

Rhizome rot of ginger-management through non-chemical approach

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ABSTRACT

Ginger (*Zingiber officinale* Rosc.) an important spice crop grown in different states of India especially Himachal Pradesh, a hilly area situated in northern Himalayas. *Pythium* and *Fusarium* are the main fungus which affect the crop in a drastic manner. Ginger rhizome diseases are both rhizome seed and soil borne and their chemical management leads to notorious effect on environment and ecosystem. Therefore, an attempt to work out for isolation of *Pythium* and *Fusarium* sp. (major pathogens causing ginger rot) from Sirmaur and Solan areas of the state and research emphasized on non-chemical management of these fungal diseases. Hot water treatment of ginger rhizomes at different temperatures excluded the maximum rhizome borne inoculum through eradication. Among the biocontrol agents *T. harzianum* was found more effective for pathogenic fungal inhibition recorded as (50.28%) followed by *T. hamatum* (44.94%) and *Streptomyces* sp. (40.11%).

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INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an important spice crop producing country (3, 82,600 tonnes) in the world, accounting for about one-fourth of the total world production (1,615,974 tonnes). (Dohroo *et al.*, 2015). Crop is affected by a number of diseases leading to great crop damage and yield loss (Sharma and Jain, 1977). Among all diseases of ginger rhizome rot of ginger caused by *Pythium* and *Fusarium* sp. was first reported in India by Mehrotra (1952), is a major bottleneck, causing

huge crop losses upto 70 per cent in a cropping season (Dohroo, 2001). Although disease can be controlled through various fungicides (Gangawane and Shaikh, 1988) but it become non-sustainable and cost-effective management. Also the use of fungicidal applications leads to soil pollution which affect the crop and thus, causing carcinogenic type problems in human beings. Keeping in view the huge loss (> 50%) caused by rhizome rot of ginger a non chemically and ecofriendly experiment was conducted for management of *Pythium* and *Fusarium*

fungus using biocontrol agents viz., *Trichoderma* spp. and *Streptomyces* sp.

MATERIAL AND METHODS

Survey and surveillance :

Different ginger growing pockets of Himachal Pradesh, a northern hilly state of India which include dist Solan and Sirmour were surveyed during the months of August and October. Occurrence of rhizome rot of ginger, disease incidence and prevalence were recorded during these peak periods. No specific permissions were required for these locations and the field studies did not involve endangered or protected species.

Collection, isolation and identification of the pathogen :

Diseased samples of ginger rhizomes were collected in sterilized polybags and plant samples were rinsed thoroughly under running tap water. Specimens were cut into 0.5 cm long segments, blotted dry on paper towels, and placed on to 2 per cent water agar (Vander, 1981). Cultures were incubated at room temperature (20-24°C) and observed daily for the emergence of fungal mycelium from the tissue. Pure culture was maintained on PDA at 4°C. For studying the cultural and morphological characters, the fungus was grown on PDA medium. Cultural characters viz., colour and growth of colony were determined by visual observation. Morphological characteristics of the fungus like mycelia, conidiophores and conidia were microscopically studied (Carl Zeiss Axiovert 25 inverted microscope).

Pathogenicity test :

Pathogenicity tests of causal organisms of rhizome rot of ginger (*Fusarium* sp.) was conducted according to Koch's postulates under pot culture conditions. Pathogen was then again re-isolated from infected ones and compared with original ones.

Non-chemical disease management :

- Physical method
- Hot water treatment

Mycelial bits (5 in no.) of different non-pathogenic and pathogenic fungi of ginger viz., *Trichoderma harzianum*, *Trichoderma harzianum*, *F. oxysporum* and *Pythium* sp. were subjected to hot water treatment (10 ml) at different temperatures viz., 40, 45, 47, 50 and

55 °C for different intervals of time (15, 30, 45 and 60 minutes). After then bits were dried in sterilized filter paper to drain off excess moisture content and transferred to PDA with an incubation of 25°C for fungal growth.

