BioControl (2008) 53:555–567
DOI 10.1007/s10526-007-9098-9

Use of plant extracts and biocontrol agents for the management of brown spot disease in rice

Sankarasubramanian Harish · Duraiswamy Saravanakumar · Ramalingam Radjacommare · E. G. Ebenezar · K. Seetharaman

Received: 6 January 2005 / Accepted: 7 May 2007 / Published online: 7 June 2007

Abstract Fifty plant extracts, four oil cakes and eight antagonistic organisms were tested against *Bipolaris oryzae* (*Cochliobolus miyabeanus*), the causal agent of brown spot disease of rice. *In vitro* studies indicated that two leaf extracts, *Nerium oleander* and *Pithecolobium dulce* exerted the higher percent inhibition to mycelial growth (77.4, 75.1%) and spore germination (80.3, 80.0%) of *B. oryzae*. Among the four oil cake extracts tested *in vitro* against *B. oryzae*, neem cake extract showed the maximum inhibition percent to mycelial growth (80.18%) and spore germination (81.13%) of the pathogen followed by mahua cake extract, castor and gingelly cake extract. *Trichoderma viride* (Tv2*)* was significantly effective in inhibiting the mycelial growth (62.92%) and spore germination (77.03%) of the pathogen followed by *Trichoderma harzianum* (Th5) and *Trichoderma reesei* (Tr3). The promising leaf extracts, oil cake extracts and antagonistic microorganisms were further evaluated for their efficacies in disease management under glasshouse and field conditions. In glasshouse studies, post-infectional spraying of rice plants with neem cake extract, *N. oleander* leaf extract and *T. viride* (Tv2) was significantly effective in reducing the incidence of brown spot of rice by 66, 52 and 45 percent respectively. Two rounds of spraying of rice plants with neem cake extract, *N. oleander* leaf extract and *T. viride* (Tv2) in the field at initial appearance of disease and 15 days later reduced the incidence of brown spot (70, 53 and 48% disease reduction respectively) and increased the yield by 23, 18 and 15 percent respectively.

S. Harish (✉) · E. G. Ebenezar · K. Seetharaman
Department of Plant Pathology, Agricultural College and Research Institute, Madurai 625104, India
e-mail: sankarshari@yahoo.co.in

Present Address:
S. Harish
Molecular Plant Virology Lab, Department of Plant Pathology, College of Agriculture and Natural Resources, National Chung Hsing University, Taichung 402, Taiwan R.O.C

D. Saravanakumar · R. Radjacommare
Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 041, India

Keywords *Bipolaris oryzae* · Brown spot · Plant extract · Antagonistic microorganisms · Oil cake extract

Introduction

Rice brown spot caused by *Bipolaris oryzae* Breda de Hann (formerly, *Helminthosporium oryzae*) (Teleomorph = *Cochliobolus miyabeanus*) is considered as important production constraint of rice and it occurs in all rice-growing areas of the world, especially under semi-dry conditions (Ou 1985). The disease was a major factor for the ''Great Bengal Famine'' during 1942–1943 (Padmanabhan 1973). The disease is reported to cause loss upto 67% (Kohls et al. 1987) both quantitatively and qualitatively in various rice growing areas. Recently, Nyvall and Percich (1999) reported that depending upon disease severity, losses vary from slight to 75% with anecdotal evidence ascribing losses of 100% in fields where disease was especially severe. Besides causing brown spot on the leaves, the fungus is also responsible for the grain discolouration of rice, which is one of the serious problems in many rice-growing areas. The seedling vigour of rice is also affected adversely due to seed discolouration (Ou 1985; Zulkifi et al. 1991). Although, chemicals are available for the management of brown spot disease, continuous, inappropriate and non-discriminative use of chemicals is known to cause undesirable effects such as residual toxicity, development of resistance, environmental pollution, health hazards to humans and animals and increased expenditure for plant protection. Instead, plant pathologists have focussed their attention to develop environmentally safe, long-lasting and effective biocontrol methods for the management of plant diseases. Interestingly, extracts of certain plants contain alkaloids, tannins, quinones, coumarins, phenolic compounds and phytoalexins, which are known for antifungal activity (Fawcett and Spencer 1970; Kagale et al. 2005).

Previously, Fiori et al. (2000) reported that crude leaf extracts of *Eucalyptus citriodora* and *Ageratum conyzoides* were more effective in inhibiting the mycelial growth and spore germination of *Didymella byroniae*. Similarly, extracts from flowers, stem and leaves of *Euphorbia macroclada* were found to be effective against *Verticillium dahliae*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Penicillium italicum*, *Rhizoctonia solani* and *Pythium* spp. *in vitro* (Al-Mughrabi 2003). On the other hand, complex plant protection measures that use biological preparations, especially those based on phytopathogen growth suppressors, are increasingly used in agriculture. The use of fungal and bacterial antagonists for the control of various plant diseases has attracted considerable interest among phytopathologist. Among various fungal and bacterial biocontrol agents, *Bacillus*, *Pseudomonas* and *Trichoderma* were most frequently used against various plant diseases (Nakkeeran et al. 2005; Saravanakumar et al. 2007). Similar to previous studies, to obtain an effective disease management strategy for the control of brown spot disease in rice, different plants were collected and studied for their disease suppression activity under *in vitro*, glasshouse and field conditions. In addition, different oil cakes and antagonistic microorganisms were also tested for their efficacy against brown spot disease under different conditions.

