*Pak. J. Agri. Sci., Vol. 59(1), 29-34; 2022*
*ISSN (Print) 0552-9034, ISSN (Online) 2076-0906*[1]
*DOI: 10.21162/PAKJAS/21.1558*
http://www.pakjas.com.pk

# Potentiality of native *Trichoderma harzianum* in controlling damping off and foot rot of chilli and its viability in different storage conditions

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The potentiality of native *Trichoderma harzianum* against damping off and foot rot of chilli caused by *Sclerotium rolfsii* and *Fusarium oxysporum*, respectively as well as its shelf life as formulated product in different storage conditions was evaluated. In dual culture, native *T. harzianum* inhibited the mycelial growth of *S. rolfsii* by 60.56% and *F. oxysporum* by 81.67% over control. In green house conditions, soil received both formulated *T. harzianum* (20 g/kg of soil) and *T. harzianum* suspension ($2.45\times10^7$ CFU/mL) resulted reduced severity of damping off by 81.80% and foot rot by 84.61% over control; however, the combined application of both the treatments was also resulted the highest seed germination (70.28%). The formulated *T. harzianum* (@ 50 g/polyethylene bags) was stored in table top, wooden shelve, tin container, wooden locker at room temperature, and in refrigerator at 4 °C. The spore density was constantly increased from the initial amount ($22\times10^6$ CFU/g) in all the treatments and reached the peak after 45 days in wooden locker ($73\times10^6$ CFU/g) and tin container ($66\times10^6$ CFU/g) where, it reached the peak at 60 days in table top ($126.3\times10^6$ CFU/g), wooden shelve ($109\times10^6$ CFU/g) and refrigerator ($89.67\times10^6$ CFU/g). However, the maximum population density ($126.3\times10^6$ CFU/g) was observed in table top storage at 60 days which also remained highest even after 120 days ($51.67\times10^6$ CFU/g) in comparison to other treatments. The findings of the study revealed that the native *T. harzianum* can be exploited as an effective bio-control agent for the successful disease control program in chilli as it showed extended shelf life in common storage facility.
**Keywords***:* Native *Trichoderma*, chilli, damping off, foot rot, shelf life.

## INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the most important spices and cash crops in the world having high nutritive value rich in vitamin C, A, and E (Asaduzzaman *et al*., 2010; Song *et al*., 2019). In Bangladesh, the maximum yield of chilli is not achieved because of several factors where diseases play a major role. About 83 different diseases were reported in chilli and among them, damping off and foot rot are considered as the most deleterious caused by soil borne fungi like *Sclerotium* sp. *Fusarium* sp. *Rhizoctonia* sp. and *Pythium* sp (Steinkellner *et al*., 2008; Kevan & Shipp, 2011). Recently, damping off and foot rot of chilli are increasing dramatically in Bangladesh leading to huge yield loss **(**Alam *et al*., 2014). The fungi causing damping off and foot rot are soil born in nature, hence, it is very difficult to control. However, farmers traditionally try to control these kind of diseases by using costly chemicals. Indiscriminate use of a huge amount of chemicals for a long period resulted in environmental pollution and health hazard. In addition to the environmental and health problem, repeated and excessive use of chemicals also developing resistance in pathogens against different chemicals (Jayaraj *et al*., 2016). Therefore, researchers around the world are continuously trying to develop eco-friendly and cost-effective management practices alternative to the chemicals.

