



e-ISSN: 2319-8753 | p-ISSN: 2320-6710

IJRSET

International Journal of Innovative Research in
SCIENCE | ENGINEERING | TECHNOLOGY

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 9, Issue 12, December 2020

ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA

Impact Factor: 7.512

9940 572 462

6381 907 438

ijirset@gmail.com

www.ijirset.com

Antifungal Activity of *Trichoderma* spp. Against *Helminthosporium sativum*

Shaikh Farah T¹, Sahera Nasreen²

Assistant Professor and Head, Department of Botany, B. S. Patel College, Pimpalgaon. Kale. Buldhana, India¹

Professor and Head, Department of Botany, Govt. Institute of Science, Nagpur, India²

ABSTRACT: The most destructive pathogens causing the plant diseases results into yield losses by attacking many economic crops, were the fungal pathogens which affect many countries economy. In India, due to excessive use of pesticides, socioeconomic and environmental pollution issues have been resulted, which demand the alternative method to reduce content of chemical pesticides. Biological control is an eco-friendly method employed to control the plant diseases, with the aim of developing a sustainable system in agriculture. In the present study three species of *Trichoderma* was used, *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma koningii* were evaluated for their antagonistic activity against *Helminthosporium sativum* which is the most devastating fungi causing number of diseases in vegetables. Three biocontrol agents were tested against *H. sativum* by different methods. The Non Volatile Metabolites released from *T. koningii* were tested and found effective and showed 84.24% inhibition of mycelial growth over control. Cell free metabolites of *T. viride*, *T. harzianum* inhibited the growth of pathogen in vitro and appeared to be fungicidal in its activity.

KEYWORDS: *Trichoderma*, *Helminthosporium sativum*, Non Volatile Metabolites, Fungicidal Activity.

I. INTRODUCTION

In South Asia, spot blotch and tan spot (caused by *Pyrenophora tritici-repentis*) form a disease complex which is often referred to as *Helminthosporium* leaf blight. Losses due to this disease complex amounted to 16% in India, 20% in Nepal, and 23% in Bangladesh [1, 2]. The fungus is one of the most important constraints to the normal growth and development of the crops in warm and humid conditions, and usually the infection becomes severe after inflorescence emergence [3]. The global distribution of common phytopathogenic species of *Bipolaris* may have resulted from the transfer of agricultural commodities including plants and seeds across geographical borders [4, 5]. Biological control has attracted attention from researchers for over 30 years, primarily because of interest in developing more 'environmentally friendly' means of disease management in the absence of agricultural pesticides. Despite considerable effort in the area of biological control, few practical applications have become established in agriculture for the control of plant diseases [6]. Research on biological control agents has utilized naturally occurring saprophytic soil fungi to compete with and/or destroy soil borne pathogens. Emergent of fungal antagonistic has made it a promising biological control strategy to control the plant diseases. The major factors which hinder the efficiency of the biocontrol agents to control the plant diseases need to be considered during the formulation of the biocontrol procedure, biocontrol agent, and its application time. The antagonistic potential of *Trichoderma harzianum*, *T. viride*, and *T. koningii* [7], is used to control pathogens of tomato, sunflower, rice, potato, pepper, cabbage, etc. The major problems of biocontrol agents are that they are difficult to use in the field. Biochemical and molecular biology studies carried out to explore the mechanisms involved in biological control revealed that *Trichoderma* is a rather specific mycoparasite. Lectins were found to be involved in the recognition between *Trichoderma* and its host fungi, whereas chitinase is involved in the degradation of the host cell wall [8].

II. MATERIAL AND METHODS

Infected vegetables were separately collected, isolated, purified and pathogenicity test was done. The targeted pathogen was collected and maintained in laboratory. *Trichoderma* species were isolated from soil. One gram of the soil sample was taken and added to 1ml of sterilized distilled water to make a dilution of 10^{-1} . This suspension was then subjected to serial dilutions and a dilution of 10^{-5} was attained. One milliliter of each dilution viz., 10^{-3} to 10^{-4} was poured on to plate containing fresh PDA and spreaded smoothly by sterile spreader. Then these plates were incubated for 28±2°C for 7 days. After incubation period they were identified by using soil manual of [9]. Cultures were identified according to conidiophore, shape of the phialides and emergence of phialophores and phialospores. The purified and identified

cultures of *Trichodermaspp.* were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further use.

A) Antifungal assay by antagonistic fungi:

For the assessment of antifungal activity against targeted fungal pathogen by following two techniques: Dual culture method and Slide culture method.

