have been well-known reported to induce systemic resistance in host plants against various pathogens that ultimately leads to lessening in disease incidence in many crops (Ramamoorthy *et al*., 2002) [38]. The use of biocontrol agent as an alternative to fungicides could be recommended for use as application particularly to manage hazardous fungicide residues. Most species of *Trichoderma* exhibit significant suppression of mycelia growth against many pathogenic fungal diseases by showing the capability to compete for nutrients, space and ability for speedy growth promotion (Amira *et al*., 2017) [4]. These findings sturdily support our results. Further, it was reported that significant reduction of disease severity was observed while *T. viride* was applied against *Alternaria solani*, (Udhav, 2013) [46].

Amongst the antagonists' treatments, *Trichoderma* sp. (PF2) followed by *Bacillus subtilis* (MNB2) exhibit highest plant height. Biomass of fresh and dry weight shoot-root also obtained maximum in *Trichoderma* sp. (PF2) followed by *Bacillus subtilis* (MNB2). However, the minimum biometric observations of shoot and root biomass was recorded in plants inoculated with pathogen alone followed by control. Fresh and dry biomass of shoot-root was significantly higher in all treatments compared to pathogen inoculated alone. Dry weight of root was significantly observed from *Trichoderma*sp. (PF2) (2.51 g) followed by *Bacillus subtilis* MNB2 (2.36g).

Shoot dry biomass was significantly affected in all the bioagents treatments ranging from (0.42 - 0.59 g) over control (0.27 g). Several soil borne pathogens such as *Trichoderma harzianum* are mostly controlled by activating induced systemic resistance resulting rapid colonization and mycelium growth inhibition of the pathogens (Kamala and Devi, 2012) [25]. The phylloplane bacterium *Ochrobactrum anthropi* BMO-111 was reported to be significantly effective against disease causing severe blister blight of tea of *Exobasidium vexans* (Sowndhararajan *et al*. 2013) [45]. According to Ahluwalia *et al*., (2015) [2], *Trichoderma harzianum* produced secondary metabolites having antimicrobial effects such as harzianopyridone, pyrone, palmitic acid, furanone, stigmasterol and anthraquinone in most of the soil borne pathogens. *Trichoderma viride* has been widely used against many fungal plant pathogens and also act as an effective bioagent due to its potential antagonistic activity (Mohamed and Gomaa, 2019) [30]. In addition *Trichoderma longibrachiatum* is considered to be as a promising biocontrol agent which has been broadly recognized because of its secretion of huge amount of peptaibols, small peptides residues, with wide rangingspectra of biological activities (Elegbede and Lateef, 2019) [15].

In the present study, the indigenous isolates were observed extremely effective with respect to plant growth promotion against the pathogen *Alternaria solani.* Results showed that the biomass of shoot and root, number of leaves and plant height in pathogen inoculated alone were significantly reduced presented in (Fig 3, 4, 5 and 6). Remarkably, the infected plants treated with bioagents revealed the most potent effect in plant height, compared to the plants treated with pathogen alone and the control. In addition, results showed foliar application of bioagents showed as effective results in number of leaves per plant. With respect to the control and pathogen inoculated alone, it is obvious that minimum plant height were obtained with (65.30 cm, 195.27 cm) and (56.73, 149.10 cm) respectively at first and second month after transplanting (MAT) (Figure 4). The highest number of leaves was obtained from *Trichoderma* sp. (PF2) (11 and 22.63) followed by the plants treated with *Bacillus subtilis* (MNB2) (10 and 22) at first and second month after transplanting (MAT). In general, inoculations of *Alternaria solani* affected more on shoot growth than parameters of root growth. Reduction in growth attributes was increased in control and pathogen alone with disease progression, while other treatments did not have same effect. Plant height was statistically and significantly different in pathogen inoculated compared to corresponding control at periodic inoculations. In the pathogen alone, inoculation significantly reduced plant height by 56.73 cm and 149.10 cm over control. However, maximum plant height was observed from treatments *Trichoderma* sp. (PF2) followed by *Bacillus subtilis* (MNB2) and *Pseudomonas fluorescens* (KAU) by 211.82 cm, 207.33 cm and 206 cm respectively (Figure 4). However, the Propineb (0.2%) recorded 61.92 cm and 187.37 cm at first and second month after transplanting respectively. Chowdappa *et al*. (2013) [12] observed strains of *Trichoderma harzianum* OTPB3 and *Bacillus subtilis* OTPB1 exhibit positive antagonistic activity towards *Alternaria solani* and *Phytophthora infestans* resulting in induced systemic resistance against early and late blight of tomato seedlings.

