Assessing the potential of combined Bio control agents (Fungi and Bacteria) and AM fungi for enhancing turmeric resistance to *Pythium aphanidermatum*: A pot study with focus on Biometric, Disease and Enzymatic parameters

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Abstract

Turmeric (Curcuma longa L.) is one of the important spice crops grown in India since time immemorial and has a potential to earn foreign exchange because of its wide utilization in Ayurvedic industry. Turmeric belongs to the family Zingiberaceae. Turmeric is the third largest spice produced in the country and it accounts for about 14 % of total spices produced in India. Though it is well known for its medicinal value, its cultivation is hindered by several diseases. Among the various diseases, rhizome rot caused by Pythium sp. is a major problem in all turmeric growing areas of India. Its damage leads to 90-100% yield loss. The present studies emphasised on AM fungi (Glomus mossae) and antagonists (Trichoderma asperellum and Bacillus subtilis) against the rhizome rot of turmeric caused by P. aphanidermatum.

In pot culture experiments, the combined application of T. asperellum RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and B. subtilis RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus G. mosseae @ SA (20 g pot^{-1}) –(T_7) recorded the minimum Rhizome rot incidence and maximum plant growth promotion of turmeric. The same treatment showed earlier induction and increased levels of defence enzymes viz. PO, PPO, PAL and β1,3- glucanase.In field trial, the combined application of T. asperellum RT (10 ml kg^{-1}) + SA (2. 5 lit ha^{-1}) and B. subtilis RT (10 ml kg^{-1}) + SA (2.5 lit ha^{-1}) plus G. mosseae @ SA (20 kg ha^{-1})– (T_7) reduced rhizome rot incidence to the minimum and increased the biometrics of turmeric to the maximum.

Keywords: Rhizome rot of Turmeric, AM fungi, Defense Enzyme, Biometric (Pot culture).

Introduction

Turmeric (*Curcuma longa* L.) is a Zingiberaceae-family perennial, cylindrical, cross-pollinated rhizomatous plant. *Curcuma longa, Curcuma aromatic, Curcuma amada, Curcuma algustifolia and Curcuma zidoria* are the most common variations, with around 40 distinct genera and 400 species[7]. Turmeric is found in subtropical and tropical places across the world including India, South-East Asian nations and North Australia. India is the world's largest turmeric grower, accounting for 75-80% of worldwide output, followed by China, Myanmar, Nigeria and Bangladesh[23,24].

The rhizome contains an active compound “curcumin” which is said to have a wide range of effects like antioxidant, antitumor, antibacterial and antiviral activities, antiprotozoal, antiviral, anti-fibrotic, antivenin, antiulcer, hypotensive and hypocholesteremic activities. It is also an important spice that has become an integral component of several unique recipes. This plant is known as the "Queen of Spices" because of its amazing nutritional qualities and caustic flavour[5]. The most damaging disease of turmeric plants in India is rhizome rot caused by *Pythium aphanidermatum*, which diminishes its economic and commercial value[4]. Several studies have found enhanced disease management employing biocontrol organisms such as *Trichoderma* sp.[1] and *Pseudomonas* sp.[22] because they have antifungal, plant growth stimulating and plant defense inducing capabilities[27].

In addition to these species, Arbuscular mycorrhizal fungi (AMF) have been shown to battle soilborne illnesses by activating plant defence proteins such as PR proteins[23,25,26]. When afflicted by phytopathogens, plants have the innate potential to produce defence mechanisms. Arbuscular Mycorrhizal (AM) fungi form a mutually beneficial connection with plant roots. AM fungus promoting plant development and production in terrestrial habitats and their interaction with plant roots are particularly beneficial to plant resilience and tolerance during biotic stressors[6]. In the light of certain constraints on management practices, biological control has been advocated as the most promising strategy.

Trichoderma is a potent biocontrol agent and used extensively for disease management. Experts recommend that rhizome treatment with bioagent like *Trichoderma spp.* can effectively control this disease. *Trichoderma spp.* produces several antibiotics and lytic enzymes effective against soil-borne pathogens. *Trichoderma harzianum* was found to be antagonistic to *P. aphanidermatum* and *P. myriotylum*. *Bacillus* species are the most common bacteria

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isolated from the soil, which accounted up to 36% of the bacterial population. *B. subtilis* is bestowed with the ability to endure stress which is an advantage over other root colonising bacteria. This bacterium also can produce antifungal metabolites and antibiotics in soil. It can well be exploited for the control of many plant pathogens.

Plants have natural ability to develop defense mechanisms when they are infected by phytopathogens. Arbuscular Mycorrhizal (AM) fungi show mutually beneficial association with the roots of plants. AM fungi influence plant growth and productivity in terrestrial ecosystems and its association with plant roots is highly supportive for the plants with respect to resistance as well as tolerance during biotic stresses[6].

Material and Methods

Effect of Rhizome treatment and soil application with antagonists and AM fungi on the plant growth and rhizome rot incidence of turmeric (Pot culture): The pot culture experiments were conducted under controlled conditions at the glasshouse, Department of Plant Pathology, Annamalai University, to evaluate the efficacy of bio inoculants and VAM against *P. aphanidermatum*, the incitant of turmeric rhizome rot. The earthen pots (30 cm dia.) disinfected with 5 per cent of copper sulphate solution were filled with the autoclaved potting mixture (soil: sand: FYM @ 2:1:1) sterilized at 121^0 C, 15 psi for two consecutive days. The test pathogen culture, mass multiplied on sand: maize medium was incorporated in the soil (@ 50g/kg mixture) watered adequately and incubated in a screen house for two weeks prior to sowing to proliferate the test pathogen.

