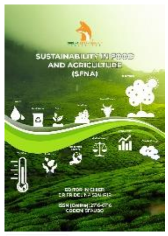
ZIBELINE INTERNATIONAL™ PUBLISHING
ISSN: 2716-6716 (Online)
CODEN: SFAUBO

Sustainability in Food and Agriculture (SFNA)

DOI: http://doi.org/10.26480/sfna.02.2021.92.98



CrossMark

RESEARCH ARTICLE

EVALUATION OF *TRICHODERMA HARZIANUM* IN CONTROLLING LATE BLIGHT OF POTATO

Husniara Khatun, Nusrat Sabiha Joya, AKM Amdadul Hoque and Mohammad Shahjahan Monjil*

Bangladesh Agricultural University, Mymensingh, Bangladesh
****Corresponding Author Email: smonjil@bau.edu.bd***

ARTICLE DETAILS

Article History:

Received 05 April 2021
Accepted 20 May 2021
Available online 01 June 2021

ABSTRACT

Evaluation of *Trichoderma harzianum* in reducing mycelial growth of *Phytophthora infestans* as well as in controlling late blight of potato was done in Microbiology and Biocontrol laboratory and Net-house, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during 2019-2020, Bangladesh. The experiment was conducted using popular potato variety Diamant and native variety Challisha. *T. harzianum* was evaluated in net-house and *in-vitro* condition. Fungicide Indofil M-45 (Mancozeb) was used as positive check for comparison. In Net-house experiment*, T. harzianum* increased plant height, number of tubers and fresh weight of tubers over control treatment for the both of the varieties of potato. *T. harzianum* on late blight of potato (var. Diamant) showed a significantly better management over control treatment. In comparison to non-treated control treatment, reduced infection of late blight was found in the *T. harzianum* treated plants, whereas increase disease severity was calculated in control treatment. After harvesting, tuber infection in *T. harzianum* treated plants was not observed. Percent inhibition of tuber infection over control was 100%. Indofil M-45 suppressed Disease severity but comparatively less effective than *T. harzianum* in the long run. In case of variety Challisha similar trend of results were observed. In dual culture assay, *T. harzianum* showed highest inhibitory effect in suppressing mycelial growth of *P. infestans*. *T. harzianum* showed 86.67% inhibition of mycelial growth of *P. infestans* over control. Thus, it can be concluded that *T. harzianum* is effective to control late blight of potato and sometimes comparable to chemical fungicide in net-house condition.

KEYWORDS

Potato, T*richoderma harzianum,* Late blight, *Phytophthora infestans*, Disease severity

1. INTRODUCTION

Potato (*Solanum tuberosum*) belongs to the Family-Solanaceae is one of the largest grown vegetable crops in the world (Barnett 2013). It is the fourth main food crop after rice, wheat and maize in respect of cultivated area and total production in the globe, and more than a billion people worldwide eat potato (Andre et al., 2014). According to FAOSTAT (2019) 388 million tons of potato were produced in 2017 throughout the world. Potato remains an essential crop in Europe, where per capita production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in southern and eastern Asia. As of 2017, China led the world in potato production and together with India, produced 26.3% of the world's potatoes (FAOSTAT, 2019). The countries having the high yield of potatoes worldwide were New Zealand (49.31 ton/ha), United states of America (48.23 ton/ha), Belgium (47.56 ton/ha), Germany (46.78 ton/ha), Netherlands (45.97 ton/ha), Ireland (44.82 ton/ha), Denmark (43.68 ton/ha), Australia (38.95 ton/ha), Turkey (33.60 ton/ha), Brazil 30.98 ton/ha, Japan (29.27 ton/ha), Egypt (26.38 ton/ha) and India (22.30 ton/ha) (FAOSTAT, 2019).

In Bangladesh, total cultivated areas of potato were 1157000 acres (468421.03 hectares), production of potatoes were 9655000 tons and average yield was 20.61 ton/ha in 2018-19 (BBS, 2019). Compared to other countries in the world, yield of potato was low in Bangladesh due to absence of proper management system as well as prevailing highly favourable condition for disease development. Diseases play a vital role in reducing the potato tuber yield.

Potatoes suffer from various diseases viz., Late Blight, Early Blight, Black Scurf, Stem Rot, Scab, Hallow heart, Black heart, Soft rot, Common rust, Bacterial wilt, Verticillium wilt, Powdery mildew, Potato yellow mosaic, Potato yellow dwarf etc. (Jassica et al., 2017; Rahim et al., 2017). Among the diseases, Late blight is the most devastating and economically important disease of potato caused by *Phytophthora infestans* (Agrios, 2005). In Bangladesh, yield of 2017-18 was significantly decreased and the average loss of tuber yield of potato production each year due to late blight is 15–30% (BBS, 2018). If the weather during the vegetation period is favourable for *P. infestans*, then the average loss of yield can reach up to 50% (Ronis et al., 2005).

There are different strategies to combat late blight disease of potato however; the most widely used approach at a global level is fungicide. In Bangladesh, farmers often use excessive fungicides available in the local markets. Indiscriminate use of fungicides encourages the development of resistance in *P. infestans*, increases the production cost but more important being it is detrimental to the environment as well as human health (Siddiqui et al., 2016). Because of the worsening problems in disease control, a serious search is needed to identify alternative methods for plant protection, which are less dependent on chemicals and are more

Quick Response Code	Access this article online	
	Website: www.sfna.org.my	**DOI:** 10.26480/sfna.02.2021.92.98

Cite the Article: Husniara Khatun, Nusrat Sabiha Joya, AKM Amdadul Hoque and Mohammad Shahjahan Monjil. (2021). Evaluation of *Trichoderma Harzianum* in Controlling Late Blight of Potato. *Sustainability in Food and Agriculture, 2(2): 92-98.*

eco-friendly. Biological control of plant pathogens has been considered as a potential control strategy in recent years (Sharma et al., 2019). Monjil and Ulfat (2020) showed that in weather conditions of Bangladesh is favourable for the development of the late blight disease. Skrabule (2010) reported that in an organic potato production field, where no fungicides were used, was infected two weeks earlier than a conventional production field with applied bio-agents.

