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STUDY ON EFFICACY OF TRICHODERMA IN BIOLOGICAL CONTROL AGAINST PURPLE BLOTCH OF ONION

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Abstract

The efficacy of the antagonistic microorganism, *Trichoderma harzianum* in controlling purple blotch of onion (*Alternaria porri)* was investigated in the laboratory and field of Plant Pathology Division of the Bangladesh Institute of Nuclear Agriculture, Mymensingh. The antagonistic activity of three isolates of *T. harzianum* was observed against *A. porri* through dual culture technique and the superior one was used in the field experiment. Five treatments were given viz. T_1: Seed treatment with *T. harzianum*, T_2: Seedling treatment with *T. harzianum*, T_3: Foliar application with *T. harzianum*, T_4: Seed treatment and seedling treatment with *T. harzianum* and T_5: Control (*A. porri* in absence of *T. harzianum*). The variety BARIpiaj-1 was used as tested crop. All treatments significantly reduced purple blotch disease intensity compared to the control. The least disease intensity on leaf and flower stalk was found in the treatment T_4 (Seed and seedling treatment with *T. harzianum*) followed by the treatment T_1 (Seed treatment with *T. harzianum*). The highest disease reduction over control (63.1% on leaf and 47.18% on flower stalk) was observed in the treatment T_4 (seed and seedling treatment with *T. harzianum*) followed by the seed treatment only (T_1) which gave disease reduction 58.7% on leaf and 37.03% on flower stalk over control. Therefore, application of *T. harzianum* both in seed and seedling was more effective in reducing disease intensity than individual application of *T harzianum* in seed or seedling.

Key words: *Trichoderma harzianum*, biological control, onion, purple blotch

Introduction

Onion (*Allium cepa* L.), family Alliaceae is economically an important horticulture crop cultivated all over the world. In Bangladesh it is one of the most important spices crop and an integral part of Bangladeshi diet. It is known as protective food because of its special nutritive value. Onions are rich in sulphur, fibres, potassium, iron, calcium, vitamin B, vitamin C but low in fat, cholesterol. Apart from being used as food it is also famous for its medicinal values. Onion contains anticancer compounds that assist in inhibiting the growth of cancer cells and thus protect against the development of colon and liver cancer (Rahim, 1991). Antioxidants are also provided by onion with its sweet flavor and distinct aroma.

In Bangladesh, onion is commercially cultivated in the greater district of Faridpur, Rajshahi, Jessore, Pabna and Kushtia. The average yield of onion is 9.71 t ha^{-1} (BBS, 2020) which is very low as compared to other leading onion growing countries like China, India,

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Egypt and USA. Onion may suffer from 22 fungal diseases among which purple blotch caused by *Alternaria porri* (Ellis) is noted as the major disease in many onion growing countries including Bangladesh (Meah and Khan, 1987). Purple blotch can infect all above ground parts of the plant as well as the bulb. Initial symptoms appear on older leaves as small water soaked lesions that quickly develop purple center with yellow margin under favorable conditions (Verma and Sharma, 1999). In Bangladesh, it is reported that the disease can reduce bulb yield up to 41-44% (Islam *et al.*, 2020). In favorable condition there may cause complete failure of onion bulb and seed production due to the disease (Sharma, 1986). Damage of foliage and breaking of floral stalks due to purple blotch is considered as the major factor for the reduction of true seed production of onion in our country.

The foliar fungicide Rovral (dicarboximide group) was found to be effective against the disease (Rahman, 1990). However synthetic fungicides have adverse effect on environment, human health and these also have negative impact on the beneficial microorganisms in the soil (Mimbs *et al*, 2016). Thus it is appropriate to minimize the use of the fungicides in crop production. Biological control for the management of plant pathogens is considered as an alternative environment friendly strategy for sustainable agriculture where *Trichoderma* sp. has gained attention as an effective fungal antagonist against foliage and soil borne pathogens (Amin *et al.*, 2010). The antagonist is able to produce compounds that can induce resistance in host plant against the pathogen. In addition to disease suppression, treatment with the antagonistic organisms can increase root growth, uptake of nutrients, productivity of plants (Harman, 2006). The efficacy of *T. harzianum, T. pseudokoningii* and *T. virens* on the inhibition of mycelial growth and spore germination of *A. porri* were observed by other researchers (Imtiaz and Lee, 2008 and Tyagi *et al.*, 1990). With this view, the present study was undertaken to investigate the efficacy of the antagonistic microorganism *T. harzianum* in controlling purple blotch of onion.

Materials and Methods

The experiment was conducted in the laboratory and field at the Plant Pathology Division of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during 2017-18.

