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Influence of organic amendments on arbuscular mycorrhizal fungi in relation to rice sheath blight disease

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Abstract The effect of various organic soil amendments on arbuscular myorrhizal (AM) fungal activity on rice plants was tested under greenhouse and field conditions with reference to sheath blight (ShB) disease caused by *Rhizoctonia solani.* AM spore density, per cent infection, and intensity of infection were increased by organic amendments, whilst ShB disease was decreased. Certain amendments, especially green leaf manure, stimulated arbuscule development in rice plants. Mycorrhiza formation and sporulation were higher with healthy rice plants than with rice plants infected with *R. solani.* Our results indicate the possibility of using selective organic amendments to enhance development of native AM fungi and thus reduce disease incidence.

Key words Organic amendments · AM intensity · Spore density · Rice sheath blight disease

Introduction

Arbuscular mycorrhizal (AM) fungi are influenced by agricultural practices and their frequency and diversity can vary between cultivated and uncultivated soils (Mosse and Bowen 1968; Kruckelmann 1975; Baltruschat and Dehne 1988; An et al. 1993; Land et al. 1993). Besides increasing plant growth (Mosse 1972; Cooper 1983; Manjunath and Bagyaraj 1986) under low fertility conditions, AM fungi can improve tolerance towards stresses such as drought (Allen and Boosalis 1983), heavy metals, salinity (Marx and Schenck 1983) and root pathogens (Schenck 1981; Caron 1989).

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Both upland (Gangopadhyay and Das 1982; Ammani et al. 1985; Brown et al. 1988) and lowland rice (Sivaprasad et al. 1990) normally develop AM (Baby and Manibhushanrao 1992), but cultivation methods can have an impact on root colonization by AM fungi and on associated spore numbers in soils (Pozzebon et al. 1992). The growth of rice plants can be increased by inoculation with AM fungi (Sanni 1976), and Dhillion (1992) observed increases in dry weight of rice plants irrespective of the associated fungal type. The impact of some organic substrates on the development of AM fungi has been reported previously (Harinikumar and Bagyaraj 1988; Calvet et al. 1993; Soedaraj and Habte 1993). However, no information is available on the influence of organic substrates on AM development and subsequently on disease resistance. In the present investigation we studied the effects of various organic amendments on AM fungi in relation to the severity of rice sheath blight (ShB). ShB is caused by *Rhizoctonia solani,* which infects rice plants at all stages of growth causing heavy yield loss in tropical and temperate regions of the world.

Materials and methods

Organic amendments

The organic manures used in this study were: oilseed cakes [marotti (*Hydnocarpus wightiana,* neem (*Azadirachta indica*), punna (*Calophyllum inophyllum*) and rubber (*Hevea braziliensis*)], green leaf manures [eupatorium (*Chromolaena odorata*), gliricidia (*Gliricidia maculata*), cassia (*Cassia siamea*) and neem] and agro-industrial wastes [bonemeal, coconut pith (*Cocos nucifera*), poultry manure and sawdust]. The experimental soil was clay with pH 7.3, organic matter content 1.3%, available N, P, K 172.9, 66.7, 172.9 kg/ha, respectively, and a water-holding capacity of 43%. The organic amendments were incorporated into soil at a concentration of 0.5% in the greenhouse (15×28-cm earthenware pots) and in the field (1-m^2 microplots) and the soil was sown with a highly ShB-susceptible rice, cv. TKM 9. In the greenhouse, 3 g of surface-sterilized seeds was sown in each pot, while in the field the seeds were sown in 20×20 cm spacing and thinned on day 15 to 6–8 seedlings per hill, with 25 hills per plot. The microplots were laid out in a completely randomized block design. All experiments were set up in triplicate. Under both conditions, the plants were irrigated daily (without flooding) to give upland conditions. Shoot height and dry weight were measured after 60 days.

Spore extraction and assessment of mycorrhizal colonization

AM fungal spores were isolated by a wet sieving and decanting method (Gerdemann and Nicolson 1963) and quantified following the method of Khan (1971). Spores were also separated from the remaining debris with transfer needles for identification (Schenck and Perez 1987).