Use of biocontrol agents :

To study the efficacy of bioagents against *Pythium* sp. and *F. oxysporum*, an experiment was conducted with three bioagents viz., *Trichoderma harzianum*, *Trichoderma hamatum* and *Streptomyces* sp. which were obtained from the Department of Mycology and Plant Pathology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. These were tested using Dual Culture Technique (Huang and Hoes, 1976). Culture discs of 5 mm from the margins of seven days and four days old pure culture of pathogen and biocontrol agents, respectively, were transferred on PDA under aseptic conditions to 90 mm diameter Petri plates on opposite sides with the help of sterilized cork borer and needle. The disc of pathogen on the PDA served as control. The plates were incubated at 25°C. The linear growth of the bioagents and pathogen from the centre of the disc towards the centre of the plates was recorded after the control plate was completely covered by the pathogen. Per cent inhibition was calculated as described by Vincent (1947):

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

where,

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm).

Field studies :

The field experiments were conducted at the Experimental Farm of the Department of Vegetable Science of Dr Y.S. Parmar University of Horticulture and Forestry. For preparation of the beds, the land was cleared of weeds and soil was then tilled with a tractor mounted disk harrow, puddled to a fine tilth and leveled using a soil leveler. Raised beds of size 3 × 1 × 0.30 m (l × b × h) were made and a spacing of 40 cm was allowed between the beds for drainage. Small shallow pits for planting seed rhizome were then made on the beds at a spacing of 30 x 20 cm. In this experimental design RBD (Randomized Block Design) experiment with four replications, *T. harzianum*, *T. hamatum* were applied @400g per plot 15 days before planting rhizomes

of ginger. Cabbage and pea residues were spread @ 7kg/plot one month before rhizome plantation. The plots were covered with polythene sheets to develop a biodisinfestation effect.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Disease incidence and prevalence :

Maximum disease incidence (28.26%) and prevalence of disease (100 %) was recorded in distt Sirmour whereas the disease incidence of 19.64 per cent with the prevalence of 98.44 per cent was recorded in distt Solan.

Isolation, purification and identification of fungus :

The fungus grown on PDA medium produced slow growing snow-white colonies. The strain also produced three types of spores called macroconidia and microconidia (Fig.1). Most of the morphological characters of the first pathogen agree with the known features of *Fusarium* sp. On the basis of morphological characters second pathogen causing rhizome rot was identified as *Pythium* sp.

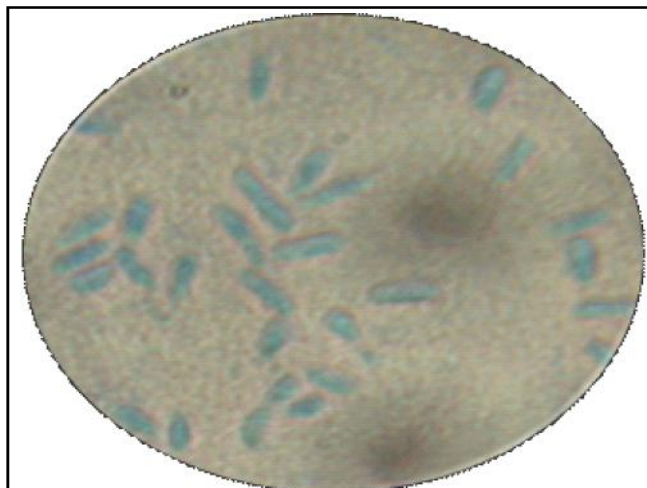


Fig. 1: Macroconidia and microconidia of *Fusarium*

Pathogenicity test :

For approval of Koch's postulates soil was inoculated with population of *Pythium* (31.3×10^3) and *Fusarium* ($105 \times 10^5 \text{ cfu ml}^{-1}$) and symptoms were recorded during rhizome rot development. Fig. 2 showed effect of fungal attack on ginger root, rhizome and leaves with subsequent incubation days and it was found that inocula mixture of both *Fusarium* and *Pythium* was much

