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Determination of Interaction between *Trichoderma asperellum* and *Fusarium oxysporum* sp. by Digital Light Microscopy and Confocal Microscopy

Preeti Sonkar*

Mahatma Gandhi Chitrakoot Gramoday Vishwavidyalaya, Chitrakoot, Mandhya Pradesh, India

Abstract

This present works were carried out on interaction between *Trichoderma asperellum* against *Fusarium oxysporum* sp. This experiment was monitored by digital light microscope and confocal microscope. Results were observed coiling structure of *Trichoderma asperellum*, attachment on cell wall of *Fusarium oxysporum* sp. and disintegration of cell wall by conidia and bioactive compound. *Trichoderma asperellum* hyphae was around of *Fusarium oxysporum* sp. Conclusively, different type of interactions were revealed during this experiment i.e. coiling structure, mycoparasitic activity by conidia, mycoparasitic activity by bioactive compound, mycoparasitic activity by attachment, around of pathogen.

Keywords: Interaction; Antagonistic behavior; Mycoparasitic activity

Introduction

Genus *Trichoderma* prevailed in soil as saprophytic fungi which are culturable and BCAs (Biocontrol agents) at world- wide [1-3]. Importantly, Antagonistic behavior of *Trichoderma* strain is applied in sustainable development of agriculture and commercialized with ecofriendly practice. Therefore, during Antagonistic behaviors are fallowed different mode of interaction as well as Sharma (2011) was classified sequence of interaction event as such two types one is direct and second is indirect [4,5]. Various researchers are classified mechanism of *Trichoderma* spp. in controlling the plant diseases as direct and indirect antagonism. In direct type, strain of *Trichoderma* played significant role and indirect type strain of *Trichoderma* secreted a kind of metabolite and enzyme which is killed plant pathogen i.e. *Aspergillum*, *Rhizoctonia*, *Fusarium* sp. as such provided defence to host and improve health with quality in plant [4,6,7].

Further present work was undertaken as determination of interaction between *Trichoderma* strain-*Fusarium oxysporum* sp. by digital light microscope and confocal microscope. Morever, we focused to find way of interaction in dual culture where are characterized to antagonistic behavior of *Trichoderma* strain against *Fusarium oxysporum* strain on PDA Plate as well as we founded kinds of interaction during this experiment [1,4,7]. Obviously this finding should be a weapon to understand of Nature of *Trichoderma* against *Fusarium oxysporum* sp.

Materials and Method

Further experiment, materials were used from dual culture plate (Media was on potato dextrose agar) near about14 days old. *Trichoderma* strains were isolated from soil (on RBA media) and identified according to Grondona et al., method [8]. Species was confirmed from ITCC, New Delhi and NFCC, Agarkar research institute, Pune (Maharashtra), India. Pathogen *Fusarium oxysporum* sp. was collected from Chandra Shekhar Azad University of Agriculture and Technology (U.P.), India.

Studies of interaction for digital light microscope

During study of interaction of *Trichoderma asperellum* with *Fusarium oxysporum* sp., sample was placed on slide and stain by lectophenol cotton blue, observed by Digital light microscope as Siameto and Bhat observed at 100X [7,9].

Studies of interaction for confocal microscope

Here, mycelia were plug to interact part, placed on slide and stain by different dye i.e. red dye, orange dye, acridine dye. Thus hyphae were visualized using a zeiss CLSM710 Confocal Lasser Scanning Microscope (Gottingen, Germany). The image were acquired by excitation at 488nm and emission with a long pass 506nm filter according to Lahlali, Hijri and Lu Z [10,11].

Results and Discussion

Further experiment was examined to interaction between *Trichoderma asperellum* with *Fusarium oxysporum* strain by digital light microscope (100X) and confocal microscope as well as we revealed vary type interaction which is mention in Figure 1:

During studied of interaction, we differentiated to *Trichoderma asperellum* strain and *Fusarium oxysporum* strain from each other by digital light microscope at 100X in Figure 1. Strain of *Trichoderma asperellum* was thin, long, without septets and consisted conidia. Conidia were yellow with round in shape according figure. Other hand strain of *Fusarium oxysporum* was septet, spores i.e. macroconidia, chitinous cell wall, ogonium condition, and was observed based on digital light microscope micrography.