Materials and methods

Plant materials and pathogen

Susceptible cultivar (ADT 36) of rice to brown spot was obtained from Central Farm, Agricultural College and Research Institute, Madurai, India. *B. oryzae* was isolated from

brown spot infected rice leaves. The fungus was purified by single spore isolation technique and identified based on the description given by Ou (1985). Pathogenicity was tested and a highly virulent isolate was selected and used throughout the experiment.

Preparation of leaf and oil cake extracts

One hundred g (fresh wt) of mature leaves of 50 various plants (Table 1) were homogenized separately in a pre-chilled pestle and mortar using chilled, sterilized distilled water. The extract was filtered through four layers of moistened muslin cloth. The final volume was adjusted to 100 ml with distilled water. The filtrate was centrifuged at 8000 rpm, 4°C for 15 min. The supernatant thus obtained was designated as concentrated leaf extract. The oil cake extract was prepared by suspending 100 g of the respective 4 oil cake types (Table 2) in sterile distilled water (100 ml). Serial 1:10 dilutions were made from this concentrated extract.

Effect of leaf and oil cake extracts on the growth of *B. oryzae*

The effect of leaf extracts on the growth of *B. oryzae* was determined by the Poisoned food technique (Schmitz 1930). The leaf and oilcake extract solutions were mixed with Potato dextrose agar (PDA) medium to obtain 10-fold dilution in the final medium. The assay was carried out in 9 cm diameter Petri dishes containing 15 ml PDA medium with 10% of leaf or oil cake extracts. A 9 mm actively growing PDA culture disc of *B. oryzae* was cut by means of a sterilized cork borer and placed at the centre of the medium. The plates were incubated at room temperature (28 ± 2°C). PDA without leaf or oilcake extract served as control. For comparison mancozeb (0.2%) was used by following the same procedure. Three replications were maintained for individual treatment. The radial growth of the mycelium was measured in treatments in 7 days when the fungus was fully grown (9 cm) in the control plate. The results were expressed as percent growth inhibition compared to the control.

In vitro effect of fungal and bacterial antagonists against pathogen

Effect of fungal and bacterial antagonists against *B. oryzae* was estimated by means of dual plate technique (Dennis and Webster 1971). The fungal antagonists *viz., Trichoderma viride* (Tv2), *T. harzianum* (Th5), *T. reesei* (Tr3), *T. longibrachiatum* (Tl1), *Gliocladium virens* (Gv2) and *Chaetomium globosum* (Cg3) were maintained on PDA slants and multiplied on PDA medium in Petri dishes. Similarly bacterial antagonists *P. fluorescens* (Pf5) and *Bacillus subtilis* (Bs3) were maintained on Nutrient agar (NA) slants and streaked on to PDA medium in Petri dishes. The fungal and bacterial biocontrol strains were obtained from the Biocontrol Unit, Department of Plant Pathology, Agricultural College and Research Institute, Madurai, India.

A 9 mm PDA culture disc of the pathogen from a 7-day old culture was placed on PDA medium approximately 1.5 cm from the periphery of the plate. Simultaneously, a 9 mm culture of the antagonistic fungus was placed opposite to the pathogen. In case of bacterial antagonists, these cultures were streaked onto the opposite side. Three replicates were maintained for each treatment. The plates were incubated at room temperature (28 ± 2°C)

Table 1 *In vitro* assay of the inhibiting effects of leaf extracts from various plant species on the growth of *B. oryzae*

