WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Developing Sustainable Disease Management Strategies for Black Pepper: Evaluating Trichoderma spp. for Controlling Phytophthora Foot Rot.

Anna Maria Augustine[1], Prejish C[1], Jomol P J[1] , Amal Jayadev [1,] Anakha Vijayan[1] , Bernaitis L[2]

[1]Microbiologist, CML Biotech Ltd, Angamaly, Ernakulam, Kerala – 683573.

[2]Head – R & D microbiology CML Biotech Ltd, Angamaly, Ernakulam, Kerala – 683573.

Address of Correspondence Author

Dr. Bernaitis L,Head – R & D Microbiology, CML Biotech Ltd, INKID Industrial area, Angamaly south, Ernakulam Kerala- 683573. Mobile- 9488358873 Email – bernaits_87@yahoo.co.in

ABSTRACT :

Black pepper, a significant spice crop, suffers from various fungal diseases including Phytophthora foot rot. This study investigated the potential of Trichoderma spp. as a biocontrol agent against Phytophthora capsici, the causal agent of foot rot. The research involved isolating and culturing both the pathogen and Trichoderma spp. Subsequently, growth inhibition of Phytophthora by Trichoderma was assessed using dual culture plate methods. Results revealed that Trichoderma, a fast-growing fungus, significantly suppressed the growth of Phytophthora in dual culture. This inhibition is likely due to the production of diffusible substances by Trichoderma. Additionally, Trichoderma's rapid growth allows it to compete for space and resources, further limiting the pathogen's growth. This study concludes that Trichoderma spp. is a promising biocontrol agent for managing Phytophthora foot rot in black pepper, offering a sustainable alternative to chemical control methods.

Keywords: Black pepper, Phytophthora, Trichoderma, Foot rot, Biocontrol agent, Antagonist.

INTRODUCTION

Black pepper (*Piper nigrum)* is flowering vine in the family *piperaceae.* The dried fruit of the plant is used as a spice (1). Black pepper is native to Kerala, India, and extensively cultivated in the tropical regions. Black pepper is the world's most traded spice and is one of the most common spices added to cuisines around the world. Black pepper suffers considerably from various diseases; one of the major diseases is Phytophthora foot rot/quick wilt, caused by *Phytophthora capsici (2).* The leading procedures for controlling the phytophthora root rot on *piper nigrum* include chemical methods, cultural practices, and the use of fungicides. Chemical methods and fungicides are harmful to the normal flora of the environment. Fungicides are expensive and not completely effective against the pathogen (3). The use of fungicides can also lead to the development of resistant strains. The use of biological methods instead of fungicides is less expensive and more environmentally friendly. Trichoderma sp. is a fungal biocontrol agent that is used against plant disease (4). The use of Trichoderma sp. against phytophthora can be an alternative method for controlling the phytophthora root rot on black pepper. The success of a biocontrol agent depends on the ability of the antagonist to proliferate under the conditions of a given environment, so native fungal strains have a greater advantage (5). The native strains do not possess any foreign material and thus do not endanger the natural biodiversity (6).

Our study is focused on the biocontrol potential of native Trichoderma sp. against *P. capsici*, the causal agent of phytophthora root rot in black pepper (7).

MATERIALS AND METHOD

Isolation, Identification and maintenance of Pathogen

Infected Phytophthor*a* disease samples of black pepper were observed at Kunchithanny fields, Myladumpara and brought to the laboratory. The symptoms of affected plant were closely observed and recorded. For the isolation of casual organisms, affected leaves were collected. The leaves were cut in to small pieces with scalpel or blade. These where thoroughly washed with distilled water. The pathogen were isolated by surface sterilizing the affected piece in 0.1% mercuric chloride solution for 1 minutes followed by repeated washing with distilled water. The surface sterilized piece is then plated on PDA medium under aseptic conditions using laminar air flow equipment. The plates were incubated at $28°C$ for 7 days (8).

The isolated organism was identified by using LPCB (Lacto Phenol Cotton Blue) Staining method (9).

The fungal growth obtained was sub cultured and purified to get pure culture of the organism. Isolated organism was cultured and maintained in PDA medium at 25°*c* in petriplates and slants (10).

