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RESEARCH ARTICLE

A COMPARATIVE STUDY OF FUNGICIDES AND BIOCONTROL AGENTS ON THE GROWTH AND SCLEROTIAL PRODUCTION OF *Rhizoctonia solani* AND *Sclrotium oryzae*

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ABSTRACT

The Phytopathogenic fungi *Rhizoctonia solani and Sclrotium oryzae* were isolated from the diseased rice plants. Nine fungicides and three biological controls were evaluated against the phytopathogenic fungi. Among nine fungicides screened eight fungicides were found to be 100% inhibition to both the test fungi, except the fungicide copper oxychloride could not inhibit the growth of the pathogenic fungi. However, the fungi could not produce Sclerotia at all. Among the bioagents, *T. hamatum and T. harzianum* could be completely overgrown *R. solani*. While all the biocontrol agents could grow 75% over *S. oryzae T. viride* could not inhibit completely the sclerotial production of *R. solani*. However *S. oryzae* failed to produce sclerotia in all the three biocontrol agents treated plates. Hence here is a scope to integrate bio agents for eco- friendly management instead of chemicals of stem rot and sheath blight disease of rice.

INTRODUCTION

In the context of globalization and WTO scenario it becomes immensely important to maintain the standards of export produce free of pesticides residues and grown with minimum interfere of inorganic chemical inputs. Biological control is the use of specific micro organism to inhibit or kill plant pathogen and pest. It is nature of friendly, ecologically approach to overcome the problems caused by standard chemical methods of plant protection. Biocontrol is an integral part of disease management to achieve this objective. Among the commonly used biocontrol agents *Trichoderma* species are the most tested against soil/seed borne disease. The potential of *Trichoderma* species as biocontrol agents of plant diseases was first recognized in the early 1930's (Weindling, 1932). Dennis and Webster (1971) found that many isolates of *Trichoderma* Spp. produced volatile and non- volatile antibiotics against wide range of fungi and indicating antibiosis as a mode of action (Kapoor, 2007). This has culminated in the commercial production of several *Trichoderma* species for the protection and growth enhancement of a number of crops in the United States, and in the production of *Trichoderma* species and mixtures of species in India, Israel, New Zealand, and Sweden (Howell 2003). The use of pesticide for disease control should be a component of an integrated system that uses cultural and biological controls.

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Soil borne pathogens have a broad host range and persist for longer periods in soil by resistant resting structures. Chemical control of soil borne pathogens provides certain degree of control but at the same time have adverse effects on environment affecting the beneficial soil microorganisms (Faheem *et al.*, 2010). Sheath blight (*Rhizoctonia solani)* in rice is a soil borne disease that cause devastating looses to tune of over 70% in endemic areas (Naidu, 1992). Phenomenal reduction in yield levels is possible if timely control measures are not initiated. Chemical control of the sheath blight disease is a success story at level in majority of the cases (Kandhari *et al.,* 2003*)*. However the chemical fungicides that effectively control the disease under field conditions should be able to vitiate the enduring effect of the sclerotial bodies that thrive, germinate and cause further infections on the host. Although satisfactory control of disease by using various chemicals has been documented in the literature, the continuous use of these agrochemicals for controlling the disease may pose several problems like toxicity to non- target organisms, development of resistance among population of pathogen and environmental pollution. Therefore, as an alternative to fungicide application it is desirable to exploit other environmentally safe means. There are much information on the management of diseases with biocontrol agents and chemical fungicides but there is little information on the management of sheath blight and stem rot of rice in the state. Stem rot of rice is caused by the fungus *S. oryzae* catt and it is widespread in over the world of rice producing areas and has been reported from most rice growing countries.

MATERIALS AND METHODS

Isolation of *Rhizoctonia solani and Sclerotium oryzae*

The phytopathogenic fungus *Rhizoctonia solani* and *Sclerotium oryzae* were isolated from the diseased sample of sheath blight and stem rot of rice respectively. The tissue segment method were used for isolating the organism that can grow on artificial media. For this method infected host tissues from the advancing margin of the lesions was selected and cut into small pieces of about 2-5 mm and transferred to sterile petridish from sheath blight infected rice. The phytopathogenic fungus *Sclerotium oryzae* was isolated from the diseased sample of stem rot of rice. The sclerotia present inside the rice stem was isolated in PDA slant and purified by hyphal tip cut method. The pieces were surface sterilized with 0.1% sodium hypochlorite solution for about half to one minute. List of the fungicides used as follows:

