

**RESEARCH ARTICLE :**

Studies on disease incidence and efficacy of fungicides, herbicides and antagonists micro flora against stem rot of rice (*Sclerotium oryzae*) along with integrated management

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SUMMARY : Survey studies conducted in the rice growing districts of Telangana, revealed that incidence of stem rot disease varied from field to field (0 to 15%) in the surveyed districts of Khammam and Warangal. The disease incidence was varied from location to location. Highest disease incidence was recorded in late transplanted as compared to early transplanted crop. Maximum stem rot incidence (14.8%) was recorded in rice-rice followed by rice-groundnut (3.8%), rice-maize (2.8%) and sunhemp-rice-maize-vegetables (2.1%). Similarly, highest disease incidence was found in clay loam soils (14.0%), followed by clay soils (8.7%), loamy soils (7.8%), while lowest incidence of stem rot was noted in fields with sandy soils (2.7%) and sandy loam soils (2.6%). The fields received with 151-180 kg N ha⁻¹ showed highest disease incidence (11.1%) followed by 120-150 kg N ha⁻¹ (8.1%) and 100-120 kg N ha⁻¹ (2.5%). Maximum disease incidence of stem rot was recorded in the fields which received pretilachlor (7.9%) as compared to the fields received butachlor (2.5%). Studies conducted in the rice growing districts of Andhra Pradesh, revealed that incidence of stem. The mycoflora and bacteria were isolated from rhizosphere soil associated with diseased rice plants during the survey on Martin medium and soil extract agar medium, respectively. Mycoflora viz., *Aspergillus flavus*, *A. niger*, *Cladosporium*, *Trichoderma viride* isolate-1 and 2 while bacterial isolates viz., *Pseudomonas fluorescens* (BI-1), isolate-2 (BI-2), isolate-3 (BI-3), isolate-4 (BI-4), isolate-5 (BI-5) were found to be antagonistic to test pathogen *S. oryzae*. The detected mycoflora and bacterial isolates were further screened following dual culture technique and the results indicated that among mycoflora screened, *T. viride* (T₁) was found to have most potential antagonistic effect with maximum inhibition (75.3 %) of test pathogen. Similarly among antagonistic bacterial isolates screened *P. fluorescens* (BI-1) was found to be highly effective in inhibiting the test pathogen by 77.2 per cent. These potential biocontrol agents can be exploited as an integrated approach in the management of stem rot of rice. The compatibility studies between *T. viride* (T₁) and *P. fluorescens* (BI-1) following dual culture technique under *in vitro* conditions indicated that the per cent inhibition of *T. viride* (T₁) by *P. fluorescens* was 5.0 per cent, while no inhibition was observed in the growth of *P. fluorescens*. Out of six fungicides tested, Hexaconazole @ 200 ppm and Propiconazole @ m100 ppm completely inhibited *S. oryzae* in poisoned medium. Out of two herbicides tested, the inhibition of test pathogen was high (97.1%) in Butachlor (400 ppm) and least (28.0%) in Oxadiargyl (150 ppm). In compatibility studies, *T. viride* (T₁) was incompatible with fungicides Propiconazole (100 ppm) and Hexaconazole (200 ppm). However, it was 60.6 per cent inhibited

by Butachlor (400 ppm). Similarly, *P. fluorescens* was least (7.9%) compatible with Propiconazole and highly (3.6%) compatible with Butachlor (400 ppm). Integrated management against stem rot of rice was attempted with twelve treatments under pot culture. Of the twelve treatments combined soil application of butachlor (400 ppm) 8-10 days after inoculation by the pathogen followed by application of *T. viride* @ 10 g kg and *P. fluorescens* @ 10 ml kg⁻¹ just at the appearance of the disease followed by spraying of propiconazole (100 ppm) were found superior over other treatments in reducing the disease and promoting the plant growth parameters like root length, shoot height, dry shoot and root weight of rice plants.

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BACKGROUND AND OBJECTIVES

“Rice is life” aptly describes the importance of rice in food and nutritional security particularly for Asian countries. The crop is prone to the attack of many diseases caused by fungi, bacteria and viruses. Of which, stem rot disease caused by *Sclerotium oryzae* hitherto considered as a minor disease is now prevalent in most popular cultivars of rice causing considerable loss in quality and quantity of the produce. In Telangana and Andhra Pradesh the disease is reported from rice growing areas of East Godavari, West Godavari, Khammam and Warangal districts. The yield losses upto a maximum of 80 per cent in different rice cultivars has been reported by several workers from varied agro climatic regions in India and abroad (Ou, 1985 and Cother and Nicol, 1999). Continuous and contiguous cultivation of rice during different seasons under high dosages of nitrogenous fertilizers and prevalence of many graminaceous weed flora in rice fields which serve as collateral host of *S. oryzae* and lack of proper irrigation and drainage facilities have progressively aggravated the stem rot disease in recent years. Although control of stem rot through fungicides examined, induction of host resistance through antagonists has not received attention. Hence the present study was undertaken to record disease incidence in various rice growing areas and the effect of fungicides, herbicides and antagonist microflora against *S. oryzae*.

