Biocontrol of Rhizome Rot Disease of Ginger (*Zingiber officinale* Rosc.)

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Abstract

The efficacy of some biological control agents were tested for their antagonistic ability against *Fusarium oxysporum* f.sp *zingiberi* both *in vitro* and *in vivo*. Among the biological control agents assayed, *Trichoderma viride* (68.3%) and *Trichoderma harzianum* (66.7%) exhibited the maximum mycelial growth inhibition in dual culture under *in vitro*. Under field condition, seed treatment with *T.viride* @ 4g 10 ml^{-1} of water kg^{-1} of seed resulted in maximum reduction in plant mortality (4.2.%) with consequent increase in disease control (84.9%), plant stand over control (32.8.%), plant height (48.9 cm), number of tillers (18.0) and yield (10.5 kg $plot^{-1}$), respectively.

1. Introduction

Ginger (*Zingiber officinale* Rosc.) occupy an important position among the cultivated spices in the country and is next only to black pepper and cardamom. It is popular not only for its use as spices and condiments but also for its use in perfumery and food flavouring and is credited with multifarious medicinal properties. In Nagaland, it is one of the most valuable cash crops grown. The crop is however severely affected with rhizome rot caused by *Fusarium oxysporum* f.sp *zingiberi* and poses perpetual problem to its production and cultivation in the state. The disease has been found to appear as pre-emergence or post-emergence rotting of rhizomes causing heavy losses even up to 92% in some local cultivars (Daiho and Upadhyay, 2004). The pathogen is both seed as well as soil borne. Efforts have been made to control this disease by several workers and the efficacy of different chemicals against the disease is well documented (Das et al., 1990; Haware and Joshi, 1974; Sharma and Rana, 2000; Singh et al., 2004: Sterling, 2004). However, their negative impacts on environment and health outweigh their efficacy. Moreover, organic ginger cultivation is very much a feasible proposition in the state where chemical use is minimum or absolutely nil in some areas. Biological control of plant pathogens is considered safe and durable; the need to explore the potentialities of biological strategies specific to the environmental conditions of Nagaland is therefore imperative. In this paper, we report the effect of seed and soil treatment with two fungal antagonists' viz., *Trichoderma viride* and *Trichoderma harzianum* on rhizome rot pathogen of ginger.

2. Materials and Methods

2.1. Source of pathogen and bioagents

Fusarium oxysporum f.sp *zingiberi,* the causal organism of rhizome rot of ginger, was isolated from diseased tissues of infected standing plants in the field and further purified and maintained in PDA at 28±1°C. Cultures of *Trichoderma virens*, *Trichoderma harzianum, Trichoderma viride and Trichoderma koningii*, was obtained from the Department of Plant Pathology, Nagaland University, School of Agricultural Sciences and Rural Development, Medziphema campus and Biocontrol Laboratory, Department of Agriculture, Government of Nagaland, Medziphema respectively. The cultures were maintained on PDA at pH 6.0. Sub culturing was done periodically to maintain the purity of the cultures.

2.2. In vitro test

Interactions between the antagonists and the pathogens were

studied in dual culture. *Fusarium oxysporum* f.sp *zingiberi* and the antagonistic fungi were grown separately in petridishes containing PDA for 72 hr. Two mycelial discs (5 mm diam.) of each antagonist, *T. virens* , *T. harzianum, T. viride* and *T. koningii*, were placed one opposite the other in separate petri dishes (90 mm diam.) approximately at 1 cm from the periphery of the plate. In the centre of each petri dish a disc of the pathogen, *F. oxysporum* f.sp *zingiberi* was placed. A control having the test pathogen only was kept for comparison. The Petri dishes were incubated at 28±1°C maintaining five replications for each treatment. Observation on radial growth of *F. oxysporum* f.sp *zingiberi* was recorded at 24 hr interval till the control dish was completely covered *F. oxysporum* f.sp *zingiberi*. The% growth inhibition of the test pathogen as compared to that of control was also calculated using the following formula:

$PI=(A_1-A_2)\div A_1\times 100$

Where, PI=% inhibition; A_1=Radial growth of *F. oxysporum* f.sp *zingiberi* in control plates and A_2=Radial growth of *F. oxysporum* f.sp *zingiberi* in treated plates.