Sl. No.	Leaf extracts (10%)	Mycelial growth (cm)[a]	Growth inhibition (%)	Spore germination (%)	Germination inhibition (%)	Sl. No.	Leaf extracts (10%)	Mycelial growth (cm)[a]	Growth inhibition (%)	Spore germination (%)	Germination inhibition (%)
1.	*Abutilon indicum* L.	4.7	46.9	39.7	55.5	27.	*Nerium oleander* L.	2.0	77.4	17.6	80.3
2.	*Acalypha indica* L.	4.6	47.7	39.2	56.1	28.	*Ocimum basilicum* L.	6.2	29.6	62.8	29.6
3.	*Achyranthus aspera* L.	4.8	45.8	40.1	55.1	29.	*Pergularia extensa* Forssk.	6.3	28.8	63.9	28.4
4.	*Aloe vera* Mill.	5.6	36.4	55.7	37.6	30.	*Phyllanthus emblica* L.	6.2	30.3	61.2	31.4
5.	*Alternanthera sessilis* Lam.	6.0	32.2	58.5	34.5	31.	*Phyllanthus niruri* L.	3.7	58.6	25.3	71.0
6.	*Amaranthus viridis* L.	4.8	45.4	45.2	49.4	32.	*Pithecolobium dulce* L.	2.2	75.1	18.0	80.0
7.	*Astercantha longifolia* L.	7.0	20.6	69.5	22.1	33.	*Polygala grinersis* L.	5.7	36.4	54.8	38.6
8.	*Bauhinia purpure* L.	5.8	34.1	56.3	36.9	34.	*Pongamia glabra* L.	5.8	34.8	55.9	37.4
9.	*Calotropis gigantea* R.Br.	2.8	68.9	20.2	77.3	35.	*Punica granatum* L.	4.9	44.4	46.3	48.1
10.	*Carum roxburgianum* L.	5.3	40.5	50.4	43.6	36.	*Ricinus communis* L.	5.5	38.2	53.1	40.5
11.	*Centella asiatica* Urb.	4.2	52.2	35.6	60.1	37.	*Sesbania grandiflora* L.	5.9	33.3	57.2	35.9
12.	*Cleome viscosa* L.	3.9	55.9	30.1	66.3	38.	*Solanum indicum* L.	2.5	71.4	19.2	78.5
13.	*Clitoria ternatia* L.	6.5	26.2	64.5	27.7	39.	*Solanum trilobatum* L.	5.1	42.4	48.1	46.1
14.	*Coccinia indica* W&A	4.9	44.3	47.7	46.6	40.	*Solanum xanthocarpum* L.	5.3	39.8	51.5	42.4
15.	*Curcuma longa* L.	3.7	57.9	27.9	68.8	41.	*Tabernaemontana divaricata* Bl.	6.7	24.3	66.2	25.8
16.	*Cyanodon dactylon* L.	5.4	39.0	52.6	41.1	42.	*Tamarindus indica* L.	4.4	50.0	36.9	58.7
17.	*Cyperus rotundus* L.	6.0	31.9	59.6	33.2	43.	*Tephrosia purpuria* Pers.	3.1	65.0	24.8	72.2
18.	*Delonix regia* Raf.	4.8	45.8	44.2	50.5	44.	*Thespesia populnea* Cav.	5.6	36.7	53.6	39.9
19.	*Ficus bengalensis* L.	4.1	54.1	33.6	62.3	45.	*Thevitia neerifolia* Juss.	6.9	22.4	68.7	23.1
20.	*Ficus religiosa* L.	4.8	45.8	42.2	52.7	46.	*Vettiveria zyzanoides* L	4.2	52.9	34.1	61.3
21.	*Leucas aspera* Willd.	4.6	48.0	38.1	57.3	47.	*Vinca rosea* L.	3.0	65.8	23.6	73.7
22.	*Mimordica charantia* L.	2.8	68.1	21.1	76.4	48.	*Vitex negundo* L.	3.7	58.2	27.0	69.9

Table 1 continued

Sl. No.	Leaf extracts (10%)	Mycelial growth (cm)[a]	Growth inhibition (%)	Spore germination (%)	Germination inhibition (%)	Sl. No.	Leaf extracts (10%)	Mycelial growth (cm)[a]	Growth inhibition (%)	Spore germination (%)	Germination inhibition (%)
23.	*Mimosa pudica* L.	6.1	31.1	60.2	32.5	49.	*Vitis quandrangularis* L.	3.0	66.6	22.7	74.6
24.	*Morinda tinctoria* Roxb.	4.5	48.2	37.8	57.7	50.	*Wrightia tinctoria* Roxb.	3.8	56.78	28.6	67.9
25.	*Moringa tinctoria* Roxb.	3.8	57.4	28.1	68.5	51.	Mancozeb (0.2%)	0.6	93.2	7.1	92.0
26.	*Murraya koenigii* Lim.	5.2	41.2	49.2	44.9	52.	Control	8.9	0	89.3	0

[a] Mean of three replications. Plant extracts were assessed for their effect on the mycelial growth and spore germination of *B. oryzae*. Mycelial growth was measured in cm and spore germination by examining 100 spores in a cavity slide under microscope. Mycelial growth [SED = 0.213, LSD (5%) = 0.422, LSD (1%) = 0.558]

Spore germination [SED = 0.225, LSD (5%) = 0.431, LSD (1%) = 0.564]

