J. Mycopathol. Res. **40**(2) : 153–158 (2002)

Management of sheath blight of rice caused by *Rhizoctonia solani* by oilcake amendment of soil

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Sheath blight of rice, caused by *Rhizoctonia solani*, is one of the most serious and widespread diseases of rice. Soil amendment with oilcake not only reduced sheath blight incidence in rice, but also improved plant health. Amendment of soil with oilcakes (mustard and peanut) resulted in significant increase in zthe population of *Rhizoctonia Solani*, the causal organism of sheath blight of rice, in soil in the early stages of inoculum incubation (7-10 days). With increased incubation period, however, the population greatly reduced. The population of some saprophytic antagonistic fungi in soil, such as *Aspergillus* spp. and *Penicillium* spp. increased greatly in amended soil. *Trichoderma harzianum*, a mycoparasite of *Rhizoctonia solani*, was isolated from amended soil only. Total bacterial population also substantially increased in amended soil. Soil amendment resulted in thickning, swelling and lysis of some cells of *Rhizoctonia solani* hyphae, particularly due to soil bacterial activity. In water and n-butanol extracts of oilcake amended soils growth of Rhizoctonia solani was very much reduced. Volatile gaseous substances emitted by decomposing oilcakes in amended soil completely suppressed the growth of *Rhizoctonia solani*.

Key words : *Rhizoctonia solani*, rice sheath blight management, oil cake amendment of soil, soil microorganism, antagonistic effects

INTORDUCTION

Sheath blight of rice, caused by *Rhizoctonia solani,* is one of the most serious and destructive diseases of rice. The disease is reported to perennate in soil in the form of sclerotia (Ou, 1985). One of the most promising method of attaining control of soil with oilcakes and reduction of sheath blight infection by organic amendment of soil has been reported by Rajan (1980) and Kannaiyan and Prasad (1983), while Voland and Epsteun (1994) demonstrated suppressiveness of *Rhizoctonia solani* diseases in soils amended with organic manures. Such reduction in infection is apparently due to the reduction in the population of *Rhizoctonia solani* in amended soil.

In the present paper the effect of oilcake amendment of soil on the incidence and severity of sheath blight of rice and the suppessive effect of oilcake amendment on *Rhizoctonia solani* population has been studied in pot culture and an attempt has been make to analyse the factors affecting this suppressiveness.

MATERIALS AND METHODS

Soil for these investigations was collected from the University Experimental Farm, Kalyani (gangetic alluvial : pH 7.1 organic carbon 0.1 ; total N (%) 5.1; C/N ratio 10.36 ; clay (%) 30.0). The soil upto plough depth (15 cm) was collected, air dried, ground and seived through a 2 mm seive to remove all course particles. A rice isolate of *Rhizoctonia solani* (isolate No. 14), obtained from the stock culture collection of Department of Plant Pathology, BCKV, was used. The inoculum was prepared in sand maize meal medium (crushed maize 25 g, fine sand 75 g, distilled water 40 ml ; incubated for 10 days at 28°C). In the soil the fungal inoculum was

added @ 3% and thoroughly mixed up with the soil. The moisture holding capacity of the soil, if not otherwise mentioned, was maintained at 40%. Finely powdered cakes of mustard (*Brassica juncia* Coss.) and peanut (*Arachis hypogia* L.) were thoroughly mixed with the soil 72 h after inoculation with *Rhizoctonia solani.*

For plant inoculation, inculated amended soil was taken in 20 cm earthen pots, each pot containing 3 kg of soil. For studying the seedling infection 25 seeds of cv swarna masuri, a highly susceptible cv were surface sterilized with sodium hypochlorite solution (1%) and sown in the pots. Records were taken on the percentage of seeds germinated and percentage of seedlings infected after 21 days. For studying the disease in the transplanted plants, seedlings (21 days old) were collected from the seed bed, surface sterilized by dipping in sodium hypochlorite solution (1%) for 1 min and seedlings were transplanted in each pot. Data were collected on disease incidence (%) following the formula given by Yoshimura (1954) 30 days after transplanting and on root and shoot growth after 60 days. Six replications were taken for each treatment.

