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Effect of known antagonists on the growth of the *S. oryzae in vitro* a causal agent of rice grain discolouration

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Abstract
Eight known antagonists were screened *in vitro* for their antagonism to *S. oryzae* by three methods *viz*., dual culture, pathogen at periphery and pathogen at centre. In all the three methods, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* proved as strong and potent antagonists to *S. oryzae*.

Keywords: *Antagonism, Sarocladium oryzae*

Introduction
Rice (*Oryza sativa* L.) is the staple food of more than 60 per cent population of the world. Many hybrids and improved high yielding varieties have been developed in several countries in recent years, but they are susceptible to diseases due to narrow genetic makeup.
Glume discolouration or dirty grain discolouration or black grain disease first time was reported from Navsari, (Gujarat), caused by various saprophytic and pathogenic organisms (Anon; 1988). During pathogenecity test it is found that *S. oryzae* is closely associated with grain discolouration. Notable success of disease management through the use of antagonistic bioagents in the laboratory, glass house and field has been achieved during past several years. On the basis of this information, there is possibility of development of biological control for plant diseases. Now a day, the commercial formulation of some of the biocontrol agents has already become available in the market. In the present study, attempts have been made to identify antagonistic bioagents against *Sarocladium oryzae*.

Material and Method
Now a day the commercial formulation of some of the bio-control agents are already available in the market. However, inadequate information on the performance of the antagonists under varying conditions is a major constraint in the large scale adoption of this technology. Therefore, eight known antagonists *viz*., *Trichoderma viride, T. harzianum, T. longibrachyatum, Aspergillus niger, Gliocladium virens, Chaetomium globosum, Pseudomonas fluorescens* and *Bacillus subtilis* were tested *in vitro* for their antagonistic effect against *Sarocladium oryzae* recorded as one of the main pathogen of GD. For this study three inhibition methods of fungal growth were employed.

A. Dual culture method (Dennis and Webster, 1971)
The petriplates containing 20 ml PDA medium were inoculated aseptically with the pathogen *S. oryzae* and the test organism (antagonist) by placing 5 mm diameter culture blocks at 50 mm apart from each other. Three repetitions of each treatment were kept and the petriplates with only pathogen at centre served as control. All the plates were incubated in B.O.D. at 25 + 0.5 ^{0}C temperature. Observations on colony diameter were recorded up to the complete coverage of plates, which was inoculated only with pathogen. The per cent growth inhibition (PGI) was worked out using the following formula given by Vincent (1927) [8].

$$PGI = \frac{100\ (DC\text{-}DT)}{DC}$$

Where

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony of control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

B. Pathogen at periphery (Asalmol *et al*., 1990)

Mycelial discs of 5 mm diameter of the pathogen *S. oryzae* and the test organism (antagonist) were cut uniformly with the help of a cork borer and inoculated aseptically by placing these block in a Petriplate containing 20 ml PDA, keeping four mycelial discs of the pathogen 35 mm away radially from the centre of the Petriplates and one culture disc of the test organism was inoculated in the centre of the same Petriplates, simultaneously. Three repetitions of each treatment were kept and the Petriplates with only pathogen at centre served as control. Plates were incubated in B.O.D. at 25 + 0.5 ^{0}C temperature and the radial growth of the test organisms and pathogen was recorded after the complete coverage of plates with fungal growth in plate, inoculated only with pathogen. The per cent growth inhibition (PGI) was worked out by using the formula given by Vincent (1927) [8].

C. Pathogen at centre (Asalmol *et al*., 1990)

Each petriplate containing 20 ml PDA was inoculated aseptically in the centre by transferring a mycelial disc of the pathogen *S. oryzae* and four discs of the test organism (antagonist) were placed 35 mm away radially from the centre in the same were petriplate, simultaneously. Each treatment was repeated three times. The plates inoculated in the centre with only pathogen served as control. The plates were incubated in B.O.D. at 25 + 0.5˚C temperature. Observations on radial growth of the pathogen and the test organism were recorded after the complete coverage of plates with fungal growth in plate. The per cent growth inhibition (PGI) was worked out using the formula given by Vincent (1927) [8].

