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Use of *Trichoderma* in Biological Control of Collar Rot of Soybean and Chickpea

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MKH and MAK designed the experiment, performed the statistical analysis, develop the protocol, managed the literature searches and wrote the first draft of the manuscript. Author FA check and improve the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

An *in vitro* and field experiments for two consecutive years were conducted at Bangladesh Institute of Nuclear Agriculture, Mymensingh, aiming to investigate the efficacy of *Trichoderma harzianum* against *Sclerotium rolfsii* causing collar rot disease of soybean and chickpea. In *in vitro* the antagonistic activity of *T. harzianum* against *S. rolfsii* was observed through dual culture. In field experiment *Trichoderma* was applied as soil treatment and seed treatment. The percent inhibition of *S. rolfsii* induced by *T. harzianum* was found upto 78.9% in *in vitro*. The maximum reduction of collar rot disease incidence over control was 82.4% in soybean and 77.6% in chickpea which was recorded in the plot where *T. harzianum* was applied in the soil. The highest seed germination: 86.3% in soybean and 84.8% in chickpea, maximum fresh shoot weight: 94.5 g plant^{-1} in soybean, 62.5 g plant^{-1} in chickpea, maximum fresh root weight: 10.7 g plant^{-1} in soybean, 9.3 g plant^{-1} in chickpea and the highest yield: 2830 kg ha^{-1} in soybean, 1836 kg ha^{-1} in chickpea were obtained by the application of *Trichoderma* in soil. The study indicated that the tested isolate of *T. harzianum* had potential in controlling collar rot disease of soybean and chickpea. For the reduction of collar rot incidence application of *T. harzianum* in soil was found more effective than seed treatment.

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1. INTRODUCTION

Soybean (*Glycine max* L.) is a leguminous crop that is grown in tropical, sub-tropical and temperate climate. This crop has a tremendous value in agriculture for source of high quality plant protein and vegetable oils and also capable to fix nitrogen in soil. Soybean covers more than 50% of total vegetable oil production in the world. [1]. Soybean seed contains about 40-45% protein and 20-22% oil, 20-26% carbohydrate and a high amount of Calcium, Phosphorus and vitamins [2]. Chickpea (*Cicer arietinum* L.) is also a leguminous crop and one of the oldest cultivated crops for consumption in the world. Being a subtropical and drought resistant crop, it grows well in cooler and dry climate.

It is a vital source of protein augmented human food and animal feed, mainly for the low-income population of Southeast Asia [3]. It offers a range of health benefits. It helps to increase digestion, keeps blood sugar level stable and increases protection against diseases. The grain of chickpea is highly nutritious containing 45% starch, 25% proteins, 6% sugars, 6% crude fiber, 5% fat, 3% ash, 0.19% calcium and 0.01% minute quantities of some important vitamins and minerals [4,5].

Among different natural constraints towards the low production of crop, chiefly diseases are the most significant. Many phytopathogenic soil-borne as well as seed borne fungi are responsible for disease development which attack plants during seedling to maturity stages. Collar rot caused by *Sclerotium rolfsii* Sacc. is a fungal disease affecting crops all over the world [6]. This soil-borne pathogen causes rot at collar region on a wide range of plant species belonging to families Compositae and Leguminosae whereas members of Graminae are less susceptible to this disease [7]. The most common hosts are legumes, crucifers and cucurbits. The initial symptom of collar rot of soybean and chickpea was recorded on the leaves in form of slight paleness followed by yellowing of leaves and loss of vigour of plant. Infection usually occurs at the collar region as brownish black discoloration. Gradually the discoloration is found to spread 3-5 cm both upward and downward along the stem and tap root, respectively. In advanced stage of infection, all the leaves shed, turn brown dry and often cling to dead stem. The mycelium of pathogen grows over the diseased tissue and surrounding the soil forming a white mat of mycelial thread with the typical brown to chocolate brown mustard seed sized sclerotia. Collar rot is a serious threat, which under conducive conditions causes 55-95% mortality of the crop at seedling stage [8]. It is very difficult to manage the disease as the causing organism *S. rolfsii* survives in the soil as sclerotia and chlamydospores [9]. The sclerotia is considered as the primary inoculum of the pathogen as well as its principle means of dispersal [10]. As there is no effective fungicide or resistant variety for the management of the disease the farmers cannot maintain the desire plant population in the field and consequently the yield is reduced. In this context bioagent can be an alternative source for controlling soil-borne diseases [11]. Several strains of *Trichoderma* spp. have been found to be effective as biocontrol agents of various soil borne plant pathogenic fungi such as *Fusarium, Pythium, Rhizoctonia* and *Sclerotium* and also for seed borne fungi [12,13,14]. The biological control of *S. rolfsii* on bean plants by using *Trichoderma* spp. had been investigated [15]. Therefore, the present study was undertaken to investigate the efficacy of *Trichoderma harzianum* against *S. rolfsii* causing collar rot disease of soybean and chickpea.