Pot culture experiment was laid out using two effective bio-control agents viz, *T. asperellum* (Ta_5) and *B. subtilis* (Bs_6); one *G. mosseae* and Metalaxyl was used for chemical comparison. The variety erode local was used in this study. The formulations of these products were delivered as rhizome dip and as soil application. Metalaxyl @ 0.1% was used for comparison and pathogen alone inoculated pots served as control. The experiment was conducted with three replications in a randomized block design. The treatment schedule is mentioned as follows:

T1 - *T. asperellum* RT (10 ml kg^{-1}) + SA (5ml kg^{-1})
T2 - *B. subtilis* RT (10 ml kg^{-1}) + SA (5ml kg^{-1})
T3 - *G. mosseae* SA (20 g pot^{-1})
T4 - T1+T2
T5 - T1+T3
T6 - T2+T3
T7 - T1+T2+T3
T8 - Metalaxyl 0.1%(SA)
T9 - Inoculated control
(RT-Rhizome Treatment; SA-Soil Application)

All the observations *viz*. plant height, no. of. leaves per plant, stem grith, yield per plant and the incidence of rhizome rot were recorded. The incidence of rhizome rot was recorded at 120 days after sowing. The percent disease incidence was calculated as per the standard formula. Best results obtained from pot culture experiments were used for enzyme studies.

$$\text{(\%) Rhizome rot incidence} = \frac{\text{Number of rhizomes rotted}}{\text{Total number of rhizomes sown}} \times 100$$

Effect of Rhizome treatment and soil application with antagonists and AM fungi on the plant growth and rhizome rot incidence of turmeric (Field trial): Based on the best result obtained from the pot culture, field trial was conducted in rhizome rot prone farmers field at Ramanayakampatti in Namakkal district, Tamil Nadu during 2021- 2022 representing irrigated condition by integrating the best treatments identified in the pot culture experiments. The blanket fertilizer schedule of 100:50:30 NPK/ ha recommended by the State Agricultural University was followed. A plot size of 5 × 4 m was used for each treatment. Each treatment was replicated thrice and suitable control was also maintained. The variety erode local was used in this study. Metalaxyl @ 0.1% as soil drenching was used for comparison.

T1 - *T. asperellum* RT (10 ml kg^{-1}) + SA (2. 5 lit ha^{-1})
T2 - *B. subtilis* RT (10 ml kg^{-1}) + SA (2.5 lit ha^{-1})
T3 - *G. mosseae* SA (20 kg ha^{-1})
T4 - T1+T2
T5 - T1+T3
T6 - T2+T3
T7 - T1+T2+T3
T8 - Metalaxyl 0.1%(SA)
T9 - Inoculated control
(RT-Rhizome Treatment; SA-Soil Application)

Disease incidence: Observation was made on plants for development of *P. aphanidermatum* after 30 days of inoculation. The severity of *P. aphanidermatum* was measured as per the standard evaluation system (SES) for turmeric upto 270 days.

Biometric assessment: Biometric assessment *viz.* number of leaves, stem grith, plant height and yield t/ha were assessed and recorded.

Effect of selected bio-controls and AM fungi on the induction of defense enzymes in turmeric challenge inoculated with *P. aphanidermatum* (Pot culture): Three months old turmeric plants were treated with bio control and AM fungi by Rhizome treatment + soil application with selected biocontrol products and challenged with *P. aphanidermatum* mass multiplied in sand maize medium. Plants neither treated with eco-friendly products nor challenged by the pathogen were used as controls. Three replications were maintained for each treatment. Turmeric plants along with rhizomes were carefully removed from the pots after 0, 3, 5 and 7 days and washed several times with

sterile distilled water before enzyme extraction. The enzymes were extracted from the rhizomes at the ice-cold condition (5^0C). The samples were homogenized with phosphate buffer [1 g of rhizome with 1 ml of sodium phosphate buffer (0.1M) pH 7.0]. The homogenates were centrifuged at 10,000 rpm for 15 min. The supernatant was used as an enzyme source for peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and β-1-3 glucanase.

T1 - *T. asperellum* RT (10 ml kg^{-1}) + SA (5ml kg^{-1})
T2 - *B. subtilis* RT (10 ml kg^{-1}) + SA (5ml kg^{-1})
T3 - *G. mosseae* SA (20 g pot^{-1})
T4 - T1+T2
T5 - T1+T3
T6 - T2+T3
T7 - T1+T2+T3
T8 - Metalaxyl 0.1%(SA)
T9 - Inoculated control
(RT-Rhizome Treatment; SA-Soil Application)

Peroxidase (PO)[10]: The activity of peroxidase was measured spectrophotometrically. The reaction mixture contained 2.5 ml of guaiacol (0.25 percent v/v) in 0.01M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. To begin the reaction, 0.1ml of enzyme extract was added which was then calorimetrically monitored at 470nm at 0.1 to 0.2 absorbance units/min. The boiling enzyme preparation was used as a control. Changes in absorbance at 470 nm min^{-1} g-1 of fresh $tissue^{-1}$ were used to calculate activity.

Polyphenol oxidase (PPO)[12]: Polyphenol oxidase activity was measured using the method described by Mayer et al[12]. 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 l of enzyme extract were used in the procedure. The reaction was started by adding 200 l of 0.01 M catechol and the activity was measured as changes in absorbance at 470 nm min-1 g^{-1} fresh $weight^{-1}$ of tissue.

Phenylalanine ammonia-lyase (PAL)[8]: The PAL test was carried out according to the technique given by Ross and Sederoff[18]. For 60 minutes, an assay mixture containing 100 l of enzyme, 500 l of 50 mM tris HCl (pH 8.8) and 600 l of 1 mM L-phenylalanine was incubated. By adding 2 N HCl, the process was stopped. Later, 1.5 ml of toluene was added, vortexed for 30 seconds, centrifuged (1000 rpm, 5 minutes) and the trans-cinnamic acid-containing toluene fraction was isolated. The toluene phase was measured at 290 m against a toluene blank. As previously reported, a standard curve was produced using graded quantities of cinnamic acid in toluene. The enzyme activity was measured in moles of cinnamic acid per minute per gramme of fresh $tissue^{-1}$.

