EVALUATION OF ANTAGONISTIC ACTIVITY OF *TRICHODERMA HARZIANUM* AGAINST *SCLEROTIUM ROLFSII* OF SOME SELECTED VEGETABLES

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ABSTRACT

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Different treatments of formulated and conidial suspension of *Trichoderma herzianum* were used against *Sclerotium rolfsii* causing damping-off and foot rot diseases of bean, eggplant, tomato, cabbage and indian spinach. Following the dual culture method, the isolated *Trichoderma harzianum* showed antagonistic effect by inhibited the growth of *S. rolfsii* up to 92% over control. In pot experiments, three treatments viz. formulated *Trichoderma harzianum* @ 10 g/kg, 15 g/kg and 20 g/kg soil along with a sterilized soil as control were tested against *S. rolfsii*. Among the treatments, soil application with black gram based substrate of *Trichoderma harzianum* @ 20 g/kg soil reduced pre-emergence death (66.27-82.29%), damping-off (70.33-81.82%) and foot rot (76.93-92.56%) over control in all five different crops used in this study. Soil application with black gram based substrate of *Trichoderma harzianum* @ 20 g/kg also increased the germination by 46.09-59.68% as compared with control. Soil application of formulated *Trichoderma harzianum* @ 20 g/kg followed by spraying of conidial suspension of *Trichoderma harzianum* showed better results as compared with soil application alone. However, soil application of formulated *Trichoderma harzianum* @ 20 g/kg followed by spraying of conidial suspension of *Trichoderma harzianum* increased germination by 48.34-54.89% and reduced damping-off and foot rot by 66.71-90.27% and 85.37-100%, respectively over control.

Keywords: Damping-off, Foot rot, Formulation, *S. rolfsii*, *Trichoderma harzianum*

INTRODUCTION

Sclerotium rolfsii is an important soil-borne plant pathogen and a major constraint for vegetables production in Bangladesh (Ahmed and Hossain 1985). The pathogen mainly causes nursery diseases like damping-off and foot rot of vegetables and has a very extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers, and cucurbits (Chupp and Sherf 1960). The funguses survive in soil mainly as sclerotia, which is the main source of inocula and remain viable in soil for several months. *S. rolfsii* attacks the plants at any growth stage but more devastating at seedling stage (Bag and Sinha 1997). Farmers try to overcome this problem through different cultural practices and use of chemical fungicides. But the control of *S. rolfsii* with chemicals is neither cost effective and nor environment friendly. So as alternative of chemical method, bio-fungicides are included in the concept of biological control. Available reports revealed that some isolates of *T. harzianum* have been found to be effective biocontrol agent against various plant pathogenic fungi including *S. rolfsii* (Hadar *et al*. 1979; Elad *et al*. 1983). In native soils *Trichoderma* are present in low population to combat the pathogen. Formulation of the *Trichoderma* spp. to reduce the incidence of the soil-borne pathogens in the field is of great importance for biological management of such diseases. So far, sufficient information on formulation of *Trichoderma* isolated from northern part of Bangladesh to control *S. rolfsii* is not available. Therefore, it was aimed to isolate *Trichoderma* from the soil of Dinajpur district and also to make a suitable formulation of the isolated *Trichoderma* for the controlling of damping-off and foot rot diseases of vegetables caused by *S. rolfsii*.

MATERIALS AND METHODS

Isolation of *Trichoderma harzianum* and *Sclerotium rolfsii*

The fungus *Trichoderma harzianum* was isolated from the rhizosphere soil following dilution plate technique (Subba 2003). In brief, one gram of soil sample was taken in a test tube containing 9 ml of sterilized water to make 1:10 dilution. Then 1 ml suspension was taken in another test tube containing 9 ml of sterilized water to make 1: 100 dilutions. Similarly, a series of dilution process were continued

until the samples were diluted to 1:10000. One ml soil suspension from sample was placed in each petri-plate and incubated at 25 ± 2^0 C. After 3 days of incubation, hyphal tip/mycelial block of *Trichoderma harzianum* were transferred to PDA for purification and the isolate was identified according to the key of Kubicek and Harman (1998). The pathogen (*Sclerotium rolfsii*) was isolated from naturally infected eggplant (*Solanum melongena* L.). The fungus was purified by hyphal tip culture technique (Tuite 1969) and identified following the keys outlined by Aycock (1996) and Barnett (1960).