It is clearly evident from the results that *Alternaria solani* significantly reduced height of plant. Interestingly, after applied treatment with different isolates and bioagents, the plant height increased significantly. Moreover, the results showed that, the infection in *Alternaria solani* caused decreasing in the number of leaves per plant. The tested treatment upon isolates, the number of leaves significantly increase compared with the infected plants and the maximum effect obtained in *Trichoderma* sp. (PF2) as presented in (Figure 3). It is worth to mention that, all the treatments showed significant effect on number of fruit compared to pathogen inoculated alone as presented in (Figure 7). It was also observed that, all the isolates was found responsible in significant increase of tomato weight compared to control and pathogen alone, while the effect of both *Trichoderma* sp. (PF2) and *Bacillus subtilis* (MNB2) was slightly higher than *Pseudomonas fluorescens* (KAU). Number and weight of fruits was assessed at sixty days after inoculation. Early blight disease revealed significantly affect investigated yield attributes. However, lowest fruit number is obtained from the treatment inoculated with pathogen alone (1.67). While treatments treated with *Trichoderma* sp. (PF2), *Bacillus subtilis* (MNB2) and *Pseudomonas fluorescens* (KAU) yielded maximum average number of fruit (9.00, 8.68 and 8.00) per plant and weight of fruits (48.03 g, 47.17 g and 46.60 g) of tomato respectively. It was clearly indicate that *Alternaria solani* significantly reduced the yield of plant inoculated with pathogen alone. In addition, all treatments have significantly increased the number of fruit except in pathogen alone and control. Moreover, the yield results presented in (Figure 8) revealed maximum in *Trichoderma* sp. (PF2), *Bacillus subtilis* (MNB2) and *Pseudomonas fluorescens* (KAU) by 410 g, 389.33 g and 376.67 g respectively per pot. While the Propineb (0.2%) yield 253.33 g which response higher as compared to control. Hence, it is important to mention that, treatment with the tested bioagents has significant effect on biometric attributes and yield parameters. It was also noticed that, all the tested bioagents resulted in a significant increase in the weight of tomato compared with the control and pathogen alone. Therefore in general, the selected antagonists and biocontrol agents' treatments play a significant role in plant growth promotion and the yield while providing a minimal disease occurrence in all the treatments except control and the treatment inoculated with pathogen alone.

Induction of defense enzymes against *Alternaria solani* by phylloplane antagonists in tomato

Plants constitute with various defenses related genes. It is acknowledged that the defense genes are often latent genes due to which, an appropriate signals or stimuli are required to activate them. In the current investigation, the plants treated with bioagents as foliar spray recorded higher activity of defense-related enzymes and pathogenesis related proteins (PRPs) *viz*., peroxidise (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL). Therefore, the increase in resistance against early blight disease of tomato plants treated with bioagents as foliar spray can be credited to the direct inhibitory effect as well as their capability to induced systemic resistance against pathogen *Alternaria solani.* The current study reveals that plants challenge with the pathogen and inoculated with bioagents showed enhances stimulation of enzymes PO. The increased activity of PO has been noted to be correlated with resistance and these enzymes represent in the polymerization of lignin and proteins or precursor of plant cell wall, hence build up a physical barrier that could halt the process of pathogen penetration of cell walls (Bradley *et al*., 1992) [8]. Peroxidases have been concerned in the function of phenol oxidation, plant cell elongation, polysaccharide cross-linking, cross-linking of extension monomers, IAA oxidation, and oxidation of hydroxyl-cinnamylalcohols into free radical intermediates and wound curing (Vidhyasekaran *et al*., 1997) [47].

The results clearly indicate that accumulation of polyphenol oxidase (PPO) was higher in treated bioagents tomato plants and challenged with early blight pathogen *Alternaria solani*. In PPO, the results revealed that the *Trichoderma* sp. (PF2) treatment was the most efficient followed by *Bacillus subtilis* (MNB2) among selected antagonists in establishing induced systemic resistance against the early blight. The observations in reducing disease incidence might be probably due to increased activity of PAL in plants with bio agents and challenge with the pathogen alone. The maximum regulation of PAL activity at 5th day constituted for increasing the resistance in tomato plants against early blight disease because PAL is the key enzyme to synthesize of salicylic acid (SA), which in most plants induces systemic resistance responsible for plant health and development. The PO, PPO and PAL enzyme activity was noticed significant increased in all bioagents treatments and act as effective potential antagonists in tomato leaves compared to the absolute control as shown in (Figure 9, 10 and 11). Amongst antagonists' treatments, *Trichoderma* sp. (PF2) yields the highest PO activity, followed by *B. subtilis* (MNB2). PO activity increased over time, peaked at 5th day after inoculation, and then began to reduce gradually. On the other hand, the absolute control has the lowest PO activity, followed by treatment inoculated with pathogen alone. The treatment with *Pseudomonas fluorescens* (KAU) result the maximum of PO activity at 3rd day, followed by *Bacillus subtilis* (MNB2). The absolute control on the other hand, was the least thriving followed by pathogen alone. Meanwhile, the graph illustrated in (Figure 10) showed that PPO activity rose with increasing days of pathogen inoculation, peaked highest at 5th day in all treatments, and then declined gradually. The fungicide Propineb (0.2%) considerably enhance enzyme activity, while the potential isolates as systemic resistance inducer was the most effective in all the treatments compared to absolute control. At 5th day after pathogen inoculation, *Trichoderma* sp. (PF2) gain the highest peaked followed by *Pseudomonas fluorescens* (KAU) and *Bacillus subtilis* (MNB2), all of which enhanced PPO enzyme activity significantly as compared to the absolute control.

