J. Mycopathol. Res. **40 (1)** : 57- 62 (2002) *Printed in India*

A current approach to the management of root diseases in bast fibre plants with conservation of natural and microbial agents

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Management of root diseases in bast fibre plants pose problem of resurgence of pesticide resistant pathogens and environmental pollution from indiscriminate use of fertilizer and chemicals. Organic soil amendments, rhizosphere microorganisms and biofertilizer application prevented root-infecting fungi. Neem cake 1-0.1 ton with 25-40 kg zinc sulphate per hectare and seed inoculation with symbiotic, commensalic and plant growth promoting rhizobacteria viz. *Rhizobium japonicum*, *Azospirillum brasilense*, *Pseudomonas striata*, fungal antagonists *Aspergillus niger*, *Trichoderma viride*, *in-furrow* soil application of VA-Mycorrhiza (*Glomus mossae*) and post emergence seedling inoculation of Rice necrosis mosaic virus singly or in compatible synergistic combinations, significantly (P 0.05) reduced sunnhemp wilt and jute root rot caused by *Fusarium udum* f.sp. *crotalariae* and *Macrophomina phaseolina* respectively, influencing root nodulation in sunn hemp and plant biomass promotion in both. *Pseudomonas*, *Trichoderma* and *Aspergillus* strains respectively were strong antagonist in plant inhibition, zone formation and overlapping pathogen colony *in-vitro*. Fungitoxic properties of botanical pesticide neem, ascribed to the presence of oleic acid, sulphur and flavonoids, as well as extracelluar enzyme, toxin, siderophore and phytohormone compounds of the potential bioagents, may be exploited in biological control leading to an eco-friendly low cost technology for developing an appropriate integrated management system.

Key words : Soil amelioration, neem cake, seed pelleting, biofertilizer, biocontrol, *Azotobactor*, *Azospirillum*, *Pseudomonas*, *Aspergillus*, *Trichoderma*, VAM, PGPR, RNMV, growth promotion.

INTRODUCTION

Root diseases of bast fibre plants due to infection of soil borne vascular wilt and cortical rot pathogens of diverse taxonomic identity are persistent problem for management. Indiscriminate use of chemical fertilizer and synthetic pesticides for achieving high yield pose problem of resurgence giving rise to pesticide resistant strain of pathogen, lysis of beneficial organisms and environmental pollution. Heterogeneous organic matter amelioration to soil, rhizosphere microfloral population and management tactic integration play vital role in preventing root infecting fungi, recycling nutrient elements and scavenging xenobiotics depending upon ameliorant decomposition products, suppressive bioagents and physiology of host tolerance. Natural control through conservation and manipulation of bioagents is by far more important in the niche of biodiversity. These are often inadequate or non-persistent in effective level under high disease pressure unless supplied exogenously. Certain oil cakes exhibited efficacious result against phytophagus nematodes and fungi (Mukherjee *et al.*, 1992). Some antagonistic microorganisms, mostly rhizobacteria of fluorescent *Pseudomonas* group and hyperparasitic fungi are successful as biocontrol agents against infection of different soil borne plant pathogens (Bhattacharya and Pramanik, 1998; Biswas and Sen, 2000). Some other agents of bacterial and fungal origin including VA-Mycorrhiza are utilized as biofertilizer for supplying phosphorus and other nutrients to potential host (Subba Rao and Gaur, 2000) and certain virus being explored as growth promoter and energizer of non-conventional hosts in general and bast fibre plants in particular (Ghosh, 1995). Application of such type of beneficial bioagents have shown potentiality for controlling soil-borne root infecting fungi and other pathogens through diverse mechanism (Pandey and Kumar, 1990; Bandopadhyay and Dasgupta, 1999). Search for a comprehensive practice of eco-friendly natural control through manipulation of organic amendment and introduced bioagents against

vascular wilt of sunn hemp and jute root rot diseases caused by *Fusarium udum f.sp. crotalariae* and *Macrophomina phaseolina* respectively exhibited encouraging result.