β-1, 3-glucanase[14]: The enzyme activity was measured calorimetrically. A crude enzyme extract of 62.5 l was mixed with 62.5 l of 4% laminarin and incubated at 40°C for 10 minutes. The reaction was terminated by adding 375 l of dinitro salicylic acid (DNS) and heating it for 5 minutes on a boiling water bath. DNS was made by adding 300 ml of 4.5 percent NaOH to 880 ml containing 8.8 g of DNS and 22.5 g potassium sodium tartrate. The coloured solutions were diluted with distilled water, vortexed and the absorbance at 500 µg was measured. At zero-time incubation, the crude extract preparation was combined with laminar in. µg equivalents of glucose min^{-1} g fresh $weight^{-1}$ were used to express enzyme activity.

Results

Effects of bio control and AM fungi on Rhizome rot in turmeric, challenge inoculated with *P. aphanidermatum* (Pa1) (Pot culture): The data presented in the table.1 revealed that soil application with antagonists and AM fungi either alone or in combination showed the significant influence on rhizome rot incidence of turmeric. Among the various treatments, combined application of *T. asperellum* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus *G. mosseae* @ SA (20 g pot^{-1}) – (T_7) recorded the minimum rhizome rot incidence of 15.28 per cent. The same treatment recorded maximum plant biometrics *viz.* plant height, stem grith, no. of leaves / plant and yield/ plant of 80.48 cm, 8.73cm ,13.35 cm and 498.58g respectively. It was followed by (T_8) Metalaxyl treatment @0.1% which recorded the disease incidence, plant height, stem grith no. of leaves /plant and yield/plant of 6.39 per cent, 78.24 cm, 8.32cm,12.17cmand 465.12 g respectively. The disease incidence was maximum in control (68.25%).

Effects of bio control and AM fungi on turmeric rhizome rot, challenge inoculated with *P. aphanidermatum* (Pa1) (Field trail): The result presented in the table 2 revealed that the combined application of *T. asperellum* RT (10 ml kg^{-1}) + SA (2. 5 lit ha^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 lit ha^{-1}) plus *G. mosseae* @ SA (20 kg ha^{-1})– (T_7) recorded the minimum rhizome rot incidence of 6.14 per cent. The same treatment recorded maximum plant biometrics *viz.* plant height, stem grith, no. of leaves / plant and yield/ plant of 169.20cm, 16.54 cm, 19.54 cm and 50.35t/ha respectively. It was followed by (T_8) Metalaxyl treatment @ 0.1% which recorded the disease incidence, plant height, stem grith no. of leaves/plant and yield/plant of 12.54 per cent, 160.42cm, 15.87cm, 18.91 cm and 48.49 t/ha respectively. The disease incidence was maximum in control (28.94%).

Changes in peroxidase (PO) activity in *P. aphanidermatum* (Pa1) challenged turmeric crop treated with different formulations: The results presented in the fig. 1 revealed the increased activity of peroxidase (PO) in all treatment challenge inoculated with the pathogen when compared with pathogen alone inoculated control in turmeric plants. Among the various treatments, the higher peroxidase activity (10.86) was recorded in *T. asperellum* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus *G. mosseae* @ SA (20 g pot^{-1}) – (T_7) on 5th day which was followed by Metalaxyl 0.1% (T_8) recorded 10.32 on 5th day while the lower peroxidase

activity was recorded 8.10 in control (T_9). Further, the enzyme activity was gradually decreased on 7th day.

Changes in polyphenol oxidase (PPO) activity in *P. aphanidermatum* (Pa1) challenged turmeric crop treated with different formulations: The results presented in the fig. 2 revealed the increased activity of polyphenol oxidase (PPO) in all treatment challenge inoculated with the pathogen when compared with pathogen alone inoculated control in turmeric plants. Among the various treatments, the higher peroxidase activity 8.94 was recorded in *T. asperellum* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus *G. mosseae* @ SA (20 g pot^{-1}) –(T_7) on 5th day which was followed by Metalaxyl 0.1% (T_8) recorded 8.67 on 5th day while the lower peroxidase activity was recorded 6.89 in control (T_9). Further, the enzyme activity was gradually decreased on 7th day.

Changes in phenylalanine ammonia lyase (PAL) activity in *P. aphanidermatum* (Pa1) challenged turmeric crop treated with different formulations: The results presented in the fig. 3 revealed the increased activity of phenylalanine ammonia lyase (PAL) in all treatment challenge inoculated with the pathogen when compared with pathogen alone inoculated control in turmeric plants. Among the various treatments, the higher peroxidase activity 15.09 was recorded in *T. asperellum* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus *G. mosseae* @ SA (20 g pot^{-1}) –(T_7) on 5th day which was followed by Metalaxyl 0.1% (T_8) recorded 14.83 on 5th day while the lower peroxidase activity was recorded 7.03 in control (T_9). Further, the enzyme activity was gradually decreased on 7th day.

Changes in β-1,3-glucanase activity in *P. aphanidermatum* (Pa1) challenged turmeric crop treated with different formulations: The results presented in the fig. 4 revealed that the increased activity of β-1,3-glucanase in all treatment challenge inoculated with the pathogen when compared with pathogen alone inoculated control in turmeric plants. Among the various treatments, the higher peroxidase activity 41.47 was recorded in *T. asperellum* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus *G. mosseae* @ SA (20 g pot^{-1}) –(T_7) on 5th day which was followed by Metalaxyl 0.1% (T_8) recorded 41.09 on 5th day while the lower peroxidase activity was recorded 38.73 in control (T_9). Further, the enzyme activity was gradually decreased on 7th day.