Bio-control of the disease through antagonists is an eco-friendly approach apart from better alternative to the use of chemicals. Bio-agents will remain the best option to reduce risks of crop losses due to late blight and increase tuber yield. Naggar et al. (2016) observed that foliar spray of fungal bioagent suspensions significantly reduce the infection of late blight. The integration between induction of systemic resistance treatment and antagonist treatment by each of bio-agent showed a stronger effect in reducing the severity of late blight (Naggar et al.,2016).

Trichoderma is a genus of fungi in the family Hypocreaceae that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts (Harman et al., 2004). This refers to the ability of several *Trichoderma* species to form mutualistic endophytic relationships with several plant species (Bae et al., 2011). Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants (Harman 2006). The various mechanisms include antibiosis, parasitism, inducing host-plant resistance, and competition. Most biocontrol agents are from the species *T. harzianum, T. asperellum, T. viride* and *T. hamatum.* The biocontrol agent generally grows in its natural habitat on the root surface, and so affects root disease in particular, but can also be effective against foliar diseases.

Keeping these views in mind, the present study was designed with the following objectives:

1. To study the effect of *T. harzianum* in reducing mycelial growth of *Phytophthora infestans* in *in-vitro* condition.
2. To evaluate the effect of *T. harzianum* against late blight of potato in control net-house condition.

2. METHODOLODY

The present study was conducted to evaluate *Trichoderma harzianum* in controlling late blight of potato and to estimate the late blight disease severity of potato under net house and laboratory condition. The pot experiments was conducted in the Net-house, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and Laboratory experiment was conducted in the Microbiology and Biocontrol Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The experiments were carried out during the period July 2018 to September 2019.

Diamant, a popular potato variety in Bangladesh, and Challisha (a popular native variety) were selected for the experiment. Apparently good looking and healthy potato tubers were collected form market for planting in pot soil. Three treatments were selected for pot experiment. Three pots were prepared for each treatment.

Treatment No.	Treatment Name	Concentration
T_1	*Trichoderma harzianum* (BAU-Biofungicide)	30g/L
T_2	Indofil M-45 (Mancozeb)	2g/L
T_3	Control (water spray)	-

Trichoderma harzianum isolated from BAU-Biofungicide which was collected from Eco-Friendly Plant Disease Management Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh BAU-Biofungicide formulated from naturally occurring fungus, *Trichoderma* by growing on an organic substrate (Agro-wastes). It protects crops from different diseases (root rot, foot rot, wilt etc.) caused by different harmful fungi (*Fusarium, Sclerotium, Rhizotonia, Pythium, Macrophomina,* etc.). BAU-Biofungicide protects crop from both seed borne and soil borne diseases (Hossain, 2011). Isolation of *T. harzianum* from BAU-Biofungicide was done for *in-vitro* dual culture test against *P. infestans*. Otherwise, in spray experiment formulated product of BAU-Biofungicide (30g/L) prepared by Eco-Friendly Plant Disease Management Laboratory was applied.

Apparently healthy and good looking potato seed tubers were taken. The tubers were washed very carefully firstly with tap water to clean the dirt presents on the tuber and then washed it with distilled water. A clean sterilized knife was used to cut those tubers. Tubers were cut carefully so that in every piece of tuber contains at least two eyes or buds. For treating the tubers with the selected treatments, a working solution was made separately into 500 ml conical flask. Some tubers were soaked into the solution and some tubers were kept untreated as control. Two pieces of potato tubers were planted in each pot.

Selected treatments with suggested concentrations were made separately into 500 ml conical flask. Then the solutions were poured into spray bottle and spread over the foliage for five times. At 15 days after sowing (DAS), first times treatments were spread over the potato plants. Then second, third, fourth and fifth spray was done at 12 days interval. Disease severity was determined by observing disease symptoms on leaves of potato. The mean values of rating (blight %) were determined to get rating score of the material under the following scale with little modification (Henfling, 1987).

Disease severity grade	Description
0	No late blight observable
1	Late blight present. Maximum 10 lesions per plant
2	Foliage affected, not more than 15 leaves are affected
3	Plant looks healthy, but lesions are easily seen at closer distance. Maximum foliage area affected by lesions or destroyed corresponds to more than 20 leaflets
4	Plant looks diseased.15-20% of foliage covered with late blight
5	Late blight easily seen on most plants. About 25% of the foliage is covered with lesions or destroyed
6	All plants are affected. Lower leaves are dead. About half the foliage area is destroyed
7	About 75% of each plant is affected. Leaves of the lower half of plants are destroyed
8	Only top leaves are green. Many stems have large lesions.
9	A few top leaves still have some green areas. Most stem have lesions or are dead
10	All leaves and stems dead

Disease severity was calculated by the following formula (Yaganza et al., 2004)

$$\text{Disease severity} = \frac{\text{Total number of rating}}{\text{Total rating} \times \text{Maximum grade}} \times 100$$

After harvesting Potato tubers were examined and sorted into infected and uninfected tubers. Tuber infection was calculated using following formula (Taylor et al., 2012)

$$\%\,\text{Tuber infection} = \frac{\text{Number of infected tuber}}{\text{Total number of tubers counted}} \times 100$$

Percent inhibition of tuber infection over control was calculated using formula by Onyeani et al*.* (2015) is given below

$$\text{Inhibition } \% = \frac{C-T}{C} \times 100$$

Where, C= Tuber infection in control, T= Tuber infection in treatment

2.1 Preparation of CSA-culture media (Carrot Sucrose Agar media) for culturing *P. infestans*

One liter of Carrot Sucrose Agar media (carrot-220g, Sucrose-20g, Agar-10g and water up to 1000 ml) was prepared following the method described by Kumbar (2017). Briefly, fresh red carrots of 220g were taken and cut into small pieces. Cut pieces of carrots were autoclaved in conical flask containing 500ml of double distilled water at 121°C under 15 PSI pressure for 30 minutes. After autoclaving, carrot pieces were poured in a blender and blended the warm carrot. The homogenate mixture was squeezed with 3-4 layered muslin cloth. Volume of the homogenate mixture was made up to 1 liter with double distilled water. Then 20 g sucrose and 10 g Agar were added in 1 liter carrot solution. Thus, 1000 ml Carrot Sucrose Agar (CSA) media was prepared. The flasks were plugged with cotton and sterilized by autoclaving at 121°C under 15 PSI pressure for 20 minutes. When the media reached to 45-50°C was poured into the Petri-dishes.

