EVALUATION OF *TRICHODERMA* SPECIES AS BIO-FERTILIZER ON GROWTH AND YIELD OF ASHWAGANDHA [*WITHANIA SOMNIFERA* (L.) DUNAL]

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ABSTRACT

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The effect of three *Trichoderma* species viz. *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431, and *T. harzianum* IMI-392433 were evaluated as biofertilizer on growth, yield, and yield components of Ashwagandha. A pot experiment was conducted with randomized block design with three replications at Forest Protection Division Nursery, Bangladesh Forest Research Institute, Chattogram, Bangladesh from July 2017 to December 2017. The experiment consisted of fourteen treatments viz. T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *harzianum* IMI-392433 + NPK and T_{13}: *harzianum* IMI-392433 + CDM + NPK. The result exhibited that a combination of *T. harzianum* IMI-392433, cow dung manure and NPK fertilizers (T_{13}) had a significant effect and increased the germination percentage, growth traits and yield contributing characteristics of Ashwagandha. The correlation analysis revealed that the root yield/plant of Ashwagandha had significant and positive correlation with growth and yield contributing characters. Integrated application of *T. harzianum* IMI-392433, cow dung and NPK fertilizers showed better performance and gave the highest yield. The results recommend that inorganic fertilizers (NPK) and cow dung with *T. harzianum* IMI-392433 is suitable for better production of Ashwagandha that may increase soil fertility and this integrated approach could be contributed to improve crop production. Hence, *T. harzianum* IMI-392433 can be a growth promoter and this strain may be used as an effective bio-fertilizer for the production of Ashwagandha.

Key words: *Trichoderma*, Bio-fertilizer, Ashwagandha, Growth, Yield.

INTRODUCTION

Ashwagandha [*Withania somnifera* (L). Dunal.] is an important medicinal plant native to Bangladesh. It is belonging to the family Solanaceae. It is widely distributed in the tropical climate of Indian sub-continent, South Africa, the Mediterranean and Middle East regions (Agarwal *et al*. 2003). In traditional medicine systems, drugs made from Ashwagandha are used to treat general/sexual/nervine debility, stress, bone weakness, anxiety, high blood pressure, constipation, aging, muscular weakness, insomnia and as an everyday health supplement to cope with modern day stress. Leaves are used for curing fever, lesions, swelling, sore eyes and syphilitic sores. Green berries are used for treating ringworm infection, animal sores and horse's girth galls (Singh and Kumar 1998). The roots are credited with several medicinal properties. Ashwagandha is cultivated for food-supplement, herbal and medicine industries. The ease of cultivation and high price for the roots is attracting farmers for large scale cultivation. Nowadays, Ashwagandha is cultivated for medicinal purposes in fields and open grounds throughout Bangladesh. Due to high demand of raw materials of this medicinal plant and also market assurance of some reputed pharmaceuticals companies, the farmers of the northern districts of Bangladesh have come forward to cultivate this important medicinal plant commercially. At present farmers are commercially cultivating Ahwagandha in different districts of Northern part of Bangladesh such as Natore, Bogra, Gaibandha, Joypurhat and Naogaon (Rahman *et al*. 2019). At this time, farmers are facing various diseases at the field level, resulting in reduced yields. Application of the fungicides is not economical in the long time because they pollute the environment, leave harmful residues and can lead to the development of resistant strains of the pathogen with repeated use (Vinale *et al*. 2008). Farmers also apply chemical fertilizers for cultivation this plant in the field. Furthermore, Ashwagandha plant parts is used directly as a medicine. Uses of chemical fungicides and fertilizers are extremely harmful to the human body. Replacement of chemical fungicides and fertilizers with bio-control agents are an alternative to manage the plant pathogens, produce safe food and reduce the environment pollution (Barakat and Al-Masri 2005). The use of bio fertilizer as a biological agent could be cost-reducing and effective strategy. *Trichoderma* is a filamentous soil fungus that functions as a bio-control agent for a wide range of economically important aerial and soil borne plant pathogens (Harman *et al*. 1998). *Trichoderma* spp. also is commercially marketed as bio pesticides, bio-fertilizers, and soil amendments. The application of *Trichoderma* fungi in agriculture can provide numerous advantages such as; colonization of the root and rhizosphere of plant, control of plant pathogens by different mechanisms, improvement of the plant health by promote plant growth and stimulation of root growth (Lorito *et al*. 2010). Akladious and Abbas (2014) investigated that the application of *T. harzanium* increased all measured parameters such as growth parameters, chlorophyll content, starch content, nucleic acids content, total protein and phytohormone of maize plant. In another study, Doni *et al*. (2014) found that *Trichoderma* was able to enhance rice growth components such as plant height, leaf number, tiller number, root length and shoot fresh weight. They also reported that elicitors released by *Trichoderma* are involved in triggering expressions of defence protein within the plant to induce plant immunity against pathogens and, in turn, improve plant growth. In Bangladesh, *Trichoderma* species are mainly used as biocontrol of soil and seed borne pathogens. Therefore, the purpose of this study was to evaluate the three *Trichoderma* species as bio fertilizer alone or in combinations with cow dung compost and chemical fertilizers on growth, yield and yield contributing characters of Ashwagandha under field condition.