Concerning of PAL graph in (Figure 11) showed that PAL enzyme activity extensively increased in all bioagents treatments on tomato. *Bacillus subtilis* (MNB2) showed the highest enzyme activity followed by *Trichoderma* sp. (PF2) and *Pseudomonas fluorescens* (PF2) at 3rd day after inoculation. However, the *Pseudomonas fluorescens* (KAU) exhibit the most efficient on increase of PAL activity followed by *Trichoderma* sp. (PF2) and *Bacillus subtilis* (MNB2) at 5th day after inoculation. The graph also showed that high level of PAL activity was obtained from 5th day in most treatments and gradually decreases as the days of pathogen inoculation increases. Inhibition growth of plant

pathogenic fungi and release of antifungal compounds by *Pseudomonas* spp. is also documented suggested by (Gupta *et al*., 2001; and Bhowmik *et al*., 2002) [20, 7] as *Pseudomonas* has the capacity to act as strong elicitors of plant defense reactions. Among the plant growth promoting microbes, *Pseudomonas fluorescens* are also known to play major role in disease suppression and plant growth promotion as an active constituent of organic farming according to the study proposed by Yegesh *et al*. (2008) [51].

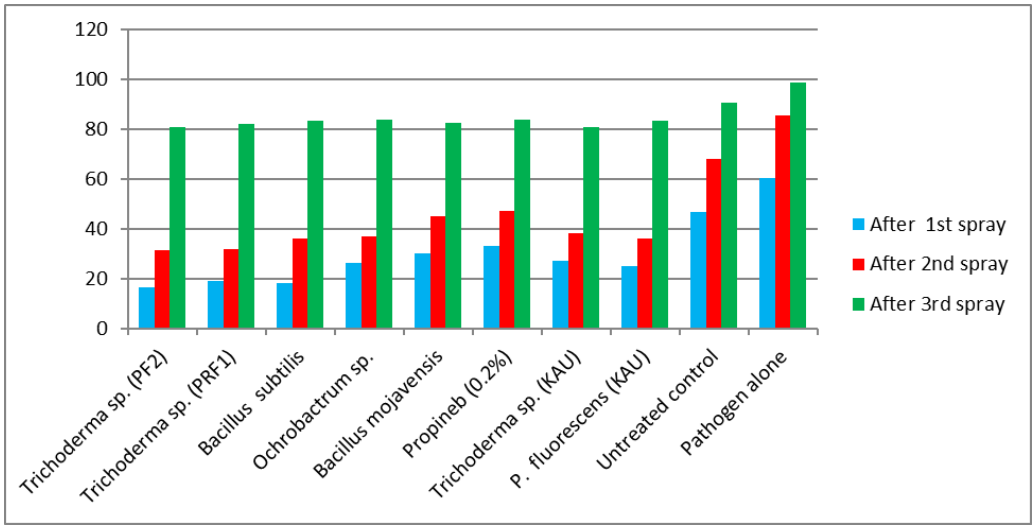

Fig 1: Effect of treatments on early blight disease incidence of tomato after inoculation with *Alternaria solani*

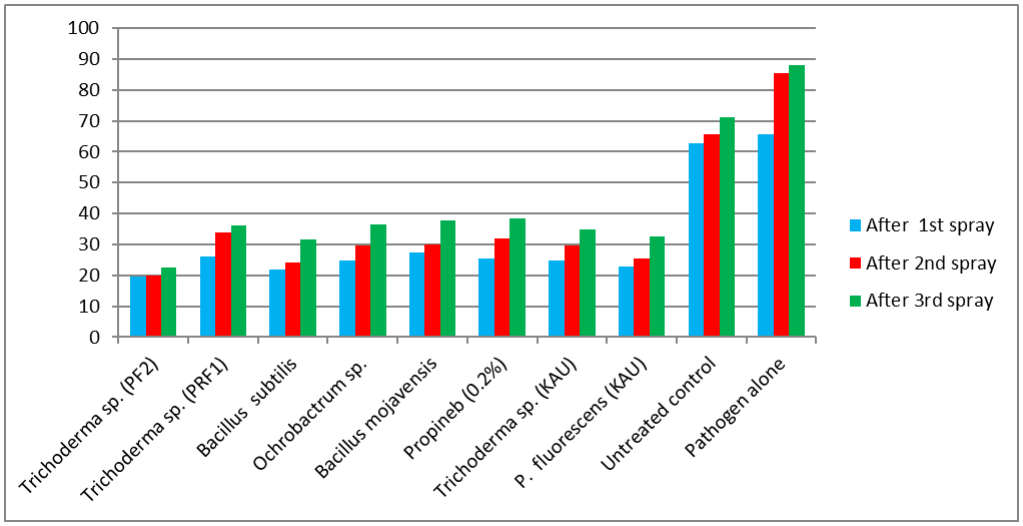

Fig 2: Effect of treatments on early blight disease severity of tomato after inoculation with *Alternaria solani*

