East African Scholars Journal of Agriculture and Life Sciences
Abbreviated Key Title: East African Scholars J Agri Life Sci
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online)
Published By East African Scholars Publisher, Kenya

Volume-4 | Issue-4 | April-2021| DOI:10.36349/easjals.2021.v04i04.04

OPEN ACCESS

Research Article

Biological Control of Damping off and Foot Rot of Chilli Using an Indigenous *Trichoderma harzianum*

Md. Mukul Islam[1], Md. Abu Zafor[2], Md. Emran Khan Chowdhury[3], A.T.M. Shafiqul Islam[1], Md. Mohidul Hasan[1*]

[1]Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh
[2]Department of Soil Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh
[3]Senior Scientific Officer, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh

Article History
Received: 26.02.2021
Accepted: 08.04.2021
Published: 25.04.2021

Journal homepage:
www.easpublisher.com

Quick Response Code

Abstract: Damping off and foot rot caused by *Sclerotium rolfsii* and *Fusarium oxysporum*, respectively are the two deadly diseases in chilli occur at the seedling stage. In the present study, the formulated indigenous *Trichoderma harzianum* and its spore suspension alone or in combinations were used to control damping off and foot rot diseases of chilli in net house conditions. The combined application of chickpea based formulated *T. harzianum* and its spore suspension was found to reduce damping off by 75.02-86.96% and foot rot by 74.29-89.28% over control caused by both the fungi. The combined application of the treatment was also found to increase seed germination by 66.23-90.57% over control. Furthermore, the combined application of formulated *T. harzianum* and its spore suspension also resulted higher shoot length (9.33 cm and 7.88 cm), root length (4.03 cm and 3.95 cm), and vigor index (1097% and 1010%) at 14 days after sowing over *S. rolfsii* and *F. oxysporum* inoculated plants, respectively. Based on the present investigation, the chickpea bran-based *Trichoderma harzianum* formulation and suspension might be exploited for the management of damping off and foot rot diseases of chilli.

Keywords: Damping off, Foot rot, Chilli, Biological control, *Trichoderma.*

INTRODUCTION

Chilli (*Capsicum annum* L.) is the second-ranked spice crop after onion in Bangladesh cultivated year round. In Bangladesh, the annual production of chilli was 1, 05,764 and 31,108 metric tons in the Rabi and Kharif season, covering 2, 11,824 and 43,637 acres of agricultural land, respectively [1]. However, the potential yield of chilli in Bangladesh is far low in comparison to other chilli producing countries. For the low yield of chilli, diseases play the most vital role and about 15 diseases of chilli including 12 fungal were reported in Bangladesh [2]. Among the fungal diseases, damping off and foot rot are the prominent and deleterious diseases caused by different soil-borne pathogens like *Sclerotium rolfsii*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium* sp. etc. [3].

Traditionally the growers use chemicals to control crop diseases and the long time unjudicial using of chemicals showed hazardous impacts on the environment and human health [4]. However, now the consumers are very concern about the health and environmental impact of the chemicals and therefore, researchers are moving to explore alternative ways of controlling the crop diseases without chemicals.

Biological control of plant diseases using different beneficial fungi and bacteria has gained significant interest as an effective alternative to chemical due to their durability, cost-effectiveness, and environmental safety nature [5, 6]. *Trichoderma* is one of the most studied and available fungus popularly used as a biological control agent against different crop diseases including damping off and foot rot caused by *Sclerotium*, *Fusarium*, *Rhizoctonia*, *Pythium*, *Phytophthora* etc. [7-9]. *Trichoderma* sp. employs various mechanisms like competition, antibiosis, parasitism, mycoparasitism, secretion of various enzymes, and hyphal interaction to combat different plant pathogens [10]. Along with the antagonistic efficacy against the respective pathogen, *Trichoderma* also plays the role in the increasing of seed germination, crop growth, and yield of the crop plants [11]. However, the repeated and long time using of the same strain of *Trichoderma* leads to the loss of its potentiality as a biocontrol agent. Hence, it needs to keep searching

***Corresponding Author:** Mohidul Hasan

for new potential strains with good efficacy of controlling damping off and foot rot diseases. Therefore, the present study has been designed to investigate the bio-efficacy of indigenous *Trichoderma* sp. against damping off and foot rot in chilli in the northern region of Bangladesh.