MATERIALS AND METHODS

A pot experiment was conducted with randomized block design with three replications at Forest Protection Division Nursery, Bangladesh Forest Research Institute, Chattogram, Bangladesh during July 2017 to December 2017.

Sources of Trichoderma

Three *Trichoderma* species, including *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431 and *T. harzianum* IMI-392432 were used in this study which were collected from the Biotechnology and Microbiology Laboratory, Department of Botany, Rajshahi University, Bangladesh. These strains were previously verified by CABI Bioscience, Surrey, UK.

Collection and sowing of Seeds

Seeds of Ashwagandha were obtained from FPD, BFRI, Chattogram, Bangladesh. Seeds were first disinfected superficially in 0.05% sodium hypochlorite solution for 3 min then washed three times in sterilized distilled water. These moistened seeds were spread over the polythene sheet for two hours and these seeds were sown 10 seeds/pot.

Preparation of Inocula

For preparation of *Trichoderma* inocula, Erlenmeyer flasks containing 100 g of wheat grains and 100 ml of water were autoclaved at 121 ºC for 1 h on three successive days. After cooling, about 5-7 small plugs of seven- day-old culture of each *Trichoderma* species individually were dropped into each flask under sterilized condition. The flasks were then kept at 25 ºC for 7 days. After that, colonized wheat grains were then transferred into paper pockets, and were dried and ground. Ten gm of this inoculum (10^8 CFU/gm) was used to add to 1 Kg of pot soil (Ommati and Zaker 2012).

Preparation and application of spore suspensions for seed treatment

Mycelial discs (5 mm diameter) of three *Trichoderma* species were obtained from 4-5 days old culture and separately transferred to 50 mL PDA in a 250-mL conical flask and incubated at 28^o C. After incubation, 30 mL of sterile distilled water was added to each culture and the flasks were shaken at 50 rpm for 30 min in an orbital shaker. Then the content of each conical flask was filtered through sterile muslin cloth. The culture filtrate, containing the spores, was collected, and a concentration of 5×10^5 spores/mL was obtained by dilution with sterilized distilled water. For seed treatment, 10 to 15 seeds were dipped in the spore suspension (5×10^5 spores/mL) of 4-5 days old of three *Trichoderma* for about 20 min, and the treated seeds were dried by laminar air flow. After germination of the treated seeds, the pot soil was treated with 30 mL of conidial suspension of three *Trichoderma* species according to respective treatment. The treatment was continued up to harvesting with ten days intervals.

Sterilization and application of soil

Soil was collected from the research field of FPD Nursery of BFRI, Bangladesh and sterilized with formaldehyde (formalin: water; 1:5 V/v) and covered with polythene. After 30 days of sterilization, sterilized soil and cow-dung manure (1:1) were put in the earthen pot (30 × 20 cm) each of 5 kg capacity. Fifty gm of prepared each *Trichoderma* inoculum powder singly and in combination with NPK (0.54 gm urea, 0.08 gm TSP, and 0.48 gm MoP) fertilizers was mixed well in each pot soil. Only soil filled pot used as control.

Treatments

Fourteen treatments were used in the experiment viz. T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 +CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *harzianum* IMI-392433 + NPK; T_{13}: *harzianum* IMI-392433 + CDM + NPK.

Data recording

Percent seed germination, growth, yield and yield attributes were recorded at 90 days after sowing. For determination of seedling vigor index after 10 days of sowing 5 seedlings were randomly selected from each pot and their individual shoot and root length were measured. The vigor of the seedlings was determined by following the formula of Abdul-Baki and Anderson (1973).

Vigor index = [mean of root length (cm) + mean of shoot length (cm)] × percentage of seed germinations.

Experimental design and statistical analysis

The experiment was carried out following Randomized Block Design with three replications and ten plants were used for each replicate. Data were analyzed by the MSTATE-C program and means were separated by DMRT. Correlation matrix were done with the help of SPSS (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Seed germination percentage and vigor index

The significant (P=0.05) variation was found on percent seed germination in different treatments (Figure 1-5). The highest seed germination (93.28 %) and vigor index (755.57) was recorded in treatment T13 (*T. harzianum* IMI-392433 + CDM + NPK) and the lowest seed germination (69.39 %) and vigor index (294.22) was found in control (T_0). From the above findings it may be decided that combination of *T. harzianum* IMI-392433, cowdung manure and NPK fertilizer (T_{13}) can increase seed germination % and vigor index of Ashwagandha. The similar results were found by Rahman *et al*. (2010) who stated that application of *Tricho*-compost with NPK fertilizers significantly increased the seed germination percentages and vigor index of chili. In another study, Kumar *et al*. (2019) observed that maximum root length (6.84 cm), shoot length (62.19 cm), number of branches (5.82) was depicted in seed treatment coupled with its three foliar sprays of T_2 (*T. harzianum*) isolate of *Trichoderma* in seedling stage of chili.