Sources and maintenance of *Trichoderma harzianum* and *Alternaria porri*

The isolates of *T. harzianum* were obtained from the Plant Pathology Division, BINA. Pure culture of *T. harzianum* was made in PDA plates following hyphal tip culture technique (Tuite, 1969) and preserved at 5ºC for further use. The isolate of *A. porii* was obtained from onion leaf showing typical symptom of purple blotch collected from the experimental field of BINA at Mymensingh. Infected plant parts were cut into 3 mm segments including the advancing margins of infection. The segments were surface disinfected with 0.5% sodium hypochlorite solution for 2 minutes, washed thoroughly with sterilized water and dried between folds of filter paper. Then the segments were transferred in PDA plates and incubated for 7 days at 28ºC. Pure culture was obtained by sub-culturing

for three times. Pathogenicity test on the crop was carried out following the method as described by Abdel Hafez (2014). Pure culture of the final isolate was maintained on PDA plates and kept in the refrigerator (5ºC) until used.

In vitro evaluation of *T. harzianum* against *A. porri*

The antagonistic activity of three isolates of *T. harzianum* was evaluated against *A. porri* through dual culture technique. Both *T. harzianum* and *A. porri* were cultured individually on PDA media in petridishes. A disc (5mm diameter) of five days old culture of *T. harzianum* was inoculated on one side of PDA plate (9 cm) and another disc of *A. porri* of the same size from ten days old culture was inoculated at the opposite side in the same plate. The distance between the discs was approximately 5 cm. In the control treatment, a sterile PDA disc (5mm diameter) was placed instead of *T. harzianum* only. The plates were incubated at 27±1ºC for 7 days. The design used in *in vitro* experiment was Completely Randomized Design (CRD) with five replications. The percentage of inhibition by *T. harzianum* in the growth of the pathogen was calculated according to the following formula (Rini and Sulochana, 2007):

$$\text{Percentage growth inhibition} = (C-T)/C \times 100$$

Here,

C = the radial mycelia growth of *A. porri* in control plate (mm)

T = the radial mycelia growth of *A. porri* in presence of *T. harzianum* (mm)

Preparation of mass inocula of *T. harzianum* and *A. porri*

Inoculum of *T. harzianum* was made in chickpea bran following the method of Dubey and Patel (2002). Chickpea bran was soaked in water for 12 hours. Around 20g chickpea bran was taken in a conical flask of 500 ml and was autoclaved at 120ºC under 15 lbs for 30 minutes. The sterilized substrate in the conical flask was inoculated with 5 mycelial discs (5mm diameter) from 3 days old culture of *T. harzianum* previously grown on PDA. The flasks were incubated at 25ºC for 15 days with intermittent hand shaking at 5 days. For the inoculum of *A. porri,* the pathogen was grown on petri plates (9 cm diameter) containing PDA for ten days at 26ºC. The mycelial growth was carefully scraped with a sterilized needle after adding 10 ml sterilized water to each plate. The obtained conidial suspension was used for inoculation.

Field experiments

To test the efficacy of *T. harzianum* against *A. porri* in the field condition, an experiment was conducted during rabi season of 2017-18 at BINA farm, Mymensingh. The variety BARIpiaj-1 was used in the experiment. Five treatments were given viz. T_1: Seed treatment with *T. harzianum*, T_2: Seedling treatment with *T. harzianum*, T_3: Foliar application with *T. harzianum*, T_4: Seed and seedling treatment with *T. harzianum* and T_5: Control (*A. porri* in absence of *T. harzianum*).

Seed treatment with *T. harzianum* was done following the method of Begum *et al.* (1998). The surface of seeds was moistened with sterilized water. The seeds were taken in petri dishes having 7 days old culture of *T. harzianum* growing in PDA. The seeds were stirred gently with a sterilized glass rod so that the whole surface of the seeds was coated with the culture of *T. harzianum*. Then the coated seeds were air dried for 1 hour. The number of conidia on treated seeds was counted in a haemacytometer and $2x10^6$ conidia $seed^{-1}$ was estimated. For seedling treatment, the inoculum of *T. harzianum* (grown in chickpea bran) was suspended in water (@ 4g inocula L^{-1} water) and sieved. Seedlings of one month old were dipped in the inocula suspension (10^8conidia ml^{-1}) for half an hour before transplanting. For foliar treatment the same inocula suspension (10^8conidia ml^{-1}) was applied at 30 days after transplanting. The onion plants were inoculated by spraying with conidial suspension of *A. porri* (10^6 ml^{-1}) after two days of application of *T. harzianum* inoculants.

