

Identification of Fungal Biocontrol Agents against *Sclerotium oryzae* Causing Stem Rot of Rice (*Oryza sativa* L.)

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The present investigation was undertaken to assess the influence of the biocontrol agents *Trichoderma harzianum* and *T. viride* for the management of stem rot of rice caused by *Sclerotium oryzae*. Eight isolates of *Trichoderma* out of these eight isolates, four isolates of *Trichoderma harzianum* and 4 isolates of *Trichoderma viride* were collected from different sources and used in this study. *Trichoderma viride* was slow growing, while isolates of *Trichoderma harzianum* were fast growing. Fungal antagonists (*Trichoderma* spp.) were tested by dual culture technique to study the inhibitory effect on mycelial growth of pathogen *S. oryzae*. The inhibition of mycelial growth of *S. oryzae* was measured up to 6 days. The visible mycelial contact between fungal antagonists and test fungus occurred on 3rd day of inoculation. The inhibition of mycelial growth of the pathogen firstly started by *Trichoderma harzianum* Pantnagar isolate followed by *Trichoderma harzianum* New Delhi isolate, Meerut isolate and rests other isolates. The fungal antagonists completely inhibited the growth of pathogen *S. oryzae* and covered the entire plates within six days of inoculations. However *Trichoderma harzianum* (Pantnagar isolate) showed maximum inhibition and covered the entire plate on 4th day of inoculation followed by New Delhi and Meerut isolate on 5th day while all other isolates covered the entire plate on 6th day. *Trichoderma harzianum* (Pantnagar) isolate showed maximum inhibition of the *S. oryzae*.

Keywords: Stem rot, *Trichoderma* spp., *Sclerotium oryzae*, Rice, Bioagents, fungal antagonists.

Rice has served human as a life giving cereal since the dawn of Civilization.¹ Rice (*Oryza sativa* L.) is the world's most important staple food crop of Asian origin. About 90 percent of world rice is produced and consumed in Asian region. It contains approximately 6-12% protein, 70-80% carbohydrate, 1.2-2.0% mineral matter and significant content of fats and vitamins. Rice supplies 23% of global human/ capita energy and it fulfil 16% of per capita protein requirements.²

Globally it is cultivated in an area of 153.51 m ha with an annual production of about 650.19 million tonnes and an average productivity of 2.96

t ha⁻¹. Among the rice producing countries, India ranks second in total production (90.00 million tonnes) next to China (184.25 million tonnes) with an average productivity of 3.09 t/ha⁻¹.³ According to the forecast, the government of India has revealed that 2015-16 rice production is at around 90.6 million tons, which is slightly less from around 90.86 million tons of production in 2014-15.⁴ Rice is the major crop in Uttar Pradesh and grown in about 5.63 mha with a production of 11.94 mmt in 2010-2011.⁵

To meet the growing need of increasing population in the country and more so in the State of Uttar Pradesh, there is an urgent need to raise rice productivity in the region. Efforts for enhancing the productivity are limited by a number of biotic and abiotic stresses, 80% of our country human

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depend fully or partially on rice as their main cereal food and staple diet. The crop suffers from a number of devastating diseases, caused by fungus, bacteria, viruses, nematodes, phytoplasma and a number of environmental stress.⁶ Major fungal diseases of rice crop are Blast (*Magnaporthe grisea* Briosi and Cavara), Brown spot (*Helminthosporium oryzae* Brenda de Haan), False smut (*Claviceps oryzae*), Bunt (*Neovossia horrida*), Sheath rot (*Sarocladium oryzae* Sawada), Sheath blight (*Rhizactonia solani*), Stem rot (*Sclerotium oryzae* Cattaneo), Bakane disease or Foot rot (*Fusarium moniliforme*) and seedling blight (*Corticium rolfsii* Curzi).⁷

Among these diseases, stem rot caused by *Sclerotium oryzae* Catt. is one of the most serious diseases of rice in India causing significant reduction in yield.⁸ Paracer and Luthra has estimated 50-75 percent losses in yield in very badly infected field while crop losses of 5-15 percent occur almost every year in Punjab.⁹ Loss in yield as high as 72% have been recorded in rice growing areas of the Punjab and Pakistan.¹⁰ The disease is soil borne and remains, mainly confined to stem but it also attacks all the aerial plant parts. Globally, more than 3 billion people have rice as staple food, and it accounts for 50 to 80% of their daily calorie intake.¹¹ Over the next 20 years it is expected that demand for rice will grow by 2.5% per year.¹²

