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IN VITRO ANTAGONISTIC PROPERTIES OF SELECTED *TRICHODERMA* SPECIES AGAINST TOMATO ROOT ROT CAUSING *PYTHIUM* SPECIES

Anita Patil, Aarti Laddha, Ashwin Lunge, Hariprasad Paikrao and Shubhada Mahure
Department of Biotechnology, Sant Gadge Baba, Amravati University, Amravati (MS) India - 444602

Abstract: The effective *in vitro* screening tests of three *Trichoderma* species for antagonism against *Pythium* species isolated from *Lycopersicon esculentum* –Mill root rot infection, together with its diffusible and volatile metabolites production capability, which in turn mycoparasitic abilities. This informs its selection as the most promising candidate for the biocontrol of isolated Pythium pathogens. Treatment with the antagonist in variable culture technique resulted in a remarkable reduction in terms of percent inhibition of pathogens. Four species of *Pythium* causing root in tomato plant were isolated, characterized macro and microscopically.
Trichoderma species by using diffusible and volatile metabolites against test pathogen, volatile metabolites gives the most acceptable and significant results as compared to the diffusible metabolites, which can be further exploited can biocontrol tool for seed infection.
Key words: *Trichoderma* species, Biocontrol, Pythium root rot pathogens, diffusible and volatile metabolites.

Introduction

Trichoderma spp. is the most widely studied biocontrol agents (BCAs) against plant pathogens because of their ability to reduce the population of soil borne plant pathogens (Papavizas, 1985). They are soil borne fungi and show significant activity against a wide range of plant pathogenic fungi (Elad et al., 1982). Mechanism used by *Trichoderma* spp. for control of plant pathogen include competition, mycoparasitism, antibiosis and induced resistance of the plant host (Chet, 1987; Schirmbock et al., 1994). Moreover, Harman (2000) reported that *T. viride* could colonize a root of plants and promote plant growth. These mechanisms are useful for *T. viride* to control pathogens.

Some *Trichoderma* strains are known to show acceptable promise for the control plant pathogen in soil, but having low efficiency for competition in the rhizosphere or low ability to produce cell wall lytic enzymes (Cook and Baker, 1983). Weindling and Emerson (1936) observed that they could excrete an extracellular compound which was named gliotoxin. Many antibiotics and extracellular enzymes were isolated and characterized later, and the biocontrol mechanisms became clearer (Haran et al., 1996; Zhihe et al., 1998).

Pythium root rot is a familiar crop disease caused by a genus of organisms called *Pythium,* which are commonly called water moulds. *Pythium* damping off is a very common problem in fields and greenhouses, where the organism kills newly emerged seedlings. This disease complex usually involves other pathogens such as *Phytophthora* and *Rhizoctonia*. Pre - and post-emergence damping-off disease caused by *Pythium* spp. in vegetable crops which are economically very important worldwide (Whipps and Lumsden, 1991). Rapid germination of sporangia of *Pythium* in 1.5–2.5 h after exposure to exudates or volatiles from seeds or roots (Osburn et al., 1989) followed by immediate infection makes management of the pathogen very difficult (Whipps and Lumsden, 1991). Many *Pythium* species, along with their close relatives, *Phytophthora* species are plant pathogens of economic importance in agriculture. *Pythium* spp. tends to be very generalistic and unspecific in their host range, which causes extensive and devastating root rot and is often very difficult to prevent or control. (Jarvis et al., 1992).

Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity and fungicide residues are serious problems leading to environmental pollution and human health hazards. In this context, the great task now facing scientists is to develop, one such alternative, which has been proposed for biological control of several plant pathogens, involves the introduction of selected microorganisms such as *Trichoderma* spp. to the soil. However, while laboratory experiments and biological control field trials document the ability of some *Trichoderma* strains to reduce *Pythium* Inoculum in soil, a clear answer to the process by which these fungal antagonists contribute to biological control of *Pythium* spp. has not yet emerged, although mechanisms of antagonism, including mycoparasitism, antibiosis and competition have been suggested

(Benhamou et al., 1997). The present study addresses the bio control mechanisms and application of *Trichoderma* spp. with particular emphasis on biological control of *Pythium* using diffusible and volatile metabolites in variable culture techniques.