Evaluation of antagonistic activity of isolated *Trichoderma* against *S. rolfsii* by dual culture method

The *in-vitro* test was conducted to find out the antagonistic effect of the isolated *Trichoderma harzianum* against *Sclerotium rolfsii* on PDA by dual culture method (Dhingra and Sinclair 1985). One mycelial disc of 5 mm size picked by sterilized cork borer from 7 days old culture of *Trichoderma* spp. and one disc (same size and age) of a *Sclerotium rolfsii* were placed simultaneously on the edge of each petri-plate at opposite direction. The plates which received only discs of *Sclerotium rolfsii* was treated as control and incubated at room temperature. Thereafter, percentage inhibition of *Sclerotium rolfsii* was calculated based on the growth of the pathogen on PDA plate following the formula of Sundar *et al.* (1995).

% growth inhibition = X-Y/X×100

Where,

X = Mycelial growth of pathogen in absence of *Trichoderma* spp.

Y = Mycelial growth of pathogen in presence of *Trichoderma* spp.

Preparation of *Sclerotium rolfsii* inocula

Inocula of *Sclerotium rolfsii* were prepared by growing the pathogen on sterilized wheat grains in 500 ml Erlenmeyer flask. Wheat grains (100 g) were soaked in water for 12 hours and autoclaved at 121°C with 15 psi for 15 minutes. The conical flask containing autoclaved wheat grains were allowed to cool at room temperature and then 10 mycelial blocks (5 mm) cut from the edge of 3 days old of pure culture of *Sclerotium rolfsii* were added to the flask and incubated at room temperature for 7 days. The flasks were shaken thoroughly by hand at every 3 days for proper distribution of fungal mycelia throughout the entire mass of the inoculated wheat grains. The colonized wheat grains were air dried and stored for further use.

Preparation of *Trichoderma harzianum* formulation and suspension

Black gram bran and water in 1:2 ratios were explored for the multiplication and formulation of *Trichoderma harzianum*. The requisite amount of materials for each substrate (Black gram bran: water =1:2) were thoroughly mixed in a 500 ml Erlenmeyer flask and autoclaved at l21°C for 15 minutes for sterilization. The sterilized substrate allowed to cool down and then inoculated with 5 mm mycelial disc of 7 days old *Trichoderma* culture. Ten discs for each flask were used for inoculation. Inoculated flasks were then incubated at room temperature (25±2 °C). After incubation for 20 days; the contents were taken out from the flasks and air dried in laminar airflow cabinet. The air dried materials were kept in polythene bag with labeling and treated as formulated. *Trichoderma* suspension was prepared by scraping the spore masses taken in a beaker containing 400 ml water and one drop of Tween-20 was added and stirred for 15 minutes. Then the number of conidia per ml was determined by using haemocytometer following the procedure of Ashrafuzzaman (1976).

Treatments and design of experiments

Three different doses of formulated *Trichoderma* were used viz. *Trichoderma* @ 10 g/kg, 15 g/kg and 20 g/kg soil to find out the effect of *Trichoderma* spp. against *S. rolfsii* and only inoculation of the plant with pathogen was maintained as control. Conidial suspension of *Trichoderma* was prepared and used in the combinations as soil treating with *Trichoderma* suspension, soil treated with formulated *Trichoderma* 20 g/kg soil and soil treated with formulated *Trichoderma* 20 g/kg followed by spraying of *Trichoderma* suspension. Each treatment was replicated three times and the experiment was set in completely randomized design (CRD).