Table 2 *In vitro* assay of oil cake extracts against *B. oryzae*

Sl. No.	Oil cake extracts (10%)	Mycelial growth (cm)[a]	Growth inhibition (%)	Spore germination (%)	Germination inhibition (%)
1.	Neem	1.77[b]	80.18	16.94[b]	81.13
2.	Mahua	3.20[b]	64.16	25.66[b]	71.41
3.	Castor	4.73[b]	47.03	31.58[b]	64.82
4.	Gingelly	4.90[b]	45.13	47.24[b]	47.37
5.	Mancozeb (0.2%) (positive control)	0.60[b]	93.28	5.13[b]	94.28
6.	Control (distilled water)	8.93	0.00	89.76	0.00

[a] Means of three replications. Mycelial growth was measured in cm and spore germination by examining 100 spores in a cavity slide under microscope. Mycelial growth [SED = 0.147, LSD (5%) = 0.242, LSD (1%) = 0.442] Spore germination [SED = 0.125, LSD (5%) = 0.331, LSD (1%) = 0.464]

[b] The values are significantly different from the control

for 7 days. The diameter of the mycelial growth was measured and the results were expressed in terms of percent inhibition of the mycelium compared to the control.

In vitro evaluation of leaf extracts, oil cake extracts and antagonistic microorganisms by spore germination assay

Leaf extract, oil cake extract and antagonistic microorganisms (0.5 ml) respectively was pipetted into the cavity of a depression slide and allowed to air dry. Conidial suspension (0.5 ml) at 4×10^6 spores ml^{-1} of *B. oryzae* (prepared in sterile distilled water) was added to the dried extract and thoroughly mixed. The cavity slide was placed in a Petri dish containing moistened cotton and was incubated at 25°C for 48 h, three replicates for each treatment. For comparison mancozeb (0.2%) was used in the same manner. A spore suspension in sterile distilled water served as control. The germinated spores were determined by microscopic inspection of 100 spores for evidence of germ tube emergence.

Preparation of talc-based formulation of *Trichoderma* sp.

Trichoderma was multiplied in the molasses yeast medium (30 g molasses, 5 g yeast and 1 l water). After proliferation, the biomass was homogenized and mixed with talc at a 1:2 (fungus:talc) ratio. To the mixture, 5 g of carboxy methyl cellulose was added as sticker and dried in the shade for 72 h, powdered and stored in polypropylene bags (Jeyarajan et al. 1994). The population of *Trichoderma* at the time of preparation was 2.5×10^8 cfu g^{-1}. The talc-based formulation was used after one month of preparation and the number of *Trichoderma* propagules during application was 3.0×10^7 cfu g^{-1}.

Effect of leaf extracts, oil cake extracts and antagonistic microorganisms on brown spot of rice in glasshouse studies

On the basis of the performance of plant extracts, oil cake extracts and antagonistic organism in the preceding *in vitro* studies, two promising leaf extracts *viz*., *N. oleander* and *P. dulce* leaf extract, two oil cake extracts *viz*., neem cake and mahua cake extract and two

antagonists *T. viride* (Tv2) and *T. harzianum* (Th5) in the form of talc based formulation each at 2 g l^{-1} were tested against *B. oryzae* in pot culture under glasshouse condition. Mancozeb (0.2%) was used as positive control. Potting soil (red soil:clay:decomposed cow dung at 1:1:1 (w/w/w); available N, P, K, Ca and Mg of red soil were 160, 18, 280, 0.28 and 0.2 kg/ha, respectively, and pH was 7.0) was autoclaved and sterilized for 1 h and placed in earthen pots (diameter 0.3 m, height 0.5 m, volume of soil 0.04 m^{-3}) to plant brown spot susceptible cultivar ADT 36. When the seedlings were 60 day old, one set of rice plants was spray inoculated with the spore suspension of *B. oryzae* (4×10^6 conidia ml^{-1}). Water congestion was provided both 24 h before and after inoculation by covering the plants with polythene bags and spraying inside with sterile distilled water profusely. Forty-eight hours after inoculation with the pathogen at high humidity, the plants were sprayed with the leaf extracts, oil cake extracts and antagonistic organisms respectively with three replicates. Unsprayed plants served as control. Observations on the disease severity were recorded 10 days after inoculation. Five plants were selected at random per treatment and the severity of brown spot symptom was assessed following Standard Evaluation System for rice (IRRI 1988). Symptom development was observed after 7 days and graded on a 0–9 scale based on leaf area affected. The percent disease index was calculated by using the formula: Disease index (PDI) = (Total grade points/Number of leaves observed) × (100/Maximum grade).

Effect of leaf extracts, oil cake extracts and antagonists microorganisms on brown spot of rice in the field

A field experiment was conducted during November 2000-February 2001 in B block of the farm at Agricultural college & Research Institute, Madurai, Tamil Nadu (India) to assess the efficacy of leaf extracts, oil cake extracts and antagonistic microorganisms against brown spot disease in a randomized block design with eight treatments and three replications. This field site has a history of continuous rice cultivation since 1980 and had a record of brown spot disease. The susceptible rice CV. ADT 36 was raised in the nursery and later transplanted with a spacing of 15 × 10 cm in plots of 4 × 3 m^2 in size. The standard fertilizer recommendation for rice (Farmyard manure (FYM) 12.5t, N-150 kg, P-50 kg and K-50 kg ha^{-1}) was followed.