Trichoderma asperellum strain was wrapped around hyphae of *Fusarium oxysporum* as such spiral which was presented in Figure 2. Therefore, this type interaction called as coiling structure and represented mycoparasitic behavior of *Trichoderma asperellum* strain against *Fusarium oxysporum*. Siameto EN was observed in *Rhizoctonia solani*. Lu Z revealed parasitic behavior in coiling form [7,11].

***Corresponding author:** Preeti Sonkar, Mahatma Gandhi Chitrakoot Gramoday Vishwavidyalaya, Chitrakoot, Mandhya Pradesh, India, E-mail: Preetisonkar1@gmail.com

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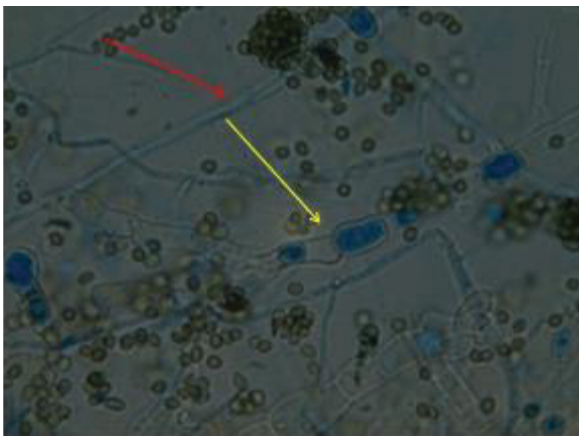

Figure 1: Included both structure i.e. *Trichoderma asperellum* and *Fusarium oxysporum* sp. strain, Red arrow represented *Trichoderma* strain and yellow arrow represented *Fusarium oxysporum* sp. strain. Conidia of *Trichoderma asperellum* was spread in yellow and round shape, observed at 100X.

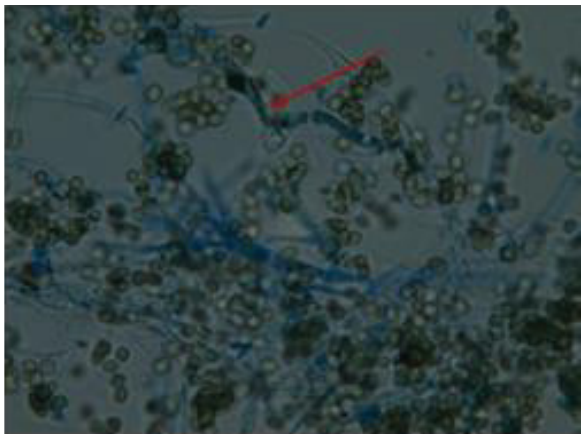

Figure 2: Included coiling structure, red arrow was shown position of coiling structure. In coiling structure, *Trichoderma* was coiled as spiral binding to *F. oxysporum* strain.

Bioactive compounds were released and attached on *Fusarium oxysporum* strain, as shown black patch which was represented by yellow arrow in Figure 3. As its degenerated to strain of *Fusarium oxysporum* and kill as well as indicated to mycoparasitic behavior of *Trichoderma asperellum* strain for hyphae of *Fusarium oxysporum* This was classified in indirect type interaction.

Conidia was played crucial role in mycoparasitic behavior as such bioactive compound in Figure 4. Conidia was attached on hyphae of *Fusarium oxysporum* and disintegrate them and thus kill it. Thus it was represented a mycoparasitic behavior. Siameto EN also observed this condition in *Rhizoctonia solani* by micrograph of light microscope [7]. de Lima FB reported that *Trichoderma* was released lysis enzymes further mycoparasitic action [12].

Trichoderma asperellum was attached on hyphae of *Fusarium oxysporum* in Figure 5 as well as penetrated to cell wall, entered and killed to fungi. We can differentiate to strain of *Trichoderma asperellum* and *Fusarium oxysporum* sp. according as figure. Thus it was a mycoparasitic activity. Bhat KA was observed mostly this mycoparasitic behavior [13].