Table 1
Effects of bio control and AM fungi on Rhizome rot in turmeric, challenge inoculated with *P. aphanidermatum* (Pot culture)

T. No.	Treatment	Plant height (cm)	Stem grith(cm)	No of leaves(cm)	Rhizome rot incidence (%)	PROC*	Yield(g/pot)
T_1	*Trichoderma asperellum* (Ta_5) RT (10 ml kg^{-1}) + SA (5ml kg^1)	68.46^c	6.87^c	9.39^{cd}	8.45^{cd} (16.89)	75.32(60.21)	374.32^d
T_2	*Bacillus subtilis* (Ba_6) RT (10 ml kg^{-1}) + SA (5ml kg^1)	63.98^e	6.54^d	8.26^e	9.27^{ef} (17.72)	72.93(58.64)	347.61^e
T_3	*Glomus mosseae* SA (20 g pot^{-1})	61.57^e	6.01^e	7.94^e	9.89^f (18.32)	71.12(57.49)	315.84^f
T_4	T1+T2	70.79^c	7.24^b	9.84^c	7.98^{bc} (16.40)	76.70(61.13)	385.89^c
T_5	T1+T3	73.12^b	7.93^b	10.27^c	7.35^b (15.73)	78.54(62.40)	414.56^b
T_6	T2+T3	66.24^d	6.62^{cd}	8.52^d	8.92^{de} (17.37)	73.95(59.31)	365.29^{de}
T_7	T1+T2+T3	80.48^a	8.73^a	13.35^a	6.14^a (14.34)	82.07(64.94)	498.58^a
T_8	Metalaxyl 0.1%(SA)	78.24^a	8.32^a	12.17^b	6.39^a (14.64)	81.34(64.40)	465.12^a
T_9	Control	46.24	3.11	4.67	68.25^g	-	214.55

RT: -Rhizome treatment, SA: -Soil Application, DAP: -Days after planting, PROC: -Percent reduction over control
*Mean of three replications
*In a column, mean values are followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

