



Evaluation the Growth Changes in Two Lettuce (*Lactuca sativa*) Cultivars at Different Concentrations of *Trichoderma harzianum*

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Abstract

In recent years, the world's growing population and growing demand for food on the one hand, and the importance of environmental issues and sustainable agriculture on the other, have led researchers to use biomass to increase agricultural productivity per unit area. Therefore, the effect of TBI extracts from *Trichoderma harzianum* fungal species in increasing lettuce growth in greenhouse conditions and in the soilless cultivation system in the form of a completely randomized design with invoice arrangement was performed in 6 replications. To perform this study, 4 concentrations of 0, 5, 10, and 15% of the extract were used for each source, as well as 2 cultivars of Siah and Gridelik. The results showed that different levels of the extract of this fungus had different effects on the growth characteristics of lettuce. Among the various levels used, the concentration level was 5% with an increase of 26.1% in a fresh weight of aerial shoot, 13.2% of the dry weight of aerial shoot, 22.29% of w fresh et root weight, 16.66% of dry root weight, 5.5 49% leaf number, 31.36% fresh leaf weight, 17.98% dry leaf weight, 20.91% leaf area, 15.78% lettuce stem diameter had the highest growth effects. Gridelik was superior in all growth factors to Siah. Research shows that the type of plant and the type and amount of secondary metabolites secreted by different nodes and species of *Trichoderma* can affect the rate of their growth effects in plant interaction with *Trichoderma*.

Keywords: Concentration; Fungus; Soilless cultivation; *Trichoderma harzianum*

Introduction

Lettuce is an annual plant that has moved from coastal Europe or Central Asia to other parts of the world. However, other scholars believe that India was the origin of lettuce [1]. 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 [2]. Lettuce is divided into two large groups: *Lactuca sativa* var capitata which has two types of Butterhead 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 [3]. *Trichoderma* fungi and its different species are among the biological tools used by researchers in sustainable agriculture. Many formulations of

Trichoderma have been commercially available in the market and are used as biocontrol factors against plant pathogens at small levels [4]. The antagonistic ability of different species of *Trichoderma* has been demonstrated against some economically important diseases. It also has the ability to occupy the supernatant and has the potential to produce extracellular enzymes and many other useful enzymes, all of which play a role in the biological inhibition of pathogens [5]. Biological inhibitors have been introduced against a wide range of pathogenic living agents such as bacteria, protozoa, nematodes and even viruses [4]. The ability of these fungal species to produce a variety of antagonistic metabolites and plant growth regulators leads to their increased biological inhibitory efficiency. The activity of *Trichoderma* fungi as a bio-inhibiting agent is influenced by soil physical and chemical environmental parameters [6].

Different species of *Trichoderma* have beneficial effects on plants. The secretion of various chemicals, high stability, and neutralization of defense compounds produced by plants and other microscopic organisms, induction of plant resistance, and defense and growth mechanisms are other factors contributing to the success of *Trichoderma*. It also coexists with other microscopic living organisms such as rhizobia and mycorrhizal. The ability of different extracellular enzymes in soil (high colonization power, root coexistence power, high sporulation ability and high tolerance to the presence of field heavy metals) have been recognized as a successful agent against many plant diseases [7-9]. Another advantage of *Trichoderma* is the improvement of photosynthesis in plants under different stresses [10]. Generally, under adverse conditions, this fungus improves plant growth and reduces stress. In addition, *Trichoderma* reduces production costs and adverse environmental effects [11]. Studies have shown different growth rates in plants treated with *Trichoderma* fungi in different hosts. On the other hand, the species of *Trichoderma* fungi, different strains of one species and the amount of concentration used also cause differences in the effects.

