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In vivo efficacy of different native *Trichoderma* isolates and fungicides against rhizome rot of ginger in Manipur

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Abstract
Ginger is widely grown spice in India as well as in North East Region for its aroma and medicinal value. Even though it is one of the important spices in India, its cultivation is hampered by many biotic and abiotic factors. Among the biotic factors, rhizome rot or soft rot is the most devastating and destructive one. A field trial was conducted using ten native *Trichoderma* isolates (*T. harzianum*, ThCAUNCIPM-61, ThCAUNCIPM-41, ThCAUNCIPM-6, ThCAUNCIPM-44, *T. viride*, ThCAUNCIPM-25, ThCAUNCIPM-76, ThCAUNCIPM-18, ThCAUNCIPM-4) and one fungicide (copper oxychloride) to assess the disease and yield and yield contributing factors against *F. oxysporum*. Findings showed significant disease reduction as compared with the control. Besides chemical fungicide, copper oxychloride, *T. viride* treated plots showed lowest incidences (20.72 percent at Langol and 35.66 percent at Sawombung). In both the locations highest germination was observed in *T. harzianum* treated plot (96.67 percent at Langol and 97.78 percent at Sawombung) and the highest yield was found in copper oxychloride (20.11 tonnes / ha at Langol and 18.28 tonnes / ha at Sawombung). Among the *Trichoderma* spp., maximum yield was obtained with *T. harzianum* (18.60 tonnes / ha at Langol and 17.75 tonnes / ha at Sawombung). Higher yield was also observed in all other *Tichoderma* isolates treated plots and found to be better than the untreated plot.

Keywords: Rhizome rot, *Trichoderma*, disease, fungicide

Introduction
Ginger (*Zingiber officinale* Rosc.) is a herbaceous tropical perennial belonging to the family Zingiberaceae. India is considered as 'The land of spices and largest producer and exporter of ginger in the world followed by China, Nigeria, Indonesia, Bangladesh and Thailand. The North Eastern Hill (NEH) region of India is accounting 49% of India's ginger area and 72% of production. Among the various diseases affecting in ginger crop, rhizome rot or soft rot is a highly destructive disease and economically important which can destroy up to 80 to 90% of the crop (Lawrence, 1984) [9]. The disease is soil-borne and caused by *Pythium* sp., *Fusarium* sp., *Ralstonia* sp., etc. *Fusarium* yellows is probably the most serious more widely spread than bacterial wilt. It has been found in most commercial plantings in Hawaii and plants infected by the fungus, *Fusarium oxysporum* f.sp. *zingiberi* (Trujillo, 1963) [19]. Several fungicides and biocontrol agents have been recommended for the control of *Fusarium* sp. (Lawrence, 1984; Selvan *et al.*, 2002) [9, 16]. However, indiscriminate use of fungicide is harmful to human beings, animals and other beneficial microorganisms present in the ecosystem. Use of environment friendly biological agents effectively controlled many soil borne pathogens (Nam *et al.*, 1988; Park, 1989) [10-11].

Trichoderma, a filamentous soil borne saprophytic fungus is known to be one of the best candidates of biocontrol agents for the management of soil borne plant pathogens. The growing interest in non-chemical methods of pest and disease management are solely due to environmental and health hazards. Biological control of pathogens has proven not only ecologically but also economically sound as seen in the benefit/cost ratio (Salami *et al.*, 2005) [14]. *Trichoderma* species as potential biocontrol agents during the last few decades has enabled non-chemical plant disease management system and organic agriculture in particular. In India, 40 percent crop losses due to soft rot disease (Dohroo *et al.*, 2012) [6], 7.43 to 40.25 percent in North East India (Singh *et al.*, 2018) [17] and in Manipur soft rot of ginger disease incidence was reported to be ranged from 30.97 to 56.07 percent (Devi, 2012) [5].