Inoculation of tray soil with *S. rolfsii* and recording of data

Tray soil was thoroughly mixed with *S. rolfsii* inocula @ 10g/kg soil and covered with polythene sheet to maintain moisture for proper growth of *Sclerotium rolfsii*. After 3 days of inoculation of *S. rolfsii*, different doses of formulated *Trichoderma harzianum* (10 g/kg soil, 15 g/kg soil and 20g/kg soil) were mixed with the soil. For conducting another experiment different treatments of formulated and spraying of conidial suspension of *Trichoderma* were added in the soil. Hundred seeds of bean, eggplant, tomato, cabbage and indian spinach were sown in each tray and disease incidence was observed regularly and data were recorded at 14 days after sowing to evaluate the antagonist effect of

Trichoderma harzianum against *S. rolfsii* on a) Seed germination (%), b) Pre-emergence death, c) Damping-off (%) and d) Foot-rot (%).

All the recorded data were analyzed by using MSTAT-C program and mean difference was evaluated by Duncan's Multiple Range Test (DMRT) at 0.05 levels.

RESULTS AND DISCUSSION

Identification of *Trichoderma* and *Sclerotium rolfsii*

The fungus was isolated from rhizosphere soil of vegetable following dilution plate technique and identified based on morphological characters. Mycelial growth and colony characters of *Trichoderma* isolate were studied using 7 days old PDA cultures incubated at 25 ± 2^0 C. The nature of mycelial growth was fast and initially the colony colour was observed as whitish to light green, gradually became deep green and formed a concentric ring. The conidiophores were branched which terminated with one or a few phialides. Phialides were flask-shape and cylindrical or nearly subglobose which born conidia. The conidia were ellipsoidal or globose shaped. Based on the observed characteristics, the isolate was identified as *Trichoderma harzianum* according to the key of Kubicek and Harman (1998) (Figure 1A). Patel and Patel (2014) identified *Trichoderma* having the same characteristics of hyphae, conidiophores and conidia. *S. rolfsii* isolated from naturally infected eggplant (*Solanum melongena* L.) and identified according to the key of Aycock (1996) and Barnett (1960). Whitish mycelial growth was observed that produced sclerotia after 7 days of incubation. The sclerotia were small, uniformly round and dark brown at mature stage (Figure 1B). The finding of present investigation was similar with the report of Darakhshanda-Kokub *et al.* (2007).

