between the reef units for supporting growth of seaweeds. Over a period of time (usually 1–2 years), a large number of seaweeds, abalone, shellfish, lobsters, etc. colonize on these artificial reefs (Figure 2 *a–d*). The abundance and biomass of these plants and animals are usually more in comparison to the natural substrata. Techniques for the construction of artificial reefs have already been standardized and are in practice, for many years. A large number of concrete-making companies have come up in recent years for this purpose creating more job opportunities. So artificial reef construction is not only economical but also ecological.

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Biological management of patchouli (*Pogostemon cablin*) wilt caused by *Rhizoctonia solani*

Amongst the various fungal diseases of patchouli (*Pogostemon cablin*), wilt caused by *Rhizoctonia solani* is one of the most destructive[1]. Patchouli is one of the most important aromatic plants which yield an essential oil known as patchoulol. This crop has been recently introduced in the Tarai of UP. Wilt incidence has been reported from various fields and accounts for about 1–10% losses. Synthetic chemicals (fungicides) do not provide adequate control of the pathogen, besides being toxic to both human beings and animals. The use of ecofriendly antagonistic biological agents can check the spread of the pathogen and disease effectively[2,3]. Several soil-borne Deuteromycetous fungi including *Trichoderma harzianum* and *Gliocladium virens* are known to inhibit the growth and the sclerotial production of *Rhizoctonia solani*[4]. This phenomenon of antagonism has been exploited to control plant diseases. Several workers have reported the interaction between AM fungi (*Glomus aggregatum*) and soil-borne plant pathogens[5,6]. These AM fungi make the host more resistant to plant pathogens and thus help in biological control of plant diseases[7–9]. In the present work, efficacy of *T. harzianum*, *G. virens* and AM fungi was tested separately and in combination to find out the suitable biological control of *Rhizoctonia* wilt of patchouli.

R. solani, the causal organism of wilt of patchouli, was isolated from infected plants of patchouli in the fields. *T. harzianum* and *G. virens* were tested as biological antagonistic agents to control the wilt of patchouli separately and in combination with AM fungi. The mass inoculum of antagonist, i.e. *T. harzianum*, *G. virens* and the pathogen *R. solani* was prepared on a sand–maize medium (sand and broken maize in 1 : 3 ratio). The medium was sterilized at 15 psi (121°C) for an hour. Pathogens as well as antagonists were inoculated aseptically and were incubated for 21 days at 27 ± 2°C. Colony forming unit (CFU)/g was calculated after 21 days of incubation.

Maize (*Zea mays*) was used as a trap plant for the multiplication of AM fungi in pots (3 kg sandy loam soil, pH 6.8, available phosphorus 10.2 ± 0.64 ppm, available potassium 42.5 ± 0.68 ppm and available nitrogen 39.25 ± 0.86 ppm taken in 20 cm diameter earthen pots). The pot soil was examined after three months for the sufficient AM spores (450/100 g soil).

Antagonistic activity of *T. harzianum* and *G. virens* was tested against *R. solani* by using the dual culture technique[10]. Pathogens as well as antagonists were inoculated at the opposite ends of petri dishes containing potato dextrose agar (PDA). The growth of the pathogen was tested against the antagonist. From the zone of interaction in the dual culture, mycelial fragments were taken periodically and were observed for hyphal interaction[11]. Another *in vitro* test was conducted on the viability of sclerotia of *R. solani* after being subjected to attack by antagonists separately. Ten sclerotia were inoculated in a row at the periphery of petri dishes and the antagonist was

Table 1. Effect of different treatments on the wilt of patchouli

Treatment	Average height of healthy plants (cm)[b]	Average height of diseased plants (cm)	Average shoot fresh weight of healthy plants (g)	Per cent infection
TH + RS	52 ± 2.12 (8.33)[c]	45 ± 2.16 (15.38)	63 ± 1.94 (10.52)	20 (47.36)[a]
GV + RS	54 ± 1.98 (12.5)	46 ± 2.22 (17.94)	66 ± 2.24 (15.78)	16 (57.89)
GA + RS	61 ± 2.16 (27.08)	49 ± 2.34 (25.64)	72 ± 2.38 (26.31)	32 (15.78)
TH + GA + RS	63 ± 2.28 (31.25)	51 ± 1.98 (30.76)	75 ± 2.33 (31.57)	12 (68.42)
GV + GA + RS	65 ± 2.36 (35.41)	54 ± 2.34 (38.46)	78 ± 2.24 (36.84)	9 (76.31)
Control	48 ± 2.24	39 ± 1.82	57 ± 2.16	38

[a]Per cent decrease over control; [b]Average of five replicates; [c]Per cent increase over control; TH, *Trichoderma harzianum*; GV, *Gliocladium virens*; GA, *Glomus aggregatum*; RS, *Rizoctonia solani*; ±, standard error.