Biological control by the use of different beneficial fungi and bacteria has been considered as an effective and environmentally acceptable tool alternative to chemicals for the successful management of plant diseases (O'Brien, 2017; Shahzad *et al*., 2018). *Trichoderma*, a filamentous soil borne fungi have been using as one of the most common and effective biocontrol agents against various plant pathogens including *Sclerotium, Fusarium, Rhizoctonia, Pythium,* etc. (Al-Ani, 2019). *Trichoderma* inhibits or suppresses the growth of the plant pathogens by several strategies like antibiosis, competition, mycoparasitism, inducing host-plant resistance, secretion of chitinolytic enzymes, and production of inhibitory compounds (Harman *et al*., 2004; Braun *et al*., 2018). However, repeated use of the same *Trichoderma* or

Islam, M. M., A.T.M. S. Islam, M. M. Hasan, M. M. Rashid and Sk. M. M. Hossain. 2022. Potentiality of native *Trichoderma harzianum* in controlling damping off and foot rot of chilli and its viability in different storage conditions. Pakistan Journal of Agricultural Sciences. 59: 29-34
[Received 20 Dec 2020; Accepted 31 Dec 2021; Published 18 Mar 2022]

improper formulations leads to the losing of antimicrobial potentiality (Singh *et al*., 2007; Cumagun, 2014). Several *Trichoderma* based formulations are available in Bangladesh, but their field efficacy yet to reach the desired level because of improper formulations with a lack of new potential strain. Hence, it is urgent to continue the searching for new native *Trichoderma* strains, develop cost-effective formulations with long shelf life properties. Therefore, the present investigations were aimed to evaluate the antifungal capability of the new native *Trichoderma* spp., develop a new formulation that leads to the long shelf life of the biocontrol agent, and their potentiality to control the damping off and foot rot diseases of chilli in green house conditions.

## MATERIALS AND METHODS

To serve the purposes of the present study, a set of three experiments were carried out in the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during 2017-2018.

***Collection of the biocontrol agent:*** The antagonist, *T. harzianum* was obtained from the Department of Plant Pathology, HSTU, Dinajpur which was formerly isolated and identified.

***Isolation and identification of test pathogens:*** *S. rolfsii* and *F. oxysporum* causing damping off and foot rot of chilli were isolated following the techniques of Dubey *et al*. (2009). Chilli plants infected with *S. rolfsii* and *F. oxysporum* showing characteristics damping off and foot rot symptoms were collected and washed under running tap water. The infected parts of chilli plants were cut into small pieces, surface sterilized by using 0.1% mercuric chloride for a minute followed by washing with sterilized double-distilled water three times. Thereafter, the pieces were transferred aseptically to potato dextrose agar (PDA) using sterilized forceps and incubated at 28±2 ˚C for 7 days in an incubator. Fungal growth was observed under the compound microscope and identified morphologically as the keys outlined in illustrated genera of imperfect fungi (Barnett and Hunter, 1998**)** and *Fusarium* laboratory manual book **(**Leslie and Summerell, 2006) and preserved at 4 ˚C until further use.

***Antimicrobial assays of T. harzianum:*** The *in vitro* bioefficacy of *T. harzianum* against *S. rolfsii* and *F. oxysporum* was evaluated following the techniques of Morton and Stroube (1955). A mycelial disc having 6 mm dia of the actively emerging colonies of both *T. harzianum* and test pathogens was placed at opposite sides on 90 mm Petri plates containing PDA leaving 1 Cm distance from the periphery. Whereas, some Petri plates were inoculated only with the test pathogen which served as control. All the Petri plates were replicated thrice and incubated at 28±2 ˚C for 7 days. The percent inhibition of radial mycelial growth was measured by following the formula (Edington *et al*., 1971):

$$L = [(C - T)/C] \times 100$$

Where L is inhibition of radial mycelial growth of pathogen; C is radial growth of the pathogen in control; T is radial growth of the pathogen in the presence of *Trichoderma* isolate.

***Preparation of T. harzianum formulation:*** In previous study it was observed that chickpea (*Cicer arietinum*) brans were the best substrate for the formulation of *T. harzianum*. Hence, chickpea bran was selected to prepare *T. harzianum* formulation. In brief, 200g of overnight soaked brans were taken in 500 mL Erlenmeyer flasks and autoclaved at 121 °C with 15 Psi for 15 minutes. Later, 10-12 mycelial discs (6 mm in dia) of actively growing culture of *T. harzianum* were transferred into each of the flasks in aseptic conditions, mixed thoroughly, and left undisturbed for 3 weeks in an incubator at 28±2 $^0$C. Then colonized brans were taken out, dried on sterilized brown paper, packed by using the sterile poly bag and kept at 4 °C until further use.