I) Dual culture Technique:

To determine the effect of *Trichodermaspp.* on mycelial growth of targeted pathogens, dual culture method was used. The dual culture of three *Trichodermaspp.* (*T. harzianum*, *T. viride* and *T. pseudokonigii*) and targeted pathogen was studied on Potato dextrose agar (PDA). 20 ml of PDA medium was poured in plates (9 cm) and was allowed to solidify. Discs (6mm diameter) of mycelium cut from the margin of 6 days old culture of each *Trichodermaspp.* were placed at the edge of each plate 10 mm from the periphery. Then disc of 6mm diameter of mycelium cut from the growing edge of 7 days old cultures of targeted fungal pathogen was placed on each plate, opposite to the mycelial discs of *Trichodermaspp.* In control plates, a sterile disc whatman No.1 filter paper of 6mm diameter was placed at opposite side of targeted fungal pathogen in complete aseptic condition. Three replications were maintained for each *Trichodermaspp.* and targeted fungal pathogen separately. All the plates were incubated at 25±1°C for about 7 days after inoculation. The radial growth of all fungi was measured, when the *Trichodermaspp.* in control plates show complete growth. The colony diameter of both *Trichodermaspp.* and targeted fungal pathogen was measured at two locations, right angle to each other and the average diameter was calculated. Percent inhibition of mycelial growth of targeted fungal pathogen over control was calculated by following equation given by [10].

$$\% \text{ Inhibition} = \frac{D1 - D2}{D1} \times 100$$

D1 = Colony diameter in the control.

D2 = Colony diameter in treated.

II) Slide culture Technique:

For slide cultures, a clean slide was placed on an L-shaped glass rod in a 9cm diameter petridish and autoclaved. Then a small amount of molten water agar was poured and evenly spread over the slide to make a thin agar film. One end of the slide was kept free of the medium to facilitate handling. Inocula from each *Trichoderma* isolate were placed separately on the slide 1cm apart from each other. A few ml of sterile water was added to the petridish to prevent drying, and the slide incubated at 22°C for 3-5 days. At the end of incubation period, regions where the hyphae of *Trichoderma* met the hyphae of the pathogen were observed under a light microscope for the presence of coil formation and penetration structures, or wall disintegration.

B) Detection of antifungal activity by Volatile metabolites from antagonistic fungi:

The effect of volatile metabolites released by *Trichoderma* species was evaluated against growth of targeted fungal pathogen of vegetables. For antifungal activity of volatile a petriplate containing PDA medium was inoculated with 6mm diameter disc of *Trichoderma* isolates growing on PDA. A second petriplate containing PDA was inoculated with a 6mm disc of the targeted pathogens in the center of the plate and inverted over the *Trichoderma* culture. The two plates were sealed together with nescofilm and incubated at 28°C for 6 days. This ensured that both organisms were growing in the same atmosphere. For control instead of *Trichoderma* one plug of PDA was placed on Agar surface. All the experiments *in vitro* were arranged as Randomized complete design with three replications. The surface areas of the colonies of targeted pathogen was recorded compared with controls and the percentage of growth inhibition was calculated by using [10].

C) Detection of antifungal activity by Non-volatile metabolites from antagonistic fungi:

To determine the effect of the non-volatile metabolites on mycelia growth of pathogen poisoned food technique was used. For the production of non-volatiles, three discs of mycelial agar plugs (6 mm diameter) obtained from edges of 7 days old culture of *Trichoderma* species were inoculated in 100 ml sterilized potato dextrose broth (PDB) in 250 ml conical flasks and incubated at 25 ± 1°C on a rotary shaker at 100 rpm for 14 days. The control conical flasks were inoculated with sterile PDA plugs respectively. After incubation, the culture was

filtered through Millipore filter for removing spores for collecting non-volatile metabolites from *Trichoderma* species.

Collect the transparent supernatant containing non-volatile metabolites. For poisoned food assay the liquid formed non-volatile was added to molten PDA medium (at $40 \pm 5^\circ\text{C}$) to obtain a final concentration of 10% (v/v). The medium was poured in Petri dishes at 20 ml per plate and inoculated with 6mm mycelial plugs of the pathogens in the centre of the plates and incubated at $25 \pm 2^\circ\text{C}$ for 7 days or until the colony reached the plate edge in control plate. Triplicates were maintained for each treatment and radial growth of the pathogen was recorded by using formula of Vincent (1927).