Table 2. Effect of different treatments on root infection and oil yield

Treatment	Per cent root infection by GA	No. of VAM spores/100 g soil	Oil content (%)
TH + RS	–	–	1.90 (4.39)[a]
GV + RS	–	–	1.92 (5.49)
GA + RS	68	428	1.92 (5.49)
TH + GA + RS	71	456	1.94 (6.59)
GV + GA + RS	73	482	1.96 (7.69)
Control	–	–	1.82
CD ($P = 0.05$)	6.4	37.0	0.68
CD ($P = 0.01$)	8.6	49.4	0.82

TH, *Trichoderma harzianum*; GV, *Gliocladium virens*; GA, *Glomus aggregatum*; RS, *Rizoctonia solani*; CD, Critical difference; [a]Per cent increase over control.

inoculated in the opposite end. After ten days the sclerotia were taken from the petri dishes, surface sterilized with 0.1% $HgCl_2$ solution, washed with distilled water and re-inoculated in the petri dishes to observe the sclerotial germination. The same process was repeated after 15, 20, 25 and 30 days. For the pot trials, earthen pots (45 cm diameter) were filled with native garden soil and sand in the ratio 3 : 1 after thorough mixing and sterilization with 5% formaline solution. Pots were watered to maintain adequate moisture. Mass inoculum of antagonists, *T. harzianum* and *G. virens* was thoroughly mixed in the upper layer of the pot soil at a rate of 100 g/pot (CFU 4.5–6×10^8, 6.5–8×10^8, respectively). Sand-grown plantlets of patchouli procured from WIMCO Seedlings, Rudrapur, India were planted singly per pot. At the time of plantation, 100 g culture soil of AM fungi containing 450–480 spores was filled in the hole. Mass inoculum of the pathogen *R. solani* (CFU 3–4.5×10^6) was added in the pots after a week at a rate of 100 g/pot. In the control sets only the pathogen was inoculated. All treatments were done in five replicates and the pots were kept in a glasshouse and were watered when required. Data were recorded on the following parameters after 125 days: average height of the healthy as well as diseased plants, average shoot fresh weight of the healthy plants, per cent infection, per cent root colonization by AM fungi, number of AM spores/100 g soil and per cent oil content. Per cent infection was calculated using the following formula.

$$\text{Per cent infection} = \frac{\text{Total no. of diseased plants}}{\text{Total no. of seedlings transplanted}} \times 100.$$

In the dual culture, the pathogen continues to grow until it comes in contact with the leading edges of *T. harzianum* and *G. virens*. In both cases antagonist showed over-growth on the advancing mycelial zone of the pathogen. The colony of *R. solani* was either suppressed by the antagonist or the mycelial strands of the pathogen got lysed when they came in contact with the antagonist. Attempts to re-isolate the pathogen from such test assay petri dishes failed and only the *T. harzianum* and *G. virens* were found, suggesting that the pathogen was completely destroyed by the antagonists. Microscopic observation of the mycelium from the zone of interaction showed coiling of the hyphae of *R. solani* around the hyphae of *T. harzianum* and *G. virens*. Results obtained from *in vitro* experiments on the sclerotial germination of pathogens, showed that *G. virens* was an effective antagonist which suppressed the sclerotial germination and killed the sclerotia completely within 15 days. *T. harzianum* on the other hand, did the same within 20 days. The result obtained from pot trials (Tables 1 and 2) showed that the application of *G. virens* in combination with *G. aggregatum* was found to be most effective and the percentage infection due to *R. solani* was found to be 9% compared to 38% in the control. The infection was 12% in the pots treated with *T. harzianum* in combination with *G. aggregatum*, and 20 and 16% when the pots were treated only with *T. harzianum* and *G. virens*, respectively. Maximum percentage infection was found to be 32% when the pots were treated with *G. aggregatum*. The average height of the healthy and diseased plants was maximum in the pots inoculated with *G. aggregatum* and in combination with *G. virens*.

The use of ecofriendly bioagents in controlling plant diseases is efficient compared with other control measures, because it reduces environmental and health hazards incurred by the use of chemicals. The success of biocontrol depends on the selection of suitable antagonist, method of application, the right environment and other factors. Biocontrol research on rhizosphere specialized micro-organisms has been going on for the past few years[12,13]. Commercial formulation of *G. virens* (GL21) has been marketed as 'Gliogard' and more recently as 'Soilgard'. These products have been registered with the US Environmental Protection Agency[14]. Production of secondary metabolites such as gliovirin and gliotoxin, has been reported as the main biocontrol mechanism[15].