Growth characters

After 90 days plant height, leaf number, primary branches, secondary branches, fresh shoot weight, dry shoot weight and number of flowers at maximum flowering time were significantly ($p<0.05$) highest in combination of *T. harzianum* IMI-392433, cow dung manure and NPK treatment (T_{13}) and lowest in T_0 (Table 1 and Figure 6). The results are in agreement with the findings of Rahman *et al*. (2010) who stated that application of *Tricho*-compost with NPK fertilizers significantly increased the growth characteristics of chili. The same result was found by Kabir (1998) and Azad (2000) who stated that combine application of manures and chemical fertilizers performed the highest plant height of cabbage. Mahato *et al*. (2018) observed that, *T. viride* display antagonism with inorganic fertilizer in wheat, where *T. viride* and NPK were accompanied with farmyard manure showed the highest growth and yield parameter of wheat. They also stated that, *T. viride* increase several growth and yield parameters of wheat that can be used as biofertilizer.

Yield and yield contributing characters

The significant ($p<0.05$) variation was found in yield and yield contributing characters of different treatments. The highest total number of pod, total number of seed, 100 seed weight, seed yield/plant, root length, root diameter, fresh root weight and root yield/plant were recorded in treatment T_{13}(*T. harzianum* IMI-392433, cow dung manure and NPK) and lowest in control. (Table 2). From this result it was observed that combine treatment with *T. harzianum* IMI-392433, cow dung manure and NPK fertilizer increased the yield of Ashwagandha. These increases might be related to the positive effect of compost and microorganisms in increasing the root surface are per unit of soil volume, water-use efficiency and photosynthetic activity, which directly affects the physiological processes and utilization of carbohydrates. Mahato *et al*. (2018) found that *T. viride* has a growth-promoting effect that increase yield of wheat. Halifu *et al*. (2019) stated that seed inoculated with *T. harzianum* E15 and *T. virens* ZT05 significantly increased seedling biomass, root structure index, soil nutrients, and soil enzyme activity of pinus seed compared with the control.

Correlation matrix

The correlation matrix among different parameters is presented in Table 3. The correlation matrix showed that root yield/plant of Ashwagandha had significant and positive correlation with plant height (r = 0.961**), number of leaf (r = 0.949**), number of primary branch (r = 0.816**), number of secondary branch (r = 0.974**), fresh shoot weight (r = 0.936**), dry shoot weight (r = 0.954**), number of flower at maximum flowering time (r = 0.915**), number of pod/plant (r = 0.946**), total number of seed (r = 0.944**), hundred seed weight (r = 0.894**), root length (r = 0.957**), root diameter (r = 0.917**), fresh root weight (r =0.943**) and seed yield/plant (r = 0.981**). These results indicated that yield of Ashwagandha depends on plant height, number of leaf, number of primary and secondary branch, number of flower, root length, root diameter, fresh shoot weight, number of pod, total number of seed, fresh shoot weight, dry shoot weight and hundred seed weight. In the similar study, Rahman *et al*. (2012) reported that growth and yield of chili were significantly increased with the application of bio compost and NPK fertilizer and that was significantly and positively correlated with total plant height, leaf number, number of primary branch, number of secondary branch, root number, root length, total number of flower at maximum flowering time, total number of fruit, fruit length, fresh fruit weight, dry fruit weigh, number of seed/ fruit, and hundred seed weight.

Conclusion

The results revealed that the integrated application of *T. harzianum* IMI-392433, cow dung manure, and NPK fertilizers showed better performance and gave the highest yield. The results suggest that inorganic fertilizers (NPK) and cow dung compost with *T. harzianum* IMI-392433 is suitable for better production of Ashwagandha that may increase soil fertility and this integrated approach could be contributed to improving crop production. Hence it is indicated that *T. harzianum* IMI-392433 can be a growth promoter and this species may be used as an effective bio-fertilizer for the production of Ashwagandha.