MATERIALS AND METHODS

Management of vascular wilt disease of sunn hemp (*Crotolaria juncea L.*) and root rot of jute (*Corchorus olitorius L.*) was attempted by organic soil amendment and application of biofertilizer and biocontrol agents singly or in various combinations under field conditions. Total ten treatments with three replication plots under each were designed in CRBD. Oil cake of neem (*Azadirachta indica Juss.*) at 1, 0.5 and 0.1 ton ha^{-1} dose was added to soil 2, 4, 6, weeks before showing or drenched between rows a week after sowing with or without 0.2% bavistin seed treatment. Seed inoculation with biofertilizer and biocontrol agents was done by mixing aliquots of 100 g seed with 10 g each of soil based culture inoculants of symbiotic nitrogen fixing bacteria *Rhizobium (Bradyrhizobium) japonicum* for sunn hemp, and non-symbiotic N_2 fixer *Azotobacter chrococcum* for jute singly or combined in a consortium with *Azospirillum brasilense* and phosphorus solubilizer *Pseudomonus striata*. 10 ml spore suspension each of fungal antagonist *Aspergillus niger*, *Gliocladium virens* and *Trichoderma viride* were added. Inoculated seeds were pelleted by mixing well with 5 g each of molasses and methylcellulose powder in 10 ml distilled water. Concentration of bioagent inoculants was kept at 10^7 cfu g^{-1}/ml^{-1}. Rice necrosis mosaic virus (RNMV) inoculation of plants was done by rubbing two weeks old seedling leaf tip with inoculum suspension prepared by crushing 3 g infected rice leaf with 100 ml water. Phosphorus mobilizer VAM fungus was applied *in-furrows* as 40 g soil based inoculum after sowing. Root nodulation in sunn hemp and plant biomass (dry weight) of both jute and sunn hemp plants were studied at 30 days age of plants. Disease incidence was recorded at pod formation stage after 60 days, calculated as percent mortality of plants. Laboratory assay of neem cake was conducted by standard method of extraction, filtration and sterilization through bacteriological grade G-5 filter. Inhibition of pathogen colony diameter and sporulation ml^{-1} of Czapek Dox Agar medium supplemented with neem cake extract was studied by poisoned food after 2 weeks growth. *In-vitro* antagonistic effect of the bioagents was observed in the amalgamated Potato Dextrose and Tryptone-Yeast Extract Agar medium (1:1) in dual culture plates with modification of techniques followed by Biswas and Sen (2000). Percent inhibition of pathogen, zone of inhibition and the overlapped area of pathogen colony by the antagonist was calculated after seven days. Result has been expressed in percent increase/decrease over control with the formula $\frac{A-B}{A}$, values for the data appended in parenthesis in the Tables.

RESULT

Sunn hemp wilt

Neem cake soil amelioration at 1-ton per ha. dose with 25 kg zinc sulphate and *Rhizobium* seed inoculation significantly (P=0.05) controlled wilt incidence over 40%, increased 12% root nodulation and 46% plant biomass. *Rhizobium* seed inoculation and 0.1-ton neem cake with 25 kg $ZnSO_4$ per ha. soil drenching between rows exhibited high degree of protection above 40% against *Fusarium* wilt, promoted root nodulation above 15% and plant biomass 40% till harvest over the *Rhizobium* seed inoculation alone, which could be a low cost technology (Table 1). Fungal lyses occurred at all levels of neem cake depending upon dose and speed of decomposition influenced by presence of a chemical principle and rhizosphere microflora population. Synergistic effect of *Rhizobium* and *Azospirillum* combined seed inoculation appeared well in reducing *Fusarium* wilt up to 31%, increasing 34% nodulation and 32% biomass. *Rhizobium* and *Pseudomonus* together was best in wilt management up to 55%. Promote nodulation by 44% and biomass 61%. *Aspergillus niger* strain was highly effective in reducing wilt by 37%, promoting 48% nodulation and 61% biomass. *Trichoderma* and *Rhizobium* also in successful synergistic association decreased wilt by 34%, increased 44% nodulation and 41% biomass. VAM exerted effect equal to *Azospirillum* by 31% wilt control. RNMV and *Rhizobium* combined reduced 39% wilt, enhanced nodulation and S1 the highest 125% plant biomass (Table 1).