III. DISCUSSION

The fungal antagonistic of *Trichoderma* species were screened for their antagonistic activity against fungal pathogen which was isolated from vegetables. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods [11]. *Trichoderma* is an exceptionally good model of biocontrol because it is ubiquitous, easy to isolate and culture, grows rapidly on many substrates, affects a wide range of plant pathogens, acts as mycoparasite, competes well for food and site, produce antibiotics and has an enzyme system capable of attacking a wide range of plant pathogens [12]. *Trichoderma* species have been known since 1930s to show antifungal activity and there have been extensive efforts to use them for plant disease control since then [13]. *Trichoderma* spp. also is commercially marketed as biopesticides, biofertilizers and soil amendments. The use of *Trichoderma* fungi in agriculture can provide numerous advantages; colonization of the root and rhizosphere of plant, control of plant pathogens by different mechanism, improvement of the plant health by promoting plant growth and stimulation of root growth.

Mycoparasitism of *T. harzianum* involves action of antibiosis, nutrient competition and cell wall degrading enzymes, such as chitinases and glucanases [14]. The early report support this findings that *Trichoderma viride* and *T. harzianum* as the best antagonists against several soil and seed borne plant pathogens [15]. [16] He demonstrated that proteases from *T. harzianum* play an important role in biological control.

The antagonistic effect of three tested *Trichoderma* species was also studied by using slide culture method in which specially *T. harzianum* and *T. koningii* revealed high chemical antagonism by producing cell wall degrading enzymes which causes disintegration of cell wall of targeted pathogens with mycoparasitism activity by creating coiling structure around the mycelium of targeted pathogens. In this study *T. harzianum* had recorded the greater coiling structures followed by *T. viride*. *H. sativum* were more resistant and showed averagely 24 coiling structure with 8 number of disintegrated mycelium. [17] They have reported that the enzymes may be produced that digest the mycelial walls and septal walls or antibiotics may be formed that inhibit growth or cause endolysis. Coiling is characteristic of the interaction between mycoparasitic and phytopathogenic fungi, leading to penetration of the cell wall [18]. *Trichoderma* is a promising candidate for the biological control of plant pathogenic fungi [19,20].

IV. EXPERIMENTAL RESULTS

In Dual culture experiment, all three tested *Trichoderma* species like *T. viride*, *T. harzianum* and *T. koningii* had a marked significantly inhibitory effect on the growth of the targeted pathogen in relation to their control. When the pathogenic fungi was tested in combination with *T. viride*, *H. sativum*, revealed lowest percent inhibition as 48.25%, whereas *T. harzianum* showed 56.92% inhibition of mycelial growth of targeted pathogen. *T. koningii* showed 47.47% of mycelial growth. In this agar diffusion experiment, the non-volatile compounds released by *T. viride* were highly effective against *H. sativum* with 76.92% mycelial inhibition. *T. harzianum* and *T. koningii* with 68.31% and 84.24% inhibition of mycelial growth over control.



Table1.:Antagonistic effect of *Trichodermaspp*against *H. sativum* by Different method

Method	Biocontrol agent	Mycelial growth in control (mm)	Growth rate in <i>Trichodermaspp</i> +Pathogen combination (mm)	% inhibition of mycelial growth over control	Radius of inhibitory Zone (mm)
Dual Culture Technique	<i>T. viride</i>	77.3	40	48.25±0.27	0±0.00
	<i>T. harzianum</i>	77.3	33.3	56.92±0.71	1±0.12
	<i>T. koningii</i>	77.3	40.6	47.47±0.00	4±0.09
Effect of Volatile metabolites	<i>T. viride</i>	80.6	53.3	33.87±0.54	-
	<i>T. harzianum</i>	80.6	49.3	38.83±4.13	-
	<i>T. koningii</i>	80.6	34	57.81±0.94	-
Effect of Non-Volatile metabolites	<i>T.viride</i>	54.6	12.6	76.92±0.27	-
	<i>T. harzianum</i>	54.6	17.3	68.31±0.71	-
	<i>T. koningii</i>	54.6	8.6	84.24±0.71	-

Value expressed in mean ± S. E. M of triplicates

Table 2: Antagonistic effect of *Trichodermaspp.* on mycelium of pathogen of vegetables by slide culture method.

Pathogens	Biocontrol agent	Number of Coiling Structure	Number of disintegrated mycelia
<i>H. sativum</i>	<i>T. viride</i>	20	07
	<i>T. harzianum</i>	24	08
	<i>T. koningii</i>	29	10

V. CONCLUSION

From the above study it is concluded that, *Trichoderma* is a promising candidate for the biological control of plant pathogenic fungi. The non-volatile metabolites released by the three tested *Trichoderma* species was used for the fungal pathogen which were isolated from vegetables for their fungitoxicity. These non-volatile metabolites which were released by *T. viride* and *T. koningii* had pathogens, recorded above 75% of inhibition.

