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Potential Role of *Trichoderma asperellum* T42 Strain in Growth of Pea Plant for Sustainable Agriculture

Bansh Narayan Singh[1,2], Archana Singh[2], Gopal Shankar Singh[2] and Padmanabh Dwivedi[1]*

[1]Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221 005, India.
[2]Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi - 221 005, India.

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***Trichoderma* species are endophytic plant opportunistic symbionts and ubiquitous in nature. They have potential as biocontrol agents of various plant diseases as well as help in improving crop production. *Trichoderma* species shows symbiotic association with apoplastic part of plant roots and has direct effect on plants. It has positive effects on seed germination, plant nutrient uptake and inorganic fertilizer efficiency, thus helps in improving plant growth and yield. In the present study, *Trichoderma asperellum* T42 strain was used to investigate its effect on pea (*Pisum sativum)* plant and biomass in the soil. *Trichoderma* treated pea seeds showed increased percentage of germination rate as compared to control plants. Apart from that, induced root and shoot development, increased photosynthetic pigment (chlorophyll), carotenoid, total sugar and protein content as compared to control plants were observed. Our study suggested that *Trichoderma asperellum* T42 can be used as plant growth promoting fungus similar to other *Trichoderma* species for sustainable agricultural practices. Therefore, it could be helpful in minimizing the rampant use of chemical fertilizers for improving agricultural and horticultural practices thus improving sustainability of agriculture.**

Key words: Chlorophyll content, *Pisum sativum*, Seed germination, Sustainable agriculture, *Trichoderma asperellum* T42.

In the modern agriculture practices, use of chemical fertilizers is primary method for increasing crop productivity. But, it has potential negative impact on agriculture fields, soil microbial biomass and environmental pollution. Excess use of chemical fertilizer causes reduction of soil fertility, soil microbial biomass and pH of soil leading to alkalinity[1]. Now-a-days, for enhanced crop productivity, soil fertility and disease management, bio-fertilizers are most commonly used in the agricultural field in place of chemical fertilizers. Over the past 30 years, microorganisms have been described, characterized and tested for use as plant growth regulators and bio-control agents. Among them, *Trichoderma* spp. is effective common filamentous imperfect saprophytic fungi in soil and rhizosphere ecosystem, exploited as plant growth promoting fungus[2,3] for suppression of different soil borne pathogens[4] by various mechanism such as myco-parasitism, MAP kinase pathway heterotrimeric G protein pathway and cAMP pathway[5]. It is colonized in the rhizospheric zone of plant roots with beneficial effects on the plant. Plant roots secrete highly hydrated form of mono and di-saccharides into the rhizosphere which encourage growth and persistence of the fungi in their rhizospheric zone. *Trichoderma* use plant derived sucrose as carbon source to facilitate

* To whom all correspondence should be addressed.
E-mail: pdwivedi25@rediffmail.com

root colonization, the coordination of defence mechanisms and enhancing rate of photosynthesis[6]. Several phyto-stimulation mechanisms associated with *Trichoderma* spp. including improved root development and auxin production[7], siderophore production[8,9], increased drought tolerance[10], expressions of defense protein within the plant[11], phosphate-solubilizing[12], releasing elicitors[13] and increased salt resistance[14] have been reported. Some *Trichoderma* rhizosphere competent strains have been shown to have direct effects on plants increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences against biotic and abiotic damage[15].

The taxonomy of *Trichoderma* species is very complex and has been the subject of many recent taxonomic studies[16] apart from having a high level of genetic diversity[17]. Thus it is likely that only a few of the species available have been utilized as plant growth regulator. In this present investigation, we used the *T. aperellum* T42 to study the beneficial effect on plant growth.

MATERIALS AND METHODS

Microbial strain and treatment

T. asperellum T42 was procured from the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi, India. The strain was routinely cultured on potato dextrose agar (PDA) at 28±2°C on slants for 6-7 days. The seed treatment was done according to method described[18]. The fungal spores of strain were separated with inoculation loop and suspended to 10^6 spores (1.41 A_{600}) per ml in 0.2% carboxy methyl cellulose (CMC) solution. Seeds of *Pisum sativum* were sterilized with 0.1% $HgCl_2$ and thoroughly shaken in spore suspension to ensure uniform coating of fungal spores on the seed surface. Seeds were then dried at room temperature (20–25°C) overnight before sowing. Six seeds were sown in each plastic pot (20 cm diameter, 5L) containing sterilized agricultural soil (sandy loam, pH 7.4). Non-treated seeds sown separately served as the control. Field experiments were conducted in the poly house, Department of Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi India.

Plant growth measurement

Percentage of pea seed germination was measured four days after fungal treatment. Pea plants were grown in pots and harvested; root and shoot length and number of leaves were determined after seedlings and vegetative stage. Pea biomass (g) was measured after root and shoot were dried in the oven at a temperature of 60°C for five days. The fourth and fifth leaves of each plant were detached from treated and control plants for estimation of biochemical test. Each treatment consisted of three replicates (each replicate containing six individual plants) and the experiment was repeated twice.