For the assessment of AM fungal colonization, 1-cm root segments were treated as described by Phillips and Hayman (1970). One hundred segments were observed under a compound microscope for each treatment and the number mycorrhizae given as per cent infection. The intensity of AM infection, i.e. the density of mycelia within infected roots was assessed on a 1–3 scale (Kormanik et al. 1980).

Disease incidence

Disease indicence was scored using a standard evaluation system (SES) for rice on a 0–9 scale (IRRI 1980) and was computed as suggested by Loganathan and Ramaswamy (1984):

$$DI = [(n \times v)/(N \times V)] \times 100$$

where n and N represent the number of tillers at each infection level and the total number of tillers observed, respectively, and v and V represent the grade for each group of tillers and the highest grade allotted in the score chart, respectively.

One-way analysis of variance was used to detect significant differences among mean effects of amendments observed with plant age. T-tests were performed to determine the significance of differences between effects on healthy and *R. solani* infected plants.

Results

Mycorrhizal colonization and spore density

The periodic assessment of AM fungal colonization of rice roots and estimations of spore density in soils revealed that AM fungi were influenced by certain organic substrates. In general, spore density, per cent infection and intensity of infection increased with plant growth and the progressive decomposition of the amendment (Tables 1, 3). The predominant AM fungal spores in all the experimental soils were *Glomus fasciculatum* (Thaxter sensu Gerdemann), *Glomus mosseae* (Nicolson & Gerdemann), *Glomus aggregatum* (Schenck & Smith), *Glomus fulvum* (Berk.) Pat., *Gigaspora candida* (Battacharji et al.) and *Gigaspora gigantea* (Nicolson & Gerdemann). Among other spore types observed, yellow vacuolate and honey-coloured spores of *Glomus* spp. were abundant at all stages.

Under greenhouse conditions, increases in spore density over the control were observed from day 30 onwards with marotti and rubber seen cake amendments, whilst with agro-industrial wastes an increase was observed at 20 days (Table 1) . The per cent infection in contrast increased progressively with plant age (Table 2) and the response was maximal with oilseed cakes (marotti and neem), cassia leaf and coconut pith.

In the field, spore density was maximal at the booting to grain-filling stage (Table 3), except with oilseed amendment. However, spore number was highest with some of the oilseed cakes (neem and punna) and with eupatorium leaf at 45 days. The mean fungal spore density with different amendments differed significantly at booting, except with bonemeal and coconut pith. In general, both per cent infection and intensity of infection were low up to 45 days and increased thereafter, reaching a maximum at the grain-filling stage (Table 4), except with cassia leaf and bonemeal amendments. Under field conditions, the intensity of infection was at a minimum (rating 1) with all amendments and in controls up to 45 days. However, higher ratings (2 and 3) were observed from 60 days onwards with most of the amendments (Table 4). Furthermore, some of the amendments (marotti and punna cakes, eupatorium, cassia and neem leaves, bonemeal, coconut pith and

Table 1 Influence of organic amendments on AM fungal spore density under greenhouse conditions. The results are expressed as number of spores per 50 g soil after an initial inoculum of 52 spores/50 g dry soil. Means followed by the same letter in a given column do not differ significantly (level of significance set at $P = 0.05$)

Amendment	Plant age (days)					
	5	10	20	30	40	50
Control (unamended soil)	58b	75bc	112c	165c	190f	205h
Marotti cake	60ab	56d	92e	180b	213e	280b
Neem cake	52cd	68c	66f	138e	240c	315a
Punna cake	50d	54de	102d	96j	186fg	232f
Rubber seed cake	62a	70c	106d	176b	224d	219g
Eupatorium leaf	36e	48ef	72f	105i	158h	244e
Gliricidia leaf	28f	56d	54h	125gh	248b	286b
Cassia leaf	22g	42f	60g	128fg	240c	230f
Neem leaf	25fg	46f	68f	98j	146i	215g
Bonemeal	65a	78ab	136b	120h	160h	186i
Coconut pith	60ab	83a	148a	156d	190f	252d
Poultry manure	57bc	53de	140b	201a	258a	266c
Sawdust	59ab	70c	140b	132f	182g	215g