Table 1. Effect of *Trichoderma* strains on growth attributes of Ashwagandha under field conditions

Treatments	Plant height at 90 DAS (cm)	Number of leaf at 90 DAS	Number of primary branch at 90 DAS	Number of secondary branch at 90 DAS	Fresh shoot weight at 90 DAS (gm)	Dry shoot weight at 90 DAS (gm)	Number of flower at the maximum flowering stage
T_0	24.84 k	8.52 k	2.12 ab	3.18 d	157.37 k	42.53 l	6.96 k
T_1	26.52 j	14.93 j	1.19 b	4.52 cd	159.82 j	45.36 k	8.54 k
T_2	28.92 i	18.72 i	2.54 ab	5.43 bc	169.12 h	49.15 i	25.49 j
T_3	31.74 g	23.37 g	2.65 ab	5.91 bc	176.48 f	58.38 f	32.76 h
T_4	36.83 d	30.75 d	2.98 ab	6.93 b	182.39 d	62.18 d	40.63 e
T_5	42.12 b	36.94 b	3.84 a	8.98 a	188.75 a	70.36 b	49.74 b
T_6	27.53 ij	16.42 j	2.49 ab	5.18 bc	162.42 i	47.28 j	24.64 j
T_7	30.62 gh	22.83 g	2.61 ab	5.87 bc	173.68 g	54.26 g	29.18 i
T_8	34.53 e	28.62 e	2.84 ab	6.75 b	180.29 e	60.78 de	38.74 f
T_9	40.86 b	35.83 b	3.29 a	8.87 a	186.68 b	69.42 b	45.18 c
T_{10}	29.39 h	20.62 h	2.58 ab	5.74 bc	172.95 g	52.42 h	27.58 i
T_{11}	32.74 f	26.93 f	2.79 ab	6.64 b	178.63 e	59.79 ef	36.53 g
T_{12}	38.73 c	32.83 c	3.12 ab	8.82 a	184.72 c	65.39 c	42.35 d
T_{13}	44.24 a	38.72 a	3.96 a	9.18 a	189.27 a	72.37 a	52.18 a

In a column same letters are not significantly different by DMRT at 5% level.

T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *harzianum* IMI-392433 + NPK; T_{13}: *harzianum* IMI-392433 + CDM + NPK.

Table 2. Effect of *Trichoderma* strains on yield and yield contributing characters of Ashwagandha under field conditions

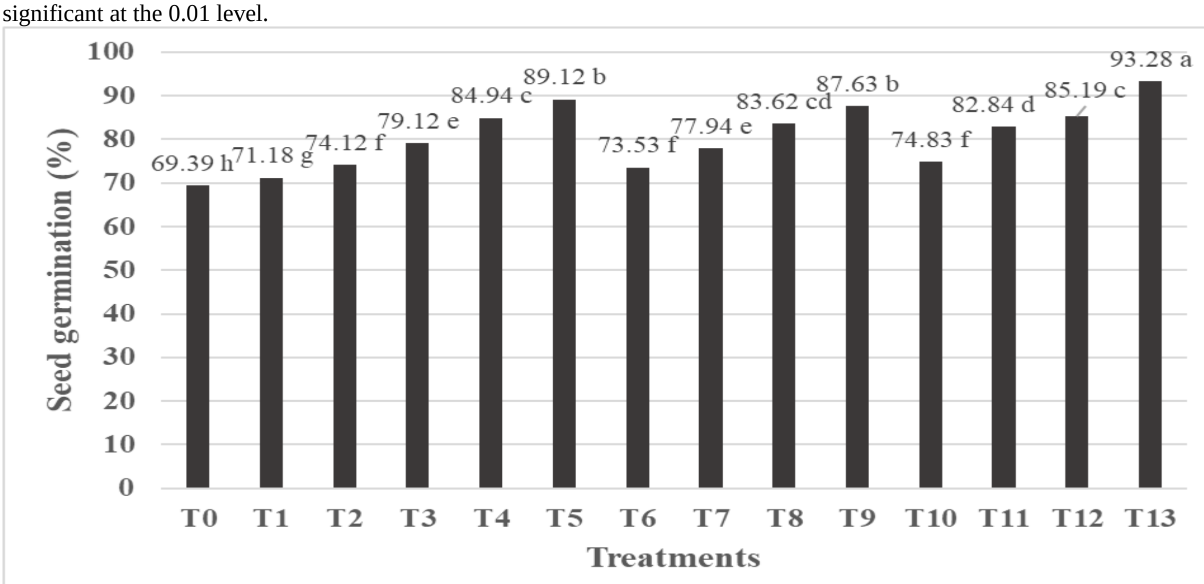
Treat-ments	Number of pod /plant at first harvest time	Total Number of seed (count 10 pod /plant)	100 seed weight (gm)	Seed yield /plant (gm) at first harvest time	Root length /plant at 180 DAS (cm)	Root Diameter /plant at 180 DAS (cm)	Fresh root weight /plant at 180 DAS (gm)	Root yield /plant (gm) at 180 DAS
T_0	7.19 m	25.65 l	0.97 c	2.34 g	6.24 n	1.14 e	22.25 k	2.96 h
T_1	14.38 l	30.25 k	1.53 bc	2.98 g	8.63 m	2.75 e	28.48 j	3.28 gh
T_2	18.38 j	34.19 j	1.98 bc	3.42 g	12.68 k	3.48 cd	32.12 i	4.28 efgh
T_3	25.24 g	43.28 g	2.58 abc	3.98 efg	18.53 g	3.98 bcd	40.68 f	5.93 de
T_4	32.62 d	52.86 d	2.85 ab	5.82 bcd	24.96 d	4.53 abcd	46.38 d	7.12 bcd
T_5	39.74 a	59.62 b	3.87 a	7.29 ab	30.28 b	5.85 ab	51.78 b	8.96 a
T_6	16.59 k	32.73 j	1.84 bc	3.16 fg	10.76 l	3.36 cd	30.89 i	3.86 fgh
T_7	23.42 h	39.23 h	2.41 abc	3.86 efg	16.64 h	3.85 cd	38.52 g	5.31 def
T_8	29.51 e	49.13 e	2.74 abc	5.21 cde	22.74 e	4.36 bcd	44.65 e	6.86 cd
T_9	37.64 b	56.39 c	3.18 ab	6.98 abc	28.31 c	4.75 abc	49.96 c	8.73 ab
T_{10}	20.63 i	36.25 i	2.16 abc	3.74 efg	14.85 g	3.76 cd	36.32 h	4.97 efg
T_{11}	27.49 f	47.18 f	2.63 abc	4.94 def	20.78 f	4.12 bcd	42.28 f	6.12 cde
T_{12}	35.86 c	54.98 c	2.98 ab	6.14 bcd	26.68 c	4.63 abcd	47.62 d	7.84 abc
T_{13}	41.28 a	62.37 a	3.96 a	8.32 a	32.17 a	6.29 a	53.83 a	9.29 a