Table 1. List of the fungicides used

Sl.No.	Name of the Fungicides Used	Con.
1	Score (Difenoconazole, 25% EC) Systemic fungicide	0.1%
2	Indolfil M-45 (Mancozeb, 75% W.P) Contact fungicide	0.2%
3	Beam (Tricyclazole, 75% W.P) Systemic fungicide	0.1%
4	Blitox (Copper oxychloride,50% W.P) Contact fungicide	0.2%
5	Topsin (Thiophanate methyl 70%W.P) Broad Spectrum Systemic fungicide	0.1%
6	Contaf Plus (Hexaconazole, 5% SC) Systemic fungicide	0.1%
7	Basvinstin (Carbendazim, 50% EC) Systemic fungicide	0.1%
8	Tilt (Propiconazole, 25% E C) Systemic fungicide	0.1%
9	Ridomil (Metalaxyl 8% + Mancozeb, 64%,WP) Mixture of systemic and Contaf Fungicide	0.2%

The above fungicides were incorporated in 50 ml of potato dextrose agar after autoclaving to give the desired concentration. Each treatment was replicated thrice. A 5 mm mycelia discs were cut from a 3 day old actively growing culture with the help of sterilized Cork borer and placed at the centre of each plate by a sterilized needle. The inoculated plates were incubated at 25 ± 1^0c .The medium without fungicide served as control. The radial growths of the fungus in each plate were recorded at every 24 hours interval till the control plates were fully covered with mycelium. The percentage of inhibition on growth was calculated by the following the method described by Vincet (1927) as given below:

$$I = \frac{C-T}{C} \times 100$$

Where,

I=percentage of inhibition
C= growth in control
T= growth in treatment

Biocontrol Agents

Three biocontrol agents namely *Trichoderma viride, Trichoderma hamatum, Trichodrema harzianum* were obtained from Plant Pathology Department, Central Agricultural University - Imphal for the experiment under the *in vitro* condition. Dual culture plate technique described by Bell's *et al.* (1982) using potato dextrose agar medium was followed. 5 mm mycelial discs of both of three antagonists and test pathogens from the three day old actively growing culture were taken and aseptically transferred to petridishes containing PDA and placed 3 cm apart from each other .The seeded plates were then incubated at 25 ± 1^oC. Each treatment was replicated thrice. Recording was started after a close watch. All ratings were done after contact between the pathogen and antagonist using a modified Bell's scale (Bell *et al*., 1982; class 1-5) developed as class 1= the antagonist completely overgrew the pathogens (100% overgrowth), class2=the antagonist overgrew at least $2/3^{rd}$ of pathogen surface (75% overgrowth), class 3= the antagonist colonized on half of the growth of the pathogen

Table 2. Results showing efficacy of the fungicides on growth and formation of sclerotia of *Rhizoctonia solani*

Name of fungicides	Conc. %	Linear growth (cm) after incubation(hrs)			% Inhibition on growth	No. of Sclerotia produced	% Inhibition Sclerotia production
		24	48	72			
Propiconazole	0.1	0	0	0	100	0	0
Thiophanate methyl	0.1	0	0	0	100	0	0
Ridomil	0.2	0	0	0	100	0	0
Mancozeb	0.1	0	0	0	100	0	0
Hexaconazole	0.1	0	0	0	100	0	0
Tricyclazole	0.1	0	0	0	100	0	0
Difenoconazole	0.1	0	0	0	100	0	0
Carbendazim	0.1	0	0	0	100	0	0
Copper oxychloride	0.2	0	0.5	1.2	86.66	0	0
Control (PDA)	-	2.5	8.3	9	0	95	-

Table 3. Results showing efficacy of the fungicides on growth and formation of sclerotia of *Sclerotium oryzae*

Name of fungicides	Conc. %	Linear growth (cm) after incubation(hrs)			% Inhibition on growth	No. of Sclerotia produced	% Inhibition Sclerotia production
		24	48	72			
Propiconazole	0.1	0	0	0	100	0	0
Thiophanate methyl	0.1	0	0	0	100	0	0
Ridomil	0.2	0	0	0	100	0	0
Mancozeb	0.1	0	0	0	100	0	0
Hexaconazole	0.1	0	0	0	100	0	0
Tricyclazole	0.1	0	0	0	100	0	0
Difenoconazole	0.1	0	0	0	100	0	0
Carbendazim	0.1	0	0	0	100	0	0
Copper oxychloride	0.2	1.5	2.0	2.5	72.2	0	0
Control (PDA)	-	3.7	6.3	9	0	Abundant	-

(50% overgrowth) class 4= the pathogen and antagonist locked at the point of contact and class 5= the pathogen overgrew the mycoparasite. Per cent inhibition on sclerotial production was calculated after 15 days.

