Scanning Electron Microscopy confirmation of mycoparasitism exhibited by *Trichoderma* spp on coconut pathogens, *Ganoderma applanatum*, *Glucidum* and *Thielaviopsis paradoxa*

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Abstract

Basal stem rot (*Ganoderma* wilt) caused by *Ganoderma applanatum* (Pers) Pat and *G.lucidum* (Leys) Karst. and stem bleeding disease caused by *Thielaviopsis paradoxa* (de Seynes) Von Hohnel are the important fungal diseases of coconut causing serious constraints to the production and productivity of the crop. The present study focuses on the mechanism of action of *Trichoderma* spp viz., *T. viride*, *T. harzianum* and *T. hamatum* on *Ganoderma applanatum*, *Ganoderma lucidum* and *Thielaviopsis paradoxa*. Spectral analysis by scanning electron microscopy was carried out to have an insight over the mycelial interactions between the *Trichoderma* spp and the *Ganoderma* spp as well as *Trichoderma* spp and the *Thielaviopsis paradoxa*. Scanning electron microscopy revealed sparse and intense coiling of *Trichoderma* spp hyphae over hyphae of *Ganoderma applanatum*, *G. lucidum* and *Thielaviopsis paradoxa*. Draining of protoplasmic contents after formation of frequent adpressing zones of the antagonistic hyphae on the mycelia of the test pathogens was evident. The present study confirms the mycoparasitism of *Trichoderma* spp on coconut pathogens, *G. applanatum*, *G. lucidum* and *T. paradoxa*.

**Key words**: Scanning Electron Microscopy, Coconut, Mycoparasitism, Biocontrol.

Introduction

Coconut palms are grown in India in an area of 1.84 million hectares with a production of 23,597 million nuts and a productivity of 6,847 nuts/ha annually. Among different states, Andhra Pradesh ranks fourth in area and production ranks fourth in area and production with respect to coconut cultivation in our country. Among the fungal diseases that affect coconut production in India, basal stem rot (*Ganoderma* wilt) caused by *Ganoderma applanatum* (Pers) Pat. and *G.lucidum* (Leys) Karst. and stem bleeding disease caused by *Thielaviopsis paradoxa* (de Seynes) Von Hohnel are important diseases of coconut (1,3, 6). *Trichoderma* spp viz., *Trichoderma viride*, *T.harzianum*, *T.hamatum* inhibited the mycelial growth of *Ganoderma applanatum* and *Ganoderma lucidum* and *Thielaviopsis paradoxa* under in vitro conditions (7). Srinivasulu and Rao (6) developed biocontrol based IDM approach against basal stem rot and stem bleeding diseases of coconut by exploiting the native *Trichoderma* spp. Present investigation was carried out to confirm the mycoparasitism of *Trichoderma* spp on *Ganoderma applanatum*, *G. lucidum* and *Thielaviopsis pradoxa* through Scanning Electron Microscopy.


**Material and Methods**

**Isolation of pathogens**

Infected root bits were collected from palms showing typical bleeding symptoms on the stem and root bits were surface sterilized with mercuric chloride (0.1%) followed by three washes in sterile distilled water and finally the bits were plated on PDA followed by incubation at 28°C for about a week. Stem bleeding infected bits were collected during survey and cut into small bits of 0.1 cm size and surface sterilized with 0.1 percent mercuric chloride for 1 mm. Then root bits were washed thoroughly on three changes of distilled water and plated out on PDA and incubated at 29 ± 1°C temperature for period of 7 days.

**Isolation of Trichoderma spp**

Soil dilution and plate count method was used for isolation of antagonistic mycoflora from the rhizosphere of coconut palms. Soil samples were collected from different coconut gardens of coastal agro ecosystem of Andhra Pradesh. The collected samples were subjected to serial dilutions using sterile distilled water and 0.5 ml of each sample at $10^{-4}$ and $10^{-4}$ dilutions were spread on Petri dishes containing Trichoderma specific medium (TSM) (2). Two plates were maintained for each dilution. The plates were then incubated at 28°C and were examined after four days.

**Methodology of Scanning Electron Microscopy**

Scanning Electron Microscopy studies were conducted at Ruska Labs, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad using Scanning Electron Microscope (SEM) model JEOL JSM 5600, Japan. Fungal mycelia of the individual pathogens and Trichoderma spp were cut from the petri plate and the pathogen growing towards the zone of interaction after 4-5 days were cut from the zone of interaction and placed on cover glasses. These were exposed to osmium tetraoxide (2%) for 24 h at 20°C and transferred to copper stubs over double adhesive tape, coated with gold in polaron, AU/ PD sputter coater and scanned in SEM and photomicrographed.

