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BIOCONTROL EFFICACY OF THE ISOLATED *Trichoderma harzianum* AGAINST DAMPING OFF AND FOOT ROT DISEASES OF COUNTRY BEAN

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ABSTRACT

Sarkar MNH, Islam MM, Habib MA, Islam ATMS, Hasan MM (2021) Biocontrol efficacy of the isolated *Trichoderma harzianum* against damping off and foot rot diseases of country bean. *Int. J. Sustain. Crop Prod.* 16(2), 20-25.

The biocontrol efficacy of the isolated *Trichoderma harzianum* was evaluated against damping off and foot rot diseases of Country bean in the net house conditions. Chickpea bran based formulated *T. harzianum* and its spore suspension alone or in combination was found to reduce damping off by 42.780-83.84% and foot rot by 46.70-86.56% over control; where Provax 200 WP showed a reduction of damping off and root rot by 74.46% and 77.06%, respectively over control. The combined application of formulated *T. harzianum* and its spore suspension was also found to increase seed germination by 18.93-65.98% over control. Moreover, the same treatment combination also yielded increased shoot length (19.77-68.42%), root length (25.73-57.80%), and vigor index (47.23-175.08%) over *Sclerotium rolfsii* inoculated plants at 14 days after sowing. Considering the biocontrol ability in net house conditions, the isolated *T. harzianum* could be used in the field condition to control different soil borne diseases of vegetable crops.

Key words: *biocontrol, Trichoderma harzianum, country bean, damping off, foot rot*

INTRODUCTION

Country bean (*Lablab purpureus* L.) is an important pulse as well as a fodder crop in Bangladesh which is commonly known as sheem. The green pods of the crop are consumed as vegetables for their rich source of protein, carbohydrates, vitamins, and minerals (Magalingam *et al*. 2013). The annual production of Country bean was 0.137 million metric tons covering an area of 20.9 thousand hectares of agricultural land in Bangladesh (BBS 2018). However, the average production of Country beans in Bangladesh is reportedly very low (3-5 t/ha) as compared to the world production (10 t/ha) (Salim *et al*. 2013). Several factors are responsible for the lower yield of Country bean in Bangladesh where diseases are the most concerning issue. About 24 diseases of Country bean were reported in Bangladesh and among the diseases, damping off and foot rot are the most common and destructive diseases caused by various soil borne pathogens including *Sclerotium rolfsii*, *Fusarium solani*, *Rhizoctonia solani*, *Pythium* sp. etc. (Al-Abdalall 2010; Hossain *et al*. 2010).

Conventionally, farmers used chemicals for controlling diseases but the indiscriminate use of costly chemicals has grave impacts on the environment as well as human health. Besides the hazardous impact on human health and the environment, the long-term, and repeated use of chemicals also creating resistance in pathogens against various chemicals (Jayaraj *et al*. 2016). Therefore, peoples around the world are looking for the best alternative to chemicals for managing crop diseases.

Biological control with beneficial microorganisms could offer eco-friendly and sustainable management of crop diseases as a part of the Integrated Disease Management Program (IDM) (Mokhtar *et al*. 2011; O'Brien 2017). A beneficial fungus named *Trichoderma* is the most studied and effective biocontrol agent against various soil borne pathogens including *S. rolfsii* (Singh *et al*. 2013; Singh *et al*. 2019). *Trichoderma* can inhibit or suppress the growth of pathogenic fungi by their fast growth ability along with the secretion of various antifungal metabolites. In addition to the inhibition of the pathogenic fungal growth, *Trichoderma* can also trigger seed germination, growth, and development of the crop plants (Harman *et al*. 2004; Abd-El-Kader *et al*. 2015). However, repeated and long time use of the same strain of *Trichoderma* leads to loss of antifungal potentiality, hence, it is urgent to keep searching for new potential strains for the successful management of crop diseases. Therefore, the present investigation was designed to evaluate the disease management ability of the isolated *Trichoderma* against damping off and foot rot diseases of Country bean.

MATERIALS AND METHODS

Disease management ability of the isolated *T. harzianum* against damping off and foot rot of Country bean was carried out in net house conditions in the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the 2018-2019 crop season.

Collection of the biocontrol agent

T. harzianum was collected from the Department of Plant Pathology, HSTU which was previously isolated and identified morphologically and preserved at 4^0C for further use.