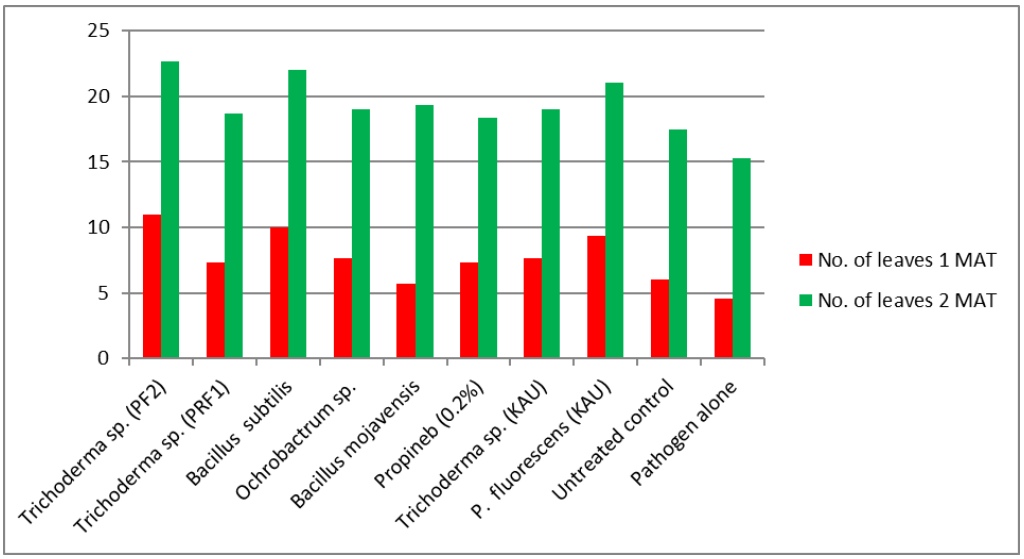

Fig 3: Effect of treatments on number of leaves at 1 and 2 month after transplanting (MAT) under rain shelter

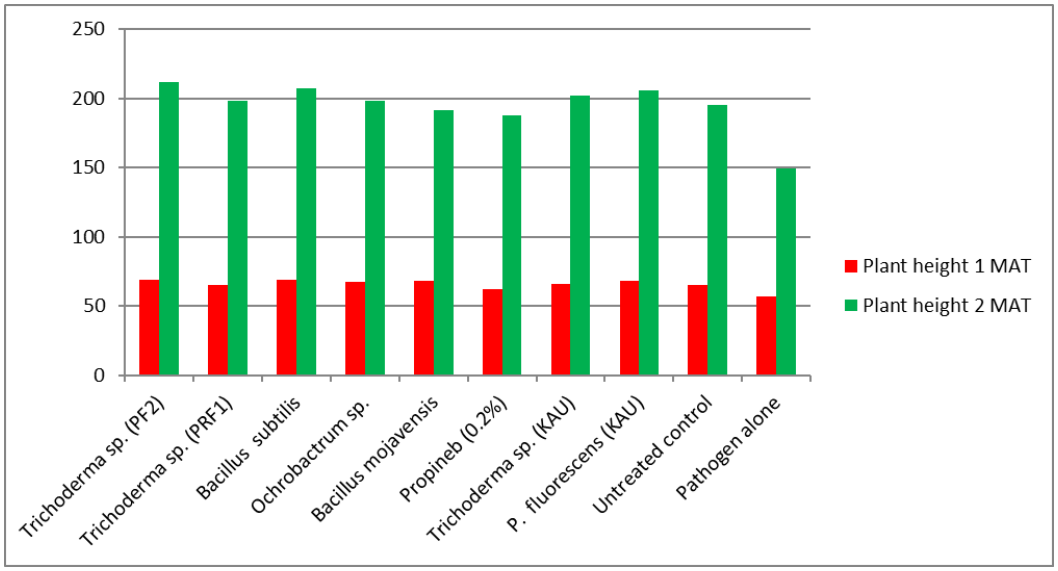

Fig 4: Effect of treatments on plant height at 1 and 2 month after transplanting (MAT) under rain shelter