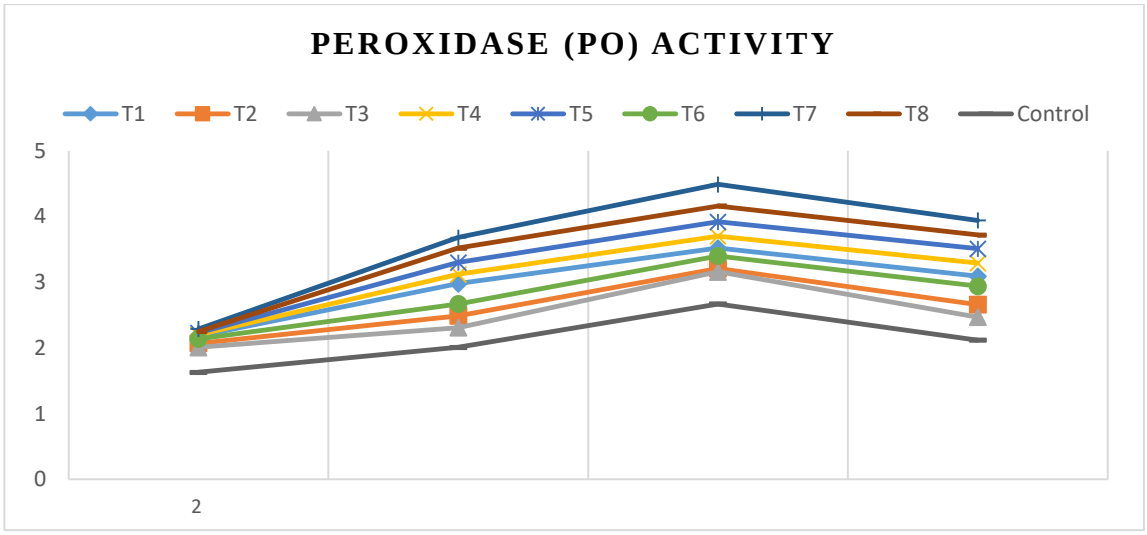

Fig. 1: Graphical value of Peroxidase (PO) Enzyme

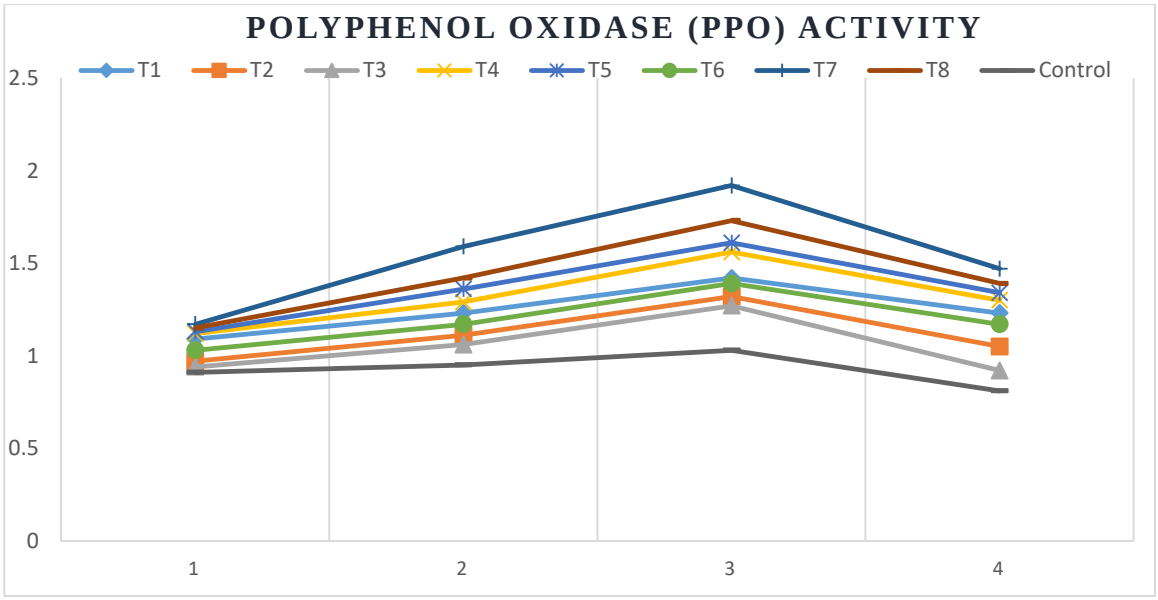

Fig. 2: Graphical value of polyphenol oxidase (PPO) enzyme

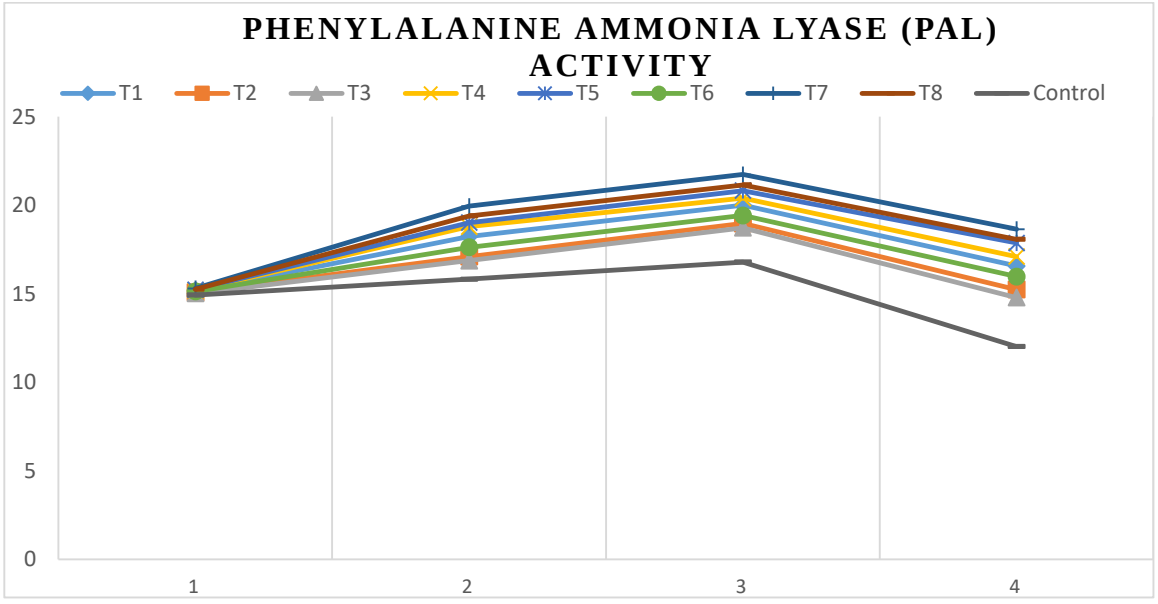

Fig. 3: Graphical value of Phenylalanine ammonia lyase (PAL) Enzyme

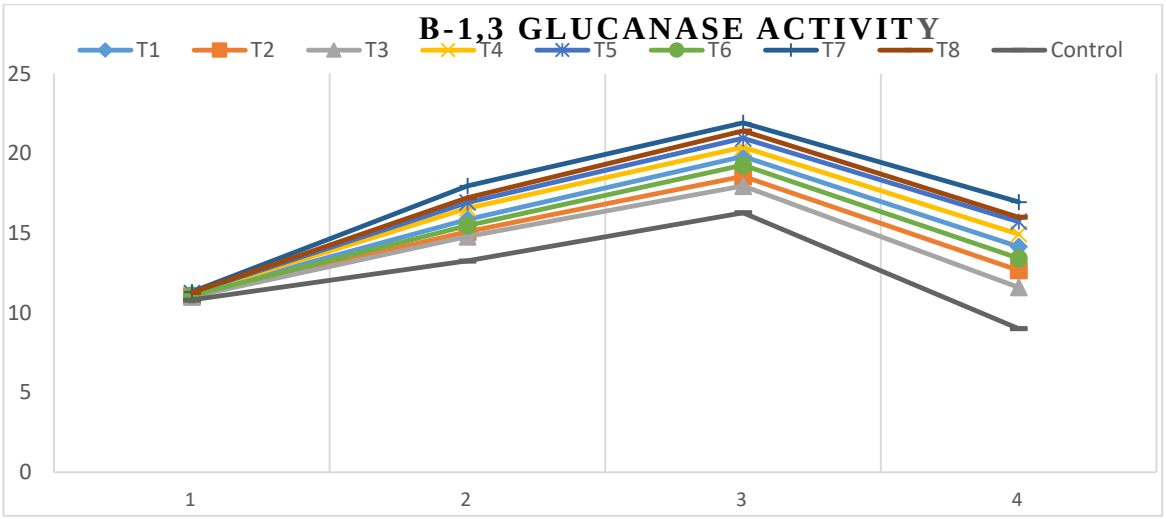

Fig. 4: Graphical value of β-1,3 glucanase Enzyme

T1- T. asperellum (Ta_5) RT (10 ml kg^{-1}) + SA (5 ml/kg^{-1}), **T2**- *Bacillus subtilis* (Ba_6) RT (10 ml kg^{-1}) + SA (5ml kg^{-1}), **T3**- *Glomus mosseae* SA (20 g pot^1), **T4**- T1+T2, **T5**- T1+T3, **T6**- T2+T3, **T7**- T1+T2+T3, **T8**- Metalaxyl 0.1%(SA), **T9**-Control.

