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Efficacy of *Trichoderma* isolates in enhancement of growth dynamics in soybean

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Abstract
The isolates of *Trichoderma* obtained from various regions of Pantnagar and Dehradun were screened for their ability to enhance germination and other growth parameters in soybean under *in vitro* as well as under *in vivo* conditions. All the fifteen isolates (PT-1, PT-2, PT-3, PT-4, PT-5, PT-6, PT-7, PT-8, PT-9, PT-10, DDNT-1, DDNT-2, DDNT-3, DDNT-4 and DDNT-5) were mass multiplied on the sorghum grains and grounded into fine powder. The soybean seeds were treated with the powdered *Trichoderma* formulation @ 10g/kg seed and kept in paper towel and incubated at 28±1°C for 7 days under *in vitro* condition. For *in vivo* screening, the *Trichoderma* formulation were applied to the soil @ 10g/ 2kg pot soil, seed treated with *Trichoderma* powder @ 10g/kg seed and foliar spray of *Trichoderma* powder @10g/l of water at 15 days after sowing was done. Under *in vitro* condition the isolate PT-2 (15.50 cm) recorded maximum plumule length while DDNT-4 (15.50 cm) showed highest radical length. However, under *in vivo* condition DDNT-4 (34.50 cm) recorded maximum shoot length and PT-5 (19.00 cm) showed maximum root length.

Keywords: *Trichoderma*, soybean, biocontrol, growth promotion

Introduction
Soybean is one of the most important oilseed crops sharing about 25 per cent in edible oil production globally. Among nine edible oilseed crops, it ranks fifth in world and third in India (Anonymous, 2018) [1]. Soybean (*Glycine max* L.) is a leguminous oilseed annual crop. Being the richest source of protein and oil it is also known as "Golden bean" or "Miracle crop". It serves as an excellent source of oil and protein consisting about 20-22% oil and 40-42% protein respectively. It also consists of high levels of amino acids like lecithin, lysine, leucine and large amount of phosphorus.

Soybean crop is very sensitive to change in the climatic conditions and thus hampering the productivity of the soybean leading to reduced germination and the yield in adverse climatic conditions. The successful cultivation of soybean requires high quality seeds which ensure better germination and yield (Mertz *et al*., 2009) [9]. The productivity of soybean is also affected by the incidence of the pest and diseases leading to huge economic losses. The *Trichoderma* species are well known for its disease suppression and growth promotion activities (Bai *et al.*, 2008 and Savazzini *et al.*, 2009) [2, 12]. The species of *Trichoderma* are ubiquitous in soil and root ecosystem and their application to the soil and seed may contribute to the enhanced plant growth, nutrient uptake and yield by employing several strategies like parasitism, competition for space and nutrients, stimulators for plant health and induced defense responses in the plants.

The sole application of *Trichoderma* to seed, soil or foliar spray is not giving the satisfactory results in soybean to enhance the plant growth and combating the pathogens. The present study was carried out to find out the efficiency of *Trichoderma* in plant growth promotion of soybean by applying the *Trichoderma* as soil treatment, seed treatment and foliar spray.

Material and Methods
Sample collection and isolation: The soil samples were collected from different locations of Pantnagar and Dehradun from the rhizospheric region of Rice, Soybean and Mungbean. The isolation of *Trichoderma* isolates was carried out using serial dilution method on *Trichoderma* Selective Medium. These isolates of *Trichoderma* were purified using the single spore isolation.

Mass multiplication of *Trichoderma* isolates: The *Trichoderma* isolates were mass multiplied on sorghum grains. The grains were soaked in water for 12 hrs and then filled in Erlenmeyer flask and autoclaved at 15 lb psi for 30 min.

Thereafter, the flasks were inoculated with *Trichoderma* and incubated for 12 days at 28±1 °C. These colonized sorghum grains were dried under shade for 4-5 days and grounded and sieved to obtain the fine powder.

Evaluation for growth promotion activity: The fifteen isolates of *Trichoderma* were evaluated under *in vitro* and *in vivo* conditions for their growth promotory activities. The soybean seeds were treated with powdered *Trichoderma* formulation @ 10g/kg seed except for the control. The seeds were kept in towel paper and incubated at 28±1°C for 7 days for *in vitro* evaluation. The observations were recorded for the seed germination, plumule and radical length. The *in vivo* evaluation was carried out by applying *Trichoderma* formulations to the soil @ 10g/2kg pot soil (10^8 spores/g), seed treatment with *Trichoderma* @ 10g/kg seed as well as the foliar spray of *Trichoderma* was done at 15 days after sowing @10g/l of water. The observations were recorded for the germination per cent, root and shoot length at 21 days after sowing. The Vigour index was calculated by following formula:

Vigour index = (shoot length + root length) × germination per cent

Statistical Analysis: The statistical data analysis of both lab and glasshouse experiments were carried out using STPR by using one way ANOVA.

Results and Discussion

In vitro all the fifteen (PT-1, PT-2, PT-3, PT-4, PT-5, PT-6, PT-7, PT-8, PT-9, PT-10, DDNT-1, DDNT-2, DDNT-3, DDNT-4 and DDNT-5) isolates of *Trichoderma* were evaluated for their growth promoting effect on soybean. Results presented in Table 1 and Plate 1 showed that all the isolates recorded 100 per cent germination under *in vitro* condition. The highest plumule length was recorded in case of isolate PT-2 (15.50 cm) which was statistically at par with the isolates PT-7, PT-8, PT-10, and DDNT-2 with plumule length of 15.50 cm, 14.50 cm, 15.30 cm and 14.50 cm respectively, but significantly higher than other isolates. However, isolates PT-1, PT-9, PT-3 and PT-6 with plumule length of 12.10 cm, 12.00 cm, 11.50 cm and 11.50 cm respectively were at par with the control (11.00 cm). In case of radical length, isolate DDNT-4 showed highest radical length of 15.50 cm followed by PT-4 (12.30 cm) which was significantly same with isolate DDNT-2 (11.50 cm). However, the isolates PT-2, PT-8, PT-3, PT-9 and PT-1 with radical length of 6.90 cm, 6.80 cm, 6.70 cm, 6.60 cm and 6.50 cm respectively were statistically at par with the control (6.40 cm). The isolates PT-1 and PT-3 were at par with control to both plumule and radical length.