T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *harzianum* IMI-392433 + NPK; T_{13}: *harzianum* IMI-392433 + CDM + NPK.

Table 3. Correlation matrix among different parameters of Ashwagandha as influenced by treatments

Para meter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1														
2	0.981**	1													
3	0.665**	0.620**	1												
4	0.931**	0.924**	0.823**	1											
5	0.952**	0.983**	0.619**	0.895**	1										
6	0.982**	0.991**	0.626**	0.911**	0.984**	1									
7	0.940**	0.971**	0.633**	0.885**	0.976**	0.966**	1								
8	0.980**	0.998**	0.612**	0.921**	0.982**	0.991**	0.972**	1							
9	0.983**	0.992**	0.609**	0.903**	0.977**	0.993**	0.965**	0.993**	1						
10	0.774**	0.757**	0.931**	0.902**	0.739**	0.752**	0.733**	0.751**	0.738**	1					
11	0.986**	0.995**	0.640**	0.917**	0.986**	0.995**	0.971**	0.995**	0.997**	0.760**	1				
12	0.832**	0.836**	0.884**	0.937**	0.810**	0.814**	0.822**	0.831**	0.807**	0.958**	0.826**	1			
13	0.965**	0.994**	0.616**	0.907**	0.989**	0.989**	0.977**	0.995**	0.988**	0.756**	0.992**	0.836**	1		
14	0.951**	0.919**	0.834**	0.968**	0.882**	0.916**	0.869**	0.912**	0.915**	0.905**	0.925**	0.927**	0.900**	1	
15	0.961**	0.949**	0.816**	0.974**	0.936**	0.954**	0.915**	0.946**	0.944**	0.894**	0.957**	0.917**	0.943**	0.981**	1

1= Plant height, 2=Number of leaf, 3=Number of primary branch, 4= Number of secondary branch, 5= Fresh shoot weight, 6 = Dry shoot weight, 7 = Number of flower at maximum flowering time, 8 = Number of pod/plant, 9 = Total number of seed, 10 = Hundred seed weight, 11 = Root length, 12 = Root diameter, 13 = Fresh shoot weight, 14 = Seed yield/plant, 15 = Root yield/plant. **. Correlation is significant at the 0.01 level.



Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis

T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *T. harzianum* IMI-392433 + NPK; T_{13}: *T. harzianum* IMI-392433 + CDM + NPK.

Figure 1. Seed germination (%) under different treatments at 12 DAS.