Culturing and maintenance of Antagonists

The standard isolate of Trichoderma sp. was collected from the laboratory of ICRI (INDIAN CARDAMOM RESEARCH INSTITUTE), Myladumpara ,Idukki. Trichoderma sp.was cultured and maintained in PDA plates and slants and allowed to grow at room temperature (11).

Mono Culture Method

This was carried out to measure the growth rate of the antagonism in individual cultures of Petri plates. 5mm size mycelial disc of the fungal antagonist, Trichoderma sp. was cut out from the PDA plates using a cork borer and placed at the center of individual plates. These plates were incubated at 28℃ and measured the diameter and recorded for 6 days (12).

Dual Culture Method

Dual cultures were done in Petri plates containing PDA to test the interaction between pathogens and Antagonists (13). The growth rate or diameter of fungi in the dual culture plate was measured and recorded daily. In dual culture plates also the antagonists grew faster and covered the entire area. However, growth of the pathogen was restricted and after 2 to 3 days of inoculation, no further growth was recorded. The antagonists completely covered the pathogen colony after three days and prevented the latter from further growth (14).

RESULTS AND DISCUSSION:

This study investigates the isolation, identification, and effect of fungi in black pepper, focusing on foot rot caused by *Phytophthora capsici* . Phytophthora species, a heterothallic oomycete, cause root and crown rot, aerial blight of leaves, fruit, and stem, and are spread through soil, water, root contact, movements of people, slugs, snails, and use of contaminated implements.

Isolation of Phytophthora capsici: Only one isolate of Phytophthora capsici was successfully isolated from the four infected black pepper samples collected.

Fig 1: Black pepper plant

Fig 2: Infected black pepper plant

Fig 3: Infected black pepper leaf

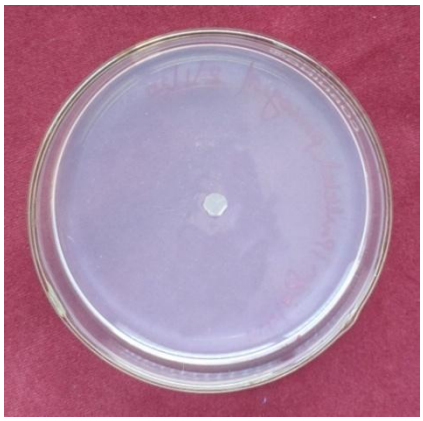
ANTAGONISTIC PROPERTY OF THE BIOAGENTS

Trichoderma spp. exhibited rapid growth, quickly colonizing the media surface and limiting the available space for Phytophthora capsici. This resulted in a restricted growth pattern of the pathogen.

FUNGAL ANTAGONISM

Observations revealed that Trichoderma spp. eventually overgrown the Phytophthora capsici colony, turning dark green due to sporulation. However, under microscopic examination, faint hyphae of the pathogen were still visible, suggesting a potential for limited survival.

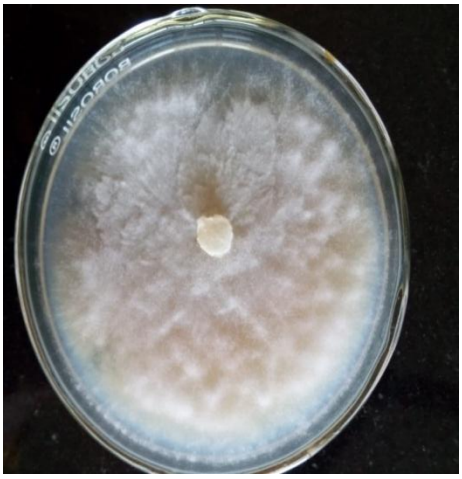
GROWTH AND CULTURAL STUDIES



Phytophthora sp. on 1st day of growth

b) Phytophthora sp. on 3th day of growth

C) Phytophthora sp. on 5th day of growth



d) Phytophthora sp. on 7th day of growth

Fig 10 : a,b,c,d – Phytophthora sp. in PDA medium

Table 1: Isolation chart

Samples Number	Sample	Pathogen Isolated	Remarks
1	Leaf	Bacteria	Contamination
2	Leaf	*Phytophthora capsici*	Required Organism
3	Leaf	No growth	No growth
4	Leaf	No growth	No growth

MICROSCOPIC STUDIES -STAINING

The isolated organism is identified as *Phytophthora capsici* by the LPCb staining technique.