Pure culture of a virulant race of *Phytophthora infestans* was provided by Prof. Dr. M. S. Monjil, Microbiology and Biocontrol Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. For experimental purpose, Carrot Sucrose Agar (CSA) medium was used to multiply *P. infestans* for further use. In solidified CSA media, a block of mycelia of *P. infestans* was transferred to CSA medium in each Petri-dish with the help of sterile forceps. After growth of mycelium, the culture was used for further work. Actively growing hyphae of the fungus were cut into 10 mm discs using sterilized cork borer and were placed at the centre of each plate and incubated at 24°C. Thus, necessary multiplication of the pathogen was done. To induce sporulation, the fungus was sub cultured in Carrot Sucrose Agar (CSA) culture media at 24°C.The plates were then maintained in Carrot Sucrose Agar (CSA) plate in refrigerator at 4°C for further studies.

2.2 *In-vitro* evaluation of *T. harzanium* in suppressing mycelial growth of *P. infestans*

The effective fungal isolates were tested for their ability to suppress the growth of *P. infestans in-vitro* using dual culture method. A plug of 10 mm diameter containing mycelium taken from 7 days old *P. infestans* was placed at the side of CSA plates and a plug of 10 mm diameter containing fungal colonies were placed opposite side of *P. infestans*. This method was done by using the treatment of *T. harzianum*. There were five replicates per treatment. In all cases these plates were incubated at 24°C for 4 days. Data on mycelial growth (cm) were recorded at one day to four days after inoculation.

Chemical fungicide Indofil M-45 (Mancozeb) was added to CSA media following the modified poison food technique described by (Hossen et al., 2017). For each treatment five replications were used. Inoculated (treated) plates were incubated at 24°C for 4 days. Data on mycelial growth (cm) were recorded at one day to four days after inoculation due to first growth of inoculated *Trichoderma*, which compared the whole plate by days. The control plate only contained mycelial plug at the middle of Petri dish. There were five replicates per treatment. In all cases these plates were incubated at 24°C for 4 days. After incubation period, when the growth in control plate reached maximum (9 cm diameter), the redial mycelial growth of pathogen was measured.

Percent inhibition of growth over control was calculated using equation of Vincent (1927) given below.

$$\text{Inhibition } \% = \frac{C-T}{C} \times 100$$

Where, C= Growth in control, T= Growth in treatment

2.3 Data Analysis

The recorded data were analyzed statistically with MSTAT-C programming software to find out the level of significance and the variations among the respective data which were compared following Duncan's New Multiple Range Test (DMRT) according to Gomez and Gomez, (1984)

3. Results

3.1 Efficacy of *T. harzianum* on plant height (cm) of potato var. Diamant and Challisha

Table 1: Effect of *Trichoderma harzianum* on plant height (cm) of potato var. Diamant and Challisha

Treatment	Plant height (cm)											
	Diamant						Challisha					
	10 DAS	15 DAS	20 DAS	25DAS	30DAS	35 DAS	10 DAS	15 DAS	20 DAS	25 DAS	30DAS	35 DAS
T_1	9.72 a	13b	25.6a	34.83 a	40.5a	46 ab	27.72 a	38.43a	45.6 a	50.27b	54.5 ab	56ab
T_2	5.80 b	14a	23.18 b	27.33 c	35 b	46.33 a	22.80 b	37.36 b	43.18 b	51.75 a	55 a	56.33 a
T_3	4.19 c	10.33 c	21.44 c	29.33 b	34.33c	42.17 b	14.19 c	33.38c	36.44 c	41.14c	44.33 b	49.17 b
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	3.24	4.85	4.33	2.73	3.54	2.21	1.13	3.39	4.5	0.83	4.30	2.00

T_1: *Trichoderma harzianum*, T_2: Indofil M-45, T_3: Control, DAS= Day after sowing

Data were subjected to Duncan's Multiple Range Test (DMRT) using a statistical computer package (MSTAT C). Each value represents the mean and standard deviation of five replications. In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). **P < 0.01 versus control treatment (Significant at 1% level of probability).

Efficacy of *T. harzianum* on plant height of potato was calculated from 10 to 35 days after sowing (DAS) with five-day intervals. Significant variation was observed in Diamant variety among the different treatments (Table 1). At 10 DAS, highest plant height was observed in *T. harzianum* (9.72cm) followed by Indofil M-45 (5.80cm). On the same times, lowest plant height was recorded in Control (4.19cm). At 15 DAS, highest plant height was observed in Indofil M-45 (14cm) followed by *T. harzianum* (13cm). The lowest plant height was recorded in Control (10.33cm). At 20 DAS, highest plant height was observed in *T. harzianum* (25.6cm) followed by Indofil M-45 (23.18cm). On the same day, lowest plant height was recorded in Control (21.44cm). At 25 DAS, highest plant height was observed in *T. harzianum* (34.83cm) followed by Control (29.33cm). The lowest plant height was recorded in Indofil M-45 (27.33cm). At 30 DAS, highest plant height was observed in *T. harzianum* (40.5cm) followed by Indofil M-45 (35cm). Lowest plant height was recorded in Indofil M-45 (34.33cm). At 35 DAS, highest plant height was observed in *T. harzianum* (46cm) and Indofil M-45 (46.33cm). On the same times, lowest plant height was recorded in Control (42.17cm).