Material and Methods

Experiments on the effect of ten isolates of *Trichoderma* spp. *viz.*, *T. harzianum*, ThCAUNCIPM-61, ThCAUNCIPM-41, ThCAUNCIPM-6, ThCAUNCIPM-44, ThCAUNCIPM-4, *T. viride*, ThCAUNCIPM-25, ThCAUNCIPM-76, ThCAUNCIPM-18 and one chemical fungicide, copper oxychloride on the control of soft rot of ginger was conducted in the field at two locations *viz.*, Langol and Sawombung with the ginger local cultivar Nonae as test plant. Rhizome treatment was done by mixing 40 gm of *Trichoderma* per kg of ginger rhizome and soil treatment was done by mixing 40 gm of *Trichoderma* per plot. The size of the plot was 1 × 3 m² area with 30 × 30 cm plant to plant spacing. Experiment was design in randomized block design (RBD) and three replications were kept for each treatment. Untreated plots served as control. Observations on germination percentage and disease incidence and yield (kg / plot) of ginger were taken right from germination till harvest.

Results

1. Effect of *Trichoderma* isolates and fungicide on the germination of ginger under *in vivo* conditions / field conditions

Efficacy of ten potent *Trichoderma* isolates *viz.*, *T. harzianum*, ThCAUNCIPM-61, ThCAUNCIPM-41, ThCAUNCIPM-6, ThCAUNCIPM-44, *T. viride*, ThCAUNCIPM-25, ThCAUNCIPM-76, ThCAUNCIPM-18, *Th*CAUNCIPM-4 and one fungicide (copper oxychloride) on the control of soft rot of ginger was conducted in the field at two locations namely Langol (hill) and Sawombung (plain) (Plate 1a and 1b). Observations on the germination percentage of ginger are presented in Table 1. The results show that germination percentage ranged from 77.78 to 96.67 percent in Langol and 76.67 to 97.78 percent in Sawombung. The maximum germination percentage at Langol location was observed in *T. harzianum* treated plots where the germination percentage was 96.67 percent followed by ThCAUNCIPM-61 (94.44 percent), ThCAUNCIPM-44 (94.44 percent), copper oxychloride (94.44 percent), ThCAUNCIPM-18 (93.33 percent), ThCAUNCIPM-41 (92.22 percent), ThCAUNCIPM-6 (92.22 percent), ThCAUNCIPM-4 (92.22 percent), ThCAUNCIPM-76 (92.22 percent), *T. viride* (91.11 percent) and ThCAUNCIPM-25 (87.78 percent). There was 77.78 percent germination in untreated control plots. At Sawombung also maximum germination percentage was observed in *T. harzianum* treated plot with 97.78 percent germination followed by ThCAUNCIPM-44 (95.56 percent), ThCAUNCIPM-76 (95.56 percent), copper oxychloride (95.56 percent), ThCAUNCIPM-6 (94.44 percent), ThCAUNCIPM-25 (94.44 percent), ThCAUNCIPM-18 (94.44 percent), ThCAUNCIPM-41 (93.33 percent), *T. viride* (93.33 percent), ThCAUNCIPM-4 (92.22 percent), ThCAUNCIPM-61 (91.11 percent) as compared to 76.67 percent in untreated control plots. Regarding pool mean, germination percentage of ginger ranged from 77.22 to 97.22 percent. *T. harzianum* showed maximum germination percentage with 97.22 percent followed by ThCAUNCIPM-44 (95.00 percent), copper oxychloride (95.00 percent), ThCAUNCIPM-76 (93.89 percent), ThCAUNCIPM-18 (93.89 percent), ThCAUNCIPM-6 (93.33 percent), ThCAUNCIPM-61 (92.78 percent), ThCAUNCIPM-41 (92.78 percent), ThCAUNCIPM-4 (92.22 percent), *T. viride* (92.22 percent), ThCAUNCIPM-25 (91.11 percent) as compared to 77.22 percent in untreated control plots. All the treatments are significantly increased germination percentage as compared with control.