Result and Discussion

In this study, pure culture of bio-agents *T. Viride*, *T. longibrachyatum*, *A. niger*, *C. globosum*, *G. virens*, *B. subtilis* and *P. fluorescens* were obtained from Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University Navsari-396 450. The effect of these antagonists was tested against *S. oryzae* by three methods *viz*., dual culture, by placing pathogen at periphery and pathogen at centre.

1. Dual culture technique

The results presented in Table-1 revealed that all the antagonists were found significantly superior in checking the growth of the pathogen than the control. Out of eight antagonists tested, significantly least growth of the pathogen was recorded in *Trichoderma viride* (10.00 mm) which was statistically at par with *Pseudomonas fluorescens* (12.67 mm) followed by *Bacillus subtilis* (16.17 mm) Next best in order of merit was *Trichoderma longibrachyatum* (21.17 mm), *Trichoderma harzianum* (25.67 mm), *Aspergillus niger* (26.50 mm), and *Gliocladium virens* (26.50 mm), *Chaetomium globosum* (34.50 mm).

T. viride produced maximum growth inhibition (80.86%) of the pathogen after 10 days of incubation and appeared to be the most superior over all the antagonists tested. Next best in order of merit was *P. fluorescens* (75.76%) followed by *B. subtilis* (69.05%), *T. longibrachyatum* (59.48%), *T. harzianum* (50.95%), *A. niger* (49.32%), *G. virens* (49.32%), and *C. globosum* (33.95%).

Table 1: Efficacy of antagonists against *S. oryzae in vitro* by dual culture method

Sr. No.	Test organism	Average Colony diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma viride*	18.42***** (10.00) ******	80.86
2	*Trichoderma harzianum*	30.44 (25.67)	50.95
3	*Trichoderma longibrachyatum*	27.39 (21.17)	59.48
4	*Aspergillus niger*	30.98 (26.50)	49.28
5	*Pseudomonas fluorescens*	20.85 (12.67)	75.76
6	*Gliocladium virens*	30.98 (26.50)	49.32
7	*Chaetomium globosum*	35.97 (34.50)	33.95
8	*Bacillus subtilis*	23.70 (16.17)	69.05
9	Control	46.34 (52.33)	
	S.Em. ±	0.452	
	C. D. at 5%	1.34	
	CV %	2.66	

***** Figures those out side are arcsine transformed values

** Figures in parenthesis are original values

2. Pathogen at periphery

In this method, test organism was kept at centre surrounded by the pathogen provided upper hand to the pathogen and real antagonistic properties of the test organism was exhibited. The result presented in Table-2 revealed that all the antagonists tested proved significantly superior than the control. Among these, *T. viride* showed significantly lower mycelial growth (13 mm) which was at par with *P. fluorescens* (13.67 mm) followed by *B. subtilis* (17.33 mm). Next best in order of merit was *T. longibrachyatum* (25.00 mm), *A. niger* (26.50 mm), *T. harzianum* (28.00 mm), *G. virens* (32.33 mm) and *C. globosum* (34.17 mm).

Maximum growth inhibition was found in *T. viride* (77.61%). Next best in order of merit was *P. fluorescens* (76.45%) and it was followed by *B. subtilis* (70.11%), *T. longibrachyatum* (56.90%), *A. niger* (54.28%), *T. harzianum* (51.75%), *G. virens* (44.24%) and *C. globosum* (41.10%).

Table 2: Efficacy of antagonists against *S. oryzae in vitro* by placing pathogen at periphery method.