Jute root rot

Neem cake 1-ton per hectare and $ZnSO_4$ 25 kg soil amelioration before sowing with *Azotobacter*

Table 1. Effect of some bio-agents on *Fusarium* wilt, root nodulation and plant growth in Sunn hemp (*Crotolaria juncea* L.)

Bio-agent	*Fusarium* wilt control (%)	Root nodulation promotion (%)	Plant biomass increase (%)
Seed Inoculation			
Rhizobium japonicum	11.7 (12.8)	14.6 (47.0)	7.1 (8.1)
Rhizobium + *Azospirillum brasilense*	31.0 (10.0)	34.0 (53.0)	32.8 (10.1)
Rhizobium + *Pseudomonas striata*	55.8 (6.4)	43.9 (59.0)	61.0 (12.3)
Rhizobium + VAM (Field application)	31.7 (9.9)	12.0 (46.0)	8.7 (8.3)
Rhizobium + *Aspergillus niger* AN- 27	37.9 (9.0)	48.7 (61.0)	61.8 (12.3)
Rhizobium + *Trichoderma viride*	34.4 (9.5)	43.9 (59.0)	41.3 (10.8)
Rhizobium + *Gliocladium virens*	27.5 (10.5)	41.4 (58.0)	47.9 (11.3)
Rhizobium + RNMV (Plant inoculation)	39.3 (8.8)	51.2 (62.0)	125.0 (17.2)
Rhizobium + Neem cake 0.1 ton + $ZnSO_4$ 25 kg ha soil drench	40.6 (8.6)	15.1 (47.2)	40.0 (10.7)
Control	--- (14.5)	--- (41.0)	--- (7.6)
L.S.D. (0.05)	8.3	23.5	6.2

seed inoculation declined root rot incidence by 45% and increased plant growth (dry weight of biomass) by 25% over control. Dual inoculation of seed with plant growth promoting bacterial biofertilizer *Azotobactor*, *Azospirillum*, *Pseudomonas striata* and biocontrol fungi *Aspergillus niger* and *Trichoderma viride* envisaged *Azotobacter* along with *Pseudomonas striata* to be compatible in effectiveness for root rot control up to 22%, followed by pre-sowing *Azotobactor* seed pelleting and post sowing in-furrows VAM application 20%. *Azotobactor* and *Azospirillum* combined 19%, *Azotobactor* and *Aspergillus niger* 17% and that with *Trichoderma viride* 11%. At 90 days plant biomass dry weight enhanced above 25% with both

Table 2. Effect of different bio-agents on *Macrophomina* root rot and plant growth in jute (*Corchorus olitorius* L.)

Bio-agents	Root rot Control (%)	Biomass increase (%)	Yield increase (%)
Azotobacter chroococcum	10.4 (8.6)	7.0 (12.1)	18.4 (29.5)
Azotobacter chroococcum + *Azospirillum brasilense*	18.8 (7.8)	25.2 (14.2)	18.4 (29.5)
Azotobacter chroococcum + *Pseudomonas striata*	22.9 (7.4)	24.0 (14.0)	20.4 (30.0)
Azotobacter chroococcum + VAM	19.8 (7.7)	25.1 (14.1)	25.3 (31.2)
Azotobacter chroococcum + Aspergillus niger AN 27	16.6 (8.0)	23.9 (14.0)	22.9 (30.6)
Azotobacter chroococcum + *Trichoderma viride*	11.4 (8.5)	12.0 (12.7)	15.6 (28.8)
Azotobacter chroococcum + *Gliocladium virens*	10.5 (8.4)	22.1 (13.1)	20.1 (29.9)
Azotobacter chroococcum + RNMV	16.8 (8.0)	44.8 (16.4)	25.2 (31.0)
Azospirillum brasilense + Neem cake 1 ton + $ZnSO_4$ 25 kg ha soil drench	40.0 (5.5)	25.0 (14.1)	25.3 (31.2)
Control	--- (9.6)	--- (11.3)	--- (24.9)
L.S.D. (0.05)	3.7	7.5	9.6