Table 2
Effects of bio control and AM fungi on turmeric rhizome rot, challenge inoculated with *P. aphanidermatum* (Field trail)

T. No.	Treatment	Plant height (cm)	Stem grith(cm)	No of leaves(cm)	Rhizome rot incidence (%)	PROC*	Yield(t/ha)
T_1	*Trichoderma asperellum* (Ta_5) RT (10 ml kg^{-1}) + SA (2. 5 lit ha^{-1})	126.58^{d}	13.25^{c}	16.41^{c}	20.14^{e} (26.66)	30.40 (33.46)	36.24^{d}
T_2	*Bacillus subtilis* (Ba_6) RT (10 ml kg^{-1}) + SA (2.5 lit ha^{-1})	107.22^{e}	12.07^{d}	14.61^{e}	22.37^{g} (28.22)	22.70 (28.45)	34.44^{e}
T_3	*Glomus mosseae* SA (20 kg ha^{-1})	98.45^{f}	11.24^{e}	13.83^{f}	23.88^{h} (29.25)	17.48 (24.71)	31.70^{f}
T_4	T1+T2	134.73^{c}	13.92^{b}	17.07^{b}	18.87^{d} (25.37)	34.79 (36.14)	37.49^{d}
T_5	T1+T3	142.64^{b}	14.89^{b}	17.62^{b}	16.09^{c} (23.64)	44.40 (41.78)	40.52^{c}
T_6	T2+T3	116.39^{d}	12.65^{d}	15.32^{d}	21.01^{f} (27.28)	27.40 (31.56)	34.92^{e}
T_7	T1+T2+T3	169.20^{a}	16.54^{a}	19.54^{a}	12.52^{a} (20.72)	56.66 (48.82)	50.35^{a}
T_8	Metalaxyl 0.1% (SA)	160.42^{a}	15.87^{a}	18.91^{a}	14.97^{b} (22.76)	48.27 (44.00)	48.49^{b}
T_9	Control	72.98	6.14	9.24	28.94^{i}	-	25.86

RT: -Rhizome treatment; SA: -Soil Application, DAP: -Days after planting, PROC: -Percent reduction over control
*Mean of three replications
*In a column, mean values are followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Discussion

Effect of rhizome treatment and soil application with antagonist against rhizome rot incidence of turmeric. (Pot trial): Among the various concentration of antagonists tested, it was revealed that rhizome treated with *T. asperellum* (Ta_5) @10 ml kg^{-1} of rhizome showed the minimum rhizome rot incidence as same because *B. subtilis* (Bs_6) @10 ml kg^{-1} showed the considerable minimum incidence of rhizome rot while comparing other lower concentration while soil application of *T. asperellum* (Ta_5) @ 5 ml kg^{-1}, *B. subtilis* (Bs_6) @ 5 ml kg^{-1} and *G. mosseae* @ 20 g pot^{-1} also showed the minimum disease incidence respectively. Rhizome treatment of turmeric rhizome by

Trichoderma viride (RT) (43.51 %) showed minimum disease incidence.

The minimum disease incidence (%) was recorded. *Trichoderma* application in compost amended with *Trichoderma* spp. (12%) was compared to treated soil and untreated control under field condition. Plants treated with AM Fungi showed significant reduction of stem rot disease severity in infected peanut plants around 34.28% - 57.15%. Combination of a fungal and a bacterial antagonist of (T7 *Trichoderma viride* and *Pseudomonas fluorescens*) as rhizome dip and soil application after 3rd and 5th month of planting recorded the least disease incidence of 17.67 percent.

Effect of rhizome rot and soil application with antagonists and AM fungi against the rhizome rot incidence and biometrics of turmeric plant under pot culture and field test: Among the various concentration of antagonists and AM fungi tested, it was revealed that the *T. asperellum* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 ml kg^{-1}) plus *G. mosseae* @ SA (20 g pot^{-1}) –(T_7) were found to be effective. It also revealed that the combined applications of AM fungi and antagonists recorded the minimum rhizome rot incidence and maximum plant growth activity. The result revealed that the combined applications of *T. asperellum* RT (10 ml kg^{-1}) + SA (2. 5 lit ha^{-1}) and *B. subtilis* RT (10 ml kg^{-1}) + SA (5 lit ha^{-1}) plus *G. mosseae* @ SA (20 kg ha^{-1})– (T_7) recorded the minimum rhizome rot incidence of 12.54 per cent. The same treatment recorded maximum plant biometrics viz. plant height, stem grith, no. of leaves / plant and yield/ plant of 169.20cm, 16.54 cm, 19.54 cm and 50.35t/ha respectively.

The disease incidence was maximum in control (34.25%). The combination of organic substrates and biocontrol agents suppressed plant pathogens through different mechanisms which can be summarized as direct interactions. Maximum percent reduction in pre- emergence rhizome rot was recorded with the treatment *T. viride* and *T. harzianum* (76.66%). Different aspects of mycorrhizal function are conserved within clades of AM fungi. All the bacterial isolates exhibited positive responses for the oxidase test except *B. subtilis*. All the tested bacterial isolates showed nitrate reduction, except for *Flavobacterium* spp. and *P. libanensis*. All the tested bacterial isolates showed gelatin hydrolysis activity except for *Flavobacterium* spp. In a similar study, rhizobacterial strains were differentiated based on morphology and biochemical traits. Singh et al[20] and Pandey et al[15] stated that among various concentration of *Trichoderma* applied as seed treatment, the minimum disease incidence was recorded in seed treated with 107cfu/ml[25,26].

The combined application of biocontrol agents' treatment (Soil application of *T. viride* @ 2.5kg/ha. + Soil application of *P. fluorescens* 2.5kg/ha + Soil application of *G. mosseae* @ 12.5 kg/ha) (Table 1 and 2) was more effective than individual treatments, which might be due to the additive and interactive effect of the bio agents. The treatment (T7) maintains its superiority over other treatments in reducing the basal rot incidence by recording an incidence of 2.00, 3.48 and 5.25 per cent basal rot incidence at 30, 45 days and at harvest respectively. It was followed by T4 with 2.12, 5.25 and 9.23 percent disease incidence at 30, 45 days and at harvest respectively.

