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Effect of bioinoculants on rhizome rot disease of turmeric (*Curcuma longa* L.)

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Abstract
Pythium sp. induced rhizome rot is a serious constraint throughout all turmeric-growing regions. Application of fungicides for rhizome rot has decreased the soil microbial biodiversity, which has resulted in the emergence of pathogen strains that are resistant to management. Under glasshouse conditions, the potential of bioagents isolated from the rhizosphere soil to control rhizome rot in turmeric was assessed. The lowest incidence of rhizome rot disease (14.60%) resulted by applying bioformulation *P. chlororaphis* PA23 and *B. subtilis* CBE4 as rhizome dip and soil on 3rd and 5th month. Turmeric height, girth and number of leaves per clump were all significantly improved by applying the consortial bioformulation. When *B. subtilis* CBE4 and *P. chlororaphis* PA23 were applied as consortial formulation against *P. aphanidermatum*, defense-related gene products including chitinase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, β-1,3 glucanase, phenols, and proteins were induced which led to suppression of the incidence of rhizome rot disease.

Keywords: Biocontrol, *Pseudomonas, Bacillus, Trichoderma,* rhizome rot, turmeric, ISR

Introduction
The world's largest producer, consumer and exporter of spices is India, often referred as the land of spices. Cardamom and black pepper are the two commodities that yield the most foreign exchange, followed by turmeric (*Curcuma longa* L.). About 80 percent of global production and 60 percent of exports are attributed to India, which is the leading producer of turmeric worldwide. 5,34,000 tonnes of turmeric are produced annually on 1,34,000 hectares of land in India (Ravikumar, 2002) [15]. Diseases such as rhizome rot, anthracnose and leaf blight affects turmeric. Ramarethinam and Rajagopal (1999) [13] indicated that rhizome rot incited by *Pythium* sp. is a major constraint in all areas of India that grow turmeric. According to Rathiah (1987) [14], the disease caused up to 95% of crop yield loss due to symptoms such as collapse of tillers, rotting roots and the hollow rhizome leaving only fibrous tissues.
According to Nirmal (1992) [9], the disease results in 50–80% yield loss during storage and more than 60% seedling mortality in both nursery and field conditions. Rhizome rot also causes a 50% yield loss (Rajalakshmi *et al*., 2016) [12]. Rhizome rot has become more common as a result of intensive turmeric farming. Fungicide-induced rhizome rot control has decreased soil microbial biodiversity and led to the emergence of pathogen strains that are resistant to the disease. The need for alternate plant protection techniques has arisen from growing concerns about the effects of pesticide misuse on the environment. The present research thus focuses into the use of antagonistic bioinoculants to manage turmeric rhizome rot in a way that's environmentally friendly.

Materials and Methods
The experiment was conducted at Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore under completely randomized block design. Under glass house conditions, the bioinoculants *Pseudomoans chlororaphis* PA23, *Bacillus subtilis* CBE4, and *Trichoderma viride* MNT7 were investigated for their potential for biocontrol. The antagonistic bacterial and fungal formulation, which was based on talc, was applied to the soil on the third and fifth months following planting and as a rhizome dip (RD) at 10 g/lit.

Under glass house conditions, *P. aphanidermatum* multiplied on sand maize medium was added at a rate of 5% (w/w). Data on the percentage of rhizome rot occurrence was recorded during harvest. Additional growth characteristics such as plant height, leaf count and stem girth were also recorded.

Eighteen treatments were used in the experiment under CRD design which was replicated three times. The particulars of each treatment include:

T_1 – *P. chlororaphis* PA23 (RD) ;
T_2 – *P. chlororaphis* PA23 (RD) + SA on 3rd Month
T_3 – *P. chlororaphis* PA23 (RD) + SA on 5th Month
T_4– *P. chlororaphis* PA23 (RD) + SA on 3rd and 5th Month
T_5- *B. subtilis* CBE4 (RD)
T_6- *B. subtilis* CBE4 (RD) + SA on 3rd Month
T_7- *B. subtilis* CBE4 (RD) + SA on 5th Month
T_8- *B. subtilis* CBE4 (RD) + SA on 3rd and 5th Month
T_9- *P. chlororaphis* PA23 + *B. subtilis* CBE4 (RD)
T_{10}– *P. chlororaphis* PA23 + *B. subtilis* CBE4 (RD) + SA on 3rd Month
T_{11}– *P. chlororaphis* PA23 + *B. subtilis* CBE4 (RD) + SA on 5th Month
T_{12}– *P. chlororaphis* PA23 + *B. subtilis* CBE4 (RD) + SA on 3rd and 5th Month
T_{13}- *T. viride* MNT7 (RD)
T_{14}- *T. viride* MNT7 (RD) + SA on 3rd Month
T_{15}- *T. viride* MNT7 (RD) + SA on 3rd Month
T_{16}- *T. viride* MNT7 (RD) + SA on 3rd and 5th Month
T_{17}- Drenching of 0.2% Copper oxy chloride
T_{18}- Inoculated Control