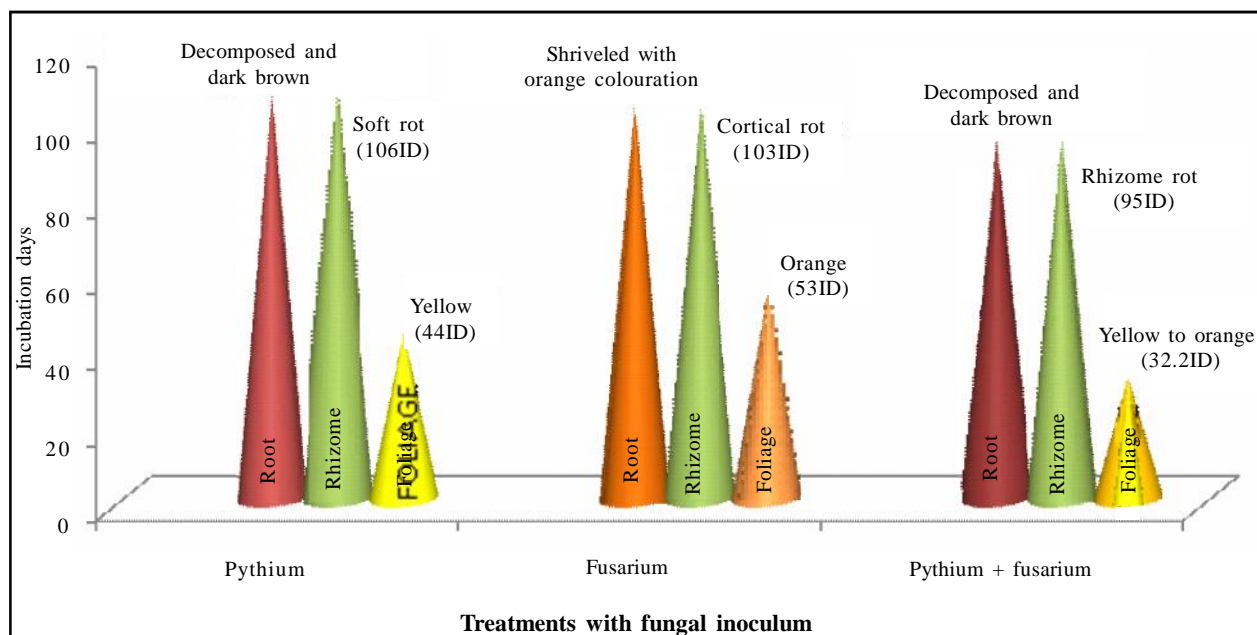


Fig. 2 : Symptoms developed on ginger plant treated with fungal inoculums (Symptoms were recorded in root, rhizome and foliage with respective ID [Incubation days] after spore inoculation of *Pythium*, *Fusarium* and *Pythium*+ *Fusarium*)

effective in disease development at early incubation days (95 ID for rhizome rot and 32 ID for foliage decoloration).

Non- chemical disease management :

Hot water treatment :

Data revealed in Table 1 evidenced that all test non-pathogenic and pathogenic fungi were inhibited at 40°C. Maximum overall mean growth inhibition was observed in *Fusarium* (24.18%) followed by *Pythium* sp. (22.01%) and *T. hamatum* (14.60%) whereas the least inhibition was found in *T.harzianum* (10.54%). Data revealed in Table 2 evidenced that all test non-pathogenic and pathogenic fungi were inhibited at 45°C maximum overall mean growth inhibition was observed in *Fusarium* (27.88%) followed by *Pythium* sp. (24.71%) and *T. hamatum* (20.68%) whereas the least inhibition was found in *T. harzianum* (14.57%). Maximum overall mean growth inhibition was observed in *T. hamatum* (46.47%)

followed by *Pythium* sp. (36.99%) and *Fusarium* (35.64%), whereas the least inhibition was found in *T. hamatum* (20.12%) during hot water mycellial treatment at 50°C as evident from Table 3. Exposure of hot water treatment was studied at 55°C for mycellial inhibition. Table 4 justified maximum overall mean growth inhibition was recorded in *T. hamatum* (90.00%) followed by *Pythium* sp. (46.56%) and *Fusarium* (45.21%), whereas the least inhibition was found in *T. harzianum* (22.94%).

