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PLANT PATHOLOGY

# Bio-control Efficacy of *Trichoderma* spp. Against the Major Diseases of Rice (*Oryzae sativa* L.)

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**ABSTRACT**

Rice (*Oryza sativa* L.) is the most widely cultivated food crop and is being cultivated in 114 countries over the world. The majority of the rice (90%) is being produced in Asia with China and India being the major producers. In nature, plants are simultaneously exposed to a combination of biotic and abiotic stresses that limit crop yields. Rice blast caused *by Pyricularia oryzae* is one of the major rice diseases that hamper rice production globally. The extent of damage caused by *P. oryzae* in rice production resulting losses of 10-30% of the global yield. Rice sheath blight, caused by the soil-borne fungal pathogen *Rhizoctonia solani,* is an economically important disease in rice. Depending upon the severity of the disease, it may cause 25-100% yield losses. Chemical control of disease leads to increase environmental toxicity hence the biological control is one of the best method to manage rice diseases. *Trichoderma* is a very effective biological mean for plant disease management. It is a free living fungus which is common in soil and root ecosystems. It is highly interactive in root, soil and foliar environments. It reduces growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion. *Trichoderma* have been found effective in controlling rice blast and sheath blight of rice. Hence, in this work we have attempted to *in vitro* management of rice blastand sheath blight by potential *Trichoderma* isolates, and found different per cent growth inhibition.

**Highlights**

- Three isolates of Trichoderma were found effective to inhibit the growth of Rhizoctonia solani *in vitro.*

**Keywords:** Rice blast**,** *Rhizoctoniasolani, Trichoderma, Mycoparasitism, Chemical control*

The major food crop of the world comprises of the cereals of which rice (*Oryzae sativa* L.) is the most important among all the cereals and it is the crucial food staple for more than half of the world. In India also major portion of the diet of the population comprises of rice making it a very essential crop for feeding the ever-increasing population. It is rated comparatively higher than other cereals on the basis of nutritional value and also plays a key part in the nutrition (Alina *et al*. 2002). The diseases of the rice plant are the major causes of the decline in the production and the productivity of the crop. Among the fungal diseases of the rice crop, the two major diseases which had more deleterious effect on the crop stand are blast disease caused by *P. oryzae* and sheath blight caused by *R. solani*. These two diseases are bound to occur in all the rice-growing areas of the world. In the temperate rice-growing area of the world, the blast disease of rice is one of the most frequent and the costly diseases (Wang and Valent 2009). The loss in the yield of the rice crop due to the blast disease is dependent on the susceptibility of variety, the timing of the fungicide application, and the degree of infection. The rice sheath blight pathogen *R.solani* is the most widespread soil-borne pathogen which causing major economically important diseases in many crops (Adams 1988). It is the also one of the major diseases of rice known to occur worldwide and leading to yield loses considerably (Sudhakar *et al*. 1998). The over-adoption of the susceptible new varieties, high-yielding cultivars with more number of tillers, and the cultural practice changes has led to the favorable conditions for the incidence of the sheath blight pathogen (Groth *et al*.1991; Rush and Lee 1992).

After the advent of the green revolution, the non-discriminative and continuous application of the synthetic chemicals has led to the abominable effects which include the toxicity due to the residues, pollution of the environment, human and animal health hazards, and increase in the expenditure of plant protection. These unacceptable effects have brought attention of the plant pathologists in developing the effective bio-control measures to manage the diseases which are environmentally safe and effective as well. The biological control of the diseases is a promising tool which helps in maintaining the current level of crop production along with reduction in the release of chemical products which pollute the environment. The process of biological control of the plant diseases is complex and comprises of several successive steps which includes the interaction between the microbial antagonist and the host (pathogen) surface (Dikshit *et al*. 2011).