Effect of bioinoculants on defense-related enzyme induction in turmeric plants

The bioinoculants viz., *B. subtilis* CBE4, *P. chlororaphis* PA23 and *T. viride* MNT7 were applied to three-month-old turmeric plants through soil and they were then challenged with *P. aphanidermatum*. After six days of challenge inoculation with *P. aphanidermatum*, the turmeric leaves were carefully removed from the pots and repeatedly rinsed with distilled water in order to extract enzymes. The green leaves were chilled at 5 °C and homogenized using 0.1 M phosphate buffer (pH 7.0) and further centrifuged at 5 °C for extracting the enzymes. The supernatant served as source for analysis of defense related enzyme for colorimetric estimation of chitinase (Boller and Mauch, 1988) [2], β-1, 3-glucanase (Pan *et al*., 1991) [10], peroxidase (Hammerschmidt *et al.*, 1982) [6], poly phenol oxidase (Mayer *et al.*, 1965) [7]. phenyl alanine ammonia lyase (Dickerson *et al*., 1984) [5], phenols (Zieslin and Ben Zaken, 1993) [20] and protein (Bradford 1976) [4]. The data were statistically analyzed using the OP STAT software.

Results and Discussion

The effectiveness of the bioinoculants in managing turmeric rhizome rot was assessed in glass house system. The outcomes are summarized in Table 1. Delivering the bioformulation *P. chlororaphis* PA23 and *B. subtilis* CBE4 as rhizome dip and soil treatment on 3rd and 5th month (T_{12}) exhibited the lowest disease incidence (14.60 percent). Rhizome dip and soil treatment on the third and fifth months of planting using *B. subtilis* CBE4 (T8) and *P. chlororaphis* PA23 (T4) resulted in disease incidence of 20.00 and 20.83 percent respectively which are statistically comparable. The maximum disease incidence of 52.66 percent was observed in the control plants. The results align with the findings of Muthulakshmi and Saveetha (2009)[8], that introducing consortia of *Trichoderma viride* and *Pseudomonas flourescens* by seed and soil application was an efficient way to reduce the severity of turmeric rhizome rot disease with maximizing yield. In both glasshouse and field conditions, the rhizome rot disease incidence was lowest when *Pseudomonas fluorescens* FP7 liquid formulation was used as rhizome dip and soil drench (Prabhukarthikeyan *et al*., 2017) [11]. Ginger rhizome rot can be effectively managed by applying *T. harzianum* as rhizome dip and soil at 30, 60, and 90 days post-planting (Shanmugam *et al*., 1999) [17]. According to Ramarethinam and Rajagopal (1999) [13], rhizome rot of turmeric was suppressed by applying Trichoderma.

Application of bioinoculants significantly increased the yield, plant height, stem girth and no. of leaves/ clump of turmeric plants. Treatment T_{12} (*P. chlororaphis* PA23 + *B. subtilis* CBE4 (RD) and SA on 3rd and 5th Month), recorded the highest rhizome yield of 510 g/plant followed by 450 g/plant in T_8 and T_4 whereas in the control plants recorded yield of 250 g/plant. Maximum height of 79.16 cm, stem girth of 8.43cm and 12.33 leaves/clump was recorded in the T_{12} treatment (*P. chlororaphis* PA23 + *B. subtilis* CBE4 RD + SA on 3rd +5th Month). Application of *Trichoderma* as rhizome and soil treatment considerably enhanced plant height and fresh rhizome yield/plant and decreased the rhizome rot disease occurrence (Vinayarani *et al*., 2019 and Anitha *et al.*, 2021) [19, 1].

Instead of applying bioinoculants individually through rhizome dip or soil treatment on 3rd and 5th Month, application of bioinoculants as a consortial formulation was effective. Due to the pathogen's mobility and potential to re-infect seed at a later stage of treatment, soil application of bioinoculants was shown to be more successful than treating rhizomes. However, the application of bioinoculants in soil led to a significant increase in the antagonistic population, which significantly slowed the growth of the pathogen. This could be the cause for the decreased occurrence of rhizome rot in treated plots.

