Indian J. Nat. Rubb. Res. 4(2): 142–145, 1991

EFFECT OF FUNGAL ANTAGONISTS ON *PHELLINUS NOXIUS* CAUSING BROWN ROOT DISEASE OF *HEVEA*

Brown root disease caused by *Phellinus noxius* (Corner) G. H. Cunn. is considered the only significant root disease of *Hevea* in India. The control of this disease is mainly dependent on chemicals used for drenching the plant base and for painting the affected roots (Rajalakshmy, 1980; Tan, 1990). Cover crops grown in rubber plantations promote antagonistic micro-organisms besides themselves acting as decoy hosts of root disease pathogens of rubber, thus reducing disease incidence (Fox, 1965). The antagonism of *Trichoderma* spp. against *Rigidoporus lignosus* and their use in integration with fungicides for the control of white root disease of rubber have been explored (Jollands, 1983; Hashim, 1990). In the present study, isolation and evaluation of potential antagonists of *P. noxius* were attempted and the effect of their introduction to rhizosphere of rubber nursery seedlings in the pathogen infested soil was observed.

Rhizosphere soils of nursery seedlings were collected from different rubber growing areas and antagonistic fungi isolated by double layer technique. Soil dilution (1:1000) plates were poured with rose bengal agar (RBA) medium. After incubation for five days at 28±2°C, a second layer of pathogen-seeded potato dextrose agar was poured above the first layer and incubated for another five days. The colonies of fungi on the RBA, above which a zone of inhibition was formed or those which overgrew the pathogen were marked, picked up by inverting the plates, purified and given code numbers. The candidate fungi so selected were screened for their antagonism to *P. noxius* by dual culture method (Dennis and Webster, 1971) and the more promising isolates selected. The antagonism of these isolates were compared with other species of *Trichoderma*. The radial growth of the antagonistic fungi and the pathogen was recorded and the percentage inhibition of the pathogen calculated using the formula:

$$\frac{\text{Growth (in control} - \text{dual culture)}}{\text{Growth in control}} \times 100$$

where the control was axenic culture of the pathogen.

The antagonistic fungal isolates were mass-multiplied on sand-sorghum (19:1) medium. A culture of the pathogen, multiplied on the same medium and mixed with the soil (225 g per bag), was used for filling the top 15 cm of the polythene bags (25 x 50 cm). The bags were arranged in nursery rows, watered and the inoculum allowed to stabilize for three days.

Bold rubber seeds were sprouted in sand beds and seeds with uniform sprouts selected. Small planting holes were made on the top soil in the polybags to a depth of 2.5 cm to accommodate the sprouted seeds. Ten grams of the antagonist inocula were introduced into each planting hole over which the sprouted seeds were placed The soil was then packed around the seeds. The plants were watered regularly. Treatments with only the pathogen inoculum introduced in top soil and also those without inocula served as controls.

The height of the plants and girth at 2.5 cm

from collar were recorded after 90 days. The population of the antagonists in the rhizosphere soil was assayed four months after introduction, by dilution plate technique using *Trichoderma* selective medium (Elad and Chet, 1983).

When the candidate fungi isolated from rhizosphere soils were evaluated in dual culture for their antagonism to *P. noxius*, the growth of the antagonist was more in isolate S_4F_1 followed by S_1F_3 and R_1F_2 which did not differ significantly. The inhibition of pathogen also was more when S_4F_1 was grown against. Isolate R_1F_2 showed similar inhibition pattern. But the inhibition was markedly less with S_1F_3 when compared to S_4F_1 while all other isolates showed poorer growth as well as inhibition of the pathogen (Table 1).

