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In Vitro Evaluation of *Trichoderma Harzianum* (Rifai.) Against Some Soil and Seed Borne Fungi of Economic Importance

Suraiya Yasmin[1], Sabiha Sultana[2], S. K Adhikary[3], Nusrat Jahan[4], Sanzida Rahman[5], Md. Imranur Rahman[6]

[1, 4, 5]Ex-student, [2]Assistant professor, [3]professor, Agrotechnology discipline, [6]Ex-student, Urban and Rural Planning discipline, Khulna University, Khulna- 9208, Bangladesh

Abstract: *To investigate the antagonistic potentiality of Trichoderma harzianum against Fusarium oxysporum, Bipolaris sorokiniana and Sclerotium rolfsii. Dual culture technique was followed to evaluate the effect of antagonist. The inhibition percentage of Trichoderma harzianum was substantially affected and differed significantly ($p<0.01$). In case of antagonist the highest percent inhibition of Trichoderma harzianum was found 89.20% against Bipolaris sorokiniana followed by 88.69% in Fusarium oxysporum but statistically dissimilar with Sclerotium rolfsii 76.76%. In every case inhibition was more than 75%.*

Key word*: Trichoderma harzianum*, dual- culture, soil and seed borne fungi

I. Introduction

The members of genus *Trichoderma* are free-living fungi that are common in soil and root ecosystems. They are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi (Harman et al.,2004). The *T. harzianum* are very promising against phytopathogenic fungi like *Fusarium oxysporum, Bipolaris sorokiniana and Sclerotium rolfsii* (Manczinger et al., 2002). These pathogenic fungi when associated with soil in different way and affect on seed germination, seedling mortality, seedling growth (Mustafa et al., 2004). *T. harzianum* and several other fungal antagonists are inhibitory to *B. sorokiniana* causing leaf and seed diseases of rye, wheat, barley (Biles and Hill 1988; Fokkema 1973). *T. harzianum* also found effective against *S. rolfsii* (Hadar et al,.(1979), Elad et al., (1980& 1981), Harman et al., (1980), Lewis and Papavizas (1984), Aziz et al., (1997) and *F. oxysporum* (Sarhan et al., 1999). It colonizes *S. rolfsii* hyphae, disrupts mycelial growth and kills the organism. Therefore systematic researches are needed to explore the potential of *T. harzianum* as bio-control agent against seed borne and soil borne fungal pathogens. Considering the above fact the present investigation was undertaken to investigate the antagonistic potentiality of *T. harzianum* against *F. oxysporum, B. sorokiniana and S. rolfsii*.

II. Materials and Method

An isolate of *T. harzianum* was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Three isolates of *B. sorokiniana, F. oxysporum, S. rolfsii* were collected from the preserved isolates of Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna.

Preparation of Potato Dextrose Agar (PDA) Medium

PDA was prepared following the standard procedure (Anonymous, 1968). 200 gm potato slice was boiled in 1000 ml distilled water. After that it was sieved and 20 gm dextrose was mixed with it. Then 15 gm agar was mixed with slowly and melted on hot plate magnetic stirrer. After preparation of PDA medium, it was poured in to 500 ml conical flasks then the conical flask was plugged with cotton plug and covered by brown paper. Finally the medium was sterilized in an autoclave at 121ºC temperature for 15 minutes.

Multiplication of *T. harzianum, B. sorokiniana, F. oxysporum* and *S. rolfsii*

PDA was poured in sterilized petridishes, 20ml in each plate. After solidification, discs were cut with flame sterilized cork borer (5 mm diameter), then the plates were inoculated by placing 5 mm discs of PDA culture of isolated pathogens. The inoculated petridishes were kept in the growth chamber (25±2 ºC) for observation. All the works were undertaken under aseptic condition.

In vitro* antagonistic effect of *T. harzianum* on mycelial growth of *B. sorokiniana, F. oxysporum, S. rolfsii

Antagonist *T. harzianum*, was tested against three seed and soil borne fungi following dual culture method (Dennis and Webster, 1971).