Pre sowing Seed Inoculation : *Azotobacter chroococcum*, *Azospirillum brasilense*, *Pseudomonas striata*, *Aspergillus niger* strain 27, *Trichoderma viride*, *Gliocladium virens*;

Post sowing Soil Application : VAM – Vesicular Arbascular Mycorrhiza (*Glomus mossae*), Neem cake + Zinc sulphate drench

Table 3. *In-vitro* effect of neem cake extract and zinc sulphate amelioration on the growth and sporulation of vascular wilt fungus *Fusarium udum* Esp. *crotalariae*

Treatment	Concentration (%)	Percent Inhibition of			
		Growth (mat diam)	Sporulation (Conidia $\times 10^5$)	Conidial germination	Germ tube growth(μ)
NC Extract	1.0	10.9 (93.0)	16.3 (123.0)	34.7 (65.3)	60.6 (42.0)
"	2.0	15.4 (79.5)	50.3 (73.0)	37.0 (63.0)	66.7 (35.5)
"	3.0	20.0 (75.5)	46.2 (79.0)	30.0 (70.0)	60.0 (42.6)
"	4.0	37.2 (59.0)	53.7 (65.0)	39.0 (61.0)	47.1 (56.3)
NC Extract /$ZnSO_4$	2.0 0.01	40.0 (57.0)	83.6 (24.0)	88.0 (12.0)	95.7 (4.6)
"	4.0 0.01	41.6 (55.0)	72.9 (40.0)	64.0 (36.3)	90.6 (10.0)
/$ZnSO_4$	0.1	21.2 (74.0)	0.6 (146.0)	53.0 (47.3)	67.8 (34.3)
Control	---	--- (94.5)	--- (147.0)	--- (100.0)	--- (106.6)
L.S.D. (0.05)		27.5	5.0	18.8	14.0

Azotobacter and *Azospirillum* as well as VAM. 24% with *Aspergillus niger* and *Pseudomonas striata*, and 12% with *Trichoderma* combinations. *Azotobacter* seed inoculation followed by RNMV plant inoculation could check 16.8% root rot infection and promote 44.8% plant biomass dry weight which were similar in effect with that of *Azotobacter* and *Aspergillus niger* and *Azotobacter* and VAM treatments respectively, being the highest biomass producer. At harvest, RNMV promoted 25% fibre yield at per with VAM as well as neem cake and *Azotobacter* combinations. *Pseudomonas*, *Aspergillus* and *Trichoderma* were also promising to that effect (Table 2).

Laboratory assay

In laboratory assay, 2-4% extract of 1-3 days decomposed neem cake and 0.01% $ZnSO_4$ combined, was highly inhibitory to growth and sporulation of fungal pathogen from 40-42% and 73-83% that even checked the conidial germination and germ tube growth up to 88% and 95% respectively (Table 3). Neem cake amended rhizosphere microflora was dominated by *Bacillus*, *Clostridium*, *Rhizobium*, *Azotobacter* and a fluorescent *Pseudomonus* species of bacteria, species of *Penicillium*, *Aspergillus*, *Trichoderma* and *Gliocladium* as fungal antagonist, saprophytic species of *Rhizopus*, *Mucor* and *Rhizoctonia chorcorum* and Actinomycetes. In dual culture, *in-vitro* inhibition of fungal pathogen was maximum up to 55% with *Pseudomonas striata*, 33% with *Azospirillum* and 13% with *Rhizobium* strains. Highest inhibition of 78%, formation of inhibition zone between 23-18 mm and overlapped area on pathogen colony by the antagonist bioagent up to 48 and 23 cm^2 was revealed with *Trichoderma viride* and *Aspergillus niger* respectively which appeared as strong antagonist (Table 4).