Table 2 Influence of organic amendments on AM fungal infection of roots in greenhouse-grown rice plants. The results are expressed as per cent infection and intensity (in parentheses). Means followed by the same letter in a given column do not differ significantly

Amendment	Plant age (days)					
	5	10	20	30	40	50
Control (unamended soil)	28.7b (1)	32.0cd (1–2)	31.3d (1)	40.7fg (1–2)	63.3bcde (1–2)	78.0ef (2–3)
Marotti cake	22.0c (1–2)	39.3b (1–3)	53.3b (1–2)	60.7b (1–2)	66.7bc (3)	89.3bc (1–2)
Neem cake	36.7a (2)	44.6a (3)	59.3a (2–3)	74.0a (2–3)	82.0a (3)	94.0ab (2–3)
Punna cake	26.0b (1)	38.7b (1)	45.3c (1–2)	52.7cd (1–2)	61.3cdef (1–2)	80.0e (1–2)
Rubber seed cake	21.3c (1)	32.7c (1–3)	49.3bc (1–2)	52.0cde (1–2)	65.3bcd (1–2)	70.0g (1–2)
Eupatorium leaf	6.7f (1)	12.0fg (1)	29.3d (1)	46.7def (2–3)	57.3f (1–2)	68.0g (1–2)
Gliricidia leaf	7.3ef (1)	22.7ef (1–2)	32.7d (1–2)	44.0f (2)	60.7def (1–2)	81.3e (2–3)
Cassia leaf	27.3b (2)	48.0a (1–2)	58.7a (1–2)	53.3c (1–2)	65.3bcd (2–3)	86.7cd (3)
Neem leaf	10.0ef (1)	27.3de (1–2)	50.0bc (1)	50.7cde (1–2)	58.0ef (1–2)	70.0g (2–3)
Bonemeal	11.3e (1)	21.3f (1–2)	26.7d (1)	46.0ef (1–2)	60.0def (2–3)	68.7g (1–2)
Coconut pith	19.3cd (1)	25.3ef (2–3)	31.3d (1–2)	51.3cde (3)	78.7a (2–3)	97.3a (2–3)
Poultry manure	16.0d (1–2)	26.7e (1)	32.7d (1–2)	38.0g (2–3)	68.7b (2)	82.7de (1–2)
Sawdust	8.7ef	16.0g	18.7e	43.3fg	61.3cdef	73.3g

Table 3 Influence of organic amendments on AM fungal spore density under field conditions. The rsults are expressed as number of spores per 100 g soil after an inoculum of 115 spores/100 g dry soil. Means followed by the same letter in a given column do not differ significantly

Amendment	Plant age (days)					
	15	30	45	60	Booting	Grain filling
Control (unamended soil)	249c	407b	380k	253j	323l	370j
Marotti cake	170d	383d	452j	480d	396i	363j
Neem cake	118g	247f	713c	546b	560b	385i
Punna cake	133f	235g	734a	526c	549c	368j
Rubber seed cake	162e	172i	308l	370g	425h	394h
Eupatorium leaf	92i	211h	723b	566a	680a	805a
Gliricidia leaf	89i	266e	550f	327h	446g	626c
Cassia leaf	108h	249f	530g	466e	498e	590d
Neem leaf	95i	131j	578e	425f	515d	698b
Bonemeal	250c	234g	584e	551b	379j	570e
Coconut pith	307a	396c	597d	310i	387j	448g
Poultry manure	292b	507a	517h	331h	482f	632c
Sawdust	290b	228g	472i	329h	353k	496f

sawdust) favoured arbuscule development (data not shown).

