Antagonistic Potential of *Trichoderma simmonsii* isolated from Bihar against pathogenic fungus *Alternaria brassiceae*

SHWETA KUMARI AND CHOUDHARY SHARFUDDIN

J. Mycopathol. Res. 60(2) : *245-249, 2022;*
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. **60(2)** : 245-249, 2022; (ISSN 0971-3719)

Antagonistic Potential of *Trichoderma simmonsii* isolated from Bihar against pathogenic fungus *Alternaria brassiceae*

SHWETA KUMARI* AND CHOUDHARY SHARFUDDIN
Plant Pathology and Microbiology Lab, Department of Botany, Patna University, Patna – 800005, Bihar, India

Received : 27.01.2022 Accepted : 30.04.2022 Published : 27.06.2022

Among all biological control agents, *Trichoderma* is most important ubiquitous soil inhabiting fungi due to their ability to suppress many plant pathogens. The present study was done to find out the antagonistic potential of *Trichoderma simmonsii* against *Alternaria brassiceae* which is one of the important fungal pathogen that cause several pre and post harvest damage to agricultural products. *In vitro* assay of *Trichoderma simmonsii* against the mycelia growth of *Alternaria brassicae* was evaluated. For this, different isolates of *Trichoderma* and *Alternaria* were isolated. All collected soil and pathogen samples were inoculated in PDA media. The plates were incubated at 26±2ºC for 5 to 7 days. The growth of pathogen in control and dual culture were recorded. Percent growth inhibition rate (PGIR) of the test pathogen over control was calculated. Based on the result obtained in dual cultures, it was clearly visible that *Trichoderma simmonsii* had 78% efficacy against *Alternaria brassicae* and mycelia growth was completely inhibited at spore concentration. In best of my knowledge, this is the first report of antagonistic effect of *Trichoderma simmonsii* against *Alternaria brassiceae*.

Key words: *Alternaria*, biocontrol, fungal pathogen, spore concentration*Trichoderma,*

INTRODUCTION

More than seventy five percent population of Bihar depends on agriculture. As agriculture is the backbone of Bihar economy, it is important to protect agriculture from several issues. Agriculture faces several problems in which, soil borne pathogenic fungi are of major concern. Pathogens cause heavy crop losses all over the world. The fungal pathogens play a major role in the development of diseases on many important field and horticultural crops, resulting in severe plant yield losses. Many fungal pathogens like, *Fusarium*, *Alternaria*, *Phytophthora*, *Colletrichum*, *Ustilago* and *Aspergillus* etc. play a major role in loss of yield. Most of these are saprophytic in nature which are present in soil and harm the plants when found the specific host.

Among all pathogens which affect the production, *Alternaria* is considered one of the most limiting factors for low productivity of many vegetables, cereal grains and fruits. Brassicaceae family is mostly affected by *Alternaria* sp.The genus *Alternaria* is widely distributed ubiquitous fungi with numerous species that cause pre and post harvest damage to agricultural products. It contaminates the aerial parts of the plants mostly. Since these fungi grow well at low temperatures, they are responsible for spoilage of crops after harvest and during storage.

Several crops of agricultural value are susceptible to infection by different *Alternaria* species and can contribute to the entry of *Alternaria* mycotoxins in the food chain. *Alternaria* spp. can produce a wide variety of toxic metabolites that play an important role in plant pathogenesis (Patriarca and Fernandez, 2018). Early blight of potato, Leaf spot of brassica, Stem canker of tomato, Blossom blight in cumin, Black rot on citrus, Ring spot of pear are some disease which decrease the quantity, quality and market value of agricultural products.

Chemical control of these pathogens disturbs the environment, subverts ecology, degrades soil productivity and mismanages water resources

*Correspondence: shwetakri33@gmail.com

(Ayala and Rao, 2002; Deshwal, *et al*. 2003). Intensified use of fungicides has not only resulted in accumulation of toxic compound potentially hazardous to human and environment but also in the buildup of resistance of the pathogens (Khandelwal *et al*. 2012).

