J. Mycopathol. Res. **44**(2) : 243-247 (2006)

Effect of soil amendments on soil microflora with special reference to rice sheath blight pathogen (*Rhizoctonia solani*)

M. SURULIRAJAN AND JANKI KANDHARI
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012

The mean fungal and bacterial population from pots and field soil after the addition of soil amendments (Sawdust 1% and FYM 1%) was increased significantly over the control two weeks after the addition of soil amendments. It further increased after 10th week of addition of soil amendments. However fourteen weeks after addition of soil amendments, the population level drastically declined while the population of *R. solani* had significantly reduced over the control two weeks after soil amendments. However ten weeks later, it declined further and after fourteen weeks, there was no significant reduction in the population level of *R. solani*.

Key words : Microbial assay, rice, *R. solani*, sheath blight, soil amendments

INTRODUCTION

Rhizoctonia solani Kuhn is the cause of sheath blight disease of rice. The disease is widespread and causes significant economic losses to farmers (Rajan, 1987). Since sheath blight is soil-borne disease hence it is difficult to control. Various approaches such as chemical, biological and cultural are used for control for sheath bight of rice, however each has its limitations. Soil amendment is found as a possible means of controlling soil borne pathogens. Davey and Papavizas (1960)listed 30 cases where soil borne diseases have been suppressed by organic amendments. They noted population of rice sheath blight organism *R. solani* can be minimized by amending the soil with green manure *Sesbania aculeata*. There are many reports of various soil amendments which reduce sheath blight of rice and total soil saprophytes viz., fungi, bacteria and actinomycetes (Rajan and Alexander, 1987; Padmakumari and Balakrishna, 1987; Manibhushan rao and Baby, 1991; Prasad *et al.*, 1998; Meena and Muthusamy, 1999). In the present studies, the efforts were made to see the effect of soil amendments on soil microflora along with *Rhizoctonia solani*, the causal agent of sheath blight of rice.

MATERIALS AND METHODS

Mass multiplication of Rhizoctonia solani

Highly virulent isolate or *R. solani* was multiplied on sand maize meal medium (Riker and Riker, 1936). The inoculum thus produced was used to inoculate the soil in pots as well as in the field.

Microbial assay

Random soil samples were collected from pots and plots at interval of 2, 6, 10 and 14 weeks for studying microbial assay with various amendments.

The number of propagules of *R. solani* in soil incorporated with different amendments was analyzed using *R. solani* specific medium (Ko and Hora, 1971).

Estimation of fungal and bacterial population

Assay of fungal flora in soil was done on PDA with Rose Bengal and Streptomycin. One ml of each of 10^{-4} soil dilution was pipetted to sterile petridise along with PDA. Observations were taken from 4th day onwards. The population of bacteria was

estimated in a similar manner except soil dilution used was 10^{-6} on King's B medium. The colonies were counted from seventh day onwards.

Soil amendments

The pot and field experiments were conducted for two years i.e. 1999-2000 and 2000-2001 *Kharif* season with sixteen treatments. Three replications of each treatment were maintainted including control. Twentyfive days old seedlings were transplanted.

In pots, the inoculum of a highly virulent isolate (RS-4500) was mixed in the soil @ 20% of the soil weight while in field it was applied to each plot uniformly @ 1 kg of culture/plot. The amendments FYM and Sawdust each @ 1% was mixed with the inoculated soil one week after inoculation. Two weeks after treatment, twenty-five days old rice seedlings of cv. Pusa Basmati-1 were transplanted @ five hills per pot.

The experimental field was ploughed twice and soil was brought to a fine tilth. The plot size was 3 × 2 m (6 m^2). The experiment was laid out in Randomized Block Design (RBD). The seedlings were transplanted at spacing of 20 × 10 cm. Controlled irrigation was given uniformly throughout the cropping seasons. Three weeks after transplanting one insecticidal spray of Ekalux @ 0.2% was given against rice hispa in all plots. Before amending, soil samples were collected, dried in shade and were utilized for the assay of fungi, bacteria and the test pathogen *R. solani*.

Statistical analysis

The population of micro-organisms viz., total fungi, bacteria and *R. solani* were analyzed after logarithmic transformation for statistical analysis. The data was subjected to Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Pot and field experiments

Effect of soil amendments on the population of fungi

In Pots (1999) the mean fungal flora was maximum (19.45 cfu/plate) in treatment T3 (FYM 1 % + Sawdust 1 %) followed by 17.29 cfu/plate in treatment T2 (Sawdust 1 %) as against 15.45 cuf/plate in control (Table 1). Same trend was observed in the year 2000. The population of soil fungi was increased in 2 weeks after addition of soil amendments.

