

## MICROSCOPIC INTERACTION BETWEEN *TRICHODERMA ATROVIRIDE* STRAIN TV3 AND PATHOGEN *ALTERNARIA MALI*

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### ABSTRACT

*Trichoderma* spp. is well-known for their biocontrol efficacy against a variety of plant diseases. A particular isolate of *Trichoderma* spp. has the capacity to limit the growth of *Alternaria mali*, which causes *Alternaria* leaf spot of apple. In this study, the interaction between *T. atroviride* and mycelia of *Alternaria mali* was studied (microscopy 40X). *Trichoderma* spp. was isolated from the phyllosphere of an apple tree. Primers (ITS 1 and ITS 4) identified this putative bioagent as *Trichoderma atroviride* isolate TV3. Sequence were submitted to the NCBI and assigned the accession number OI694002. A severe mycoparasitism was noticed in the form of coiling and firmly attaching, with the occasional production of appressoria-like structures. The biocontrol agent multiplied abundantly, forming a dense mycelium with some appressorium like structures that penetrated the mycelium of pathogen.

**KEY WORDS** : Mycoparasitism, Biocontrol, Microscopy, Coiling, Appressorium

### INTRODUCTION

Apples, like other fruit crops, are susceptible to a variety of diseases that reduce the quality and quantity of the fruit. Fungal infections, on the other hand, are responsible for the preponderance of crop losses. Scab, *Alternaria* leaf blotch, powdery mildew, collar rot, root rot, sooty blotch, canker, and fly speck are some of the most common fungal diseases that affect apples (Shahzad *et al.*, 2002). Small, somewhat sunken, light to medium brown

dots on mature fruit are indicative of fruit spot symptoms. During the growing seasons, the disease produces significant defoliation, reduced tree vigour, and lower yields (Sharma, 2000). Crop management strategies have been utilized, such as the use of clean seeds, early planting dates, and soil tillage, although their efficiency can be hampered. As a result, fungicides have become the most common approach of controlling *Alternaria* diseases, and they have been used successfully on a commercial basis. Although fungicide treatment is

far more effective than cultivation management, it has negative environmental consequences and its effectiveness diminishes with time. Several species of *Trichoderma* (Persoon) have been investigated extensively in the biocontrol of diseases (Druzhinina *et al.*, 2011). Benitez *et al.* (2004) and Druzhinina *et al.* (2011) define their antagonistic capabilities as a combination of many strategies, including nutritional competition and direct mycoparasitism, which includes the synthesis of antifungal metabolites and cell wall-degrading enzymes. Mycoparasitism is a multi-stage process in which a mycoparasite, such as *Trichoderma* sp., exerts its influence. *Trichoderma* to the host's chemotropic development is driven by chemicals from the same (Chat, *et al.*, 1981). Recognition is the first stage when *Trichoderma* and the host come into contact. Following recognition, *Trichoderma* coils or sticks around the host hyphae forming appressoria-like structures (Elad *et al.*, 1983). *Trichoderma* next secretes hydrolytic enzymes, principally Chitinase, glucanases, and proteases, which dissolve the host's cell wall (Haran *et al.*, 1996), allowing the absorption of the cell wall's components and cellular contents (Elad *et al.*, 1983). In this study, the interaction between *T. atroviride* and mycelia of *Alternaria mali* was studied (microscopy 40X). Main aim of the technique investigates the real interaction between biocontrol agent and pathogen, specifically how the biocontrol agent was able to combat the pathogen *Alternaria mali*.

## MATERIALS AND METHODS

### Isolation of biocontrol agent

Isolation of native fungal biocontrol epiphyte was made from apparently disease free leaves of apple orchard. 30 leaves were collected, put in pre-sterilized Erlenmeyer flasks of 1000 ml capacity containing 250 ml sterile distilled water. Flasks was shaken for half an hour on a shaker/rotator and one milliliter of the suspension was spread over potato dextrose agar (PDA) medium in petriplates and then incubated at  $25\pm 2^\circ\text{C}$  for the growth of fungal colonies. The resultant fungal colonies was sub-cultured or subjected to purification.

### Causal pathogen

The pathogen *Alternaria mali* was obtained from mycology laboratory, Division of Plant Pathology, SKUAST-Kashmir, India.