Due to the disadvantages of fungicides, integrated disease management programs are applied, in which judicious and recommended use of fungicides and their integration with biocontrol agents is favoured. Since fungicides may have deleterious effect on the pathogen as well as the antagonists, an understanding of the effect of fungicides on the pathogen and antagonists, would provide information on the selection of selective fungicides and fungicides resistant antagonists for compatibility studies.¹³ Majority of work done on plant disease biocontrol relate to soil borne diseases using either bacteria or fungal antagonists.¹⁴⁻¹⁶ However, the use of antagonistic fungi, especially *Trichoderma* and *Gliocladium* spp. has been more extensive than their bacterial counterparts.¹⁷⁻²⁰

There is considerable pressure from environmental scientists to give lesser emphasis on chemical control and promotion of biological

method for management of plant pests and diseases. Though the use of fungicides is necessary at present, however their use can be minimized as a long term solution to the crop health problem because they are hazardous and also eliminate natural enemies. So, the present investigation was carried out on the screening of different isolates of *Trichoderma* spp. against *S. oryzae* to find out the most effective strains for their further evaluation.

MATERIALS AND METHODS

Eight isolates of *Trichoderma* spp. were collected and used in this study. Among these four isolates of *Trichoderma harzianum* and 4 isolates of *Trichoderma viride* were collected from different sources. *T. harzianum* and *T. viridae* were also isolated from rhizospheric soil collected from rice field of Crop Research Centre (CRC) at S.V.B.P.U.A&T Meerut. The morphological and cultural characteristics of these biocontrol agents are given below.

Isolation of fungal biocontrol agents from rice field

The dilution plate method was applied for isolating fungal antagonists from soils, collected from rice field and rhizospheric soil of rice using a selective medium for the isolation of *Trichoderma* spp.²¹

Ten gm of soil was added to 100 ml of sterilized water in Erlenmeyer flask to give a dilution of 10^{-1} and shaken for half an hour. Ten ml aliquot of soil suspension was first transferred to a flask containing 90 ml of sterilized water. The process was repeated until required dilution (10^{-3} and 10^{-4}) was obtained. The entire process was done in an inoculation chamber under aseptic condition. The dilution used for *Trichoderma* spp was 10^{-3} and for other fungi it was 10^{-4} . The prepared dilution was taken with the help of a 1 ml sterilized pipette and poured into Petri plates which were containing 25 ml of *Trichoderma* selective medium (TSM). The plates were incubated at $28 \pm 1^{\circ}$ C for 3-5 days in B.O.D. incubator.

Identification of the fungal culture

The fungal culture was identified on the basis of their cultural and morphological characteristics. Slides were prepared with cotton blue and examined under compound microscope

for morphological characteristics of fungus (*Sclerotium oryzae* as well as antagonist (*Trichoderma* spp.)

Monoculture screening

For comparison of radial growth rates of fungal biocontrol agents in monoculture screening, twenty ml of sterilized PDA medium was aseptically poured in sterilized Petri plates and allowed to solidify. Five mm. mycelial discs of fungal isolates (biocontrol agents) cut from 4 days old culture plates, were placed at a centre of Petri plates. These plates were incubated at $28 \pm 1^{\circ}$ C. Periodic observations on the radial growth of fungal isolates were recorded.

Dual culture screening

Fungal isolates (biocontrol agents) were screened for their antagonistic potential against the pathogen following dual culture technique.²² Twenty ml of sterilized melted PDA medium was aseptically poured in a sterilized 90 mm diameter Petri plates and allowed to solidify. Five mm mycelial disc of pathogen *S.oryzae* and test bio control agents *Trichoderma* spp. cut with the help of sterilized cork borer from the edge of 4 days old culture plates, were placed on solidified PDA in such a manner that they lie just opposite to each other (approximately 6 cm apart from each other). Inoculated Petri plates were incubated at $28 \pm 1^{\circ}$ C. The process was repeated 6 times for seven consecutive days. During final observation the plates were 24, 48, 72, 96, 120, 144 hrs. old. Periodic observation on the growth of biocontrol agents and the ability of biocontrol agents to colonize the pathogen were recorded.