*Trichoderma* spp. has been successfully utilized as the biocontrol agents against the plant pathogens on the surface of plants among the antagonistic agents for the cruciferous, solanaceous, and graminaeous plants (Bishen *et al.* 1981; Bryan 2015; Elad and Kirschner, Rai and Singh 1980; Scharen and Kumar and Singh 1985; Trosnmo 1986; 1993; Sutton and Peng 1993; Michereff *et al*. 1995). *Trichoderma* is an anamorphic fungal genus (*Hypocreales, Ascomycota*) which contains the soil-inhabiting cosmopolitan fungi which comprises a major portion of the soil mycoflora in different ecosystems (Harman *et al*. 2004; Singh *et al.* 2017). The members of this genus are fast growing in culture and produce numerous green spores which are also known to occur worldwide and are often associated with the root, soil and plant debris (Howell *et al*. 2003). They have been recognized as the biocontrol agents since the first application of *Trichoderma* spp. to protect the crops against the plant pathogens all over the world in 1930s (Ha 2010). The researches have shown that *Trichoderma* can act by different modes in control of the plant pathogens which includes fungal parasitization, antibiotics production, and competition for food and space along with the induction of defence responses in the host plants. *Trichoderma* spp. has been shown to be effective for the control of the rice diseases including blast, and sheath blight (Ramsy 1991; Harman 2006). The formulations of *Trichoderma* spp. are now a days available readily in market as the commercialized biocontrol agents. The objective of this study was to evaluate the potential of the *Trichoderma* isolates which are indigenous to the region against the isolates of all the two plant pathogens *viz*: *P. oryzae*, and *R. solani* of rice, *in vitro*.

## MATERIALS AND METHODS

**Isolation of the pathogens:** The plant pathogen of the two diseases were isolated during the period of 2016-2017, from the naturally diseased leaves of various rice varieties infected with varying degrees of blast, and sheath blight diseases from a Varanasi and nearby regions in Uttar Pradesh, India. About 10 isolates of these fungal pathogens were isolated and purified using the single spore and hyphal tip techniques and maintained on potato dextrose agar (PDA) medium. The identification of the purified cultures was carried out as per the cultural properties, morphological, and microscopic characteristics described for the three fungal pathogens.

**Table 1:** Composition of *Trichoderma* selective media (TSM)

| | |
|---|---|
| $MgSO_4$ | 0.2g |
| $K_2HPO_4$ | 0.9g |
| KCl | 0.15g |
| $NH_4NO_3$ | 3g |
| Glucose/ Dextrose | 3g |
| Chloremphenicol | 0.25g |
| Fenaminosulf | 0.305g |
| PCNB | 0.20g |
| Rose Bengal | 0.15g |
| Agar | 18g |
| Distilled water | 1000ml |
| pH | 7 |

**Isolation of *Trichoderma*:** Rhizospheric soil samples were carefully collected in aseptic poly bags from agricultural fields of different region of Varanasi, Uttar Pradesh, India. 1g soil was mixed with 9ml of sterilized distilled water and serial dilutions were prepared. 100µl inoculum from the 4th and 5th dilution series were plated on the *Trichoderma* selective medium (TSM) developed by Elad *et al.* (1981) for isolating the *Trichoderma* spp. Single colony for each isolate was collected and cultured on PDA (Potato Dextrose Agar) plates at 27±2°C and were timely revived after every 30 days for further studies.

**Dual culture assay:** Selected isolates of *Trichoderma* spp. were used against the *P. oryzae* for evaluating antagonistic activities. "Dual culture Technique" as described by Morton and Stroube (1955) under *in vitro* condition. For the experiment 20ml of sterilized PDA medium were poured aseptically in sterilized petri-plates under laminar air flow. Five mm disc of antagonist and test pathogen were cut form one week old culture by sterilized cork borer, and was placed in petri-plates containing PDA medium. The disc was placed in straight line at distance of one cm from the corner of Petri-plates. Three replications were maintained for each treatment including control. Then inoculated Petri plates were placed in BOD for incubation at 25±2°C temperature and Radial growth of *Trichoderma* and *P. oryzae* were recorded for next seven days, inhibition zone was estimated on the basis of formula given by Vincent (1947), per cent inhibition

$$\text{Percent inhibition (PI) \%} = \frac{C-T}{C} \times 100$$

Where, C = Pathogen radial growth in cm in control.

T = Radial growth in cm in treated plates.