Two sprays of leaf extracts (10%), and oil cake extracts (10%) were given first at the initial appearance of the disease symptom and repeated 15 days later. For foliar spraying with the fungal biocontrol agents, the talc-based product was thoroughly mixed in water (1 kg ha^{-1}), allowed to settle for 1 h, filtered through muslin cloth and the filtrate was sprayed. Mancozeb (0.2%) was applied by spraying as a positive control. Untreated control plots were sprayed with sterile water. Natural incidence of diseases was recorded in each plot, twenty leaves were selected at random in each plot and scored for disease observation by following the Standard Evaluation System for rice (IRRI 1988). The PDI was calculated as described above. The yield data were recorded and percent difference in yield compared to the control was calculated for each individual treatment.

Statistical analysis

The data generated from various experiments of this study were statistically analysed following the procedure described by Gomez and Gomez (1984). The data were analyzed using IRRISTAT version 92-1 programme developed by biometrics unit at the

International Rice Research Institute, The Philippines and means were compared by least significant difference (LSD) at $P = 0.05$ and 0.01.

Results

Effect of leaf extracts against *B. oryzae in vitro*

Among the 50 leaf extracts (in water) tested against *B. oryzae*, the extracts of *N. oleander* and *P. dulce* were on par expressing the highest reduction in the mycelial growth of the pathogen by recording 2.0 cm (77% growth reduction) and 2.2 cm (75% growth reduction) as against 8.9 cm growth in the control (Table 1). With other leaves extracts from *S. indicum*, *Calotropis gigantean*, *Mimordica charantia, Vitis quadrangularis* and *Vinca rosea* inhibitions between 60 and 70% were observed. The mycelial growth inhibition was at least 21% in *Astercantha longifolia* leaf extract. Mancozeb caused the highest (93%) growth reduction, with the mycelial growth of only 0.6 cm. Leaf extract effect on spore germination showed that *N. oleander* had the highest inhibition of the spore germination of *B. oryzae* (80%) followed by leaf extract of *P. dulce*, *S. indicum* and *C. gigantea* (80, 78 and 77% respectively). The lowest inhibition (22%) of spore germination was recorded by leaf extract of *A. longifolia*. Mancozeb caused 92% inhibition.

Effect of oil cake extracts against pathogen *in vitro*

Oil cake extracts of neem (*Azadirachta indica*) and mahua (*Bassia latifolia*) reduced the mycelial growth of the pathogen by 80% and 64% respectively while mancozeb reduced it 93%. The extracts of *A. indica*, *B. latifolia*, *R. communis* and *S. indicum* inhibited spore germination of the pathogen by 81, 71, 64 and 47%, respectively (Table 2).

In vitro evaluation of antagonistic microorganism against pathogen

Tv2 and Th5 recorded similar inhibition of 60%. The rest of the fungi showed somewhat lesser inhibition (40–55%) (Table 3). The spore germination data followed the trend for hyphal inhibition with 77% as the highest inhibition (Table 4).

Effect of leaf extracts, oil cake extracts and antagonistic microorganisms against brown spot of rice in glasshouse studies

Post-inoculation spraying with neem cake extract significantly reduced the intensity of brown spot of rice by PDI 31 (66% disease reduction) compared to the untreated control. *N. oleander* leaf extract ranked next which recorded 52% disease reduction while mancozeb (0.2%) reduced disease severity by 82% compared to untreated control (Table 5).

Effect of leaf extracts, oil cake extracts and antagonistic microorganisms in the management of brown spot of rice in the field

Two sprays of neem cake extract given on rice cv. ADT 36 reduced the brown spot disease to 27 PDI as against 90 PDI in the control. *N. oleander* leaf extract ranked second (42 PDI)

Table 3 *In vitro* assay of antagonistic organisms against *B. oryzae*

Sl. No	Antagonistic organisms	Mycelial growth (cm)[a]	Growth inhibition (%)
1.	*Trichoderma viride* (Tv2)	3.30[b]	62.92
2.	*T. harzianum* (Th5)	3.50[b]	60.67
3.	*T. reesei* (Tr3)	4.07[b]	54.26
4.	*Chaetomium globosum* (Cg3)	4.17[b]	53.14
5.	*Pseudomonas fluorescens* (Pf5)	4.35[b]	51.12
6.	*Gliocladium virens* (Gv2)	4.67[b]	47.52
7.	*T. longibrachiatum* (Tl1)	4.77[b]	46.40
8.	*Bacillus subtilis* (Bs3)	5.60[b]	37.07
9.	Control	8.90	0.00