2. MATERIALS AND METHODS

2.1 Source and Maintenance of *Trichoderma harzianum* and *Sclerotium rolfsii*

The isolate of *T. harzianum* was obtained from Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Pure culture of *T. harzianum* was made in PDA plates following hypal tip culture technique [16] and preserved at 5ºC for further use. The isolate of *S. rolfsii* was obtained from diseased plant samples of soybean and chickpea collected from the experimental field of BINA at Mymensingh and Magura, respectively. Diseased plants showing typical symptoms of collar rot were collected from the field. 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. These were washed thoroughly with sterilized water and dried between folds of filter paper. The sterilized segments were transferred in PDA plates and incubated for 7 days at 26ºC. Pure culture was obtained by sub-culturing three times and pathogenicity test on the crops were carried out [17]. Pure cultures of the final isolate was maintained on PDA plates and kept in the refrigerator (5ºC) until required.

2.2 *In vitro* Evaluation of *Trichoderma harzianum* against *Sclerotium rolfsii*

The antagonistic activity of *T. harzianum* was screened against *S. rolfsii* through dual culture technique [18]. Both *T. harzianum* and *S. rolfsii* were cultured individually on PDA media in petridishes (9 cm diameter) at 25ºC. A disc (5mm diameter) of five days old culture of *T. harzianum* was inoculated on one side of PDA plate and another disc of *S. rolfsii* of the same size was inoculated at the opposite side of PDA plate. The distance between the discs was approximately 5 cm. In the control treatment, a sterile agar disc (5mm diameter) was placed instead of *T. harzianum.* The plates were incubated at 25±1ºC for 8 days. The experimental design used in *in vitro* experiment was Completely Randomized Design (CRD) with five replications. The percentage of inhibition in the growth of the fungal pathogen by *Trichoderma* was calculated by the following formula [18].

Percentage growth inhibition = (C-T)/C x 100
Here, C= the radial mycelia growth of *S. rolfsii* in control plate (mm)
T= the radial mycelia growth of *S. rolfsii* in presence of *T. harzianum* (mm)

2.3 Preparation of Mass inocula of *T. harzianum* and *S. rolfsii*

Chickpea bran was soaked in water for 12 hours. Around 20 g of soaked 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 (5 mm diameter) of 3 days old culture of *T. harzianum* and *S. rolfsii* previously grown on PDA. The flasks were incubated at 25ºC for 15 days with intermittent hand shaking at 5 days.

2.4 Field Experiments

To test the efficacy of *T. harzianum* against *S. rolfsii* for collar rot of chickpea and soybean, field trials were conducted during rabi season of 2016-17 in the field of Magura substation (23.4855º N, 89.4198º E) of Bangladesh Institute of Nuclear Agriculture (BINA) and 2017-18 in the field of BINA Head Quarter, Mymensingh (24.7471º N, 90.4203º E). The cultivar Binasola 5 for chickpea and Binasoybean 3 for soybean were used in the experiments.

The land was prepared by four ploughings and cross ploughings. The field experiments were laid out in a randomized complete block design with three replications. The unit plot size was 2.0 m x 1.5 m with plant spacing of 20 cm for soybean and 15 cm for chickpea and seeds were sown in rows. The recommended dose of fertilizer and cowdung were applied in the plots. There were three treatments: (i) T_1 = Soil treatment with *T. harzianum*, (ii) T_2 = Seed treatment with *T. harzianum* (iii) T_3 = Control (only *S. rolfsii)*. The inoculum of *T. harzianum* was applied in the field soil in rows 10g/m before three days of seed sowing. Seed treatment with *T. harzianum* was done following a described method [19]. 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 was estimated. The inocula of *S rolfsii* was applied in the field soil in rows 5g/m during seed sowing. Soil was moistened when necessary. Weeding was done three times during the crop growing period. No pesticide was used. Data on collar rot disease incidence was recoded at 10, 20, 30 and 40 DAS (Days After Sowing), plant growth and yield attributes were recorded at 3.5 months after sowing.

