Research Article

Biocontrol Efficacy of *Trichoderma SPS* against Rhizome Rot Disease of *Zingiber officinale Rosc*

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ABSTRACT

In the present study, the organisms were isolated and identified from disease infected ginger and soil sample, namely *Aspergillus awamori, Aspergillus conicus, Aspergillus luchensis* and *Pythium aphanidermatum.* The pathogenicity test was performed as two treatments i.e control and treatment. In pot culture experiment, plants were grown, control plants were not infected but treatment plants were infected by pathogen and the symptoms were observed. *Trichoderma koningii, Trichoderma virens, Trichoderma harzianum* and *Trichoderma viride* grew quickly and dominate the *Pythium* spp within 15 days. After 21 days, the pathogen was completely inhibited by antagonist *T.koningii*. The culture filtrate (non volatile) from *Trichoderma koningii* inhibits the growth of test microorganisms. *Trichoderma koningii* which is inhibitory the growth of test fungi (*Pythium aphanidermatum*) by 31.10% at 15% concentration.

Keywords: Infected ginger, *Trichoderma sps,* Fungicides, *Pythium aphanideratum*, Antagonistic activity.

INTRODUCTION

Ginger (*Zingiber officinale Rosc*) is one of the most important spice crops of the family Zingiberaceae. It is an important tropical horticultural plant, valued all over the world as an unparallel spice in culinary preparations and for its medicinal properties. The use of rhizomes is a routine vegetative propagation method for *Zingiber officinale*, and many rhizomes are required because the efficiency of vegetative propagation is low. In addition, during storage and cultivation, rhizomes used for vegetative propagation are susceptible to diseases that cause tissue senescence and degeneration. Heavy losses have been reported because of infection with *Ralstonia solanacearum* formerly (*Pseudomonas solanacearum*), soft rot (*Pythium aphanidermatum*) and nematodes (*Meloidogyne* spp)[1]

Ginger (*Zingiber officinale Rosc*) is among the important and widely used spice crops throughout the world. In India, it is grown on an area of 1.06 lakh hectares with annual production of about 3.76 lakh tones (NHB, 2008). Ginger is an herbaceous perennial, rhizomatous spice crop containing volatile oil, fixed oil, pungent compounds, resins, starch, protein and minerals[2].

Edible ginger (*Zingiber officinale Rosc*) is a popular spice crop grown in Hawaii, primarily on the island of Hawaii, with annual production at approximately 6.35 million kg (14million lbs) and value at about $4.3 million[3].

The efficacy of *Trichoderma* species on soil borne fungal disease higher than fungicides and maintain longer.

The value obtained through development, exploitation and use of *Trichoderma* products are not only plant disease control but also gave the local people opportunities to reduce health risks, costs and environmental damage due to over fungicide usages.

India is the largest producer of ginger accounting for about 1/3rd of total world output so it is basic need to develop high yielding varieties with better quality to increase the production and productivity of ginger in India[4].

MATERIALS AND METHODS

Description of the study area

The study area is situated in Thanjavur district of Tamil Nadu state. The present investigation was carried out with the collection and examination of rhizome rot disease infected samples of ginger fields of Papanasam Taluk. The *Trichoderma koningii, Trichoderma virens, Trichoderma harzianum and Trichoderma viride* procured from Sri Amman Biocare, Thirukkanurpatti, Thanjavur district, Tamil Nadu. Soil samples were collected from the disease infected ginger filed, Thirukkanurpatti, Thanjavur.

Isolation and Identification of fungi

After sample collection, the fungal organisms were isolated and identified using appropriate method.

Pathogenicity test

Inoculum preparation

The healthy ginger rhizomes were planted in pots filled with sterile potting mixture containing soil, sand and farm yard manure in the ratio of 1:1:1 and grown under greenhouse conditions. *P. aphanidermatum* was cultured in potato dextrose broth in Roux bottles using mycelial plugs (3 mm) taken from the advancing margin of 7 days old culture of the isolate.