MATERIALS AND METHODS

The net house study of the bio-efficacy of *Trichoderma* sp. against damping off and foot rot of chilli was carried out in the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during 2017-2018.

Collection of the antagonist

T. harzianum was collected from the Department of Plant Pathology, which was previously isolated, identified, and preserved at 4 ^{O}C.

Collection, isolation, and identification of *Sclerotium rolfsii* and *Fusarium oxysporum*

Chilli plants having characteristic damping off and foot rot symptoms were collected, washed thoroughly with running tap water, and cut into small pieces with the help of scissors. The cut pieces were then surface sterilized with 0.1% mercuric chloride solution for a min. following three times washing with sterilized double-distilled water. The pieces were then aseptically placed on Petri plates containing potato dextrose agar (PDA) and incubated for 7 days at 28±2 ^{0}C. After colonization, the superficial growth of the fungi was sub-cultured for purification and identified morphologically under a compound microscope [12, 13] and preserved at 4 ^{0}C until further use.

Preparation of *T. harzianum* formulation

The commonly available substrate, chickpea brans were used to formulate the indigenous *T. harzianum*. In brief, 200g of overnight soaked chickpea bran were taken in 500 mL Erlenmeyer flasks and autoclaved for 15 min at 121 ^{0}C with 15 psi. Subsequently, chickpea bran was inoculated with actively growing 10-12 mycelial discs of *T. harzianum* (6 mm in dia). After vigorous mixing, the flasks were left undisturbed for 21 days at 28±2 ^{0}C. Finally, the colonized substrates were taken out and kept in a laminar air flow chamber for drying and stored in sterile poly bags at 4 ^{O}C until further use.

Preparation of *T. harzianum* suspension

For the preparation of spore suspension, 10 mL sterilized double distilled water was poured onto full-grown *T. harzianum* Petri plates and the surface was scrapped smoothly with the help of a sterilized glass rod. The suspension was then sieved carefully using a double-layered sterile muslin cloth and taken in a 400 mL beaker containing double distilled water. Later, a drop of Tween-20 was added to the suspension and the spores were counted to make 2.45×10^{7} conidia/mL using a hemocytometer [14].

Preparation of *S. rolfsii* and *F. oxysporum* inoculum

Overnight soaked wheat grains were used for the preparation of both *S. rolfsii* and *F. oxysporum* inoculum. In brief, 100 g moisten wheat grains were taken in a 500 mL Erlenmeyer flask and autoclaved for 15 min at 121 ^{0}c with 15 psi. After that, 7-10 mycelial discs (6 mm in dia) of vigorously growing culture of both the fungi were transferred aseptically to the flasks, mixed, and left undisturbed for 21 days at 28±2 ^{0}C. After colonization, the flasks were taken out and allowed to dry in a laminar air flow chamber and stored in a refrigerator at 4 ^{O}C for further use by using polybags [15].

Net house study to control damping off and foot rot of chilli

To evaluate the bio-efficacy of native *T. harzianum* against damping off and foot rot of chilli, two sets of experiments were conducted separately for *S. rolfsii* and *F. oxysporum*. A mixture of 1:2 ratios of sterilized soil (4 kg) and well-decomposed cow dung was used to fill the individual pots ($18\times12\times4$ cm^{3}). The treatment combinations used for both the fungal pathogens were as follows: healthy control (without fungal pathogen and *Trichoderma*); control (only fungal pathogen); formulated *T. harzianum* + fungal pathogen; *T. harzianum* spore suspension + fungal pathogen and formulated *T. harzianum* + *T. harzianum* spore suspension + fungal pathogen. Fifty chilli seeds were sown in each pot and all the treatments applied thrice following a completely randomized design. Formulated *T. harzianum* and the fungal pathogens were applied (@ 20 g/kg of soil) before 7 days of sowing where *T. harzianum* spore suspension was sprayed (@ 2.45×10^{7}cfu/mL) after three days of sowing. Data were recorded on damping off (%), foot rot (%), and seed germination (%) at 7 and 14 days after sowing (DAS). While data on different growth attributes [shoot length (cm), root length (cm), and vigor index (%)] were collected at 14 DAS. Vigor index was computed following the equation [16]:

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

STATISTICAL ANALYSIS

Collected data were analyzed using the MSTAT-C package program. The means were estimated by DMRT (Duncan Multiple Range Test) at 5% level of significance [17].

RESULTS AND DISCUSSION

The disease management potentiality of indigenous *T. harzianum* was evaluated against damping off and foot rot diseases of chilli caused by both *S. rolfsii* and *F. oxysporum*. The used formulated

T. harzianum alone or in combination with its spore suspension were found to reduce both damping off and foot rot of chilli. Besides, the application of *T. harzianum* also increased the seed germination and other agronomic traits that have a direct link to the yield of chilli.

When the plants inoculated with *S. rolfsii*, the combined application of formulated *T. harzianum* and its spore suspension was found to show minimum damping off (1% and 2.33%) and foot rot (1.33% and 3%) in comparison to formulated *T. harzianum* (3.67% and 4.67% of damping off and 3.67% and 5.67% of foot rot); *T. harzianum* spore suspension (5.33% and 6% of damping off and 7% and 7.67% foot rot) alone at 7 and 14 DAS, respectively. The combined treatment also yielded the maximum seed germination (80.67% and 82%) in comparison to formulated *T. harzianum* (59% and 61.33%) and *T. harzianum* spore suspension (51% and 54.33%) alone at 7 and 14 DAS, respectively (Table 1 and Table 2).

Table-1: Effect of different combinations of T. harzianum on the germination (%), damping off (%) and foot rot (%) of chilli at 7 DAS as affected by S. rolfsii and F. oxysporum

Treatments	*S. rolfsii*			*F. oxysporum*		
	Damping off (%)*	Foot rot (%)*	Germination (%)*	Damping off (%)*	Foot rot (%)*	Germination (%)*
T. harzianum formulation	3.67^{c}	3.67^{c}	59.00^{c}	7.67^{bc}	5.00^{c}	62.33^{c}
T. harzianum suspension	5.33^{b}	7.00^{b}	51.00^{d}	8.67^{b}	7.33^{b}	54.67^{d}
T. harzianum formulation + suspension	1.00^{e}	1.33^{d}	80.67^{a}	2.00^{d}	1.00^{d}	83.33^{a}
Control (without pathogen)	2.33^{d}	3.00^{c}	71.33^{b}	6.33^{c}	3.67^{c}	73.33^{b}
Control (only pathogen)	7.67^{a}	9.67^{a}	42.33^{e}	13.33^{a}	9.33^{a}	49.00^{d}
LSD	1.24	1.409	5.40	1.69	1.627	6.63
CV %	17.08	15.70	4.87	12.25	16.98	5.64

*The average of three replications; means followed by the same superscript letter in a column are not significant at 5% level by Duncan's multiple range test (DMRT).

In case of *F. oxysporum* inoculated plants, the combined application of formulated *T. harzianum* and its spore suspension was found to show minimum damping off (2% and 2.67%) and foot rot (1% and 2.33%) in comparison to the single application of formulated *T. harzianum* (7.67% and 9.33% of damping off and 5% and 6.33% of foot rot); *T. harzianum* spore suspension (8.67% and 10.67% of damping off and 7.33% and 8.67% foot rot) at 7 and 14 DAS, respectively. The combined treatment also resulted the higher seed germination (83.33% and 85.33%) in comparison to formulated *T. harzianum* (62.33% and 64.67%) and, *T. harzianum* spore suspension (54.67% and 59.67%) alone at 7 and 14 DAS, respectively (Table 1 and Table 2).