To minimize the use of chemical pesticide, an alternative method of disease control can be used instead of chemicals. Biological control is best alternative to reduce the use of chemicals in agriculture with high yield and low ecological impact.*Trichoderma* is very promising method of biocontrol against soil borne phytopathogenic fungi. The biocontrol activity of theses fungi is through their abilities to induce systemic disease resistance; even "classical" biocontrol by antibiosis and classic mycoparasitism, when analyzed by genetic or mutational approaches, were found to be due solely to induced resistance (Howell, 2006). It not only protect the plants from diseases but establishment of root colonization and chemical communication by *Trichoderma* strongly affect plant physiology by changing plant gene expression, as documented by several groups(Alfano *et al.* 2007; Bae *et al*.2011; Djonovic *et al*. 2007; Marra *et al*. 2006; Shoresh and Harman, 2008). The advantage of using *Trichoderma* in managing soil borne plant pathogens are eco-friendly, effective, ease of mass culturing with less cost of production and growth promoting effects. Considering the above facts, the present investigation was undertaken to evaluate the antagonistic performance of different isolates of*Trichoderma* on pathogen.

MATERIALS AND METHODS

Collection of soil samples

One hundred fifty soil samples were collected from different agricultural fields of Bihar. Soil samples were collected from a depth of 10-15cm into clean polythene bags. These soil samples were air dried at room temperature for 24 hours.

Soil dilution

One gram of each soil sample was weighed out and placed into a sterile falcon tube with 9ml of sterile distilled water and again transfer 1ml of this dilution into another tube and repeat this step to prepare 10^{-3} dilution of the sample.

Isolation and identification of Trichoderma

From the serial dilution, soil samples of 1ml each was poured in Czapek's and PDA media. The plates were incubated for 5-7 days at 27±2°C for growth. After 3 to 4 days several colonies of different fungi were observed. Now, the individual colony of *Trichoderma* was cultured onto fresh media. Morphological and microscopic identification were done with the help of authentic laboratory manual of Burnett and Hunter.For identification at species level, ITS sequencing was done by sequencing service of CSIR NCL lab. The species of *Trichoderma* was identified as *simmonsii*on the basis of ITS sequencing.

Isolation of pathogen

The infected leaf samples were collected for the isolation of fungal pathogen. Infected leaves were cut into small pieces and washed thoroughly under running tap water. After this, the samples were surface sterilized with 0.1% $HgCl_2$ for 1 minute and washed thoroughly with sterile distilled water for 2-3 times and blotted in sterile blotters. These were maintained on PDA media for growth of fungal colony. The plates were incubated for 4-5 days at 25±2°C.

Dual culture of pathogen with biocontrol agent

Pairing on solid medium

In vitro evaluation of *Trichoderma,* obtained from the soil for their biocontrol efficacy against *Alternaria* was done by dual culture method. Small pieces of inoculawere taken from the margin of 7 day old culture of *Trichoderma* and *Alternaria* with the help of sterile needle at equal distance from periphery on the same day. Both, the control plates and dual culture plates were incubated at 27±2ºC for 7 days. The diameters of radial growth of colony were measured after each day of incubation period after 48 hours. Percent inhibition of radial growth(PIRG) was calculated by the formula,

$PIRG = [(R_1 – R_2)/R_1] \times 100$, Where,$R_1$ = Diameter of pathogen in control andR_2 = Diameter of pathogen in dual culture

Spore germination

The effect of *Trichoderma* on spore germination of *A brassiceae* was tested in sterile Potato Dextrose Broth (PDB). A volume of 150µl of antagonist suspension already prepared and adjusted to various concentrations is mixed with 150µl of *A brassiceae* of suspension into 10ml tubes containing 5ml PDB liquid medium. The tubes are incubated at 26±2ºc for 24 hr. Percent spore germination in control and antagonist treated samples were determined.

RESULTS

Pathogen from the diseased leaves was identified as *Alternaria brassiceae* on the basis of cultural and microscopic characters (Fig.1).