Materials and methods

Collection of *Trichoderma* strains – The available, pure and most efficient *Trichoderma* strains of *T. harzianum, T. flavofuscum* and *T. viride* were collected from Microbial Biocontrol Laboratory, Department of Biotechnology, SGB Amravati University, Amravati. They are further checked for purity and are used for experimentation.

Isolation of *Pythium* species - *Pythium* species were isolated from the soil sample of by using root trapping method with some modifications. For the isolation of plant pathogen, soil sample was collected from top of 15cm surface soil of marshy area and mixed thoroughly in a polythene bag. Samples were usually assayed within 10days after collection. *Pythium* cultures were isolated by using root trapping method with seeds of cucumber as trapping substrate. About 10, seeds embedded in each 20-50gm soil sample in a 9cm petri dish incubated at 26°C for 1 day or 10 °C for 5-10 days respectively. Seeds were removed from soil, washed under running tap water for 1 hr, air dried and placed on water agar (2 seeds per plate). After further incubation at 26 °C for 1-3 days, pure isolates of *Pythium* species grown out of cucumber seeds on water agar and can be obtained by single hyphal tipping method. As the *Pythium* species usually grow more rapidly than other fungi, hyphal tips can be transferred to another medium after 2-3 days. Similarly, for further purification of isolated *Pythium*, a cornmeal and potato-carrot agar medium containing an antibiotic substance to suppress the development of bacteria and the growth of other fungi was used.

Characterization of *Pythium* species –The *Pythium* isolates were characterized at the genus level based on their cultural characteristics such as growth rate, sporangia color and sporulation rate, etc. Further species-level identification and characterization were carried out by using multiple identification key such as oogonia wall, antheridia position, swollen hyphae and sporangia size, etc.

Key of identification:

1) Oogonial wall with numerous spines or blunt projections, oogonial wall smooth or with few irregular projections.
2) Antheridia hypogynous or paragynous, antheridia monoclinous or diclinous
3) Size of oogonia
4) Sporangia elongated, slightly swollen at tip, sporangia filamentous

Biocontrol of Pythium: Diffusible metabolites - Antifungal activity of diffusible metabolites secreted by used *Trichoderma* species was carried out by using various cultural techniques such as dual culture and pathogen at centre culture technique.

i) Dual culture technique: About 5-day old culture, mycelial disc (5mm) from a *Trichoderma* and test pathogens were placed on the plate opposite to each other equidistant from the periphery and were incubated at 25°C. After 6 days of the incubation period, radial growth of pathogen was recorded and percentage inhibition calculated in relation with control (Hajieghrari *et al.,* 2008).

$$L= (C-T)/C*100;$$

L = inhibition of radial mycelial growth; C= radial growth measurement of pathogen in control; T = radial growth measurement of pathogen in the presence of antagonists.

ii) Pathogen at the centre: In this type of culture technique, disc of pathogen from the periphery of a colony was placed ascetically in the centre of the agar plate. Four discs of each antagonistic *Trichoderma* species were placed at the distance 35mm away from pathogen disc. The plates were incubated at 25^0C for 6 days and radial growth of pathogen along with control was measured. Percentages of growth inhibition were calculated in relation to the growth of control (Henis et al., 1979).

b) **Volatile metabolites** - Effect of volatile metabolites produced by *Trichoderma* species against test pathogens were evaluated by the method of Dennis and Webster (1971b). The mycelia disc (5mm) of *Trichoderma* species as well as test pathogens were centrally placed on separate RBA plates and incubated for 25^0 C for 4 days. After the completion of an incubation period, lid off all plates was placed with each other's bottom (sealed) so as test pathogen was directly exposed to the antagonistic environment created by *Trichoderma*

species. Radial growth of pathogens was recorded after 4 days of incubation and percentage inhibition was calculated in relation to control by the above mentioned formula.

