Biological control of damping-off disease of cotton seedling

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Biological control of damping-off disease of cotton seedling caused by the pathogen *Rhizoctonia solani* Kühn, by *Penicillium pinophilum* Hedge. is reported along with details on its antagonistic potential including isolation of pathogen, soil amendment, etc. *P. pinophilum* isolated from soil suppressed *R. solani*. Mycoparasitism and cell lysis are reported as mechanisms of antagonism. Amendment of soil with *P. pinophilum* cultures protected the seedlings from post-emergence damping-off. The antagonist amendment showed no phytotoxic effect on the growth of cotton seedlings. Application of the antagonist through seed coating was equally effective in controlling damping-off of cotton.

BIOLOGICAL control of plant diseases with fungal antagonist is gaining importance[1] as chemical control of soil-borne pathogen is difficult, expensive and results in accumulation of hazardous toxic compounds. The present study is an attempt to isolate and test a biocontrol agent to control *Rhizoctonia solani*, the causal organism of pre- and postemergence damping-off of cotton in soils. An attempt has also been made to study the mode of action of the antagonist.

The pathogen *Rhizoctonia solani* Kühn was isolated from the infected seedlings of MCU-5 cotton variety (*Gossypium hirsutum* L., Malvaceae) collected from Coimbatore. The infected tissues were cut into small bits, surface-sterilized with 0.1% mercuric chloride solution for 2 min, washed in three changes of sterile distilled water, plated on PDA medium and inoculated under laboratory conditions (28 ± 3°)C. On day 4, the fungal growth from the infected tissues was transferred to PDA slants. The isolate was further purified by hyphal tip method, identified and maintained on PDA medium with periodic sub-culturing.

The antagonistic potential of different soil fungi to *R. solani* was studied on PDA medium using co-culture technique. The test antagonist was then placed at the periphery directly opposite to the disc of *R. solani* on agar medium. At the zone where both the fungal colonies meet, mycelia were picked up using sterile inoculation needle, mounted in lactophenol-cottonblue and observed under light microscope for possible mycoparasitism.

The selected antagonist was grown in 60 ml aliquotes of Czapek (DOX) liquid medium dispensed in 250 ml Erlenmeyer flasks. Ten-day-old culture filtrates, both autoclaved and non-autoclaved, were amended separately in PDA medium to obtain 5% and 10% amended media and poured in petri plates, which were inoculated in the centre with 5 mm discs from 5-day-old *R. solani* culture. PDA medium without culture filtrate served as control.

Garden soil (1.5 kg) was taken in 24 cm wide earthern pots and autoclaved for 2 h for two successive days. Fifty grams of rice–sand medium (river sand : rice : water = 10 : 1 : 2 and autoclaved) inoculated with 4-day-old *R. solani* culture and kept for 10 days was mixed with sterile soil incubated for a week. MCU-5 cotton seeds were sown in *R. solani* infested moist soil (about ten seeds per pot).

Biological control of damping-off was tried through soil amendment with the selected antagonist *Penicillium pinophilum*. For this, 25 g of the 10-day-old cultures of *P. pinophilum* grown over rice–sand medium was transferred to *R. solani*-infested soil during sowing of the cotton seeds. Seeds sown in pots without amendment and pots with only *P. pinophilum* grown over rice–sand medium were transferred to the *R. solani* infested soil during sowing of the cotton seeds. Seeds sown in pots without amendment and pots with only *P. pinophilum* amendment served as controls.

In another set of experiment the *P. pinophilum* amendment was replaced by coating the conidia of *P. pinophilum* over the cotton seeds before sowing. Cowdung was used as a carrier-sticker of conidia. Conidia were harvested from petri plate cultures in minimum quantity of water and mixed with cowdung to form a paste which was used in seed coating. The coated seeds were dried under shade for 24 h before sowing.

Five pots were maintained for each treatment. Growth parameters such as root length, shoot length, leaf length, width and seedling dry weight were measured for the seedlings under each treatment on days 20 and 30 of sowing.

A total of 33 fungi were isolated from garden soil. When tested for antagonism against *R. solani* on culture, only one suppressed *R. solani* markedly, which was identified as *P. pinophilum* and used for further study. In co-culture studies, *P. pinophilum* grew over *R. solani* without any sign of antibiosis (Figure 1). When left undisturbed for 10 days, the antagonist *P. pinophilum* covered the *R. solani* colony completely and killed the hyphae (Figure 2). The sclerotia formed by *R. solani* were also colonized by the antagonist rendering them non-viable and ineffective.

Microscopic observation of mycelium in the meeting zone of the colonies revealed clear hyperparasitism by *P. pinophilum*. Often, the antagonist hyphae grew parallel to the hyphae of *R. solani* and produced many minute, protuberance-like haustoria along its length (Figure 3).