In Challisha variety, at 10 DAS, highest plant height was observed in *T. harzianum* (27.72cm) followed by Indofil M-45 (22.8cm). On the same times, lowest plant height was recorded in Control (14.19cm). At 15 DAS, highest plant height was observed in Control (38.43cm) followed by Indofil M-45 (37.36cm). The lowest plant height was recorded in Control (33.38cm). At 20 DAS, highest plant height was observed in *T. harzianum* (45.6cm) followed by Indofil M-45(43.18cm). On the other hand, lowest plant height was recorded in Control (36.44cm). At 25 DAS, highest plant height was observed in Indofil M-45 (51.75cm) followed by *T. harzianum* (50.27cm). On the same times, lowest plant height was recorded in Control (41.14cm). At 30 DAS, highest plant height was observed in *T. harzianum* (54.5cm) and Indofil M-45 (55cm). On the other hand, lowest plant height was recorded in Control (44.33cm). At 35 DAS, highest plant height was observed in *T. harzianum* (56cm) and Indofil M-45 (56.33cm). Lowest plant height was recorded in Control (49.17cm).

3.2 Efficacy of *T. harzianum* on total number of tubers / plant and weight of tubers / plant of potato var. Diamant and Challisha

Table 2: Effect of *Trichoderma harzianum* on total number of tubers / plant, weight of tubers / plant of potato var. Diamant and Challisha

Treatments	Diamant		Challisha	
	Total no. of tubers/plant	Weight of tubers/plant (g)	Total no. of tubers/plant	Weight of tubers/plant (g)
T_1	2.08a	11.46 a	2.68 b	4.42 a
T_2	1.56b	8.94 b	3.19 a	3.73 b
T_3	1.33 c	8.61 c	2.56 c	3.11 c
Level of significance	**	**	**	**
CV (%)	2.20	4.71	3.12	0.83

T_1: *Trichoderma harzianum*, T_2: Indofil M-45, T_3: Control

Data were subjected to Duncan's Multiple Range Test (DMRT) using a statistical computer package (MSTAT C). Each value represents the mean and standard deviation of five replications. In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). **P < 0.01 versus control treatment (Significant at 1% level of probability).

Efficacy of T. *harzianum* on total number of tubers/plant and weight of tubers/plant of potato differed significantly among the different treatments (Table 2). In case of Diamant variety, highest number of tubers per potato plant was observed in *T. harzianum* (2.08) followed by Indofil

M-45 (1.56). The lowest number of tubers per potato plant was recorded in Control (1.33). In case of weight of tuber per plant, highest weight of tubers per plant was observed in *T. harzianum* (11.46g) followed by Indofil M-45 (8.94g). The lowest weight of tubers per plant was recorded in Control (8.61g).

In case of Challisha variety, highest number of tubers per potato plant was observed in Indofil M-45 (3.19) followed by *T. harzianum* (2.68). The lowest number of tubers per potato plant was recorded in Control (2.56). In case of total weight of tubers per plant, highest weight of tubers per plant was observed in *T. harzianum* (4.42g) followed by Indofil M-45 (3.73g). The lowest weight of tubers per plant was recorded in Control (3.11g).

3.3 Efficacy of T. *harzianum* on tuber infection (%) of variety Diamant and Challisha

Table 3: Effect of *Trichoderma harzianum* on tuber infection (%) of variety Diamant and Challisha

Treatment	Diamant		Challisha	
	Tuber infection (%)	% Inhibition of Tuber infection over control	Tuber infection (%)	% Inhibition of Tuber infection over control
T_1	0.00 e	100	15.29 c	86.67
T_2	16 b	60	21.56 b	63.33
T_3	38.46 a	0	39.47 a	0
Level of significance	**	-	**	-
CV (%)	2.93	-	3.93	-

T_1: *Trichoderma harzianum,* T_2: Indofil M-45, T_3: Control

Data were subjected to Duncan's Multiple Range Test (DMRT) using a statistical computer package (MSTAT C). Each value represents the mean and standard deviation of five replications. In a column, figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). **P < 0.01 versus control treatment (Significant at 1% level of probability).

Efficacy of *T. harzianum* on tuber infection (%) differed significantly among the different treatments (Table 3). In case of Diamant variety, highest percent of tuber infection was observed in Control (38.46%) followed by Indofil M-45 (16%). Lowest tuber infection recorded in *T. harzianum* (0%). Highest percent inhibition of tuber infection over control observed in *T. harzianum* (100%) followed by Indofil M-45 *(60%)*. In case of Challisha variety, highest percent of tuber infection was observed in Control (39.47%) followed by Indofil M-45 (21.56%). Lowest tuber infection was recorded in *T. harzianum* (15.29%). Highest percent inhibition of tuber infection over control was observed in *T. harzianum* (86.67%) and lowest percent inhibition of tuber infection over control was observed in Indofil M-45 (63.33%).

3.4 Effect of *T. harzianum* on disease severity of late blight of potato var. Diamant

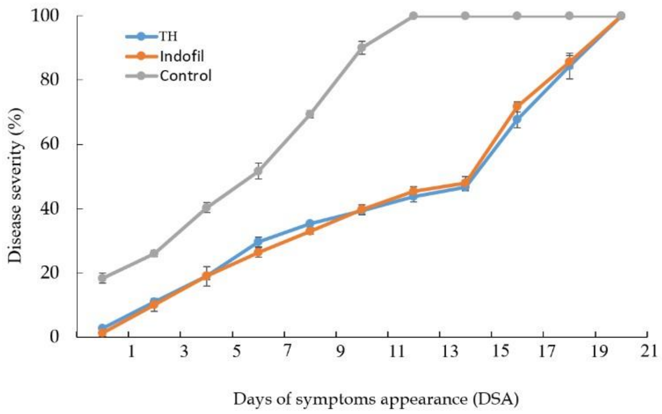
Figure 1: Effect of *Trichoderma harzianum* on disease severity of late blight of potato var. Diamant Treated and untreated control plants at 1, 7, 15 days of symptoms appearance (DSA) respectively