Isolation and identification of *Sclerotium rolfsii*

The naturally infected Country bean plants showing damping off and foot rot symptoms were collected, brought to the lab., washed thoroughly, and cut into small pieces using a sterile scissor. The cut pieces were then dipped

in 0.1% mercuric chloride solution in Petri plates for a min. following three times washing with sterilized double-distilled water. The pieces were then aseptically placed on potato dextrose agar (PDA) and incubated at 28±2^{0}C for 7 days. After colonization, the mycelial growth of the fungus was sub-cultured for purification and morphologically identified using a compound microscope (Barnett and Hunter, 1998) and kept at 4^{0}C until further use.

Preparation of *T. harzianum* formulation

Cheap and easily available Chickpea brans were used as a substrate for the preparation of *T. harzianum* formulation. In brief, chickpea brans were soaked overnight and 200 g were taken in each of 500 mL Erlenmeyer flasks for autoclaving at 121^{0}C with 15 psi for 15 min. The chickpea brans were then aseptically inoculated with full-grown 10-12 mycelial discs (6 mm in dia) of *T. harzianum*. The flasks were then mixed vigorously and left undisturbed at 28±2^{0}C for 21 days. After colonization, the flasks were taken out, dried under a laminar air flow chamber, and finally packed in sterile polythene bags at 4^{0}C until further use.

Preparation of *T. harzianum* spore suspension

The full-grown *T. harzianum* Petri plates were poured with 10 mL of double distilled sterilized water and then scrapped gently by using a glass rod. A double layered sterile muslin cloth was used to sieve the suspension to a beaker having 400 mL of double distilled water. Finally, a drop of Tween-20 was added to the beaker to sparse the suspension and the spore suspension was adjusted to 2.47×10^8 conidia/mL by using a hemocytometer (Navaneetha *et al*. 2015).

Preparation of *S. rolfsii* inoculum

Overnight soaked wheat grains (100 g) were taken in 500 mL Erlenmeyer flasks and autoclaved at 121^{0}C with 15 psi for 15 min for the preparation of *S. rolfsii* inoculum. Actively growing 7-10 mycelial discs (6 mm dia) of the fungus were aseptically transferred to the flasks, mixed vigorously, and incubated at 28±2^{0}C for 21 days. Finally, the well colonized flasks were taken out, dried in a laminar air flow chamber, packed in sterile poly bags, and kept in a refrigerator at 4^{0}C for further use (Islam *et al*. 2007).

Net house bioassay of *T. harzianum* to the management of damping off and foot rot diseases of Country bean

A net house experiment was conducted to evaluate the biocontrol ability of *T. harzianum* against damping off and foot rot diseases of Country bean caused by *S. rolfsii.* Each earthen pot (18×12×4 cm^3) was filled with 4 kg sterilized soil mixed with well decomposed cow dung (1:2). Before sowing of seeds (50 seeds/pot), the soil was inoculated with *S. rolfsii*. Following inoculation, the soil also received the treatments are as follows: control (no treatment); formulated *T. harzianum*; *T. harzianum* spore suspension; formulated *T. harzianum* + *T. harzianum* spore suspension; and Provax 200 WP (Carboxin 17.5% + Thiram 17.5%). Formulated *T. harzianum* and *S. rolfsii* inoculum were applied @ 20 g/kg of the soil before 7 days of seed sowing; *T. harzianum* spore suspension was sprayed @ 2.47×10^8 cfu/mL after 3 days of seed sowing, and Provax 200 WP was applied @ 2.5 g/kg seed. All the treatments were replicated thrice following a completely randomized design.

Collection and analysis of data

Data on damping off (%), foot rot (%), and seed germination (%) were recorded both at 7 and 14 days after sowing (DAS) while growth parameters *viz*., shoot length (cm), root length (cm) and vigor index (%) were recorded at 14 DAS. Vigor index was calculated following the formula (Abdul-Baki and Anderson 1973):

