

Effect of different isolates of *Trichoderma harzianum* fungus on Lettuce (*Lactuca sativa*) seedling growth traits in soilless culture

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Abstract— In this study, the effects of five isolates of *Trichoderma harzianum* fungal species on Lettuce plant growth rate under greenhouse conditions and soilless culture were evaluated in the CRD in four replications. To conduct the research, only the 10% concentration of the extract per total amount of irrigation (water + chemicals) of each pot was used. Wet and dry weights of shoot and root were used as growth indices in this experiment. The test results showed that the isolates had different effects on the lettuce plant growth indices, and indicated a significant statistical difference at 1% level using the LSD test. Among the isolates, the T_{Bi} isolate showed the strongest growth effect with the following features: 66.66% increase in fresh shoot weight, 45.21% increase in dry shoot weight, 87.81% increase in fresh root weight, and 51.12% increase in dry root weight. The possible use of isolated strains as plant growth promoting factors is discussed in this paper.

Keywords— *Trichoderma harzianum*, soilless culture, growth promoter, lettuce

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I. INTRODUCTION

Based on this study, it was demonstrated that the use of *Trichoderma spp.* inoculants as biomass mixed with soil increased the dry weight of horseradish shoots more than using them as spore suspension [1]. Osli et al. [9] showed that some isolates of *T. harzianum* only by 1% of the concentration in the soil increased the growth of lettuce shoots and roots. However, the reaction of various plants to the growth effects of *Trichoderma* fungal isolates would be different. Research has shown that the type and rate of metabolites produced by *Trichoderma* isolated strains can negatively or positively contribute to the growth of

different plants. In a greenhouse experiment, adding conidial suspension of *Trichoderma spp.* to the soil caused a significant increase in dry weight of tomato, pepper and cucumber plants, but made no increase in the growth of bean and radish plants [3]. Considering the proven antagonistic effects of *Trichoderma* species, especially *T. harzianum* (compared with bacterial species with similar properties, and even many of antagonistic fungi) against many soil pathogens [11], it should also be noted that this species of *Trichoderma* fungus is common in Iran's soils, and in other words, it is considered as the most frequent species of *Trichoderma* in Iran's soils [15]. Lettuce is the annual plant which has been transferred from coastal Europe or Central Asia to other parts of the world. Some of the researchers believe that India is the main source of lettuce [11]. Lettuce has Vitamins A, B, C and other substances such as iodine, iron, phosphorus, magnesium, zinc, manganese and copper in terms of nutritional value. Today, lettuce is cultivated to extract oil from its corn and consume fresh fruit [5]. Lettuce is divided into two large groups: *Lactuca sativa* var *capitata* which has two types of Butter head and Crisphead and is produced almost in greenhouse conditions as hydroponic cultivation or soil greenhouse cultivations and *Lactuca sativa* var *Crispa* and *Lactuca sativa* var *longifolia* (which is known as Romain or Coshead) are cultivated in open space [6]. The lettuce is mostly grown in transplants. Thus, it was tried to study the developmental and growth effects of 5 isolates of the fungus *T. harzianum* on lettuce plant aimed at evaluating the impact of isolated strains of T₆₅, T₂₀, T₁₄, T_{Bi} and T₉₅ and the mentioned fungus on important growth factors of lettuce seedling, including fresh and dry weight of shoot and root.

II. MATERIAL AND METHODS

An experiment was planned in the form of a completely randomized design with 4 replications. The strains were prepared from the Phytopathology department of Faculty of Agriculture, which included T₆₅, T₂₀, T₁₄, T_{Bi} and T₉₅. Each of the isolates was maintained within PDA medium in Petri dishes 10 cm in

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Growth of fungus in medium Davet:

To prepare extract of fungi from the Davet selective culture medium which included 1gram of nitrate calcium, 1 gram of chloride calcium, 250 mg of nitrate potassium, 250 mg of phosphate Monopotassium, 50 mg of citric acid, 2 grams of sucrose, 25 grams of agar, 30 mg of Streptomycin sulfate for each liter of distilled water and culture medium with 0.2 grams of magnesium sulfate, 0.9 grams of phosphate di-potassium, 1.5 grams of potassium chloride, 3 grams of glucose, 20 grams of agar for each liter of distilled water were prepared [14]. This culture medium was poured in 2-liter Erlenmeyer which had been sterilized before for 20 min with autoclave at 120 °C under pressure of 10 atm. Now, it is time to transfer the grown biomass of fungus into these containers. In this way, scalpel was used and pieces of

the fungus with approximate dimensions of 2*2 cm along with culture medium of PDA were transferred to the containers. The containers were aerated with aquarium pipes which were connected to an air pump and kept for 8 days at 25°C. The Erlenmeyer flasks were kept on shaker for aeration for 8 days at 25 °C. After this period, the solid phase was isolated from the liquid phase using tiffany that fiberglass put at its bottom, and the liquid phase was kept in the refrigerator for the next steps. The lettuce seeds, variant Siah, were transferred into pots with a 10 cm span filled with coco peat and

The studies results indicate that the plant type and the type and amount of secondary metabolites secreted by different isolates and strains of *Trichoderma* species can affect their growth effects in plant - *Trichoderma* interactions [12].