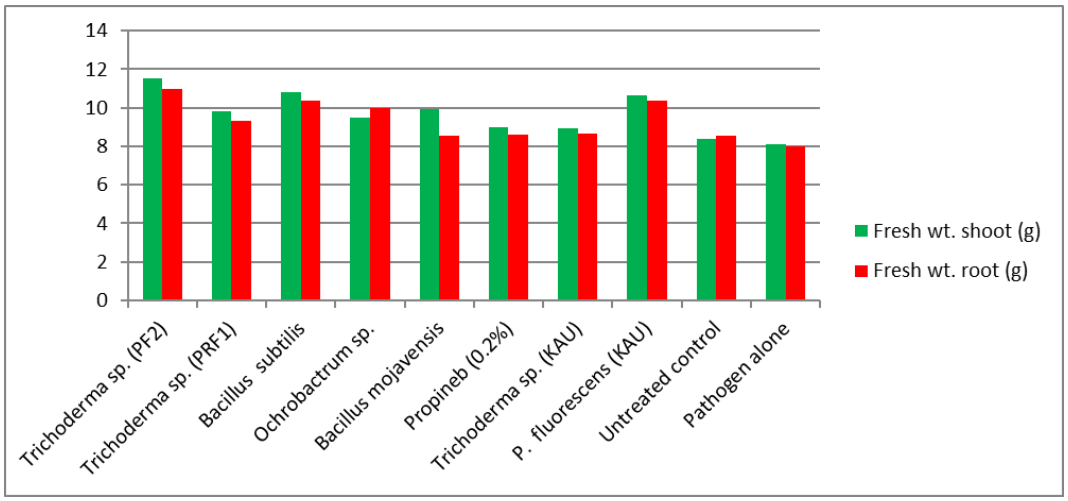

Fig 5: Effect of treatments on fresh weight of shoot and root biomass of tomato

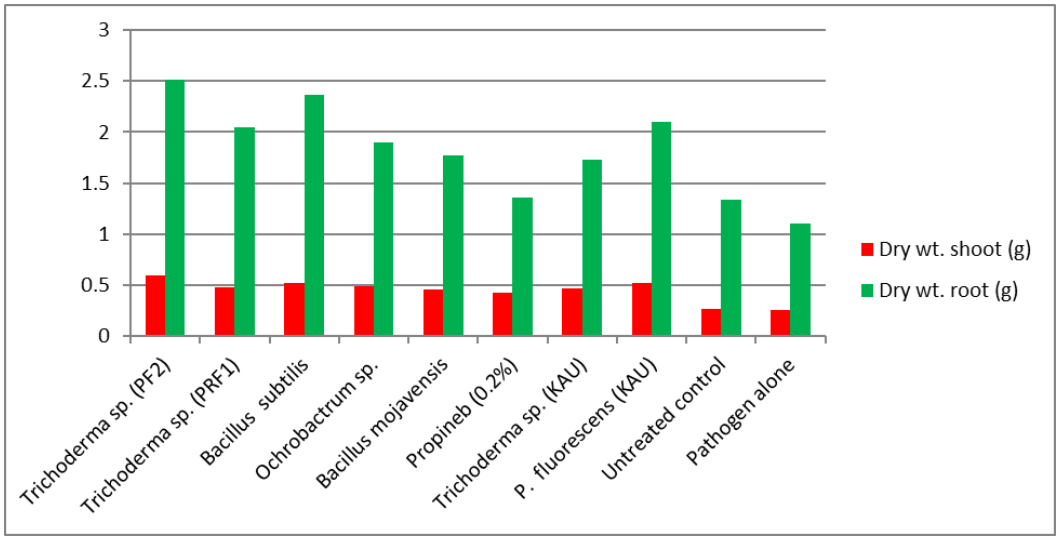

Fig 6: Effect of treatments on dry weight of shoot and root biomass of tomato

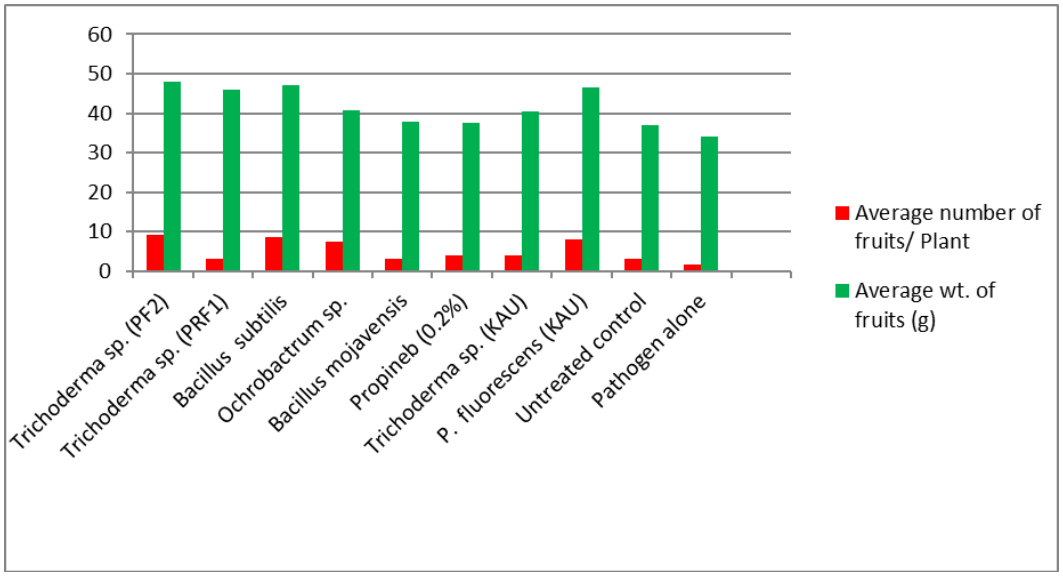

Fig 7: Effect of treatments on average number of fruit per plant and average weight of fruit in pot experiment under rain shelter