In field also same trend was observed in both the years (1999 & 2000). The maximum fungal population (19.49 cfu/plate) in 1999 was observed in treatment T3 (FYM 1 % + Sawdust 1 %) followed by 17.83 cfu/plate in treatment T2 (Sawdust 1 %) as against 15.92 cfu/plate in control (Table-4).

Table 1 : Effect of soil amendments on the population of soil fungi in pot

Treatment	Mean population (c.f.u.) Week after addition of soil amendments					
	Year 1999					
	0	2	6	10	14	Treatment Mean
T1	9.10	14.73	16.90	17.50	19.00	15.45c
T2	9.90	16.26	18.60	21.00	20.70	17.29b
T3	8.90	17.80	23.30	22.90	24.30	19.45a
T4	8.60	15.80	17.80	17.90	15.70	15.45c
	Year 2000					
T1	8.90	11.40	16.10	18.00	19.10	14.70d
T2	9.70	18.00	18.30	21.00	20.40	17.48b
T3	8.40	20.40	23.70	23.40	23.20	19.82a
T4	8.30	16.40	18.30	19.40	15.10	15.50c

Duncan Multiple Range Test (DMRT)-Treatment with same alphabetical letters – not significant

TI-FYM 1%, T2-Sawdust 1 %, T3-FYM 1% + Sawdust 1%, T4-Control

Table 2 : Effect of soil amendments on the population of bacteria in pot

Treatment	Mean population (c.f.u.) Week after addition of soil amendments					
	Year 1999					
	0	2	6	10	14	Treatment Mean
T1	17.23	19.46	24.60	28.20	24.70	22.84b
T2	18.33	20.33	22.80	24.70	22.40	21.91c
T3	17.83	23.63	29.80	34.40	24.40	26.81a
T4	18.73	19.83	22.60	22.30	21.90	21.07c
	Year 2000					
T1	18.20	18.60	22.80	28.00	28.00	22.36b
T2	19.70	19.30	22.40	25.20	25.20	21.80c
T3	19.30	23.70	28.70	38.20	38.20	26.86a
T4	20.70	21.10	25.40	22.50	22.50	22.32b

Duncan Multiple Range Test (DMRT)-Treatment with same alphabetical letters – not significant

TI-FYM 1%, T2-Sawdust 1 %, T3-FYM 1% + Sawdust 1%, T4-Control

Effect of soil amendments on the population of bacteria

In pots (1999), the mean bacterial population was maximum (26.81 cfu/plate) in treatment T3 (FYM 1 % + Sawdust 1 %) followed by 22.84 cfu/plate in treatment T1 (FYM % as against 21.97 cfu/plate in control (Table 2). Same trend was observed in the year 2000. The population of soil bacteria was increased upto 10 weeks and then declined.

In field (1999) the mean bacterial population was maximum (26.13 cfu/plate) in treatment T3 (FYM 1% + saw dust 1%) followed by 22.03 cfu/plate in treatment 1 (FYM 1%) as against 20.25 cfu/plate in control (Table 5). Similar trend was observed in the year 2000. The population of soil bacteria increased up to 10 weeks and then declined.

Effect of soil amendments on the population of R. solani

In pots, the mean population of *R. solani* in all treatments significantly declined from the control and it was just opposite to fungal and bacterial population. The mean population of *R. solani* in the year 1999 was minimum (14.15 cfu/plate) in treatment T3 (FYM 1 % + Sawdust 1 %) followed by 16.87 cfu/plate in treatment T1 (FYM 1 %) as against 21.31 cfu/plate in control (Table 3). Same trend was observed in the year 2000. The population of *R. solani* decreased upto 10 weeks and then increased.

In field, the same treand was observed. The mean population of *R. solani* in the year 1999 was minimum (14.00 cfu/plate) in treatment T3 (FYM 1 % + Sawdust 1 %) followed by 16.63 cuf/plate in treatment T1 (FYM 1 %) as against 20.90 cfu/plate in control (Table 6). Same trend was observed in the year 2000. The population of *R. solani* decreased up to 10 weeks and then increased.

