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## *In vitro* efficacy of bio-agents against *Pythium aphanidermatum* causing rhizome soft rot of ginger

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### Abstract

Rhizome soft rot disease caused by *Pythium aphanidermatum* is one of the most widely distributed and destructive disease of Ginger (*Zingiber officinale* Rosc.) causing about 50 to 80 per cent yield losses. Five fungal and two bacterial antagonists were evaluated under *in vitro* condition against *P. aphanidermatum* causing rhizome soft rot disease of ginger. All antagonist were found fungistatic / fungicidal action against test pathogen. Amongst the antagonist used *T. asperellum* was found most effective and showed significantly highest mycelial inhibition (81.72%) followed by *T. harzianum* and *T. koningii* with inhibition of 76.66 and 61.74 per cent, respectively. While the antagonist *Bacillus subtilis* showed least inhibition (33.33%) against the test pathogen.

**Keywords:** Rhizome soft rot, antagonist, bio-mix, mycelial inhibition

### Introduction

India is considered as 'The land of spices' and enjoys from time immemorial a unique position in the production and export of ginger. Ginger (*Zingiber officinale* Rosc.) is an important spice crop belongs to family Zingiberaceae. It is originated from south East Asia and cultivated in several parts of the World including India, China, Thailand, Nigeria, Indonesia, Bangladesh, Australia, Fiji, Nepal and Sierra Leone (Dohroo et al., 2012) [7]. Among them, India and China are the dominant suppliers to the world market. The area under ginger cultivation during the year 2017-18 was 164.70 thousand hectare with production of 1081.40 thousand metric tonnes with 6.57 MT/ha productivity (Anonymous, 2017) [1]. In India Karnataka, Assam, Orissa, West Bengal, Madhya Pradesh, Maharashtra, Sikkim, Meghalaya and Arunachal Pradesh are the major ginger growing states.

Ginger crop cultivated for underground rhizomes, which are used in many ways. The refreshing aroma, biting taste and carminative property of ginger make it an indispensable ingredient of food processing throughout the World. Fresh ginger, ginger powder, oleoresin and oil are all used for this purpose. Fresh ginger is unique for its flowery flavour and spicy taste that said as flavouring agent, a preservative, used in pickling and ginger oil in soft drinks. It is also used in the production of ginger bread.

There are many biotic as well as abiotic factors adversely affect the yield and quality of ginger crop. Among those biotic factors diseases caused due to fungi, bacteria and nematodes was major factor infect ginger cultivation. Rhizome soft rot disease of ginger was a prominent and destructive fungal disease which infects ginger and causing huge losses of crop. Ginger crop is affected with various diseases, the major diseases diagnosed were rhizome soft rot disease caused by *Pythium aphanidermatum* (Edson) Fitzp. with losses upto 100% (Rajan and Agnihotri, 1989) [13]; disease causes 50 to 80% in ginger during storage (Nirmal et al., 1992) [11] rhizome rot resulted in yield loss of 50% (Rajalakshmi et al., 2016) [12]. The disease name indicates that, ultimately rotting of rhizomes, which part of ginger is economically.

In recent days, peoples are inclining towards organic farming due to awareness of ill-effects caused by indiscriminate use of chemical fertilizers, fungicides and pesticides in crop cultivation and in turn their residual effects in plant products resulting in adverse effects on human health. In this context, the use of biofertilizers and organic manures is now a day gaining more importance in crop cultivation to get higher crop yield and quality produce. There for, looking into importance of bio control agents in disease management present investigation was undertaken.

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## Materials and Methods

### 1. Source of pathogen and antagonists

Typical diseased samples of rhizome soft rot were collected from local markets and fields. The isolation of pathogen from infected tissue were made on *Pythium* selective medium. The pathogen was identified as *P. aphanidermatum* based on microscopic and pathogenic studies. The culture was multiplied on PDA media for further studies.

Seven fungal antagonists viz; *Trichoderma asperellum*, *T. harzianum*, *T. hamatum*, *T. koningii*, and *T. (Gliocladium) virens* procured from Department of Plant Pathology, COA, Latur and bacterial antagonists viz; *Pseudomonas fluorescens* and *Bacillus subtilis* procured from spawn production-cum bio control Laboratory, Department of Plant Pathology, VNMKV, Parbhani.

### 2. In vitro evaluation of antagonists

Five fungal and two bacterial bioagents were evaluated *in vitro* against pathogen *P. aphanidermatum*, applying by dual culture technique (Dennis and Webster, 1971) [5]. Seven days old cultures of the test bio agents and test pathogen (*P. aphanidermatum*) grown on PDA were used for the study. Two culture discs of 5 mm size, one was test pathogen and second was test bio agents were cut out with sterilized cork borer and placed at equidistance, exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and plates were incubated at  $28 \pm 2$  °C in BOD incubator. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as untreated control. The experiment is designed in CRD and all treatments replicated thrice.

Observations on linear mycelial growth of the test pathogen and test bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen with the test bioagent, over untreated control was calculated by applying following formula (Arora and Upadhyay, 1978).

$$\text{Percent Growth Inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

The present experiment was carried out with 3 replications & 8 treatments in Completely Randomized Design.