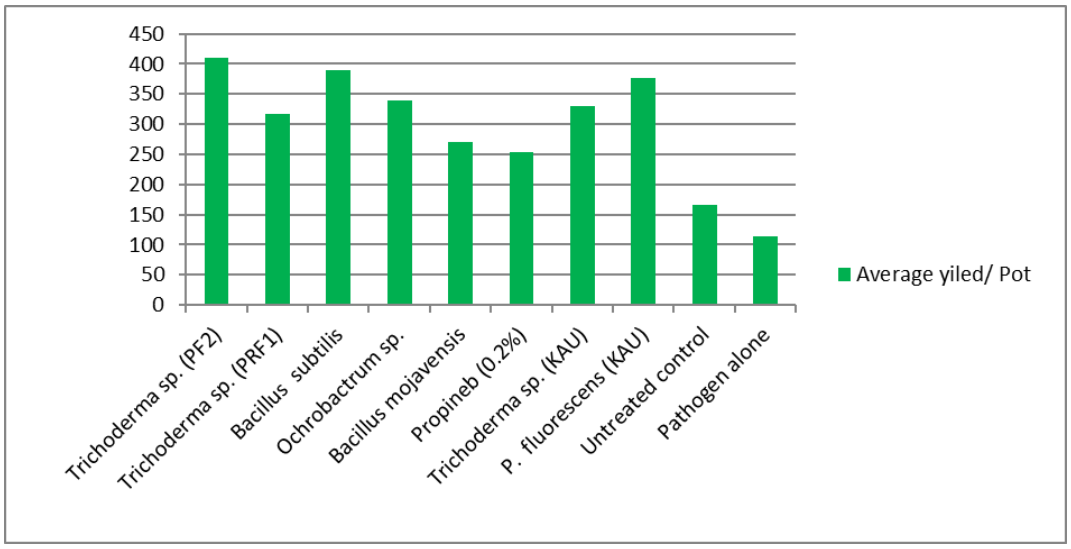

Fig 8: Effect of treatments on yield in pot experiment under rain shelter conditions

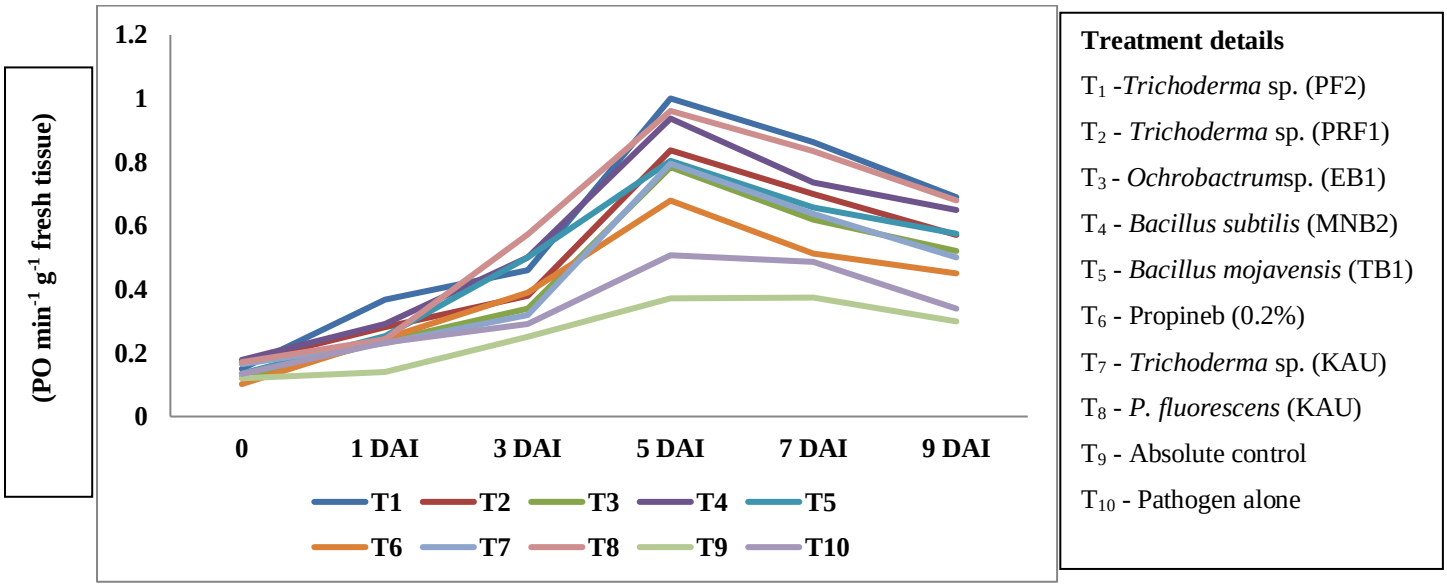

Fig 9: Effect of the treatments on PO enzyme activity of tomato leaves infected with *Alternaria solani*