[a] Mean of three replications. Mycelial growth was measured in cm. SED = 0.138, LSD (5%) = 0.281, LSD (1%) = 0.411

[b] The values are significantly different from the control

Table 4 *In vitro* assay of antagonistic organisms against spore germination of *B. oryzae*

Sl. No.	Antagonistic organisms	Spore germination (%)[a]	Germination inhibition (%)
1.	*Trichoderma viride* (Tv2)	20.67[b]	77.06
2.	*T. harzianum* (Th5)	25.41[b]	71.79
3.	*T. reesei* (Tr3)	36.44[b]	59.55
4.	*Chaetomium globosum* (Cg3)	38.60[b]	57.15
5.	*Pseudomonas fluorescens* (Pf5)	41.25[b]	54.21
6.	*Gliocladium virens* (Gv2)	42.76[b]	52.54
7.	*T. longibrachiatum* (Tl1)	45.62[b]	49.36
8.	*Bacillus subtilis* (Bs3)	54.29[b]	39.73
9.	Mancozeb (positive control)	6.42[b]	92.87
10.	Control	90.09	0.00

[a] Mean of three replications. Mycelial growth was measured in cm. SED = 0.137, LSD (5%) = 0.276, LSD (1%) = 0.404

[b] The values are significantly different from the control

followed by Tv2 (Table 6). Mancozeb recorded significantly highest disease reduction of 83% with the least PDI (15) as compared to 90 PDI in the control. The yield was the highest in mancozeb (4800 kg ha^{-1}) treatment followed by neemcake spray (4675 kg ha^{-1}) while *P. dulce* gave the lowest response (4017 kg ha^{-1}) among the treatments. However untreated control recorded the lowest yield (3808 kg ha^{-1}) compared to all treatments (Table 6).

Discussion

The exploitation of plant products and biocontrol agents for the management of plant diseases have achieved greater significance in recent times due to its readily available nature, antimicrobial activity, easy biodegradability, non-phytotoxicity, besides inducing resistance in host. In this study, among the 50 leaf extracts tested *in vitro* on *B. oryzae*,

Table 5 Effect of leaf extracts, oil cake extracts and antagonistic microorganisms against brown spot of rice in glasshouse studies

Sl. No.	Treatments	PDI[a]	Disease reduction (%)
1.	Neem cake extract (10%)	31.1[b]	66
2.	*Nerium oleander* extract (10%)	44.4[b]	52
3.	*Pithecolobium dulce* extract (10%)	68.9[b]	26
4.	Mahua cake extract (10%)	65.9[b]	29
5.	*T. viride* (Tv2) (2 g l^{-1})	51.1[b]	45
6.	*T. harzianum* (Th5) (2 g l^{-1})	63.7[b]	31
7.	Mancozeb (0.2%) (positive control)	17.0[b]	82
8.	Control (distilled water)	92.6	0

[a] Mean of three replicates. Percent disease index was calculated based on disease grade following SES of rice developed by IRRI. SED = 0.210, LSD (5%) = 0.444, LSD (1%) = 0.612

[b] The values are significantly different from the control

Table 6 Effect of leaf extracts, oil cake extracts and antagonistic microorganisms against brown spot of rice in the field

Sl. No.	Treatments	PDI[a]	Disease reduction (%)	Yield (kg ha^{-1})	Increase in yield (%)
1.	Neem cake extracts (10%)	27[b]	70	4675[b]	23
2.	*Nerium oleander* extract	42[b]	53	4475[b]	18
3.	*Pithecolobium dulce* extract (10%)	66[b]	26	4017[b]	6
4.	Mahua cake extract (10%)	62[b]	31	4142[b]	9
5.	*T. viride* (Tv2) (1 kg ha^{-1})	47[b]	48	4383[b]	15
6.	*T. harzianum* (Th5) (1 kg ha^{-1})	60[b]	33	4242[b]	11
7.	Mancozeb (0.2%) (Control)	15[b]	83	4800[b]	26
8.	Control (untreated)	90	0	3808	0

[a] Mean of three replications. Percent disease index was calculated based on disease grade following SES of rice developed by IRRI. Grain yield for individual plot was measured and converted to kg ha^{-1}. PDI [SED = 0.184, LSD (5%) = 0.395, LSD (1%) = 0.548] Yield [SED = 0.18, LSD (5%) = 0.400, LSD (1%) = 0.55]