The soil in the seed bed was well prepared and levelled. The bed size was 1.5m x 3m and it was 6 cm high from the field level. Urea 95g, TSP 75g and 10 kg cowdung were applied in the bed during final land preparation. About 120g seeds of BARIpiaj-1 were soaked in water for 12 hours. The wet seeds were taken in cotton bag and kept it at room temperature (24ºC) for 48 hours. The sprouted seeds were sown in the seed bed. Beds were covered with gunny bags for 6 days and after that the bags were removed. The field was prepared by four ploughings and cross ploughings. The experiments were laid out in a randomized complete block design with three replications. The unit plot size was 1.5m x 1.0m. The row to row and plant to plant spacing was 25 cm and 15 cm, respectively. The recommended dose of fertilizer and cowdung were applied in the field. One month old seedlings were transplanted to the well prepared plot. Inoculation of *T. harzianum* and *A. porri* was done as mentioned earlier. Weeding was done two times during the growing period. Irrigation was given to maintain the soil moisture as and when necessary. Disease assessment was done on leaf and flower stalk at 15 days intervals started just after the onset of disease symptoms in the experimental plots. Disease scoring was recorded according to 0-5 scale (Sharma, 1986):

Score 0	No symptom of disease
Score 1	A few spots towards the tip covering less than 10% of leaf area/ flower stalk
Score 2	Several dark purplish brown patches covering less than 20% of leaf area/ flower stalk
Score 3	Several dark purplish brown patches covering less than 40% of leaf area/ flower stalk
Score 4	Long streaks, covering up to 75% of leaf area/flower stalk or breaking of the leaves/ flower stalk from the center
Score 5	Complete drying of the leaves/flower stalk or breaking of the leaves/flower stalk from the base

The percent disease intensity (PDI) was calculated by using the following formula (Wheeler, 1969):

$$PDI = (TNR \times 100) \div (TIL \times MDR)$$

Where, PDI = percent disease intensity, TNR = Total sum of numerical ratings, TIL = total number of infected leaves observed and MDR = maximum disease rating.

Data on plant height (cm), bulb diameter (cm), bulb fresh weight (g) and yield (t ha^{-1}) were also recorded.

Results and Discussion

Table 1. Percent inhibition of growth of *A. porri* induced by *T. harzianum* in PDA plates

Trichoderma isolates	Inhibition (%)		
	3DAI	5DAI	7DAI
TI-1	16.5a	53.2a	62.7a
TI-2	8.7b	33.0c	45.8c
TI-3	9.8b	35.2b	48.5b
LSD (P≥0.05)	2.04	1.96	1.61

DAI= Days after inoculation. In a column data followed by the same letter are statistically similar at 5% level of significance.

In the *in vitro* evaluation of *T. harzianum* against *A. porri*, the three isolates of the antagonist inhibited the mycelial growth of the pathogen (Table 1). The isolate TI-1 inhibited the growth of *A. porii* up to 62.7% at 7 days after inoculation (DAI) followed by TI-3 (48.5%). The least inhibition was found in TI-2 (45.8%). The *in vitro* test indicated that the isolate TI-1 was superior to other ones in suppressing the growth of *A. porri* in PDA media. Prakasam and Sharma (2012) recorded significant inhibition of *A. porri* by *T. harzianum* up to 61.5% in the *in vitro* evaluation. In another study, among five biocontrol agents *T. harzianum* gave maximum inhibition (79.5%) of *A. porri* (Chethana *et al.*, 2013). The inhibition of the pathogen by *Trichoderma* on PDA medium may be due to diffusible antibiotic production and mycoparasitism (Kumar, 2013). Protease and fungal cell wall degrading enzymes make *Trichoderma* an attractive biocontrol agent for plant pathogenic fungi (Elad, 2000).

In the field experiment the disease intensity of purple blotch of onion on leaf and flower stalk due to the application of different treatments is shown in the Table 2 and the Table 3. The percent disease intensity (PDI) on leaf was recorded from 45 days after transplanting (DAT) to 90 DAT and it was found that PDI gradually increased in all treatments with time (Table 2). However, all treatments significantly reduced purple blotch disease intensity compared to the control. At 90 DAT, the disease intensity on leaf ranged from 29.21-79.16%. The least PDI (29.21%) was found in the treatment T_4 (Seed and seedling treatment with *T. harzianum*) followed by the treatment T_1 (Seed treatment with *T. harzianum*). The highest disease reduction over control (63.1%) was observed in the treatment T_4 (Seed and seedling treatment with *T. harzianum*) followed by the treatment T_1 (Seed treatment with *T. harzianum*) which was 58.7%. On flower stalk the percent disease intensity (PDI) was recorded from 90 DAT up to 120 DAT. At 120 DAT, the disease intensity ranged from 41.36-78.30% (Table 3). At 90 DAT, The least percentage of disease intensity (41.36%) and the highest disease reduction over control (47.18%) on flower stalk was recorded with the application of *Trichoderma* in seeds and subsequently seedlings. The