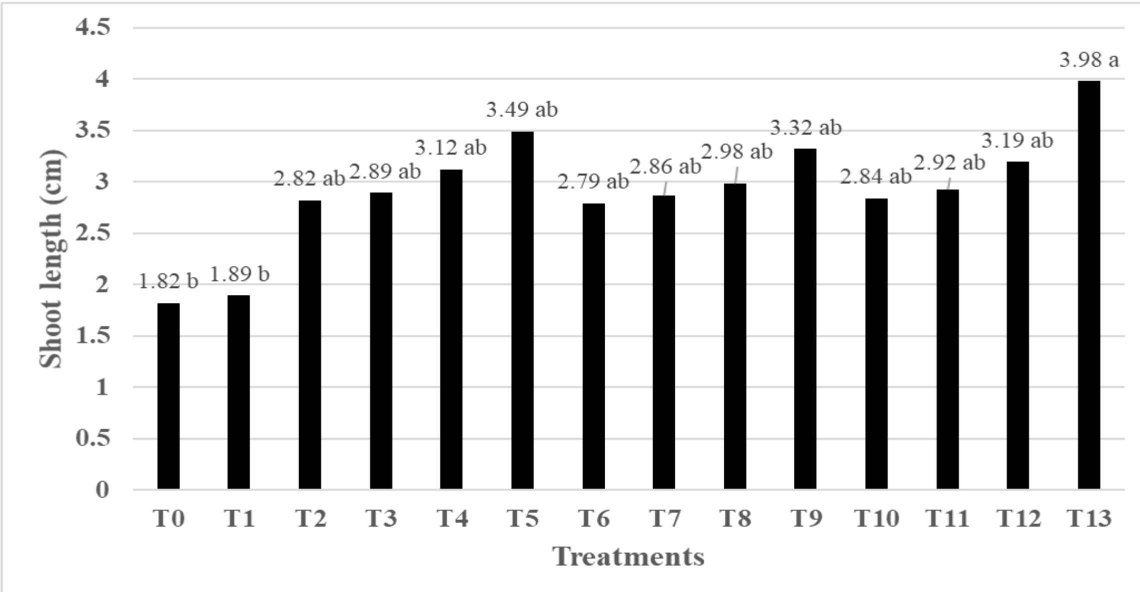


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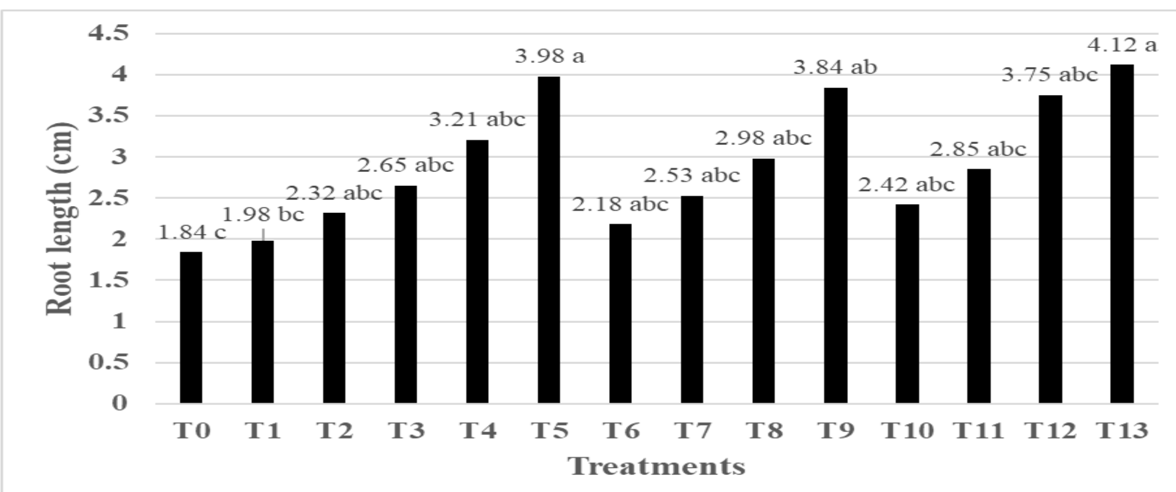
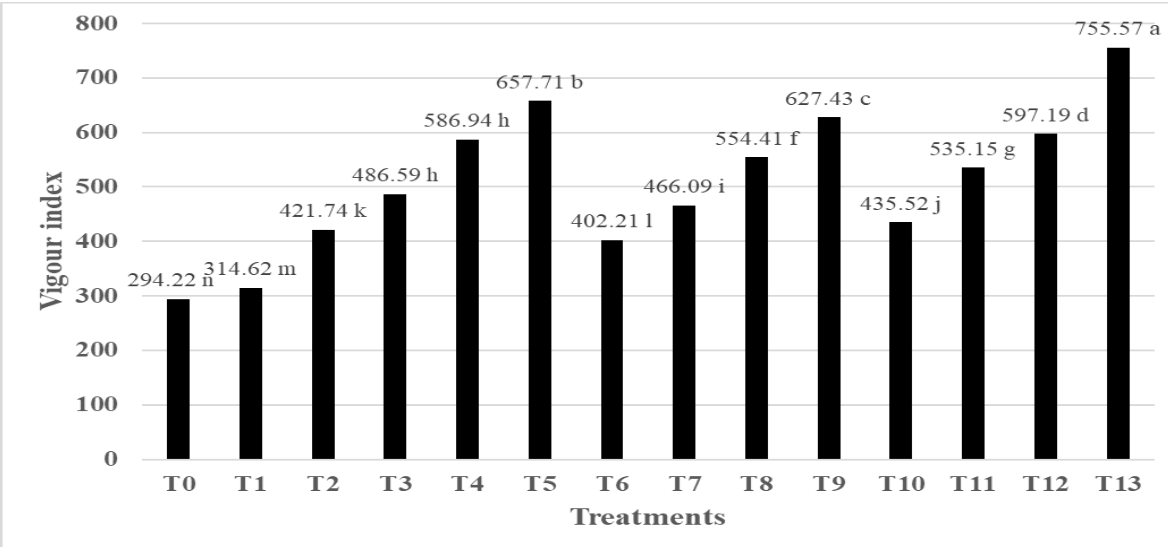
Figure 2. Shoot length (cm) under different treatments at 12 DAS.



Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis
T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *T. harzianum* IMI-392433 + NPK; T_{13}: *T. harzianum* IMI-392433 + CDM + NPK.

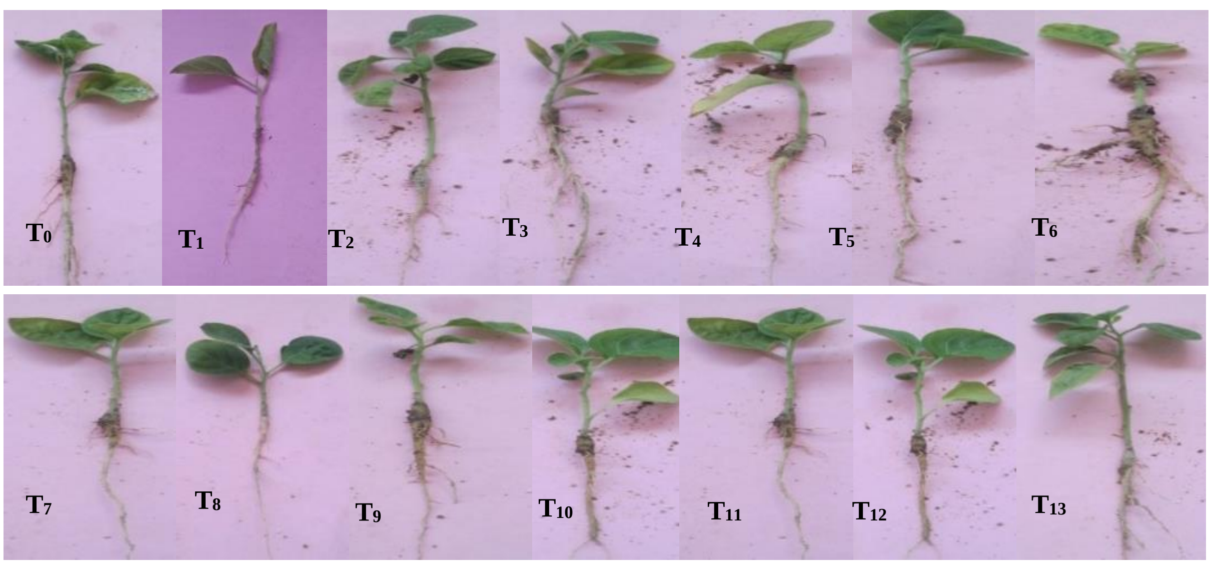
Figure 3. Root length (cm) under different treatments at 12 DAS.



Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis

T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *T. harzianum* IMI-392433 + NPK; T_{13}: *T. harzianum* IMI-392433 + CDM + NPK.

Figure 4. Vigor index under different treatments at 12 DAS.



T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *harzianum* IMI-392433 + NPK; T_{13}: *harzianum* IMI-392433 + CDM + NPK.

Figure 5. Effect of individual treatments of *Trichoderma* species on Shoot and Root length of Ashwagandha seedlings after 12 DAS.

A: Mass production of *Trichoderma* species; T_0: Control (Only soil); T_1: Soil + NPK; T_2: *T. virens* IMI 392430; T_3: *T. virens* IMI 392430 + CDM; T_4: *T. virens* IMI 392430 + NPK; T_5: *T. virens* IMI 392430 + CDM + NPK; T_6: *T. pseudokoningii* IMI-392431; T_7: *T. pseudokoningii* IMI-392431 + CDM; T_8: *T. pseudokoningii* IMI-392431 + NPK; T_9: *T. pseudokoningii* IMI-392431 + CDM + NPK; T_{10}: *T. harzianum* IMI-392433; T_{11}: *T. harzianum* IMI-392433 + CDM; T_{12}: *harzianum* IMI-392433 + NPK; T_{13}: *harzianum* IMI-392433 + CDM + NPK.