Growth enhancers of *Trichoderma* fungi occur on various plants. Also, the ability to enhance growth in *Trichoderma* depends on the type of isolate used [12]. According to available reports, the effects of plant growth-promoting by different species of *Trichoderma* have been proven in a number of different plants including crops, ornamental and horticultural and have been effective on greenhouse products such as beans, eggplant, lettuce, chickpeas, radishes, and peppers [13]. The inoculum of *Trichoderma harzianum* T22 improves growth, crop yield, leaf width and root length of maize [14]. Numerous reports on the healing effect of different species of *Trichoderma harzianum*. T and viride. T has been proposed in plant growth including lettuce [15,16]. Studies have shown that the production of various hormones by *Trichoderma* stimulates plant growth, induces growth hormones indoleacetic acid and auxin, and also improves root and shoot growth of plants [17]. *Trichoderma harzianum*. increases germination rate, plant height and dry weight of maize, tomato, tobacco, and radish [18]. Report on the application of *harzianum*. T root growth of tomato increased growth and yield of tomato, tobacco, and strawberry seedlings by producing growth-promoting compounds. *Trichoderma* by infiltrating the host root caused sub-root development, plant fresh weight gain, leaf width, and yield a plant [19]. Today, due to the excessive use of nitrogen-containing chemical fertilizers to accelerate vegetative growth, many vegetables, especially leafy vegetables, have a high percentage of nitrate, which in many cases exceeds established standards [20]. Lettuce is one of the leafy vegetables that have a relatively high production and consumption in Iran and is mostly consumed in fresh food. For this reason, the effects of TBI isolate from *Trichoderma* fungi and two cultivar on the growth factors of lettuce, including lettuce fresh and dry weight for shoot and

root, stem diameter, leaf area, a number of leaves, the amount of chlorophyll and nitrate accumulation were investigated.

Materials and Method

Plant Materials and Growing Conditions

In this study, the effect of TBI isolate from a fungal species, *Trichoderma harzianum*, was investigated in a greenhouse experiment in a completely randomized design with a factorial arrangement with 4 replications of lettuce in Ferdowsi University of Mashhad. Four concentrations (0, 5, 10 and 15%) were considered for the fungus and two varieties of Siah and Gridelik were also used. A system was designed with a water pump (0.5 horsepower), 9 check valves, 6 digital timers, 5 water tanks (50 liters), tape (20 cm) and required fittings and executed in re-search greenhouse of Faculty of Agriculture of the Ferdowsi University of Mashhad. This system was designed such that each row was fed only with one of the tanks. Seed of two cultivars of lettuce called Gridelik and siah was transplanted in seedling trays and the seedlings were prepared for being transferred to the main bed after 40 days. Vases with openings of 30 °C were filled with 20% of cocopeat and 80% of perlite so that roots can be separated in this bed. Vases were picked in the system and seedling was transferred.

Growth of fungus in medium Davet

To prepare an 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 g of sucrose, 25 g of agar, 30 mg of Streptomycin sulfate for each liter of distilled water and culture medium with 0.2 g of magnesium sulfate, 0.9 g of phosphate di-potassium, 1.5 g of potassium chloride, 3 g of glucose, 20 g of agar for each liter of distilled water were prepared [21]. This culture medium was poured in 2-liter Erlenmeyer which had been sterilized before for 20 min with an autoclave at 120 °C under the pressure of 10 atm. Now, it is time to transfer the grown biomass of fungus into these containers. In this way, a 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 that were connected to an air pump and kept for 8 days at 25 °C. The Erlenmeyer flasks were kept on a 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.

Nutrition

5 sources were used, and no extract was added to source A and was regarded as control. Five volume percent of the source was added to source B, 10 volume percent of the source was added to source C and 15 volume percent of extract source was added

to source D. the remaining volume of sources was filled with Hoagland Ousley [18] nutrient solution. Source E was filled with iron and calcium and separated entered into the system. In the early days, only pure water was given to the shrubs. After ensuring full placement of shrubs in the early days, 80 ml of water and food were provided for each shrub and in the next days, they were provided considering temperature and light. P^H, KOH and HCL were set about 6.5. The feeling was controlled like commercial cultivation.