Results and Discussion

Purification and characterization of *Pythium:* After the isolation of *Pythium*, it was further purified by subculturing it on fresh 2% PDA agar plates. The *Pythium* isolates were isolated successfully from the water logging soil, cucumber seeds with the help of root trapping method on water agar plates.

The isolated culture by root trapping method contains mixed culture. Therefore, it was repeatedly subcultured on 2% potato dextrose agar until pure culture of *Pythium* was isolated. The uncontaminated culture of *Pythium* was maintained on 2% potato dextrose agar. Further the macroscopic and microscopic study was done, to characterize the *Pythium* isolates. Growth characteristics of isolated *Pythium* isolate, and *Trichoderma* species was studied on Rose Bangal Agar media, as shown in Table- 1 and shown Fig 1 a and b respectively.

Table 1: Growth (in mm) after 5 days of *Trichoderma* and *Pythium* isolates.

Pythium isolates	Diameter (mm)	*Trichoderma* species	Diameter (mm)
B	90	*T. harzianum*	90
C	65	*T. flavofuscum*	60
D	90	*T. viride*	60
F	90	----	----

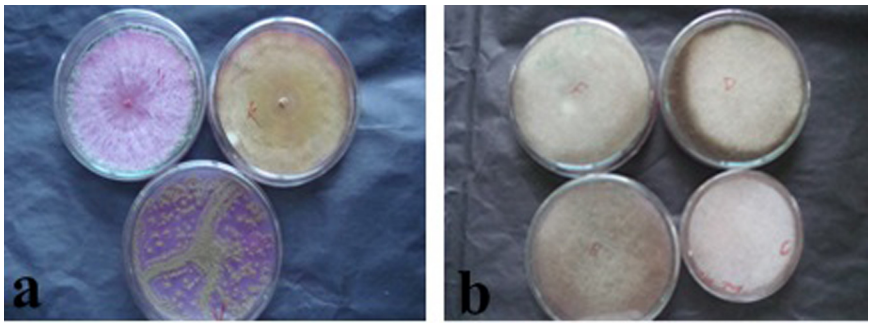

Fig. 1.a. Pure culture of *T. harzianum, T. flavofuscum, T. viride* and b. Morphological characteristic of pure cultures of isolated *Pythium* sp.

The microscopic characterization and for species-level identification, slides of isolated cultures were prepared and stained with lactophenol cotton blue. The slides were observed under 40X and 100X light microscope. Their microscopic characteristics are noted and were presented in Table 2 and shown in Fig 2, i- (culture B-*Pythium aphanidermatum*), ii- (culture C-*Pythium vexans*), iii (culture D-*Pythium ultimum*) and iv- (culture F-*Pythium viniferum*) respectively.

Table 2 – Macroscopic and microscopic characteristics of isolated *Pythium* species

Unknown *Pythium* isolates	Macroscopic characteristics	Microscopic characteristic
B	On PDA it shows fast dense hairy growth; On CMA and PCA slow and less dense hairy growth. It shows brown sporangium with white filament. Spores starts appearing within 2 days.	Sporangia consisting of a terminal complex of swollen hyphal branches of varying length, broadly sac shaped antheridia, 24-29 µm sporangia.
C	On PDA, CMA and PCA it shows slow less dense hairy growth; it shows brown sporangium with white filament. Spores starts appearing after 2 days.	Proliferating sporangium, cylindrical intercalary oogonium, Swollen hyphae, terminal and intercalary oogonium, 2 oogonium fusing, sporangium 25µm
D	On PDA it shows fast dense hairy growth; On CMA and PCA slow and less dense hairy growth. *Pythium* shows brown sporangium white filament. Spores starts appearing within 2 days.	Circular pyriform oogonium, thick walled intercalary oospore, 3 oogonium in chains, pyriform oospore.
F	On PDA, CMA and PCA it shows slow and less dense hairy growth; it shows brown sporangium with white filament. Spores starts appearing within 2 days.	oospore with papillae, swollen hyphae, double oospores in oogonium.