Population of *Rhizoctonia solani* in soil was studied by taking 200 g of inoculated amended soils in each of the plastic boxes (6 cm diam, 8 cm height) and inserting rice straw pieces (0.5–1.0 cm long) in the soil in plastic boxes as baits and incubated at room temperature for 48 hrs after which the straw pieces were removed, thoroughly washed in distilled water and seeded on sterilized 2% water agar supplemented with 50 ppm streptomycin in petridishes. Observations were recorded after 3-5 days and the percentage of straw pieces yielding *Rhizoctonia solani* was taken as the parameter of the population of the fungus. The population of other fungi in the soil was determined by dilution plate count technique using Martin's Rose Bengal agar. Number of bacterial colonies in the soil was also determined by dilution plate count technique using soil extract agar (peptone, 0.5 g, yeast extract 0.5 g, KH_2PO_4, 0.4 g, $MgSo_4$, 7HO 0.05 g, NaCl 0.1 g, $FeCl_3$, 0.01 g, soil extract 250 ml, agar 15g, dist water 750 ml).

For extraction of antifungal substances from amended soil, to 150 g of air dried powered soil finely ground mustard cake was thoroughly mixed (2% w:w) in glass tumblers (7 cm diam, 10 cm height) covered with a Petridish lid and incubated at 28°C for 10 days. MHC of the soil was maintained at 50%. Unamended soil was taken as control. Extraction of the antifungal substances was done in distilled water as well as in water saturated n-butanol. The soil samples were taken in 250 ml Erienmeyer flasks, 100 ml of distilled water or n-butanol (water saturated) added and shaken vigorously for 30 min in an electric shaker. The extracts were collected by filtering through two folds of cheese cloth. The filtrates were centrifuged at 5000 rpm for 5 min. The supernatants were collected. The water extract was passed through a bacteria proof sintered glass filter (B-5, Pyrex) and the volume made upto 100 ml by adding required amount of sterile distilled water. The n-butanol fraction of the n-butanol extract was evaporated in flash evaporator. The residual extract was make upto 100 ml by sterile distilled water and passed through a bacteria proof sintered glass filter and the filtrate collected as above. the volume was made upto 100 ml by addition of sterile distilled water, if required to 100 ml of sterilized PDA (agar 4%), which had previously been melt and cooled to 50°C, 100 ml of water or n-butanol extract of soil was added separately and aseptically in 250 ml Erlenmeyer flasks and shaken throughly for proper mixing. The contents of the flasks were plated in Petriplates (20 ml medium per plate). PDA + sterile distilled water was taken as control. The Petriplates were inoculated at the center with 6 mm discs, aseptically cut by a sterilized cork borer, of the test fungus *Rhizoctoni solani* and incubated at 28°C. After 72 hrs radial growth of the fungus was measured. Four replications were taken for each treatment.

In vitro fungitoxic effect of soil amendments on *Rhizoctonia solani* was studied following Chinn's (1953) slide burial technique with some modifications. The test isolate of *Rhizoctonia solani* was grown in 100 ml potato dextrose broth in 250 ml Erienmeyer flasks for 10 days at 28°C. The mycelial growth on the top of the medium was taken out and the sclerotia formed were removed. The mycelial growth was washed twice in sterile distilled water

and homogenised in a Waring blender with 100 ml sterile distilled water. Twenty ml of the mycelial supspension was added to 80 ml of sterilized 2% water agar (40°–45°C). Sterilized microscopic slides were momenterily dipped into this mycelial suspension in water agar, removed and kept horizontally on two glass rods in sterilized moist chambers made in Petriplates. Air dried soil (150 g), supplemented with mustard cake (@ 2%), was taken in 250 ml beaker and the glass slides with mycelial fragments in agar film were inserted in the amended soil in beaker, two slides per beaker. The soil moisture was adjusted to 20 and 60% MHC respectively. Unamended soil was taken as control. At the ends of specific incubation periods at room temperature the slides were carefully removed from soils, gently rinsed in water to remove adhearing soil particles and the agar film wipped off from one side of the slides. The slides were stained with lactophenol– cotton blue and examined under a microscope for observing the growth pattern of the test fungus, *Rhizoctonia solani.*