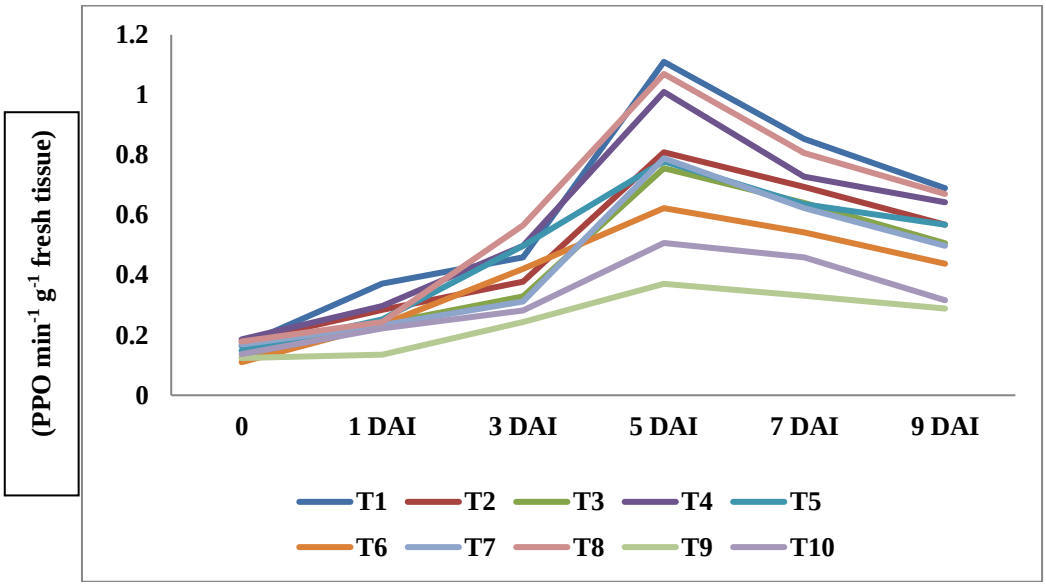

Fig 10: Effect of the treatments on PPO enzyme activity of tomato leaves infected with *Alternaria solani*

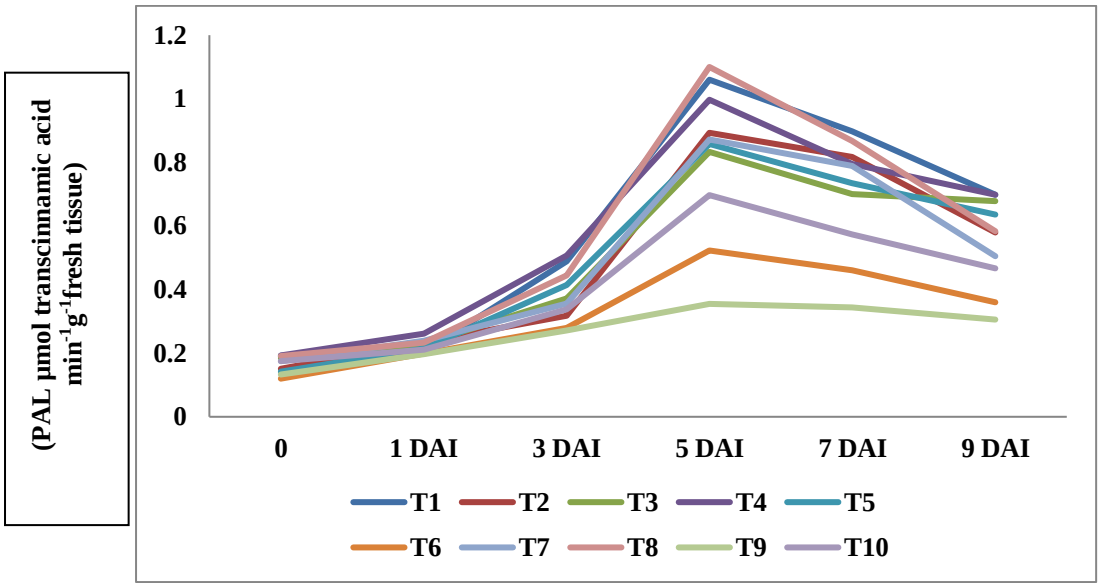

Fig 11: Effect of the treatments on PAL enzyme activity of tomato leaves infected with *Alternaria solani*

Induced systemic resistance (ISR) is a proficient defense mechanism inducing changes in physiology of plants, such as modifications of cell wall structure and accumulation of antimicrobial compounds like phytoalexins and pathogenesis related proteins (PRPs) resulting in weakening the activities of pathogens for further spread (Filippi *et al*., 2011) [17]. Results in graph clearly indicate that, the alterations in the activities of PO and PPO oxidative enzymes in infected plants exhibit changes with respect to the control. However, the significant increase in PO and PPO enzymes activity was achieved generally by provision of potential antagonists such as *Trichoderma* sp. (PF2) on the infected plants followed by *Bacillus subtilis* (MNB2) and *Pseudomonas fluorescens* (KAU) compared to the non-treated tomato plants as illustrated in (Figure 9 and 10). Whereas in PAL, the treatment with *Pseudomonas fluorescens* (KAU) followed by *Trichoderma* sp. (PF2) and *Bacillus subtilis* (MNB2) recorded the higher enzyme activities compared to control and pathogen alone.