The data presented in the Table 1 clearly revealed that under *in vivo* conditions the highest germination per cent (96.67%) was recorded in isolate PT-10 which was statistically at par with the DDNT-4 and DDNT-5 with germination percentages of 96.67% and 96.67% respectively. This was followed by PT-5 having germination per cent of 93.33% which did not differ significantly from PT-7 with germination per cent of 93.33 per cent. The highest shoot length was recorded for the isolate DDNT-4 (34.50 cm) followed by PT-10 (33.00 cm) which was at par with PT-5 (32.00 cm). The isolates PT-3, PT-6, PT-1 and PT-9 with shoot length 21.50 cm, 22.00 cm, 22.00 cm and 22.00 cm which did not differ significantly from control (21.50 cm).

Table 1: Efficacy of *Trichoderma* isolates on seedling and plant vigour under *in vitro* and *in vivo* conditions

S. No.	*Trichoderma* isolates	*In Vitro* (7 Days after inoculation)				*In Vivo* (21 Days after sowing)			
		Germ (%)	Plumule length* (cm)	Radical length* (cm)	Vigour index	Germ (%)	Shoot length** (cm)	Root length** (cm)	Vigour index
1.	PT-1	100.00	12.10	6.50	1860.00	86.67	22.00	12.50	2990.12
2.	PT-2	100.00	15.50	6.90	2240.00	86.67	26.00	16.50	3683.48
3.	PT-3	100.00	11.50	6.70	1820.00	90.00	21.50	18.00	3465.00
4.	PT-4	100.00	12.30	12.30	2460.00	86.67	25.00	14.00	3380.13
5.	PT-5	100.00	12.30	8.50	2080.00	93.33	32.00	19.00	4759.83
6.	PT-6	100.00	11.50	10.50	2200.00	90.00	22.00	16.00	3420.00
7.	PT-7	100.00	15.50	10.00	2550.00	93.33	27.00	16.00	4013.19
8.	PT-8	100.00	14.50	6.80	2130.00	90.00	25.00	12.50	3375.00
9.	PT-9	100.00	12.00	6.60	1860.00	90.00	22.00	13.00	3150.00
10.	PT-10	100.00	15.30	8.90	2420.00	96.67	33.00	16.00	4736.83
11.	DDNT-1	100.00	14.00	8.70	2270.00	86.67	24.50	16.00	3510.14
12.	DDNT-2	100.00	14.50	11.50	2600.00	90.00	28.00	18.00	4140.00
13.	DDNT-3	100.00	13.00	9.50	2250.00	90.00	31.00	14.00	4050.00
14.	DDNT-4	100.00	13.00	15.50	2850.00	96.67	34.50	18.50	5123.51
15.	DDNT-5	100.00	12.40	10.30	2270.00	96.67	27.50	15.50	4156.81
16.	Control	100.00	11.00	6.40	1740.00	83.33	21.50	9.00	2541.57
CD (5%)		-	1.19	0.84	-	1.58	1.21	1.27	-
S.Em±		-	0.41	0.29	-	0.55	0.42	0.44	-

*Mean of ten seedlings
**Mean of five plants

Plate 1: Growth promotion under *in vitro* condition

In case of root length highest was recorded with isolate PT-5 (19.00 cm) which was statistically same with DDNT-4 (18.50 cm), DDNT-2 (18.00cm) and PT-3 (18.00 cm). The minimum root length was observed for isolate PT-1 (12.50 cm) which was at par with PT-8 (12.50 cm) but significantly better than control (9.00 cm). This increase in root and shoot length may be attributed to production of plant hormones (Chang *et al*., 1986; Baker, 1988) [4, 3], production of vitamins and conversion of unavailable plant nutrients into available form for easy uptake by plants (Kleifeld and Chet, 1992) [7], release of soil minerals and nutrients (Ousley *et al*., 1994) [11], suppression of plant pathogens (Inbar *et al*., 1994) [5].

These findings are supported by the results obtained by Maisuria and Patel (2009) [8]. They observed *T. viride* resulted an increase in the root length, shoot length and germination per cent of soybean seeds. The germination per cent varied from 76.00 to 96.00 when seeds were treated with six different *Trichoderma* species. Mukhtar *et al*. (2012) [10] reported increased seed germination of *Trichoderma* treated soybean seeds. Tancic *et al*. (2013) [13] evaluated the effect of seed treatment with *Trichoderma* isolates on soybean seedlings. They observed significant positive effect in treatment with four *Trichoderma* isolates - K114, K132, K150 and K160 on both germination and root length in glasshouse as compared to control. Joshi *et al.* (2018) [6] assessed the effect of seed treatment with phosphorous solubilizing bacteria, *T. viride* and *Pseudomonas flurescence* on soybean seedlings. They observed increased in growth parameters i.e., germination per cent, shoot length, root length, leaf area and seedling vigour index in case of phosphorous solubilizing bacteria (PSB) followed by *T. viride*.

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