Examined Traits

Fresh and dry weight (root, shoot, leaf)

The fresh weight was measured using a digital scale as 0.01 g. Next, the samples were placed in an Oven device at 74 °C for 4 days, the dry weight of the samples was also measured using a digital scale as 0.01 g ultimately.

Number of leaves, Leaf area and Stem diameter

The stem diameter was measured by a caliper with 0.01 mm accuracy from the upper and lower parts of the stem. Finally, the mean of these two parts was used for statistical analysis. Leaves were also counted at the end of the period and after harvest. Leaf area was measured using Leaf Area Meter (Model LI-3100c) device and Image j software [22].

Statistical analysis

The experiment was performed in a complete randomized block design with factorial arrangement of (2×4) in six replications. To analyze the results, Minitab 16-2001 software was used. The LSD test was used for data average comparison.

Table 1: Variation analysis of the effect of Trichoderma Bi fungal isolation at levels of 0, 5, 10 and 15% on the growth of Gridelik and Siahio lettuce cultivars.

C.V	df	Mean Square					
		Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Leaf fresh weight	Leaf dry weight
Concentration of Fungi		11376**	7.4958**	65.834**	0.07169**	14670**	5.8619**
variety	1	165205**	34.8504**	204.6**	3.27085**	141484**	47.0052**
Concentration × variety	3	388 ^{ns}	3.979 ^{ns}	3.979 ^{ns}	0.03712 ^{ns}	2354 ^{ns}	0.5491 ^{ns}
Error	35	1195	3.096	3.096	0.02513	3364	0.6514

Table 2: Variation analysis of the effect of Trichoderma Bi fungal isolation at levels of 0, 5, 10 and 15% on the growth of Gridelik and Siahio lettuce cultivars.

C.V	df	Mean Square		
		Leaf number	Leaf area	Stem diameter
Concentration of Fungi	3	167.97**	1552.7**	26.172**
variety	1	1887.52**	36630.8**	158.05**
Concentration × variety	3	20.47 ^{ns}	62.6 ^{ns}	2.2 ^{ns}
Error	35	14.84	80.8	3.319

Results

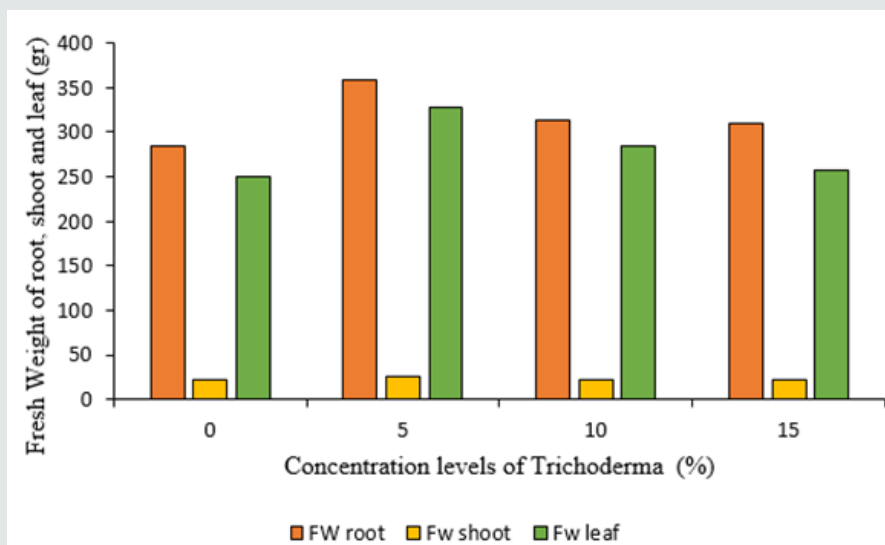
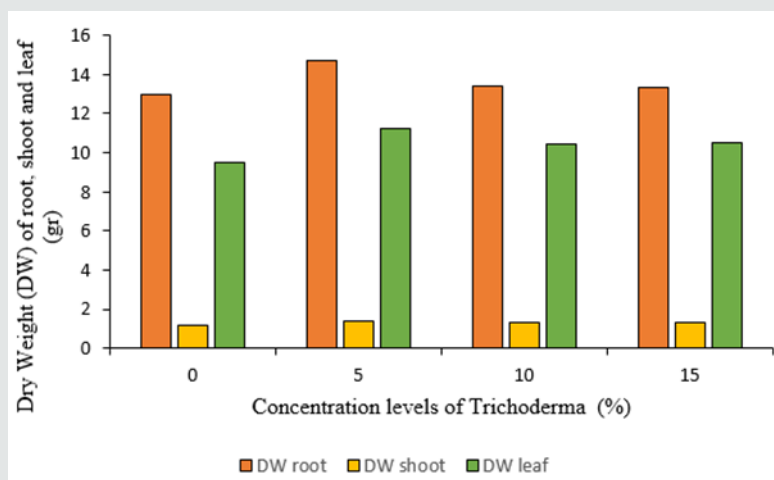
Fresh and dry weight (root, shoot, leaf)