RESOURCES AND METHODS

Disease survey :

Extensive roving survey was carried out in farmers' fields covering the major rice growing areas of Warangal and Khammam districts of Andhra Pradesh to assess

the incidence of stem rot disease during *Kharif* 2009. In each field, a plot size of 1.0 x 1.0 m was selected diagonally at five locations for recording the disease incidence. The plants were visually examined for characteristic stem rot lesions during mid tillering, flowering, maturity and harvesting stage of the crop following Ou (1985) and Standard Evaluation System (SES) of Rice formulated by IRRI. In each plot, 10 hills were selected at random to count number of diseased and healthy plants and the per cent disease incidence was calculated by using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total number of plants per ten hills}} \times 100$$

In addition to assessing disease incidence the various agronomic practices followed by farmers in different rice eco-systems were recorded by using a common data format. The data on different parameters *viz.*, date of sowing, name of the cultivars, cropping sequences, soil texture of the field, usage of herbicides and nitrogenous fertilizers were recorded and the pre-disposing factors involved in the development of stem rot incidence was analysed based on the data collected.

Isolation and compatibility of native microflora :

Rhizosphere of healthy and diseased samples collected from Khammam and Warangal districts were isolated by following serial dilution technique (Johnson and Curl, 1977). Composite soil sample (50 g) was collected from rhizosphere of healthy plants and stem rot infected rice plants. The soil was shade dried and then used for serial dilution.

Rhizosphere mycoflora were isolated on rose bengal agar medium by using a dilution of 10⁻⁴ and bacteria were

isolated on soil extract agar medium by using dilution of 10^{-6} . Three days old colonies of mycoflora were picked up and purified by single hyphal tip method whereas, one day old colonies of bacteria were picked up and purified by streak plate method. Compatibility / incompatibility between potential native antagonistic fungus and bacterium were determined using dual culture technique.

Efficacy of fungicides and herbicides against *S. oryzae* in vitro :

In vitro efficacy of fungicides and herbicides against the test pathogen was evaluated by poisoned food technique (Nene and Thapliyal, 1993). Six commercial fungicides Carbendazim 50% WP (Bavistin), Propiconazole 25% EC (Tilt), Metaminostrobin 20% SC (Varisma), Tebuconazole 80% WP (Raxil), Hexaconazole 5% SC (Contaf), Azoxystrobin 25% SC (Amister), and two herbicides oxadiargyl 80 % WP (Topstar), butachlor 50EC (Hunter) were tested at their recommended dosages. Fifty ml of double strength PDA was mixed with 50 ml of double concentrated fungicidal solution to obtain final concentrations of recommended ppm of each fungicides and herbicides. Twenty ml of this medium was plated in 9 cm Petri plates. A six mm mycelial disc of five days old pathogen was inoculated at the centre and then incubated at $28 \pm 2^\circ\text{C}$ for 7 days. A control was maintained without fungicide. Three replications were maintained for each treatment and per cent inhibition was calculated.

Compatibility in vitro of antagonistic microflora with effective fungicide and herbicide :

The compatibility of the potential native antagonistic fungus *i.e.* *Trichoderma* isolate-1 (T_1) and bacteria *P. fluorescence* (BI-1) with the effective fungicide and herbicide was determined using poisoned food technique.

Glass house studies :

To study the effect of integration of potential antagonists, fungicides and herbicide on stem rot incidence incited by *S. oryzae*, an experiment was conducted in pot culture using susceptible rice cv. MTU 3626 with twelve treatments and three replications under glass house. Earthen pots of 9 inch diameter and un-sterilised soil containing red, black and FYM mixture @ 3 kg per pot (1:1:1) were used for this experiment. Five gm urea was applied in each pot as basal followed by split application

of nitrogen through urea @ 5 g per pot for 2 - 3 times, at 15 days interval after establishment of seedlings.

Preparation and inoculation of the pathogen :

Seven day old culture of *S. oryzae* grown on PDA medium was inoculated on to the sand maize meal medium and incubated at $23.5 \pm 2^\circ\text{C}$ for 30 days. 22 days old seedlings of susceptible rice cv. MTU -3626 grown in plastic trays were transplanted in earthen pot @ six seedlings per pot. Fifteen days after transplanting of rice seedlings, thirty days old culture of *S. oryzae* (containing mycelium and sclerotia) grown on sand maize meal medium was inoculated into the soil @ 50 gm kg^{-1} and mixed in upper layers of 1-2 cm soil and then the pots were moistened with water.

Spraying of fungicides :

The fungicides *viz.*, hexaconazole (200 ppm) and propiconazole (100 ppm) were sprayed by using automizer just at the appearance of the disease at mid tillering stage (55-60 days old seedlings).