The culture B was found to be *Pythium aphanidermatum* by the following characteristics- The culture was without a special pattern on all the three media. Sporangia consisting of a terminal complex of swollen hyphal branches of varying length; oogonia terminal, globus smooth with 24-29µm diameter. Antheridia terminal and intercalary of broadly sac shaped.

The culture C was found to be *Pythium vexans* by the following characteristics- The culture was without a special pattern on all the three media. Sporangium was found to be pyriform. Antheridium was found to be monoclinous. Oospores was aplerotic.

The culture D was found to be *Pythium ultimum* by the following characteristics- Colonies on corn meal agar forming cotton aerial mycelium. Hyphae upto 11 µm wide. A thick oospore wall was found. Oospore single, aplerotic, globus with a diameter of about 2µm thick.

The culture F was found to be *Pythium viniferum* by the following characteristics- Smooth walled oospore. 2 oospores were found in the oogonium. Oospore with pappilae, Oogonium with monocinous and diclinous antheridia, Monoclinous and hypogynous antheridia was found. Cylindrical sporangium was found.

Biocontrol of *Pythium* by using various metabolites of *Trichoderma*:

In dual culture technique, while studying the interaction between *Trichoderma* and isolated Pythium species the range of inhibition was observed ranging from 27.78-69.23%.

In case of the study of diffusible metabolites secreted by *T. harzianum*, percentage inhibition ranges from 38.89 - 56.92%. Highest percentage inhibition was observed against *P. vexans* (C) i.e 56.92% followed by *P. aphanidermatum* (B)-55.56% and the least inhibition was observed against *P. ultimum* (D) i.e 38.89% respectively.

In study with *T. flavofuscum*, percentage inhibition ranges from 27.78% - 57.78%. Highest percentage inhibition was observed against *P. viniferum* (F)-57.78% followed by *P. aphanidermatum* (B) -55.56%. While the least percentage inhibition was observed against *P. ultimum* (D)- 27.78%. The diffusible metabolites secreted by *T. viride*, showed percentage inhibition ranges from 38.89–69.23%. Highest percentage inhibition was observed against *P. vexans* (C)-69.23% followed by *P. viniferum* (F)- 66.69%. While the least percentage inhibition was observed against *P. ultimum* (D) -38.89%. The results were shown in Table -3.

Table 3: Effect of diffusable metabolites (% inhibition) of *Trichoderma* species on *Pythium* isolates in dual culture

Pythium isolates	Growth inhibition by *T. harzianum*	Growth inhibition by *T. flavofuscum*	Growth inhibition by *T. viride*
P. aphanidermatum (B)	55.56%	55.56%	44.44%
P. vexans (C)	56.92%	32.31%	69.23%
*P. ultimum (*D)	38.89%	27.78%	38.89%
P. viniferum (F)	50%	57.78%	66.69%

It has been observed that as the concentration of Inoculum of biocontrol agent increases, increase in % inhibition can be observed. Therefore during the pathogen at centre technique, *Pythium* (pathogen) disc was placed at centre while four *Trichoderma* (biocontrol) disc were placed at periphery and more percentage inhibition was observed as compared to the results of dual culture technique.