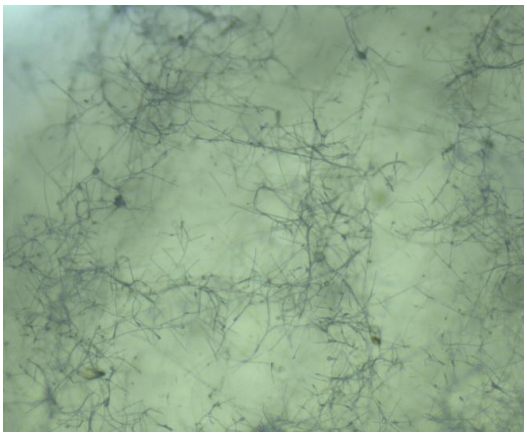


Fig 11: Mycelia of *Phytophthora capsici* (day1)

Fig.12: Mycelia with spores (Slide culture)

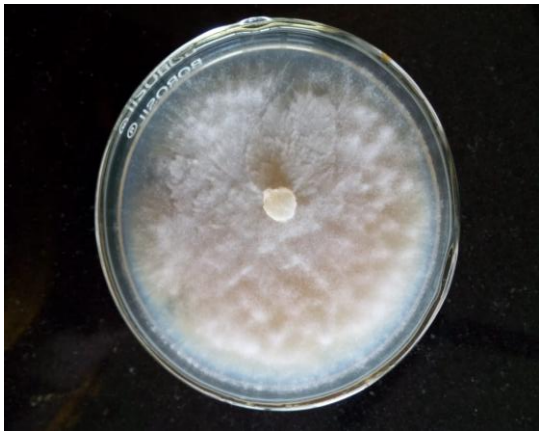


Fig.13: *Phytophthora capsici* (Control plate) Culture (Control)

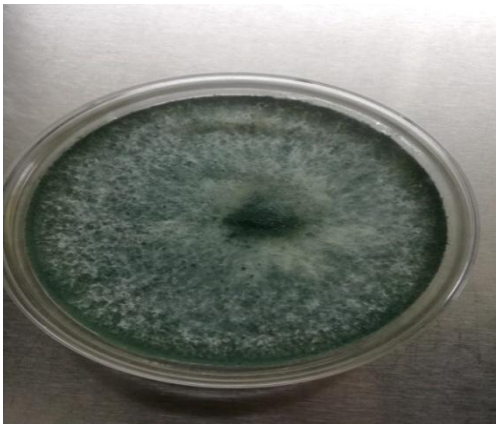


Fig.14: *Trichoderma-ICRI Stock*

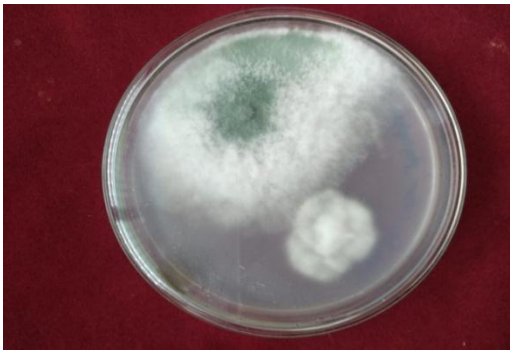


Fig.15: Dual culture (Day 2)

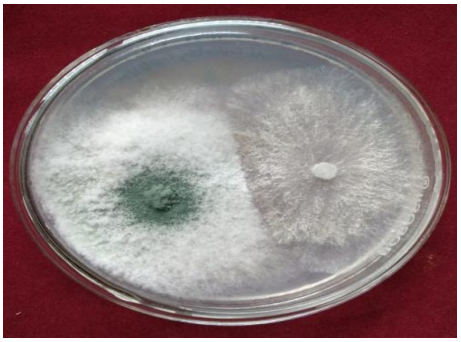


Fig.16: Dual culture (Day 5

Table. 2: Inhibition chart

Day	Growth of *Phytophthora capsici* in control (mm) (C)	Growth of *Phytophthora capsici* in dual culture (mm) R_1	Growth of *Phytophthora capsici* in dual culture (mm) R_2	Mean growth of *Phytophthora capsici* in dual culture (mm)	Growth of inhibition (%) (I)