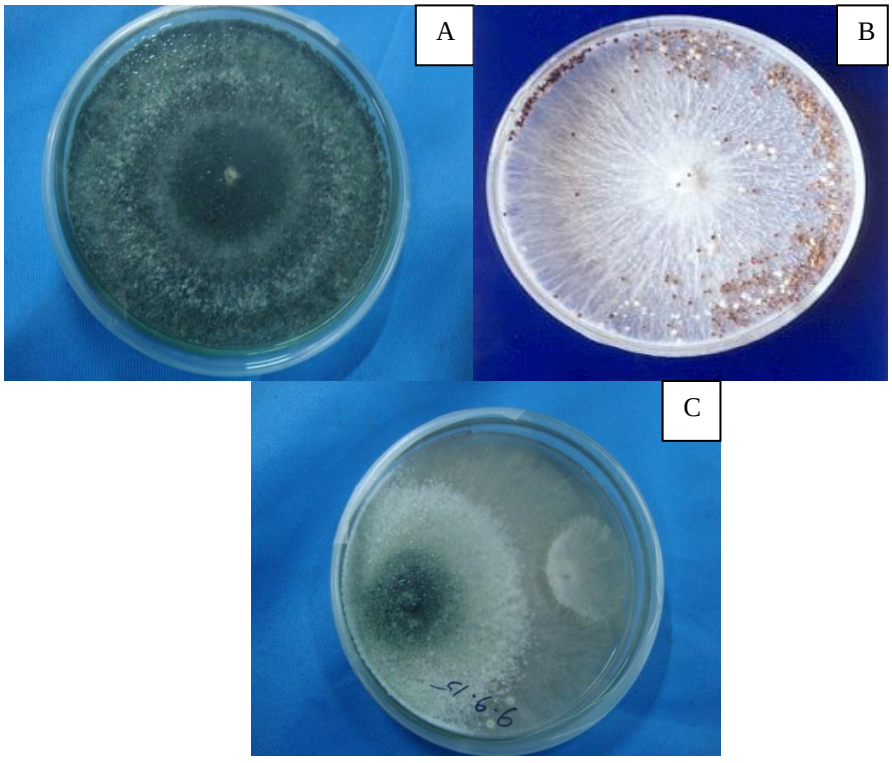

Figure 1. (A) *Trichoderma harzianum* in dark green colour (B) Mycelial growth of *Sclerotium rolfsii* with mature Sclerotia (C) Mycelial growth inhibition of *Sclerotium rolfsii* by *Trichoderma harzianum*

Mycelial growth inhibition assay

Antagonistic activity of *Trichoderma harzianum* against *S. rolfsii* was tested using dual culture method. The studies indicated that *Trichoderma harzianum* significantly inhibited the mycelial growth of *S. rolfsii* and the percent inhibition of *S. rolfsii* was observed

92 % (Figure 1C). *T. harzianum* significantly reduced the radial colony growth of *S. rolfsii* in dual culture on PDA (Faruk *et al*. 2002). Prasad *et al*. (1999) found the similar findings and reported 61.4 % inhibition of *S. rolfsii* with *T. harzianum* while Yogendra and Singh (2000) found maximum of 64.44 % inhibition of *S. rolfsii* by *T. harzianum* at 4 DAI.

Effect of soil treatments with formulated *Trichoderma harzianum* on germination of bean, eggplant, tomato, cabbage and indian spinach

In-vitro conditions, soil treatments with different doses of formulated *Trichoderma harzianum* significantly influenced germination of bean, eggplant, tomato, cabbage and indian spinach (Table 1). Application of formulation of *Trichoderma harzianum* @ 20 g/kg soil gave the highest germination of cabbage (88.67 %) followed by bean (83%), eggplant (82.67%), tomato (81%) and indian spinach (79%). The lowest germination was observed in control treatment in case of all crops.

Table 1. Effect of different doses of formulated *Trichoderma harzianum* on germination of bean, eggplant, tomato, cabbage and indian spinach

Treatments	Germination % of seeds				
	Bean	Eggplant	Tomato	Cabbage	Indian spinach
Control	35	33.33	43.67	36	32
Formulated *Trichoderma* @ 10g/kg Soil	49.67	39	54.33	42.67	41.33
Formulated *Trichoderma* @ 15g/kg Soil	57	62	65.67	60.33	62
Formulated *Trichoderma* @ 20g/kg Soil	83	82.67	81	88.67	79

Effect of soil treatments with formulated *Trichoderma harzianum* on pre-emergence death, damping-off and foot rot diseases of bean, eggplant, tomato, cabbage and indian spinach