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

All the collected data were subjected to analysis by using MSTAT-C computer program. The means were compared by Duncan Multiple Range Test (DMRT) at 5% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Formulated *T. harzianum* and its spore suspension were used alone or in combination for the eco-friendly management of damping off and foot rot disease of Country bean in net house conditions. Data recorded at 7 DAS revealed that the combined application of formulated *T. harzianum* and its spore suspension reduced maximum damping off (83.84%) and foot rot (86.56%) diseases over control. The plants treated with Provax 200 WP was also found to reduce damping off and foot rot severity by 74.46% and 76.82%, respectively over control. The reduced damping off by 56% and 44% and foot rot by 71.01% and 46.70% were also recorded with a single application of formulated *T. harzianum* and its spore suspension, respectively. In addition to the reduction of disease severity, the combined application of formulated *T. harizianum* and its spore suspension was also increased seed germination (64.11%) over control followed by the single application of formulated *T. harzianum* (33.62%), *T. harzianum* spore suspension (18.93%), and Provex 200 WP (12.34%) (Table 1).

Table 1. Bioefficacy of different combinations of formulated *T. harzianum* and its spore suspension against damping off and foot rot diseases along with seed germination of Country bean at 7 DAS

Treatment combinations	Damping off		Foot rot		Germination	
	Damping off (%)	Reduction (%)	Foot rot (%)	Reduction (%)	Germination (%)	Increased germination (%)
S. rolfsii inoculum + *T. harzianum* formulation	2.86 bc	56.00	2.50 c	71.01	62.11 b	33.62
S. rolfsii inoculum + *T. harzianum* suspension	3.64 b	44.00	4.60 b	46.70	55.28 c	18.93
S. rolfsii inoculum + *T. harzianum* formulation and suspension	1.05 d	83.84	1.16 c	86.56	76.28 a	64.11
S. rolfsii inoculum + Provax 200 WP	1.66 cd	74.46	2.00 c	76.82	52.22 d	12.34
Control (only *S. rolfsii* inoculum)	6.50 a		8.63 a		46.48 e	-
LSD	1.628		1.339		2.162	
CV%	27.52		18.83		1.96	-

Each value is an average of 3 (three) replications. In a column, values having the same letter do not differ significantly at P≥ 0.05 level.

Similar to 7 DAS, the combined application of formulated *T. harzianum* and its spore suspension was also found to reduce maximum damping off (78.66%) and foot rot (79.82%) diseases over control at 14 DAS. The plants treated with Provax 200 WP was also reduced damping off and foot rot severity by 67.09% and 77.06%, respectively over control. The reduced damping off by 51.02% and 42.80% and foot rot by 61.31% and 48.03% were also recorded when the plants were treated with a single application of formulated *T. harzianum* and its spore suspension, respectively. The combined application of *T. harzianum* and its spore suspension also resulted in increased seed germination (65.98%) over control followed by the single application of formulated *T. harzianum* (41.48%), *T. harzianum* spore suspension (28.90%), and Provex 200 WP (9.53%) treated plants (Table 2).

Table 2. Bioefficacy of different combinations of formulated *T. harzianum* and its spore suspension against damping off and foot rot severity along with seed germination of Country bean at 14 DAS

Treatment combinations	Damping off		Foot rot		Germination	
	Damping off (%)	Reduction (%)	Foot rot (%)	Reduction (%)	Germination (%)	Increased germination (%)
S. rolfsii inoculum + *T. harzianum* formulation	3.81 c	51.02	3.93 c	61.31	69.33 b	41.48
S. rolfsii inoculum + *T. harzianum* suspension	4.45 b	42.80	5.28 b	48.03	63.16 c	28.90
S. rolfsii inoculum + *T. harzianum* formulation and suspension	1.66 e	78.66	2.05 d	79.82	81.33 a	65.98
S. rolfsii inoculum + Provax 200 WP	2.56 d	67.09	2.33 d	77.06	53.67 d	9.53
Control (only *S. rolfsii* inoculum)	7.78 a		10.16 a		49.00 e	-
LSD	0.569		0.603		4.470	-
CV%	7.46		6.75		3.75	

Each value is an average of 3 (three) replications. In a column, values having the same letter do not differ significantly at P≥ 0.05 level.

In addition to the reduced damping off and foot rot diseases of Country bean, the combined application of formulated *T. harzianum* and its spore suspension was also found to maximize shoot length (68.42%), root

length (57.80%), and vigor index (175.08%) over control. The single application of formulated *T. harzianum* also increased shoot length, root length, and vigor index by 27.59%, 27.42%, and 80.53%; single application spore suspension by 19.77%, 25.73%, and 47.23%; application of Provex 200 WP by 10.37%, 5.90%, and 19.70%, respectively over control (Table 3).