Based on the results of the analysis of variance Table 1, the application of different percentages of Trichoderma fungus had a significant effect on fresh and dry weight traits of the shoot, roots, and leaves (Table 1). The best effective concentration in fresh and dry weight of the shoot parts is 5% concentration, which is 358.8 and 14.7 gr, respectively. As the concentration used increased, there was no change in the amount of fresh and dry weight of the lettuce shoot parts (Figures 1&2) (Table 2). Also, among the different cultivars, Gridelik had the highest fresh and dry weight compared to Siahio cultivar, which increased by 24.05% and 11.8% of the fresh and dry weight of shoot organs (Table 3). The highest root weight was observed in Trichoderma treatment with a concentration of 5% to 26.4 gr (Figures 1&2), which was not significantly different from other treatments; similarly, the highest dry root weight in Trichoderma treatment was 5% to 1.4 g (Figures 1&2). There was no significant difference between 10 and 15% concentrations in dry root weight, but the lowest weight was related to control treatment (1.2 gr). Also, in related, with the cultivars used, the highest amount of fresh and dry root weight was in Gridelik cultivar compared to Siahio cultivar (Table 3). The highest fresh and dry weight of leaves were observed in Trichoderma 5% treatment at 327.2 and 11.2 gr (Figures 1&2), respectively, and the lowest rates were observed in the control treatment (249.3 and 9.5 gr), as well as Gridelik cultivar. It had the highest fresh and dry weight of the leaves compared to the Siahio variety (Table 3).

Table 3: Comparison of the mean effects of cultivar type in two varieties of lettuce.

Measured traits	Cultivar	
	Siaho	Gridelik
Shoot fresh weight	285.1 ^b	375.4 ^a
Shoot dry weight	12.7 ^b	14.4 ^a
Root fresh weight	20.8 ^b	24.9 ^a
Root dry weight	1 ^b	1.5 ^a
Leaf fresh weight	225.4 ^b	334 ^a
Leaf dry weight	9.4 ^b	11.4 ^a
Leaf number	25.1 ^b	37.6 ^a
Leaf area	171 ^b	226.3 ^a
Stem diameter	17.4 ^b	21.1 ^a

Numbers with dissimilar letters in each row have significant difference at the 5% probability level by LSD test.

**Figure 1:** Comparison of the effect of Trichoderma on Fresh weight (FW) of root, shoot and leaf ($p \leq 0.05$). Different letters indicate significant differences between treatments by LSD test.**Figure 2:** Comparison of the effect of Trichoderma on Dry Weight (DW) of root, shoot and leaf ($p \leq 0.05$). Different letters indicate significant differences between treatments by LSD test.