Application of *T. viride* and *P. fluorescence* :

15 day old culture of *T. viride* multiplied on sorghum grains was added to the top 1-2 cm of soil @ 10 g kg^{-1} just at appearance of disease. It was followed by incorporation of seven day old culture of *P. fluorescence* multiplied on nutrient broth was added @ 10 ml kg^{-1} soil.

Application of butachlor :

Butachlor was added to the soil @ 1.5 ml per pot of soil 8-10 days after inoculation by the pathogen after moistening the pots.

Application of *T. viride*, *P. fluorescence* and fungicides:

15 day old culture of *T. viride* multiplied on sorghum grains was added to the top 1-2 cm of soil @ 10 gm kg^{-1} soil. After 48 h of incorporation of *T. viride*, seven day old culture of *P. fluorescence* multiplied on nutrient broth was added @ 10 ml kg^{-1} soil and this was followed by spraying of fungicides (propiconazole @ 100 ppm and hexaconazole @ 200 ppm) just at the appearance of disease.

Application of butachlor, *T. viride* and *P. fluorescence*:

Butachlor was added to the soil @ 1.5 ml per pot

of soil 8-10 days after inoculation by the pathogen after moistening the pots. This was followed by application of *T. viride* @ 10 g kg⁻¹ and *P. fluorescens* @ 10 ml kg⁻¹ just at appearance of the disease.

Application of butachlor and fungicides :

Butachlor was added to the soil @ 1.5 ml per pot of soil 8-10 days after inoculation by the pathogen after moistening the pots, while the fungicides (propiconazole @ 100 ppm and hexaconazole @ 200 ppm) was sprayed on the stem just at the appearance of the disease.

Application of butachlor, *T. viride*, *P. fluorescens* and fungicides :

Butachlor was added to the soil @ 1.5 ml per pot of soil 8-10 days after inoculation by the pathogen after moistening the pots. This was followed by application of *T. viride* @ 10 g kg⁻¹ and *P. fluorescens* @ 10 ml kg⁻¹ just at appearance of the disease and fungicide (propiconazole @ 100 ppm and hexaconazole @ 200 ppm) was sprayed just after appearance of the disease.

Inoculated control :

Inoculated control was maintained by mixing of unsterilised soil with *S. oryzae* mycelial mat along with sclerotia @ 15 g per pot.

Disease incidence was recorded at the time of maximum disease development (*i.e.*, at mid tillering to booting stage) by following disease index formula given by Krause and Webster (1973).

$$\text{Disease index (DI)} = \frac{(H^n) + 2(L^n) + 3(M^n) + 4(M^n) + 5(S^n)}{\text{Total no. of tillers examined}}$$

where,

Hⁿ = Number of healthy tillers with no symptoms.

Lⁿ = Number of slightly infested tillers with disease lesions only on the outer leaf sheath.

Mⁿ = Number of mildly infested tillers with lesions extending through the sheath to the culm.

Mⁿ = Number of moderately infested tillers with lesions penetrating the culm.

Sⁿ = Number of severely infected tillers with mycelium and /or sclerotia formed within the culm.

The growth parameters *viz.*, plant height (cm), root length (cm), dry shoot weight (g) and dry root weight (g) were also recorded to know the inhibitory or stimulatory effect of the treatments imposed.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Survey for the incidence of Stem rot of rice, *Sclerotium oryzae* :

Out of 76 rice fields surveyed in two districts of Warangal and Khammam of Andhra Pradesh, eight fields recorded the disease incidence ranging from 10.1 – 15.0 per cent. In 59 fields incidence of stem rot was 5.1 - 10.0 per cent and in rest of the 9 fields the disease incidence was 0-5 per cent (Table 1). Krishnaveni and Laha (2009) noted that rice crop grown over 45,000 ha in East Godavari was infected by stem rot disease in just three days during the *Kharif* season 2007. Kumar *et al.* (2009) claimed that stem rot was the great limiting factor in rice cultivation in upland rice fields of Imphal, Thoubal, Bishanpur, Senapati districts of Manipur and Haryana.

During the survey it was observed that the rice crop transplanted (31 fields) between September 8-24 showed stem rot incidence of 13.1 per cent while incidence was slightly less in crop transplanted (36 fields) from August 8-21 (8.1%) (Table 2). Lowest stem rot incidence (2.7%) was recorded in crop transplanted (9 fields) between July 8-24. None of the cultivars were found free from stem rot infection. Out of the 13 cultivars surveyed, maximum per cent disease incidence (14.8%) was recorded in MTU 1061 and least in RNR 2465 (2.5%), NLR 34449 (2.5%) and PT 1042 (2.5%). A definite trend was observed in the reaction of varieties with transplanting time, where in late transplanted crop more succumbed to heavy rains occurred in the month of September due to North East monsoon than the early transplanted crop *i.e.*, July 8 – 24. It was observed that the disease incidence was more in late transplanted crop irrespective of the cultivars and soil type. This is mainly attributed that in the fields transplanted late in the season (September 8 – 24) with aged seedlings of long / medium duration varieties *viz.*, BPT 5204, MTU 1075, WGL 44 etc, the farmers followed close planting with 4 – 5 plants / hill duly applying higher doses of nitrogen (150 – 180 kg N ha⁻¹) in two splits instead of 3 – 4 splits and this might have favoured higher disease incidence. Laha (2009) emphasized that, changes in cultivation practices, high relative humidity (>80%), high temperature (30 – 35°C) and water logged conditions have led to alarming

