Hyphal interaction studies between *Thielaviopsis paradoxa* and its antagonistic fungi

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*Thielaviopsis paradoxa* (de Seynes) von Hohnel infects coconut stem through growth cracks or wounds that occur on the stem near ground level and cause stem bleeding disease of coconut. Ramanujam *et al.* (4) reported the antagonistic effect of four fungi viz., *Gliocladium virens* (=*Trichoderma virens*), *Trichoderma harzianum*, *T. viride* and *T. hamatum* on *T. paradoxa* in dual culture and *in vivo* tests. In the present study, light microscopic observations on hyphal interactions of four antagonistic fungi on *T. paradoxa* were recorded to understand the mode of antagonistic action.

The pathogen isolate of *T. paradoxa* (ITCC No.4567) used in the study was isolated from stem bleeding affected coconut palm from Kudlu village in Kasaragod district of Kerala. Two of the antagonistic fungal isolates viz. *G. virens* (ITCC No.4570) and *T. harzianum* (ITCC No.4572) were isolated from rhizosphere soils of coconut palm from Uduma and Kallangai villages respectively in Kasaragod district. *T. viride* (IISR isolate) was obtained from Indian Institute of Spices Research, Calicut, Kerala and *T. hamatum* was obtained from ITCC, IARI, New Delhi. Dual cultures of *T. paradoxa* and *G. virens*/ *T. harzianum*/ *T. viride*/ *T. hamatum* were grown on 2% malt agar for a period of five days. Small mycelial fragments from interaction areas in dual cultures and from monocultures of these fungi were observed under microscope (400-2500 X) at 12 h intervals for five days. Morphological differences observed in the hyphae and spores of *T. paradoxa* and antagonistic fungi in the dual culture plates were recorded in comparison with the hyphae and spores of monocultures.

**Hyphal interaction between *G. virens* and *T. paradoxa***

No contact between *G. virens* and *T. paradoxa* colonies was established in the dual culture till 12 h. During this period, *T. paradoxa* colony showed several hyphal abnormalities like stunted growth, blunt appearance of tips, excessive dichotomous branching (Fig.1), shrinkage and withdrawal of mycelium.
protoplasm from the tips and periphery of the walls and bulging of tips. During 12-24 h, breakage of hyphal tips, release of protoplasm out side (Fig.2) and lysis of protoplasm of \textit{T. paradoxa} were noticed. Contact between \textit{G. virens} and \textit{T. paradoxa} colonies was established after 36 h and lysis of protoplasm of \textit{T. paradoxa} continued. After 72 hours of co-culturing, the hyphae of \textit{T. paradoxa} appeared empty indicating complete lysis of protoplasm. \textit{G. virens} hyphae were intact and healthy till the fifth day.

**Fig.2.** Bulging, breakage of hyphal tips and release of protoplasm of \textit{T.paradoxa} (\textit{G.virens} vs. \textit{T.paradoxa}).

**Hyphal interaction between \textit{T. harzianum} and \textit{T. paradoxa}**

No contact between \textit{T. harzianum} and \textit{T. paradoxa} colonies was established in the dual cultures till 24 h of interaction and during this period normal intact hyphae of pathogen and antagonist were observed without any abnormalities. Contact between \textit{T. paradoxa} and \textit{T. harzianum} was established after 36 h of incubation. After contact, the hyphae of \textit{T. harzianum} were found coiling around the hyphae of \textit{T.paradoxa} by forming hook-like structures and entangling chlamydospores and endoconidia. After 48 h of interaction, lysis of protoplasm in the hyphae, endoconidia and chlamydomspores of \textit{T. paradoxa} was noticed. After 60 h of interaction the hyphae of \textit{T. harzianum} were found to penetrate into the chlamydomspores (Fig.3). Complete disintegration of hyphae, endoconidia and chlamydospores of \textit{T. paradoxa} were observed after 72 h of interaction.

**Fig.3** Coiling and penetration of \textit{T.harzianum} hyphae into the chlamydomspores of \textit{T.paradoxa} and lysis (\textit{T.harzianum} vs. \textit{T.paradoxa}).