In vitro efficacy testing of *Trichoderma simmonsii* against *Alternaria brassicae* was carried out by dual culture methodand percent inhibition of radial growth of dual culture and control were calculated for a reliable result. The colony growth of *Alternaria brassicae* was found to be completely covered by the growth of *Trichoderma simmonsii* on the fourth day of incubation. The result of the determination and the effect of *T simmonsii* at different

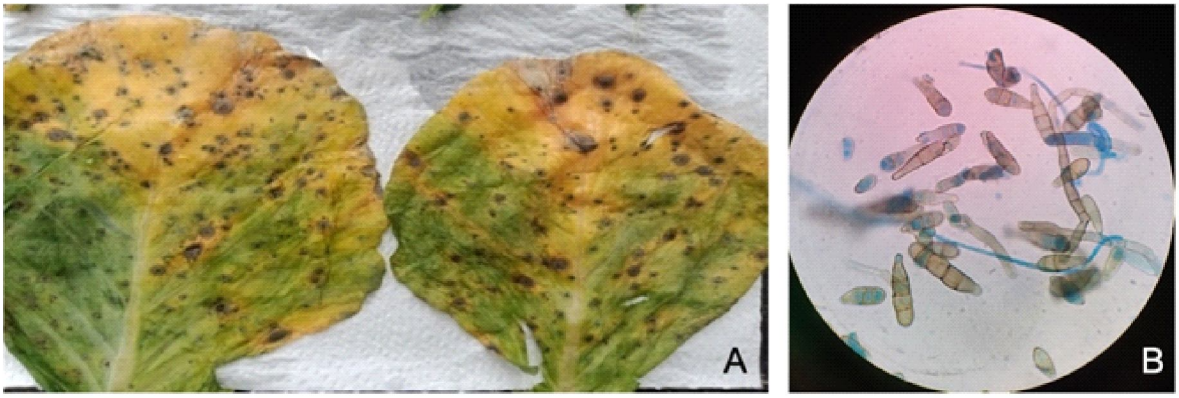

Fig.1 Diseased leaves (A); Microscopic view of spores of *Alternaria brassiceae* (B)

The isolated *Trichoderma* species was identified on the basis of morphological, microscopic and molecular studies as *Trichderma simmonsii* (Figs. 2 &3, Table 1).

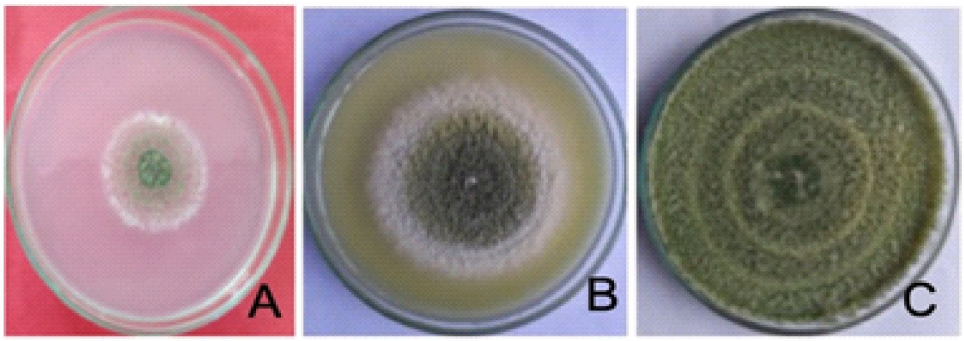

Fig.2 . Mycelial growth of T.simmonsii after 3 (A), 5 (B) and 7 (C) days of growth in Petri plate