Impact of bioinoculants on induction of defense related enzyme in turmeric plants

One novel method of protecting plants is to use biological stimulants to activate the plant's defense mechanisms. Table 2 presents the findings of the induction of defense enzymes, phenol and protein following the challenge inoculation of *P. aphanidermatum* in soil after bioinoculants treatment.

When exposed to pathogens in plants treated with bioinoculants, the turmeric plants exhibited increased chitinase activity. The pathogen's challenge inoculation significantly increased the activity. When *P. chlororaphis* PA23 + *B. subtilis* CBE4 were applied to turmeric plants followed by challenge inoculation with *P. aphanidermatum*, the plants' chitinase activity was increased (400 n mol). In the healthy control, chitinase activity (250.00 n mol of) which remained at low level. Turmeric plants' resistance to *P. aphanidermatum* infection was considerably enhanced by a significant rise in chitinase activity (Sathiyanarayanan and Muthukrishnan, 2014) [16]

Table 1: Effect of bioinoculants on the rhizome rot of turmeric under glass house condition

Treatments	Plant height(cm)	Stem girth (cm)	No. of leaves/clump	Rhizome rot incidence (%)	Percent reduction over control	Yield (g/plant)	Percent increase over control
T_1	69.66	7.26	10.33	30.2 (33.32)	42.65	320	21.87
T_2	72.93	7.46	10.00	27.33 (31.51)	48.10	330	24.24
T_3	75.16	8.16	11.00	24.33 (29.54)	53.79	350	28.57
T_4	75.5	8.16	10.33	20.00 (26.55)	62.02	450	44.44
T_5	69.00	7.96	11.33	30.66(33.61)	41.77	320	21.87
T_6	70.5	7.73	11.66	27.66 (31.72)	47.47	340	26.47
T_7	75.76	8.23	10.66	23.66 (29.09)	55.07	390	35.89
T_8	77.96	8.43	11.66	20.83(27.14)	60.44	450	44.44
T_9	72.00	7.93	11.33	28.33(32.14)	46.20	350	28.57
T_{10}	71.5	7.16	10.33	23.66 (29.09)	55.07	380	34.21
T_{11}	76.16	7.33	10.66	22.00(27.96)	58.22	450	44.44
T_{12}	79.16	8.43	12.33	14.60 (22.45)	72.27	510	50.98
T_{13}	70.33	7.30	11.33	30.66 (33.61)	41.77	330	24.24
T_{14}	76.83	7.76	10.66	27.66 (31.72)	47.47	350	28.57
T_{15}	71.66	7.30	11.66	23.33 (28.87)	55.69	380	34.21
T_{16}	74.50	7.33	11.66	24.50(29.66)	53.28	420	40.47
T_{17}	55.33	6.60	9.33	30.66 (33.61)	41.77	300	16.66
T_{18}	53.66	6.26	8.66	52.66 (46.51)	-	250	-
CD(0.05)	3.325	1.247	NS	0.998		17.458	
SEM	1.155	0.433	0.693	0.346		6.062	

values are mean of three replications. Figures in the parentheses represent arcsine transformed values

Following the application of bioinoculants, the turmeric plants exhibited increased β-1, 3, glucanase activity in response to pathogen stimulation. The healthy control group showed reduced β-1,3 glucanase activity with a level of 112.15 µmol. When *P. chlororaphis* PA23 and *B. subtilis* CBE4 were applied together followed by a challenge infection with

P. aphanidermatum, the activity of β-1, 3 glucanase was increased (322.58 µmol). *P. aphanidermatum* inoculated plants that were pretreated with bioinoculants showed a greater concentration of this hydrolytic enzyme.

When the plants were primed with bioinoculants and challenged with pathogen the turmeric plants showed increased PO activity. Plants treated with bioinoculants alone also showed increased peroxidase activity. However, plants that had been pretreated with bioinoculants and *P. aphanidermatum* challenge inoculation showed an additional rise in activity. The findings showed that turmeric plants pretreated with *P. chlororaphis* PA23 + *B. subtilis* CBE4 and challenge inoculation with *P. aphanidermatum* had a considerably higher induction of PO enzyme (4.083). However, the healthy control plants had very less PO activity (1.520).

When exposed to a pathogen, the turmeric plants shown increased PPO activity irrespective of the fact they were previously treated with bioinoculants. Compared to the uninoculated control, the aforesaid treatment resulted in a two-fold higher induction of PPO. *P. chlororaphis* PA23 + *B. subtilis* CBE4 pretreatment of turmeric plants, followed by *P. aphanidermatum* challenge inoculation, resulted in the highest PPO activity (2.317). However, in the healthy control, the activity was incredibly low (1.117). Nevertheless, the activity in bioinoculants pretreated plants that were not challenged with a pathogen did not significantly decrease.