These haustoria penetrated the host hyphae and formed intracellular parasite hyphae. Once within the host, *P. pinophilum* grew along the length without branching and killed the host hyphae (Figure 4). No coiling of

Figure 1. Growth of *Penicillium pinophilum* (dark colony) over *Rhizoctonia solani* in PDA culture in early stage.

Figure 2. Complete suppression of *Rhizoctonia solani* colony by *Penicillium pinophilum*.

the antagonist over *R. solani* hyphae was observed. The mycelia taken from the zone where the colonization of antagonist over the host was complete revealed more than 25% of *R. solani* hyphae lysed.

When the culture filtrate of *P. pinophilum* was amended with the culture medium, the radial mycelial growth of *R. solani* was inhibited by more than 30%. The inhibition was slightly greater when the non-autoclaved culture filtrate was amended when compared with autoclaved filtrate. *R. solani* attached cotton seedlings and caused post-emergence damping-off. The incidence of damping-off incited by *R. solani* was scared

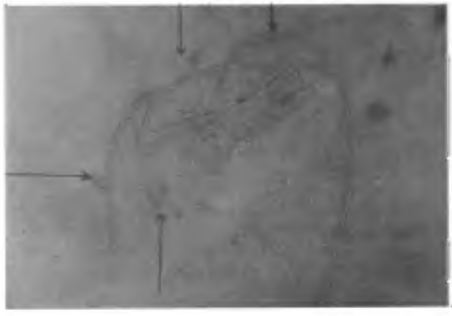

Figure 3. Minute, protuberance-like haustoria produced by *Penicillium pinophilum* for penetration in host *Rhizoctonia solani* (indicated in some places by arrow) (× 500).

Figure 4. The intracellular parasitic hyphae of *Penicillium pinophilum* in the host hyphae of *Rhizoctonia solani* (× 1000).

on the 20th day (Figures 5, 6). The addition of *R. solani* to soil resulted in 60% seedling mortality. However when *P. pinophilum* was added to soil the survival increased by over 50% (from 39.5 to 92.7). The controlled pots where there was no amendment and the other pots which received only *P. pinophilum*, exhibited 100% seedling survival.

To study the effect of soil amendment on the growth of cotton seedlings, various growth parameters such as shoot, root and leaf length and width and seedling dry weight were measured on days 20, 30 and 40 of sowing. The observations indicate that soil amendment with the antagonist *P. pinophilum* did not adversely affect the growth of seedlings as measured by root, shoot and leaf lengths and width, seedling weight (Tables 1–3) and the number of leaves produced (Tables 2, 3). Results on the survival of cotton seedlings as influenced by

Figure 5. Treatment pot which received only *Rhizoctonia solani* culture showing killed seedlings by damping-off disease.

Figure 6 Treatment pot which received both *Rhizoctonia solani* and *Penicillium pinophilum* cultures showing protected healthy seedlings.

seed coating with *P. pinophilum* conidia are presented. In controls the seedling survival was total. *R. solani* amended soil showed a high seedling mortality of 60%. However, seed coating with the antagonist improved the survival of cotton seedlings from 40% to 73%. The

Table 1. Effect of soil amendment on the growth of cotton seedlings as measured on day 20

Treatment	Shoot length (cm)	Root length (cm)	Leaf length (cm)	Leaf width (cm)	Seedling dry wt (mg)
Control	11.6 (±2.87)	5.0 (±1.73)	2.33 (±0.42)	4.51 (±0.92)	55 (±9.09)
P. pinophilum	12.5 (±2.02)	6.6 (±2.96)	2.44 (±0.28)	4.40 (±0.44)	54 (±8.11)
R. solani	–	5.7 (±2.15)	–	–	36 (±11.98)
P. pinophilum + *R. solani*	12.2 (±2.93)	9.3 (±3.32)	2.44 (±0.18)	4.34 (±0.37)	53 (±6.38)

± indicates the standard deviation from mean values.

Table 2. Effect of soil amendment on the growth of cotton seedlings as measured on day 30

Treatment	Shoot length (cm)	Root length (cm)	Number of leaves	Seedling dry wt (mg)
Control	14.1 (±1.50)	10.7 (±1.96)	5.1 (±0.57)	153 (±13)
P. pinophilum	13.2 (±2.32)	8.87 (±2.18)	5.4 (±0.99)	143 (±16)
R. solani	–	8.87 (±2.18)	–	124 (±52)
P. pinophilum + *R. solani*	14.0 (±1.95)	10.71 (±1.91)	5.6 (±0.73)	160 (±18)

± indicates the standard deviation from mean values.

Table 3. Effect of soil amendment on the growth of cotton seedlings as measured on day 40

Treatment	Shoot length (cm)	Root length (cm)	Number of leaves	Seedling dry wt (mg)
Control	18.2 (±1.82)	12.19 (±1.21)	6.75 (±0.83)	195 (±12)
P. pinophilum	16.4 (±1.45)	11.49 (±1.68)	6.38 (±0.48)	188 (±34)
R. solani	–	8.11 (±1.68)	–	156 (±53)
P. pinophilum + *R. solani*	17.46 (±1.46)	12.04 (±1.51)	6.43 (±0.48)	189 (±26)

± indicates the standard deviation from mean values.

antagonist *P. pinophilum* was repeatedly isolated from the rhizosphere soil of the cotton seedlings where the

seeds were coated with conidia.