Table 4: Percent inhibition of mycelial growth of *Phytophthora infestans* over control by using *Trichoderma harzianum* in dual culture assay

Treatment	% inhibition of mycelial growth (cm) of *P. infestans* over control			
	1 DAI	2 DAI	3 DAI	4 DAI
T_1	48.4	78.57	80.49	82.11
T_2	48	77.78	81.7	81.44
T_3	0	0	0	0

DAI=Days After Inoculation

T_1: *Trichoderma harzianum,* T_2: Indofil M-45, T_3: Control



TH= *Trichoderma harzianum*, Indofil=Indofil M-45

Figure 2: Effect of *Trichoderma harzianum* on disease severity of late blight of potato var. Diamant, Data represent in line graph. Each value represents the mean and standard deviation of 5 replications.

Effect of *T. harzianum* on disease severity of late blight of potato was evaluated and presented in Fig. 2 & 3. In comparison to non-treated control treatment, *T. harzianum* showed a significant result against late blight of potato. The first symptoms were observed at 4 weeks after planting. In control treatment, disease symptoms rapidly increased and within thirteen days disease severity reached to 100%, whereas *T. harzianum* it was 43.67%. In *T. harzianum* treated plants, disease severity was lower in comparison to control treatment. At 1st day after symptoms appearance disease severity of *T. harzianum* treated plants was 2.67%, whereas in control it was 18.33%. From the next count of disease severity, reduced infection was found in the *T. harzianum* treated plants. From 5 days after disease appearance, late blight severity increased slowly and reached to maximum level of disease infection within 21 days. Thus, Treatment of *T. harzianum* significantly reduced the disease progress of late blight disease in potato and it delayed 9 days for reaching to maximum disease severity than control treatment.

In comparison to non-treated control treatment, Indofil M-45 showed a significant result against late blight of potato. The first symptoms were observed at 4 weeks after planting. In control treatment, disease symptoms rapidly increased and within thirteen days disease severity reached to 100%, whereas Indofil M-45 was 45.33%. In Indofil M-45 treated plants, disease severity was lower in comparison to control treatment. At 1st day after symptoms appearance disease severity of Indofil M-45 treated plants was 1.33%, whereas in control it was 18.33%. From the next count of disease severity, reduced infection was found in the Indofil M-45 treated plants. Thus, Treatment of Indofil M-45 significantly reduced the disease progress of late blight disease in potato.

3.5 Effect of *T. harzianum* on disease severity of late blight of potato var. Challisha

Effect of *T. harzianum* on disease severity of late blight of potato was evaluated and presented in Fig. 4 & 5. In comparison to non-treated control treatment, *T. harzianum* showed a significant result against late blight of potato. The first symptoms were observed at 4 weeks after planting. In control treatment, disease symptoms rapidly increased and within thirteen days disease severity reached to 100%, whereas *T. harzianum* it was 59.67%. In *T. harzianum* treated plants, disease severity was lower in comparison to control treatment. At 1st day after symptoms appearance disease severity of *T. harzianum* treated plants was 4.33%, whereas in control it was 20.33%. From the next count of disease severity, reduced infection was found in the *T. harzianum* treated plants. From the next count of disease severity, reduced infection was found in the *T. harzianum* treated plants. From 5 days after disease appearance, late blight severity increased slowly and reached to maximum level of disease infection within 21 days. Thus, Treatment of *T. harzianum* significantly reduced the disease progress of late blight disease in potato and it delayed 10-11 days for reaching to maximum disease severity than control treatment.

Figure 3: Effect of *Trichoderma harzianum* on disease severity of late blight of potato var. Challisha, Treated and untreated control plants at 1, 7, 15 days of symptoms appearance (DSA) respectively.

In comparison to non-treated control treatment, Indofil M-45 showed a significant result against late blight of potato. The first symptoms were observed at 4 weeks after planting. In control treatment, disease symptoms rapidly increased and within thirteen days disease severity reached to 100%, whereas Indofil M-45 was 59.67%. In Indofil M-45 treated plants, disease severity was lower in comparison to control treatment. At 1st day after symptoms appearance disease severity of Indofil M-45 treated plants was 4.67%, whereas in control it was 18.33%. From the next count of disease severity, reduced infection was found in the Indofil M-45 treated plants. Thus, Treatment of Indofil M-45 significantly reduced the disease progress of late blight disease in potato.

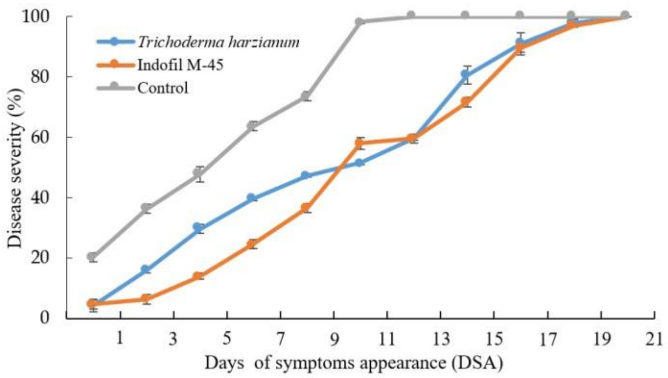
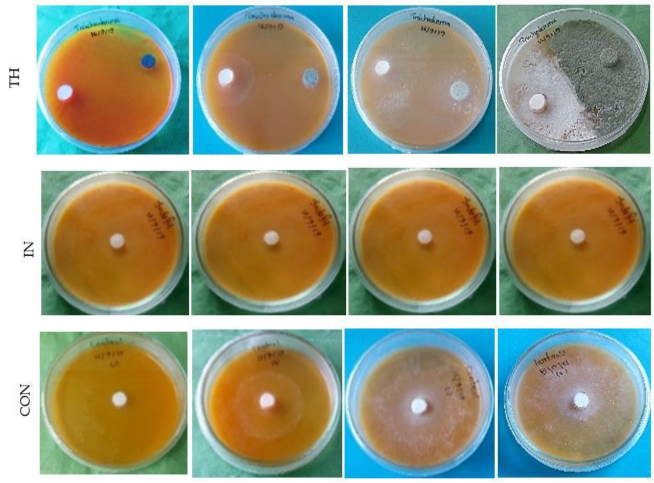


Figure 4: Effect of *Trichoderma harzianum* on disease severity of late blight of potato var. Challisha, Data represent in line graph. Each value represents the mean and standard deviation of 5 replications.