Thus the application of *G. virens* in combination with *G. aggregatum* has a potential control over wilt of patchouli besides growth promoting activities. The wilt of patchouli caused by *R. solani* has been successfully controlled by using *G. aggregatum* in combination with *G. virens*. Commercial application of this combination to control soil-borne fungal diseases in various crops in the fields is now in progress.

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Auxin modulation of differentiation-specific polypeptides in the protonema cultures of the wild strain and auxin mutants of *Funaria hygrometrica*

Maintenance of chloronema state and the onset of caulonema state and of caulonema differentiation in the protonema of *Funaria hygrometrica* are regulated by endogenous cyclic 3′,5′-AMP and indole-3-acetic acid (IAA), respectively[1–6]. The role of these growth regulators is further controlled through an inverse relationship between the activities of cyclic nucleotide phosphodiesterase (cNPDE) and IAA oxidase in the protonema[7]. One of the significant subcellular changes during the transition from chloronema to caulonema involves a change in the shape, structure and distribution of chloroplasts[3]. There are some reports on the detection of caulonema-specific proteins in the whole tissue protonema extracts of *Funaria*[8–10]. In the present work, a comparative analysis of the age-dependent and auxin-triggered changes in the polypeptide profile in the soluble fractions of whole tissue extracts and chloroplast fraction of the wild strain and two auxin mutants, has been undertaken in order to detect the possible changes in the location and distribution of auxin-induced differentiation-specific proteins, between chloroplasts and extrachloroplast cytoplasm.

Mutants were isolated at the Botanisches Institut, University of Heidelberg (Germany) by UV irradiation of protoplasts, as described earlier[11]. Cultures raised on Knop's nutrient medium solidified with 2% agar, were kept under continuous illumination (4.32 Watts m^{-2}) at 25 ± 2°C and maintained by regular subculturing after every 8–10 days. The data presented were recorded 48 and 120 h after subjecting the 7-day-old protonema cultures to various treatments. For SDS PAGE of polypeptides, protonema tissue was homogenized in an ice-cold grinding medium (30 mM Tris–MES, pH 8, containing 0.4 M sucrose, 10 mM KCl, 1 mM EDTA.Na_2, 0.1 mM $MgCl_2$, 1 mM β-mercaptoethanol and 1.5 mM phenylmethylsulphonyl fluoride). The supernatant obtained after centrifugation at 5500 *g* was used to precipitate soluble proteins by mixing with 8 volumes of ice-cold acetone for 3 h at – 20°C. Chloroplasts were isolated by homogenizing protonema (50 mg fw) in 1 ml of buffer (0.33 M sorbitol, 0.2 mM $MgCl_2$ and 20 mM MES, brought to pH 6.5 with Tris)[12]. The resultant slurry was recentrifuged at 500 *g* for 1 min. The supernatant was recentrifuged at 2700 *g* for 1 min to get crude chloroplast fractions. The pellet thus obtained was resuspended in a cation-free medium (0.33 M sorbitol, brought to pH 7.5 using Tris), for purification by repeated centrifugation (3 times) at 2700 *g* for 1 min each. This procedure resulted in a preparation consisting of more than 90% intact and active chloroplasts, as determined by their activity in Hill's reaction. For precipitating the chloroplast proteins, the chloroplast pellet was resuspended in 0.33 M sorbitol (pH 7.5) followed by acetone precipitation. The precipitated proteins were pelleted by recentrifugation at 5500 *g* for 10 min. The pellet was air-dried and redissolved in Laemmli buffer (0.06 M Tris, 2% glycerol and 5% β-mercaptoethanol). After removing an aliquot for the estimation of total protein content by Bradford assay[13], the remaining protein sample was subjected to SDS treatment[9]. About 20 µg protein was loaded in each well of the 1.5 mm thick, 12.5% polyacrylamide gel, from the whole tissue samples and 15 µg from each of the chloroplast preparations. The polypeptide profiles were visualized by silver staining[14].

Under the above-mentioned culture conditions, wild strain protonema of *Funaria* exhibits normal growth and development in terms of chloronema formation, followed by spontaneous caulonema differentiation after 8 days of growth on hormone-free Knop's medium (basal medium). Wild strain protonema exhibits further promotion of caulonema differentiation by IAA (50 µM) treatment.