2.3. Field test

The field experiment was laid out in Randomized Block Design with three replications. A highly susceptible ginger cultivar, *Nadia* was planted by maintaining a spacing of 20×30 cm plant to plant and row to row in a plot size of 1×3 m^2 under a total net area of 14×42 m^2. Farm Yard Manure was applied 1 week before planting as basal dressing @ 3 kg in 1×3 m^2 plots. The different treatments included seed and soil treatment with *T. viride, T. harzianum* and Bavistin (Carbendazim 50 WP). Two controls *viz*., inoculation with and without *F. oxysporum* were also maintained for comparison. For seed treatment, the rhizomes were dipped in slurry of talc based (@ 2% kg^{-1} of rhizome) *T. viride* and *T. harzianum* @ 4 g 10 ml^{-1} water kg $seed^{-1}$ for one hour and with Carbendazim 50 WP @ 0.2% for 30 minutes respectively. The treated rhizomes were then air dried for 24 hours at room temperature before planting. Soil treatment was done after planting by drenching the soil around the seed rhizome with suspension containing *T. viride* and *T. harzianum* (talc based with water) @ 5 g lit^{-1} 40m^{-2} area and @ 100 ml $plant^{-1}$ with Carbendazim (0.2%). Mass cultures of *F. oxysporum* prepared in MSM was directly inoculated in the soil @ 200 g $m^{2\ -1}$ and thoroughly mixed with the top 10 cm of the soil profile. Inoculation of *F. oxysporum* was carried out in all the treatment plots except uninoculated control plot and incubated for three days before planting. Observations on% plant mortality, disease control, and increase in plant stand over control was recorded 60 days after sowing (DAS) at 30 days interval till harvest and calculated following De and Mukhopadhyay (1994). Plant height, number of tillers $hill^{-1}$ and yield were also recorded.

3. Results and Discussion

3.1. In vitro test

In dual culture, *T. virens* , *T. harzianum, T. viride* and *T. koningii* inhibited the growth of *F. oxysporum* showing considerable increase in their biocontrol potency with time (Table 1). It was observed that the different *Trichoderma* species exerted varied degree of stress on *F. oxysporum* in culture. Significant variations in the inhibitory properties of *Trichoderma* species tested were discernible when assessed at 120 hours of inoculation (Figure 1). Among the antagonists, *T. viride* at 68.3% was observed to be more aggressive and hence superior to *T. harzianum* at 66.7%, *T. virens* at 61.1% and *T. koningii* at 57.2% as evidenced by the higher% inhibition at 120 hr of incubation. In culture, besides the reduction of the radial growth, overgrowths of the pathogen and colony degradation by the antagonists were observed. Microscopic examination also showed that the hyphae of *T.viride* and *T.harzianum* coiled around the hyphae of *F. oxysporum* followed by cell wall degradation and cellular coagulation of the pathogen. Mycoparasitic behaviours involving envelopment and coiling around of the hyphae of *F. oxysporum* by *Trichoderma* species has been reported by Otadoh et al., (2011). Microscopic examination, in the present study also showed constrictions of the mycelia of *Fusarium* with marked differences in

Table 1: Effect of antagonists on radial growth of *F. oxysporum* f.sp *zingiberi* in culture, bioassayed in PDA at 28 ± 1°C at different hours of incubation

Treatments	24 hrs (mm*)	GI%	48 hrs (mm*)	GI %	72hrs (mm*)	GI%	96 hrs (mm*)	GI%	120 hrs (mm*)	GI %
F. oxysporum+T. harzianum	4.5	10	20.0	20	25.5	43.3	28.5	56.2	30.0	66.7
F. oxysporum+T. virens	5.0	-	22.0	12	28.0	37.8	33.5	48.5	35.0	61.1
F. oxysporum+T. viride	4.3	14	22.5	10	24.0	46.7	27.0	58.5	28.5	68.3
F. oxysporum+T. koningii	5.0	-	24.0	4	28.5	36.7	35.0	46.2	38.5	57.2
Control	5.0	-	25.0	-	45.0	-	65.0	-	90.0	-
CD (p=0.05)	0.5		2.9		4.1		5.9		7.1	

*Average of five replications, GI=Growth inhibition.

the morphology between *F. oxysporum* in control and *F. oxysporum* in treatment similar to the observation made by Sharma (2011).