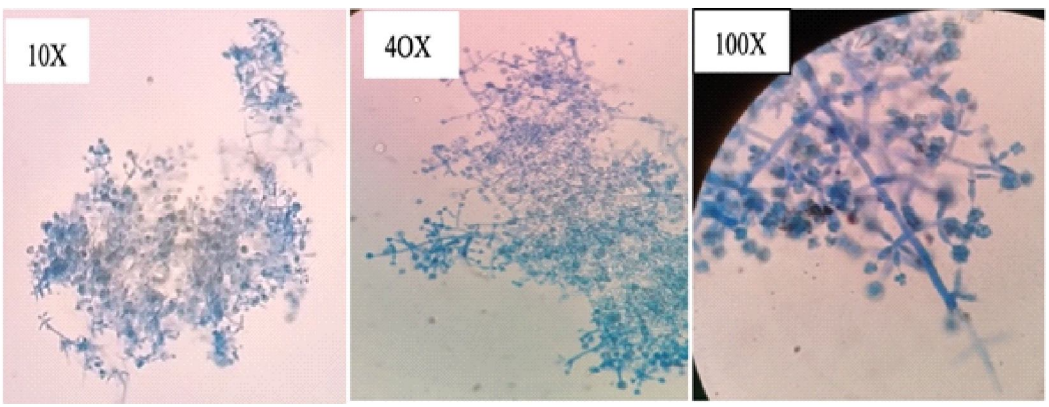

Fig.3 Spores of *T.simmonsii* at 10x, 40x and 100x magnification

Table 2: ITS Sequencing result of *Trichoderma isolate*.. NCBI-BLASTn hits (top 5)

Description	ScientificName	Max Score	Total Score	Query Cover	E value	Per.Ident	Acc.Len	Accession
Trichoderma simmonsii CBS130431IT Sregion; from TYPE material	*Trichoderma simmonsii*	1090	1090	98%	0.0	98.86%	626	NR_137297.1
Trichoderm apleuroticola CBS 124383 ITS region; fromTYPE material	*Trichoderma pleuroticola*	1075	1075	99%	0.0	98.06%	628	NR_134420.1
Trichoderma atrobrunneum CBS 548.92IT Sregion; fromTYPE material	*Trichoderma atrobrunneum*	1072	1072	98%	0.0	98.52%	618	NR_137298.1
Trichoderma lixii CBS 110080 IT Sregion; from TYPE material	*Trichoderma lixii*	1072	1072	98%	0.0	98.37%	623	*NR*_131264.1
Trichoderma rifaii CBS130746 IT Sregion; from TYPE material	*Trichoderma rifaii*	1070	1070	97%	0.0	98.68%	602	NR_137305.1

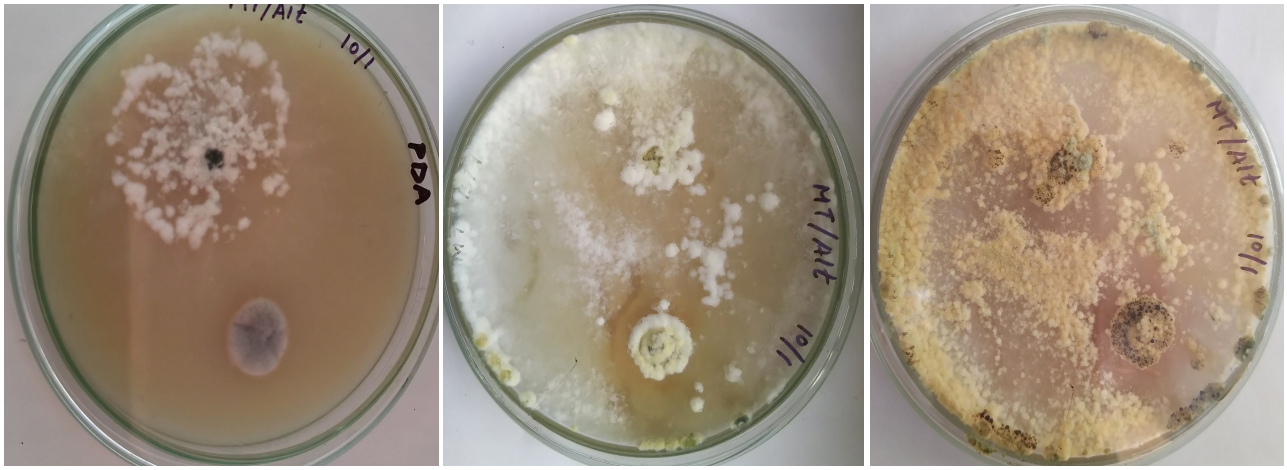

Fig.4 Antagonistic effect of *T.simmonsii* against *A.brassiceae* 3,5 and 7 days of incubation