Table 1: Effect of different *Trichoderma* isolates and fungicide on the germination of ginger at different locations under field condition

Sl. No.	Treatments	Germination percentage (Langol)	Germination percentage (Sawombung)	P-value of t-test	Pool mean
1	*T. harzianum*	96.67 (9.86) *	97.78 (9.91)	≥ 0.05	97.22 (9.86)
2	ThCAUNCIPM-61	94.44 (9.74)	91.11 (9.56)	≥ 0.05	92.78 (9.63)
3	ThCAUNCIPM-41	92.22 (9.62)	93.33 (9.69)	≥ 0.05	92.78 (9.63)
4	ThCAUNCIPM-6	92.22 (9.62)	94.44 (9.74)	≥ 0.05	93.33 (9.66)
5	ThCAUNCIPM-44	94.44 (9.74)	95.56 (9.80)	≥ 0.05	95.00 (9.74)
6	ThCAUNCIPM-4	92.22 (9.62)	92.22 (9.63)	≥ 0.05	92.22 (9.60)
7	*T. viride*	91.11 (9.57)	93.33 (9.69)	≥ 0.05	92.22 (9.60)
8	ThCAUNCIPM-25	87.78 (9.39)	94.44 (9.74)	≥ 0.05	91.11 (9.54)
9	ThCAUNCIPM-76	92.22 (9.63)	95.56 (9.80)	≥ 0.05	93.89 (9.69)
10	ThCAUNCIPM-18	93.33 (9.69)	94.44 (9.74)	≥ 0.05	93.89 (9.69)
11	Copper oxychloride	94.44 (9.74)	95.56 (9.80)	≥ 0.05	95.00 (9.75)
12	Control	77.78 (8.85)	76.67 (8.78)	≥ 0.05	77.22 (8.79)
	S.E. (d)±	0.24	0.18		0.14
	C.D. (5%)	0.49	0.37		0.28

P – Value ≤ 0.01, the test is highly significantly different.
P – Value ≤ 0.05, the test is significantly different.
P – Value ≥ 0.05, the test is not significant.
* Mean of three replications.
Figures in parenthesis are Square Root Transformed value

2. Effect of *Trichoderma* isolates and fungicide on the incidence of soft rot of ginger under *in vivo* conditions / field conditions

The results showed that percent disease incidence at Langol ranged from 17.75 to 37.14 percent in Langol and 24.47 to 55.08 percent in Sawombung (Table 2). Among the different treatments beside the chemical fungicide copper oxychloride (17.75 percent), *T. viride* was found to be the best where the disease incidence was 20.72 percent followed by *T. harzianum* (21.82 percent), ThCAUNCIPM-6 (22.88 percent), ThCAUNCIPM-41 (22.92 percent), ThCAUNCIPM-25 (23.91 percent), ThCAUNCIPM-61 (24.67 percent), ThCAUNCIPM-18 (24.94 percent), ThCAUNCIPM-76 (25.26 percent), ThCAUNCIPM-44 (28.18 percent) and

ThCAUNCIPM-4 (28.92 percent). Disease incidence under untreated control was 37.14 percent at Langol. At Sawombung also minimum percent disease incidence was observed in copper oxychloride (24.47 percent) and amongst the *Trichoderma* isolates, *T. viride* showed least disease incidence (35.66 percent) followed by *T. harzianum* (36.35 percent), ThCAUNCIPM-18 (36.45 percent), ThCAUNCIPM-76 (39.45 percent), ThCAUNCIPM-25 (40.01 percent), ThCAUNCIPM-61 (40.25 percent), ThCAUNCIPM-41 (41.57 percent), ThCAUNCIPM-6 (42.34 percent), ThCAUNCIPM-4 (44.48 percent) ThCAUNCIPM-44 (45.31 percent), as compared to 55.08 percent in untreated control plots. Regarding pool mean, minimum percent disease incidence was observed in copper oxychloride (21.11 percent) and amongst the *Trichoderma* isolates, *T. viride* showed least disease incidence (28.19 percent) followed by *T. harzianum* (29.08 percent), ThCAUNCIPM-18 (30.69 percent), ThCAUNCIPM-6 (31.50 percent), ThCAUNCIPM-25 (31.96 percent), ThCAUNCIPM-76 (32.36 percent), ThCAUNCIPM-41 (32.25 percent), ThCAUNCIPM-61 (32.46 percent), ThCAUNCIPM-4 (36.70 percent) and ThCAUNCIPM-44 (36.75 percent) as compared to 46.11 percent in untreated control plots. All the treatments are significantly reduced disease incidence as compared to untreated control.