3. RESULTS AND DISCUSSION

The data obtained from dual culture showed that *Trichoderma* inhibited the growth of *S. rolfsii* in PDA plates (Table 1). The percent inhibition induced by *Trichoderma* at 2, 4, 6 and 8 days after inoculation was 20.0%, 66.7%, 75.3% and 78.9%, respectively. Numerous reports indicated that *T. harzianum* was effective to suppress the growth of *S. rolfsii* in *in vitro* [18,20]. This antagonistic nature might be due to antibiosis, nutrient competition and/or action of cell wall degrading enzymes [21]. It is reported that *T. harzianum* could release B-1-3 Gluconase and Chitinase on the hyphal wall of *Sclerotium rolfsii* resulting in disintegration of the host mycelium which assist the penetration, growth, absorption, lysis and bursting of the host hyphae by mycoparasite [22].

In soybean and chickpea, collar rot disease incidence was significantly influenced by the application *T. harzianum* in soil (Table 2).The minimum disease incidence (6.8-7.2% in soybean and 8.0-9.2% in chickpea) was observed in the plot where *T. harzianum* was incorporated in the soil while the maximum disease incidence (38.8-39.2% in soybean and 35.7-40.2% in chickpea) was recorded in the control plot. The maximum decrease of collar rot incidence over control (82.4-81.6% in soybean and 77.6-77.1% in chickpea) was observed in the plot where *T. harzianum* was applied in the soil followed by the plot where seeds were treated with *T. harzianum* (78.4-75.2% in soybean and 70.8-75.8% in chickpea).

Seed germination (%) and plant stand (%) of soybean and chickpea were significantly influenced by the application *T. harzianum* in soil (Table 3). In soybean, the highest seed germination (86.3-85.5%) was recorded in the plot incorporated with *T. harzianum* followed by the seed treatment (82.4-83%) while the lowest germination was recorded in the control plot (63.0-62.7%). The highest plant stand (83.2-83.7%) and the lowest plant stand (60.8-61.1%) were recorded in the plot incorporated with *T. harzianum* and in control plot, respectively. In chickpea, the highest seed germination (84.3-84.8%) and the highest plant stand (82.4-83.5%) was recorded in the plot where *T. harzianum* was incorporated in the soil (Table 4). The lowest seed germination (64.1-63.2%) and the lowest plant stand (62.3-60.6%) were observed in the control plot.

Table 1. Percent inhibition of the radial growth of the pathogen (*S. rolfsii*) by the antagonist (*T. harzianum*) on PDA medium

Isolate	Inhibition (%)			
Trichoderma harzianum	2DAI	4DAI	6DAI	8DAI
	20.0	66.7	75.3	78.9

DAI= Days after Inoculation, Data represent the mean of five replications

Table 2. Effect of *Trichoderma harzianum* on collar rot disease incidence (%) in soybean and chickpea

Treatments	Disease incidence (%)			
	Soybean		Chickpea	
	2016-17	**2017-18**	**2016-17**	**2017-18**
Soil treatment with *Trichoderma harzianum*	6.8 (-82.4)	7.2 (-81.6)	8.0 (-77.6)	9.2 (-77.1)
Seed treatment with *Trichoderma harzianum*	8.4 (-78.4)	9.7 (-75.2)	10.4 (-70.8)	9.7 (-75.8)
Control	38.8	39.2	35.7	40.2
LSD (P≥0.05)	13.3	15.6	11.8	17.4

Data in parenthesis indicate per cent decrease (-) of collar rot incidence over control
Data represent the mean of three replications

Table 3. Effect of *Trichoderma harzianum* on seed germination (%) and plant stand (%) in soybean

Treatments	Seed germination (%)		Plant stand (%)	
	2016-17	**2017-18**	**2016-17**	**2017-18**
Soil treatment with *Trichoderma harzianum*	83.3 (+ 36.9)	82.5 (+40.5)	83.2 (+36.8)	83.7 (+36.9)
Seed treatment with *Trichoderma harzianum*	82.4 (+37.3)	83.0 (+41.4)	81.4 (+33.9)	80.6 (+31.9)
Control	60.0	58.7	60.8	61.1
LSD (P≥0.05)	7.6	6.3	7.3	8.0

Data in parenthesis indicate per cent increase (+) over control
Data represent the mean of three replications

Table 4. Effect of *Trichoderma harzianum* on seed germination (%) and plant stand (%) in Chickpea

Treatments	Seed germination (%)		Plant stand (%)	
	2016-17	2017-18	2016-17	2017-18
Soil treatment with *Trichoderma harzianum*	84.3 (+31.5)	84.8 (+34.2)	82.4 (+32.3)	83.5 (+37.8)
Seed treatment with *Trichoderma harzianum*	82.7 (+29.1)	81.3 (+28.6)	80.0 (+28.4)	80.6 (+33.0)
Control	64.1	63.2	62.3	60.6
LSD (P≥0.05)	7.8	7.3	6.9	7.0