Table 1: Incidence of stem rot of rice in different villages of Warangal during Kharif 2009

Villages/District	No. of fields	Area (ha)	No. of fields/varieties showing different levels of disease incidence					
			0-5.0%		5.1-10.0%		1.1-15.0%	
			Fields	Varieties	Fields	Varieties	Fields	Varieties
Warangal								
Hasahparthy, Kommala, Geesukonda, Chalaparthy, Duggondi, Madannapeta, Narsampeta	15	14.3	3	RNR 2465, NP 360	9	BPT 5204, MTU 1001, MTU 1010, JGL 384, WGL 14	3	WGL 44
Ameerabad, Chennarpet, Nekkonda, Battuthanda, Parvathagiri	11	13.3	-	-	11	BPT 5204, MTU 1001, MTU 1010, JGL 384	-	-
Turkalasomaram, Parvathagiri, Upparpally, Vardhnnapeta, Katryal, Station Ghanpur	15	7.0	-	-	15	BPT 5204, MTU 1001, JGL 384, WGL 14	-	-
Sub Total	41	34.6	3	-	35	-	3	-
Khammam								
Jakkepally, Kusumanchi, Rajeshwarapuram, Nelakondapally	12	16.4	2	NLR 34449	8	BPT 5204, MTU 1010	2	MTU 1061
Tutipudi, Vemsur, Guvvalagudem, Vyra, Medipally, Mudigonda, Tumburu, Sattupally, Madapuram	13	45.8	1	RNR 2465	9	BPT 5204, MTU, MTU 1010	3	MTU 1061
Madapuram, Byyaram, Khammam (R)	10	9.7	3	MTU 1078, NLR 34449	7	BPT 5204, MTU 1001, MTU 1010, WGL 14	-	-
Sub total	35	71.9	6	-	24	-	5	-
Grand total	76	106.5	9	-	59	-	8	-
% fields showing different levels of Disease incidence	-	-	11.8	-	77.6	-	10.5	-

increase in the stem rot disease. According to review made by Nevertheless, the present findings strongly suggest that time of transplanting and maturity group influence the occurrence of stem rot disease in a given location.

The crop sequence played an important role in reducing stem rot incidence as maximum stem rot incidence of (14.8%) was recorded in rice-rice sequence followed by rice-groundnut (3.8%), rice-maize (2.8%) and sunhemp-rice-maize-vegetables (2.1%). The results clearly indicates that monocropping of rice, season after season definitely increases the incidence of the disease whereas, other crop sequences do not provide congenial conditions required for growth, development and infection of *Sclerotium oryzae*. Konthoujam and Chhetry (2005) reported that stem rot of rice is a potential threat to mono cropped rice and may eventually become endemic and wide spread sooner or later.

Highest disease incidence was recorded in clay loam soils (14.0%), followed by clay soils (8.7%), loamy soils (7.8%), while lowest incidence of stem rot was noted in fields with sandy soils (2.7%) and sandy loam soils (2.6%). The difference in incidence. These floating sclerotia easily come in contact with rice leaf sheaths and germinate to form approsoria or infection cushion

leading to severe infestation in clay/ loam rice fields. It may therefore be argued that the level of water maintained in the field based on the soil type influence the disease incidence as described by Konthoujam *et al.* (2007). The low intensity of disease in sandy and sandy loam soils is mainly attributed to prevalence of saturated moist conditions due to alternate wetting and drying. According to Keim and Webster (1974), alternate wetting and drying reduced sclerotial viability of *Sclerotium oryzae* sclerotia in rice soils (Sandy / Sandy loams) which supports the results of present study.

Minimum disease incidence (2.5%) was noted where rice fields were applied with 100-120 kg N ha⁻¹ followed by 121-150 kg N ha⁻¹ (8.1%) and 151-180 kg N ha⁻¹ (11.1%). Konthoujam *et al.* (2007) have reported that application of NPK @ 120-80-30 kg ha⁻¹ enhanced the stem rot disease incidence by 57.7 per cent and disease severity by 54.3 per cent and resulted to an abrupt decline in the yield by 13.6 per cent. A comprehended picture of the effect of N P K on stem rot of rice during the survey is clearly reflected. This finding confirms the observations made by Sharma and Verma (1985), who reported 54.1 and 60.0 per cent yield reduction respectively, due to the disease under Indian conditions with increased dosage of nitrogen from 120-180 kg ha⁻¹.