## RESULTS AND DISCUSSION

### Antagonism test against *P. oryzae* and *R. solani* with *Trichoderma*

A total 14 isolates of *Trichoderma* spp. were used for the antagonistic test against rice blast pathogen *P. oryzae*. Out of 14 isolates, only 4 isolates PT-15 (Fig. 2), PT-14 (Fig. 1), PT-6 (Fig. 3) and PT-5 (Fig. 4) were showed maximum growth inhibition against *P. oryzae* in dual culture assay, which were further carried for the field experiments. Rest 10 isolates also showed a different degree of growth inhibition up to 67% (Table 2). Similarly 14 isolates of *Trichoderma* spp. were also used for the antagonistic test against rice sheath blight pathogen *R. solani*. Out of 14 isolates of *Trichoderma*, only three isolates BHU-8 (Fig. 5), T-4 (Fig. 6) and BHU-11 (Fig. 7) showed maximum per cent growth inhibition against *R. solani* in dual culture assay (Table 3), and these *Trichoderma* isolates were also carried for field experiment.

**Table 2:** Percent growth inhibition of *Trichoderma* isolates against rice blast pathogen (*P. oryzae*). Results indicated mean of three replications with SD

| Sl. No. | *Trichoderma* isolates | Radial growth (mm) | % growth inhibition |
|---|---|---|---|
| 01 | PT-15 | 30.84±1.3 | 68.9±1.02 |
| 02 | PT-14 | 29.9±1.9 | 69.8±1.09 |
| 03 | PT-11 | 30.7±1.31 | 67.2±0.93 |
| 04 | PT-6 | 26.3±1.12 | 70.3±0.91 |
| 05 | PT-5 | 26.21±1.5 | 70.4±1.11 |
| 06 | PT-2 | 32.4±1.5 | 63.6±1.21 |
| 07 | PT-1 | 31.2±0.9 | 65.5±1.12 |
| 08 | T3V | 32.3±1.1 | 62.3±1.21 |
| 09 | T4V | 30.4±1.5 | 67.2±0.95 |
| 10 | T1V | 31.2±1.7 | 65.2±1.1 |
| 11 | T2V | 31.9±1.9 | 64.5±0.89 |
| 12 | T28A | 32.4±1.4 | 63.8±1.21 |
| 13 | T6A | 31.8±0.94 | 66.5±1.1 |
| 14 | T5A | 31.1±1.5 | 64.5±1.30 |

**Table 3**: Antagonistic activity of different *Trichoderma* isolates against *Rhizoctonia solani.* Results indicated mean of three replications with SD

| Sl. No. | *Trichoderma* isolates | Radial growth (mm) | % growth inhibition |
|---|---|---|---|
| 01 | BHU-1 | 59.22±1.4 | 34.20±1.3 |
| 02 | BHU-5 | 36.33±1.1 | 59.63±1.2 |
| 03 | BHU-8 | 32.56±1.8 | 63.89±1.9 |
| 04 | BHU-9 | 40.40±0.98 | 37.84±0.9 |
| 05 | BHU-11 | 25.67±1.32 | 71.48±1.5 |
| 06 | BHU-13 | 39.78±1.31 | 55.80±1.3 |
| 07 | BHU-14 | 59.22±1.91 | 34.20±0.9 |
| 08 | BHU-15 | 39.00±1.62 | 56.67±1.01 |
| 09 | BHU-16 | 42.00±0.99 | 53.33±0.91 |
| 10 | T-1 | 39.78±1.02 | 55.80±1.01 |
| 11 | T-2 | 40.89±1.34 | 54.57±1.2 |
| 12 | T-3 | 30.00±1.21 | 43.80±0.97 |
| 13 | T-4 | 29.44±0.97 | 67.28±1.32 |
| 14 | T-5 | 41.22±1.01 | 54.20±1.23 |