### Treatments detail

- T1: *T. hamatum*  
 T2: *T. koningii*  
 T3: *T. harzianum*  
 T4: *T. asperellum*  
 T5: *T. (Gliocladium) virens*  
 T6: *Bacillus subtilis*  
 T7: *P. fluorescens*  
 T8: Control

## Results

### In vitro evaluation of antagonists against *P. aphanidermatum*

Results from Table 1 and Fig. 1 revealed that, all the bio agents evaluated against pathogen *P. aphanidermatum* exhibited fungistatic as well as antifungal activity and significantly inhibited its growth over untreated control. The

antagonists tested, *T. asperellum* was found most effective with highest mycelial growth inhibition (81.72%) of the test pathogen. The second and third inhibitor antagonists were found as *T. harzianum* (76.66%) and *T. koningii* (61.74), respectively and followed by *T. hamatum* (58.56%) and *T. (Gliocladium) virens* (57.54%). Among the bacterial bio agents *P. fluorescens* were found effective and recorded 36.20 per cent mycelial inhibition whereas, *Bacillus subtilis* were found less effective with minimum mycelial inhibition of 33.33 per cent. Overall, results from present investigation stated that, among the all fungal and bacterial bio control agents *T. asperellum* was found most effective against pathogen *P. aphanidermatum*.

**Table 1:** *In vitro* bio-efficacy of bio agents against *P. aphanidermatum*

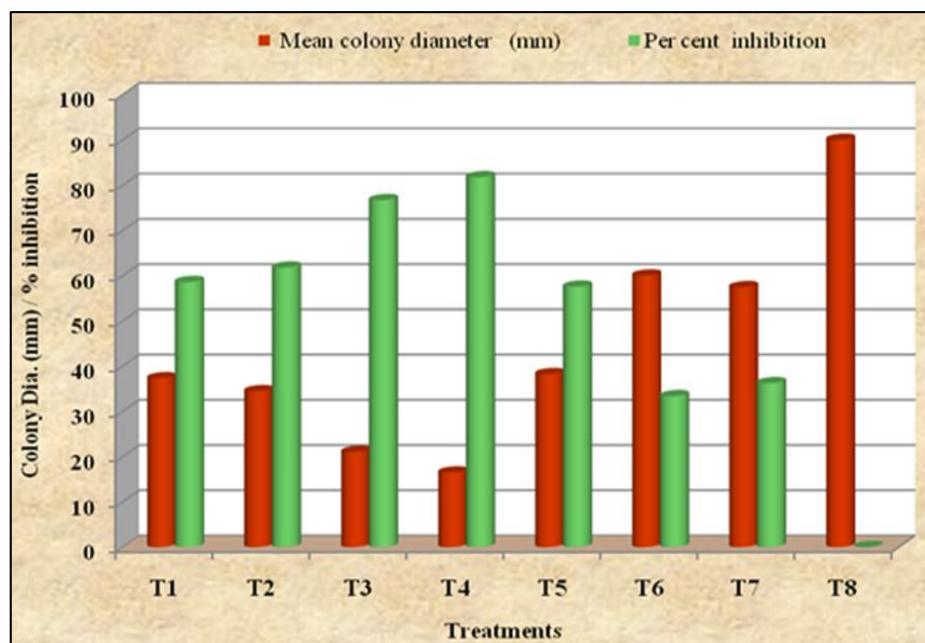
Tr. No.	Treatments	Colony Dia. of test pathogen * (mm)	% Growth inhibition
T1	<i>Trichoderma hamatum</i>	37.29	58.56 (49.92)
T2	<i>T. koningii</i>	34.43	61.74 (51.78)
T3	<i>T. harzianum</i>	21.00	76.66 (61.11)
T4	<i>T. asperellum</i>	16.45	81.72 (64.68)
T5	<i>T. (Gliocladium) virens</i>	38.21	57.54 (49.33)
T6	<i>Bacillus subtilis</i>	60.00	33.33 (35.26)
T7	<i>Pseudomonas fluorescens</i>	57.42	36.20 (36.98)
T8	Control	90.00	00.00 (00.00)
	S.E.±	0.51	0.55
	C.D. (P = 0.01)	1.50	1.62

\*-Mean of three replications, Dia.: Diameter, Figures in Parentheses are angular transformed values



**Plate 1:** *In vitro* bio-efficacy of bio agents on growth and inhibition of *P. aphanidermatum*

Tr. No.	Treatments	Tr. No.	Treatment
T <sub>1</sub>	<i>Trichoderma hamatum</i>	T <sub>5</sub>	<i>T. virens</i>
T <sub>2</sub>	<i>T. koningii</i>	T <sub>6</sub>	<i>Bacillus subtilis</i>
T <sub>3</sub>	<i>T. harzianum</i>	T <sub>7</sub>	<i>Pseudomonas fluorescens</i>
T <sub>4</sub>	<i>T. asperellum</i>	T <sub>8</sub>	Control (Untreated)



**Fig 1:** *In vitro* bio-efficacy of bio agents on mycelial growth and inhibition of *P. aphanidermatum*

### Discussion

These results are in conformity with the earlier findings of those workers who reported bioagents viz., *T. hamatum*, *T. koningii*, *T. harzianum*, *T. asperellum*, *T. (Gliocladium) virens*, *Bacillus subtilis* and *Pseudomonas fluorescens* had significantly inhibited mycelial growth of *P. aphanidermatum* infecting ginger (Rajan *et al.*, 2002; Bhai *et al.*, 2005; Sagar, 2006; Rani and Satheesh, 2007; Muthukumar *et al.*, 2010; Latha, 2012; Singh *et al.*, 2012; Kadam, 2014; Dohroo *et al.*, 2015, Apet *et al.*, 2018) [14, 4, 16, 15, 10, 9, 17, 6, 2].

### Conclusion

All of the seven antagonist's evaluated *in vitro*, *Trichoderma asperellum* bioagent was found significantly highest mycelial growth inhibition (81.72%) and most effective against *P. aphanidermatum*.

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