In study of interaction between *Trichoderma* and isolated Pythium species in pathogen at centre technique, range of inhibition was observed ranging from 56.92 -86.67%. In case of non volatile metabolites secreted by *Trichoderma* against pathogen at periphery, *T. harzianum* showed highest inhibition against *P. viniferum* (F) - 84.44%) and least against *P. vexans* (C) - 56.92%. While *T. flavofuscum* showed highest inhibition against *P. viniferum* (F) -77.78% and least against *P. vexans* (C) -69.23%. The significant inhibition was observed in case of *T. viride* against *P. viniferum* (F) -86.67% and least against *P. vexans* (C) -69.23%. The results were shown in Table no. 4.

Table 4: Effect of diffusable metabolites of *Trichoderma* species (% inhibition) on *Pythium* isolates in pathogen at centre technique

Pythium isolates	Growth inhibitio by *T. harzianum*	Growth inhibition by *T. flavofuscum*	Growth inhibition by *T. viride*
P. aphanidermatum (B)	66.67%	76.67%	83.33%
P. vexans (C)	56.92%	69.23%	69.23%
*P. ultinum (*D)	66.67%	71.11%	73.33%
P. viniferum (F)	84.44%	77.78%	86.67%

Out of all the studied *Trichoderma* species against test pathogen for diffusible metabolites *T. viride* followed by *T. harzianum* showed the acceptable growth inhibition with a significant results. As the growth inhibition characteristics mainly depend upon the growth characteristics of bio-control agent, *T. viride* was the most fast growing bio control agent along with *T. harzianum*. Which can strongly overgrew over the colony of test pathogen, penetrate into their mycelia and inhibit the further proliferation. *Trichoderma* species are known to produce a number of antibiotics, such as trichodermin, trichodermol, harzianum A and harzianolide (Dennis and Webster, 1971Simon and Sivasithamparam, 1988; Schirmbock, 1994).

Effect of volatile metabolites: Due to effect of volatile metabolites secreted by *Trichoderma* species against test pathogen, the range of inhibition was observed from 38.46 - 87.78%. The volatile metabolites secreted by *T. harzianum*, showed inhibition ranging from 74.44 – 87.78 %. Highest percentage inhibition was observed against *P. aphanidermatum* (B)-87.78% followed by *P. vexans* (C)-80.00 %. While the least percentage inhibition was observed against *P. viniferum* (F)-74.44%. In case of the study of volatile metabolites secreted by *T. flavofuscum*, percentage inhibition ranges from 72.22 - 80%. Highest percentage inhibition was observed against *P. aphanidermatum* (B) - 80.00% followed by *P. ultimum (*D) -75.56%. While the least percentage inhibition was observed against *P. viniferum* (F)-72.22%.

In case of the study of volatile metabolites secreted by *T. viride*, percentage inhibition ranges from 38.46 – 81.11%. Highest percentage inhibition was observed against *P. aphanidermatum* (B) -81.11%, followed by *P. ultimum (*D) -55.56%. While the least percentage inhibition was observed against *P. vexans* (C)- 38.46%. The results were shown in Table no. 5.

Table 5: Effect of volatile metabolites of *Trichoderma species* on *Pythium* isolates by volatile metabolites (% inhibition)

Pythium isolates	Growth inhibition by *T. harzianum*	Growth inhibition by *T. flavofuscum*	Growth inhibition by *T. viride*
P. aphanidermatum (B)	87.78%	80.00%	81.11%
P. vexans (C)	80.00%	75.38%	38.46%
*P. ultimum (*D)	75.56%	75.56%	55.56%

P. viniferum (F)	74.44%	72.22%	50.00%

Out of all the studied antagonistic activity of selected *Trichoderma* species by using diffusible and volatile metabolites against test pathogen, volatile metabolites gives the most acceptable and significant results as compared to the diffusible metabolites. Volatile metabolites of *T. harzianum* followed by *T. flavofuscum* shows the acceptable growth inhibition with significant results against all the isolated *Pythium* species.