The effect of different treatments of formulated *Trichoderma harzianum* were recorded on pre-mergence death, damping-off and foot rot of bean, eggplant, tomato, cabbage and indian spinach (Figure 2). The highest pre-emergence death was obtained from untreated control in all the five crops which was statistically identical with the application of formulated *Trichoderma* @ 10g/kg soil in eggplant (61%) and cabbage (57.33%). The lowest pre-emergence death was found when soil was treated with *Trichoderma harzianum* @ 20g/soil in cabbage (11.33%) followed by bean (17%), eggplant (17.33%), tomato (19%) and indian spinach (21%) seedlings. The maximum percent of damping-off was recorded in control trays which were statistically identical with *Trichoderma* formulation @ 10g/kg soil in bean, tomato, indian spinach. The minimum damping-off was found when formulated *Trichoderma* were used @ 20g/kg soil in all the five vegetable crops. Similarly, the highest percent of foot-rot was recorded in control trays. Likewise, other parameter, the lowest foot-rot was observed when soil treated with *Trichoderma* formulation @ 20g/kg soil in all the crops. It was reported that *Trichoderma* produced chemicals called trichodermin which is responsible for its antagonistic properties and grow topically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target pathogenic fungi (Tverdyukov *et al*. 1994). Biological treatment of tomato, potato, chickpea, lentil and peanut seeds with *Trichoderma harzianum* and *Gliocladium virens* resulted in excellent potentials against a wide range of plant pathogens including *S. rolfsii* and the treatments were consistently as effective as or better than fungicidal seed treatment (Mukhopadhyay 1989). Soil treatment with a powder formulation of *Trichoderma* spp. two weeks before planting or at the time of planting reduced significantly the incidence of damping-off and wilt diseases on Giza 3 bean cultivar (Nashwa *et al*. 2008). The results of present study was seemed to be in accordance with the finding of Bhuiya (2006) especially the role of *Trichoderma* formulation @ 20g/kg soil in controlling collar rot and regeneration of eggplants.