For the study of Mycoparasitism of biocontrol agents on pathogen, the mycelial fragments were taken out with the help of needle from the zone of interaction, stained with cotton blue mounted with lactophenol on a glass slide and teased out to separate individual hyphae. A cover slip was placed over it. Another slide was also prepared from mycelial fragment of completely over grown pathogen mycelium. Microscopic study was done with the help of microscope.

RESULTS AND DISCUSSION

Trichoderma harzianum

It grew actively on PDA medium producing hyaline, branched and septate

mycelium. Conidiophores arose within 48 hrs that were terminated with divergent phialides (verticillate type) bearing spores. The phialospores were smooth, green and sub-globose to short ovoid. It also produced thick walled, globose chlamydospores either terminal or intercalary. The fully grown culture on PDA was yellowish to green velvety.

Trichoderma viridae

The fungus grew profusely on PDA, producing hyaline, branched and septate mycelium. Conidiophores terminated into phialides. The phialospores were rough walled, green, sub-globose to short ovoid. The colonies after 48 hours emit coconut odour. The colour of full grown colony was greenish velvety.

Comparison of growth rates of fungal biocontrol agents (monoculture screening)

Eight isolates of *Trichoderma* spp. were screened in monoculture to compare the growth rates. Observations on radial growth of different isolates of *Trichoderma* showed that isolate of *Trichoderma viride* were slow growing, while isolates of *Trichoderma harzianum* were growing fastest followed by *Trichoderma viride* in general. Among four isolates only *Trichoderma harzianum* (Pantnagar isolate) attained a radial growth of 89.12 mm after 72 hours whereas *Trichoderma harzianum* (New Delhi) isolate and *Trichoderma harzianum* (Meerut isolate) attained 87.52 and 86.32 mm radial growth respectively, at 72 hrs of incubation the similar rate of growth was found at 42 hrs of incubation. (Table.1). *Trichoderma harzianum* Pantnagar isolate (89.12) was statistically at par with growth rate of Meerut isolate (86.32) at 72 hrs of incubation. Among isolates of *Trichoderma viride*, the Bangalore isolate showed fastest growth rate (68.72 mm) followed by New Delhi isolate (67.32 mm) and Meerut isolate (66.94 mm). However these were not significantly different from each other at 42 and 72 hrs of incubation.

In vitro* evaluation of fungal antagonist *Trichoderma* spp. against *S. oryzae

Fungal antagonists (*Trichoderma* spp) were tested by dual culture technique to study the inhibitory effect on mycelial growth of pathogen *S.oryzae*. The inhibition of mycelial growth of *S.oryzae* was measured up to 6 days. Observation indicated that growth of both the organisms in dual culture was visible in 24 hours of inoculations.

The visible mycelial contact between fungal antagonists and test fungus occurred on 3rd day of inoculations. The inhibition of mycelial growth of the pathogen firstly started by *Trichoderma harzianum* Pantnagar isolate followed by *Trichoderma harzianum* New Delhi isolate, Meerut isolate and rests other isolates. (Table 2).

The fungal antagonists completely inhibited the growth of pathogen *S.oryzae* and covered the entire plates within 6 days of inoculations. However *Trichoderma harzianum* (Pantnagar isolate) showed maximum inhibition and covered the entire plate on 4th day of inoculation followed by New Delhi and Meerut isolate on 5th day while all other isolates covered the entire plate on 6th day. Since *Trichoderma harzianum*

Pantnagar isolate showed maximum inhibition of the *S. oryzae*, this isolate was selected for further studies. Venkateswarlu *et al.* reported that the fungi viz., *Trichoderma harzianum*, *Fusarium* sp., *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Penicillium notatum*, *Alternaria* sp., and *Rhizopus* sp. were isolated from rhizosphere samples of paddy.²³ The antagonistic effect of these isolates was assessed based on their ability to inhibit the pathogen growth and sclerotial population by dual culture technique under *in vitro*. *Trichoderma* spp. are the most commonly used fungal biological control agents and have long been known as effective antagonists against plant pathogenic fungi.²⁴⁻²⁷ Dual culture of biocontrol agent (s) is also useful for *in vitro* screening, as it is a

Table 1. Radial growth of fungal isolates in monoculture at 42 hrs. and 72 hrs. incubation