***Preparation of conidial suspension of T. harzianum:*** Full grown *T. harzianum* was used for preparing inoculum suspension following the method of Navaneetha *et al*. (2015). In brief, 10 mL sterilized double distilled water was poured on *T. harzianum* grown Petri plates and the surface was smoothly scrapped with the help of a sterilized glass rod. The conidial suspension was then sieved by using two layered of aseptic muslin cloth and taken in a beaker containing 400 mL double distilled water. Finally, a drop of Tween-20 was added to the suspension and the spores were counted as $2.45\times10^7$ conidia/mL by using haemocytometer (Improved Neubauer Hemocytometer, Marienfeld, Germany).

***Preparation of test pathogens:*** Both *S. rolfsii* and *F. oxysporum* inoculum were prepared by using moist wheat grains. In brief, 100 g of overnight soaked wheat grains were taken in a 500 mL Erlenmeyer flask and autoclaved at 121 °C with 15 psi for 15 minutes. Later, 5-7 mycelial discs (6 mm dia) of actively growing culture of the respective pathogens were added to the flasks, mixed thoroughly, and kept in an incubator at 28±2 ˚C. After 21 days, the colonized wheat grains were air dried on sterilized brown paper, packed in a poly bag, and stored at 4 °C until further use (Islam *et al*., 2007).

***Green house experiment:*** A pot culture bioassay was conducted to assess the efficacy of formulated *T. harzianum* to control damping off and foot rot of chilli. Each pot ($18\times12\times4$ Cm$^3$) was filled with a mixture of 4 kg sterilized sand and well decomposed cow dung (1:2). Before sowing of the seed, the pot soils were infested with the antagonist and test pathogens in the following treatment combinations: treatment 1. healthy control (no test pathogens and no formulated *T. harzianum*); treatment 2. control (only test pathogens); treatment 3. test pathogens + formulated *T. harzianum*; treatment 4. test pathogens + *T. harzianum* suspension; treatment 5. test pathogens + formulated *T. harzianum* + *T. harzianum* suspension. Each treatment was replicated thrice following a completely randomized design

where both formulated *T. harzianum* and test pathogens were applied @ 20 g/kg of soil at 7 days before and *T. harzianum* suspension was sprayed on the soil adjacent to the seedlings @ $2.45\times10^7$ CFU/mL at 3 days after sowing of chilli seeds. Fifty seeds were sown in each pot and optimum moisture was maintained from inoculation to final data collection. Data were collected on the following parameters: seed germination (%), damping off (%), foot rot (%), and growth attributes including shoot length (cm), root length (cm), shoot weight (gm), root weight (gm) and vigor index (%) after14 days of sowing (DAS). For computing growth attributes, ten plants from each of the pots were randomly selected as their means, and vigor index was calculated by using the following equation (Abdul-Baki and Anderson 1973):

Vigor Index (VI) = (Mean shoot length + Mean root length) × % Germination

***Shelf life study of formulated T. harzianum:*** The viability of *T. harzianum* was assessed using the chickpea bran-based formulations which were packaged @50 g/polyethylene bags and stored in different storage conditions as treatments viz., table top (TT, room temp.); refrigerator (Rf-4 °C), wooden shelve (WS, room temp.), tin container (TC, room temp.) and wooden locker (WL, room temp.). Three replications were maintained for each treatment and their colony forming units (CFU) were counted fortnightly up to 4 months following the serial dilution plate technique (Khan *et al*., 2011). In brief, 1 g of the formulation was taken in a test tube having 10 mL of sterilized double distilled water. The test tube was shaken vigorously and allowed to stand for 30 minutes. One (1) mL of the supernatant from the upper layer was taken and made serial dilution up to$10^6$. From the final dilution, 1 mL of the suspension was spread on PDA using a glass spreader. The plates were then incubated at 28±2 ˚C in an incubator for 7 days and CFU were counted following the formula (Khan *et al.*, 2011)

$$\text{CFU of sample} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Sample (g)}}$$