RESULT AND DISCUSSION

Effect of Fungicides

Among the nine different fungicides screened *in vitro* against mycelia growth of *Rhizoctonia solani* and *Sclerocium oryzae* were hexaconazole, tricyclazole, propiconazole, thiophanate methyl, mancozeb, difenoconazole, carbendazim and ridomil and could completely inhibit the mycelial growth of both phytopathogenic fungi. However, the pathogen could grow in copper oxychloride at 0.2% with inhibition percentage over control as 86.665% & 72% respectively (Table 1 and 2). Phytopathogenic fungus could not produce sclerotia at all (100%) inhibition in all the fungicides treated plates (Figure 1 and 2). The present findings are in the agreement with Vijay *et al*., (2008) who reported that fungicides propiconazole, tebuconazole, hexaconazole and carbendazim + mancozeb completely inhibited the radial growth of *Rhizoctonia solani*. Tebuconazole is effective in controlling the sheath blight pathogen, *Rhizoctonia solani* Kuhn were carried out both under laboratory and at field levels (Krishnan *et al., 2008)*. Carbendazim and Thiophanate methyl were effective in inhibiting sclerotial germination and mycelial growth of *S. oryzae* (Singh *et al.,* 1985).

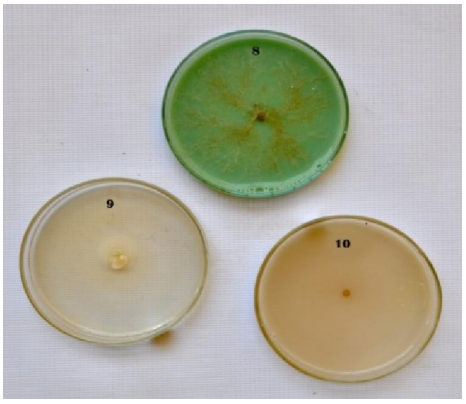

Figure 1. Effect of fungicides on linear growth of *Rhizoctonia solani*

[1]. Tricyclazole 75%WP (0.1%) [2]. Pripiconazole 25%EC (0.1)
[3]. Thiophanate Methyl 70% (0.1) [4]. Mancozeb 75%WP (0.2)
[5]. Difenoconazole 25%EC (0.1%) [6]. Hexaconazole 5% SC (0.1%)
[7]. Control (untreated) [8]. Copper oxychloride 50%WP (0.2%)
[9]. Carbendazim 50% WP (0.1%) [10]. Ridomil 72%WP (0.2)

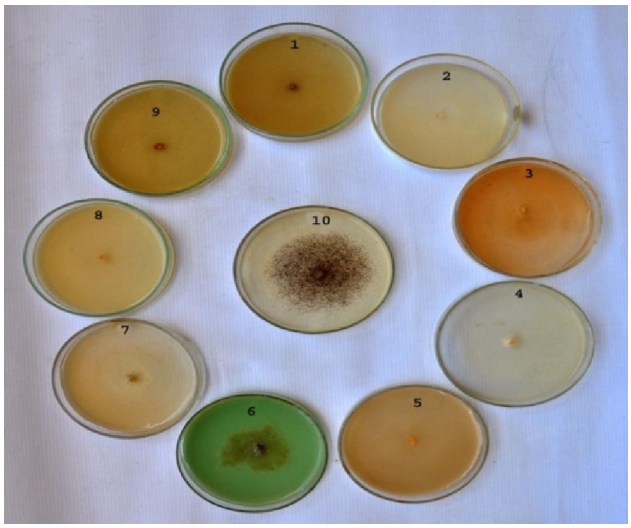

Figure 2. Effect of fungicides on the linear growth of *Sclerotium oryzae*

[1]. Propiconazole 25%EC (0.1%) [2]. Thiophanate methyl
[3]. Ridomil 72%WP (0.2) [4]. Mancozeb 75%WP (0.2)
[5]. Hexaconazole 5% SC (0.1%) [6]. Tricyclazole 75%WP (0.1%)
[7]. Difenoconazole 25%EC (0.1%) [8]. Carbendazim 50% WP (0.1%)
[9]Copper Oxychloride 50%WP (0.2%) [10]. Control (Untreated)

Figure 3. Effect of Bio-control Agents on the growth of *Rhizoctonia solani* using dual culture technique after 15 days

1. Trichoderma harzianum 2. Trichoderma hamatum 3. Trichoderma viride

Figure 4. Effect of Bio-Control Agents on the growth of *Sclerotium oryzae* using dual culture technique after 15 days

1. Trichoderma harzianum 2. Trichoderma hamatum 3. Trichoderma viride

Table 4. Efficacy of biocontrol agents on growth of *Rhizoctonia solani*

Treatment	Growth (cm) after (hr)		No. Of days required for point of contact	Bell's scale
	24	48		
1.*T. viride.*	3	6	3	Class 4
2.*T. hamatum.*	5	7	3	Class 1
3.*T. harzianum*	3.5	6.8	3	Class 1

