SUMMARY

Rice (*Oryza sativa*) is the most important cereal food crop of India. It occupies about 23.3% of gross cropped area of the country. It plays vital role in the national food grain supply. Rice contributes 43% of total food grain production and 46% of the total cereal production of the country. Rice is the staple food of more than 60% of the world's population especially for most of the people of South-East Asia.

Rice sheath blight, one of the most serious fungal diseases of rice, is caused by *Rhizoctonia solani* Kühn [teleomorph *Thanatephorus cucumeris* Donk], a ubiquitous pathogen. In view of the importance of the pathogen an investigation has been taken up to study the following aspects:

1. To isolate sheath blight pathogen *Rhizoctonia solani* from different rice growing areas of the country.
2. To assess the cultural, morphological, pathological and molecular variability among the isolates of *R. solani*.
3. To isolate *Pseudomonas* and *Trichoderma* spp. from the rice fields affected with sheath blight and to assess their biocontrol potential under *in vitro* and *in vivo* conditions against sheath blight pathogen.
4. To develop the formulations of potential bioagents and to evaluate their efficacy of biocontrol potential under field conditions.

R. solani infects rice plants at any stage of growth. Lesion started at the base of the culms near the water level on artificial inoculation and ascended to the upper parts of the plants. The lesions on sheath were first greenish-grey, ellipsoidal or oval, 2-3 cm long and gradually became greenish white with black brown margin. The sheath blight of rice is a

typical disease starting from seedling stage to harvest, indicating all the stages of a crop are susceptible to infection by *R. solani.*

The samples of affected rice sheath showing typical blight symptom collected from different geographic regions were used for isolation by standard isolation method. Repeated isolations yielded the same fungal strain which was later confirmed as *Rhizoctonia solani* that could be obtained in pure form by hyphal tip isolation and maintained in pure form on PDA.

Mycelial and sclerotial morphology of the fungus was studied from one week old culture. Colour of the rapidly grown mycelium was pale to dark brown with relatively large diameter with branching near the distal septum of the hyphal cell. Constriction of the branch hyphae was found at the point of origin and formation of the septum in the branch was observed near the point of origin. Mycelial branching was right angled and the barrel shaped cells also called monilioid cells were produced. Sclerotia produced by the fungal species varied in size, shape and texture.

Based on the morphological and cultural characters of the mycelium and sclerotia, the fungus was identified as *Rhizoctonia solani* (Kuhn). Further, the identity of the fungus was confirmed by Dr. P. N Choudhury, Principal Scientist (retired), Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi.

A total of 28 strains isolated from different geographic regions and are designated as RS- 1 to RS- 28. Diversity in morphological characters

of 28 isolates of *R. solani* was studied. Morphological characterization of different isolates indicated that, there existed much variation among the isolates, in mycelium colour, appearance, type of margin, mycelial width diameter and sclerotial number, colour, shape, growth pattern number diameter and texture. The branching of mycelium, constriction and septum remained same in all the isolates tested. The colour of the fungal colony varied from light brown to brown and it was found to be dark brown in four isolates. The right angled branching of mycelia was found with all twenty eight isolates. Surface of colony was flat in sixteen isolates and the remaining were fluffy. The margins of the colonies varied from smooth to irregular. The smooth margin was observed in the majority of isolates and only few were with irregular margin .The constriction at the point of origin and the formation of septum in the branch near the point of origin were found in all the isolates. Among the 28 isolates, maximum growth was noticed in 15 isolates with 89 mm colony diameter after 13 days of incubation on PDA, while, the least growth was recorded in RS- 3 and RS-24 (79 mm). Further, among the isolates mycelial width was larger in the isolate RS- 13 (2.76 µm) where as, it was least in RS- 2 and RS- 7 (1.81 µm).