From the above results in could be concluded that pot as well as field experiments showed increased mean population of fungal (*Trichoderma* spp., *Aspergillus* spp. and *Fusarium* spp.) and bacterial (*Pseudomonas* and *Bacillus subtilis*) population but the mean population of *R. solani* significantly declined. The population of fungi and bacteria

Table 3 : Effect of soil amendments on the population of *R. solani* in pot

Treatment	Mean population (c.f.u.) Week after addition of soil amendments Year 1999					
	0	2	6	10	14	Treatment Mean
T1	20.13	18.60	17.60	13.40	14.60	16.87b
T2	19.73	18.70	17.20	15.90	14.40	17.19b
T3	20.60	17.60	12.40	10.00	10.10	14.15c
T4	20.73	20.13	20.60	20.90	24.20	21.31a
	Year 2000					
T1	19.90	16.70	17.20	13.00	14.60	16.28c
T2	20.10	19.10	17.40	15.30	13.90	17.16b
T3	21.10	13.30	12.40	7.90	9.30	12.80d
T4	21.60	20.80	20.40	20.50	23.60	21.38a

Duncan Multiple Range Test (DMRT)-Treatment with same alphabetical letters – not significant

TI-FYM 1%, T2-Sawdust 1 %, T3-FYM 1% + Sawdust 1%, T4-Control

Table 4 : Effect of soil amendments on the population of soil fungi in field

Treatment	Mean population (c.f.u.) Week after addition of soil amendments Year 1999					
	0	2	6	10	14	Treatment Mean
T1	7.90	15.90	17.60	19.80	19.20	16.02c
T2	8.50	17.23	19.30	22.60	21.50	17.83b
T3	7.40	18.73	22.00	24.70	24.60	19.49a
T4	7.20	17.60	18.50	20.30	16.00	15.92d
	Year 2000					
T1	9.10	13.00	16.70	17.40	20.00	15.24d
T2	10.00	19.60	18.40	20.40	21.90	18.06b
T3	8.60	22.00	24.30	22.60	24.20	20.34a
T4	8.40	17.90	18.90	19.30	16.60	16.22c

Duncan Multiple Range Test (DMRT)-Treatment with same alphabetical letters – not significant

TI-FYM 1%, T2-Sawdust 1 %, T3-FYM 1% + Sawdust 1%, T4-Control

Table 5 : Effect of soil amendments on the population of bacteria in field

Treatment	Mean population (c.f.u.) Week after addition of soil amendments Year 1999					
	0	2	6	10	14	Treatment Mean
T1	16.90	18.66	22.80	27.30	24.50	22.03b
T2	17.40	19.66	22.00	24.30	22.40	21.15c
T3	17.40	23.03	27.90	37.30	25.60	26.13a
T4	18.50	18.50	20.60	21.90	21.70	20.25d
	Year 2000					
T1	17.80	19.30	23.70	28.00	24.80	22.72b
T2	19.70	20.20	22.80	25.20	22.90	22.16d
T3	19.60	24.30	29.00	38.10	24.30	27.06a
T4	20.80	21.30	26.40	22.40	21.90	22.56c

Duncan Multiple Range Test (DMRT)-Treatment with same alphabetical letters – not significant

TI-FYM 1%, T2-Sawdust 1 %, T3-FYM 1% + Sawdust 1%, T4-Control

Table 6 : Effect of soil amendments on the population of *R. solani* in field

Treatment	Mean population (c.f.u.) Week after addition of soil amendments Year 1999					
	0	2	6	10	14	Treatment Mean
T1	19.93	17.73	17.40	13.80	14.3	16.63c
T2	20.33	17.96	17.50	15.60	13.50	16.98b
T3	21.13	16.86	13.00	9.90	9.10	14.00d
T4	21.43	19.06	20.20	20.50	23.30	20.90a
	Year 2000					
T1	19.20	16.30	18.70	14.70	14.80	16.74c
T2	18.90	18.60	18.70	17.00	14.00	17.44b
T3	20.60	13.00	13.30	9.60	8.50	13.00d
T4	21.00	20.00	21.30	22.00	23.90	21.64a

Duncan Multiple Range Test (DMRT)-Treatment with same alphabetical letters – not significant

TI-FYM 1%, T2-Sawdust 1 %, T3-FYM 1% + Sawdust 1%, T4-Control

increased after two weeks and afterwards population of fungi and bacteria increased after two weeks and afterwards population level drastically declined. However, in *R. solani* it was just the opposite, it declined after two weeks and after fourteen weeks there was no significant reduction in population level. Numerous investigator had attempted suppression of plant pathogens with organic amendments in green house test, micro plots and field (Cook and Baker, 1983). Singh and Singh (1984) presented sixty-six references dealing with thirty soil-borne pathogens which could be managed by organic amendments. In amended soil there is always an intense microbial activity of saprophytic organisms, which poses a severe competition for nutrients, oxygen and space with the weakly saprophytic pathogens. As amended soil causes several fold increase in the population of soil micro-organism, the chances of parasitisation of many soil borne pathogens also increase (Lewis and Papavizas, 1980). Organic amendments may also stimulate activities of antibiotic producing fungi, actinomycetes and bacteria in soil, which have adverse effects on soil borne plant pathogens. Organic amendments in soil may inhibit fungal growth in soil through toxic decomposition products or through enhancing the growth of competitive or antagonistic organisms.