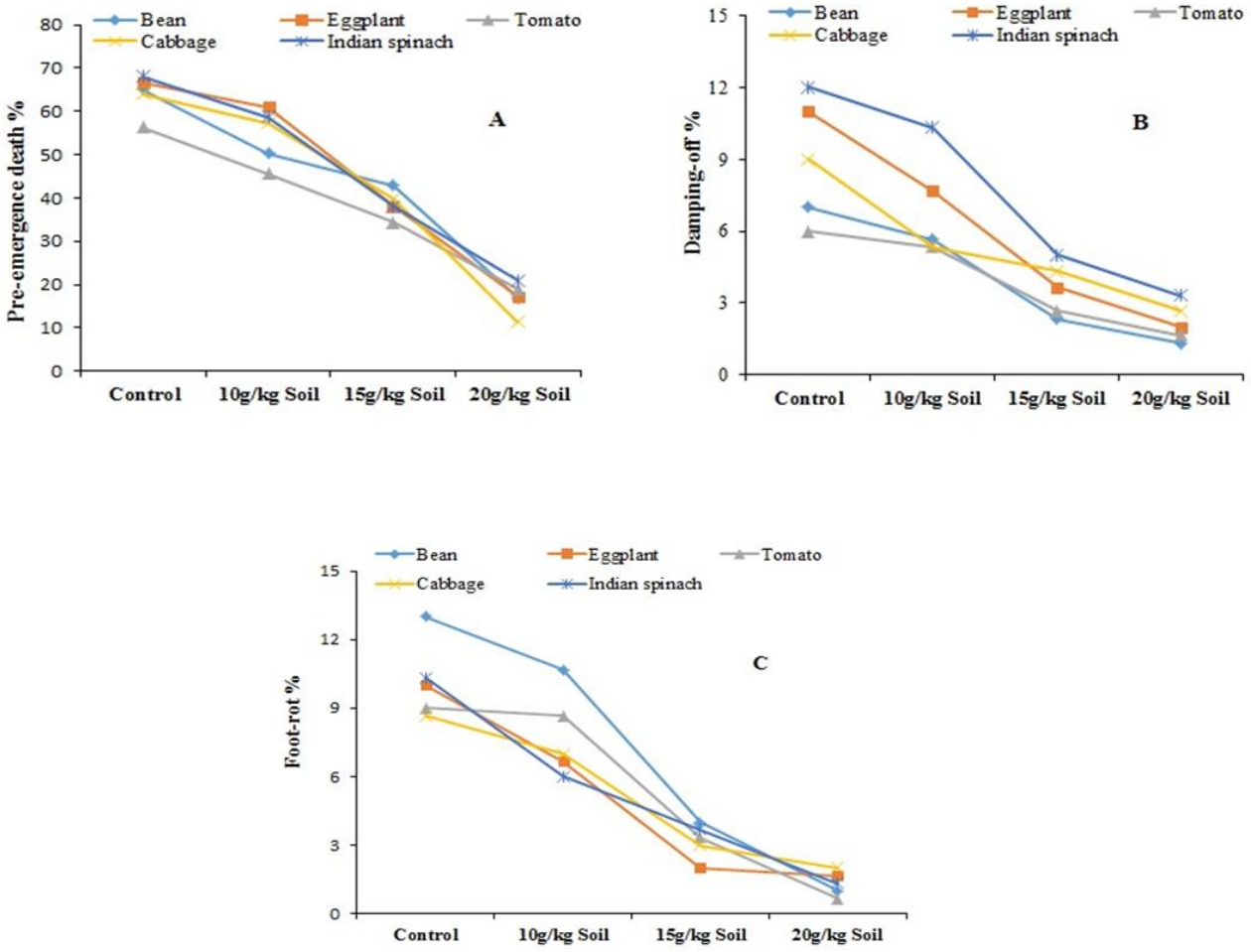

Figure 2. Effect of different doses of formulated *Trichoderma* on A) Pre-emergence death, (B) Damping-off and (C) Foot-rot percent of Bean, Eggplant, Tomato, Cabbage and Indian Spinach after *S. rolfsii* inoculated soil

Effect of formulated and conidial suspension of *Trichoderma* on germination, pre-emergence death and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach

Significant effect of different treatments was found on germination, pre-emergence death and seedling diseases of bean, eggplant, tomato, cabbage and indian spinach in *S. rolfsii* inoculated soil at 14 days (Figure 3). The highest germination was recorded where tray soil treated with *Trichoderma* formulation followed by spraying of conidial suspension of *Trichoderma harzianum* and lowest germination was found in control trays in all the five crops. Among the crops the maximum germination was observed in cabbage (83%) followed by eggplant (81.33%), indian spinach (80%), tomato (75%) and bean (71.67%) and minimum germination was recorded in control tray of bean (32.33%). The maximum pre-emergence death was observed in untreated control trays in bean (67.67%) whereas the minimum pre-emergence death was found by the application with *Trichoderma* formulation along with conidial suspension in cabbage (17%) followed by eggplant (18.67%) and indian spinach (20%). Similarly, the highest percent of damping-off was obtained from control trays of bean (13.67%) whereas the lowest was recorded from the soil treated with *Trichoderma* formulation along with suspension in eggplant (0.67%). Indian spinach was severely affected by foot rot disease whereas less incidence was observed by applying *Trichoderma* formulation followed by spraying of conidial

suspension. The results of present study were supported by the findings of other researchers. The effectiveness of *Trichoderma* might be attributed that the fungi destroyed the sclerotia of *S. rolfsii* and overlaped the pathogen and suppressed its growth (Susceelendra and Schlosser 1999, Iqbal *et al*. 1995). Seed treatment with *T. harzianum* (6×10^6 CFU/ ml) resulted higher germination, fresh shoot weight, fresh root weight and higher vigor index of cucurbits over control (Shamsuzzaman *et al.* 2003). Xu *et al.* (1993) reported that seed treatment with *Trichoderma* spore suspension (10^8c.f.u./ml) increased emergence of cucumber seedlings by 14 to 20% in the *S. rolfsii* inoculated soil.