Table 2 : Effect of various agronomic practices on incidence of stem rot of rice caused by *Sclerotium oryzae*

Agronomic practices	No. of fields	Mean disease incidence* (%)
Date of transplantation		
July 8 – July 24 (Long duration varieties)	9	2.7
Aug 8 – Aug 21 (Long and medium duration varieties)	31	8.1
Sep 8 - Sep 24 (Long, medium and short duration varieties)	36	13.1
Varieties		
Long duration varieties		
BPT 5204	28	10.0
WGL 14	6	7.7
MTU 1061	4	14.8
MTU 1075	1	13.6
WGL 44	3	13.7
MTU 1078	1	3.2
Medium duration varieties		
MTU 1010	7	8.7
RNR 2465	3	2.5
NP 360	1	2.7
NLR 34449	3	2.5
PT 1042	1	2.5
MTU 1001	14	8.6
JGL 384	4	7.2
Crop sequence		
Rice-Rice	67	14.8
Rice-Ground nut	3	3.8
Rice-Maize	3	2.8
Sunhemp-Rice-Maize-Vegetables	3	2.1
Soil texture/pH		
Clay (7.5-8.2)	7	8.7
Clay loam (7.5-7.8)	8	14.0
Loam (7.2-7.5)	52	7.8
Sandy loam (7.2-7.6)	4	2.6
Sandy (7.5-7.9)	5	2.7
Herbicides usage		
No weedicide	52	13.1
Butachlor	12	2.5
Oxadiargyl	3	3.2
Pretilachlor	9	7.9
Usage of nitrogenous fertilizers (kg N ha⁻¹)		
151-180	37	11.1
121-150	31	8.1
100-120	8	2.5

*Average of number of fields

Keim and Webster (1974) noted that the amount of stem rot increased with increasing rates of nitrogen. Thus any further increase in nitrogen dose above 120 kg N ha⁻¹ enhances stem rot disease status which consequently may reduce the grain yield. Maximum disease incidence (13.10%) was recorded in fields where no herbicides were applied followed by pretilachlor (7.9%), oxadiargyl (3.2%) and butachlor (2.5%) sprayed fields.

It was observed that the reaction exhibited by different rice cultivars to stem rot was neither restricted to their crop duration, nor the nutritional status of the soil nor climatic conditions wherever they were grown. This apparently suggests that resistance or susceptibility is an inherent character and cultivar specific, which is not influenced either by the soil status or duration of the cultivars. Ali and Singh (1994) and Konthoujam and Chhetry (2007) also opined that resistance is a varietal character and is independent of maturity period. Observations gathered during the present investigation suggests that, a few genotypes resistant to stem rot exists among medium maturity cultivars viz., MTU 1010, MTU 1001, WGL 14, JGL 384, RNR 2465, NLR 34449 etc., as compared to long duration maturity types.

Identification of native rhizosphere mycoflora and bacteria :

Mycoflora and bacteria were identified based on colony and morphological characters (Barnett and Barry, 1972 and Bergey's Manual of Determinative Bacteriology). Five fungi viz., *Aspergillus flavus*, *A. niger*, *Cladosporium* sp., *Trichoderma* sp. isolate-1 and *Trichoderma* sp. isolate-2 were isolated and five bacterial isolates viz., *P. fluorescens* (BI-1) Bacterial isolate-2 (BI-2), Bacterial isolate-3 (BI-3), Bacterial isolate-4 (BI-4) and Bacterial isolate-5 (BI-5) were detected during isolation.

Mass multiplication of *Trichoderma* sp. isolate-1(T₁) and *Pseudomonas fluorescens* (BI-1) :

Trichoderma sp. isolate-1 (T₁) was mass multiplied on sorghum seeds for 15 days and added to soil @ 10g kg⁻¹ soil, two days after the pathogen inoculation. *P. fluorescens* (BI-1) was mass multiplied on nutrient broth for 48 hours and applied to soil @ 10 ml kg⁻¹ soil, 12 hour after inoculation with *Trichoderma* sp. isolate-1 (T₁).

In vitro evaluation of all rhizosphere microflora

against *Sclerotium oryzae* :

Out of five fungal isolates tested *T. viride* (T₁) was found to be significantly superior in inhibiting the mycelial growth of *Sclerotium oryzae* by 75.3 per cent with a mean radial growth of 22.2 mm while the *Cladosporium* sp. was found to be least effective by inhibiting the pathogen to the extent of 29.2 per cent only. The percentage inhibition by other were in between 36.3 to 60.2 per cent (Table 3).