***Statistical analysis:*** Data collected on growth parameters along with diseases of chilli and shelf life of *T. harzianum* were statistically evaluated by using MSTAT-C package program. The means from all the treatments were estimated by DMRT (Duncan Multiple Range Test) at 5% level of probability (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

***In vitro evaluation of T. harzianum against test pathogens:*** The *in vitro* antagonistic potentiality of *T. harzianum* against *S. rolfsii* and *F. oxysporum* was assessed following dual culture techniques. *T. harzianum* showed remarkable antagonism against both of the test pathogens by inhibiting the growth. Maximum mycelial growth inhibition (81.67%) occurred in the case of *F. oxysporum* where *S. rolfsii* experienced 60.56% inhibition of mycelial growth after 7 days of dual culture with *T. harzianum* (Fig. 1A, 1B). Several studies also revealed the mycelial growth inhibition of *S. rolfsii* (55.00-67.91%) and *F. oxysporum* (44.00-75.70%) by the dual culture with *T. harzianum* (Parmar *et al*., 2015; Napitupulu *et al*., 2019). *T. harzianum* secrets various enzymes (chitinase, cellulase, β-1-3-glucanase, and protease) as well as secondary metabolites which offer lysis of the cell wall of the soil borne pathogens lead to the retard mycelial growth (Li *et al*., 2016; Saravanakumar *et al*., 2016). Moreover, the faster growth rate of *T. harzianum* exposing a higher amount of nutrient and space in the growth media leading starvation to death of the test pathogens might be other reasons for the growth suppression of the test pathogens (Devi *et al*., 2012).

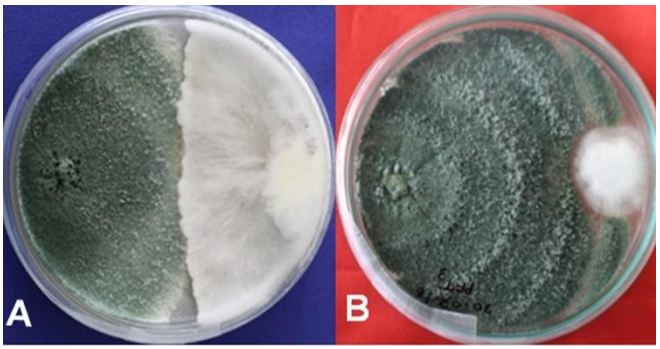


**Figure 1. Mycelial growth inhibition of *Sclerotium rolfsii* (A) and *Fusarium oxysporum* (B) by native *Trichoderma harzianum***

***Control of damping off and foot rot of chilli by formulated T. harzianum in green house conditions:*** Different combinations of chickpea bran-based formulated *T. harzianum* and conidial suspension of *T. harzianum* was applied to see their efficacy against damping off and foot rot of chilli. The results demonstrated that all form of *T. harzianum* has a significant effect to reduce the disease severity along with the increasing of plant growth parameters. The combined application of formulated *T. harzianum* and suspension was found to reduce maximum disease severity of damping off and foot rot disease by 81.80 and 84.61%, respectively over control. Interestingly, plants without exposure to test pathogens and treatments showed significantly reduced damping off (56.85%) and foot rot (59.00%) disease over control. A single application of formulated *T. harzianum* and suspensions were also found to reduce both damping off (36.40 and 22.77%) and foot rot (38.46 and 23.07%) diseases, respectively over control. Along with the decrease of disease severity, formulated *T. harzianum* and suspension alone or in combinations were found to increase the germination over control. However, the highest germination (70.28%) was recorded in the pot where both formulated *T. harzianum* and suspension were applied together followed by the application of formulated *T.*