### Dual culture Technique

Mycoparasitism was determined by Agar plate assisted slide culture technique (Bhat, 2017). 3-4mm of Potato Dextrose Agar (PDA) was placed into a 90 mm petri dish, about the thickness of a glass slide, and allowed to solidify. A sterilized glass slide was gently pressed against the solidified agar medium to leave a glass slide impression on the PDA media. Following that, the slide was eliminated. The agar medium was scraped with a sterile blade to match the imprint made by the glass slide that had been pressed on it, resulting in an agar strip that was precisely the same size as the glass slide. A sterile glass slide is inserted into the thin strip such that both of its sides touch the PDA and the upper surface of the glass slide fits the PDA's surface. Host fungus was inoculated on PDA on the margins of glass slides at different places. For 36 hours, the plate was incubated, allowing the host fungus to grow on both the medium and the glass slide. After the presence of host mycelium growth on the slide edges, the bioagent *Trichoderma* sp was inoculated on the margins of the glass slide in the area of the previously inoculated host fungus, and the petri plate was incubated for yet another 24 hours (Figure 1). After incubation, slide was carefully removed without disturbing mycelium of both bioagent and pathogen.



Fig. 1.

### Observations

Microscopic mycelial interaction (mycoparasitism) between epiphyte and pathogen was observed.

## RESULTS

Isolated bioagent was identified as *Trichoderma atroviride* strain AV1 and was submitted to NCBI under accession number 0L694002. The slides were carefully removed after a 24 to 36 hour incubation period after bioagent inoculation. The slides were stained and examined under a microscope, at low and high magnification. *Trichoderma atroviride*

microscopic capability against *Alternaria mali* was clearly displayed. *Trichoderma atroviride* a parasitic fungus, developed in close proximity to *A. mali*, with appressoria-like structures entering *A. mali* on occasion (Figure 3). *Trichoderma atroviride* hyphae have also been detected coiling frequently around *A. mali* (Figure 2). *T. atroviride* parasitized *A. mali* hyphae, growing along them and developing branches that wrapped around them and sporulate densely (Figure 4).

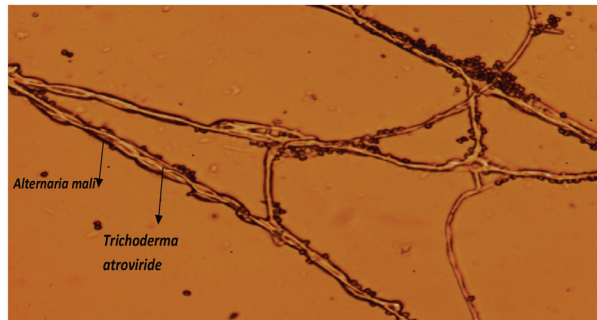


Fig. 2.

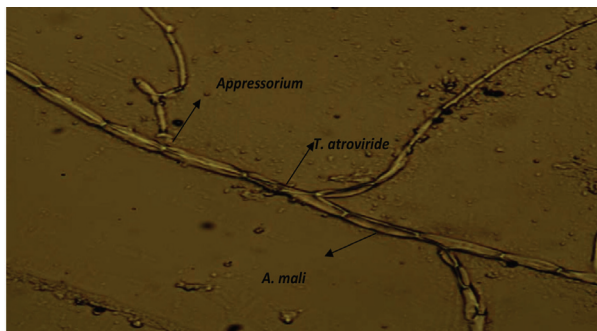


Fig. 3

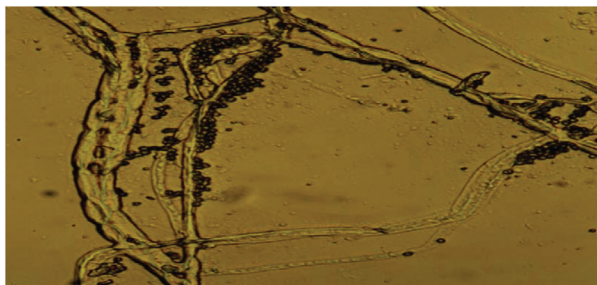


Fig. 4

## DISCUSSION

Antagonism of *Trichoderma* species to a variety of diseases has previously been described, and it varies across *Trichoderma* species and even between strains of the same species (Amin *et al.*, 2010; Dubey *et al.*, 2011; Qualhato *et al.*, 2013; Qualhato *et al.*,