Discs of mycelia of different fungi were cut from the edge of an actively growing fungal colony with a 5 mm diameter cork borer and three disc of *T. harzianum* were placed near the edges of each PDA plate (90 mm). One disc of pathogenic fungies was placed in the center. The plates only with discs of each pathogenic fungies in the center were used as control plate. Plates were incubated in growth chamber for 25±2°C until the mycelia of pathogenic funnies covered the whole plate. A triangular shaped colony of each pathogenic fungies was observed. Area of the colony of tested fungi was calculated by ½×base×height. The area of the colony of control plates (seed and soil borne fungi) also measured by $\pi \times r^2$.Thereafter inhibition percentages of the pathogenic fungies were calculated by following the formula suggested by Sunder et al. (1995).

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Where X = Mycelial growth of pathogen alone (control)

Y = Mycelial growth of pathogen along with antagonist

Experimental Design and Data Analysis

The plates were arranged in Completely Randomized Design (CRD) with six replication. The measurement of mycelium in each plate was recorded in case of *S. rolfsii, B. sorokiniana* and *F. oxysporum* after 3 days, 7 days and 10 days of incubation respectively. The data were analyzed statistically using MSTAT-C computer program and means were compared for difference following LSD Test.

III. Results and Discussion

Antagonistic effect of *T. harzianum* on mycelial growth of *B. sorokiniana, F.oxysporum, S. rolfsii*

Antagonistic effect of *T. harzianum* on *B. sorokiniana, F. oxysporum, S. rolfsii* was tested on PDA in dual culture technique. The effect was varied significantly ($p \leq 0.01$). It was found that growth of *B. sorokiniana* was inhibited maximum (89.20%) which was similar to *F. oxysporum* (88.69%) but statistically dissimilar with *S. rolfsii* 76.76%. In every case inhibition was more than 75%.

Table2. Inhibition of the growth of tested fungi by *T. harzianum* on PDA

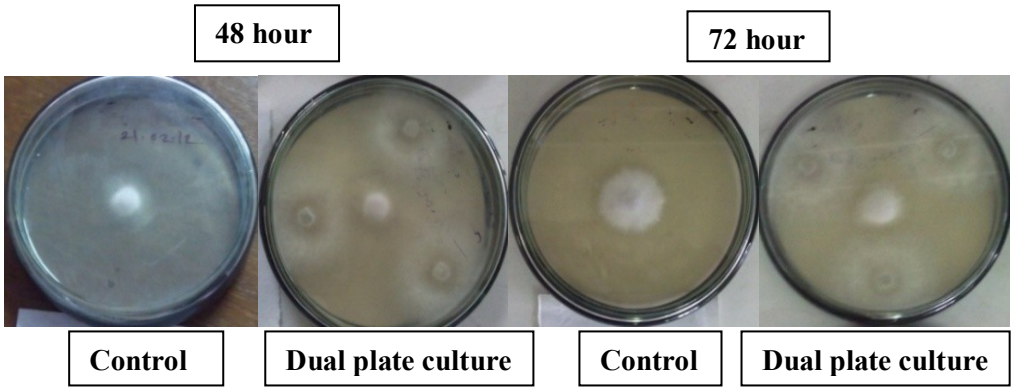
	T. harzianum
S. rolfsii	76.76
F. oxysporum	88.69
B. sorokiniana	89.20

LSD value-5.490

T. harzianum was also found effective against *S. rolfsii* by many scientists (Mathur and sarbhoy, 1978, Southworth et al. 1993, Sultana et al. 2012, Virupaksha et al. 1997, Shaigan et al., 2008, Ambra and Ferrata, 1984,Yaqub and Shahzad, 2005). Yogendra and Singh (2002) examined that *T. harzianum* inhibited *S. rolfsii* 64.44% after 96hour of incubation. Present study showed 76.76% inhibition of *S. rolfsii* by *T. harzianum*.