Induction of systemic resistance: Van Loon et al[23] stated that besides direct antagonistic activity, induction of systemic resistance by bio- control agents against diseases has been established by which the plants defend themselves from pathogen attack. Induced systemic resistance in several crops are associated with enhancement of lignification by the increased activities of hydrolytic enzymes involved in phenyl propanoid pathway and PR-protein synthesis[10].

Changes in peroxidase (PO) activity: Durgadevi et al[8] reported that bulb treatment with consortial formulation of *T. viride* (Tv1) and *B. subtilis* (Bs10) challenged with the *L. theobromae* recorded maximum induction of PO activities in tuberose plants. Yuvarani et al[25,26] stated that the combination treatment (T7) involving soil application of *T. viride* (2.5kg/ ha), *P. fluorescens* (2.5kg/ha) and *G. mosseae* (12.5kg/ ha) recording higher peroxidase (2.82). The maximum PO was observed on the 5th day in all the treatments and thereafter a gradual decrease was observed.

Changes in polyphenol oxidase (PPO) activity: Enhanced PPO activity due to the application of bio-control agents reported earlier by several workers[13,16]. Shoba et al[19] reported that the increased polyphenol oxidase activities were observed in *T. viride*, *T. asperellum* and *B. subtilis* compared to *P. fluorescens* treated black pepper vines which showed a drastic increase from 24 to 48 h and decreased the activity at 72 h after inoculation. The results in the present study corroborated with these earlier reports. Yuvarani et al[25,26] stated that the combination treatment (T7) involving soil application of *T. viride* (2.5kg/ ha), *P. fluorescens* (2.5kg/ha) and *G. mosseae* (12.5kg/ ha) recorded polyphenol oxides (2.46). The maximum PPO was observed on the 5th day in all the treatments and thereafter gradual decrease was observed.

Changes in phenylalanine ammonia lyase (PAL) activity: Muthukumar and Venkatesh[13] revealed that combined application of talc-based formulation of bio-agents and challenge inoculation with *S. rolfsii* recorded maximum induction of defense-related enzymes (PAL) as compared with individual application in peppermint plants. Shoba et al[19] reported that changes in PAL activities were observed after challenge inoculation with the target pathogen *P. capsici* up to 48 h and drastic decrease at 72 h after challenge inoculation. *T. viride* was most effective in enhancing the PAL activity compared to other strains. *T. asperellum* and *B. subtilis* also showed higher activity when compared to *P.*

fluorescens, healthy control and control. The above results add value to the present findings.

Yuvarani et al[25,26] studied the combination treatment (T7) involving soil application of *T. viride* (2.5kg/ ha), *P. fluorescens* (2.5kg/ha) and *G. mosseae* (12.5kg/ ha) recorded phenylalanine ammonia (100.42). The maximum PPO was observed on the 5th day in all the treatments and thereafter gradual decrease was observed. Phenylalanine ammonia-lyase (PAL) is typically triggered in plants by pathogen contact.

Changes in β-1, 3 Glucanase activity: Moreover, a variety of extracellular lytic enzymes such as high chitinase and β-(1,3)-glucanase activities have been reported to be produced by *T. harzianum*[11] and there may be relationship between the production of these enzymes and the ability to inhibit the pathogen[3,17,21]. Ahmed et al[2] reported that the activity of both chitinase and β-1,3-glucanase enzymes positively enhanced by means of the combined application of both fungal and bacterial bio control.

Plate 1: Symptoms of rhizome rot of turmeric

Mycelium growth **Healthy rhizome** **Rotted rhizome**

Plate 2: Rotten rhizomes of turmeric due to *Pythium aphanidermatum*

Plate 3: Effect of biocontrol and AM fungi on the percent disease incidence and plant parameters of Rhizome rot in turmeric, challenge inoculated with *P. aphanidermatum* (pot culture)
R-Replication; T-Treatment

Conclusion

Turmeric is one of the best spice crops grown in India. Its cultivation is inhibited by several diseases. Turmeric has great nutritional and medicinal properties. The application of AM fungi and Bio- Control can enhance the potential yield by providing disease resistance by the production of PR-Enzyme (Pathogen related Enzyme).

References

1. Adekunle A.T., Cardwell K.E., Florini D.A. and Ikotun T., Seed treatment with Trichoderma species of control of damping off cowpea caused by Macrophomina phaseolina, *Biochem. Sci. Technol.*, **11**, 449-457 **(2001)**

2. Ahmed A.G., Abeer H., Makhlouf and Selim Mohamed E., Efficacy of Compost and Some Biocontrol Agents in Controlling Cucumber White Mould Disease under Protected House Conditions, *Alexandria Sci. Exchange Journal*, **42**, 142 **(2021)**

3. Akhter W., Bhuiyan M.K.A., Sultana F. and Hossain M.M., Integrated effect of microbial antagonist, organic amendment and fungicide in controlling seedling mortality (*Rhizoctonia solani*) and improving yield in pea (*Pisum sativum L.*), *C. R. Biologies*, **338**, 21–28 **(2015)**

4. Anoop K. and Suseela Bhai R., Host Range Study of Turmeric Rhizome Rot Pathogen Pythium aphanidermatum on Selected Zingiberaceae Members, *International Journal of Research in Pure and Applied Microbiology*, **3**, 113-115. **(2013)**

5. Begum M.E.A., Miah M.M., Rashid M.A., Islam M.T. and Hossain M.I., Economic Analysis of Turmeric Cultivation: Evidence from Khagrachari District, *Bangladesh Journal of Agricultural Research*, **44**, 43-58 **(2019)**