For studying the effect of toxic volatile emitted from mustard and peanut cake amended soil, finelly ground cakes were thoroughly mixed separately with 50 g of powdered soil @ 2% (w:w) in glass tumblers (7 cm diam, 10 cm height). The MHC of the soil was adjusted to 50% and the tumblers covered with lids of Petridishes. After 48 hrs the lids were replaced with lower lids of Petriplates containing PDA seeded at the centre with a 6 mm disc of mycelial growth cutout by a sterilized cork borer from a 7 day old actively growing culture of *Rhizoctonia solani* and incubated at 28°C. Unamended soil was used as control. After 72 hrs the radial growth of the *Rhizoctonia solani* isolate on the Petridishes (3 replications for each treatment) was measured.

RESULTS AND DISCUSSION

Seedling infection and disease incidence in inoculated amended soil

In the studies on disease incidence and severity, it was observed that when the seeds were directly sown in inoculated amended soil, seed germination reduced to some extent, probably due to very high population of *Rhizoctonia solani* in soil during this period resulting in seed rot. Of the surviving seedlings infection was greatly reduced. In the transplanted seedlings also disease incidence reduced to a great extent in amended soil (Table 1). Different parameters for assessing disease severity caused by *Rhizoctonia solani* in seedling grown from directly sown seeds and transplanted seedlings

Table 1 : Effect of oilcake amendment in *R. solani* inoculated soil on germination and seedling infection (directly sown seeds) and disease incidence (in transplanted seedlings) of rice seeds/ seedling.

Amendment	Directly sown seeds		Transplanted seedlings
	Seed germination (%)	Seedling infection (%)	Disease incidence (%)
Mustard Cake	60.83	31.94	15.75
	(51.25)	(34.41)	(23.38)
Peanut Cake	64.15	40.25	25.00
	(53.22)	(39.38)	(30.00)
Control (unamenaded)	66.42	51.35	41.55
	(54.58)	(45.77)	(40.13)
CD (P = 0.05)	2.00	1.80	9.23

were used as in case of early stages of seedlings growing from seeds pre-emergence and post-emergence seedling rots, as well as necrosis at the basal part of the seedlings were the main symptoms, while in older seedlings manifestation of symptom was primarily as sheath blight (Acharya *et al.*, 1997). Seedling health also improved both in seedling grown both from directly sown seeds, as well as transplanted ones in amended soil as demonstrated by increased length of both roots and shoots (Table 2). Such increased root and shoot growth in oilcake amended soil was apparently due to nutritional effect of organic amendment of soil, as well as decrease in *Rhizoctonia solani* population in oilcake amended soil. Papavizas and Davey (1960) observed that the degree of control of *Rhizoctonia* diseases depended on when the susceptible host was planted after amendment incorporation and better control was achieved when plants were sown one to three weeks after incorporation. It is, therefore, presumed that better control of sheath blight would have been obtained if the seeds or seedlings were sown at a later period, as the population of *Rhizoctonia solani* in the soil would have much reduced during that period, as has been shown in the later studies.

Table 2 : Effect of oilcake amendment in *R. solani* inoculated soil on rice seedling growth and health.