The culture was allowed to grow at 25˚C±2˚C for 5 days and the mycelial mat grown was used for pathogenicity tests. The mycelial mats were harvested, weighed and homogenized in a mixer blender and made into a suspension. The suspension at 5 ml containing 1 g ml/L was inoculated over the soil surface around one month old healthy ginger plants. The plants without inoculums served as control[5]. Three replicates were maintained for each test. The plants were evaluated for the development of water soaked lesions on pseudo stem and subsequent yellowing of the leaves. The rhizome rot symptoms showed plants were observed carefully and recorded at regular intervals.

Antagonistic activity[6]

The biocontrol agents namely *Trichoderma koningii, Trichoderma virens, Trichoderma harzianum and Trichoderma viride* were selected to study the antagonistic activity against *Pythium aphanidermatum* isolated from rhizome rot disease infected ginger sample. The Potato dextrose agar medium was prepared and poured in to the Petri plate. After solidification, 6 mm diameter of pure culture of *Trichoderma koningii, Trichoderma virens, Trichoderma harzianum and Trichoderma viride* against *Pythium aphanidermatum* were placed on the PDA medium in opposite direction. The plates were incubated at 27± 2˚ C for 15 days and the results were noted at every 72 hours on 3, 6, 9, 12, 15 and 21st days respectively.

Culture filtrate method[7]

The biocontrol agents were grown in potato dextrose broth at 27°C with intermittent shaking at 150 rpm. The metabolites were collected from 12 days and filtered. The sterilized filtrate was amended in PDA to make 5%, 10%, and 15% concentration in Petri plates. The solidified agar plates in triplicates were inoculated at the centre with 6mm diameter mycelial disc of pathogen and incubated at 27°C for 7 days. The plates without filtrate served as control. The colony diameter was measured and percent inhibition of radial growth was also calculated.

The percent inhibition of growth was calculated as follows.

$$\%\ of\ inhibition\ of\ growth = \frac{Growth\ in\ control - Growth\ in\ treatment}{Growth\ in\ control} \times 100$$

Disc preparation

The Whatmann No. 1 filter paper was used to disc preparation, the disc size was 6 mm. Commercially available chemical fungicides namely Carbendazim (50% WP) and Mancozeb (75% WP), were separately prepared and maintained in hot air oven at 45˚ C. The prepared disc was used for disc diffusion method.

Effect of chemical fungicide on the growth of pathogen

Fungicidal activity of commercial fungicide was tested against *P. aphanidermatum* using disc diffusion method. The PDA medium was prepared and sterilized at 121˚C for 15 minutes and allowed to cool approximately 50˚C. Then, the medium was poured into the sterile Petri dishes. After solidification, the pathogen was swabbed over agar plate by sterile cotton buds. After preparation of the discs, they were placed over PDA medium. The plates were incubated at 27±2˚C for 48 h. After incubation, the results were recorded[8].

Statistical analysis[9]

Statistical analysis was performed by calculating Mean ± Standard deviation.

The formula for calculating standard deviation S.D $= \sqrt{\frac{\sum(X-X)^2}{N-1}}$

RESULTS

Soil sample was collected from disease infected ginger field. The result of the physico-chemical properties of the soil sample was recorded. Soil sample was collected from disease infected ginger field. Serially diluted soil sample was inoculating on the PDA medium and incubated at 27°C for 72 hours. After incubation period, the plates were observed for fungal colonies and identified using lactophenol cotton blue staining method. The following organisms were identified from sample, *Aspergillus awamori, Aspergillus conicus, Aspergillus luchensis* and *Pythium aphanidermatum.*

Pathogenicity test

The pot culture experiment was done, to prove the *Pythium aphanidermatum* was rhizome rot pathogen of ginger plant. In the pathogenicity test, two treatments were carried i.e., control and treatment.

In pot culture experiment, plants were grown, control plants were not infected but treatment plants were infected by pathogen and the symptoms were observed. In plate culture method, the *Pythium aphanidermatum* organism was observed in PDA plate where the sample taken from disease infected pots.

Antagonistic activity

After incubation period, the plates were examined and results were recorded for every 72 hours. *Trichoderma koningii* grew quickly and dominate the *Pythium* spp within 15 days.