6. Bhosale S., Jamdade R., Jadhav P. and Saha S., Comparative analysis of plasmid size of phytopathgenic Xanthomonas sp., *The Society of Agricultural Professionals*, **13(1)**, 62-64 **(2022)**

7. Damalas C.A., Potential Uses of Turmeric ('Curcuma longa') Products as Alternative Means of Pest Management in Crop Production, *Plant OMICS*, **4**, 136-141 **(2011)**

8. Durgadevi D., Prabhu S. and Sankaralingam A., Induction of defense related enzymes by bio-control agents against peduncle blight of tuberose, *Agro Technol.*, **2**, 4 **(2014)**

9. Gupta A.K., Mishra R. and Lal R.K., Genetic Resources, Diversity, Characterization and Utilization of Agronomical Traits in Turmeric (Curcuma longa L.), *Industrial Crops and Products*, **77**, 708-712 **(2015)**

10. Hammerschmidt R. and Kuc J., Induced Resistance to Disease in plants. Kluwer Academic Publishers, Dordrecht the Netherlands 182 II Production of volatile antibiotics, *Transactions of the British Mycological Society*, **57(1)**, 41-48 **(1995)**

11. Kumar K. et al, Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens, *Indian J Microbiol.*, **52(2)**, 137-144 **(2012)**

12. Mayer A.M., Harel E. and Shaul R.B., Assay of catechol oxidase critical comparison of methods, *Phytochemistry*, **5**, 783-789 **(1965)**

13. Muthukumar A. and Venkatesh A., Biological induction of systemic resistance to collar rot of pepper mint caused by *Sclerotium rolfsii*, *Acta Physiol Plant*, **36**, 1421-1431 **(2014)**

14. Pan S.Q., Ye X.S. and Kuc J., Association of ß-1,3 glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with Peranosporatabacina or leaf inoculation with tobacco mosaic virus, *Physiol Mol Plant Pathol*., **39**, 25-39 **(1991)**

15. Pandey P., Sagar G.C., Shrestha S., Manandhar H., Yadav R.K. and Devkota R., Management of collar rot disease of chickpea by *Trichoderma* species, *J Agri Search*, **7(3)**, 172-17 **(2020)**

16. Patel M. and Saraf M., Biocontrol efficacy of *Trichoderma asperellum* MSST against tomato wilting by *Fusarium oxysporum* f. sp l*ycopersici*, *Arch Phytopathology Plant Protect*, **50**, 228-238 **(2017)**

17. Ridout C.R., Coley-Smith J.R. and Lynch J.M., Fractionation of extracellular enzymes from a mycoparasitic strain of *Trichoderma harzianum*, *Enzyme Microb. Technol.*, **10**, 180-187 **(1988)**

18. Ross W.W. and Sederoff R.R., Phenylalanine ammonia lyase from loblolly pine: Purification of the enzyme and isolation of complementary DNA clones, *Plant Physiol*., **98**, 380-386 **(1992)**

19. Shobha M.S., Lakshmi Devi N. and Mahadeva Murthy S., Induction of Systemic Resistance by Rhizobacterial and Endophytic Fungi against Foot Rot Disease of *Piper nigrum* L. by Increasing Enzyme Defense Activity, *Int J Environ Agri and Biotechnol*., **4(1)**, 86-98 **(2019)**

20. Singh H.B., Singh Prachi, Singh Jyoti, Rajput Rahul Singh, Vaishnav Anukool, Ray Shatrupa and Singh R.K., Exploration of multitrait antagonistic microbes against *Fusarium oxysporum* f.sp. *lycopersici*, *J Appl Natural Sci.*, **1(2)**, 503 – 510 **(2019)**

21. Sivan B., Elad Y. and Chet I., Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*, *Phytopathol.*, **74**, 498-503 **(1984)**

22. Srivastava Rashmi, Khalid A., Singh U.S. and Sharma A.K., Evaluation of arbuscular mycorrhizal fungus, fluorescent Pseudomonas and Trichoderma harzianum formulation against Fuasrium oxysporum f. sp. lycopersici for the management of tomato wilt, *Biological Control*, **53**, 24-31 **(2010)**

23. Van Loon L.C., Rep M. and Pieterse C.M.J., Significance of inducible defence- related proteins in infected plants, *Annv. Rev. Phytopathol.*, **44**, 135-162 **(2006)**

23. Vinayarani G. and Prakash H.S., Fungal Endophytes of Turmeric (Curcuma longa L.) and Their Biocontrol Potential against pathogens Pythium aphanidermatum and Rhizoctonia solani, *World Journal of Microbiology and Biotechnology*, **34**, https://doi.org/10.1007/s11274-018-2431-x **(2018)**

24. Vinayarani G. and Prakash H.S., Growth Promoting Rhizospheric and Endophytic Bacteria from Curcuma longa L. as Biocontrol Agents against Rhizome Rot and Leaf Blight Diseases, *The Plant Pathology Journal*, **4**, 218-235, https://doi.org/10.5423/PPJ.OA.11.2017.0225 **(2018)**

25. Yuvarani R., Induction of defense-related enzymes in onion by using combined application of fungal and bacterial biocontrol agents with am fungi against *fusarium oxysporum f. Sp. Cepae*, *Plant Archives*, **20(1)**, 21-24 **(2018)**

26. Yuvarani R., Studies on the management of basal rot of onion (*Allium cepa* var. *aggregatum* G. Don) caused by *Fusarium oxysporum*f. sp. *cepae* (Hans.) using antagonists and AM fungi, M.Sc. Thesis Dept. of Plant Pathol, Annamalai University, India **(2018)**

27. Zaidi N.W., Pramila N. and Singh U.S. Biological control of plant pathogens: Status in India, In Singh S.P. and Singh S.B., eds., Eco-Agriculture with Bio augmentation: An emerging concept, DASP, Lucknow, 21-52 **(2004)**.

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