disease intensity on leaf at 90 DAT and on flower stalk at 120 DAT was higher in the treatment T_3 (foliar application with *Trichoderma*) than other treatments. The result of the present study indicates that application of *T. harzianum* in different methods was able to reduce purple blotch disease intensity significantly over control. This is in agreement with the study of other researchers where *T. harzianum* showed potential biocontrol activity against *A. porri* (Mishra, 2019 and Shahnaz *et al.*, 2012). Prakasam and Sharma (2012) reported that an isolate of *T. harzianum* was able to reduce onion purple blotch disease 67.7% under greenhouse and 64.8% under field condition. *Trichoderma* spp. might act as bio-control agent by growing and parasitizing towards the pathogen, coiling and penetrating the pathogen hyphae resulting lysis of the cytoplasm of pathogens (Howell, 2003).

Table 2. Percent disease intensity (PDI) on leaf with the various treatments at different days after transplanting

Treatments	45DAT	60DAT	75DAT	90DAT
T_1 (Seed treatment with *T. harzianum*)	12.36c (48.21%)	20.05d (55.98%)	28.41d (57.22%)	31.42d (58.70%)
T_2 (Seedling treatment with *T. harzianum*)	18.40b (22.92%)	23.08c (49.32%)	31.05c (53.25%)	36.56c (53.82%)
T_3 (Foliar application with *T. harzianum*)	16.93b (29.07%)	29.38b (35.40%)	42.47b (36.06%)	50.31b (36.45%)
T_4 (Seed and seedling treatment with *T. harzianum*)	10.06c (57.86%)	18.48d (59.60%)	26.63d (59.91%)	29.21e (63.10%)
T_5 (Control)	23.87a	45.54a	66.42a	79.16a
LSD (P≥0.05)	2.52	2.13	2.04	2.10

DAT = Days after transplanting, In a column data followed by the same letter are statistically similar at 5% level of significance. Data in the parentheses indicate percent disease reduction over control.

Table 3. Percent disease intensity (PDI) on flower stalk with the various treatments at different days after transplanting

Treatments	90DAT	105DAT	120DAT
T_1 (Seed treatment with *T. harzianum*)	22.35c (20.52%)	46.74c (24.89%)	52.42d (33.05%)
T_2 (Seedling with *T. harzianum*)	22.46c (20.13%)	48.70c (21.74%)	57.50c (26.56%)
T_3 (Foliar application with *T. harzianum*)	23.56b (16.23%)	51.39b (17.41%)	61.24b (21.79%)
T_4 (Seed and seedling treatment with *T. harzianum*)	17.40d (38.12%)	38.54d (38.07%)	41.36e (47.18%)
T_5 (Control)	28.12a	62.23a	78.30a
LSD (P≥0.05)	1.02	2.12	2.32

DAT = Days after transplanting, In a column data followed by the same letter are statistically similar at 5% level of significance. Data in the parentheses indicate percent disease reduction over control.

The growth parameters like, plant height, bulb diameter, bulb fresh weight and yield were increased by the application of *T. harzianum* in all the treatments compared to the control (Table 4). The highest plant height (41.30cm), bulb diameter (4.3cm), bulb fresh weight (23.74g) and yield (9.90 t ha^{-1}) were provided in the combined application of soil and seed treatment with *T. harzianum*. In a study, Altintas and Bal (2008) found that yield and quality characters of onion were improved with the application of *Trichoderma* sp. *Trichoderma* spp. can colonize root surface, interact with plant and exchange compounds which bring substantial changes in plant metabolism and thus plant growth and crop yield are enhanced (Gajera *et al.*, 2013).

Table 4. Yield and yield contributing characters of onion as influenced by different treatments

Treatments	Plant height (cm)	Bulb diameter (cm)	Bulb fresh weight (g)	Yield (t ha^{-1})
T_1 (Seed treatment with *T. harzianum*)	40.32ab	3.20b	21.96b	7.84b
T_2 (Seedling treatment with *T. harzianum*)	39.05b	3.60b	18.70c	7.41b
T_3 (Foliar application with *T. harzianum*)	40.37ab	3.81b	19.89c	7.03b
T_4 (Seed and seedling treatment with *T. harzianum*)	41.30a	4.30a	23.74a	9.90a
T_5 (Control)	34.61c	2.51c	13.47d	5.20c
LSD (P≥0.05)	1.50	0.70	1.23	1.41

In a column data followed by the same letter are statistically similar at 5% level of significance.

Conclusion

The results revealed that *T. harzianum* was a potential biocontrol agent in reducing the disease intensity of purple blotch of onion and application of *T. harzianum* could increase the yield of onion. However, the application of *T harzianum* in seed and subsequently seedling was more effective in disease reduction than the individual application of *T harzianum* in seed or seedling.

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