Table 3 : Efficacy of rhizosphere mycoflora isolated from Khammam and Warangal districts against *S. oryzae* in dual culture technique

Fungicides and Herbicides	Concentration (ppm)	Per cent inhibition of mycelial growth of <i>S. oryzae</i>
<i>Aspergillus flavus</i>	57.2	36.3 (37.0)
<i>Aspergillus niger</i>	53.3	40.6 (39.61)
<i>Cladosporium</i> Sp.	63.6	29.2 (32.7)
<i>Trichoderma viride</i> (T ₁)	22.1	75.3 (60.2)
<i>Trichoderma</i> isolate – 2 (T ₂)	35.7	60.2 (50.8)
Control	90.0	0.0 (00.0)
S.E. ±		0.4
C.D. (P=0.05)		1.4
CV %		1.8

All the figures are means of 3 replications

Figures in parentheses are angular transformed values

Similarly among the five bacterial isolates tested for their antagonistic activity, *P. fluorescens* (BI-1) was significantly superior in inhibiting the growth of *Sclerotium oryzae* by 77.2 per cent with a mean radial growth of 20.4 mm of pathogen as against 90.0 mm in control. The percentage inhibition by other bacterial isolates were in between 1.8 to 8.3 per cent (Table 4).

Among the microflora tested, *Trichoderma viride* (T₁) and *Pseudomonas fluorescens* (BI-1) inhibited the mycelial growth of *Sclerotium oryzae* to a maximum extent and the same were used as a potential native antagonists fungus against the test pathogen for further studies.

The dual culture studies conducted by Hemanthu (2006) revealed that *T. viride* was effectively inhibiting by 68.0 per cent growth of *S. hydrophilum* followed by *T. koningii* (63.1%), *T. harzianum* (55.5%) and *T. reesei* (55.3%). According to Banyal *et al.* (2008) *T. viride* (local strain) inhibited maximum mycelial growth of *S. rolfisii* causing tomato color rot and was significantly better than *T. harzianum* and *Gliocladium virens*. In

Table 4 : Efficacy of Rhizosphere bacteria isolated from Khammam and Warangal districts against *S. oryzae* in dual culture technique

Rhizosphere bacteria	Radial growth of the pathogen (mm)	Per cent inhibition of mycelial growth
<i>Pseudomonas fluorescens</i> (BI-1)	20.40	77.20 (61.47)
Bacterial isolate-2 (BI-2)	82.48	8.30 (16.63)
Bacterial isolate-3 (BI-3)	90.00	3.60 (10.94)
Bacterial isolate-4 (BI-4)	90.00	3.6 (10.9)
Bacterial isolate-5 (BI-5)	88.28	1.83 (7.56)
Control	90.0	0.0 (00.0)
S.E. ±		0.9
C.D. (P=0.05)		2.8
CV %		0.9

All the figures are means of 3 replications

Figures in parentheses are angular transformed values

the present investigation the two *Trichoderma* isolates (T₁, T₂) caused drastic reduction in the mycelial growth. The present finding is in agreement with the studies conducted by Laha (2009) they found that *P. aeruginosa*, *B. subtilis* and *B. pumulus* strains were effective against stem rot of rice pathogen *S. oryzae*.

In the present investigation, among the bacterial isolates, *Pseudomonas fluorescens* (BI-1) was found to be more effective against *S. oryzae* as it inhibited mycelial growth to an extent of 77.2 per cent. The mechanisms by which bacteria affect the plants involve the production of diverse metabolites including siderophores, hydrocyanic acid (HCN), phytohormones and the other associated activities including phosphate solubilization and root colonization resulting in plant growth promotion (Shivani *et al.*, 2005).

Efficacy of fungicides and herbicides against *Sclerotium oryzae* :

Six fungicides *viz.*, carbendazim (Bavistin), propiconazole (Tilt), hexaconazole (Contaf), tebuconazole (Raxil), metaminostrobin (Varisma), azoxystrobin (Amister), and two herbicides *viz.*, oxadiargyl (Topstar) and butachlor (Hunter) were tested at recommended concentrations along with a check. The results are presented in Table 5.

Out of the six fungitoxicants tested against the test pathogen, propiconazole at 100 ppm and hexaconazole at 200 ppm were significantly superior over all the other treatments and found to be most effective in inhibiting

Table 5 : In vitro evaluation of fungicides and herbicides against mycelial growth of *Sclerotium oryzae*

Fungicides and Herbicides	Concentration (ppm)	Per cent inhibition of mycelial growth of <i>S. oryzae</i>
Carbendazim	100	98.4 (82.8)
Propiconazole	100	100.0 (90.0)
Hexaconazole	200	100.0 (90.0)
Tebuconazole	100	98.5 (82.9)
Metaminostrobin	200	42.2 (40.5)
Azoxystrobin	100	98.6 (83.2)
Oxadiargyl	150	27.9 (31.9)
Butachlor	400	97.1 (80.2)
Control		0.0 (00.0)
S.E. \pm		0.37
C.D. (P=0.05)		1.10
CV %		9.98