REFERENCES

[1]Dubin, H.J. and Ginkel, V. The status of wheat diseases in warm areas of south Asia: An update. In: Wheat in Heat Stressed Environments: Irrigated Dry Areas and Rice–Wheat Farming Systems.CIMMYT, pp. 353– 359, 1991.
 [2] Saari, E.E. Leaf blight diseases and associated soilborne fungal pathogens of wheat in south and southeast Asia. In: *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot* (E. Duveiller,H.J. Dubin, J. Reeves and A. McNab, eds). Mexico, D.F., Mexico: CIMMYT, pp. 37– 51, 1998.
 [3] Couture, L., Sutton, J. C. Relation of weather variables and host factors to incidence of airborne spores of *Bipolaris sorokiniana*. Can. J. Bot. 56: 2162-2170, 1978c.
 [4] Farr D.F., Rossman A.Y. ARS, USDA; Fungal databases, systematic mycology and microbiology laboratory, 2013.
 [5] Zhang N., Rossman A.Y., Seifert K. Impacts of the international code of nomenclature for algae, fungi and plants (Melbourne Code) on the scientific names of plant pathogenic fungi, 2013.
 [6]AbhishekTripathi, Neeta Sharma, NidhiTripathi. Management of fungal plant pathogens. Chapter 10:pp121, 2010.



- [7] Dutta P and Das B C. Management of Collar Rot of Tomato by *Trichoderma* spp. And chemicals. Indian Phytopath. 55(2): 235-237, 2002.
- [8] Chet I, Inbar J. Biological control of fungal pathogens. ApplBiochemBiotechnol. 48(1):37-43,1994.
- [9] Jha, D. K. Laboratory manual on plant pathology. Pointer publisher, Jaipur, India. pp.150-152, 2004.
- [10] Vincent, J. M. Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, 59: 850, 1927.
- [11] Baker, R. and Paulitz, T. C. Theoretical basis for microbial interactions leading to biological control of soilborne plant pathogens In: Principles and Practice of Managing Soilborne Plant Pathogens. Hall, R. (ed.). The American Phytopathol. Soc. St. Paul, MN. 50-79, 1996.
- [12] Mukerji, K. G. and K. L. Garg. Biocontrol of Plant Diseases, Vol. II. CRC Press Inc., Boca Raton, Florida, 1988.
- [13] Hjeljord, L. G and A. Tronsmo. *Trichoderma* and *Gliocladium* in biocontrol: an overview, pp.135-151, 1998.
- [14] Carsolio, C., A. Gutierrez., B. Jimenez, M. Van Montagu and A. Herrera-Estrella. Characterisation of ech-42. A *Trichoderma harzianum* endochitinase expressed during mycoparasitism. Proc Natl Acad Sci USA, **91**: 10903-10907, 1994.
- [15] Poddar, R. K., D.V. Singh, and S. C. Dubey. Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f.sp. *ciceri*). *Indian J. Agric. Sci.*, **74**: 346–348, 2004.
- [16] Benitez, T., J. Delgado-Jarana, A. M. Rincon, M. Rey, M. C. Limon. Biofungicides: *Trichoderma* as a biocontrol agent against phytopathogenic fungi. In: Pandalai SG (ed). Recent Signpost Trivandrum. 129-150, 1998.
- [17] Baker, F.K. and Cook, R.J. Biological control of plant pathogens. S. Chand and Company Limited, New Delhi, India. pp.433, 1979.
- [18] Calvet, C., J. Pera and J. M. Barea. Interactions of *Trichoderma* spp. with *Glomus mosseae* and two wilt pathogenic fungi. Agriculture, Ecosystems & Environment. **29**: 59–65, 1989.
- [19] Shaikh Farah T and Sahera Nasreen. In Vitro Assessment of Antagonistic Activity of *Trichoderma viride* and *Trichoderma harzianum* Against Pathogenic Fungi. Indian Journal of Applied Research. 3(5): 57-59, 2013.
- [20] Shaikh Farah T and Sahera Nasreen. Biocontrol Efficacy of *Trichoderma koningii* Against some Plant Pathogenic fungi. Indian Journal of Research. 2(3): 9-10, 2013.



INNO SPACE
SJIF Scientific Journal Impact Factor

**Impact Factor:
7.512**

ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA



INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

 9940 572 462  6381 907 438  ijirset@gmail.com



www.ijirset.com

Scan to save the contact details