Data in parenthesis indicate per cent increase (+) over control
Data represent the mean of three replications

In the present study it was observed that the antagonist *T. harzianum* was significantly superior over control in respect of collar rot disease reduction and increasing seed germination of soybean and chickpea. In soybean, reduction of collar rot incidence caused by *S. rolfsii* and increase of seed germination was observed by other workers [23]. It is also reported that *T. harzianum* controlled 64-99% root rot of lentil due to *S. rolfsii* [24]. The percentage of seed germination in chickpea was found to be increased with the application of *Trichoderma* isolates in soil and seed treatment [25,26].

Fresh shoot and root weight, number of pod and yield were significantly influenced by the application of *Trichoderma*. In soybean the maximum shoot weight (94.5 g plant^{-1}) was recorded in case of soil treatment with *Trichoderma* followed by seed treatment with *Trichoderma* (82.8 g plant^{-1}) (Table 5). The highest root weight (10.7 g plant^{-1}) was observed in soil treatment and the lowest in the control plot (5.2 g plant^{-1}). The highest number of pod was measured in soil treatment (59 plant^{-1}) followed by seed treatment (51 plant^{-1}) and the lowest one was recorded in control plot (39 plant^{-1}). In aspect of yield the maximum was recorded in soil treatment with *T. harzianum* (2830 kg ha^{-1}) and the lowest was in the control plot (1632 kg ha^{-1}).

In chickpea, the highest shoot weight (62.5 g plant^{-1}), root weight (9.3 g plant^{-1}), the highest number of pod (69 plant^{-1}) and the maximum yield (2136 kg ha^{-1}) were recorded in the plot where *T. harzianum* was incorporated in the soil and the lowest ones were observed in the control plot (Table 6). Thus the maximum fresh shoot and root weight, number of pod and yield in chickpea and soybean were obtained in soil treatment. The growth promoting effect on plant and yield by application of *Trichoderma* sp. has been suggested in soybean [27,28] and in chickpea [29,30].

The present study indicates that soil treatment with *Trichoderma* was found to be the most effective for the reduction of collar rot disease in soybean and chickpea. The highest germination, plant stand, root and shoot fresh weight and yield were also observed in this treatment. Numerous reports suggested that broadcast application of biocontrol agents in the soil was superior to seed coating for protecting seedling diseases caused by *Fusarium, Pythium, Sclerotium* [31,32]. Soil amendment with *T. harzianum* gave 61.5% disease control of root rot of chickpea while seed treatment with *T. harzianum* gave 30% less

Table 5. Effect of *Trichoderma harzianum* on shoot weight, root weight, number of pod and yield of soybean

Treatments	Shoot weight (g plant^{-1})	Root weight (g plant^{-1})	Number of pod (plant^{-1})	Yield (kg ha^{-1})
Soil treatment with *T. harzianum*	94.5	10.7	59	2830
Seed treatment with *T. harzianum*	82.8	8.5	51	2573
Control	59.4	5.2	39	1632
LSD (P≥0.05)	6.7	2.2	10.7	880

Data are the mean of two consecutive years

Table 6. Effect of *Trichoderma harzianum* on shoot weight, root weight, number of pod and yield of chickpea

Treatments	Shoot weight (g plant^{-1})	Root weight (g plant^{-1})	Number of pod (plant^{-1})	Yield (kg ha^{-1})
Soil treatment with *T. harzianum*	62.5	9.3	69	2136
Seed treatment with *T. harzianum*	58.4	7.1	62	1920
Control	43.7	5.0	46	1202
LSD (P≥0.05)	5.4	1.9	13.5	706

Data are the mean of two consecutive years

disease control compared to the control [33]. As Trichoderma sp. is naturally soil inhabitant, therefore it has an opportunity to establish and multiply more quickly in soil than on seed surface. In addition, the inocula of *T. harzianum* that was mixed with soil was grown in chickpea bran which might be acted as “food package” to enhance the growth of the antagonist.

4. CONCLUSION

In the present study, the *in vitro* assessment of the effect of *T. harzianum* against *S. rolfsii* revealed that the antagonist could inhibit the growth of *S. rolfsii*. In field experiments, application of *T. harzianum* in soil resulted lower disease incidence of collar rot in soybean and chickpea and also increased fresh weight of plants and yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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