Amendment	Directly sown seeds		Transplanted seedlings	
	Root Length (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Length (cm)
Mustard Cake	24.66	24.53	31.00	89.68
Peanut Cake	14.00	18.00	21.50	62.80
Control (unamenaded)	12.53	13.80	15.75	55.00
CD (P = 0.05)	1.36	1.78	3.32	3.38

Fungal population in the amended soil

The population of *Rhizoctonia solani* in the amended soil significantly increased in the early stages of inoculum incubation (2–7 days) (Table 3). With increase in inoculum incubation period the population declined. The decrease in *Rhizoctonia solani* population was rapid in oilcake amended soils after 25 days incubation and after 55 days the population very much declined.

Table 3 : Effect of soil amendment with oilcakes on the population of *R. solani.*

Incubation period (Day)	P. C. of rice straw pieces yielding *R.solani* in soil amended with					
	Peanut Cake		Mustard Cake		Control (Unamended)	
2	69	(56.2)[1]	60	(50.8)	58	(49.6)
7	73	(58.8)	70	(56.79)	47	(43.3)
25	17	(21.9)	10	(8.4)	40	(49.2)
40	11	(19.4)	6	(14.2)	32	(34.4)
55	5	(12.9)	5	(12.9)	23	(28.7)

CD (P = 0.05) : Treatment – 1.70 Days = 0.87 Treatment X Days = 3.80

[1]Figures in parenthesis are transformed angular values

Table 4 : Total fungal (other than *R. solani*) and bacterial population in soil amended with oilcakes.

Incubation period (Day)	Total population in soil amended with					
	Peanut Cake		Mustard Cake		Control (Unamended)	
	Fungal propagules (1x104)	Bacterial colony (1x106)	Fungal propagules (1x104)	Bacterial colony (1x106)	Fungal propagules (1x104)	Bacterial colony (1x106)
2	52.3	36.1	64.1	32.0	10.1	10.1
7	95.0	76.0	85.0	57.4	8.9	9.8
25	32.0	39.0	17.2	29.3	11.0	14.1
40	19.1	31.0	15.0	24.4	10.5	10.8
55	18.0	20.4	10.0	22.3	8.3	11.0

CD (P = 0.05)

For fungal propagules : Days –0.90, Treatment–1.70, DayxTreatment–1.00

For bacterial colony : 0.74 1.45 4.65

The fungal population, other than *Rhizoctonia solani* in oilcake amended soils also significantly increased after 7 days incubation (Table 4) after which there was sharp decline. However, the total fungal population in amended soils was much higher than in unamended soil. Bacterial population increased after 25 days incubation, the decline in population thereafter was not so sharp.

Several saprophytic fungi were isolated from both the amended and unamended soils. The number was, however, much higher in amended soils, species of *Aspergillus* topping the list (Table 5).

Table 5 : Population of different fungi in soil amended with oilcake.

Fungal genera	Population of soil fungi (×10⁴) in different amendments		
	Peanut cake	Mustard cake	Contorl (unamended)
Aspergillus niger	2	3	1
A. flavus	2	1	1
A. ochraceus	4	3	–
Penicillium sp.	2	3	1
Fusarium sp.	2	3	1
Mucoerales (spp.)	2	3	2
Trichoderma harzianum	1	2	–
Humicola sp.	–	1	–
Unidentified (nonsporulating)	–	2	2

Chung *et al.* (1988) observed that *Rhizoctonia solani* was able to colonize fresh organic matter, but less or not at all after it had decomposed. Availability of readily assimilable nutrients in the substrate in the form of oilcake during the initial stage of colonization may explain the intensive saprophytic activity, during the initial 7 days after oilcake amendment. The rapid decline in *Rhizoctonia solani* population with increased incubation period was probably due to decomposition of the organic amendment. Various reasons suggested by different workers for decline in a fungal population during the decomposition process of organic matters are : (i) general increase in microbial population in soil (Papavizas and Davey, 1960)increasing the activity of competitors and antagonists and (ii) release of volatile toxic substances, mainly ammonia, during decomposition of oilcakes in soil (Zakaria *et al.*, 1980).