Biochemical parameters

Chlorophyll, protein and sugar contents were analyzed twice during the experimental period, at 21 (seedlings stage) and 40 days (vegetative stage) after the onset of the experiment.

Chlorophyll content

Assessment of chlorophyll content was performed as per method described[19].

Total protein estimation

Sample (500 mg) was homogenized with 5 mL of 80% ethanol and centrifuged at 4000 rpm for 20 min. Supernatant was kept aside and the residue was re-extracted twice with 4 mL of ethanol and centrifuged. The residue was hydrolysed in 5 mL of 1N NaOH for overnight and centrifuged at 4000 rpm for 20 min. This step was repeated before both the supernatants were pooled and volume was made to 10 mL. Total protein was estimated in this supernatant by Folin reagent following the described method[20].

Total sugar content

Total sugar content in the plant samples was measured by described method[21]. One gram of the leaf sample was homogenized with 10 mL ethanol till all the leaf tissues were fully digested. The homogenate was centrifuged at 4,000 rpm for 15 min. Volume of the extract was maintained to 100 mL by adding distilled water. Anthrone reagent (6 mL) was added in 1 mL of extract. The tubes were placed on a boiling water bath for 3 min after which they were allowed to cool. After some time blue colour developed in the test tubes and intensity of the blue colour was measured at 620 nm by a fluorescence spectrophotometer (Spectra-Max 2, Molecular Devices, USA). Sample without leaf was used as a blank. The amount of the sugar in the

leaf samples was calculated by standard curve.

Quantification of *Trichoderma* species from rhizospheric zone

Colony forming units (cfu) of *T. asperallum* T42 from rhizospheric soil of *Trichoderma* treated pea plant were counted following a standard method[22]. PDA containing 100 mg L^{-1} streptomycin sulphate was used for plating. Plates were incubated at 28±2°C and cfu was counted after seven days. Data were expressed per gram of dry soil.

Statistical analysis

Statistical analysis was performed by subjecting the data to ANOVA and analyzing them by the Duncan test for statistical significance at $p \leq 0.05$.

RESULTS

Trichoderma asperellum T42 treated pea seeds showed increased rate of germination; germination increased by 85-90% in *Trichoderma* treated seeds as compared to control (65-75%). Plant growth significantly ($p \leq 0.05$) increased from seedlings to vegetative stage: root length increased from 108 to 137 %, shoot length from 125 to 127 %, and number of leaves per plant from 142 to 143 % in seeds treated with *T. asperellum* T42 as compared to control (Table 1, Fig. 1). Root and shoot dry weight significantly increased from 1.23 to 4.83 g and 3.33 to 6.63 g, respectively in *Trichoderma* treated plants (Fig. 2A, B). These data indicate that *T. asperellum* T42 helps in plant growth and development. Total photosynthetic pigment (chlorophyll) content significantly increased from 3.10 to 4.30 mg per gram fresh weight as compared to control plants (2.67 to 3.59 mg per gram fresh weight) after 21 and 40 days, respectively (Fig. 2C). However, carotenoid content significantly ($p \leq 0.05$) increased in seedlings stage but not affected at vegetative stage (Fig. 2D). Apart from that, total protein content and sugar content were significantly increased by 16.93 to 20.73 mg per gram fresh weight and 0.5 to 0.69 mg per gram fresh weight from seedlings stage to vegetative stage, respectively in *Trichoderma* treated plants (Fig. 2 E, F). In the present experiment, colony forming unit was sharply increased from 2.8×10^8 to 25.7×10^8 cfu in the rhizospheric zone of infected pea plants (Table 2).

DISCUSSION

Relatively little is known about the host mechanism that connects the perception of *Trichoderma asperallum* T42 root colonization, leading to activation of developmental responses. *Trichoderma* spp. employs several mechanisms in influencing seed germination[21]. Seed germination rate and development during seed germination, plant height, number of leaves, root and shoot dry weight of seedlings are the most important indicators of seedling vigour. A number of mechanisms for plant growth promotion associated with *Trichoderma* have been proposed in several plant species[4,24,25]. It has been reported that *Trichoderma* spp. SL2 had potential capacity to increase rice plant growth[26]. In the present experiment, *Trichoderma asperellum* T42 significantly increased pea seedling's growth, thus making a potential source for high germinating seeds, root and shoot length, number of leaves per plant, total root and shoot dry weight, total photosynthetic pigment (chlorophyll) and

Table 1. Effect of *Trichoderma asperellum* T42 on growth and development of pea plants at seedling and vegetative stage