Number of leaves, Leaf area and Stem diameter

The analysis of variance table shows the significant effect of Trichoderma concentrations and different varieties of lettuce on the number and surface of leaves, as well as stem diameter (Table 2). The highest number of leaves in lettuce was 39 in the treatment of 5% Trichoderma fungus (Figure 3) and there was no significant difference between other treatments and control treatment in terms of leaf count. Among the cultivars used, the highest number of leaves was observed in the Gridelik cultivar by 37 accounts compared to the Sياهو cultivar (25 accounts) (Table

3). Similarly, at the leaf area, it was observed that the treatment of 5% of Trichoderma fungi had the highest rate (238.1) and there was no significant difference between other treatments and control treatment (Figure 4). Also, the Gridelik cultivar increased by 24.33% compared to the Sياهو cultivar (Table 3). The diameter of the stem in lettuce did not change significantly with different concentrations of Trichoderma fungi, but the lowest stem diameter was seen in the control treatment at 17.1 mm (Figure 5). Among the cultivars used, the highest stem diameter was seen in Gridelik treatment (21.1 mm) compared to Sياهو (Table 3).

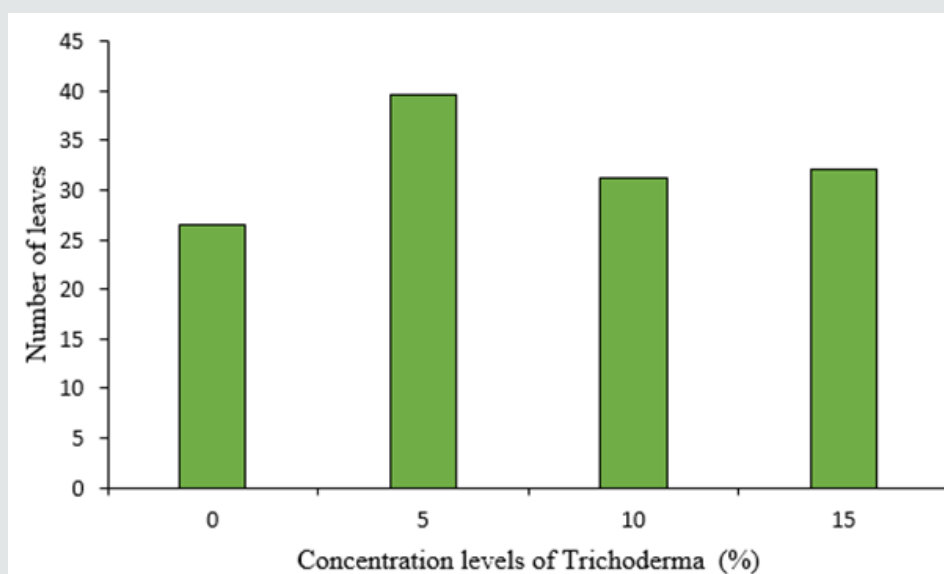


Figure 3: Comparison of the effect of Trichoderma on number of leaves ($p \leq 0.05$). Different letters indicate significant differences between treatments by LSD test.