[b] The values are significantly different from the control

N. oleander and *P. dulce* were highly inhibitory to the mycelial growth and spore germination of *B. oryzae*. In earlier, several research workers have demonstrated that some plants posses antifungal activity against several plant diseases (Wilson et al. 1997; Al-Mughrabi et al. 2001). Similar to the present findings, Chelvan and Sumathi (1994) reported the inhibitory effect of *Solanum nigrum* on the mycelial growth of *B. oryzae*. Recently, Satheesh et al. (2005) demonstrated that leaf extract of *Datura metel, Zizuphus jujuba, Ipomea carnea, A. indica* and *V. negundo* has strong inhibitory effect against *Rhizoctonia solani* and *Xanthomonas oryzae* pv.*oryzae* under *in vitro* and *in vivo*. Previous reports in this field suggested that inhibition of *B. oryzae* by *N. oleander* might be attributed to the fungitoxic principle and toxic substances present in the plant extracts. Further, in the present study neem cake extract was found to be effective in reducing the growth and germination of *B. oryzae in vitro*. Our results are in agreement with the previous findings of Eppler (1995) who reported the efficacy of *A. indica* leaf and fruit

extract against a range of pathogenic fungi, bacteria, and viruses. Also, the efficacy of *V. rosea* and *A. indica* against the mycelial growth of *B. oryzae* was documented by Ganguly (1994). From the findings of earlier work it is assumed in the present study that antifungal activity of *A. indica* might reduced the growth of *B. oryzae*.

In addition, use of biocontrol strains for the management of plant pathogens has recently been exploited by the research workers (Saravanakumar et al. 2007; Kavino et al. 2007). Conspicuously, in the present study *T. viride* significantly inhibited the growth and spore germination of *B. oryzae*. Similarly, reports from earlier studies showed that *T. viride* and *T. harzianum* were mycoparasites of fungal pathogens and caused lysis of the pathogens (Majumder et al. 1996). Further, it is reported that *Trichoderma* grew over the pathogen and caused hyphal coiling, hyphal abnormalities, reduction in sclerotial production, lysis of hyphae and sclerotia of fungal pathogens (Malathi 1996). Thus, the efficacy of *T. viride* on *B. oryzae* in the current study attributed to the production of lytic metabolites and mycoparasitism.

Our results from pot culture and field study demonstrated that post inoculation spray of neem cake extract on 60 day old rice plants resulted in significant disease reduction followed by *N. oleander* leaf extract and *T. viride* (Tv2) besides increasing crop yield. Management of plant diseases by application of plant products has previously been demonstrated by Amadioha (2000). Results of our findings are in agreement with the previous findings of Jaganathan (1984) and Unnikrishna Pillai (1988) who demonstrated the efficacy of neem oil (3%) spray against *Helminthosporium nodulosum* causing ragi blight and *B. oryzae* in pot culture study. Later, Vijayakumar (1998) reported that application of neem oil (3%) + neem cake (150 kg ha^{-1}) was the most effective treatments against brown spot of rice both in pot culture and the field. The oleandrin toxin present in *N. oleander* is a cardiac glycoside and this may be responsible for the antimicrobial activity. However, the toxin is biodegradable and it is rapidly degraded to a very low concentration in due course (Downer et al. 2003). Also, the residual toxin in the leaves is not equivalent to chemical fungicides, which persist in the plant and soil after application for a long time. Recently, the efficacy of natural products and bioagents on leaf rust severity under field conditions were evaluated which indicated that jojoba, peppermint and chenopodium oil effectively reduced wheat rust severity besides increased yield under field conditions (Eldoksch et al. 2001). Similarly, Kamalakannan et al. (2001) reported that spraying of *Prosopis juliflora* leaf extract was significantly effective in reducing blast in pot culture and field experiments as well as increasing the yield. Biochemical analysis of rice plants treated with *P. juliflora* upon challenge inoculation with the pathogen showed enhanced activities of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) apart from the increase in phenolic contents and thus inducing systemic resistance against rice blast. Biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development (Vidhyasekaran 1992). From these reports, it is assumed that besides fungitoxic principle present in plant products, induction of defense enzymes and enhanced disease resistance in the present study could also taken part in reducing the *B. oryzae* in rice plants under pot culture and field study. However, the role of plant products used in this study needs further investigation to prove their influence against *B. oryzae* to induce systemic resistance.

Interestingly the important issue that must be noticed in the present work is the effectiveness of mancozeb (0.2%), which appeared to be most effective in reducing the brown spot disease in rice under pot culture and field study. However, easy availability of plant species coupled with less phytotoxicity and environmental hazards make them a

potential alternative. Also, fungitoxicity of botanical products were considered to be the safe means of plant disease control. Furthermore, the combination studies with plant extract, oilcake and biocontrol agents need to be tested for better protection of the rice crop. Thus plant products and biocontrol agents can be well exploited in future and the active principles from the plant extract can also be isolated and formulated for the effective management of various plant diseases.