Table 2: Effect of different *Trichoderma* isolates and fungicide on the incidence of soft rot of ginger under field condition

Sl. No.	Treatments	Percent disease incidence (Langol)	Percent disease incidence (Sawombung)	P-value of t-test	Pool mean
1	*T. harzianum*	21.82 (27.83) *	36.35 (37.05) *	≤ 0.01	29.08 (32.44)
2	ThCAUNCIPM-61	24.67 (29.75)	40.25 (39.39)	≤ 0.01	32.46 (34.57)
3	ThCAUNCIPM-41	22.92 (28.58)	41.57 (40.14)	≤ 0.01	32.25 (34.36)
4	ThCAUNCIPM-6	22.88 (28.57)	40.12 (38.38)	≤ 0.01	31.50 (34.58)
5	ThCAUNCIPM-44	28.18 (32.04)	45.31 (42.30)	≤ 0.01	36.75 (37.17)
6	ThCAUNCIPM-4	28.92 (32.54)	44.48 (41.84)	≤ 0.01	36.70 (37.19)
7	*T. viride*	20.72 (27.05)	35.66 (36.64)	≤ 0.01	28.19 (31.85)
8	ThCAUNCIPM-25	23.91 (29.25)	40.01 (39.25)	≤ 0.01	31.96 (34.25)
9	ThCAUNCIPM-76	25.26 (30.15)	39.45 (38.86)	≤ 0.05	32.36 (34.51)
10	ThCAUNCIPM-18	24.94 (29.93)	36.45 (37.13)	≤ 0.01	30.69 (33.53)
11	Copper oxychloride	17.75 (24.81)	24.47 (29.60)	≤ 0.05	21.11 (27.20)
12	Control	37.14 (37.54)	55.08 (47.93)	≤ 0.01	46.11 (42.73)
	S.E. (d)±	1.23	1.41		1.35
	C.D. (5%)	2.55	2.92		2.97

P – Value ≤ 0.01, the test is highly significantly different.
P – Value ≤ 0.05, the test is significantly different.
P – Value ≥ 0.05, the test is not significant.
* Mean of three replications.
Figures in parenthesis are Arc sine Transformed value

3. Effect of *Trichoderma* isolates and fungicide on the yield of ginger under *in vivo* conditions / field conditions

Results shows that the range of yield was found from 3.93 to 6.03 kg at Langol and 3.65 to 5.48 kg at Sawombung. At Langol, maximum yield was obtained in copper oxychloride (6.03 kg) (Table 3). Besides the chemical fungicide, *T. harzianum* treated plots showed maximum yield which was 5.58 kg followed by *T. viride* (5.42 kg), ThCAUNCIPM-25 (5.40 kg), ThCAUNCIPM-41 (5.16 kg), ThCAUNCIPM-76 (5.14 kg), ThCAUNCIPM-4 (4.89 kg), ThCAUNCIPM-6 (4.80 kg), ThCAUNCIPM-18 (4.87 kg), ThCAUNCIPM-44 (4.66 kg), ThCAUNCIPM-61 (4.49 kg), as compared to 3.93 kg in untreated control plots. At Sawombung also the maximum yield was also obtained in copper oxychloride (5.48 kg). Besides the chemical fungicides, highest yield was obtained in *T. harzianum* treated plots (5.27 kg) followed by *T. viride* (5.20 kg). However, in untreated control plots yield was only 3.65 kg per plot. Regarding pool mean, the maximum yield was also obtained in copper oxychloride (5.76 kg). Besides the chemical fungicides, highest yield was obtained in *T. harzianum* treated plots (5.43 kg) followed by *T. viride* (5.31 kg). However, in untreated control plots yield was only 3.79 kg per plot. All the treatments are significantly increased the yield per plot as compared to untreated control. Size of rhizomes of healthy plants was larger than the rhizome of infected plants (Plate 2a and 2b).