Species of *Trichoderma* have been demonstrated *in vitro* to act against fungal plant pathogens by producing diffusible and volatile antibiotics. Claydon et al. (1987) reported antifungal properties of volatile metabolites (alkyl pyrones) produced by *T. harzianum*. Similarly, Rathore et al. (1992) reported volatile activity of *T. viride* against *F. solani* which vacuolated most hyphae of pathogen and that the hyphae of pathogens were comparatively thin as compared to control. Workers like Michrina et al. (1995) and Pandey and Upadhyay (1997) have also reported the effectiveness of diffusible volatile metabolites of *T. harzianum* and *T. viride in vitro.* Dal Bello et al. (1997) studied the volatile compounds produced by *Trichoderma hamatum* against various phytopathogenic fungi and suggested the inhibitory volatiles of *Trichoderma hamatum* as one of the possible mechanism of biological control.

In conclusion, four *Pythium* species were isolated from infected soil causing root rot in tomato. Application of *Trichoderma* species in variable culture based on diffusible and volatile metabolites in vitro reduced the growth of *Pythium*. Prior treatment of tomato with *Trichoderma* spp in single or in combination triggered the plant-mediated defense mechanism in response to infection by *Pythium.* Thus, it has been found that volatile metabolites of selected three species of *Trichoderma* showed broad-spectrum inhibition of *Pythium* as compared to diffusible metabolites. Further research in the characterization and usability of volatile metabolites, in which biotechnology may offer a promising solution.

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References

1. Benhamou N, Rey P, Chérif M, Hockenhull J, Tirilly Y (1997) Treatment with the mycoparasite, Pythium oligandrum, triggers the induction of defense-related reactions in tomato roots upon challenge with Fusarium oxysporum f. sp. radicis-lycopersici. Phytopathology 87:108–122
2. Chet I, Baker R (1981). Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. *Phytopathol.,* 71: 280-290.
3. Chet, I. (1987). *Trichoderma*: Application, mode of action, and potential as a biocontrol agent of soil-borne plant pathogenic fungi. in: *Innovative Approaches to Plant Disease Control.* 137-160.
4. Claydon, N., M. Allan., J.R. Hanson and A.G. Avent. (1987). Antifungal alkyl *pyrenes of Trichoderma harzianum. Trans. British Mycol. Soc.,* 88:503-513.
5. Cook, R.J. and Baker, K.F. (1983). The Nature and Practice of Biological Control of plant pathogen. *American phytological society*, 539.
6. Dal Bello GM, Mónaco CI, Cháves AR. (1997). Study of the effect of volatile metabolites of *Trichoderma hamatum* on the growth of phytopathogenic soil borne fungi. *Rev Iberoam Micol.* 14(3):131-4.
7. Dennis C, Webster J (1971b). Antagonistic properties of species groups of *Trichoderma* Production of volatile antibiotics.*Trans. Br. Mycol. Soc*. 57: 41-48.
8. Dennis C, Webster J (1971). Antagonism properties of species groups of *Trichoderma*, III. Hyphal interaction. *Transactions British Mycological Society*, 57:363-369.
9. Elad, Y., Chet, I. and Henis, Y. (1982). Degradation of plant pathogenic fungi by *Trichoderma harzianum. Canadian J. Microbiol*, 28: 719-725.