When exposed to a pathogen, the turmeric plants had increased PAL activity regardless of whether they had previously been treated with bioinoculants. The highest PAL activity (12.325 µ mol) was reported by pretreating turmeric plants with *P. chlororaphis* PA23 + *B. subtilis* CBE4 followed by challenging with *P. aphanidermatum*. The activity of PAL in the pathogen-inoculated control was 8.513 µ mol, while the activity of PAL was 6.233 µ mol in the healthy control.

Following challenge inoculation with pathogen, phenolics accumulation increased. The total phenol content of fresh tissue treated with *P. chlororaphis* PA23 + *B. subtilis* CBE4 was 600 µg g^{-1} following pathogen inoculation. However, pathogen-inoculated control the total phenol was only 420 µg g^{-1}. It was evident that, in comparison to plants treated with bioinoculants and challenged with pathogen, the accumulation of phenolic compounds was relatively lower in the healthy control (350 µg g^{-1}).

When *P. aphanidermatum* was applied to turmeric plants pretreated with bioinoculants, the plants showed increased protein accumulation. When compared to the other treatments, the activity was much higher (490 µg/g) in turmeric plants primed with *P. chlororaphis* PA23 + *B. subtilis* CBE4 and challenge inoculated with *P. aphanidermatum*. In contrast, healthy control recorded protein accumulation of 290 µg/g, the pathogen-inoculated control reported protein accumulation of 355 µg/g.

Pretreatment of turmeric plants with bioinoculants followed by challenging with *P. aphanidermatum*, induced higher accumulation of protein. The activity was significantly higher in leaves (490 µg/g) of turmeric plants pretreated with *chlororaphis* PA23 + *B. subtilis* CBE4 treatment challenge inoculated with *P. aphanidermatum* when compared to all other treatments. The pathogen inoculated control recorded protein accumulation (355 µg/g) when compared to the healthy control which recorded protein accumulation of 290 µg/g. Several plant defense enzymes were activated when FYM-based biomanure of *T. viride* was applied to turmeric plants (Ushamalini *et al.*, 2006) [18]. The results are similar to those of Vinayarani *et al.*, 2019 [19], that defense enzymes and phenolics were increased by two to three times when *T. asperellum* was pretreated in turmeric followed by challenge inoculation with pathogen when compared to the untreated control.

Table 2: Effect of bioinoculants on the induction of defense enzymes against rhizome rot of turmeric

Treatment	Chitinase activity (n mol of NAGlu/min/g of leaf tissue)	β-1, 3 glucanase activity (µmol equivalent glucose released/h/g of leaf tissue)	Peroxidase activity (Change in absorbance / min/ g of leaf tissue)	Polyphenol oxidase activity (Change in absorbance / min/ g of leaf tissue)	Phenylalanine ammonia- lyase activity (µ mol of cinnamic acid /min/g of leaf tissue)	Phenol (µg of catechol /g of leaf tissue)	Protein (µg of BSA/g of leaf tissue)
P. chlororaphis PA23+ *P. aphanidermatum*	385	300.18	3.127	2.113	9.65	500	410
B. subtilis CBE4+ *P. aphanidermatum*	360	311.00	3.583	2.200	10.36	550	420
P. chlororaphis PA23+ *B. subtilis* CBE4+ *P. aphanidermatum*	400	322.58	4.083	2.317	12.35	600	490
T. viride MNT7+ *P. aphanidermatum*	380	275.03	3.257	2.057	9.89	520	430
P. chlororaphis PA23	285	120.15	2.127	1.410	6.81	380	328
B. subtilis CBE4	275	130.50	2.2	1.320	7.00	390	325
P. chlororaphis PA23+ *B. subtilis* CBE4	290	150.84	2.25	1.520	7.21	400	350
T. viride MNT7	280	110.15	2.12	1.320	6.70	360	319
Inoculated Control (*P. aphanidermatum*)	350	240.21	2.85	1.583	8.51	420	355
Healthy Control	250	112.15	1.52	1.117	6.233	350	290
CD(0.05)	8.576	4.288	0.858	NS	3.430	39.449	16.294
SEM	2.887	1.443	0.289	0.346	1.155	13.279	5.485

values are mean of three replications.

Conclusion

The results of the present investigation revealed that application of bioinoculants *B. subtilis* CBE4 and *P. chlororaphis* PA23 as rhizome and soil application in turmeric suppressed rhizome rot and enhanced plant growth and induced the defense enzymes.