Sr. No.	Test organism	Average Colony diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma viride*	21.10***** (13.00) **	77.61
2	*Trichoderma harzianum*	31.94 (28.00)	51.75
3	*Trichoderma longibrachyatum*	30.00 (25.00)	56.90
4	*Aspergillus niger*	30.98 (26.50)	54.28
5	*Pseudomonas fluorescens*	21.68 (13.67)	76.45
6	*Gliocladium virens*	34.65 (32.33)	44.24
7	*Chaetomium globosum*	35.77 (34.17)	41.10
8	*Bacillus subtilis*	24.60 (17.33)	70.11
9	Control	49.60 (58.00)	
	S. Em. ±	0.553	
	C. D. at 5%	1.64	
	CV %	3.08	

***** Figures those out side are arcsine transformed values
** Figures in parenthesis are original values

3. Pathogen at center

In this method, the pathogen kept at centre surrounded by test organism provided upper hand to the test organism. The results presented in Table-3 showed that all the antagonists were found significantly superior in restricting the growth of the pathogen over the control. Among these, *P. fluorescens* showed significantly lower mycelial growth of the pathogen (10.00 mm) which was statistically at par with *T. viride* (11.50 mm) followed by *B. subtilis* (13.83 mm). Next best in order of merit was, *T. longibrachyatum* (23.33 mm), *T. harzianum* (24.00 mm), *A. niger* (26.33%), *G. virens* (28.67 mm) and *C. globosum* (40.17 mm).

Maximum growth inhibition by *P. fluorescens* was 81.80% after 10 days of incubation and appeared to be the most superior antagonists against *S. oryzae* over all the antagonists tested. Next best in order of merit was *T. viride* (79.11%), followed by *B. subtilis* (74.83%), *T. longibrachyatum* (57.59%), *T. harzianum* (56.36%), *A. niger* (52.12%), *G. virens* (47.86%) and *C. globosum* (26.96%).

Table 3: Efficacy of antagonists against *S. oryzae in vitro* by placing pathogen at centre method.

Sr. No.	Test organism	Average Colony diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma viride*	19.78* (11.50) **	79.11
2	*Trichoderma harzianum*	29.32 (24.00)	56.36
3	*Trichoderma longibrachyatum*	28.88 (23.33)	57.59
4	*Aspergillus niger*	30.87 (26.33)	52.12
5	*Pseudomonas fluorescens*	18.42 (10.00)	81.80
6	*Gliocladium virens*	32.37 (28.67)	47.86
7	*Chaetomium globosum*	39.33 (40.17)	26.96
8	*Bacillus subtilis*	21.83 (13.83)	74.83
9	Control	47.87 (55.00)	
	S. Em. ±	0.525	
	C. D. at 5%	1.56	
	CV%	3.04	

* Figures those out side are arcsine transformed values
** Figures in parenthesis are original values

It is evident from these studies that among all the antagonists evaluated by three methods, *Trichoderma viride* and *Pseudomonas fluorescens* proved highly antagonistic, followed by and *Bacillus subtilis* against *S. oryzae* as compared to other antagonists. Hence considered as potential antagonists. These findings are in harmony with earlier researcher's *viz*., Sakthivel and Gnanamanickam (1986) [6], Sakthivel *et al*. (1988) [7], Paneerselvam and Saravenamuthu (1996), and Bolla (2002) who observed strong antagonism of *Trichoderma viride* and *Pseudomonas fluorescens* to *S. oryzae*. Gopalakrishnann and Valluvaparidasan (2006) [4] who observed that *B. subtilis* showed strong antagonistic properties against *S. oryzae*. Our findings are in complete agreement with the findings of above researchers. Daroga *et al*. (2007) [3] reported that both sheath blight and Sheath rot would be effectively managed by integration of soil application with *T. viride* @ 5 kg/ha and foliar application of Validamaycin @ 21/ha. Akila and Ebenezar (2009) reported that spraying of neem oil 80 EC (3%) at flowering stage and ten days later reduced the grain discolouration from 21.60 to 11.45 per cent was on par with rhizome extract of *C. longa* (10%) and *P. fluorescens* (10^9cfu ml^{-1}).

Conclusion

Eight known antagonists were screened *in vitro* for their antagonism to *S. oryzae* by three methods *viz*., dual culture, pathogen at periphery and pathogen at centre. In all the three methods, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* proved as strong and potent antagonists to *S. oryzae*.

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