The colour of sclerotium varied from brown to blackish brown in 24 isolates while it was greyish in two isolates (RS-11 and RS- 13). In many of the isolates (16) distribution of sclerotia over the colony was found to be central, in some (ten isolates) they were scattered. The number of sclerotia produced also varied in different isolates. Eleven isolates produced higher number of sclerotia followed by moderate number of

sclerotia in nine isolates. Further, low level of sclerotial formation was observed in six isolates. On the contrary, isolates RS-1 and RS-6 failed to produce sclerotia on the PDA medium. Shape of sclerotia ranged from globose to irregular.The texture of sclerotia ranged from fine to coarse. Majority of the isolates (seventeen) showed coarse texture and only nine isolates showed fine texture. The maximum sclerotial size was observed in isolate RS- 23 (180 µm). On the other hand, least sclerotial size was exhibited by isolate RS- 7 (47 µm).

To investigate the variation in the virulence of different *R. solani* isolates of rice and also to identify the most aggressive isolate, inoculation of *R. solani* isolates was done on *Oryza sativa* cv. MTU 1010, which is susceptible to sheath blight. The isolates differed in their aggressiveness and the isolates were categorized into three groups viz. low aggressive (AG 1), medium aggressive (AG 2) and highly aggressive (AG 3) based on the incidence of sheath blight induced on susceptible MTU 1010 variety of rice. All the isolates except RS-20 and RS -23 were grouped in AG 2. RS -20 was grouped in AG 1 due to its low aggressiveness. RS-23 was highly aggressive inducing highest sheath blight incidence (52.3%), hence kept in AG 3. Therefore, the virulent isolate RS-23 was employed to examine the biocontrol potentiality of the biological control agents.

Isolates of *R.solani* were distinguished using the PCR protocol described by Williams *et al.* (1990) using random primers for PCR amplification. Molecular variability in twenty-eight isolates of *R.solani* on rice was studied by RAPD technique.

In a preliminary study, one isolate of *R. solani* was amplified with 30 primers of arbitary nucleotide sequence. Of these, four primers (OPC-5, OPC 2, OPA-8, and OPA-11) were selected based on amount of polymorphic amplified bands for analysis of all 28 isolates of *R. solani.* Significant differences in RAPD profiles of 28 isolates of *R. solani* were found with two primers OPC 5 and OPC 2. The primers OPC-5, OPC-2, OPA-8 and OPA-11 produced 265 PCR products of molecular weight ranging from 0.5 kb to 2.0 kb in different isolates with diverse finger printing patterns. To test the resolving ability of these primers, cumulative RAPD profiles generated by the primers were analyzed by UPGMA. The dendrogram constructed using 265 polymorphic bands obtained from 28 isolates with 4 primers was divided into 7 clusters.

Biological control, which is a part of integrated disease management and eco-friendly alternative to chemicals to control the disease, an attempt was made to isolate the antagonistic candidates including *Pseudomonas fluorescens* and *Trichoderma* spp. and examined their biocontrol potential under *in vitro*, *in vivo* and field conditions against *R. solani*

Fluorescent pseudomonads were isolated on King's Medium B(KMB). Antagonistic isolates of bacteria were identified by biochemical, physiological and biochemical tests (Schaad, 2001).

Eleven *Pseudomonas fluorescens* isolates were employed to assess their biocontrol efficiency under *in vitro* conditions using dual culture technique and screening for volatile antifungal compounds and extracellular metabolites. The effect of bioagents on the germination and

lysis of sclerotia under *in vitro* conditions and in soil was also studied.

Efficacy of the *P. fluorescens* in inhibiting growth of *R. solani* was tested by dual culture technique. In this Pfr 1 was found to outstand all other isolates followed by Pfr 2, Pfr 9 and Pfr 11. *Pseudomonas fluorescens* isolates were screened for the production of volatile antifungal compounds. The volatile compounds of the bacterial antagonists had remarkable inhibitory effect on the mycelial growth of the pathogen. Pfr 1 was found significantly superior over other isolates followed by Pfr 8, Pfr 10 and Pfr 11.

Pseudomonas fluorescens isolates were screened for the secretion of extracellular metabolites. The extracellular metabolites secreted by the bacterial antagonists had lethal effect on the rice sheath blight pathogen. The metabolites seemed to have strong lethal effect on the mycelial growth of pathogen which was reflected by the percentage reduction of mycelial growth compared to direct mycelial interaction in dual culture and volatile compounds. Pfr 1 was found superior over other isolates with 68.6% of mycelial growth reduction over control. This was followed by the isolates Pfr 12, Pfr 9, Pfr 7 and Pfr 2.