All the figures are means of 3 replications
 Figures in parentheses are angular transformed values

mycelial growth of *Sclerotium oryzae* by 100 per cent, while azoxystrobin @ 100 ppm (98.6%), tebuconazole (98.5%), carbendazim (98.4%) @ 100 ppm were on par with each other. Among the two herbicides tested, butachlor at 400 ppm was significantly superior over oxadiargyl by inhibiting the mycelial growth by 97.1 per cent as compared to oxadiargyl at 150 ppm by 28.0 per cent. Superiority of triazoles (propiconazole and hexaconazole) over there fungitoxicants (mancozeb, chlorothalonil, thifluzamide) in managing stem rot has been reported by Kumar *et al.* (2003 b). The above results differ with studies conducted by Hemanthu (2006) where in propiconazole at 500 ppm, hexaconazole @ 1000 ppm, carbendazim @ 2500 ppm were found effective in inhibiting the growth of fungus completely. Similar differences were observed by Ram Singh *et al.* (1988) indicating that thiophanate methyl followed by carbendazim and edifenphos were effective in inhibiting mycelial growth with LC 50 values of 0.048, 0.507 and 3.98 $\mu\text{g ai ml}^{-1}$, respectively. *In vitro* evaluation of fungicides against the pathogen showed that the systemic fungicides like penconazole and hexaconazole resulted in complete mycelial inhibition of *Sclerotium rolfsii* of french bean even at 50 ppm concentration (Gupta and Sharma, 2004).

Compatibility studies between potential native antagonistic fungus *in vitro* :

Compatibility between *Trichoderma viride* (T₁) and *Pseudomonas fluorescens* (BI-1) was evaluated by

using dual culture technique. *P. fluorescens* (BI-1) showed five per cent inhibition of growth of *T. viride* (T₁). The bacterial isolate -1 (BI-1) growth was not inhibited by *T. viride* (T₁). Arunasri (2003) tested the compatibility of *Trichoderma* isolate-1 (T₁) with *Pseudomonas* sp. (BI-1) by dual culture technique and found that both were compatible showing just five per cent inhibition in growth of *Trichoderma* sp., by *Pseudomonas* sp. (BI-1).

Compatibility of *Trichoderma viride* (T₁) with fungicides and herbicide *in vitro* :

The compatibility of two fungicides and one herbicide with *Trichoderma viride* (T₁) was tested by using poisoned food technique and results are presented in Table 6.

Table 6 : Compatibility of *Trichoderma viride* (T₁) with fungicides and herbicide *in vitro*

Fungicides and Herbicides	Concentration (ppm)	<i>Trichoderma viride</i> (T ₁)	
		Radial growth (mm)	Per cent inhibition of Mycelial growth
Propiconazole	100	0.0	100 (90.0)
Hexaconazole	200	0.0	100 (90.0)
Butachlor	400	35.5	60.56 (51.1)
Control		90.0	0.00 (0.0)
S.E. \pm			0.13
C.D. (P=0.05)			0.43
CV %			3.95

All the figures are means of three replications
 Figures in parentheses are angular transformed values

Both the fungicides tested *viz.*, propiconazole (100 ppm) and hexaconazole (200 ppm) were most effective in inhibiting 100 per cent mycelial growth of *Trichoderma viride* (T₁) as compared to control. Butachlor at 400 ppm inhibited the mycelial growth to the extent of 60.6 per cent with a mean radial growth of 35.5 mm as against 90.0 mm in control. Thus, butachlor was found to be highly significant and superior over other treatments in compatibility with *T. viride* (T₁). Similar incompatible studies with test antagonist was reported by Johnson *et al.* (2008) where in hexaconazole showed complete inhibition of the growth of *T. viride* inciting groundnut stem rot whereas herbicide pendimethalin showed 48 per cent inhibition on the growth of *T. viride*.

Compatibility of *Pseudomonas fluorescens* (BI-1) with fungicides and herbicide :

The compatibility of *P. fluorescens* (BI-1) with two fungicides and one herbicide was tested following inhibition zone technique. The fungicides viz., propiconazole (100 ppm) and hexaconazole (200 ppm) inhibited *P. fluorescens* (BI-1) growth upto 7.9 per cent and 6.8 per cent, respectively while herbicide butachlor inhibited *P. fluorescens* (BI-1) growth upto 3.6 per cent (Table 7). Therefore butachlor was found to be highly compatible with *P. fluorescens* (BI-1). Hari Narayana (1999) also reported that hexaconazole at 0.1 per cent concentration showed 9.6 per cent inhibition of *P. fluorescens*.

Integrated management against *Sclerotium oryzae* in glasshouse conditions :

The initial symptoms were observed at mid tillering to booting stage and disease incidence was recorded by following disease index formula. It is evident from the data that all the treatments were significantly superior over the control in reducing stem rot incidence indicating a low disease index ranging from 0.5-3.4 when compared to 7.9 in control (Table 8). All the treatments were effective in reducing disease index stem rot of rice ranging from 0.5-3.4 per cent as compared to inoculated control (7.9%). The treatment (T₁₀) consisting of propiconazole @100 ppm + *T. viride* isolate-1 (T₁) @ 10 g kg⁻¹ and *P. fluorescens* (BI-1) @ 10 ml kg⁻¹ +

Table 7 : Compatibility between *Pseudomonas fluorescens* (BI-1) with effective fungicide and herbicide in vitro