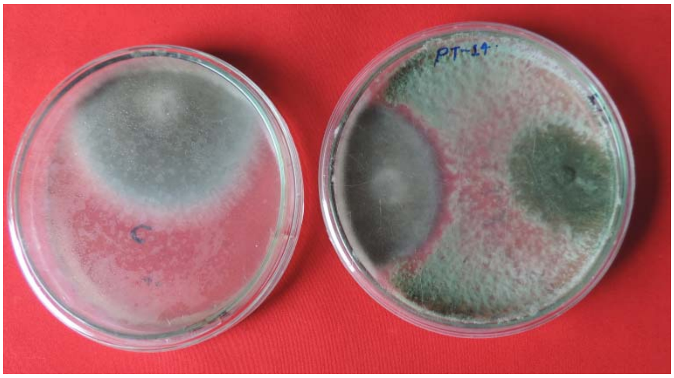


**Fig. 1:** Dual culture assay of *P. oryzae* with *Trichoerma* isolates PT-14

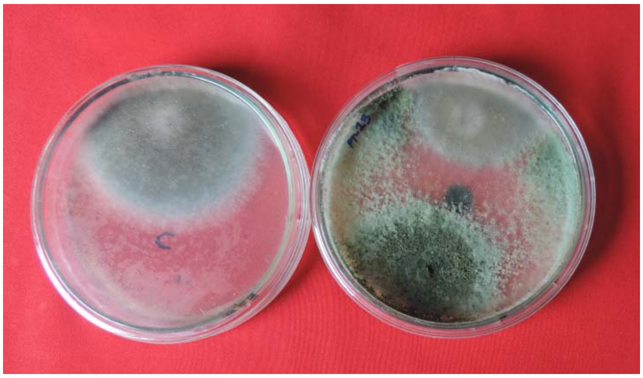

**Fig. 2:** Dual culture assay of *P. oryzae* with *Trichoerma* isolates PT-15

**Fig. 3:** Dual culture assay of *P. oryzae* with *Trichoerma* isolates PT-6

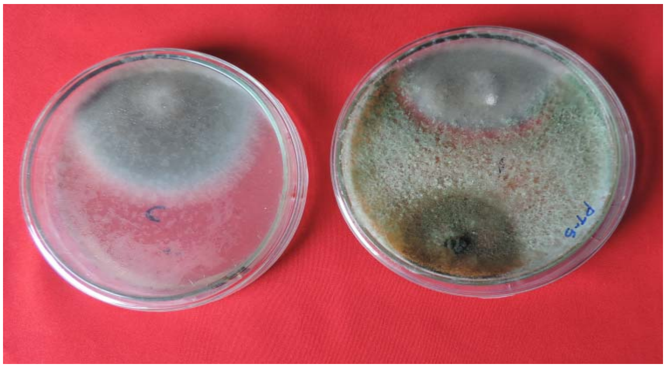

**Fig. 4:** Dual culture assay of *P. oryzae* with *Trichoerma* isolates PT-5

**Fig. 5:** Dual culture assay of *R. solani* with *Trichoderma* isolates T-4

**Fig. 6:** Dual culture assay of *R. solani* with *Trichoderma* isolates BHU-8

**Fig. 7:** Dual culture assay of *R. solani* with *Trichoderma* isolates BHU-11

With the help of dual culture technique three isolates of *Trichoderma* were found effective to inhibit the growth of *Rhizoctonia solani in vitro*.

Excess application of chemical pesticides for plant disease control leads to increase toxicity day by day, hence in current years the use of bioformulations in plant disease management has gained great interest as a safe and viable solution to the chemical alternatives (Keswani *et al.* 2016; Suryadi *et al.* 2013). Moreover our study has observed into the efficacy of *Trichoderma* in controlling Rice diseases.

## CONCLUSION

Our studies revealed that *Trichoderma* isolates isolated from different agricultural field were effective in inhibiting *P. oryzae* and *R. solani*. *Trichoderma* isolate PT-5 was found the most effective against *P. oryzae*. Similarly *Trichoderma* isolate BHU-11 showed maximum growth inhibition against *R. solani.*

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## REFERENCES

Adams, G.C. 1988. *Thanatephorus cucumeris* (*Rhizoctonia solani*) a species complex of wide host range. In: *Advances in Plant Pathology*, vol. 6. (G.S. Sidhu, ed.), Academic Press, New York, NY, USA, 535–552.

Alinia, F., Bahrami, M. and Amoogholi-Tabari, M. 2002. *Rice Significant Pests and Diseases and Their Control Methods.* Jihad Agriculture Organization of Mazandaran Publications, Iran.

Bisen, K., Keswani, C., Mishra, S., Saxena, A., Rakshit, A. and Singh, H.B. 2015. *Unrealized potential of seed biopriming for versatile agriculture.* In: A. Rakshit, H.B. Singh, A. Sen (Eds.), Nutrient Use Efficiency: from Basics to Advances, Springer, Berlin, pp. 193-206.

Elad,Y. and Kirshner, B. 1993. Survival in the phylloplane of an introduced biocontrol agent (*Trichoderma harzianum*) and population of the plant pathogen *Botrytis cinerea* as modified by abiotic conditions. *Phytoparasitica,* **21**: 303–313.