Table-2: Effect of different combinations of T. harzianum on the germination (%), damping off (%) and foot rot (%) of chilli at 14 DAS as affected by S. rolfsii and F. oxysporum

Treatments	*S. rolfsii*			*F. oxysporum*		
	Damping off (%)*	Foot rot (%)*	Germination (%)*	Damping off (%)*	Foot rot (%)*	Germination (%)*
T. harzianum formulation	4.67^{bc}	5.67^{c}	61.33^{c}	9.33^{bc}	6.33^{c}	64.67^{c}
T. harzianum suspension	6.00^{b}	7.67^{b}	54.33^{d}	10.67^{b}	8.67^{b}	59.67^{d}
T. harzianum formulation + suspension	2.33^{d}	3.00^{d}	82.00^{a}	2.67^{d}	2.33^{d}	85.33^{a}
Control (without pathogen)	3.33^{cd}	3.67^{d}	76.33^{b}	7.67^{c}	5.00^{c}	76.33^{b}
Control (only pathogen)	9.33^{a}	11.67^{a}	45.00^{e}	14.67^{a}	11.67^{a}	51.33^{e}
LSD	1.69	1.69	4.38	1.94	2.05	4.04
CV %	18.14	14.70	3.77	11.83	16.55	3.29

*The average of three replications; means followed by the same superscript letter in a column are not significant at 5% level by Duncan's multiple range test (DMRT)

Along with the reduced damping off and foot rot, the single or combined use of formulated *T. harzianum* and its spore suspension was also remarkably increased the shoot length, root length, and vigor index caused by both *S. rolfsii* and *F. oxysporum* inoculated plants. The combined application of formulated *T. harzianum* and its spore suspension was increased shoot length by 91.58% and 31.99%, root length by 65.84% and 28.66% and, vigor index by 233.13% and 117.77% in *S. rolfsii* and *F. oxysporum* inoculated plants, respectively at 14 DAS over control (Table 3).

Table-3: Effect of different combinations of T. harzianum on the growth parameters of chilli at 14 DAS as affected by S. rolfsii and F. oxysporum

Treatments	*S. rolfsii*			*F. oxysporum*		
	*Shoot length (cm)	*Root length (cm)	*Vigor index (%)	*Shoot length (cm)	*Root length (cm)	*Vigor index (%)
T. harzianum formulation	7.33[b]	3.23[b]	648.8[c]	6.83[b]	3.60[bc]	674.5[c]
T. harzianum suspension	6.90[b]	3.16[b]	546.9[c]	6.32[c]	3.50[c]	585.8[d]
T. harzianum formulation + suspension	9.33[a]	4.03[a]	1097[a]	7.88[a]	3.95[a]	1010[a]
Control (without pathogen)	7.17[b]	3.70[ab]	830.1[b]	7.17[b]	3.82[ab]	838.8[b]
Control (only pathogen)	4.87[c]	2.43[c]	329.3[d]	5.97[c]	3.07[d]	463.8[e]
LSD	0.63	0.53	107.5	0.42	0.23	59.43
CV %	4.91	8.86	8.56	3.41	3.53	4.57

*The average of three replications; means followed by the same superscript letter in a column are not significant at 5% level by Duncan's multiple range test (DMRT)

Different beneficial fungi and bacteria are considered as the major tools of biological control worldwide. However, the field application of biological controlling agents is getting popular for their effective and environmental friendly nature for the management of plant diseases in the last decade [18, 19]. Among the beneficial fungi, *Trichoderma* is one of the most effective and common biocontrol agents to manage crop diseases caused by *Fusarium*, *Sclerotium*, *Rhizoctonia*, *Pythium*, etc. [20]. *Trichoderma* can act against the plant pathogen in numerous ways including competition, antibiosis, mycoparasitism, inducing systemic host resistance, secretion, and production of chitinolytic enzymes, inhibitory compounds, etc. [21, 22]. Along with the super-fast growing ability in comparison to the other rhizospheric fungi, *Trichoderma* also secrets various enzymes like β-1-3-glucanase, indole-3-acetic acid, gibberellins, siderophores, etc., which might have the suppressing ability of growth of the pathogens results in reduced plant diseases with increased plant growth [23-25]. In addition to the suppression of plant pathogens, the single or combined use of *Trichoderma* spp. also proved to increase the percent germination, shoot length, root length, and vigor index in various crop plants [26-28]. Moreover, *Trichoderma* spp. activates the plant's inherent immunity and enhances nutrient uptake efficiency from the soil, which may trigger higher plant growth and yield in crop plants [29].

