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## Bio efficacy of bioagents against *Fusarium oxysporum f.sp. zingiberi* causing rhizome rot of ginger in Maharashtra state

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### Abstract

Five fungal antagonists were evaluated *in vitro* against *Fusarium oxysporum*, applying Dual Culture Technique (Dennis and Webster, 1971) and using PDA as basal medium all the bioagents evaluated exhibited fungistatic/fungitoxic activity against *F. oxysporum* and significantly inhibited mycelial growth of the test pathogen over untreated control. Of the seven antagonists tested, *Trichoderma viride* was found most effective and recorded least linear mycelial growth (22.00 mm) with highest mycelial growth inhibition (75.66%) of the test pathogen as compared to untreated control (90.33 mm and 00.00%). Among the different bioagents tested the maximum of 75.66 per cent inhibition was found in *Trichoderma viride*, followed by *Trichoderma harzianum* and *Trichoderma koningii*, and the least inhibition was recorded with the Bacterial bioagents *Bacillus subtilis* with 43.18 per cent.

**Keywords:** Bioagents, *Fusarium oxysporum*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma kunningii*, *Pseudomonas fluorescens*, *Bacillus subtilis*

### Introduction

Ginger (*Zingiber officinale* Rosc.) belonging to the family Zingiberaceae is an important commercial crop grown for its aromatic rhizomes which are used as a spice and a medicine (Sharma and Pandey, 2014). It is obtained from the underground stems or rhizome of *Zingiber officinale* Rosc. Ginger originated in South- East Asia, probably in India. It is usually grown as an annual. The whole plant is refreshingly aromatic, but it is the underground rhizome (raw or processed) which is valued as spice. Its medicinal value is increasingly being recognized now days the name itself supports this view.

At present, India is the largest producer of ginger and is accounting for about one-third of the total world output. It is followed by Thailand and Japan. The area under ginger is 1, 35,250 ha with a total production of 6, 82, 630 Mt and productivity of 4,870 kg/ha (Anonymous, 2014-15) [2]. The area under ginger in Maharashtra is 1060 ha with a total production 1040 Mt). In India, a large portion of the ginger produced is consumed domestically as green ginger or dried ginger. It is cultivated in many States, but major producers are *viz.*, Assam, West-Bengal, Meghalaya, Kerala, Arunachal Pradesh, Orissa, West Bengal and Mizoram. Among the major constraints for growing these crop is the rhizome rot. Even though, important foliar diseases do exist in these crops, rhizome rot is very important in view of severe crop losses.

### Material and Methods

#### Culture media

Potato dextrose Agar (PDA) was used as basal culture medium for isolation, purification and maintenance of the pure culture of *Fusarium oxysporum f.sp. zingiberi*. Sand : maize (1:2) medium was used for mass multiplication of the pathogen. For studying cultural characteristics of *Fusarium oxysporum f.sp. zingiberi*, synthetic readymade (make: Hi media) and non-synthetic (prepared) media were used. The synthetic media and ingredients of non-synthetic media were obtained from the Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri.

### Biocontrol agents

Pure culture and talk based formulations of biocontrol agents viz., *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. virens*, *T. koningii*, *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the Spawn Production-cum-Biocontrol Laboratory, Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri; were maintained and multiplied on appropriate culture media and used for present studies.

### Evaluation of bioagents against *F. oxysporum*

Dual culture technique Five fungal antagonists viz., *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. virens*, *T. koningii*, and two bacterial antagonists viz., *Pseudomonas fluorescens*, *Bacillus subtilis* were evaluated *in vitro* against *Fusarium oxysporum* f.sp. *zingiberi* (FOZ- 8 isolate), applying Dual Culture Technique (Dennis and Webster, 1971) [3]. Seven days old culture of the test bioagents and the test pathogen (*Fusarium oxysporum* f.sp. *zingiberi* (FOZ-8 isolate)) were used for the study. Culture discs (7 mm dia.) of the test pathogen and bioagents (7 mm dia.) were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagent were placed aseptically at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates and plates were incubated at  $28 \pm 2$  °C. Three plates/ treatment/replication were maintained. PDA plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

### Experimental details

Design: CRD

Replications: Three

Treatments: Eight

**Table 1:** List of bioagents tested against the *Fusarium oxysporum* f. *sp. zingiberi*

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

Observations on linear mycelial growth of the test pathogen and bio-agent 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 by the bioagent over untreated control was calculated by applying following formula (Arora and Upadhyay, 1978).

$$\text{Per cent Growth Inhibition} = \frac{\text{Linear mycelial growth in control plate} - \text{Linear mycelial growth in intersecting plate}}{\text{Linear mycelial growth in control plate}} \times 100$$

### Results and Discussion

Present studies on the Rhizome rot of ginger caused by *Fusarium oxysporum* were undertaken during Rabi, 2015-16 and 2016-2017 at Department of Plant pathology and Agril. Microbiology, MPKV Rahuri on the aspects *in vitro*

evaluation bioagents. The results obtained on all these aspects are presented in the following paragraphs.