Various species of *Aspergillus, Penicillium* and several other fungi, found to be associated with organic decomposition process of oilcakes, may suppress *Rhizoctonia solani* by antibiosis or

competetion. *Trichoderma harzianum,* a hyperparasite of *Rhizoctonia solani,* was also isolated from amended soil. Roy (1977) demonstrated parasitic activity of *T. viride* on the sheath blight fungus.

Fungitoxic effect of oilcake amendment of soil or *Rhizoctonia solani*

a. *In vitro* studies. As observed from Chinn's slide burial technique, in mustard cake amended soil deformity of hyphal cells of *Rhizoctonia solani* (Table 6), such as attenuation of some cells, bulbous growth of some cells and lysis of some cells was quite evident and was indicative of antibiotic effect of some microorganisms on *Rhizoctonia solani.* Production of bulbous cells by hyphae of *Alternaria sp.* in soil as a result of antibiotic action of *Bacillus subtilis,* a common soil bacterium, had been demonstrated by Vasudeva *et al.* (1963). Hyphal lysis was more pronounced at higher MHC (60%) of soil. Similar result was observed by Kavoor (1954) with *R.bataticola.* It was suggested that increased bacterial activity at higher MHC of soil resulted in hyphal lysis.

Table 6 : In vitro growth of *R. solani* hyphae in mustard cake (2%) amended soil.

Treatment	Hyphal Characteristics
Amended soil (20% MHC)	Attenuated cells of the hyphae with bulbous swelling of the some of the cells. Lysis of some of the cells.
Unamended soil (20% MHC)	Normal growth of the hyphae.
Amended soil (60% MHC)	Bulbous growth of some of the cells, thickening of some of the cells, lysis of a number of cells.
Unamended soil (60% MHC)	Normal growth of the hyphae.

b. Growth in fungitoxicants extracted from oilcake amended soil. Both water and n-butanol extracts of mustard cake amended soil had appreciabl suppressive effect on *Rhizoctonia solani* (Table 7), suggesting production of antibiotic substances effective against the pathogen by enhanced growth of various microorganisms during the decomposition process of the organic materials. The suppressive effect was more pronounced with n-butanol extract, indicating that the antifungal substance in mustard cake amended soil was much better extracted in n-butanol than in water. However, better growth of *Rhizoctonia solani* in n-butanol extract of unamended soil than on PDA only probably suggests the presence in soil itself some growth stimulating factors for *Rhizoctonia solani.*

c. Effect of toxic volatile substances emitted from amended soil. Although in PDA plates placed on tumblers containing unamended soil satisfactory growth of *Rhizoctonia solani* occurred, growth was completely checked in those placed on tumblers containing peanut cake and mustard cake amended soils. The inoculum also turned dark brown. Bosalis and Scharen (1959) observed that the hyphae of *Rhizoctonia solani* exposed on the surface of plant debris from soil, turned dark brown. Some of these hyphae were filamentous and dead, while others became relatively thick walled, 35% of which were

Table 7 : Effect of water and n-butanol extracts of mustard cake amended soil on growth of *R. solani.*

Treatment	Radial growth (cm) after 48 h	
	Water extract	n-butanol extract
PDA + mustard cake (2%) amended soil	2.81	0.87
PDA + unamended soil	3.60	6.32
Control (PDA)	4.32	5.00
LSD (P = 0.05)	0.21	0.24

viable. Observations by slide burial technique as discussed earlier has shown that while some of the hyphal cells of the test *Rhizoctonia solani* lysed, obviously due to antibiotic action of other soil microorganisms, some others became attenuated and thickened. These cells apparently remained viable. Production of chlamydospores and thickening of hyphal cells are defensive mechanisms for survival of some soil borne pathogens under adverse soil conditions. The deleterious effect of oilcake ammendment of soil on the biological activity of the test isolate of *Rhizoctonia solani,* thus, appears to be a complex manifestation of several factors inactivating and suppressing the population.

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(Accepted for publication July 13, 2002)