Figure 6. Effect of Individual Treatments of *Trichoderma* Species on Growth Characters of Ashwagandha after 90 DAS

LITERATURE CITED

Abdul-Baki, A.A. and Anderson, J.D. 1973. Vigor Determination in Soybean Seed by Multiple Criteria, *Crop Science*, 13:630-633.

Agarwal, M., Agarwal, M.K., Singh, P. and Gupta, A.K. 2003. Economic Evaluation of Different Treatment Combinations of Sowing Time and Spacing in Ashwagandha, *Current Agriculture,* 27(1&2): 109-110.

Akladious, S.A. and Abbas, S.M. 2014. Application of *Trichoderma harzianum* T22 as a Biofertilizer Potential in Maize Growth, *J. Plant Nutrition,* 37(1): 30-49.

Azad, A.K. 2000. Effects of Plant Spacing, Source of Nutrients and Mulching or Growth and Yield of Cabbage, M.S. Thesis, Department of Horticulture, Bangladesh Agricultural University, Mymensingh, Bangladesh: 15-40pp.

Barakat, R.M. and Al-Masri, M.I. 2005. Biological Control of Gray Mold Disease (*Botrytis cinerea*) on Tomato and Bean Plants by Using Local Isolates of *Trichoderma harzianum*. Dirasat, *Agril Sci.*, 32(2):145-156.

Doni, F., Isahak, A., CheMohd Zain, C.R. and Wan Yusoff, W.M. 2014. Physiological and Growth Response of Rice Plants (*Oryza sativa* L.) to *Trichoderma* spp. Inoculants. *AMB Express* 4:45.

Halifu, S., Deng, X., Song, X. and Song, R. 2019. Effects of Two *Trichoderma* Strains on Plant Growth, Rhizosphere Soil Nutrients, And Fungal Community of *Pinus sylvestris* var. mongolica Annual Seedlings, *Forests,* 10(9): 1-17.

Harman, G. E and Bjorkman, T. 1998. Potential and existing uses of Trichoderma and Gliocladium for plant disease control and plant growth enhancement. In: Kubicek CK, Harman GE eds. Trichoderma and Gliocladium. London, England: Taylor and Francis. pp 229 – 265.

Kabir, H.T. 1998. Effect of Sources of Nutrients on Yield and Quality of Cabbage, M.S. Thesis, Dept. of Horticulture, Bangladesh Agricultural University, Mymensingh: 13-39pp.

Kumar, A., Patel, A., Singh, S.N. and Tiwari, R.K. 2019. Effect of *Trichoderma* spp. in Plant Growth Promotion in Chilli, *Int. J. Current Microbiol. Applied Sci.,* 8(3): 1574-1581.

Lorito, M., Harman, G.E.and Mastouri, F. 2010. Translational Research on *Trichoderma*: From Omics to the Field, *Ann. Revi. Phytopath.*, 48:395–417.

Mahato, S., Bhuju, S. and Shrestha, J. 2018. Effect of *Trichoderma Viride* as Biofertilizer on Growth and Yield of Wheat, *Malaysian J. Sustainable Agril.*, 2(2): 01-05.

https://doi.org/10.1016/j.soilbio.2007.07.002.

Ommati, F. and Zaker, F. 2012. *In Vitro* and Greenhouse Evaluations of *Trichoderma* Isolates for Biological Control of Potato Wilt Disease (*Fusarium solani*), *Archives of Phytopath.Plant Protec.,* 45 (13-16): 1715-1723.

Rahman, M.A., Begum, M.F., Alam, M.F., Mahmud, H. and Khalequzzaman, K.M. 2010. Effect of Tricho-compost, Compost and NPK Fertilizers on Growth Yield and Yield Components of Chili, *I. J. Sustainable Agril. Tech.,* 6(3): 64-72.

Rahman, M.A., Moni, Z.R., Hussen, M.A. and Ansari, T.H. 2019. Prevalence of Fungal Diseases in Medicinal Plants in Northern Region of Bangladesh, *SAARC J. Agril.*, 17(2): 197-212. DOI: https://doi.org/10.3329/sja.v17i2.45306.

Rahman, M.A., Rahman, M.M., Begum, M.F. and Alam, M.F. 2012. Effect of Bio Compost, Cow Dung Compost and NPK Fertilizers on Growth, Yield and Yield Components of Chili, *Int. J.Biosci.*, 2(1): 51-55.

Singh, S. and Kumar, S. 1998. *Withania Somnifera*: The Indian Ginseng Ashwagandha. Central Institute of Medicinal and Aromatic Plants, Lucknow, India. 293pp.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R.S.L. and Lorito, M. 2008. *Trichoderma* - Plant Pathogens Interactions, *Soil Biology & Biochemistry,* 40: 1-10.