References

Al-Mughrabi KI, Abujai TA, Anfoka GH, Shahrour W (2001) Antifungal activity of olivecake extracts. Phytopathol Mediterr 40:240–244

Al-Mughrabi KI (2003) Antimicrobial activity of extracts from leaves, stems and flowers of *Euphorbia macroclada* against plant pathogenic fungi. Phytopathol Mediterr 42:245–250

Amadioha AC (2000) Controlling rice blast in vitro and in vivo with extracts of *Azadirachta indica*. Crop Protect 19:287–290

Chelven PTK, Sumathi L (1994) A fungitoxic substance from *Solanum nigrum*. Indian Phytopathol 47(4):424–426

Dennis C, Webster J (1971) Antagonistic properties at species groups of *Trichoderma* I Production of non-volatile antibiotics. Trans Brit Mycol Soc 57:25–39

Downer J, Craigmill A, Holstege D (2003) Toxic potential of oleander derived compost and vegetables grown with oleander soil amendments. Vet Hum Toxicol 45(4):219-221

Eldoksch HA, Atteia MF, Abdel-Moity SMH (2001) Management of brown leaf rust, *Puccinia recondita* of wheat using natural products and biocontrol agents. Pakistan J Biol Sci 4(5):550–553

Eppler JC (1995) Effects on viruses and organisms In: Schumutterer H (ed) The neem tree Sources of unique natural products for integrated pest management, medicine, industry and other purposes. Weinheim, New York, Basel, Cambridge, Tokyo: VCH, pp 575–581

Fawcett GH, Spencer DM (1970) Plant chemotherapy with natural products. Annu Rev Phytopathol 8: 403–418

Fiori ACG, Schwan-Estrada KRF, Stangarlin JR, Vida JB, Scapim CA, Cruz MES, Pascholati SF (2000) Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. J Phytopathol 148:483–487

Ganguly LK (1994) Fungitoxic effect of certain plant extracts against rice blast and brown spot pathogen. Environ Ecol 12(3):731–733

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. 2nd edn John Wiley and Sons, New York, pp 680

IRRI (1988) (International Rice Research Institute) Standard Evaluation System for rice (Revised) 3rd edn. Manila, Philippines

Jaganathan R (1984) Studies on the effect of plant products on fungal diseases of finger millet (*Eleusine coracana* Gaerth) MSc (Ag) Thesis Tamil Nadu Agricultural University, Coimbatore, 280 p

Jeyarajan R, Ramakrishnan G, Dinakaran D, Sridar R (1994) Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot diseases. In: Dwivedi BK (ed) Biotechnology in India Bioved Research Society, Allahabad, pp 25–36

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2005) Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv *oryzae*. Physiol Mol Plant Pathol 65:91–100

Kamalakannan A, Shanmugam V, Surendran M (2001) Effect of plant extracts on susceptibility of rice seedlings to blast disease and consequent biochemical changes in rice plants. J Plant Dis Protect 108(5):536–543

Kavino M, Harish S, Kumar N, Saravanakumar D, Damodaran T, Soorianathasundaram K, Samiyappan R (2007) Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. Soil Biol Biochem 39:1087–1098

Kohls CL, Percich JL, Huot CM (1987) Wild rice yield losses associated with growth stages specific fungal brown spot epidemics. Plant Dis 71(5):419–422

Majumdar VL, Jat JR, Gour HN (1996) Effects of biocontrol agents on the growth of *Macrophomina phaseolina* the incitent of blight of moth bean Indian. J Mycol Plant Pathol 26(2):202–203

Malathi P (1996) Biocontrol of groundnut (*Arachis hypogaea* L.) dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid PhD Thesis Tamil Nadu Agricultural University, Coimbatore

Nakkeeran S, Renukadevi P, Marimuthu T (2005) Antagonistic potentiality of *Trichoderma viride* and assessment of its efficacy for the management of cotton root rot. Arch Phytopathol Plant Protect 38:209–225

Nyvall RF, Percich JA (1999) Development of fungal brown spot and spot blotch on cultivated wild rice in Minnesota. Plant Dis 83:936–938

Ou SH (1985) Rice diseases.2nd edn. Commonwealth Mycological Institute Kew, Survey, England, p 380

Padmanabhan SY (1973) The great Bengal famine. Annu Rev Phytopath 11:11–24

Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R (2007) PGPR-induced defense responses in the tea plant against blister blight disease. Crop Protection 26:556–565

Schmitz H (1930) Poisoned food technique. Indust Engin Chem Analyst 2:333–361

Unnikrishna Pillai, KP, (1988) Studies on the effect of plant extract/products and antagonist bacterium against rice brown spot pathogen *Drechslera oryzae* (Breda de Hann) Subram and Jain MSc (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 195 p

Vidhyasekaran P (1992) Principles of plant pathology. CBS Printers and publishers, New Delhi

Vijayakumar S (1998) Use of plant oils and oil cakes in the management of rice diseases MSc (Ag) Thesis Tamil Nadu Agricultural University, Coimbatore

Wilson CL, Solar JM, El Ghaouth A, Wisniewski ME (1997) Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis 81(2):204–210

Zulkifi EJ, Clap Castano J (1991) Effect of grain discolouration in upland rice on some yield components. Int Rice Res Newsl 16: 20