S.N.	Fungal isolates with location	Radial growth *(mm)	
		42hr.	72 hr.
1	<i>Trichoderma harzianum</i> (New Delhi isolate)	55.26	87.52
2	<i>Trichoderma harzianum</i> (Kanpur isolate)	45.63	79.46
3	<i>Trichoderma harzianum</i> (Pantnagar isolate)	59.72	89.12
4	<i>Trichoderma harzianum</i> (Meerut isolate)	48.53	86.32
5	<i>Trichoderma Viride</i> (Bangalore isolate)	41.35	68.72
6	<i>Trichoderma viride</i> (Meerut isolate)	38.68	66.94
7	<i>Trichoderma viride</i> (New Delhi isolate)	39.43	67.32
8	<i>Trichoderma viride</i> (Kanpur isolate)	46.75	90.00
	CD at 5%	3.35	4.09

Table 2. *In vitro* evaluation of fungal antagonists against *S.oryzae* in dual culture

S. N.	Treatments	Observation on the growth of organism in dual culture days after inoculation					
		1	2	3	4	5	6
1	<i>S. o</i> + <i>T harzianum</i> (New Delhi)	+	+++			++++	
2	<i>S. o</i> + <i>T harzianum</i> (Kanpur)	+	++	+++			++++
3	<i>S. o</i> + <i>T harzianum</i> (Pantnagar)	+	+++		++++		
4	<i>S. o</i> + <i>T harzianum</i> (Meerut)	+	+++	+++		++++	
5	<i>S. o</i> + <i>T viride</i> (Banglaur)	+	++	+++			++++
6	<i>S. o</i> + <i>T viride</i> (Meerut)	+	++	+++			++++
7	<i>S. o</i> + <i>T viride</i> (New Delhi)	+	++				++++
8	<i>S. o</i> + <i>T viride</i> (Kanpur)	+	++	+++			++++

S o = *Sclerotium oryzae*

T = *Trichoderma*

+

++ = Contact of both organisms and contact zone

+++ = Inhibition of the mycelial growth of test fungus (*S.oryzae*)

++++ = Overlapping and biocontrol agents occupy full growth

measurement of relative saprophytic survival ability of biocontrol agents.²⁸

Knowledge of the mechanism is must and would prove very useful for the effective disease control. The mechanism of mycoparasitism was critically investigated. mycoparasitism includes both hyphal interaction and sclerotial parasitization. Many scientists are in favour of mycoparasitism as principal mechanism of biological control.²⁹⁻³⁰ Light microscopic study on hyphal interaction between species of *Trichoderma* and the pathogen indicated that biocontrol agent parasitized the mycelium of *S. oryzae*. Studies of hyphal interaction between biocontrol agent and *S. oryzae* indicated attraction of parasite towards their host, hyper parasitic coiling, penetration and finally resulting in to lysis or collapse of *S. oryzae* hyphae. Cambell demonstrated that the parasitized hypha of *Sclerotium* (revealed by penetration hole) by a parasitic fungus, *Trichoderma* spp. later has also been observed by many other scientists.³¹⁻³⁴ The enzymes can digest cell wall components which suggest that they are involved in lysis, penetration and/or in the contact region of the mycoparasite and its host. At later stages of parasitism host cell was completely lysed and protoplast protrudes out of host cell wall. Similar result was observed by Prakash and Puri.⁸ Results are in accordance to the previous findings and indicate that *Trichoderma harzianum* has maximum antagonistic potential against *Rhizoctonia solani* in comparison to *Trichoderma viride* and *Trichoderma virens*.³⁵ Prasanthi *et al.* reported that among eight antagonistic micro organisms in suppressing *Rhizoctonia bataticola* under *in-vitro* conditions, *T. viride* and *T. harzianum* could overgrew the pathogen.³⁶

CONCLUSION

Plant diseases caused by pathogenic fungi may result in significant yield losses of agricultural crops. Farmers, in general still rely on the use of synthetic fungicides to control plant diseases. However, the misuse of these chemicals may cause serious environmental and health problems. Microbial antagonists are potential agents that can be explored to provide effective and safe means to manage plant diseases. Several microorganisms have been tested and proven to

posses antagonistic properties against plant pathogenic fungi. Present recent study showed that the *Trichoderma* species apparently suppressed the growth of *Sclerotium oryzae*, the cause of rice stem rot disease. The potential of these agents can be improved by continual improvement in isolation, formulation and application methods, particularly in the field.

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