Growth of rice plants and sheaht blight incidence

In terms of the growth of rice plants, there was a significant increase in shoot length and dry matter content with most of the various amendments (Table 5). This was particularly high with oilseed cake. Sheath blight incidence was considerably reduced in soils treated with the various organic amendments (Table 6) under both greenhouse and field conditions. Gliricidia leaf amendment gave the highest (62%) protection from ShB under both conditions. There was no clearcut rela-

Table 4 Influence of organic amendments on AM fungal infection of roots in field-grown rice plants. The results are expressed as percent infection and intensity (in parentheses). Means followed by the same letter in a given column do not differ significantly

Amendment	Plant age (days)					
	15	30	45	60	Booting	Grain filling
Control (unamended soil)	40.2c (1)	30.0g (1)	16.7e (1)	35.2e (1)	55.0e (1–2)	62.9de (1–3)
Marotti cake	8.3i (1)	35.2e (1)	24.1d (1)	40.3bc (1–2)	62.9d (1–3)	65.9cd (2–3)
Neem cake	49.8a (1)	37.8d (1)	24.0d (1)	41.1bc (2)	62.9d (2–3)	73.8a (2–3)
Punna cake	35.2e (1)	29.3g (1)	25.7d (1)	44.3ab (1–2)	52.3ef (2–3)	69.4abc (1–3)
Rubber seed cake	30.0g (1)	29.3g (1)	35.2bc (1–2)	42.6a (1–2)	48.3g (1–2)	65.9cd (1–2)
Eupatorium leaf	32.7f (1)	37.8d (1)	32.7bc (1)	39.1cd (1–2)	52.2ef (1–3)	65.9cd (1–3)
Gliricidia leaf	35.2e (1)	30.3g (1)	35.2bc (1)	37.8d (1–2)	52.2ef (2–3)	54.8f (1–3)
Cassia leaf	49.8a (1–2)	20.7h (1)	24.1d (1)	35.2e (1–2)	78.3b (2–3)	62.9de (1–3)
Neem leaf	45.0b (1)	32.7f (1)	24.1d (1)	37.8d (1–2)	69.3c (2–3)	69.4abc (1–3)
Bonemeal	30.0g (1)	42.6b (1–2)	37.8b (1–2)	32.6f (1–3)	95.8a (1–3)	68.9bc (1–2)
Coconut pith	30.4fg (1)	45.0a (1)	42.6a (1–2)	32.4f (1–2)	52.2ef (1–3)	60.1e (1–3)
Poultry manure	37.8d (1)	30.5g (1)	16.7e (1)	20.7g (1–2)	51.4f (3)	73.3ab (1–3)
Sawdust	27.1h (1)	40.2c (1)	35.2bc (1)	42.6ab (1–2)	66.0d (1–3)	69.4abc (1–3)

Table 5 Growth response of rice plants in soils treated with various organic manures under greenhouse and field conditions. Means followed by the same letter in a given column do not differ significantly

Amendment	Shoot height (cm)		Dry weight (mg)	
	Greenhouse	Field	Greenhouse	Field
Control (unamended soil)	28g	33f	340l	650i
Marotti cake	36ab	46a	570f	1260b
Neem cake	37a	42c	785a	1265a
Punna cake	34cd	44b	520h	1185d
Rubber seed cake	37a	44b	720b	1220c
Eupatorium leaf	32ef	38d	655e	800g
Gliricidia leaf	34cd	41c	525g	1120e
Cassia leaf	31f	35e	390k	600j
Neem leaf	32ef	36e	410i	650i
Bonemeal	35bc	44b	715c	1110f
Coconut pith	33de	36e	400j	650i
Poultry manure	37a	32fg	675d	780h
Sawdust	24h	31g	280m	450k

tionship between organic amendment, plant growth, mycorrhiza development and disease reduction. Assessment of AM colonization in healthy and *R. solani*-infected rice plants revealed that both the spore density in the rooting zone and percent infection were significantly higher in healthy plants than in diseased pants, at the booting and grain-filling stages (Table 7). The intensity of infection was rated at 2–3 in healthy plants but only 1–2 in diseased plants. Furthermore, the number of vesicles formed was significantly higher in roots of healthy plants than in diseased plants at the booting and grain-filling stages.