The effect of *Pseudomonas fluorescens* isolates on lysis of sclerotia of *Rhizoctonia solani* was studied *In vitro*. The bacterial antagonists caused lysis of sclerotia of the pathogen. Significantly highest lysis was brought by two isolates, Pfr 1 and Pfr 2 compared to other isolates.

The effect of *Pseudomonas* isolates on germination of sclerotia of *Rhizoctonia solani in vitro* was studied in KMB and also in soil. In KMB, Pfr 1 significantly inhibited the sclerotial germination of the pathogen when

compared to other isolates. It was followed by Pfr 9 and Pfr 10. The effect of *P. fluorescence* on the germination of sclerotia was tested according to the method of Knudsen and Eschen (1991). In soil also almost same level of implication of antagonists was observed on the sclerotial germination of the pathogen. The isolate Pfr 1 was significantly superior over other isolates with highest inhibitory effect on the germination of sclerotia followed by Pfr 9 and Pfr 8.

Overall, the isolates *viz.*, Pfr 1, Pfr 2, Pfr 9 and Pfr 11 were found common in checking the pathogen's activity using *in vitro* techniques employed to screen the isolates. Also these isolates were found to have significantly highest lethal impact on the pathogen. The isolate Pfr 1 was consistently observed to posses inhibitory effect against the rice sheath blight pathogen. Therefore, these four isolates were selected for further screening studies in pot culture experiments.

Trichoderma strains were isolated by the soil dilution-plating technique of Johnson *et al.* (1989). Identification of antagonistic *Trichoderma* spp. was done according to the key proposed by Rifai (1969). Nine isolates of *Trichoderma viridae* and two isolates of *T. harzianum* were assessed for their biocontrol efficiency against *R.solani* under *in vitro* conditions using dual culture technique and screening for production of volatile compounds. Their potentiality for the production of lytic enzymes and also in solubilization of inorganic phosphate was also recorded.

All the isolates of *Trichoderma* were individually tested for their antagonistic property against *R. solani* causing sheath blight using the dual culture technique. In this all the isolates showed inhibitory action

against *R. solani.* The isolates, *T. viride* 4, *T. viride* 33 and *T. viride* 12 were potent to inhibit the mycelial growth of *R.solani* than the other isolates. The mycelia of *R.solani* and *Trichoderma* spp. with in overlapping areas in dual culture plates were observed after 4 days of incubation using scanning electron microscope (SEM). The SEM micrographs showed the mycelium of *Trichoderma* spp. were found to coil around *R. solani.*

Trichoderma isolates were screened for the production of volatile antifungal compounds. All the isolates inhibited the mycelial growth of the pathogen through the production of volatile compounds. *T. harzianum* S12 was found to have significantly highest inhibitory ability followed by *T. viride* 5, *T. viride* 2 and *T. viride* 16.

Quantification of lytic enzymes Chitinase, β-1,3-glucanases and β-1,4-glucanases in selected *Trichoderma* spp. was made. *Trichoderma harzianum* S12 produced large amount of chitinase followed by *T. viride* 3 and *T. harzianum* 10. *T. harzianum* S12 produced large amount of β-1,3-glucanase (50.67U/ml) and β-1,4-glucanase (1.25U/ml) followed by *T. viride* 3 and *T. harzianum* 10.

Trichoderma isolates were evaluated for the solubilization of inorganic phosphate. P_i content in the was estimated by phosphomolybdic blue colour method. The P content released into the medium from TCP was 120.7µg P $ml^{-1}$by *T. harzianum* S12 followed by *T. harzianum* 10 (116.0 µg P ml^{-1}). The amount of P solubilized by the reference strain *Aspergillus niger* was 146.9 µg P ml^{-1}.