The biocontrol potential of *Trichoderma* spp. is a result of a number of qualities which include antagonism, antibiotics and degrading enzymes which digest the cell wall (Brian and Hemming, 1945; Elad et al. 1982; Elad et al., 1983; Jones and Hancock, 1988; Harman and Björkman, 1998). Henis et al., (1983) observed *T. harzianum* to secrete large amount of chitinase and ß-(1,3)-glucanase while Claydon et al. 1987) reported the production of a pyrone compound, 6-n-pentyl-2H-pyron-2-one by *T. harzianum* which has antibiotic properties. Vinale et al. (2008) also reported the mycoparasitic prowess of *Trichoderma* species involving specific high molecular weight compounds and low molecular weight degradation products that are released by the host cell walls triggering the myco-parasitic gene expression cascade of the antagonists. The intense inhibitory activity of the antagonist observed in dual cultures and the frequency of mycoparasitic activities and the antibiosis by the antagonists against *F. oxysporum* in the present investigation is probably due to the production of these toxic metabolitics, antibiotics, volatile gases and cell wall degrading enzymes.

3.2. Field test

The results obtained from the field test showed that% mortality and disease control were significantly influenced by all the treatments as compared to inoculated control (Table 2). Amongst all the treatment, seed treatment with *T. viride* recorded maximum reduction in plant mortality (4.2%) with consequent increase in disease control (84.9%) and plant stand over control (32.8%) respectively (Figure 2).

Table 2: Effect of different treatments on mortality and disease control at 180 DAS

Treatments	Mortality (%)*	Disease control (%)*
T_1	27.8	0
T_2	19.4	30.2
T_3	16.9	75.4
T_4	5.5	79.4
T_5	4.2	84.9
T_6	5.5	79.4
T_7	5.5	79.4
T_8	4.2	84.1
CD (p=0.05)	5.2	16.8

*Average of three replications

T_1=Inoculated control; T_2=Uninoculated control; T_3=Seed treatment with *T. harzianum*; T_4=Soil application with *T. harzianum*; T_5=Seed treatment with *T. viride*, T_6=Soil application with *T. viride*; T_7=Seed treatment with Carbendazim; T_8=Soil application with Carbendazim

Seed treatment with *T. viride* also recorded maximum height (48.9 cm) at 180 days DAS (Table 3). Maximum number of tillers at 180 DAS was recorded when Carbendazim was applied as seed treatment (17.2). It was however statistically at par with *T. viride* when applied as seed treatment (16.7). The highest yield was observed when Carbendazim was applied as soil treatment (10.7 kg plot^{-1}) and with *T. viride* when applied as seed treatment (10.6 kg plot^{-1}). Incidence of rhizome rot was recorded at regular intervals. Among the treatments, the minimum disease incidence of 2.8, 5.5 and 6.9% were observed on 60th and 120th and 180th DAS respectively when *T. viride* was applied as seed treatment which was at par with Carbendazim when also applied as seed treatment. All the treatments significantly decreased disease incidence as compared to control and was statistically at par (Table 3).

The efficacy of *Trichoderma* spp, as observed in the present investigation, in suppressing *Fusarium* induced diseases with increased growth parameters and yield have been reported by different workers (Bhardwaj et al., 1988; Srivastava, 1994; Rajan et al., 2002; Daiho and Upadhyay, 2004; Sangle, and Bambawal, 2004). Ram et al. (2000) 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. Moreover, research in recent decades, has