### *In vitro* evaluation of bioagents against *F. oxysporum*

Five fungal viz., *T. viride*, *T. harzianum*, *T. koningii*, *T. hamatum* and *T. virens* and two bacterial *P. fluorescens* and *B. subtilis* antagonists were evaluated *In vitro* against *F. oxysporum*, applying Dual Culture Technique (Dennis and Webster, 1971) [3] and using PDA as basal medium as detailed under.

**Table 2:** *In vitro* evaluation of different bioagents on linear mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *zingiberi*

Sr. No.	Biocontrol agents	Mean colony diameter (mm)* (after 7 days)	Per cent (%) inhibition
1	<i>Trichoderma viride</i>	22.00 (27.96)	75.66
2	<i>Trichoderma harzianum</i>	23.20 (28.79)	74.31
3	<i>Trichoderma koningii</i>	25.43 (30.28)	72.11
4	<i>Trichoderma hamatum</i>	28.50 (32.26)	68.70
5	<i>Trichoderma virens</i>	32.22 (34.58)	64.33
6	<i>Pseudomonas fluorescens</i>	42.19 (40.50)	52.29
7	<i>Bacillus subtilis</i>	51.32 (45.76)	43.18
8	Control	90.33 (71.88)	00.00
	S.E. $\pm$	0.35	
	C.D. at 1%	1.06	
	CV %	1.57	

\* = Average of three replications.

Figures in parenthesis are angular transformed values

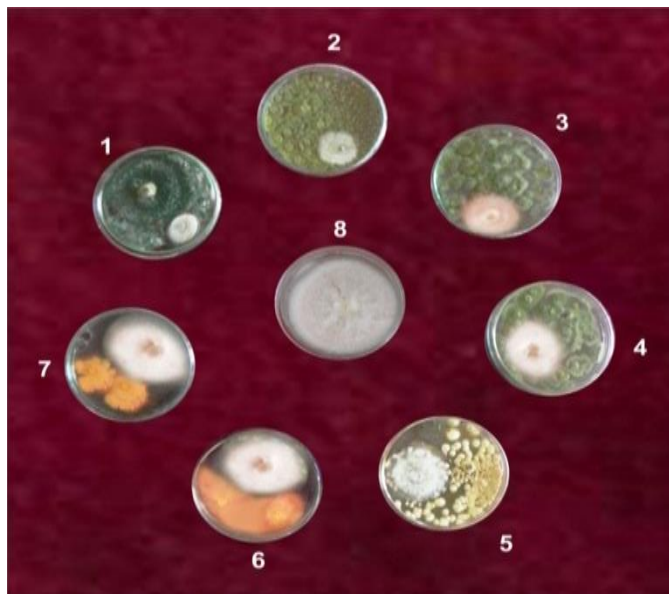
Result (Table 2) revealed that all the bioagents evaluated exhibited fungistatic/fungitoxic activity against *F. oxysporum* and significantly inhibited mycelial growth of the test pathogen over untreated control. Of the seven antagonists tested, *T. viride* was found most effective and recorded least linear mycelial growth (22.00 mm) with highest mycelial growth inhibition (75.66%) of the test pathogen as compared to untreated control (90.33 mm and 00.00%). The second and third best antagonists found were *T. harzianum* and *T. koningii*, which recorded mycelial growth of 23.20 mm and 25.43 mm, respectively and inhibition of 74.31 and 72.11 per cent, respectively. This was followed by *T. hamatum* (col. dia.: 28.50 mm and inhibition: (68.70%). The fungal antagonist *T. virens* was found least effective which recorded 32.22 mm and 64.33 per cent linear mycelial growth and inhibition, respectively. Bacterial antagonist *P. fluorescens* (col. dia.: 42.19 mm and inhibition: 52.29%) and *B. subtilis* (col. dia.: 51.32 mm and inhibition: 43.18%). was also found least effective per cent linear mycelial growth and inhibition, respectively.

Thus, all the fungal and bacterial antagonists/bioagents evaluated *In vitro* were found fungistatic/fungitoxic against *F. oxysporum* and caused significant reduction in the linear mycelial growth of the test pathogen over untreated control. The inhibitory effect of *Trichoderma* spp. and *P. fluorescens* and *B. subtilis* against *F. oxysporum* may be attributed to the mechanisms viz., antibiosis, lysis, mycoparasitism, competition and production of volatile and non-volatile substances.

Results of the present study on antagonistic effects of the *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus subtilis* against *F. oxysporum* are in conformity with those reported earlier by several workers (Philippe and Claude (1991); Amara *et al.* (1996); Ram *et al.* (1997) ; Meena and Mathur (2003) [5, 1, 6, 4]

## Conclusion

Among the different bioagents tested the maximum of 75.66 per cent inhibition was found in *T. viride*, followed by *T. harzianum* and *T. koningii*, and the least inhibition was recorded with the Bacterial bioagents *B.subtilis* with 43.18 per cent.



**Plate 1:** *In vitro* evaluations of different bioagents on linear mycelial growth and inhibition of *Fusarium oxysporum f.sp. zingiberi*

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

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