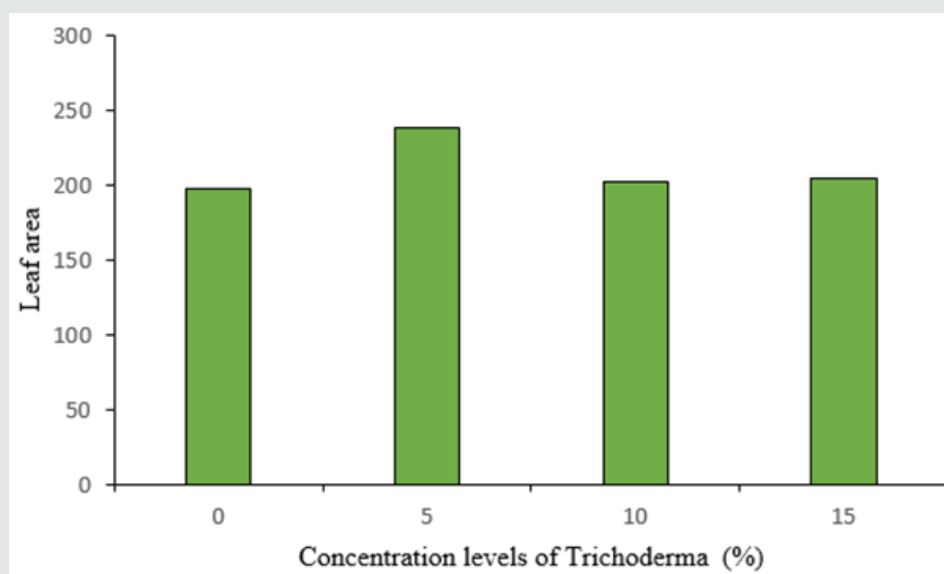


Figure 4: Comparison of the effect of Trichoderma on leaf area ($p \leq 0.05$). Different letters indicate significant differences between treatments by LSD test.

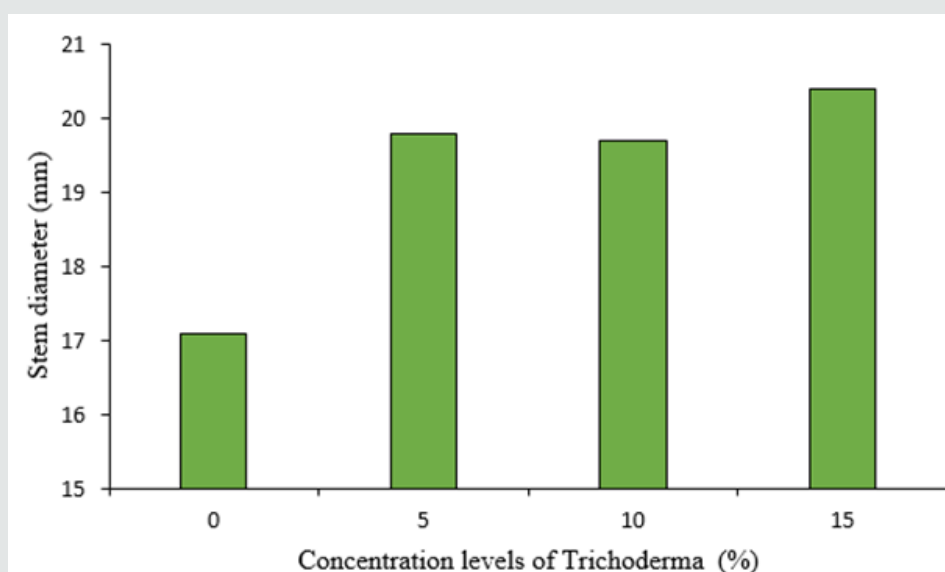


Figure 5: Comparison of the effect of Trichoderma on stem diameter ($p \leq 0.05$). Different letters indicate significant differences between treatments by LSD test.

Discussion

The results of this study compare the growth effects of *Trichoderma harzianum* and the use of two different varieties of lettuce on important growth factors including the weight of dry shoots, roots, and leaves, as well as the number and leaf area and stem diameter, chlorophyll content and nitrate accumulation. Although different percentages of *Trichoderma* are mainly used as antagonists against many plant pathogens, their beneficial effects on the growth and development of many plants have been proven [18]. The results of this study were consistent with a report that the extract of the fungus *Trichoderma harzianum* isolate Bi was able to stimulate growth in plants [18]. Yedidia [16] in their research on a large number of plants, stated that increasing branch and leaf area is a general role of this fungus. The complex mechanisms of this growth increase are probably due to the production and secretion of growth stimulant compounds into the extract of this fungus, which also increases the efficiency of fertilizer [23]. Other studies have significantly increased the rate at which seedlings emerge, plant height, leaf area, and dry weight due to the inoculation of *Trichoderma* fungus and its extract [24]. This fungus was proven in two stages on lettuce, in addition to increasing growth due to the treatment of plants by this fungus in plants such as chickpeas Zheng and Shetty [25], *Mentha arvensis* Singh [26], Bean Brandão [27], tomatoes Gravel [23], Wheat Cavalcante [28], lettuce Bal and Altintas [15] have also been reported. The results of this test showed that Bi *Trichoderma harzianum* isolates at all levels of concentration compared to control treatment had increasing effects on dry and fresh weight of aerial and root organs, number of leaves, stem diameter, fresh and dry weight of leaves. In one study, the