In this experiment it was found that *T. harzianum* showed 88.69% reduction of the mycelial growth against of *F. oxysporum*. Deshmukh and Raut (1992), Rajeswari and Kannabiran (2011), Southworth et al. (1993), Biswas (1999), Prasad et al. (2002), Sarhan et al. (1999), Kaur et al. (2003), Fakhrunnisa et al. (2006) were found *T. harzianum* to be highly effective against *F. oxysporum*. Nikam et al. (2007) found the antagonistic effect by *T. harzianum* causing 83.33% inhibition of mycelial growth of *F. oxysporum* f. sp. Ciceri.

T. harzianum also showed 89.20% inhibition of the mycelial growth of *B. sorokiniana* in the experiment. Salehpour et al. (2005) were also found that *T. harzianum* highly effective against *B. sorokiniana*.



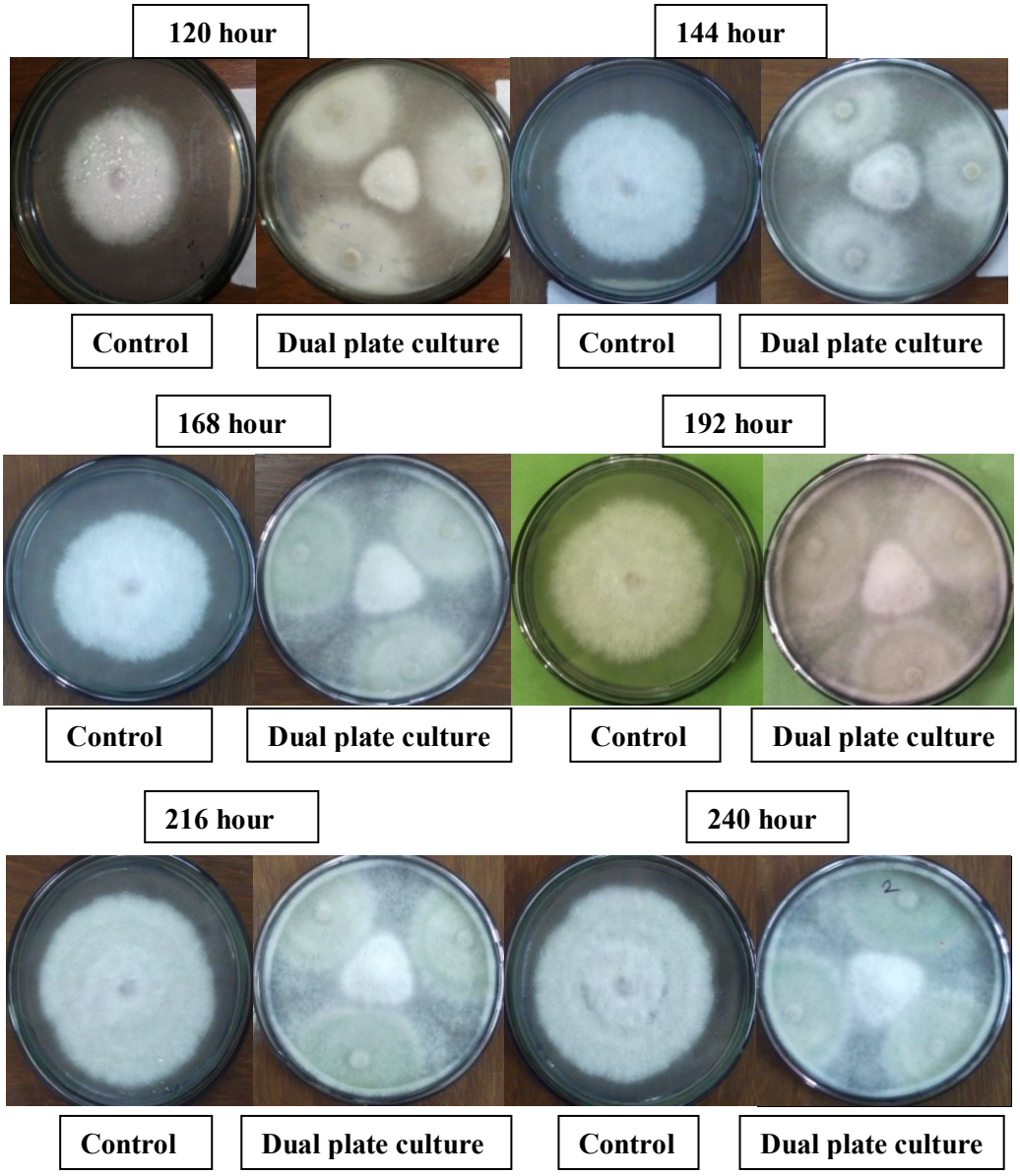
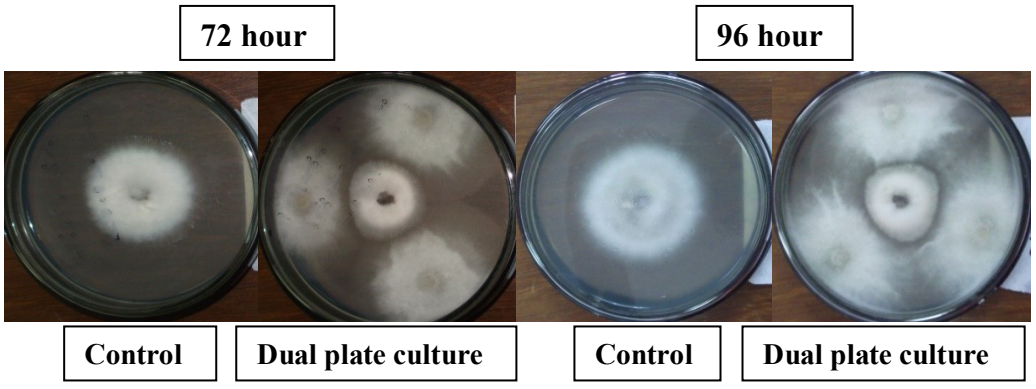