Discussion

The results of this investigation reveal favourable effects of some organic amendments on the development

Table 6 Sheath blight incidence in rice plants grown in soils with various organic amendments. Values in parentheses indicate per cent protection

Amendment	Culture condition	
	Greenhouse	Field
Control (unamended soil)	26.2	18.1
Marotti cake	13.0 (50.4)	10.3 (43.1)
Neem cake	12.5 (52.3)	10.4 (42.6)
Punna cake	13.6 (48.1)	9.4 (47.8)
Rubber seed cake	11.0 (58.1)	7.1 (60.7)
Eupatorium leaf	11.6 (55.8)	9.6 (46.9)
Gliricidia leaf	9.8 (62.6)	6.9 (61.7)
Cassia leaf	10.3 (60.6)	9.1 (49.8)
Neem leaf	8.6 (67.4)	7.5 (58.4)
Bonemeal	15.1 (42.4)	11.0 (39.1)
Coconut pith	11.4 (56.6)	9.2 (49.2)
Poultry manure	12.4 (52.6)	9.3 (48.8)
Sawdust	14.3 (45.7)	10.1 (44.3)

of AM fungi in rice plants. Although responses varied, mycorrhizal development in terms of spore density, percent infection and intensity of infection was generally higher in amended soils. The increase in the spore density in amended soils was significantly greater during the booting and grain-filling stages with most of the amendments. A relatively constant spore density during the vegetative growth phase and a sharp increase during the reproductive phase (flowering and fruit ripening) have been reported for various crops (Hayman 1970; Sutton and Barron 1972; Saif and Khan 1975; Pozzebon et al. 1992). It has been suggested that senescing and dead roots stimulate the onset of sporulation at the end of the host growing season. Incorporation of an organic amendment caused early increases in both per cent infection and intensity of infection in rice roots. These values were only equalled or exceeded in unamended controls in later stages of plant growth. These late increases could be due to increased mycorrhizal activity following nutrient depletion in the unamended control soils. The chemical composition and/or the decomposition products of the amendments may have stimulated mycorrhizal development in rice plants. Furthermore, most of the amendments, but especially green leaf manures, enhanced arbuscule development. Similar effects have been reported by Hayman (1975) and may be due to a general effect of amendments on host-fungus interactions rather than to selection of a particular fungal strain. None of the amendments appeared to favour the proliferation of a particular AM fungal species, although yellow and honey-coloured spores were abundant in the amended soils.

Correlations between AM fungal spore density and percent AM fungal infection have been reported (Hayman 1970; Bert et al. 1987). However, a relationship was not observed in the present study, in agreement with reports by Mosse and Bowen (1968), Allen and Allen (1980) and Land and Schonbeck (1991), who advocated that sporulation rates are not necessarily a function of the rate of mycorrhiza development.

In most cases, rice plants treated with the various organic substrates exhibited an increased growth response compared with controls, as well as different levels of AM fungal colonization. AM fungal-induced growth responses have been observed in rice by Dhillion (1992) and Khan et al. (1988). Furthermore, there were significant differences in ShB incidence in various treatments, as well as in AM fungal development (oilseed cakes, green leaf manures and agro-industrial wastes). The potential of organic manures to suppress the development of certain soil-borne pathogens has already been reported (Papavizas and Lumsden 1980; Lumsden et al. 1983; Manibhushanrao et al. 1989). The present results indicate the possibility of using selective organic amendments to enhance native AM fungal populations and consequently decrease fungal disease.

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Table 7 Mycorrhizal development and response to sheat blight incidence at different stages of growth of rice cv. TKM 9

	Booting			Grain filling		
	Healthy	Infected	*t* ($P<0.01$)	Healthy	Infected	*t* ($P<0.01$)
No of spores dry soil/100 g	203	186	3.02	400	340	10.47
Per cent infection	60	45	16.63	74	15	6.40
No. of vesicles per root segment	35	18	7.71	56	32	12.37
Infection intensity	2–3	1–2		2–3	1–2	

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