use of *Trichoderma* fungi significantly increased the fresh and dry weight of aerial and root organs, as well as the number of leaves in a tomato plant Newman [29], as well as the effects of this fungus on fresh and dry root weight and shoot parts, Tobacco and tomatoes, corn, and horseradish, were evaluated positively.

The association between *Trichoderma harzianum* and the plant can increase root uptake levels, which in turn can promote the exchange of nutrients between fungi and plants, similar to reports of mycorrhiza coexistence with plants [30]. In addition to the production of IAA by *Trichoderma*, which may increase root volume and subsequently increase nutrient uptake. These fungi directly increase root growth and eventually plant growth by controlling the destructive non-pathogenic microbial population as well as by digesting the toxic metabolites produced by this microflora by a number of enzymes [31]. It has been proven that one of the most important metabolites generated by *T. harzianum* 6-pentyl- α -pyrone, known as the plant growth stimulus at low concentrations. This combination at higher concentrations (3-10) caused a hindrance in the growth of wheat. The two hypotheses were proposed that the compound functions as a semi auxin compound (Auxin at lower concentrations cause growth in the various organs of the plant) or are contributing to the production of auxin production. In any case, the effect of the dose in this compound and other similar compound in the increase or inhibition of plant growth requires more studies [32]. On the other hand, in interpreting the mechanism of action of plant growth stimulants, many researchers believe that mainly different isolates of *harzianum* fungi stimulate plant growth by producing biochemicals or reduce the effects of inhibiting the growth of some compounds, existing biological and

chemical toxins the soil and even changes in the number of soluble elements [29]. The secretion of organic acids such as gluconic acid, citric acid, and fumaric acid by *Trichoderma* species reduces soil pH and ultimately increases the solubility and absorption of important micronutrients needed for plant growth such as iron, manganese, magnesium, mineral cations and phosphates [13].

In one study, the use of 0.1 g of Triatum commercial substance for each tomato plant in the carnation cultivar increased the yield by 33.34% and significantly reduced the disease of rot, which is caused by calcium deficiency and malabsorption. Calcium uptake is directly related to root volume and water uptake, and due to root formation by *Trichoderma* and increased root volume, calcium uptake increases and eventually disease of rot decay decreases [33]. Due to the above explanations, the type of plant and its root secretions, the diversity, and population of microorganisms that stimulate plant growth and their ability to colonize around the roots and the physical and chemical conditions of the soil, especially the type of soil microbial flora, ultimately create complex interactions. Any change in this interaction can change the growth conditions of the plant [29].

Conclusion

According to various studies, *Trichoderma* fungus, due to its ability to colonize and spores abundantly in the soil environment, especially around the roots of most crops and non-crops, and has the ability to compete for food and high location in addition to inducing compounds that cause plant resistance Stimulating the growth of underground or shoot organs also increases the production of secondary metabolites and improves growth. The results of this study also prove the positive effects of *Trichoderma* fungus on improving the growth factors of lettuce. Therefore, considering the above and the results obtained in the present study, it can be hoped that in addition to the biocontrol effects of *Trichoderma* fungus, which is currently used in agriculture, its positive effects on increasing plant growth factors and increasing production in greenhouses can be used considered.

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Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

a. The best cultivar used in this experiment was Gridelik, but Siah cultivar showed higher nitrate accumulation and higher levels of chlorophyll b and a than Gridelik.

b. When the concentration of the used fungus was more than 5%, there was no significant difference between the traits, so even with a lower concentration, the best result can be obtained.

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