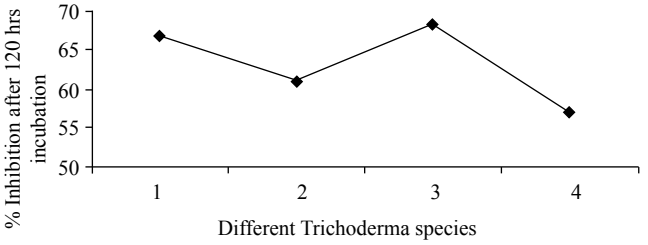
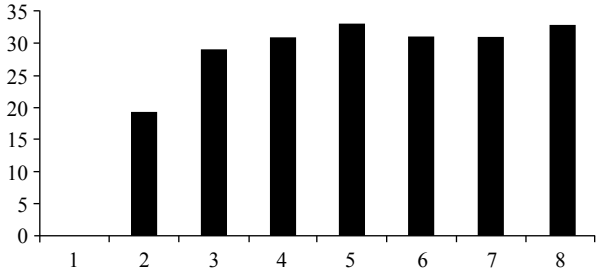


Figure 1: Inhibition of mycelial growth of *F. oxysporum* after 120 hrs of inoculation 1=*T harzianum*; 2=*T. virens;* 3=*T. viride;* 4=*T. koningii*



1=Inoculated control; 2=Uninoculated control; 3=*T. harzianum* (seed treatment); 4=*T. harzianum* (soil treatment); 5=*T. viride* (seed treatment); 6=*T. viride* (soil treatment); 7=Carbendazim (seed treatment); 8=Carbendazim (soil treatment)

Figure 2: Effect on different treatments on% increase in plant stand over control

Table 3: Effect of different treatments on germination, growth, yield and disease incidence of ginger in the field

Treatments	Germination %	Plant height (cm)	Number of tillers	Yield plot^{-1} (kg)	Disease incidence (%)		
					60 DAP	120 DAP	180 DAP
T_1	90.3	29.7	13.4	6.4	8.3	19.4	34.7
T_2	91.6	33.3	14	7.2	8.3	15.3	25
T_3	95.8	37.7	15	8.9	4.2	8.3	9.7
T_4	91.6	32.5	14.8	9.1	4.2	9.7	8.3
T_5	95.8	48.9	16.7	10.5	2.8	5.5	6.9
T_6	93	39.3	15.3	9.5	4.2	6.9	8.3
T_7	95.8	38.4	17.2	9.5	2.8	5.5	6.9
T_8	97.2	40.5	15.3	10.6	1.4	4.2	5.5
CD (p=0.05)	3.6	NS	1.9	1.8	4.3	4.8	7.7

*Average of three replications

T_1=Inoculated control; T_2=Uninoculated control; T_3=Seed treatment with *T. harzianum*; T_4=Soil application with *T. harzianum*; T_5=Seed treatment with *T. viride*, T_6=Soil application with *T. viride*; T_7=Seed treatment with Carbendazim; T_8=Soil application with Carbendazim

revealed that some Trichoderma strains can interact directly with roots, increasing plant growth potential, resistance to disease and tolerance to abiotic stresses. Hermosa et al., (2012) reviewed Trichoderma-plant interaction involving molecular dialogue between the two organisms, and reported the 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 *Trichoderma viride* in the present study is thus in agreement with the reports of the earlier workers.

Application of Carbendazim as soil and seed treatment also resulted in reduced plant mortality with subsequent increase in disease control and plant stand over control. Rana and Sharma (1993) reported that ginger rhizome treated with Carbendazim (0.1%)+Mancozeb (0.25%) effectively reduced the incidence of *F. oxysporum*. Haware and Joshi (1974) suggested the control of rhizome rot by soaking seed rhizome in fungicidal suspension of Dithane M-45 or Bavistin. The result obtained from the present experiment revealed that seed treatment with *T. virens* was found to be more effective in enhancing the growth of plants and suppressing rhizome rot disease incidence. Carbendazim applied both as seed and soil treatment was found to be equally effective in reducing the disease and enhancing plant growth. Analysis of variance of the data obtained however, indicated that application of *T. virens* and Carbendazim were statistically at par.

4. Conclusion

The need for increasing agricultural productivity and quality has led to an excessive use of chemicals, creating serious environmental pollution. Given the adverse effect of chemicals, notwithstanding their effectiveness, the prospect of use of biocontrol, as indicated in the present studies, however, offer a very promising field for further exploration in the management of rhizome rot of ginger under Nagaland condition.

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