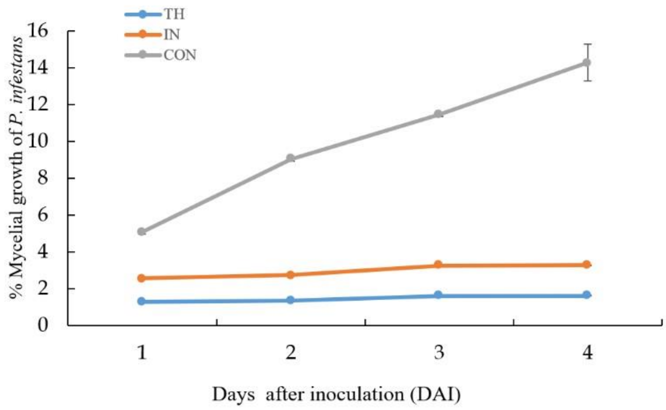
3.6 Mycelial Growth suppression of *P. infestans* over control by using *T. harzianum* in dual culture assay

The results were analyzed based on the data recorded on 2, 3 and 4 days after inoculation during conducting the experiments. Growth suppression of *P. infestans* by using T. *harzianum* in dual culture assay was shown in Table 4. The growth of mycelia of *P. infestans* was recorded from 1 cm to 9 cm in Petri dishes during dual culture assay. Average lowest growth (1.2 cm) was observed in case of Indofil M-45, whereas highest (9 cm) was observed in control plate (Fig. 6 & 7). The highest percent inhibition (82.11%) on mycelial growth over control was observed in case of the treatment of *T. harzianum* followed by Indofil M-45 (Table 4). The lowest inhibition was found in Control (Table 4).



TH=*Trichoderma harzianum,* IN= Indofil M-45, CON= Control

Figure 5: Growth suppression of *P. infestans* by *Trichoderma harzianum* in dual culture assay, Pictorial view of mycelial growth of *P. infestans*.



TH=*Trichoderma harzianum,* IN= Indofil M-45, CON= Control

Figure 6: Growth suppression of *P. infestans* by *Trichoderma harzianum* in dual culture assay, Data presented in line graph.

4. Discussion

The present research work was undertaken for evaluating *Trichoderma harzianum* against *P. infestans* and its effect on disease severity of late blight of potato. The causal organism of late blight, *P. infestans*, was collected and studied its morphological colony characters and microscopic observation of sporangium before use in experiment. Colony colour of *P. infestans* was white and lemon shaped sporangia were observed under the field microscope (Fig. 1). These results were confirmed by comparing the report of Rahim et al. (2017) who mentioned the typical symptoms of late blight of potato in Bangladesh condition and Bush et al. (2006), who published the identified characters of *P. infestans* which are white mycelial growth with lemon shaped sporangia.

Effect of *T. harzianum* on late blight of potato (var. Diamant) was recorded as a significantly enhanced management of late blight over control treatments (Fig. 2 & 3). Comparatively reduced infection was found in the *T. harzianum* treated plants, whereas increase disease severity was calculated in control treatment. No tuber infection in *T. harzianum* treated plant compare to control. Fatima et al. (2015) evaluated biocontrol potential of *T. harzianum* and their antagonistic activities including competition and colonization against *P. infestans. T. harzianum* application increased the fresh weight of potato and higher grade of potatoes. Hossain et al. (2014) reported that *Trichoderma* based formulation, BAU-

Biofungicide-treated potato seed tubers resulted lower late blight incidence and severity followed by Bavistin (Carbendazim). Late blight incidence and severity was higher in control.

Results from this study have shown that growth of potato plants is enhanced by the application of the *T. harzianum*. The number of stem as well as highest plant height were recorded where potato tubers were treated with *T. harzianum* followed by control treatment. *T. harzianum* was effective in reducing the plant mortality, promoting the plant growth, and increasing the yield at experimental field as well as at farmers' fields (Kabdwa et al., 2019). Noorulla et al. (2018) recorded low disease severity and higher yield of *T. harzianum* and *Pseudomonas fluorescens* treated plant compared to control. Naggar et al. (2016) observed that *T. harzianum* showed a stronger effect in reducing the severity of late blight. The increased growth in terms of plant height, weight and number of tubers may be as a result of production of growth hormones (Contreras-Cornejo et al., 2014), increased uptake of nutrients or enhancement of root growth (Viterbo et al., 2002). Menezes-Blackburn et al. (2014), while working on *Trichoderma* spp., reported an increase in plant growth due to the ability of the microbe to help in bioavailability of different mineral nutrients via solubilisation or chelation. Therefore, these growth promotion attributes of the *T. harzianum* may have led to the enhanced growth and production in the present study.

Indofil M-45 (Mancozeb) was used as a positive check (positive control) treatment for the comparision of the effect of bioagent used in the experiment. In Diamant variety, Disease severity was suppressed but comparatively less effective than *T. harzianum*. Das et al. (2005) reported that alternate spray of Indofil M-45 or Ridomil MZ-72 @0.25% at 15 days interval along with Mancozeb @0.2% at 8 days interval would be useful for effective and economic management of late blight and minimize the yield loss. Zarger and Rizvi (2015) reported that Indofil M-45 was effective in inhibiting the mycelial growth at all concentrations followed by Ridomil at 0.5% against *P. infestans*. In case of variety Challisha (native) similar results were observed. *T. harzianum* (86.67%) was much effective to control late blight in the native variety Challisha.