DISCUSSION

The fungitoxic properties of neem cake ascribed to the presence of low C/N sulphur containing principle in 11-18% oil content with about 1% sulphur. Fatty acids viz. behenic, arachidic, stearic, palmitic, linoleic and oleic have been isolated from neem seed kernel and neem oil. Liquid fraction of the oil contains nimbidin and crystalline components nimbin and nimbinin, the main component being oleic acid, which is regarded to be responsible for sanitary effect of neem. These compounds, collectively called Azadirachtin, a viable botanical pesticide, reported to be effective against several root infecting fungi and other pathogens (Bandopadhyay, 1983; Soon and Botterel, 1994). Direct effect of azadirachtin as a chitin inhibitor on insect has been established. Chlamydospores of *Fusarium udum* contain about 25% chitin (Jayarajan *et al.*, 1986). Thus it is hypothesized that azadirachtin of neem oil present in substantial amount in neem cake caused lysis of cell wall of the fungal spores in neem cake amended soil, and stimulation of fungal antagonists in soil may be an

Table 4. Interaction of some bacterial biofertilizer and antagonist fungi with *Fusarium udum* f sp *crotalariae* in-vitro

Bioagent	Initial distance from pathogen (cm)	Inhibition zone (mm)	Inhibition (%)	Overlapped colony area (cm^2)
Rhizobium japonicum	4.5	----	13.5	23.8
Azospirillum brasilense	"	----	33.7	13.2
Pseudomonas striata	"	----	55.0	1.5
Trichoderma viride	"	22.0	78.2	38.5
Gliocladium virens	"	19.0	76.8	36.3
Aspergillus niger	"	18.0	78.2	22.0
Control	----	0.1	0.4	100.0
L.S.D.		1.1	6.3	8.3

indirect effect.

Root nodulating nitrogen fixing bacterial symbiont *Rhizobium japonicum* (*Bradyrhizobium japonicum*) is capable of suppressing pathogenic fungi by abundant presence in the rhizosphere and antagonism through colonization and parasitism on fungal mycelium (Tu, 1979; Balsundaram and Sorbhoy, 1988). It has been found to produce a 'Rhizobiotoxine', which may influence the host plant as well as pathogen (Chakraborty and Purakayastha, 1984). Non-symbiotic N fixing bacteria *Azotobacter* and *Azospirillum*, P solubilizing *Pseudomonas striata* and the P mobilizer VAM fungus *Glomus mossae* also inhibit pathogenic fungi while supplying nutrient elements to the host plant. *Azospirillum* and *Azotobacter* also produce siderophore like compound which acts through iron chelating mechanism making it non available to the pathogen. These organisms poses plant growth promoting hormonal properties and is now included in the list of PGPR (Subba Rao and Gaur, 2000). *Trichoderma* and *Gliocladium* suppress and antagonize fungal pathogen either in competition for nutrients or mycoparasitism through mechanism of direct penetration and hyphal coiling or antibiosis by liberation of antibiotic compounds (Mukhopadhyay, 1987). Gliotoxin and possibly other antibiotic substances might have role to inhibit pathogen. *Aspergillus niger* strain AN 27 produce low molecular weight iron chelating siderophore and hormone like substances (Mondal and Sen, 1999) which can kill the pathogen in soil and exert simultaneous growth promotion in host plant. Triggered synthesis of cytokinin and IAA like hormones by RNMV in non-primary host are thought to be responsible for plant growth promoting properties of this virus (Ghosh, 1995). The fungitoxic secondary metabolites, extracellular enzymes and hormonal compounds produced by the bioagents used, singly or in association of one or more factors acted up on the sunn hemp wilt and jute root rot pathogens through direct or indirect mechanism and provided promising protection against disease while promoting host plant growth at the same time. Present experiments warrant that, fungitoxicity of the nodulation promoting, plant growth promoting rhizobacteria (PGPR), biofertilizer, VAM, *Aspergillus*, *Trichderma* and other antagonistic as well as plant growth promoting fungi and RNMV, alone or in combination with sulphur and limonoids containing organic amendments, may be suitably exploited for associative, antagonistic or antibiotic prevention of root rot pathogens with simultaneous host plant growth promotion, which is likely to be acceptable as economically sustainable eco-friendly technology for root disease management. That will go long way as new dimension toward the philosophy of biological control leading to an appropriate integrated management system in the production and quality improvement of varied crops.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the active scientific support and facilities provided by the Director, Central Research Institute for Jute and Allied Fibres, Indian council of Agricultural Research, Barrackpore, W.B. toward successful

execution of this experimental work.

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(Accepted for publication November 19, 2001)