**Hyphal interaction between \textit{T. viride} and \textit{T. paradoxa}**

Normal healthy hyphae of \textit{T. paradoxa} and \textit{T. viride} were observed up to 36 h in the dual cultures. After 36 h of interaction, protoplasm of \textit{T. paradoxa} hyphae appeared as round balls indicating shrinkage and lysis. The hyphae of \textit{T. paradoxa} appeared empty and devoid of protoplasm by 72 h. Hyphae of \textit{T. viride} were found normal during the interaction.

**Hyphal interaction between \textit{T. hamatum} and \textit{T. paradoxa}**

There was no contact between \textit{T. hamatum} and \textit{T. paradoxa} colonies in dual culture up to 36 h and either of the colonies did not show any abnormality during pre-contact period. After the establishment of contact between \textit{T. hamatum} and \textit{T. paradoxa} colonies, the hyphae of \textit{T. hamatum} were found growing along the hyphae of \textit{T. paradoxa} and coiling around it with the
production of knob-like haustoria at the point of contact (Fig. 4). Simultaneously lysis of protoplasm in the *T. paradoxa* hyphae was noticed. After 48 h of interaction, the hyphae of *T. hamatum* penetrated into *T. paradoxa* hyphae and produced hook-like structures with appressoria at their tips. Extensive growth and ramification of *T. hamatum* hyphae around the hyphae of *T. paradoxa* and disintegration of protoplasm were observed after 60 h. By 72 h, *T. paradoxa* hyphae were empty and filled with the hyphae of *T. hamatum*.

Fig. 4. Coiling of *T. hamatum* hyphae around the hyphae of *T. paradoxa* (*T. hamatum* vs. *T. paradoxa*).

In the interaction between *G. virens* and *T. paradoxa*, several hyphal abnormalities and lysis of protoplasm of *T. paradoxa* were observed even before it came in contact with the antagonist. *G. virens* was found to produce diffusible antifungal metabolites against *T. paradoxa* in antibiosis test on malt agar (4). These metabolites might have caused the hyphal abnormalities and lysis in *T. paradoxa*. Howell and Stipanovic (2) reported the production of several antifungal metabolites like gliotoxin, viridin and viridol by *G. virens* in cultures. In the interaction between *T. harzianum* and *T. paradoxa*, no hyphal abnormalities were noticed till the two colonies came in contact with each other. After the establishment of contact, *T. harzianum* showed mycoparasitic interactions. Although the hyphae of *T. harzianum* coiled around the hyphae, endoconidia and chlamydospores of *T. paradoxa*, intra-cellular penetration was noticed only into the chlamydospores. However, lysis of the protoplasm was observed uniformly in hyphae, endoconidia and chlamydospores during the interaction period. In an earlier study (4), production of diffusible antifungal metabolites by *T. harzianum* against *T. paradoxa* was observed in antibiosis test. These metabolites along with parasitization might be responsible for destruction of hyphae, endoconidia and the chlamydospores of *T. paradoxa* in dual cultures. Hyphal interaction between *T. harzianum* and *Sclerotium rolfsii* / *R. solani* by scanning electron microscopy, attachment of *T. harzianum* to the host either by hyphal coils, hooks or appressoria, lysed sites and penetration holes on the host hyphae were reported (1). Light microscopic observations in the present study indicated only hyphal coils and hooks on the host hyphae and chlamydospores. In the interaction between *T. viride* and *T. paradoxa*, hyphae of *T. paradoxa* showed lysis of the protoplasm immediately after contact with the *T. viride*. The production of antifungal compounds like trichodermin, suzukacillin and alamethicine by *T. viride* in cultures were also reported (3). These compounds might have caused lysis of protoplasm. In the interaction between *T. hamatum* and *T. paradoxa* typical mycoparasitic effect of *T. hamatum* on *T. paradoxa* was observed immediately after the two colonies came in contact with each other. Similar mycoparasitic interaction of *T. hamatum* with *S. rolfsii* and *R. solani* were observed (1).

REFERENCES


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