Several studies have identified the ability of biocontrol agents to accelerate the defense enzymes activity in host plants and foliar spraying can increase PO, PPO and PAL which are often responsible for good plant health promotion and yield. Induced systemic resistance generally involves the production of oxidative enzymes like PO, PPO and PAL which catalyzes the structure of lignin, and other oxidative phenols that support to the defenses formation (Jetiyanon, 2007 and Yasmin *et al*., 2016) [24, 50]. Apart from preventing the growth of pathogens, phylloplane antagonists have the capability to advance crop growth and encourage plant resistance against pathogen attack. *Trichoderma longibrachiatum* also found to play significant key role to induce system resistance in plants (Zhang *et al*., 2015) [53] along with enhanced crop growth, yield and vigour (Zhang *et al*., 2016; Montesinos *et al*., 2019) [54, 31]. It is mainly achieved by production of indole-3-acetic acid and ACC deaminase (Zhang *et al*., 2019) [52]. Induced systemic resistance in host plants has been investigated by the application of *Trichoderma asperellem* (Shang *et al*., 2020) [42]. The higher activity of defense-related enzymes shown by *Bacillus amyloliquefaciens* which accelerate to inducing systemic resistance were also reported by (Gowtham *et al*.,

2018) [19]. It was revealed that *Bacillus velezensis* induced systemic resistance by the production of 'acetoin' in plants which further support to release H_2O_2 and hence assist in progress activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase enzymes (Peng *et al*., 2019; Rabbee *et al*., 2019; Chen *et al*., 2020) [34, 11]. The experiment conducted in glasshouse identified that the corn flour prepared as formulation with *B. subtilis* var. *amyloliquefaciens* (FZB 24) and applied as foliar spray @ 0.2% for six times at seven days interval had revealed significant reduction of late blight disease severity of 49.7% over control. Besides, the potato plants from the above mentioned treatment showed higher expression activity of defense-related enzymes *viz.*, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase, resulting higher accumulation of total phenols in comparison to control plants (Keerthana *et al*., 2018) [26]. The tomato plants of primed *B. subtilis* IAGS 174 revealed a more production of total flavonoids and phenolics in response to pathogen infection. These compounds act as antimicrobial or antioxidant properties and aid plants to evade pathogenic infections (Kumar *et al*. 2020) [28]. Akram and his workers in 2021 demonstrated the potentiality activity of *Bacillus subtilis* IAGS174 that elicit induced systemic resistance against *Fusarium* wilt pathogens in tomato plants through several mechanisms including histological, biochemical and molecular re-modulations., which synthesized the accumulation of defense-related enzymes, cytochemical barriers, and various phenolic acids serving as precursor metabolites of phenylpropanoid pathway. Furthermore, strain of *B. subtilis* IAGS174 also revealed to induce the biosynthetic genes in transcripts of lignin. Thus, this bacterial inducer can serve as a potential biological agent candidate that can effectively control fungal diseases of tomato plants.

According to comprehensive study of this research, biological control is seen as a better alternative to especially against many soil borne fungal pathogens. One of the major advantages of biocontrol agents is that it is cost effective and offers no harm to the crop. Most species of *Trichoderma*, *Bacillus* and *Pseudomonas* are used commonly for biological control of fungal pathogens. They are broadly recognized to be considered as potential promising biocontrol agents owing to their ability in reducing the disease occurrence caused by plant pathogenic fungi (Dubey *et al*., 2007) [14]. The possible mechanisms of biocontrol agents employing antagonism generally involve for niche and nutrients competitions, antibiosis through release of volatile substances and non-volatile antibiotics that inhibit the pathogen growth. Thus, the infected plants treated with selected bioagents against *Alternaria solani* showed the most potent effect in plant growth attributes and in yield parameters. The current investigation also carried out a strategy focused on using plant growth promoting antagonists in the induction of the systemic resistance of plant diseases and confirmed to be proficient in managing early blight disease of tomato. The selected potential antagonists accelerate the induction of the systemic resistance of the tomato plant while providing minimal disease incidence and disease severity. Additionally, tomato plants with selected treatments showed a significant increase in the content of PO, PPO and PAL over control. The current findings of our study indicate the potential species of *Trichoderma*, *Bacillus* and *Pseudomonas* as an efficient biological agent against *Alternaria solani* in tomato. However, field experimentation is essential to conclusively prove the potential antagonists and plant growth promotion with good yield of the isolates for commercial exploitation.

Conclusion

The promising investigation obtained from our study reveals that the selected phylloplane antagonists considerably increase in the enzyme activities which play a vital role in plant defense mechanisms against the pathogen *Alternaria solani* infection. Amongst anatgonists treatments, foliar application of *Trichoderma* sp. (PF2) and *Bacillus subtilis* (MNB2) reported the utmost beneficial effects against early blight of tomato with significant reduction in disease occurrence. However, the induction of plant resistance by application of bioagents has emerged as a good strategy in the management of plant diseases. Therefore, they could be exploited as a promising potential biological agent in sustainable agricultural field against various pathogenic soil borne fungal pathogens.

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