Further studies of interaction was based on confocal microscope, here we assumed two type strain with differentiate in color as Figure 6. Red arrow was indicated to strain of green color which was represented to *Trichoderma asperellum* strain and white bright color was

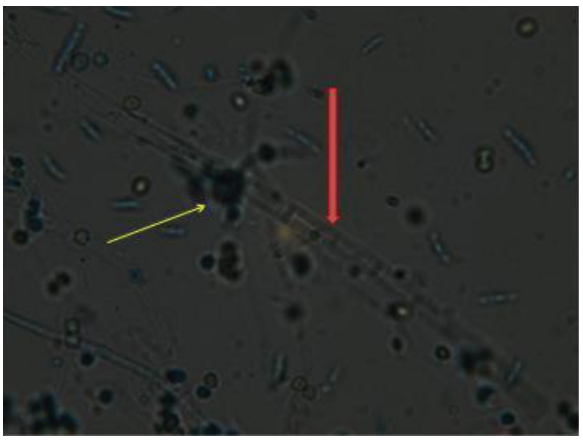

Figure 3: Shows bioactive compound action on *Fusarium oxysporum* strain. Red arrow was presented *Fusarium oxysporum* strain and yellow arrow was presented position of bioactive compound action.

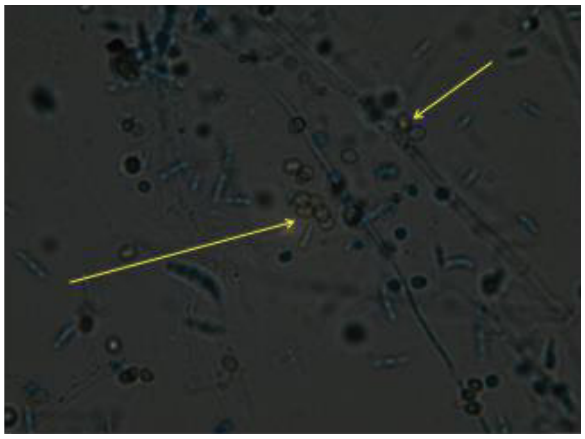

Figure 4: Shows to disintegrate of *Fusarium oxysporum* hyphae by action of conidia and some bioactive compound.

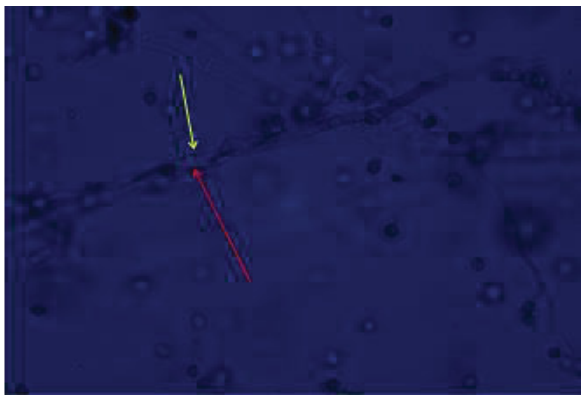

Figure 5: This pic was presented mycoparasitic activity by *Trichoderma* attach on *Fusarium oxysporum* hyphae. Yellow arrow was *Fusarium oxysporum* hyphae and red arrow was shown *Trichoderma asperellum* strain.

represented to hyphae of *Fusarium oxysporum*, shown by yellow arrow. *Trichoderma asperellum* strain was formed a net like structure around to hyphae of *Fusarium oxysporum* but was not shown mycoparasitic behavior.

Further investigation, we were revealed kind of interaction between *Trichoderma asperellum* and *Fusarium oxysporum* viz. coiling structure; bioactive compound, conidia and mycoparasitic action by attach on strain of *Fusarium oxysporum* in Figures 1-5, result was observed by digital light microscope at 100X. However, type of interaction was direct and indirect type based study of literature as well as both type of interaction was founded during studies. Khalili had been assumed disrupt the cell wall of phytopathogens due to production of volatile substances from *Trichoderma* which include acetaldehyde, isocyanide derivatives, Terpene derivatives, piperazine, polyketides, alcohols and 6pentyl-2H-pyron [14]. *Trichoderma* was released lysis enzymes during mycoparasitic action. Ranasingh reported mechanism of antagonist by competition, antibiosis, mycoparasitism, hyphal interaction and enzyme secretion [15]. Sharma (2011) observed interaction between *Trichoderma* and *Fusarium*, reported sequence of event during interaction [4].