concentrations on mycelial growth of *A. brassicae* gave several percentage grades of development inhibiting this pathogen. The comparison of the percentages of inhibition of mycelia growth based fungal concentration generally shows, inhibition of mycelial growth of *A.brassicae* decreases with decreasing *T.simmonsii* concentration. The complete inhibition of pathogen development was achieved by *T.simmonsii* at 10^7 CFU/ml. At the low concentration of 10^1 CFU/ml, *T. simmonsii* have a minimum inhibition (Fig 4).

To estimate whether there is a difference in the activity of the *T. simmonsii* over time onthe implem-entation of the spore germination of *A.brassicae*, this activity was evaluated either by mixing the antagonist and pathogen suspensions simultaneously t_0, after four hours of incubation of antagonist t_4, and by adding the conidial suspension of antagonist after eight hours of incubation t_8. Spore germination was maximum at 10^1 concentration, 8 hours of incubation (Fig.5).

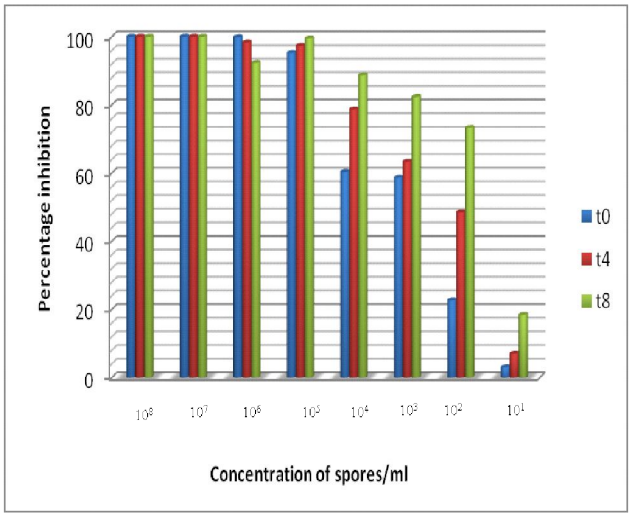

Fig.5 .Effect of *T.simmonsii* on spore germination of *A.brassiceae*

DISCUSSION

Trichoderma play a major role in microbial equilibrium and serve as powerful agent for biological control of plant disease (Anal *et al*. 2009).

The use of *Trichoderma* for the protection of commercially important crops are increasing interest of many plant pathologists and microbiologists. It was reported that several species of *Trichoderma* possess the ability to inhibit the pathogenic fungi *Alternaria* in *in vitro* conditions. Such inhibitory effect of *Trichoderma* have been recorded by Waghe *et al*. (2015). *Trichoderma* grows rapidly and occupy the growth space of pathogen, which is one of the major phenomena of their antagonistic effect (Nagy et al., 2007). Nutrients and niche competitions, production of volatile and non volatile compounds could be possible cause of antagonism (Hajieghrari *et al*. 2008). *T.asperellum* and *T. harzianum* showed effective inhibition on *graminicola* in dual culture assay (Manzar et al., 2021). In this study, it could be clearly observed that *T simmonsii* covered most parts of the plates and continue to grow on the colony of pathogen and then covered the entire colony, showing competition. The spore suspension of antagonist at high concentration also inhibit the spore germination. So, this *in vitro* study clearly shows that *T. simmonsii* have excellent efficacy against *A. brassicae*. Therefore, this can be a best alternative of chemical pesticides to reduce the incidence of diseases caused by *A. brassicae*.

On the basis of this study, the findings support the fact that biological control is a promising tool to maintain current level of agricultural production by reducing the release of polluting chemical pesticides to the environment as well as making the plants free from infecting soil borne pathogens, leading to high yield of crops (Bastakoti *et al*., 2017). By using *Trichoderma* as biocontrol agent, disease caused by pathogens can be reduced.