In vitro studies :

In the present study, efforts were made to determine the antagonistic activity of the bioagents against test pathogens (*Fusarium* and *Pythium*) so that the effective antagonists could be identified and tested for disease management in the field experiment. Data revealed in Table 5 maximum growth inhibition (52.20%) was showed by *T. harzianum* followed by *T. hamatum*

Table 1 : Hot water treatment at 40 °C					
Treatment	Per cent mycellial inhibition in hot water treatment (minutes)				Mean
	15	30	45	60	
<i>Fusarium</i>	4.93(12.66)	8.63(17.01)	18.51(25.41)	29.62(32.95)	17.91(24.18)
<i>Pythium</i>	7.01(15.15)	12.27(20.42)	21.05(27.19)	31.30(33.98)	15.43(22.01)
<i>T. hamatum</i>	2.89(9.65)	4.34(11.76)	6.51(14.65)	14.49(22.33)	7.06(14.60)
<i>T. harzianum</i>	1.64(7.25)	2.76(9.50)	4.11(11.67)	5.75(13.75)	3.56(10.54)
Mean	4.12(11.18)	7.00(14.67)	12.55(19.73)	20.29(25.75)	

Figures in parentheses are arcsine transformed values

Table 2 : Hot water treatment at 45 °C					
Treatments	Per cent mycellial inhibition in hot water treatment (minutes)				Mean
	15	30	45	60	
<i>Fusarium</i>	7.40(15.45)	18.51(25.03)	18.51(25.41)	29.62(32.95)	23.63(27.88)
<i>Pythium</i>	7.01(15.15)	15.78(23.21)	26.31(30.79)	45.43(42.38)	18.51(24.71)
<i>T. hamatum</i>	2.89(9.65)	10.86(19.19)	17.37(24.60)	23.91(29.26)	13.76(20.68)
<i>T. harzianum</i>	2.46(8.82)	4.93(12.77)	7.79(16.14)	12.34(20.55)	6.88(14.57)
Mean	4.94(12.27)	12.52(20.05)	17.50(24.24)	27.83(31.28)	

Figures in parentheses are arcsine transformed values

Table 3 : Hot water treatment at 50 °C					
Treatments	Per cent mycellial inhibition in hot water treatment (minutes)				Mean
	15	30	45	60	
<i>Fusarium</i>	22.22(28.07)	32.09(34.48)	38.27(38.20)	44.44(41.80)	34.25(35.64)
<i>Pythium</i>	21.05(27.19)	31.30(33.98)	41.83(40.28)	52.63(46.51)	36.70(36.99)
<i>T. hamatum</i>	36.95(37.43)	51.44(45.83)	56.52(48.75)	65.21(53.86)	52.53(46.47)
<i>T. harzianum</i>	5.57(13.75)	12.34(20.55)	13.58(21.61)	17.28(24.55)	12.24(20.12)
Mean	21.49(26.61)	31.79(33.71)	37.55(37.21)	44.89(41.68)	

Figures in parentheses are arcsine transformed values

(43.4%) and *Streptomyces* sp. (40.99%) against *Fusarium* sp. whereas in case of *Pythium* maximum growth inhibition (48.36%) was showed by *T. harzianum* which is statistically at par with *T. hamatum*. However, the least growth inhibition was observed in *Streptomyces* sp. (39.23%). Among these bioagents tested *T. harzianum* was best with the highest overall mean growth inhibition (50.28%) followed by *T. hamatum* (44.94%) and *Streptomyces* sp. (40.11%).

Field experiment :

The effectiveness of non-chemical soil treatments viz., *T. harzianum*, *T. hamatum*, pea biodisinfestation, cabbage biodisinfestation was tested in a field experiment and data is listed Table 6. Maximum emergence of tillers was found in case of *T. harzianum* (77.67%) which is statistically par with all other soil treatments. Maximum disease incidence on foliage was found in Pea biodisinfestation (3.87). *T. harzianum* was found to be the best treatment in control of rhizome rot (21.51%) which is statistically at par with *T. hamatum* (23.67%)

followed by Pea biodisinfestation (25.53). Highest yield of fresh rhizomes (4.231kg) was observed in *T. harzianum* and minimum yield was observed in cabbage biodisinfestation (2.962 kg).