Confocal microscope was provided 3D structure at Z-axis, hyphae of *Fusarium oxysporum* was arounded by hyphae of *Trichoderma asperellum* which was represented in Figures 6-8. Here we differentiated

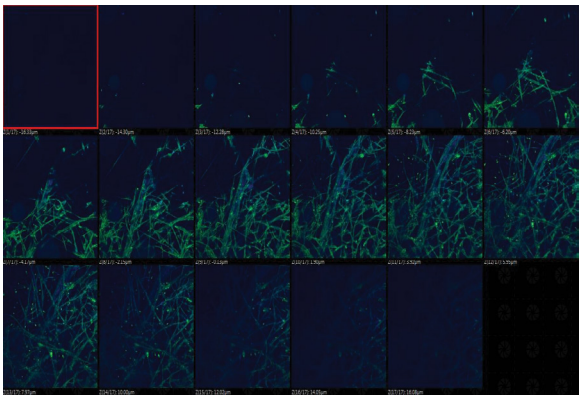

Figure 6: This picture represented interaction of *Trichoderma asperellum* with *Fusarium oxysporum* sp. at Z-axis by confocal laser scanning microscope.

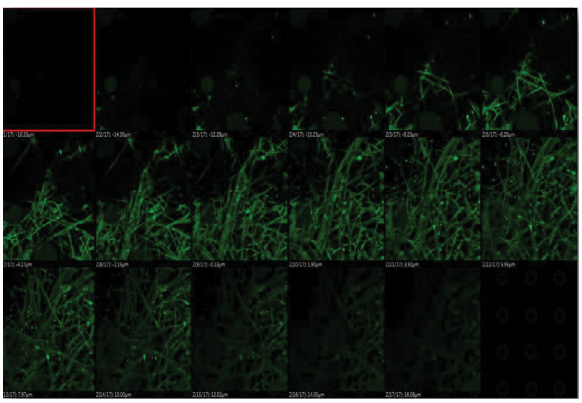

Figure 7: This picture represent interaction of *Trichoderma asperellum* with *Fusarium oxysporum* sp. in acridine dye at Z-axis by Confocal laser scanning microscope.

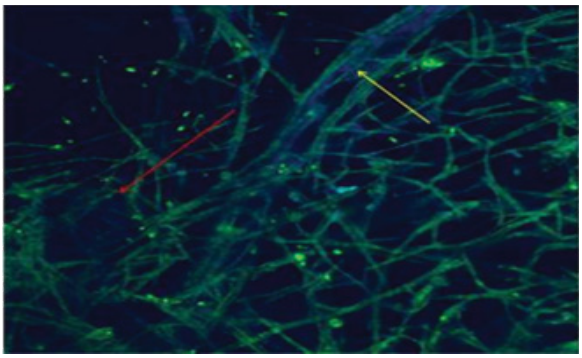

Figure 8: CLSM Image.

based on size and color as such *Trichoderma asperellum* was thin like thread in green color other hand hyphae of *Fusarium oxysporum* was thick and white bright color which was placed between thin hyphae of *Trichoderma asperellum* but was not initiated parasitic behavior in this. Czymmek (1994) reported the interaction between fungus-host by laser scanning confocal microscopy [16]. Hansen (2000) reported image of living fungal hyphae with fungal antagonist *viscosinamide* [17]. Lu Z (2004) was observed coiling and formation of specialized structures similar to hooks, appressoria, and papillae by confocal scanning laser microscope [11].