Table 1. **Growth of antagonistic fungal isolates and inhibition of *Phellinus noxius* in dual cultures**

Code	Growth of antagonist (cm)	Inhibition of pathogen* (%)
S_1F_1	6.75	40.22 (39.32)
S_1F_2	5.75	32.61 (34.80)
S_1F_3	7.35	48.91 (44.37)
S_2F_1	2.85	11.96 (20.05)
S_2F_2	3.25	22.17 (8.47)
S_3F_1	3.65	30.44 (33.43)
S_4F_1	8.05	65.21 (53.87)
S_5F_1	4.35	47.83 (43.75)
R_1F_1	2.55	10.87 (18.88)
R_1F_2	7.35	53.26 (46.88)
SE	0.49	3.45
CD	1.09	7.68

*Values in parentheses represent arc sine transformation

The three isolates selected in the primary screening were compared in dual culture along with four known isolates of *Trichoderma*. It was observed that the isolate S_4F_1 performed significantly better than all the other isolates in their growth and inhibition of the pathogen. The isolate is tentatively identified as *T. harzianum*. The inhibition of the pathogen by all the other isolates were on par (Table 2).

Table 2. **Antagonistic potential of different isolates of *Trichoderma* against *Phellinus noxius* in dual culture**

Antagonist isolates	Growth in dual culture (cm)	Inhibition of pathogen* (%)
T. hamatum	4.80	34.15 (35.75)
T. harzianum	4.10	37.40 (37.59)
T. koningii	4.30	30.08 (33.24)
T. viride	4.16	27.64 (31.63)
S_4F_1 (*Trichoderma harzianum*)	6.60	69.91 (56.81)
S_1F_8 (*Trichoderma* sp.)	4.77	34.14 (35.64)
R_1F_2 (*Trichoderma* sp.)	5.00	36.58 (37.19)
SE	0.26	3.16
CD	0.56	6.77

*Values in parentheses represent arc sine transformation

All the antagonists except S_1F_3 grew well when multiplied on sand sorghum medium and sporulated profusely. Those isolates which multiplied well were introduced in the rhizosphere of rubber seedlings. The girth of the seedlings which received the antagonists were significantly more than that of plants grown in pathogen infested soil, being on par with that of plants grown in uninfested soil when observed 90 days after planting.

The height of plants in all the treatments which received the antagonists was significantly more than that in the pathogen inoculated control. The plant height in treatments which received *T. viride* and *T. hamatum* was significantly more than that in the uninoculated control (Table 3).

Table 3. **Effect of introduction of antagonists in the rhizosphere of rubber seedlings planted in infested soil**

Treatments	Girth (mm)	Height (cm)
T. hamatum	10.62	61.88
T. harzianum	9.43	59.13
T. koningii	10.21	56.13
T. viride	9.83	65.63
S_4F_1 (*Trichoderma harzianum*)	11.40	56.25
R_1F_2 (*Trichoderma* sp.)	9.43	51.75
Uninoculated control	8.64	53.75
Inoculated control	6.69	42.63
SE	0.89	3.73
CD	1.85	7.75

The infection by the pathogen in control plants was confirmed by uprooting the control plants maintained in excess of the replications included in the trial, observing the lesions on the roots and reisolation of the pathogen from them.

The population of the antagonists monitored four months after planting showed that it was higher in all treatments than in uninoculated control R_1F_2 being an exception. The population of antagonist in the pathogen inoculated control was less than that in uninoculated control (Table 4).

Table 4. **Population of *Trichoderma* spp. four months after introduction to rhizosphere soil**

Treatments	Population of *Trichoderma* spp.
T. hamatum	7.37
T. harzianum	3.88
T. koningii	4.00
T. viride	6.25
S_4F_1 (*Trichoderma harzianum*)	6.75
R_1F_2 (*Trichoderma* sp.)	2.63
Uninoculated control	2.50
Inoculated control	0.63

Trichoderma sp. is reported to be antagonistic to root disease causing fungi in plantation crops like coffee and tea (Venkatasubbaiah and Safeeulla, 1983). The present study shows that they are effective against *P. noxius* infecting rubber. These antagonists survive well in the rhizosphere of crop plants (Papavizas, 1985). *T. harzianum* when present in the rhizosphere is known to produce a growth regulating factor which stimulates plant growth (Windham *et al.*, 1986). Similar stimulation of rubber seedlings was observed when *T. hamatum* and *T. harzianum* were introduced in the rhizosphere.

The authors thank Dr. M. R. Sethuraj, Director, RRII for the facilities extended for this study.

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