Ginger is the most important cash crop of Himachal Pradesh and for successful cultivation presence of rhizome rot become the major constraint. Surveys were conducted to record the disease incidence and its prevalence. Maximum disease incidence of rhizome rot was recorded in Sirmour (28.26%) followed by Solan (19.64%). Various surveys for ginger rhizome rot were available literature (Ekka and Prasad, 2009; Rekha *et al.*, 2015 and Moreira *et al.*, 2013). Pure culture identified from roots and rhizomes of ginger plants were *Pythium ultimum* and *Fusarium oxysporum*. Pathogenicity test revealed rhizome incubation period was longer (101) days as compared to young foliage which was associated with *P. ultimum* and *F. oxysporum*. Similar findings were supported by Robin *et al.* (2008). The hot water treatment has been known to exclude pathogenic fungi by eradication and might

Table 4 : Hot water treatment at 55 °C

Treatments	Per cent mycellial inhibition in hot water treatment (minutes)				Mean
	15	30	45	60	
<i>Fusarium</i>	37.03(37.47)	41.97(41.36)	46.91(43.22)	61.72(52.79)	46.91(45.21)
<i>Pythium</i>	36.84(37.34)	47.36(43.38)	57.89(49.56)	68.42(55.86)	52.63(46.56)
<i>T.hamatum</i>	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
<i>T.harzianum</i>	11.11(19.45)	13.58(21.61)	15.22(22.96)	21.71(27.75)	15.40(22.94)
Mean	46.24(46.07)	50.73(49.86)	55.00(51.44)	62.96(56.60)	

Figures in parentheses are arcsine transformed values

Table 5 : Evaluation of *Trichoderma* spp. and *Streptomyces* against two major pathogens of ginger

Bioagent	Per cent inhibition in mycellial growth		
	<i>Fusarium</i> sp.	<i>Pythium</i> sp.	Mean inhibition
<i>T. harzianum</i>	62.42 (52.20)	55.83 (48.36)	59.12(50.28)
<i>T. hamatum</i>	47.27 (43.44)	52.50 (46.44)	49.88(44.94)
<i>Streptomyces</i> sp	43.02 (40.99)	40.00 (39.23)	41.51(40.11)

Figures in parentheses are arcsine transformed values

Table 6 : Effect of non-chemical soil treatments on ginger

Treatments	Emergence of tillers (%)	Disease incidence (%)		Fresh rhizome weight (kg/3m ²)
		Foliage	Rhizomes	
<i>T. harzianum</i>	95.31 (77.67)	5.70(2.34)	13.50(21.51)	4.231
<i>T. hamatum</i>	93.75(71.25)	8.22(2.80)	16.12(23.67)	3.637
Pea biodisinfestation	87.50 (75.76)	15.02(3.87)	18.60(25.53)	3.327
Cabbage biodisinfestation	75.79 (62.08)	13.30(3.63)	22.35(26.79)	2.962

Figures in parentheses are arcsine transformed values

have acted by checking their mycelium growth. No mycelial growth of *T. hamatum* was recorded at 55°C temperature. Similar findings were observed by Coelho *et al.* (2000). *In vitro* screening of *Trichoderma* spp. and *Streptomyces* sp. against the pathogens were carried out for testing their efficacy against test pathogens. Maximum mycelial inhibition was found in *T. harzianum* (52.20%) followed by *T. hamatum* (43.4%) and *Streptomyces* sp. (40.9%). Under field conditions *Trichoderma* spp. were evaluated for different parameters. Maximum emergence of tillers was found in case of *T. harzianum* (77.67%). *T. harzianum* was found to be the best treatment in control of rhizome rot (21.51%) followed by Pea biodisinfestation (25.53%). The yield of fresh rhizomes was 4.31 kg/3m² and successive loss of yield was attributed to the destruction of rhizomes and root tissues which affect total photosynthetic activity and results in low accumulation of food materials in rhizomes (Tabel 6).

Conclusion :

Non-chemical management of ginger with biocontrol agents against fungal pathogens in ginger rhizome rot could be helpful to protect the crop from great loss. It is one of the ecofriendly approach of controlling the ginger disease using antagonistic pathogens. This would be useful for crop improvement programmes with high diseased free crop production.

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