1	7	6	6	6	14
2	15	8	8	8	46
3	25	12	12	12	52
4	32	18	15	16.5	48
5	43	20	19	19.5	54
6	46	23	21	22	52
7	46	25	23	24	91

Growth of inhibition (I) = Growth in Control (C) - Growth in Treatment (T) X 100
Growth in Control (C)

Table. 3: Growth of Trichoderma sp. in control

Day	Growth of Trichoderma sp. in control (mm)
1	10
2	23
3	30
4	35
5	42
6	49
7	50

Table. 4: Growth of *Phytophthora capsici* in control (mm)

Day	Growth of *Phytophthora capsici* in control (mm)
1	7
2	15
3	25
4	32
5	43
6	46
7	46

Fig.17: Graphical representation of *Phytophthora capsici* in pure culture

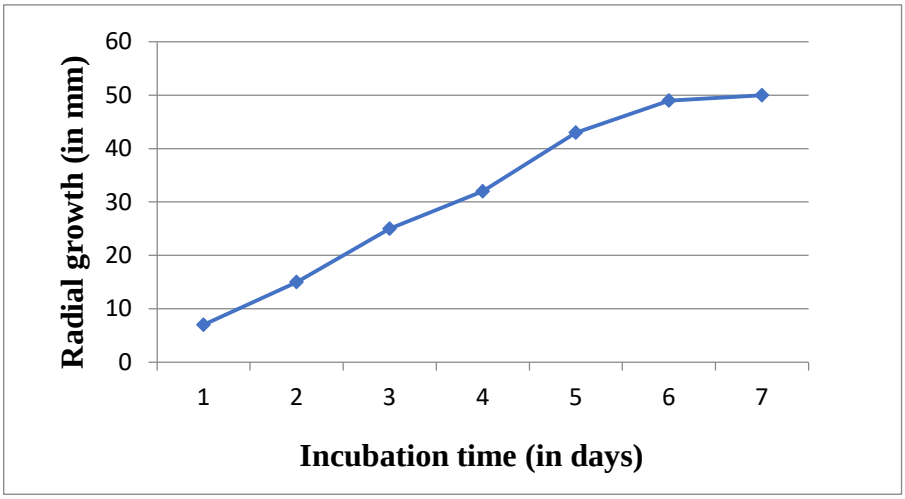
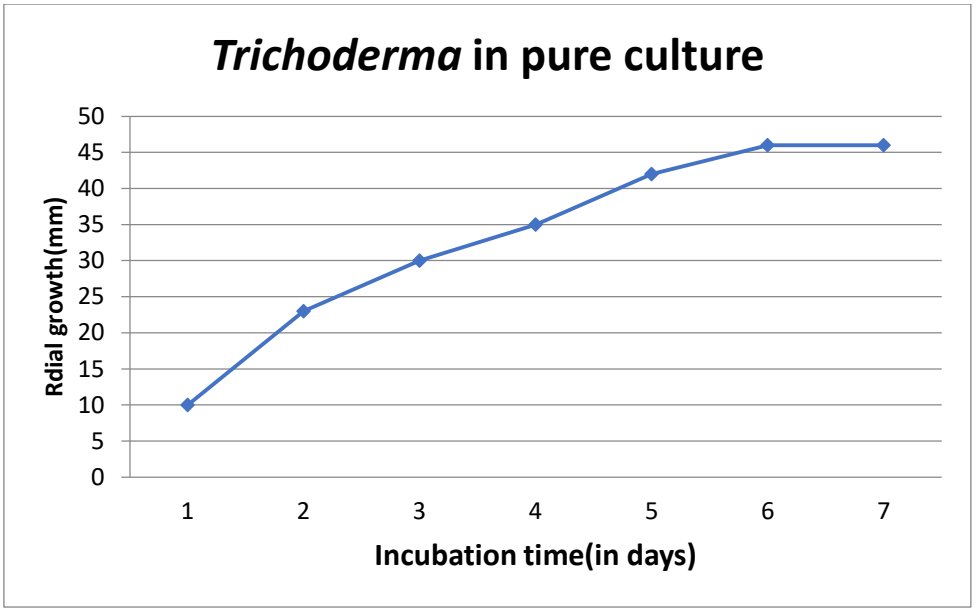
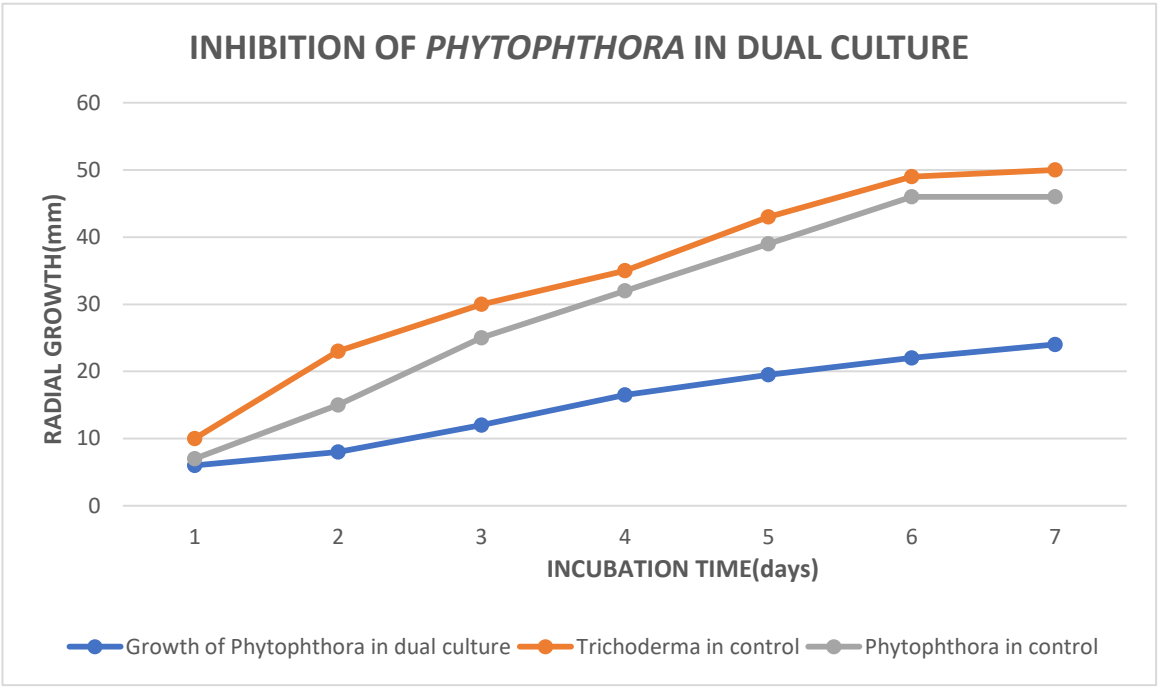


Fig.18: Graphical representation of Trichoderma sp. in pure culture



Dual Culture Assay: When Trichoderma spp. was co-cultured with Phytophthora capsici in a dual culture plate assay, the growth of Phytophthora capsici was significantly suppressed compared to the control plate where it grew alone.

Fig.19: Inhibition of *Phytophthora* in dual culture



This study successfully isolated Phytophthora capsici, the causal agent of foot rot disease, from a black pepper sample. The dual culture assay demonstrated the antagonistic potential of Trichoderma spp. against Phytophthora capsici. The observed growth suppression of the pathogen can be attributed to two possible mechanisms:

Competition: Trichoderma spp.'s rapid growth likely outcompeted Phytophthora capsici for essential nutrients and space on the culture medium. This limited resource availability can significantly hinder the pathogen's growth.

Antibiosis: The study suggests the potential role of diffusible substances produced by Trichoderma spp. These antifungal compounds might directly inhibit the growth and viability of Phytophthora capsici. Further investigation is needed to identify and characterize these potential antifungal metabolites.