Table 3. Bioefficacy of different combinations of *T. harzianum* and its spore suspension against different agronomic traits of Country bean at 14 DAS

Treatment combinations	Shoot length		Root length		Vigor index	
	Shoot length (cm)	Increased (%)	Root length (cm)	Increased (%)	Vigor index (%)	Increased (%)
S. rolfsii inoculum + *T. harzianum* formulation	8.00 b	27.59	3.02 b	27.42	763.30 b	80.53
S. rolfsii inoculum + *T. harzianum* suspension	7.51 b	19.77	2.98 b	25.73	622.50 c	47.23
S. rolfsii inoculum + *T. harzianum* formulation and suspension	10.56 a	68.42	3.74 a	57.80	1163.04 a	175.08
S. rolfsii inoculum + Provax 200 WP	6.92 c	10.37	2.51 c	5.90	506.10 d	19.70
Control (only *S. rolfsii* inoculum)	6.27 d		2.37 c		422.80 e	-
LSD	0.551		0.162		33.748	-
CV%	3.73		2.94		2.55	-

Each value is an average of 3 (three) replications. In a column, values having the same letter do not differ significantly at P≥ 0.05 level.

With the increasing of safe food and pollution-free environmental necessity, the use of beneficial microbial organisms including fungi and bacteria in alternative to chemicals has gained importance in recent years (O'Brien 2017). As a result, the commonly available rhizospheric fungus *Trichoderma* has long been using as an effective biocontrol agent against various soil borne pathogens *viz.*, *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Pythium* etc. (Al-Ani 2019; Singh *et al*. 2019). *Trichoderma* either as formulated or spore suspension act against different pathogenic fungi using various mechanisms like antagonism, antibiosis, parasitism, mycoparasitism, competition, host resistance, secretion of secondary metabolites, etc. (Harman *et al*. 2004; Braun *et al*. 2018). The faster growing ability of *Trichoderma* may also facilitate to occupy more space and nutrients which leads to the suppression of growth and starvation to death of other pathogenic fungi (Devi *et al*. 2012). During mycoparasitism, *Trichoderma* triggered Heterotrimeric G protein, MAP kinase, and cAMP pathway where, G protein and MAPK are largely taking part in the secretion of antifungal compounds and the construction of infection structure and, cAMP pathway helps to inhibit the proliferation of pathogenic fungi by coiling with *Trichoderma* mycelium (Mukhopadhyay and Kumar, 2020). *Trichoderma* also exudates numerous enzymes and secondary metabolites like cellulase, chitinase, protease, β-1-3-glucanase, gibberellins, etc. which might suppress the growth of the pathogenic fungi and increased plant growth *viz.* seed germination, shoot length, root length, shoot weight, root weight, and vigor index by assisting nutrient uptake efficacy of the crop plants (Vinale *et al*. 2008; Hajieghrari and Mohammadi, 2016; Saravanakumar *et al*. 2016; Uddin *et al*. 2018; Zhou *et al*. 2018; Islam *et al*. 2021). In comparison to the single application, the combined application of formulated *Trichoderma* and its spore suspension showed better performance to control the diseases. However, formulated *Trichoderma* composed largely with mycelia where, its suspension composed with spore only, hence, all the possible mechanism of the suppression or killing of pathogenic fungi by *Trichoderma* might be obtained from the combined application rather than the single application of formulated *Trichoderma* and its suspension.

CONCLUSION

The overall results of the study revealed that the isolated *T. harzianum* is capable of showing its biocontrol ability against damping off and foot rot diseases of Country bean. The formulated *T. harzianum* (@ 20 g/kg of the soil before 7 days of seed sowing), as well as its spore suspension (@ 2.47×108 cfu/mL after 3 days of seed sowing), showed the potentiality of controlling the diseases, however, the best performance was recorded with combined application of both the treatments. Along with the ability to reduce the disease severity, the antagonist also proved to enhance different agronomic traits of Country bean including seed germination, shoot length, root

length, and vigor index. Considering the present findings, the isolated *T. harzianum* could be a good candidate for the management of soil borne diseases of different vegetables at the field level.

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