Table 5. Efficacy of biocontrol agents on growth of *Sclerotium oryzae*

Treatment	Growth (cm) after (hr)		No. Of days required for point of contact	Bell's scale
	24	48		
1. T. viride	3.5	6.2	3	Class 2
2. T. hamatum	5.2	7.5	3	Class 2
3. T. harzianum	3.9	6.8	3	Class 2

Table 6. Efficacy of bio control agents on sclerotia production of *R. solani*

Treatment	No. of sclerotia produced		% of Inhibition over control	
	R. solani	*S. oryzae*	*R. solani*	*S. oryzae*
1. *T. viride*	0	0	100	100
2. *T. hamatum*	0	0	100	100
3. *T. harzianum*	18	0	66.6	100

Effect of Bio-Control Agents

Among the biocontrol agents *T. harzianum* and *T. hamatum* completely inhibited (100%) and sclerotial production of the pathogen *Rhizoctonia solani* (Table 5). However, 76.4% inhibition was observed in the *T. viride,* the pathogen could produce less sclerotia very far away from the point of contact (Figure 3). It was observed against *Sclerocium oryzae* that all the three biocontrol agents could contact after 48 hours of incubation with the fungus. All the three biocontrol agents showed 75% overgrowth the fungus. The pathogen could not produce any sclerotia at all due to overgrowth of bioagents (Figure 4). Similarly, Prasanthi *et al.* (2000) reported that among eight antagonistic micro organisms in suppressing *Rhizoctonia bataticola* under *in-vitro* conditions, *T. viride* and *T. harzianum* could overgrew the pathogen. Similarly Correa *et al.* (1996) reported that *T. harzianum* inhibited mycelial growth of *Sclerotium cepivorum* due to secondary metabolites trichorzianins and Prakhia and Vaishnav (1986) reported that *Trichoderma harzianum* was effective in controlling *Rhizoctonia bataticola.* Further they again reported that the growth of *R. bataticola* was stopped at the point of contact where Trichoderma *harzianum* met at culture and then overgrew. Hence, it may be concluded that for the management of phytopathogenic soil borne fungi, *Rhizoctonia solani and Sclerotium oryzae* causing sheath blight and stem rot of rice respectively, has the scope to integrate or use of effective biocontrol agents for eco friendly management.

Trichoderma isolates reduced the growth of all the two soil borne pathogens significantly and, therefore, can be incorporated for integrated disease management of soil borne plant pathogens.

REFERENCES

Bell, D.K., Wells, H.D. and Markham, C.R. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology*. 72: 379-382.

Correa, A., Robequebert, M.F, Bettuci, L. 1996. *Trichoderma* activity on mycelia growth of *Sclerotium cepivorum* under in-*vitro*. Cryptogenic, *Mycologic,* 17(21): 25-128.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species group of *Trichoderma*-III. production of volatile antibiotics. *Transactions of British Mycological Society*. 51**:** 363-369.

Faheem , A., Razdan, V.K., Mohiddin, F. A., Bhat, K. A. and Saba B. 2010. Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *Journal of Phytology*. 2(10): 38–41.

Howell, C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases. *Plant Disease Research*. 87 (1): 127-137.

Khandhari, j., Gupta, R.J. and Khandhari, J. 2003. Efficacy of fungicides and resistance inducing chemicals against sheath blight of rice. *Journal of Mycopathological Research*. 41: 67-69.

Krishnan, R.S., Vijay, K.K. and Ramabhadra, R.M. 2008. Efficacy of tebuconazole against *Rhizoctonia solani* , causal agent of rice sheath blight. *Indian Journal of Plant Protection*. 36(1): 98-101.

Naidu, V.D. 1992. Influence of sheath blight of rice on grain and straw yield in some popular local varieties. *Journal of Research*. 10: 78-80.

Prakhia, A.M. and Vaishnav, M.U. 1986. Biocontrol of *Rhizoctonia baticola. Indian Phytophatology*. 39: 439-440.

Prasanthi, S.K., Kulakrni S.N. and Anakosur, K.H. 2000. Management of sunflower root rot caused by *Rhizoctonia bataticola* by antagonistc micro-organisms. Plant Disease Research. 15(2): 146-150.

Singh, R.A. and Pavgi, P.K. 1998. Stem rot of rice in Uttar Pradesh, *Indian Phytopathology*. 57: 24-28.

Vijay, K.K., raju, S.K. and Raju, M.R. 2008. Efficacy of Tebuconazole against *Rhizoctonia solani* causal agent of rice sheath blight. *Indian Journal of Plant Protection*. 36 (1): 68-101.

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 159: 850.

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*. 22: 837- 845.

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