The application of soil amendments can increase the beneficial microbes and diminishes the pathogen population, as a result of that plant health is improved. In the present study, the fungal flora such as *Trichoderma, Aspergillus* and *Fusarium* were obtained in all the treatments amended with FYM and sawdust. The bacterial population such as *Pesudomonas* and *Bacillus subtilis* had also been obtained in all the treatments. Also in the present study, the fungal and bacterial population significantly increased over the control during two weeks after the addition of soil amendments. Further it had increased after the 10th week addition of soil amendments. Fourteen weeks after addition of soil amendments the population level drastically declined. The *R. solani* population significantly reduced two weeks after addition of soil amendments. Further it had declined after 10th week addition of soil amendments. After fourteen weeks of addition of soil amendments, there is no significant reduction in the population. Kannaiyan and Prasad (1981) observed reduction in seedling infection with organic amendments such as farmyard manure and sawdust. Rajan and Alexander (1987) studied the effect of various soil amendments including sawdust on the incidence of sheath blight and total soil saprophytes (fungi, bacteria and actinomycetes) and found that sheath blight incidence was significantly lower in amended soil. Padmakumari and Balakrishnan (1987) reported that the high microbial activity and reduction of pathogen propagules occurred in the amended soils. Manibhushan Rao and Baby (1991) studied the effect of organic manure (glyricidia leaves and neem cake) alone and combined with *T. longibrachiatum* and *Gliocladium virens* against *R. solani*. Rice sheath blight was managed better with these amendments and *Gliocladium virens* was the best. The highest grain yield was obtained with a combination of glyricidia leaves and *G. virens*. Meena and Muthusamy (1999) also reported significant disease reduction with farmyard manure.

Thus the present study validated the performance of soil amendments in pot and field experiments and which can be used for the ultimate benefit of farming community.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Division of Plant Pathlolgy, IARI, New Delhi for providing necessary facilities.

REFERENCES

Cook, R. J. and Baker, K. F. 1983. *The Nature and Practice of biological control of plant pathogens.* American Phytopathological Society, St. Paul, Minn. 539 pp.

Davey, C. B. and Papavizas, G. C. 1960. Effect of dry mature plant materials and nitrogen on *Rhizoctonia solani* in soil. *Phytopathology.* **50** : 522-525.

Kannayan, S. and Prasad, N. N. 1981. Effect of certain green manure on the saprophytic survival of *Rhizoctonia solani* in soil. *National Academy Science Letters.* **4** : 5-7.

KO, W. H. and Hora, F. K. 1971. A selective medium from the qualitative determination of *R. solani* in soil. *Phytopathology* **61** : 707-710.

Lewis, J. A. and Papavizas, G. C. 1980. Integrated control of rhizoctonia fruit rot of cucumber, *Phytopathology,* **70** : 85-89.

Manibhushanrao, K. and Baby, U. I. 1991. Managing rice sheath blight (ShB) using fungal antagonists and organic amendments. *Intl. Rice Res. Newslett.* **16** : 19-20.

Meena, B. and Muthusamy, M. 1999. Effect of organic soil amendments against rice sheath blight. *Indian Phytopath.* **52** : 92-93.

PadmaKumari, G. and Balakrishnan, S. 1987. Effect of organic amendments on the survival of *R. solani* the sheath blight pathogen. Presented in the workshop on biological control of plant diseases held at Tamil Nadu Agricultural University, Coimbatore.

Prasad, M. S., Kakshni, B. S., Mohiddin, S. and Mohiddin, S. 1998. Survival of sclerotia of *Rhizoctonia solani* in rice soils amended with oilcakes and green leaf manures. *Annals Agricultural Research* **19** : 44-48.

Rajan, C.P.D. 1987. Estimation of yield loss due to sheth blight of rice. *Indian Phytopath.* **40** : 174-177.

Rajan, K. M. and Alexander, S. 1987. Soil amendments in relation to incidence and intensity of sheath blight of rice Workshop on biological control of plant disease (Abstr.,), 10-12, March, 1987. TNAU, Coimbatore. P.1.

Riker, A. J. and Riker, R. S. 1936. *Introduction to Research of Plant Disease.* John S. Swift Co. Inc., New York. Pp. 117.

Singh, G. N. and Singh, R. S. 1984. Significance of organic amendment of soil in biological control of soil-borne plant pathogens. In : *Progress in Microbial Ecology,* Mukerji, K. G., Agnihotri, V. P. and Singh, R. P. (eds.) Print House (India), Lucknow, pp. 303-323.

(Accepted for publication June 6 2006)