References

1. Anitha B, Padma M, Seenivasan N, Lakshmi Narayana D, Sujatha M, Mahender N. Effect of biocontrol agents on growth, yield and rhizome rot incidence of turmeric varieties. The Pharma Innovation J. 2021;10(7):1341-1345.
2. Anusuya S, Sathiyabama M. Effect of chitosan on rhizome rot disease of turmeric caused by *Pythium aphanidermatum*. ISRN Biotechnol. 2014 Mar;2014:305349.
   Available from: https://doi.org/10.1155/2014/305349
3. Boller T, Mauch F. Colorimetric assay of chitinase. Methods Enzymol. 1988;161:430-435. Available from: https://doi.org/10.1016/0076-6879(88)61052-4
4. Bradford MM. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976;72:248-254. Available from: https://doi.org/10.1016/0003-2697(76)90527-3
5. Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia lyase and hydroxyl cinnamate: CoA ligase in maize mesocotyls inoculated with Helminthosporium maydis or *Helminthosporium carbonum*. Physiol Plant Pathol. 1984;25:111-123. Available from: https://doi.org/10.1016/0048-4059(84)90050-X
6. Hammerschmidt R, Kuc JA. Lignification as a mechanism for induced systemic resistance in cucumber. Physiol Plant Pathol. 1982;20(1):61-71. Available from: https://doi.org/10.1016/0048-4059(82)90024-8
7. Mayer AM, Harel E, Shaul RB. Assay of catechol oxidase: A critical comparison of methods. Phytochemistry. 1965;5:783-789. Available from: https://doi.org/10.1016/S0031-9422(00)83660-2
8. Muthulakshmi P, Saveetha K. Management of turmeric rhizome rot using eco-friendly biocontrol consortia. J Biol Control. 2009;23(2):181-184. Available from: https://doi.org/10.18311/jbc/2009/3643
9. Nirmal BK, Samsuddin K, Ratnambal MJ. *In vitro* plant regeneration from leaf-derived callus in ginger (*Zingiber officinale* Rosc.). Plant Cell Tissue Organ Cult. 1992;29:71-74.
   Available from: https://doi.org/10.1007/BF00033610
10. Pan SQ, Ye XS, Kuc J. Association of β-1,3-glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. Physiol Mol Plant Pathol. 1991;39:25-39. Available from: https://doi.org/10.1016/0885-5765(91)90029-H
11. Prabhukarthikeyan SR, Manikandan R, Durgadevi D, Keerthana U, Harish S, Karthikeyan G, *et al*. Bio-suppression of turmeric rhizome rot disease and understanding the molecular basis of tripartite interaction among *Curcuma longa*, *Pythium aphanidermatum* and *Pseudomonas fluorescens*. Biol Control. 2017;111:23-31. Available from: https://doi.org/10.1016/j.biocontrol.2017.05.003
12. Rajalakshmi J, Durgadevi D, Harish S, Raguchander T. Morphological and molecular characterization of *Pythium aphanidermatum*, the incitant of rhizome rot in turmeric. Int J Env Ecol Family Urban Stud. 2016;6(4):1-8.
13. Ramarethinam S, Rajagopal B. Efficacy of *Trichoderma* spp., organic amendments, and seed dressing fungicides on rhizome rot of turmeric. Pestology. 1999;13:21-30.
14. Rathiah Y. Control of soft rot of ginger with Ridomil. Pesticides. 1987;21:29-30.
15. Ravikumar P. Production technology for organic turmeric. Spice India. 2002;15:2-6.
16. Sathiyanarayanan A, Muthukrishnan S. Effect of chitosan on rhizome rot disease of turmeric caused by *Pythium aphanidermatum*. ISRN Biotechnol. 2014;2014:305349. Available from: https://doi.org/10.1155/2014/305349
17. Shanmugam V, Varma AS, Surendran M. Management of rhizome rot of ginger by antagonistic microorganisms. Madras Agric J. 1999;86:341-2.
18. Ushamalini C, Nakkeeran S, Marimuthu T. Induction of plant defense enzymes in turmeric plants by

Trichoderma viride. Arch Phytopathol Plant Protect. 2006;41(2):79-93. Available from: https://doi.org/10.1080/03235400600662293
19. Vinayarani G, Madhusudhan KN, Prakash HS. Induction of systemic resistance in turmeric by rhizospheric isolate *Trichoderma asperellum* against rhizome rot disease. J Plant Pathol. 2019;101:965-980. Available from: https://doi.org/10.1007/s42161-019-00303-9
20. Zieslin N, Ben-Zaken R. Peroxidase activity and presence of phenolic substances in peduncles of rose flower. Plant Physiol Biochem. 1993;31:333-339.