CONCLUSION

From the study, it can be concluded that the natively isolated *T. harzianum* possessed strong antagonism against *S. rolfsii* and *F. oxysporum* responsible for damping off and foot rot in chilli when applied alone or in combination. In addition to the suppressing ability of the soil-borne pathogens, *T. harzianum* also promoted seed germination, plant height, weight, and vigor index of the crop plant. However, it is necessary to observe the field efficacy of the isolated indigenous *T. harzianum* before final recommendation to the farmers.

REFERENCES

1. BBS (Bangladesh Bureau of statistics). (2017). Yearbook of Agricultural Statistics-2017. Statistics and Information Division, Ministry of Planning, Government of the People's Republic of Bangladesh.
2. Ahmed, H. U., & Hossain, M. M. (1985). Crop Disease Survey and Establishment of a Herbarium at BARI, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, 107.
3. Subash, N., Meenakshisundarm, M., & Sasikumar, C. (2013). In-vitro evaluation of different strains of *Trichoderma harzianum* as bio-control agents of chilli. International Journal of Biology, Pharmacy and Allied Sciences, 2(2), 495-500.
4. Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdisciplinary Toxicology, 9, (3-4), 90-100.
5. Papavizas, G. V., & Lumsden, R. D. (1980). Biological control of soil borne fungal propagules, Annual Review of Phytopathology, 18, 389-413.
6. Vinale, F., Sivasithamparam, K., Ghisalbertic, E. L., Marraa, R., Wooa, S. L., & Loritoa, M. (2008). *Trichoderma*-plant-pathogen interactions. Soil Biology and Biochemistry, 40, 1-10.
7. Weindling, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology, 22, 837-845.
8. Singh, S. P., Singh, H. B., & Singh, D. K. (2013). *Trichoderma harzianum* and *Pseudomonas* sp. mediated management of *Sclerotium rolfsii* rot in tomato (*Lycopersion esculentum* Mill.). The Bioscan, 8(3), 801-804.
9. Singh, J., Kumar, V., Srivastava, S., Kumar, A. & Singh, V. P. (2018). *In vitro* Evaluation of Trichoderma Species against *Fusarium oxysporum* f. sp. *lycopersici* Causing Tomato Wilt. Plant Pathology Journal, 17, 59-64.
10. Pal, K. K., & McSpadden Gardener, B. (2006). Biological control of plant pathogens. The Plant