Table 3: Effect of different *Trichoderma* isolates and fungicide on the yield of ginger plant

Sl. No.	Treatments	Yield (Langol) tonnes per ha	Yield (Sawombung) tonnes per ha	P-value of t-test	Pool mean (Yield in tonnes per ha)
1	*T. harzianum*	18.60	17.57	≥ 0.05	18.08
2	ThCAUNCIPM-61	14.98	14.22	≥ 0.05	14.60
3	ThCAUNCIPM-41	17.19	16.00	≥ 0.05	16.59
4	ThCAUNCIPM-6	15.99	14.94	≥ 0.05	15.47
5	ThCAUNCIPM-44	15.52	14.72	≥ 0.05	15.12
6	ThCAUNCIPM-4	16.29	14.84	≥ 0.05	15.57
7	*T. viride*	18.08	17.33	≥ 0.05	17.71
8	ThCAUNCIPM-25	18.00	15.61	≤ 0.05	16.81
9	ThCAUNCIPM-76	17.13	15.83	≥ 0.05	16.48
10	ThCAUNCIPM-18	16.22	15.61	≥ 0.05	15.92
11	Copper oxychloride	20.11	18.28	≥ 0.05	19.19

12	Control	13.11	12.17	≤ 0.05	12.64
	S.E. (d)±	0.74	0.54		0.72
	C.D. (5%)	1.54	1.14		1.51

P – Value ≤ 0.01, the test is highly significantly different.
P – Value ≤ 0.05, the test is significantly different.
P – Value ≥ 0.05, the test is not significant.

Discussion

In vivo efficacy of ten potent *Trichoderma* isolates and one fungicide *viz*., *T. harzianum*, ThCAUNCIPM-61, ThCAUNCIPM-41, ThCAUNCIPM-6, ThCAUNCIPM-44, ThCAUNCIPM-4, *T. viride*, ThCAUNCIPM-25, ThCAUNCIPM-76, ThCAUNCIPM-18 and copper oxychloride against *F. oxysporum* showed significant disease reduction at both the two locations enhancing the growth and yield of ginger as compared to control under field conditions. Besides chemical fungicide (copper oxychloride), the result of the experiment revealed that *T. viride* was found most effective in suppressing the soft rot disease incidence. The efficacy of *Trichoderma* spp. as observed in the present investigations in suppressing *F. oxysporum* with increased growth parameters and yield have also been reported by different workers (Srivastava, 1994; Rajan *et al*., 2002; Daiho and Upadhyay, 2004; Sangle, and Bambawal, 2004) [18, 12, 3, 15]. Ram *et al*. (2000) [13] also reported that *T. harzianum* could establish in ginger rhizosphere and reduce the incidence of *Fusarium* sp. that correlated well with a significant increase in yield. Hermosa *et al*., (2012) [7] reviewed *Trichoderma*-plant interaction involving molecular dialogue between the two organisms, and reported dramatic changes in plant resistance and stress tolerance induced by the *Trichoderma* strains. The reduction of the severity of plant diseases by inhibiting plant pathogens through highly potent antagonistic and mycoparasitic activity of *T. viride* in the present study is thus in agreement with the reports of the earlier workers. Dohroo *et al*. (2012) [6] reported that four periodic drenching of copper oxychloride @ 0.3% at 15 days interval during rainy season proved most efficacious in limiting the soft rot disease besides improving the yield and growth parameters of ginger plants. Khatso and Tiameren (2013) [8] tested the efficacy of some biological control agents for their antagonistic ability against *F. oxysporum* f.sp. *zingiberi* under *in vivo*.

Conclusion

In the field trial, it was also observed that soft rot disease incidence was found to be high at Sawombung (Plain) location as compared to the Langol (Hill) in all the treatments as well as in untreated control. The difference may be due to the variation in the microclimatic conditions that favours the adaptation and multiplication of the causal pathogens and also due to the difference in the soil types of the two locations. Biocontrol is a promising tool to maintain current level of agriculture production by reducing the release of polluting chemical pesticides to the environment causing health hazards. The native potent *Trichoderma* spp. will provide great antagonist potential for the management of soil borne diseases. The present investigation will give information regarding the potentiality of native *Trichoderma* spp. for the management of soft rot of ginger.

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