2013). *Trichoderma atroviride* strain AV1 was effectively antagonizes the pathogen *Alternaria mali*. *Trichoderma atroviride* is a parasitic fungus that evolved in close proximity to *A. mali* and occasionally enters it with appressoria-like structures. *Trichoderma atroviride* hyphae have also been detected coiling frequently around *A. mali* for easy acquisition of nutrients. Similar relation was demonstrated in a study by Donayre and Dalisay (2016). Mycoparasite hyphae grow along with the hyphae of *A. mali*, wrapped the mycelium and sporulate densely. Pathogen cannot thrive in crowded branches with dense spores of biocontrol agent, restricting their growth. Similar results were also observed by Cao *et al.*, 2009.

## CONCLUSION

In a dual culture, the interaction of *Trichoderma atroviride* strain TV3 with *Alternaria mali* was investigated. The biocontrol agent coils its appressorium around the hyphae and inserts it into the pathogen. It develops alongside the pathogen's mycelium and sporulates tightly around the pathogen's mycelium. It's likely that the biocontrol agent releases signalling molecules that cause it to coil around the pathogen. Such chemical signals must be investigated. It's also important to figure out which enzymes are secreted during mycoparasitism and which genes are involved for their production. Future research on how to acquire a particular figure of Biocontrol agents from epiphytic fungi to overcome problems connected with the use of epiphytic fungi in plant disease suppression.

## REFERENCES

- Amin, F., Razdan, V.K., Mohid, F.A., Bhat, K.A. and Banday, S. 2010. Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *J Phytol.* 10:38-41.
- Bhat, K.A. 2017. A New Agar Plate Assisted Slide Culture Technique to Study Mycoparasitism of *Trichoderma sp.* o on *Rhizoctonia solani* and *Fusarium oxysporium*. *Int. J. Curr. Microbiol. App. Sci.* 6(8): 3176-3180.
- Benitez, T., Rincon, A.M., Limon, M.C. and Codon, A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. *Intern Microbiol.* 7:249-260.
- Cao, R., Liu, X., Gao, K., Mendgen, K., Kang, Z., Gao, J., Dai, Y. and Wang, X. 2009. Mycoparasitism of endophytic fungi isolated from reed on soilborne

- phytopathogenic fungi and production of cell wall-degrading enzymes *in vitro*. *Curr Microbiol.* 59: 584-592. <https://doi.org/10.1007/s00284-009-9477-9>
- Chet, I., Harman, G.E. and Baker, R. 1981. *Trichoderma hamatum*: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb Ecol.* 7: 29-38.
- Donayre, D.K.M. and Dalisay, T.U. 2016. Identities, characteristics, and assemblages of dematiaceous-endophytic fungi isolated from tissues of barnyard grass weed. *Phil J Sci.* 145:153-164
- Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V. and Kubicek, C.P. 2011. *Trichoderma*: the genomics of opportunistic success. *Nat Rev Microbiol.* 9: 749-759.
- Dubey, S.C., Bhavani, R. and Singh, B. 2011. Integration of soil application and seed treatment formulations of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani*. *Pest Manag.* 67:1163-1168.
- Elad, Y., Chet, L., Boyle, P. and Henis, Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* Scanning electron microscopy and fluorescence microscopy. *Phytopathology.* 73: 85-88
- Elad, Y., Barak, R. and Chet, L. 1984. Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*. *Soil Biol Biochem.* 16: 381-386.
- Haran, S., Schickler, H. and Chet, L. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology.* 142: 2321-2331.
- Qualhato, T.F., Lopes, F.A., Steindorff, A.S., Brandaõ, R.S., Jesuino, R.S. and Ulhoa, C.J. 2013. Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production. *Biotechnol Lett.* 35: 1461-1468
- Shahzad A., Bhat, G. N, and Mir, N. A. 2002. *Alternaria mali*-A new pathogen of apple in Kashmir. *SKUAST Journal of Research.* 4:96-98.
- Sharma, J.N. 2000. Marssonina Blotch: A new disease of apple and its control. *Indian Journal of Plant Protection.* 28:100-1.
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