The observed overgrowth by Trichoderma spp. with some remaining hyphae of Phytophthora capsici suggests a potential for partial control rather than complete eradication. Future studies could explore the optimal conditions for maximizing Trichoderma spp.'s antagonistic activity and investigate its effectiveness in a controlled greenhouse setting.

Overall, this study provides evidence that Trichoderma spp. holds promise as a biocontrol agent for managing Phytophthora foot rot disease in black pepper. This approach offers a more sustainable alternative to traditional chemical control methods, potentially reducing reliance on pesticides and promoting environmentally friendly agricultural practices.

CONCLUSION :

The present study is focused on the isolation and identification of the fungal pathogen P*hytophthora capsici* as well as the antagonistic effects of native Trichoderma sp. against the pathogen. A native Trichodermal strain showed inhibitory effects on the pathogen. Our study shows that antagonism between fungal species can be used as an alternative to the drugs used to treat plant diseases. Further study can be done on the secondary metabolites produced by the antagonist against the pathogen.

REFERENCES :

1. Aziz, N.H.; Youssef, Y.A.; El-fouly, M.Z.; Moussa, L.A. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. Bot. Bull. Acad. Sin. 1998.
2. Centers for Medicare and Medicaid, Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services. Subpart K - Quality System for Non-Waived Testing. 493; 1200-1265. *www.cms.hhs.gov/clia*.
3. "Cleaner technology for white pepper production". The Hindu Business line. 27 March 2008. Archived from the original on 9 May 2008. Retrieved 29 January 2009.
4. Hajeski, Nancy J (2016). National Geographic Complete Guide to Herbs and Spices: Remedies, Seasonings, and Ingredients to Improve Your Health and Enhance Your Life. National Geographic Books. p. 236. ISBN 9781426215889.
5. Jaramillo, M. Alejandra; Manos (2001). "Phylogeny and Patterns of Floral Diversity in the Genus Piper (Piperaceae)". American Journal of Botany. 88 (4): 706–16. doi:10.2307/2657072. JSTOR 2657072. PMID 11302858.
6. "Karvy's special Reports — Seasonal Outlook Report Pepper" (PDF). Karvy Comtrade Limited. 15 May 2008. Retrieved 29 January 2008.
7. Krishnamuthry, K. S.; Kandiannan, K.; Sibin, C.; Chempakam, B.; Ankegowda, S. J. (2011). "Trends in climate and productivity and relationship between climatic variables and productivity in black pepper (Piper nigrum)". Indian Journal of Agricultural Sciences. 81(8): 729–733. Retrieved 29 January 2018.
8. Manjunath Hegde, Bomnalli (19 October 2013). "Meet the pepper queen" (Bangalore). Deccan Herald. Retrieved 22 January2015.
9. "Pepper (noun)". Online Etymology Dictionary, Douglas Harper. 2016. Retrieved 24 September 2016.
10. "Pepper (piper spp.), Production/Crops". Food and Agriculture Organization of the United Nations: Statistical Division (FAOSTAT). 2016. Retrieved 4 December 2018.
11. "Piper nigrum". Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), United States Department of Agriculture Sen, Colleen Taylor (2004).
12. Richardson, R.C. Chile Peppers and Mycotoxin Contamination: Problems and Solutions Final Report for the Agribusiness Project. United States Agency for International Development (USAID). Available online: http:// agribusiness.org.pk/wp-content/uploads/2015/04/7.-Chili-Peppers-and-Mycotoxin-Report.pd.
13. Sen, Colleen Taylor (2004). Food Culture in India - Food culture around the world. Greenwood Publishing Group. p. 58. ISBN 9780313324871. Peppers, called the king of spices, are the dried berries of a tropical vine native to Kerala, which is India's major producer.
14. Thahira Banu, A., & Aswini, M. (2023). Role of Piper nigrum in functional food and nutraceuticals. In A. Amalraj, S. Kuttappan, K. Varma A.C., & A. Matharu (Eds.), Herbs, Spices and Their Roles in Nutraceuticals and Functional Foods (pp. 41–60). Academic Press. https://doi.org/10.1016/B978-0-323-90794-1.00019-3.