In the dual culture assay, substantially rapid culture growth of *T. harzianum* isolate was observed than the *P. infestans* isolate, and *T. harzianum* speedily moved to the *P. infestans* isolate and exceeded its culture growth (Table 3). This results might be corroborated to the rapid growth ability of the *Trichoderma* species, giving it an added advantage in competing for nutrients and space with the pathogen (Adnan et al., 2019; Kariuki et al., 2020). Faruk and Rahman (2015) also reported that the Trichoderma sp. were able to suppress the pathogen's colony by rapid growth of the Trichoderma sp. This may have led to starvation of the pathogen due to the competition for the limited resources resulting to the death of the P. infestans (Sempere and Santamarina, 2007). Naggar et al. (2016) investigated biological control of *P. infestans* causing late blight of potato using *T. harzianum* which was antagonistic and highly restricted the growth of the late blight pathogen by 83.3 and 84.4 %; respectively over the control in agar assays while *P. fluorescens* restricted the growth of *P. infestans* by 75.1 to 77.6 % respectively. Thus, it can be concluded that *T. harzianum* might be effectively applied to control late blight of potato and sometimes comparable to chemical fungicide in field condition.

5. Conclusion

In comparison to positive and negative check *Trichoderma harzianum* was found superior to control late blight severity. In Diamant variety, no tuber infection was observed in *T. harzianum* treated plants, and percent inhibition of tuber infection over control was 100%. In Native variety, very low tuber infection was observed in *T. harzianum* treated plants, and percent inhibition of tuber infection over control was 86.67%. *T. harzianum* showed best result in producing total number of tubers, total number of tubers/plant, weight of tuber/plant and decreasing disease severity.In artificial media, *T. harzianum* also found effective to inhibit the growth of *P. infestans*. Thus, application of *T. harzianum* can be a good alternate management practice for late blight disease of potato.

References

Agrios, G.N., 2005. Plant Pathology. 5th Edition. Academic Press, London, New York, 922.

Adnan, M., Islam, W., Shabbire, A., Khang, K.A., Ghram, H.A., Huang, Z., Chenb, H.Y.H., Lu, G., 2019. Plant defense against fungal pathogens by antagonistic fungi with *Trichoderma* in focus. Microbial Pathogenesis, 129, Pp. 7-18.

Andre, C.M., Legay, S., Iammarino, C., 2014. The potato in human diet: a complex matrix with potential health benefits. Potato Research, 57, Pp. 201–214. doi: 10.1007/s11540-015-9287-3

Bae, H., Roberts, D.P., Lim, H.S., Strem, M.D., Park, S.C., Ryu, C.M., Melnick, R.L., Bailey, B.A., 2011. "Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms". Journal of Molecular Plant-Microbe Interactions, 24(3), Pp. 36–51. doi: 10.1094/MPMI-09-10-0221.

BBS, 2018. Statistical Year Book of Bangladesh. Statistics Division, Agriculture wing, Ministry of Planting, Govt. of the People's Republic of Bangladesh, Dhaka. 318.

BBS, 2019. Statistical Year Book of Bangladesh. Statistics Division, Agriculture wing, Ministry of Planting, Govt. of the People's Republic of Bangladesh, Dhaka. 318.

Bernett, B.A., 2013. Potato yield and nutrient acquisition are supported by the soil "bacteriome". Degree of Master of Science, Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, Colorado.

Bush, E.A., Stromberg, E.L., Hong, C., Richardson, P.A., Kong, P., 2006. Illustration of key morphological characteristics of *Phytophthora* species identified in Virginia nursery irrigation water. Journal of Plant Health Progress, 7(1), doi: 10.1094/PHP-2006-0621-01-RS

Contreras-Cornejo, H.A., Macías-Rodríguez, L.I., Alfaro-Cuevas, R., López-Bucio, J., 2014. *Trichoderma* spp. improve growth of Arabidopsis seedlings under salt stress through enhanced root development, osmolite production, and Na+ elimination through root exudates. Molecular Plant-Microbe Interaction, 27, Pp. 503-514.

Das, B.K., Gupto, V.K., Kumar, G., Singh, P.H., Baishya, L.K.U., 2005. Evaluation of Fungicides / Fungicide Combinations Against Potato Late Blight In Autumn Tps Grown Crop In Meghalaya Hills. Central Potato Research Station, Shillong-793009, Meghalaya, India 32, Pp. 3-4.

FAOSTAT, 2019. Food and Agriculture Organization of the United Nations, Statistical Pocketbook. FAO, Rome, Italy.

Fatima, K., Henni, J.E., Mebrouk, K., Noureddine, K., 2015. Antagonistic effect of *Trichoderma harzianum* against *Phytophthora infestans* in the North-west of Algeria. International Journal of Agronomy and Agricultural Research (IJAAR), 6(4), Pp. 44-53.

Faruk, M.I., Rahman, M.L. 2015. Management of tomato seedling rot disease (*Rhizoctonia solani*) in seedbed with *Trichoderma harzianum* bio-fungicide. Agricultural Science Research Journal, 5: 14-20.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research (second edition). John Wiley and Sons, New York.

Harman, G.E., 2006. "Overview of mechanisms and uses of *Trichoderma* spp". Phytopathology, 96 (2), Pp. 190–194. doi: 10.1094/PHYTO-96-0190

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. "*Trichoderma* species—opportunistic avirulent plant symbionts". Nature Reviews Microbiology, 2(1), Pp. 43–56. https://doi.org/10.1038/nrmicro797

Henfling, J. W., 1987. Late Blight of potato *Phytopthora infestans*. International Potato Center (CIP), Lima, Peru. Technical information bulatin 4.