**Results and Discussion**

Isolation studies: Isolations carried out from diseased portions of the roots (basal stem rot) and stem (stem bleeding) of diseased coconut palms. Isolation from root bits yielded in two species of the genus Ganoderma i.e. *Ganoderma applanatum* and *G. lucidum* and isolation from stem bits yielded Thielaviopsis paradoxa. Rhizosphere isolations carried out resulted in three species of Trichoderma i.e., *T. viride, T. harzianum* and *T. hamatum* and the cultures were confirmed based on the identification key of Rifai (4).

**Scanning Electron Microscopy of Ganoderma applanatum**: Septate hyphae, mycelium white, bright yellow or orange and often spreads in fan shaped growth. Primary mycelium multinuclei. Secondary mycelium is binucleate with clamp connections. Mycelium white, with bottom orange or bright yellow. Clamidospores are elongated, spindle shaped and mostly appears single (Plate-1).

**Scanning Electron Microscopy of Ganoderma lucidum**: Septate hyphae, mycelium white, bright yellow or orange and often spreads in fan shaped growth. Primary mycelium multinuclei. Secondary mycelium is binucleate with clamp connections. Mycelium initially white, later turns to brown colour with bottom orange or bright yellow. Clamidospores are round shaped and mostly appears in bunches (Plate-2).
Scanning Electron Microscopy confirmation of mycoparasitism

Scanning Electron Microscopy of *Thielaviopsis paradoxa*: *T. paradoxa* produced pale brown to brown hyphae, conidiophores are slender, arising laterally from the hyphae and produced cylindrical to oval endoconidia. Chlamydomspores are terminal in chains, oborate to oval, thick walled and brown (Plate-3).

*Trichoderma viride*: Having usually more than 2-3 phialidy, curved, pin shaped, narrower at the base, widening above the middle, attenuated into long neck. Phialospores are globose or obovoid (Plate-4).

*T. harzianum*: Phialides arised in false verticellate upto five in number, short, skittle shaped, having pointed neck. Phialospores were accumulated at the tip of the phialides, sub globose short, obovoid, often broad truncate base, much darker on mass (Plate-5).
T. hamatum: Phialides are smooth, hyaline to pale greenish, short, plump, pear shaped, narrowed at base than middle, shoot conical neck (Plate-6).

Scanning Electron Microscopy on Trichoderma spp mycoparasitism

Hyphal interactions between Trichoderma spp viz., T. viride, T. harzianum and T. hamatum and Ganoderma spp and T. paradoxa were studied through scanning electron microscopy and the results revealed that Trichoderma spp coiled round the hyphae of Ganoderma spp and T. paradoxa both sparsely (in the initial stages) and intensely (later stages). This was followed by frequent adpressions of the Trichoderma hyphae on the hyphae of both the Ganoderma spp and T. paradoxa. Later penetration of Trichoderma spp into the hyphae of test pathogens followed by replacement of its protoplasmic contents was noticed. Finally, lysis of the Ganoderma spp and T. paradoxa mycelium was observed in three species of Trichoderma followed by protuberance of the phialospores of the bioagent from within the lysed mycelia of Ganoderma spp and T. paradoxa (Plate7-9).
In consonance with our observations, Upadhyay and Mukhopadyay (8) reported that *Trichoderma harzianum* coiled round the hyphae of sclerotium rolfsii and completely parasitized the mycelia there by completely replacing the proto plasmic contents of the mycelia of the test pathogen, while working on sugar beet diseases. He further reported that *T. harzianum* penetrated the test pathogen hyphae both directly and through haustoria. It was also noticed that the viability of the sclerotia of the test pathogen was significantly reduced (8). Saweant and Mukhopadhyay (5), while working on damping off of sugarbeet reported frequent adpressing zones of *Trichoderma harzianum* on the hyphae of *Pythium aplanidermatum*. He further reported that septation in the coenocytic mycelia of the *P. aplanidermatum* mycelia as a result of the invasion of the *T. harzianum*. Based on the scanning electron microscopic studies, it is evident that the *Trichoderma spp* were proved to be effective in controlling the BSR pathogens, *G. aplanatum* and *G. lucidum* and the stem bleeding pathogen, *T. paradoxa* by their penetration and subsequent killing of mycelia of pathogens.

References