Fraceto, L.F., Maruyama, C.R., Guilger, M., Mishra, S., Keswani, C., Singh, H.B. and de Lima, R. 2018. *Trichoderma harzianum*-based novel formulations: potential applications for management of Next-Gen agricultural challenges. *Journal of Chemical Technology & Biotechnology*.

Groth, D.E., Rush, M.C. and Hollier, C.A. 1991. Rice diseases and disorders in Louisiana. *Louisiana State University, Agricultural Center, Louisiana Agricultural Experiment Station, Bulletin* No. 828. Baton Rouge, LA, USA.

Ha, T.N. 2010. Using *Trichoderma* species for biological control of plant pathogens in Vietnam. *J. ISSAAS.*, **16**(1): 17-21.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* species-opportunistic, a virulent plant symbionts. *Nature Review of Microbiology,* **2**: 43–56.

Harman, G.E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology,* **96**: 190–194.

Keswani, C., Sarma, B.K. and Singh, H.B. 2016. *Synthesis of policy support, quality control, and regulatory management of biopesticides in sustainable agriculture.* In: Singh H.B., Sarma B.K. and Keswani C. (eds.). Agriculturally Important Microorganisms. Commercialization and Regulatory Requirements in Asia. Springer, Singapore, Singapore, pp. 3-12.

Kumar, K. and Singh, H. 1985. *In vitro* antagonistic studies between *Alternaria solani* and saprophytic mycoflora isolated from potato leaves. *Indian J. Mycol. Plant Pathol*., **13**: 24–27.

Michereff, N., DaSiveira, S., Reis, A. and Mariano, R. 1995. Greenhouse screening of *Trichoderma* isolates for control of curvularia leaf spot of yam. *Mycopathology,* **130**: 103–108.

Mishra, B.K., Mishra, R.K., Mishra, R.C., Tiwari, A.K., Yadav, R.S. and Dikshit, A. 2011. Biocontrol efficacy of *Trichodermaviride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. *Archives of Applied Science Research,* **3**(2): 361-369.

Ou, S.H. 1985. *Rice Diseases*. *Commonwealth Mycological Institute,* London, USA.

Rai, B. and Singh, D. 1980. Antagonistic activity of some leaf surface microfungi against *Alternaria brassicae* and *Drechslera graminea*. *Trans. Br. Mycol. Soc.*, **75**: 363–369.

Ram, R.M., Keswani, C., Bisen, K., Tripathi, R., Singh, S.P. and Singh, H.B. *Biocontrol Technology: Eco-Friendly Approaches for Sustainable Agriculture.* In: Omics Technologies and Bio-Engineering, pp. 177.

Rasmy, M.R. 1991. *Studies on rice diseases in Egypt.* Ph.D. thesis, plant pathology dept Fac Agric Alex Univ Egypt.

Rush, M.C. and Lee, F.N. 1992. Sheath blight. In: *Compendium of Rice Diseases* (R.K. Webster, P.S. Gunnell, ed.), APS Press, St. Paul, MN, USA, 22–23.

Scharen, A. and Bryan, D. 1981. A possible control agent for net blotch of Barley. *Phytopathology,* **71**: 902–903.

Singh, H.B., Sarma, B.K. and Keswani, C. 2016. *Agriculturally Important Microorganisms,* 1st ed. Springer, Singapore.

Singh, H.B., Sarma, B.K. and Keswani, C. 2017. *Advances in PGPR Research*.

Sudhakar R., Rao, K.C. and Reddy, C.S. 1998. Studies on antagonism of *Trichoderma* and *Gliocladium* species against

*Rhizoctonia solani* of rice. *Indian Journal of Plant Protection*, **26**: 25–29.

Suryadi, Y., Susilowati, D.N., Riana, E. and Mubarik, N.R. 2013. Management of rice blast disease (*Pyricularia oryzae*) using formulated bacterial consortium. *Emir J Food Agric.*, **25**(5): 349-357.

Sutton,J. and Peng, G. 1993. Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Ann. Rev. Phytopathol*., **31**: 473–479.

Trosnmo, A. 1986. Use of *Trichoderma* spp. in biological control of necrotrophic pathogens. In: Fokkema, N., Der Heuvel, J. (Eds.), *Microbiology of the Phyllosphere,* Cambridge University Press, Cambridge.

Wang, G.L. and Valent, B. 2009. *Advances in genetics, genomics and control of rice blast disease.* Springer Science+ Business Media B.V.