Time	Seedlings stage (21 days)			Vegetative stage (40 days)		
Treatments	Root length (cm)	Shoot length (cm)	No. of leaf per plant	Root length (cm)	Shoot length (cm)	No. of leaf per plant
Control	4.33 ± 0.33 a	22.33 ± 2.18 a	16.67 ± 1.2 a	6.33 ± 0.33 a	29.67 ± 1.85 a	18.67 ± 0.33 a
Trichoderma asperellum T42	9.0 ± 0.88 b	50.33 ± 2.33 b	40.33 ± 2.9 b	15.0 ± 0.58 b	67.33 ± 0.88 b	45.33 ± 1.76 b

Data are mean value (±SE) of three independent experiments. Values with the different letters within the column were significantly different ($p \leq 0.05$) according to Duncan's test.

carotenoid. These attributes continuously increased from seedling to vegetative stage. These results indicated that *T. asperallum* T42 enhanced growth of pea plants. Similar observations were reported in tomato seedlings treated with *T. harzianum* T969 and *T. harzianum* T447[27]. Total sugar and protein content were significantly increased in *Trichoderma* treated plants in our study. This indicated that *Trichoderma asperallum* T42 was able to enhance the metabolic pathways of carbohydrate and protein. Colony forming unit observation indicated that this strain was able to symbiotically associate with roots with increasing number in rhizosphere. This also indicated that the increased growth response of pea plants, caused by *Trichoderma*, depended mainly on the ability of *Trichoderma* to survive and develop in the rhizosphere[17]. Root colonization by *Trichoderma* could be a result of not only the root exudates such as sugars and proteins, but

Table 2. Colony forming unit of *Trichoderma asperallum* T42 from rhizosheric soil after treatment

Days	Colony forming unit per gram soil sample
21	2.8×10^8
40	25.7×10^8

Fig. 1. Effects of *T. asperellum* T42 inoculation on the growth of pea plants. This strain was grown on the surface of PDA media plate at 28±2°C (A). Pea seeds treated with *T. asperellum* T42 showed enhanced effect in plant development: growth of root after 40 days (B) and shoots at 21 (C) & 40 days (D).

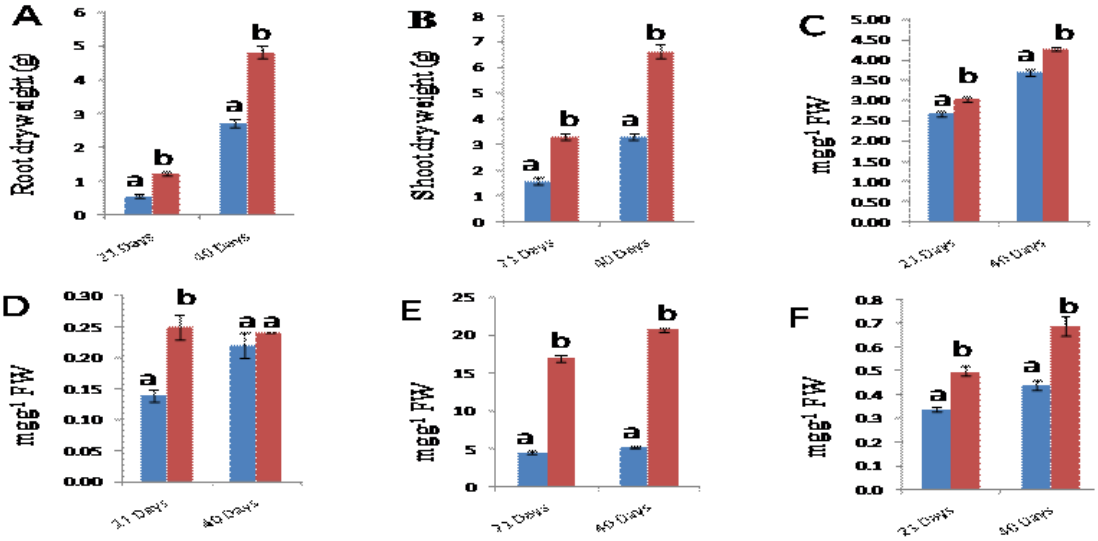

Fig. 2. Effects of *T. asperellum* T42 inoculation on growth of pea plants. Total root (A) and shoot dry weight (B) were observed in pea plants after 21 and 40 days. Comparative effect of total chlorophyll content (C), carotenoid content (D), total sugar content (E) and total protein content (F) were estimated in *Trichoderma* treated and non-treated pea plants after 21 and 40 days. Each data represented mean ± SE (n=3) at significant level ($p \leq 0.05$). Variation in letter shows significant difference between *Trichoderma* treated and non-treated plants.

also many factors that affected *Trichoderma*-plant interaction.

CONCLUSION

Trichoderma is free-living fungi that are common in soil and root ecosystems. Our recent investigations show that *Trichoderma asperellum* T42 symbiotically associated with plant roots as well as frequently enhances growth and development. Their use as a biofertilizer in agriculture is able to reduce the chemical fertilizers which have negative impact on the soil fertility. Therefore, our results show great promise for the use of *Trichoderma asperallum* T42 as bio-inoculants for plant's growth and development leading to eco-friendly and sustainable agricultural practices.

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