Biocontrol agents were tested *In vivo* for their efficacy to control sheath blight using the cultivar MTU 1010 very susceptible to sheath blight. Four *P. fluorescens* isolates (Pfr 1, Pfr 2, Pfr 9 and Pfr 11) and four *Trichoderma* isolates (Th 12, Tv 5, Tv 2 and Tv 16) were selected based on the results of *in vitro* evaluation and were assessed for their biocontrol potentiality against rice sheath blight pathogen in greenhouse conditions.

In greenhouse experiments non-formulated fresh cells of antagonistic bacteria and non-formulated spore suspension of antagonistic fungi were prepared to evaluate their efficacy against the build up of sheath blight pathogen. Non-formulated fresh bacterial cell suspension or non-formulated spore suspension of fungi was sprayed on the rice plants with hand held sprayer. There were ten treatments, which consisted of rice plants (cv. MTU 1010) inoculated with four isolates of *P.fluorescens*, four isolates of *Trichoderma*, one fungicide and one control. The experiment was arranged in a Complete Randomized Design.

The decrease in lesion length due to antagonist's effect was measured and the percent disease intensity and reduction over control were calculated. The biocontrol potentiality of the antagonists was compared with the activity of the fungicide, Benlate (0.2%). In all the treatments *P. fluorescens* and *Trichoderma* spp. exhibited inhibitory effect on the pathogen with varied level of disease incidence ranging from 15.5 to 33.3%. The lesion length was significantly low (1.3 mm) in the plants treated with *P. fluorescens* 2 followed by treatments with *T. harzianum* 12 (8.0 mm), *P. fluorescens* 1 (15.5 mm) and Benlate (15.5 mm). Lesion length was significantly high in the control which did not receive any

treatment (102.7 mm). Likewise, disease intensity was significantly low in the plants treated with *P. fluorescens* 2 (15.5%) followed by treatments with Benlate (16.9%), *T. harzianum* 12 (17.4%), *P. fluorescens* 1 (23.4%) and *T. viride* 2 (23.4%). Disease intensity was very high in the control (53.4%). Obviously reduction in disease incidence was significantly high in the plants treated with *P. fuorescens* 2 (70.5%) as compared to the other treatments. It was followed by Benlate (68.0%), *P. fluorescens* 1 (60.2%) and *T. viride* 2 (60.2%). The treatments, Benlate and *T. harzianum* 12 and *P. fluorescens* 1 and *T. viride* 2 did not differ significantly in their effect. The biocontrol agents *P. fluorescens* 2, *P. fluorescens* 1, *T. harzianum* 12 and *T. viride* 2 were found elite in control of the sheath blight pathogen. Therefore, these four biocontrol agents were considered for testing their biocontrol efficacy under field conditions.

The efficacy of the isolates of bioagents against sheath blight was investigated under field conditions at Regional Agricultural Research Station, ANGR Agricultural University, Maruteru, West Godavari District, Andhra Pradesh, India. There were six treatments in the field trial. Treatment consisted of rice plant (cv. MTU 1010) inoculated with two isolates of *P.fluorescens*, two isolates of *Trichoderma* , one chemical fungicide (Benlate @ 0.2%), one day after pathogen inoculation. Rice plants inoculated only with *R.solani* were used as a control treatment. Each treatment consisted of three replications, with twelve rice hills for one replication. The experiment was arranged in a Randomized Complete Block Design (RBD).

Significant highest yield per plot and per hectare (12.5 kg/plot and 6644.1 kg/hectare) was recorded in the plot treated with Benlate compared to other treatments. It was followed by treatment with *P. fluorescens* 1 (10.8kg/plot and 5568.0 kg/ha), *P.fluorescens* 2 (10.5 kg/plot and 5402.8 kg/ha), *T.viride* 2 (9.7 kg/plot and 5067 kg/ha) and *T. harzianum* 12 (9.6 kg/plot and 5065.7 kg/ha). The least yield was recorded in the plot treated with pathogen alone (8.2 kg/plot and 4202.7 kg/ha).

The results indicate that treatment with Benlate is highly effective in checking the pathogens growth as well as improving crop yield followed by *P. fluorescens* 1 and *T. harzianum* 12. The biocontrol agents *P. fluorescens* and *T. harzianum* can be further developed as bioformulation for large scale use in rice ecosystem.