Plate 1: Antagonistic *in vitro* effect of *T. harzianum* on mycelial growth of *F. oxysporum*



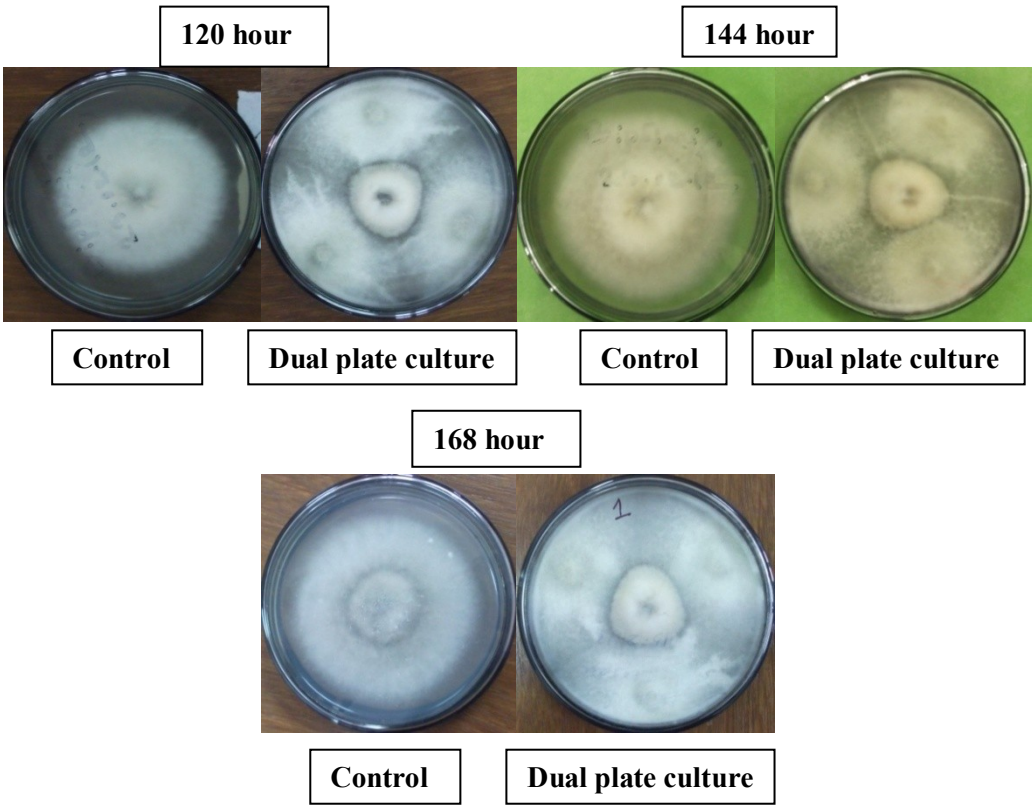


Plate 2: Antagonistic *in vitro* effect of *T. harzianum* on mycelial growth of *B. sorokiniana*

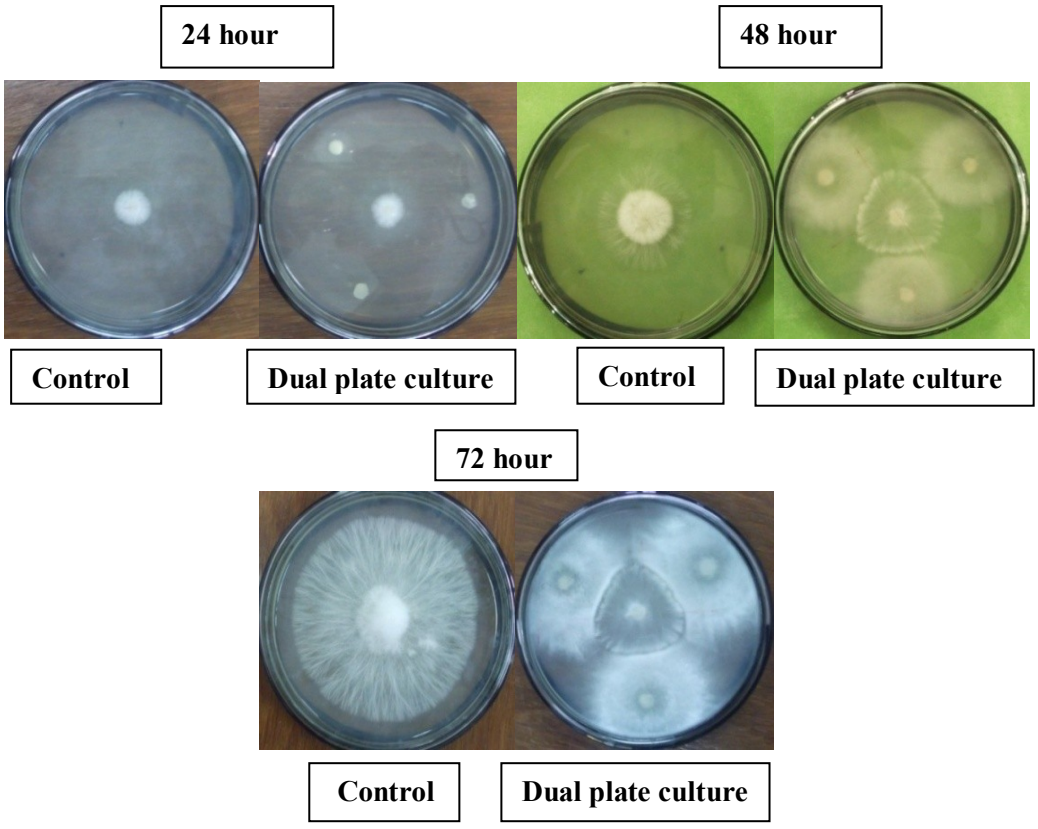


Plate 3: Antagonistic *in vitro* effect of *T. harzianum* on mycelial growth of *S. rolfsii*

IV. Conclusion

Based on the evaluation of *T. harzianum* and different botanical extracts, it can be concluded that *T. harzianum* was found to be effective against *B. sorokiniana, F. oxysporum, S. rolfsii* and that was more than 75%. Further investigation may be conducted with more treatments to confirm this result and generation of more information.

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