1 Boland, G J, *Can. J. Plant Pathol*, 1990, **12**, 295–299.

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Surface morphology of some articulated corallines from India

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Surface structures of seven species belonging to four genera of subfamily Corallinoidae (Fly: Corallinaceae) were microscopically investigated. Two distinct surface morphologies were revealed namely a *'Corallina type'* (c-type) with round to irregular cell outlines and round trichocyte bases and another *'Jania type'* with elongate polygonal cell outlines and round trichocyte bases. Trichocytes were observed in all species except in *Amphiroa anceps* and *Arthrocardia capensis*.

CALCAREOUS algae are scattered in all major divisions of algae. The method of lime deposition, type and amount of calcification vary from group to group and even from genus to genus[1]. Corallinacean forms deposit calcite which is rich in magnesium[2]. Calcite crystals are deposited among the fibrils of cell wall and show distinct organization[3] which is of great taxonomic significance[4]. Corallinoidae is further divided into two tribes on the basis of the reproductive structures. These are Janiae and Corallinae[5]. Vegetative distinction between the members of Janiae and Corallinae has been hardly studied.

All the calcified forms along the Indian coast have not been worked out for many aspects like mineralogy, taxonomy, surface morphology, etc. Since surface morphology provides additional taxonomic characters, an attempt has been made to study the structure of $CaCO_3$ of articulated corallines.

To study the surface morphological features, air-dried specimens of *Amphiroa* (*A. anastomosans*, *A. anceps*, *A. foliacea* and *A. fragilissima*), *Arthrocardia capensis*, *Cheilosporum spectabile* and *Jania rubens* were coated with gold-palladium[4]. The observations were made using an scanning electron microscope (camera Camebax model 571).

Intergenicular surfaces of *Amphiroa anastomosans*, *A. anceps*, *A. foliacea*, *Arthrocardia capensis*, *Cheilosporum spectabile*, *Jania rubens* under SEM revealed two distinct surface morphologies (Figures 1*a–h*). All species show C-type surface morphologies except *Jania rubens* which shows J-type surface features (Figure 1*h*). Cell outlines of the species of *Amphiroa*, *Arthrocardia* and *Cheilosporum* vary from round to irregular and these species show roundish trichocyte base (Figure 1*a*, *d*, *e*, *g*). Conspicuous concavities were also observed in these species (Figures 1*a* to *g*). In *A. fragilissima* and *A. foliacea* (Figure 1*a*) trichocytes were scattered randomly and quite conspicuous in *A. anastomosans* and *A. fragilissima* (Figures 1*a*, *c*). Trichocytes in all these species show a slightly raised doughnut-shaped base with a pore lacking a flange and appeared slightly smaller than the surrounding concavities. In the case of *A. foliacea*, there appeared to be additional cells produced at the juncture of other cells (Figure 1*e*). *Amphiroa anceps and Arthrocardia capensis* show absence of trichocytes (Figures 1*b*, *f*). Cells of reproductive region in *A. fragilissima* (Figure 1*d*) were smaller than those of the vegetative region. Clear termination of surface feature next to the adjoining walls was not observed in species of *Arthrocardia* and *Cheilosporum*. In *Jania rubens* regular rows of depression and a terminal ridge around the distal and proximal ends adjacent to the contiguous genicula were observed (Figure 1*h*).

Trichocytes in *J. rubens* showed a single pore with an expanded flange and trichocyte bases could be easily distinguished from the surrounding epithalial cells (Figure 1*h*).

Garbary and Johansen[4] showed vegetative distinction between Janiaeae and Corallinaeae. Corallina type surfaces occur with several modifications in all genera of the Corallinae while Jania type surface characters are limited to tribe Janiaea[6]. We have observed that all species of *Amphiroa*, *Cheilopsorum*, *Arthrocardia* show C-type surface morphology. All C-type surface cells are characterized by round to irregular cell outlines and with round trichocyte bases. All the studied species except *Amphiroa anceps* and *Arthrocardia capensis* show the presence of trichocytes and this should be considered as an additional taxonomic character for these species. Production of a single hair by trichocytes, i.e. cell and cell complexes was so far reported in *Jania rubens* and in some crustose coralline forms[7,8]. To the best of our knowledge except for *Yamadea*, *Jania* and *Haliptilon* trichocytes have not been reported in the other genera of Corallinoideae[4,9].

Our results show that C-type and J-type of surface morphological features are common in members of Corallinaeae and Jaminaeae tribes respectively. Smaller cells in the conceptacular areas in *A. fragilissima* may be