Health Instructor. https://doi.org/10.1094/PHI-A-2006-1117-02
11. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species opportunistic, a virulent plant symbiont. Nature Reviews Microbiology, 2, 43-56.
12. Barnett, H. L., & Hunter, B. B. (1998). Illustrated genera of imperfect fungi. 4th Ed. APS Press, St Paul, Minnesota.
13. Leslie, J. F., & Summerell, B. A. (2006). The Fusarium laboratory manual. 1st Ed. Blackwell Publishing Ltd, Oxford, London.
14. Navaneetha, T., Prasad, R. D., & Venkateswara, R. L. (2015). Liquid Formulation of *Trichoderma* Species for management of gray mold in Castor (*Ricinus communis* L.) and alternariaster leaf blight in Sunflower (*Helianthus annuus* L.). Journal of Biofertilizers & Biopesticides, 6, 149.
15. Islam, M. N., Shamsuddula, A. M., & Ahmed, H. P. (2007). Comparative effectiveness of Trichoderma colonized organic wastes in controlling foot and root rot (*Sclerotium rolfsii*) disease of wheat. African Crop Science Conference Proceedings, 8, 2079-2082.
16. Abdul-Baki, A. A., & Anderson, J. D. (1973). Vigor determination in soybean by multiple criteria. Crop Science, 13(6), 630-633.
17. Gomez, K. A., & Gomez, A. A. (1984). Duncan's Multiple Range Test, Statistical Procedure for Agril. Res. 2nd Ed. A Wiley Inter-Science Publication, Johan and Sons, New York.
18. O'Brien, P. A. (2017). Biological control of plant diseases. Australasian Plant Pathology, 46, 293-304.
19. Shahzad, S., Rajput, A. Q., & Khanzada, M. A. (2018). Effect of different organic substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma harzianum*. Journal of Agricultural Science and Technology, 16, 731-745.
20. Al-Ani, L. K. T. (2019). Secondary metabolites of nonpathogenic Fusarium spp.; scope in agriculture. In: Singh, H. B., Keswani, C., Reddy, M. S., Royano, E. S., & Garcı´a-Estrada, C. (Eds.) Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms - Discovery and Applications. Springer, Singapore, 59-76.
21. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species opportunistic, a virulent plant symbiont. Nature Reviews Microbiology, 2, 43-56.
22. Braun, H., Woitsch, L., Hetzer, B., Geisen, R., Zange, B., & Schmidt-Heydt. M. (2018). Trichoderma *harzianum*: Inhibition of mycotoxin producing fungi and toxin biosynthesis. International journal of food microbiology, 280, 10-16.
23. Vinale, F., Sivasithamparam, K., Ghisalbertic, E. L., Marraa, R., Wooa, S. L., & Loritoa, M. (2008). *Trichoderma*-plant-pathogen interactions. Soil Biology and Biochemistry, 40, 1-10.
24. Li, Y., Sun, R., Yu, J., Saravanakumar, K., & Chen, J. (2016). Antagonistic and biocontrol potential of *Trichoderma asperellum* ZJSX5003 against the maize stalk rot pathogen Fusarium graminearum. Indian Journal of Microbiology, 56(3), 318-327.
25. Zhou, D., Huang, X. F., Guo, J., Dos-Santos, M. L., & Vivanco, J. M. (2018). *Trichoderma gamsii* affected herbivore feeding behaviour on Arabidopsis thaliana by modifying the leaf metabolome and phytohormones. Microbial Biotechnology, 11(6), 1195-1206.
26. Mahdizadehnaraghi, R., Heydari, A., Zamanizadeh, H. R., Rezaee, S., & Nikan, J. (2015). Biological control of garlic (Allium) white rot disease using antagonistic fungi-based bioformulations. Journal of Plant Protection Research, 55(2); 136-141.
27. Hajieghrari, B., & Mohammadi, M. (2016). Growth-promoting activity of indigenous *Trichoderma* isolates on wheat seed germination, seedling growth and yield. Australian Journal Crop Science, 10(9), 1339-1347.
28. Uddin, M. N., Rahman, U., Khan, W., Uddin, N., & Muhammad, M. (2018). Effect of Trichoderma harzianum on tomato plant growth and its antagonistic activity against *Pythium ultimum* and *Phytophthora capsici*. Egyptian Journal of Biological Pest control, 28, 32.
29. Wonglom, P., Daengsuwan, W., Ito, S. I., & Sunpapao, A. (2019). Biological control of *Sclerotium* fruit rot of snake fruit and stem rot of lettuce by *Trichoderma* sp. T76- 12/2 and the mechanisms involved. Physiological and Molecular Plant Pathology, 107, 1-7.

Cite This Article: Mukul Islam *et al* (2021). Biological Control of Damping off and Foot Rot of Chilli Using an Indigenous *Trichoderma Harzianum*. *East African Scholars J Agri Life Sci*, *4*(4), 101-105.