Fungicides and Herbicides	Concentration (ppm)	Per cent inhibition of growth of <i>P. fluorescens</i> (BI-1)
Propiconazole	100	7.9(16.3)
Hexaconazole	200	6.8(15.1)
Butachlor	400	3.6(10.9)
Control		0.0(0.0)
S.E. ±		0.27
C.D. (P=0.05)		0.88
CV %		9.30

All the figures are means of 3 replications

Figures in parentheses are angular transformed values

butachlor @ 400 ppm) followed by treatment (T₁₁) consisting of hexaconazole @ 200 ppm + butachlor @ 400 ppm + *T. viride* isolate-1 (T₁) @ 10 g kg⁻¹ and *P. fluorescens* (BI-1) @ 10 ml kg⁻¹ were found to be the effective with low disease index of 0.5 and 1.1 respectively and it was on par with other treatments (T₈, T₇, T₅, T₁ treatments). However the treatments viz., T₁₀ (Propiconazole at 100 ppm + *T. viride* isolate-1 (T₁) @ 10 g kg⁻¹ and *P. fluorescens* (BI-1) @ 10 ml kg⁻¹ + Butachlor at 400 ppm) and T₁₁ (Hexaconazole at 200 ppm + *T. viride* isolate-1 (T₁) @ 10 g kg⁻¹ and *P. fluorescens* (BI-1) @ 10 ml kg⁻¹ + Butachlor at 400 ppm) besides reducing the disease index, they significantly enhanced the plant growth parameters viz., plant height (99.4 and 96.5 cm), root length (31.7 and 23.8 cm) and

Table 8: Efficacy of fungicides, herbicides and antagonists on the disease severity and growth parameters in rice cv. MTU 3626

Treatment no.	Treatments	Disease index	Plant height (cm)	Root length (cm)	Dry weight of each plant (g)	
					Shoot	Root
T ₁	Spraying of propiconazole @ 100ppm	1.2 (6.4)	52.3	16.9	5.8	6.4
T ₂	Spraying of hexaconazole @ 200ppm	3.4 (10.7)	56.1	19.4	6.4	6.9
T ₃	Soil application of butachlor @ 400ppm	2.1 (8.3)	71.4	20.1	8.8	7.2
T ₄	Soil application of <i>Trichoderma viride</i> (T ₁) @ 10 g kg ⁻¹ <i>Pseudomonas fluorescens</i> (BI-5) 10 ml kg ⁻¹	2.1 (8.5)	65.0	12.1	7.4	4.8
T ₅	T ₁ + T ₃	1.3(6.4)	74.6	9.5	9.2	3.3
T ₆	T ₂ + T ₃	1.5 (7.0)	78.5	14.0	10.4	6.0
T ₇	T ₃ + T ₄	1.3 (6.7)	68.5	8.2	7.9	3.0
T ₈	T ₁ + T ₄	1.3 (6.2)	71.5	12.3	9.0	5.2
T ₉	T ₂ + T ₄	1.8 (7.8)	62.3	14.2	7.0	6.3
T ₁₀	T ₁ + T ₃ +T ₄	0.6 (4.0)	99.4	31.7	15.4	8.3
T ₁₁	T ₂ +T ₃ + T ₄	1.81 (6.0)	96.5	23.8	13.4	7.5
T ₁₂	Control (Inoculated)	7.9	54.1	6.3	5.0	2.2
	S.E.±	0.35	0.6	0.6	5.7	7.7
	C.D. (P=0.05)	1.0	1.7	1.9	0.2	0.2
	CV	7.8	1.7	4.9	5.8	9.9

Means of three replications

Figures in parentheses are angular transformed values

dry weight of shoot (15.4 and 13.3 g) and root (8.3 and 7.4 g), respectively, when compared to individual treatments. Even though highly susceptible cv. MTU 3626 was used, besides application of more nitrogen and creating with logged conditions, the low intensity of disease pressure in the control inoculated pots may be attributed to application of insufficient quantity of inoculum and the environmental conditions prevailed in the green house might not favoured to create high disease pressure in pot culture studies. In integrated management the fungicide might have weakened the sclerotia making them more sensitive to antagonists. The present hypothesis is in agreement with the findings of Henis and Papavizas (1983) who also reported that sclerotia of *S. rolfsii* were weakened at sub-lethal concentrations of metham sodium and become sensitive to invasion and degradation by *T. harzianum*. However, the fungus alone does not degrade fresh sclerotia. In such situation, the weakened or dead cells of pathogen might have served as enrichment medium for the multiplication of antagonists. Soil application of biocontrol agents have an edge over seed treatment, as these being natural soil inhabitants, they establish and multiply more quickly in soil (Vyasa and Mathur, 2002). Liu *et al.* (2000) also observed longer shoot per root ratio in plants treated with strains of plant growth promoting rhizobacteria. Two mechanisms have been advocated most frequently to explain the increased growth response induced by certain microflora. The first hypothesis was that enhanced growth of plants induced by antagonists might be due to biological control of plant pathogens in the soil.

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