Hossain, I., 2011. BAU-Biofungicide: Unique eco-friendly means and new dimension of plant disease control in Bangladesh. Leaflet published from the Dept. of Plant Pathology, BAU, Mymensingh, Pp. 8-11.

Hossain, I., Taslima, F., Kashem, M.A., Hakim, M.A., Rafii, M.Y., Latif, M.A., 2014. Biological control of late blight caused by *Phytophthora infestans* of potato. Life Science Journal, 11, Pp. 242-247.

Hossen, M.T., Sohag, M.A.S., Monjil, M.S., 2017. Comparative efficacy of Garlic, BAU-biofungicide, Bavistin and Tilt on seed borne fungal flora in chilli. Journal of Bangladesh Agricultural University, 15(1), Pp. 41–46.

Jassica, R., Jacobsen, B., 2017. Bacterial and fungal diseases of potato and their management. Department of Plant Sciences and Plant Pathology, Montana State University, Pp. 1-9.

Kabdwa, B.C., Sharma, R., Tewari, R., Tewari, A., Singh, R.P., Dandona, J.K., 2019. Field efficacy of different combinations of *Trichoderma harzianum, Pseudomonas fluorescens,* and arbuscular mycorrhiza fungus against the major diseases of tomato in Uttarakhand (India). Egyptian Journal of Biological Pest Control, 29, Pp. 1. https://doi.org/10.1186/s41938-018-0103-7.

Kariuki, W.G., Mungai, N.W., Otaye, D.O., Thuita, M., Muema, E., Korir, H., Masso C., 2020. Antagonistic effects of biocontrol agents against *Phytophthora infestans* and growth stimulation in tomatoes. African Crop Science Journal, Vol. 28 (s1), Pp. 55-70.

Kumbar, B., 2017. Standardization of specific media for *Phytophthora infestans.* Global Journal of Bio-Science and Biotechnology, 6(2), Pp. 374-376.

Lal, M., Sharma, S., Yadav, S., Kumar, S., 2017. Management of Late Blight of Potato IntechOpen, Pp. 22-27. doi: 10.5772/intechopen.72472.

Menezes-Blackburn, D., Jorquera, M.A., Gianfreda, L., Greiner, R., Maria de la Luz, M. 2014. A novel phosphorus biofertilization strategy using cattle manure treated with phytase-nanoclay complexes. Biology and Fertility of Soils 50, Pp. 583-592.

Monjil, M., Ulfat, T., 2020. Severity of potato late blight under different weather conditions in Mymensingh region of Bangladesh. Progressive Agriculture, 31(2), Pp. 74-80. https://doi.org/10.3329/pa.v31i2.50711

Naggar, M.A., Abouleid, H.Z., Deeb, H.M., Kareem, F., 2016. Biological control of potato late blight by means of induction systemic resistance and antagonism. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(1), Pp. 1338.

Noorulla, H., Thulasiram, K., Shashidhar, K.R., Santhosha, H.M., 2018. Effective Management Strategy against Potato Late Blight Incited by *Phytophthora infestans*. International Journal of Current Microbiology and Applied Sciences, **7**, Pp. 09.

Onyeani, C.A., Amusa, N.A., 2015. Incidence and Severity of Anthracnose in Mango Fruits and its Control with Plant Extracts in South West Nigeria. International Journal of Agricultural Research, 10, Pp. 33-43.

Rahim, M., Anowar, M.M., Begum, K.S., Monjil, M.S., 2016. Management of insect pest and disease of vegetable crops in Bangladesh (Bengali). USAID/ Agro-inputs project (AIP) and Agro-input retailers networks (AIRN).

Ronis, A., Tamosiunas, K., 2005. The comparison of the decision support systems "NegFry" and "PLANT PLUS" for the control of late blight in Lithuania. Scientific works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture-Horticulture and vegetable growing, 24, Pp. 362–370.

Sempere, F., Santamarina, M., 2007. In vitro biocontrol analysis of *Alternaria alternata* (Fr.) Keissler under different environmental conditions. Mycopathologia, 163, Pp. 183-190.

Sharma, R., Sukla, N., Archana, E., Kumar, J., 2019. Present Status and Future Prospects of Bio-Agents in Agriculture. International Journal of Current Microbiology and Applied Sciences, 8, Pp. 04.

Siddique, N.A., Aktar, M.S., Swapon, N.H., 2016. Comparative Efficacy of Different Fungicides against Late Blight Diseases of Potato incited by *Phytophthora infestans* (Mont.) de Bary and its Management. Journal of Plant Pathology & Microbiology, 7, Pp. 364.

Skrabule, I., 2010. Evaluation of potato breeding clones' inorganic and conventional growing conditions. Proceedings of EUCARPIA 2nd conference of the Organic and Lowinput Agriculture section. Paris, France, Pp. 102–105.

Taylor, R. J., Pasche, J. S., Shew, H. D., Lannon, K. R., Gudmestad, N. C. 2012. Tuber rot of potato caused by *Phytophthora nicotianae*: Isolate aggressiveness and cultivar susceptibility. Plant Dis. 96 Pp. 693-704.

Vincent, J. M., 1927. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 59, Pp. 850.

Viterbo, A., Montero, M., Ramot, O., Friesem, D., Monte, E., Liobell, A., Chet, I. 2002. Expression regulation of the endochitinase chit 39 from *Trichoderma asperellum* (*T. harzianum* T203). Current Genetics, 42, Pp.114-122.

Yaganza, S.E., Arul, J. Tweddell, R.J., 2004. Effect of pre-storage application of different organic and iorganic salts on stored potato quality. Potato Research, 46, Pp. 167-178.

Zarger, S.A., Rizvi, G., Parashar, R., 2015. Studies on Leaf Spot Disease of Mango and Its Management. International Journal of pharmacy and biological sciences, 6(1)(B**)**, Pp. 769-776.