Conclusion

Trichoderma was played antagonism against *Fusarium oxysporum* by kind of way during interaction. Further study, type of interaction was presented as direct type antagonism and indirect type antagonism. However, result revealed type of interaction during antagonism as such coiling structure, attachment as mycoparasitic behavior on *Fusarium oxysporum* sp., conidia and bioactive compound which played role in disintegration of hyphae of *Fusarium oxysporum*. Hyphae of *Trichoderma* were a rounded to hyphae of *Fusarium oxysporum*. Recently, we find out to different kinds of interaction, are revealed by this present works. This study proofed type of antagonism of *Trichoderma asperellum* with *Fusarium oxysporum* sp. during interaction on potato dextrose agar media.

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References

1. Elad Y (2000) Biological control of foliar pathogens by means of Trichoderma harzianum and potential modes of action. Crop Protection 19: 709-714.
2. Freeman S, Minz D, Kolesnik I, Barbul O, Zreibil A, et al (2004) Trichoderma biocontrol of Colletotricum acutatum and Botrytis cinerea, and survival in strawberry. Eur J Plant Pathol 110: 361-370.
3. Dubey SC, Suresh M, Singh B (2007) Evaluation of Trichoderma species against Fusarium oxysporum f.sp. ciceris for integrated field against Fusarium oxysporum. African J Microb Management Chickpea wilt Biological Control 40: 118-127.
4. Sharma P (2011) Complexity of Trichoderma-Fusarium interaction and manifestation of biological control. Australian J Crop Sci 5: 1027-1038.
5. Sonkar P, Chandra R, Singh R, Kumar S (2018) Study on Management of Fusarium oxysporum through Different Mode of Action of Trichoderma spp. Inter J Curr Trends Sci Tech 8: 20192-20200.
6. Dubey SC, Tripathi A, Dureja P, Grover A (2011) Characterization of secondary metabolites and enzymes produced by Trichoderma species and their efficacy against plant pathogenic fungi. Indian J Agricul Sci 81: 455-461.

7. Siameto EN, Okoth S, Amugune NO, Chege NC (2010) Antagonism of Trichoderma farzianum isolates on soil borne plant pathogenic fungi from Embu District, Kenya. J Yeast Fungal Res 1: 47-54.

8. Grondona I, Hermosa R, Tejada M, Gomis MD, Mateos PF, et al. (1997) Physiological and biochemical characterization of Trichoderma harzianum, a biological control agent against soilborne fungal plant pathogens. Appl Environ Microbiol 63: 3189-3198.

9. Rabinal CA, Bhat S (2017) Profiling of Trichoderma k. koningiioningii IABT1252'S secondary metabolites by thin layer chromatography and their antifungal activity. The Bioscan 12: 163-168.

10. Lahlali R, Hijri M (2010) Screening, identification and evaluation of potential biocontrol fungal endophytes against Rhizoctonia solani AG3 on potato plants. FEMS Microbiol Lett 311: 152-159.

11. Lu Z, Tombolini R, Woo S, Zeilinger S, Lorito M, et al. (2004) In vivo Study of Trichoderma-pathogen-plant interactions, Using constitutive and Inducible green fluorescent protein reporter systems. Appl Environ Microbiol 70: 3073-3081.

12. de Lima FB, Félix C, Osório N, Alves A, Vitorino R, et al. (2016) Secretome analysis of Trichoderma atroviride T17 biocontrol of Guignardia citricarpa. Biological Control 99: 38-46.

13. Bhat KA (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: 3176-3180.

14. Khalili E, Javed MA, Huyop F, Rayatpanah S, Jamshidi S, et al. (2016) Evaluation of Trichoderma isolates as potential biological control agent against soybean charcoal rot disease caused by Macrophomina phaseolina. Biotechnology and Biotechnological Equipment 30: 479-488.

15. Ranasingh N, Saurabh A, Nedunchezhiyan M (2006) Use of Trichoderma in disease Management. Orissa Review 68-70.

16. Czymmek JK, Whallon HJ (1994) Confocal Microscopy in mycological research. Experimental Mycology 18: 275-293.

17. Hansen M, Thrane C, Olsson S, Sorensen J (2000) Confocal Imaging of living fungal hypae challenged with the fungal antagonist viscosinamide. Mycology 92: 216-221.