10. Hajieghrari B, Torabi-Giglou M, Mohammadi MR, Davari M (2008). Biological potential of some Iranian Trichoderma isolates in control of soil borne plant pathogenic fungi. *Afr. J. Biotechnol*. 7(8): 967-972.
11. Haran S, Schinckler H, Chet I (1996). Molecular mechanism of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology* 142: 2312-2331.
12. Harman, G.E.(2000). Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis*. 84 : 377-393.
13. Henis, Y.,Ghajfar, A., Baker, R. (1979). Factors affecting suppressiveness to rhixoctonia rolani in soil. *Phytopathology*.69 : 1164
14. Jarvis, W. R. (1992). Managing diseases in green house crops. Saint Paul, Minnesota: *APS Press*. 122-7.
15. Michrina, J., A. Michalikova., T. Rohacik and R. Kulichova. (1995). Antibiosis as a possible mechanisim of antagonistic action *Trichoderma harzianum* against *Fusarium culmorum, Ochrana Rostlin,* 31:177-184 .
16. Osburn RM, Schroth MN, Hancock JG and Hendson M (1989) Dynamics of sugarbeet colonization by *Pythium ultimum* and *Pseudomonas* species: Effects on seed rot and damping-off. Phytopathology 79: 709–716.
17. Pandey, K.K. and J.P. Uapadhyay. (1997). Selection of potential biocontrol agents based on production of volatile and non volatile antibiotics. *Veg. Sci,* 24(2):140- 143
18. Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. *Ann. Rev. Phytppath*. 23 : 23-54
19. Rathore, V.R.S; Mathur, K; Hodha, B.C and Mathur, K (1992). Activity of volatile and non volatile substances produced by Trichoderma viride on giner rhizone rot pathogens. *Indian Phytopathology*. 45, 253-254.
20. Schirmbock, M., Lorito, M., Wang, Y. –L., Hayes, C.K., Arisan-Atac, I., Scala, F., Harman, G.E. and Kubicek, C.P.(1994).Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Appl. Environ. Microbiol*. 60: 4,364- 4,370.
21. Simon, A. and K. Sivasithamparam. 1988. The soil environment and the suppression

of saprophytic growth of *Gaeumannomyces graminis* var. *tritici. Canadian Journal of Microbiology*, 1988, 34(7): 865-870, 10.1139/m88-149

22. Weindling R, Emerson H (1936). The isolation of toxic substance from the culture filtrates of *Trichoderma. Phytopathology*., 26: 1068-1070.
23. Whipps JM and Lumsden DR (1991) Biological control of *Pythium* species. *Biocontrol Science and Technology* 1: 75–90
24. Zhihe C, Qingping W, Lihong X, Xiaoyan Z, Jumei Z (1998). Advance of biocontrol of *Trichoderma* and *Gliocladium. J. Microbiol.,* 25(5): 284-286.

Figure 2i. *P. aphanidermatum* (B) a) smooth walled globose oogonium, b) terminal broadly sac shaped antheridia, c) terminal branches of swollen hyphal branches of varying length, d) swollen hyphae