After 21 days, the pathogen was completely inhibited by antagonist *T. koningii* compared with *Trichoderma virens, Trichoderma harzianum* and *Trichoderma viride* (Table–1).

Effect of culture filtrate of fungi on the growth of test pathogens

The maximum percentage of the inhibition growth in *P. aphanidermatum* as on the potato dextrose agar medium amended with 15% of the culture filtrate of *T. konongii* (13.35±0.1 mm), followed by *T. virens* (22.47±0.17mm*), T. harzianum* (31.10±0.19mm) and *T. viride* (30.2±0.11). The dominant culture filtrate of *Trichoderma koningii* was

more effective when compared to other biocontrol agents (Table-2).

Effect of chemical fungicides on the growth of test pathogens

Carbentiazim was amended with potato dextrose agar medium in various hours viz, 72, 120, 168, 216 and 264. The percentage inhibitions of *P. aphanidermatum* were expressed as follows (23±0.5mm), (21±0.7mm), (17±0.9.mm), (15±0.10mm) and (12±0.11mm) respectively. Mancozeb was suspended with potato dextrose agar medium in various hours *viz,* 72, 120, 168, 216 and 264. The percentage inhibitions of *P. aphanidermatum* were expressed as follows (22±0.3mm), (20±0.4mm), (16±0.7mm), (15±0.8mm) and (12±0.10mm) respectively.

Table 1: Antagonistic Activity of four biocontrol agents Vs *P. aphanidermatum* by dual culture method

Hours	Growth of Antagonist (mm)				Growth of Pathogen (*P.aphanidermatm*) (mm)
	T.konigii	*T.virens*	*T.harzianum*	*T.viride*	
72	22±0.5	30±0.7	35±0.13	38±0.14	18±0.11
144	54± 0.7	58±0.9	62±0.15	68±0.16	15±0.16
216	63±0.9	69±0.11	76±0.14	78±0.19	12±0.17
264	79±0.11	83±0.12	86±0.16	88±0.20	9±0.19

Values are expressed as Mean± Standard deviation

Table 2: Effect of culture filtrates against rhizome rot pathogen *P. aphanidermatum*

S. No.	% of culture filtrate	*P. aphanidermatum* growth in (mm)				% of growth inhibition
		T.koningii	*T. virens*	*T.harzainum*	*T.viride*	
1	0	61.4±0.06	58.5±0.05	55.4±0.12	50.3±0.08	--
2	5	53.2±0.09	50.2±0.07	47.9±0.14	43.2±0.11	13.35±0.15
3	10	47.6±0.012	45.4±0.08	40.4±0.15	35.4±0.13	22.47±0.17
4	15	42.3±0.014	39.6±0.011	35.2±0.16	30.2±0.15	31.10±0.19

Values are expressed as Mean± Standard deviation

Table 3: Antifungal activity of chemical fungicides against *P. aphanidermatum*

S. No.	Hours	Carbendiazim Zone of inhibition (mm)	Mancozeb Zone of inhibition (mm)
1	72	23 ± 0.5	22 ± 0.3
2	120	21 ± 0.7	20 ± 0.4
3	168	17 ± 0.9	16 ± 0.7
4	216	15 ± 0.10	15 ± 0.8
5	264	12 ± 0.11	12 ± 0.10

Values are expressed as Mean ± Standard deviation

CONCLUSION

The current status of research suggests that there are indeed alternatives to replace the synthetic fungicides for management of this notorious soil as well as seed borne fungi: *Pythium*, which causes loss of multimillion dollars. However, the farmers use the common synthetic fungicides which leads into ill effects as well as many of the commonly used synthetic fungicides are unable to control *Pythium* species as it has got resistant against these synthetic fungicides. Hence, there is need to replace the chemical fungicides by bio-fungicides, prepared from plant extracts and antagonistic microorganisms.

Bio-fungicides will also be economical to the farmers and besides this, the use of bio fungicides will not leave any ill effect in the soil, water as well as in the environment. It is possible that by combining these approaches, (use of plant extracts, antagonistic microorganisms, organic manure) an economically viable alternative for crop production system can be developed.

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