**Table 1. Effect of *T. harzianum* on the germination and incidence of damping off and foot rot of chilli at 14 days after sowing (DAS)**

| Treatment combinations | Germination | | Damping off | | Foot rot | |
|---|---|---|---|---|---|---|
| | Germination (%) | Increased germination (%) | Damping off (%) | Reduction (%) | Foot rot (%) | Reduction (%) |
| Test pathogens + *T. harzianum* formulation | 64.67±2.52 | 31.10 | 9.33±0.58 | 36.40 | 8.00±1.00 | 38.46 |
| Test pathogens + *T. harzianum* suspension | 59.00±4.00 | 19.60 | 11.3±2.08 | 22.77 | 10.0±1.00 | 23.07 |
| Test pathogens + *T. harzianum* formulation + *T. harzianum* suspension | 84.00±1.00 | 70.28 | 2.67±0.58 | 81.80 | 2.00±1.00 | 84.61 |
| Healthy control (plant only) | 81.33±2.08 | 64.87 | 6.33±0.58 | 56.85 | 5.33±0.58 | 59.00 |
| Control (Only test pathogens) | 49.33±1.53 | - | 14.7±1.53 | - | 13.0±1.00 | - |

Test Pathogens-*S. rolfsii* and *F. oxysporum*

**Table 2. Effect of *T. harzianum* on the growth attributes of chilli at 14 days after sowing (DAS)**

| Treatment combinations | Shoot length (cm) | Root length (cm) | Shoot weight (gm) | Root weight (gm) | Vigor index (%) |
|---|---|---|---|---|---|
| Test pathogens + *T. harzianum* formulation | 6.50±0.40 | 3.43±0.38 | 0.054±0.003 | 0.022±0.001 | 642.6±61.03 |
| Test pathogens + *T. harzianum* suspension | 6.67±0.76 | 3.50±0.50 | 0.053±0.003 | 0.020±0.002 | 597.8±57.89 |
| Test pathogens + *T. harzianum* formulation + *T. harzianum* suspension | 7.73±0.25 | 3.97±0.25 | 0.061±0.003 | 0.024±0.000 | 983.0±49.19 |
| Healthy control (Only plant) | 6.97±0.16 | 3.65±0.15 | 0.056±0.003 | 0.021±0.002 | 863.1±09.50 |
| Control (Only test pathogens) | 4.10±0.40 | 2.07±0.15 | 0.037±0.005 | 0.018±0.003 | 304.6±34.19 |

Test Pathogens-*S. rolfsii* and *F. oxysporum*

*harzianum* (31.10%) and suspension (19.60%) alone (Table 1).

Combined or single use of formulated *T. harzianum* and suspension were significantly increased shoot length (6.50-7.73 cm), root length (3.43-3.97 cm), shoot weight (0.053-0.061 gm), root weight (0.020-0.024 gm), and vigor index (597.8-983%) in comparison to the application of only test pathogens (Table 2).

The fungus, *Trichoderma* is one of the widely used biocontrol agents to combat plant pathogens (Al-Ani, 2019). *Trichoderma* is not only used to control different crop diseases but also acts as growth promoting agent (Srivastava *et al*., 2016). In this study, along with the suppression of damping off and foot rot disease of chilli, *T. harzianum* also found to increase seed germination, shoot length, root length, shoot weight, root weight, vigor index as shown in many different crop plants (Subash *et al*., 2013; Rajendraprasad *et al.*, 2017; Uddin *et al*., 2018). *Trichoderma* exudates various secondary metabolites including gibberellins, indole-3-acetic acid, siderophores, etc. in the rhizospheric soil which might responsible for increased plant growth with a reduced disease (Vinale *et al*., 2008; Zhou *et al*., 2018).