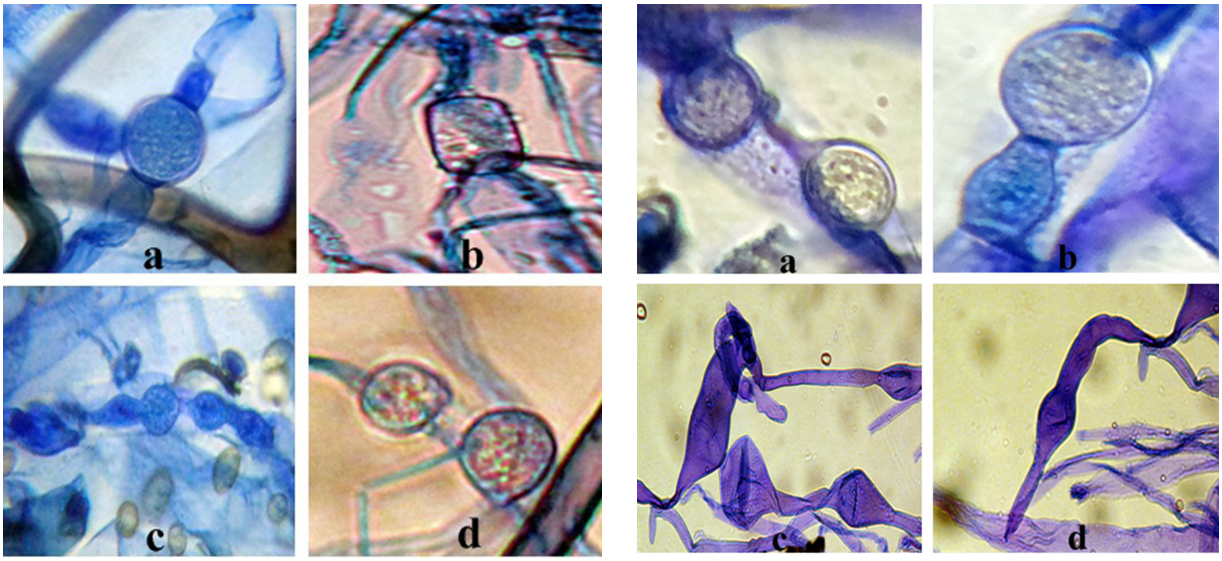


Figure 2ii. *P. vexans* (C) a) terminal monoclinous anthridia, b) pyriform sporangium, c) oogonium fusing , d) pyriform oospore

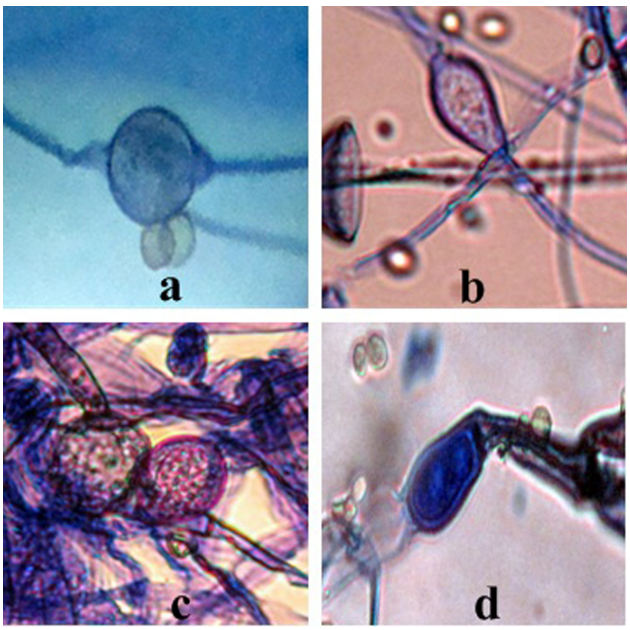
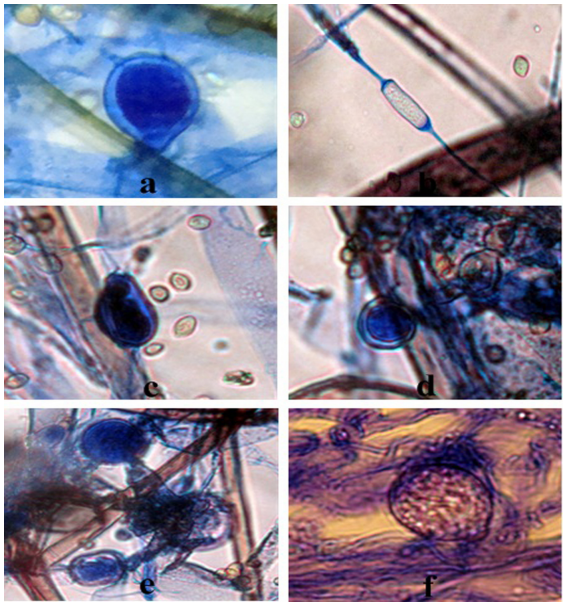


Figure 2iii *P. ultimum* (D) a) oospore with papillae, b) cylindrical sporangium, c) oogonium in chains, d) sporangium with papillae

Figure 2iv *P. viniferum* (F) a) oospore with papillae, b) cylindrical intercalary sporangium, c) double walled oospore in oogonium, d) thick walled oospore, e) sporangium with papillae, f) diclinous anthridia



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