Based on conducted studies, the developmental effects of 31 isolates from 8 common species of *Trichoderma* on tomatoes and eggplant were studied. The results showed that the highest growth effects on tomato were obtained by the strains of two species, *T. viride* and *T. harzianum*, while the highest growth effects on eggplant were achieved using three species of *T. polysporum*, *T. album* and *T. hamatum* [13]. Differences in various strains of *T. harzianum* fungus performance in this test could also possibly be attributed to differences in the secretion of biochemical substances. As an example, it was found in a research that certain values of antimicrobial compound of Viridiol produced by *T. virens* species are highly toxic for rice, and cause a significant decrease in the plant seedlings growth [6]. It has been proved that one of the most important metabolites produced by 6-pentyl- α -pyrone is *T. harzianum* which has been known as plant growth stimulant in low concentrations. This compound in higher concentrations M (10^{-3}) prevented growth of wheat coleoptiles. Here, two hypotheses were mentioned that this compound acted as an auxin like compound (auxin causes growth of different organs of the plant in

perlite by 50% ratios. The pots were kept in the research greenhouse of Faculty of Agriculture, Ferdowsi University of Mashhad. The daily requirement of seedlings was provided from 90% of Hoagland food solution and 10% of *Trichoderma* isolates extracts. The 10% concentration of extract was used for the whole solution consumption. The fresh and dry weights of shoot and root were weighed after 25 days, and data analysis was performed by Minitab16-2001 software.

III. DISCUSSION OF RESULTS

Analysis of data obtained from this test showed that *Trichoderma* fungus had different growth effects on growth parameters of lettuce. Based on analysis of variance (Table 1), the effects of isolate on fresh and dry weight of roots and shoots were significant at 1% probability level.

Comparison of means showed that the T_{Bi} isolate had the strongest growth effect with the following features: 66.66% increase in fresh shoot weight, 45.21% increase in dry shoot weight, 87.81% increase in fresh root weight, and 51.12% increase in dry root weight. It had a significant difference with other isolates 1% probability level. The strain T_{14} showed the lowest effect or in better words, the negative effect (compared with control treatments) in growth indices (Table 2).

lower concentrations and prevents growth of different organs' in higher concentrations) or it played role in production of auxin inductors. In any case, effect of this compound or other similar compounds on increase or prevention of plants growth should be studied more [4]. On the other hand, many researchers believe that different isolates of *Trichoderma spp.* stimulate growth of plants by producing biochemical materials or reduce effects of inhibiting effects of growth of some compounds, biological and chemical toxins in soil and even change rate of soil soluble elements [12], [8], [13]. Exudation of organic acids such as gluconic acid, citric acid and fumaric acid by *Trichoderma* species reduces pH of soil and finally increase solubility and absorb important micronutrients required for growth of plant such as iron, manganese, magnesium, mineral cations and phosphates [2], [12]. Thus, it can be expected that using the examined strains, optimal conditions for growth of plants such as lettuce can be better provided.

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Table 1: Analysis of variance of effect of Trichoderma fungi isolates on the growth characteristics of lettuce, cultivar Siah.

Sources of changes	Degrees of freedom	Mean squares			
		Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
Strains	3	465.46**	6.29**	3.39**	0.08**
Experimental error	15	59.66	0.53	0.22	0.01

** Significance at 1% probability level.

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Table 2: Comparison of the average effect of *Trichoderma harzianum* fungal isolates on lettuce growth traits.

Measured traits	Strain					
	Control	T ₁₄	T ₂₀	T ₆₅	T ₉₅	T _{Bi}
Shoot fresh weight (g)	15.11 ^c	15.04 ^c	16.34 ^c	21.15 ^b	17.14 ^c	24.25 ^a
Root fresh weight (g)	0.83 ^c	0.81 ^c	0.84 ^c	1.13 ^b	0.95 ^c	1.65 ^a
Shoot dry weight (g)	0.82 ^c	0.80 ^c	0.85 ^c	0.92 ^b	0.83 ^c	1.20 ^a
Root dry weight (g)	0.07 ^c	0.06 ^c	0.07 ^c	0.09 ^b	0.08 ^c	0.124 ^a

* Numbers with dissimilar letters in each row have significant differences at the 5% probability level using LSD test.