***Shelf life of formulated T. harzianum:*** The spore density and survival ability of chickpea bran-based *T. harzianum* were observed in different storage conditions (Fig. 2). The results showed that the spore density of formulated *T. harzianum* was found to increase from the initial population ($22\times10^6$ CFU/g) to the maximum ($126.3\times10^6$ CFU/g) followed by a decline with the time goes. The highest spore density was recorded at 60 days of storage in the case of TT ($126.3\times10^6$ CFU/g), WS ($109\times10^6$ CFU/g) and Rf ($89.67\times10^6$ CFU/g), whereas, WL ($73\times10^6$ CFU/g), and TC ($66\times10^6$ CFU/g) exhibited their maximum spore density at 45 days of storage. However, at the end of 120 days, the highest spore density ($51.67\times10^6$ CFU/g) was counted TT followed by WS ($30\times10^6$ CFU/g), Rf ($14\times10^6$ CFU/g), WL ($8\times10^6$ CFU/g) and TC ($4.67\times10^6$ CFU/g). *Trichoderma* has been using for a long period mixing up with various substrates and found to have extended shelf life for their successful commercialization (Kolombet *et al*., 2008; Woo *et al*., 2014). Various studies also revealed the maximum spore density at 40-90 days of storage following a decline (Mev and Meena, 2003; Rajput *et al*., 2014). In the present study, TT storage enjoyed room temperature and more available light facilities compare to other storage conditions which may facilitate the production of maximum spores as 25-30 ˚C along with abundant light are the optimum condition for the growth to *Trichoderma* (Singh *et al*., 2013). However, the decline in population density of *T. harzianum* in all the stored conditions over time might

be due to the accumulation of lytic enzymes responsible for the decaying of viable cells (Kolombet *et al*., 2008).

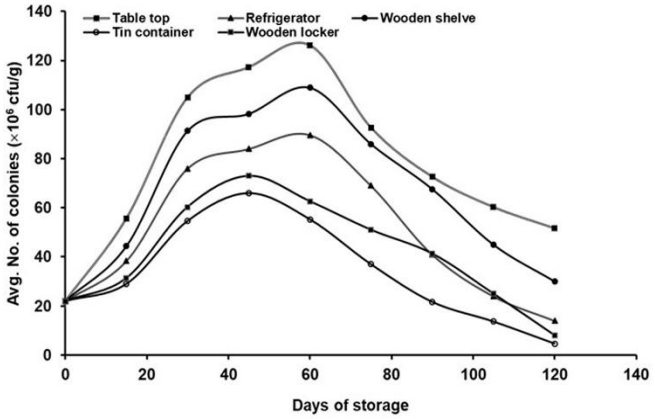


**Figure 2. Shelf life of chickpea bran-based formulated native *T. harzianum* in different storage conditions.**

***Conclusion:*** The gross results of this study reveal that the native *T. harzianum* has a strong antagonism effect against *S. rolfsii* and *F. oxysporum* both in the laboratory and green house conditions. In *in vitro* test, *T. harzianum* exhibited maximum mycelial growth inhibition of *S. rolfsii* (60.56%) and *F. oxysporum* (81.67%). Likewise, suppression of the growth of the fungal pathogen, *T. harzianum* also remarkably reduced disease severity along with the increasing of seed germination and other plant growth parameters. However, the maximum reduction of damping off (81.80%) and foot rot (84.61%) were recorded with the combined application of chickpea bran-based *T. harzianum* and its spore suspension. The native *T. harzianum* formulation with chickpea bran lives longer in convenient storage conditions offer a less expensive storage option for the farmers. Considering the bio-control ability along with extended shelf life, chickpea bran-based *T. harzianum* could be a potential candidate in the eco-friendly plant health management program.

***Authors Contributions statement:*** MMI: Preparing the layout and design, collection and/or assembly of data, analyzing data, and drafting the manuscript. ATMSI: Research concept, supervising the experiment, and reviewing the manuscript. MMH: Writing and critical revision of the manuscript. MMR: Designing and collecting the data. SkMMH: Analyzing the data. The authors read and approve the final manuscript.

***Conflict of interest:*** The authors declare that they do not have any competing interests.

***Acknowledgement:*** The authors would like to extend their sincere appreciation to the Department of Plant Pathology of Hajee Mohammad Danesh Science and Technology University, Dinajpur for providing the facilities throughout the research.

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