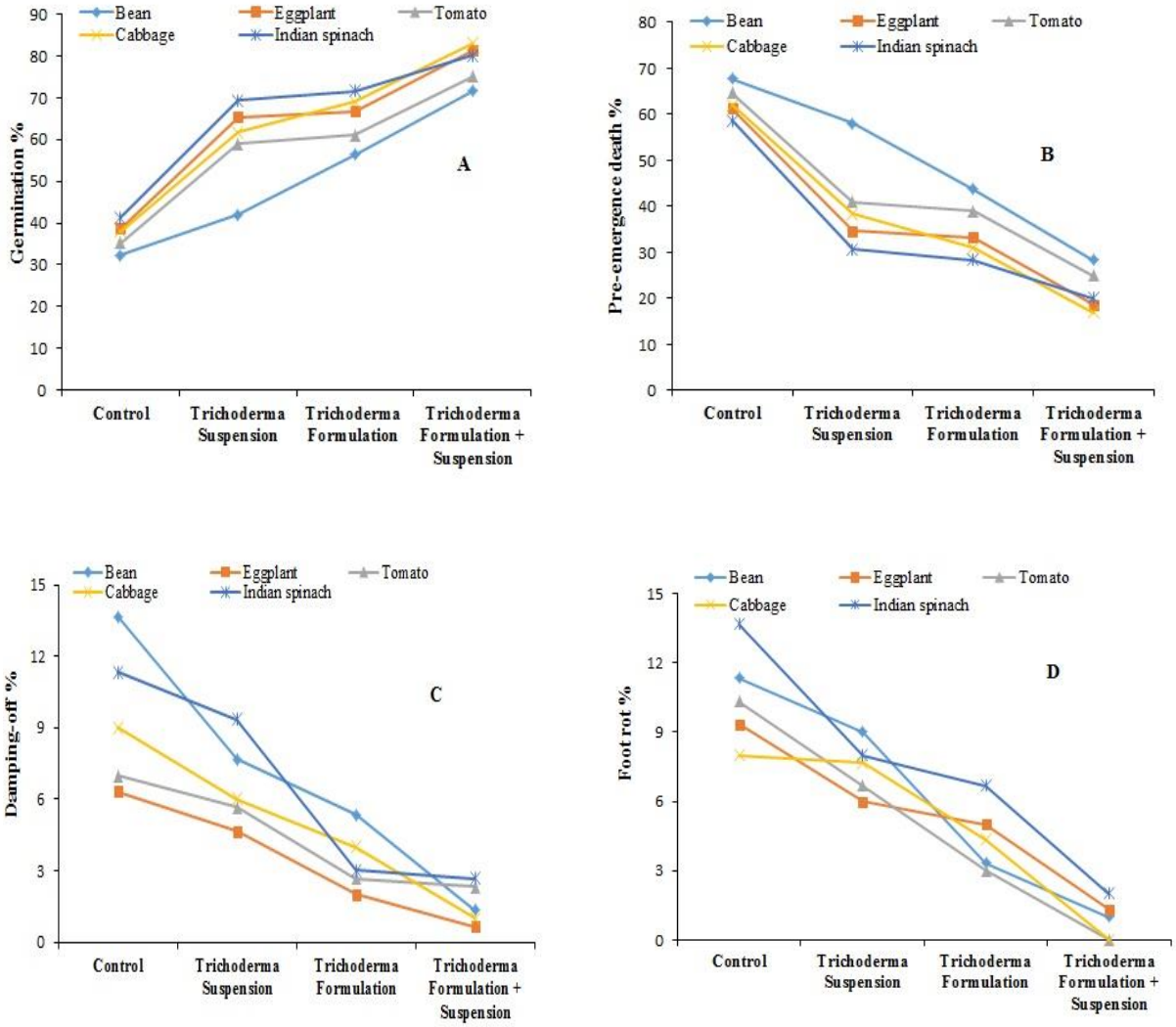

Figure 3. Different treatments effect on (A) Germination, (B) Pre-emergence death, (C) Damping-off and (D) Foot-rot of Bean, Eggplant, Tomato, Cabbage and Indian Spinach after *S. rolfsii* inoculated soil

CONCLUSION

The *Trichoderma harzianum* isolated from Dinajpur districts was potential antagonist against *S. rolfsii* causing damping off and foot rot diseases in vegetable crops. Soil treated with the black gram based *Trichoderma harzianum* followed by spraying of conidial suspension remarkably reduces the disease incidence along with the increasing of seed germination. However, the judicious use of *Trichoderma harzianum* based biocontrol agent might be incorporated in the integrated disease management program to reduce the chemical based management practices. For the development of a complete and sustainable integrated management program for controlling nursery diseases of vegetables, a continuous effort should be undertaken to find out more potential *Trichoderma* species with proper formulation.

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