REFERENCES

Alfano, G., Lewis Ivey, M. L., Cakir,C., Bos, J.I.B., Miller, S.A., Madden, L.V., Kamoun,S., Hoitink, H.A.J.2007. Systemic modulation of gene expression in tomato by *Trichoderma harzianum* 382. *Phytopathology* **97**:429-437.

Anal, E.P., Mareno, U.M., Cardo, C. 2009. Biological control of *Septorialtritici*blotchon wheat by *Trichoderma sp.* under field conditions in Argentina. *Biocontrol* **54**: 113-122.

Ayala, S., Rao,A.. 2002. EVSP: Perspective of soil fertility management with a focus on fertizer use for crop productivity. *Curr. Sci*. **82**: 797-807.

Bae, H., Roberts, D.P., Lim, H-S., Mary D Strem, M.D., Park, S-C., Ryu,C-M., Rachel L Melnick,R.L., Bryan A Bailey, B.A.2011. Endophytic *Trichoderma* isolates from tropical environments delay disease and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. *Mol. Plant Micr. Inter*. **24**: 336-351.

Bastakoti, S., Belbase, S.,Manandhar, S., Arjyal, C.2017. *Trichoderma* species as Biocontrol Agent against Soil Borne Fungal Pathogens. *Nepal J. Biotech*. **5**: 39-45.

Deshwal, V. K., Dubey, R.C., Maheshwari, D. K. 2003. Isolation of plant growth promoting strains of *Bradyrhizobium arachis* sp. with biocontrol potential against *Macrophominaphaseolina* causing charcoal rot of peanut. *Curr. Sci*. **84**: 443-448.

Djonovic, S., Vargas, W.A., Kolomiets, M.V., MichelleHorndeski, M., Aric Wiest, A., Kenerley, C.M.2007. A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for systemic resistance in maize. *Plant Physiol*. **145**: 875-889.

Hajieghrari, B., Torabi- Giglou, M., Mohammadi M.R., Devari, M. 2008. Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *Afr. J. Biotech.* **7**: 967-972.

Harman, G.E., Obregon, M.A., Samuels, G.J., Lorito, M., 2010. Changing models of biocontrol in the developing and developed world. Plant Disease. **94**: 928-939.

Howell, C.R. 2006. Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. *Phytopathology*. **96**: 178-180.

Khandelwal, M., Datta, S., Mehta,J., Naruka, R., Makhijani, K., Sharma,G.,Kumar,R. Chandra, S.2012. Isolation, characterization and biomass production of *Trichoderma viride* using various agro products – A biocontrol agent. *Adv. Appl. Sci. Res*. **3**:3950-3955.

Marra, R., Ambrosino, P.,MarraR., Patrizia Ambrosino, P., Carbone,V., Vinale, F., Woo, S.L., Ruocco, M., Ciliento, R., Lanzuise, S., Ferraioli, S., Soriente, I., Gigante, S., Turrà, D., Fogliano, V., Scala, F., Lorito, M. 2006. Study of the three-way interaction between *Trichoderma atroviride*, Plant and fungal pathogens by using a proteomic approach. *Curr. Genet*. **50**: 307-321.

Nagy, V.,Seidl,V.,Szakacs, Komon-zelazowska, M., Kubicek,C.P., Druzhinina,I.S. 2007. Application of DNA G., bar codes for screening of industrially important fungi: the haplotype of *Trichoderma harzianum*sensu strict indicates superior chitinase formation. *Appl.Environ.Microbiol*. **73**: 7048-7058.

Patriarca, A., Fernandez Pinto, V. 2018. *Alternaria*. Reference Module in Food Science. Elsevier. pp. 1-8.

Shoresh, M., Harman, G.E. 2008. The molecular basis of maize responses to *Trichoderma harzianum* T22 inoculation: a proteomic approach. *Plant Physiology*. **147**: 2147-2163.

Waghe, K.P., Waghe, S.S., Kuldhar, D.P., Pawar, D.V. 2015. Evaluation of different fungicides, bioagents and botanicals against Alternaria blight caused by *Alternaria helanthi*(Haesf) sunflower. *Afr. J.Agricult. Res*. **10**: 351-358.