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The effects of *Thrichoderma harzianom* extract on the chlorophyll rate and nitrate concentration in two varieties of Lettuce in soilless culture system

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***Abstract*—**In this study, the effect of extracts of TBi isolated strain of *Trichoderma harzianum* fungal species on increased or decreased amount of chlorophyll a and b as well as on the nitrate accumulation on lettuce in greenhouse conditions and in the soilless culture system was studied. The experiment was performed in a complete block randomized design with factorial arrangement of (2×4) in 6 replication. To conduct the research, four concentrations of 0%, 5%, 10% and 15% of the extract per each source, and also 2 varieties of Siahoo and Grade Lake were used. The amounts of chlorophyll "a" and "b" were measured by Lichtenthaler and Wellburn method; nitrate concentrations were measured using Spectrophotometer (Diazo) method. The test results showed that different levels of this fungus extract have had different effects on the parameters listed on lettuce plant. Among different levels used, the concentration of 5% included the highest concentration of nitrate and showed a significant difference at the 5% probability level with other levels. This level also had higher chlorophyll content than other levels, but the difference was not significant. The Siahoo variety showed higher nitrate accumulation as well as larger amounts of chlorophyll "a" and "b" compared to the Grade Lake variety, and the difference was significant at the 1% probability level.

***Keywords*—** *Trichoderma harzianum*, Soilless culture, Nitrate accumulation, Chlorophyll, Lettuce

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I. INTRODUCTION

Different types of microorganisms live in soil and this group includes pathogenic and nonpathogenic factors. Among the known microorganisms which are usually applied for protecting general health of the plant, Trichoderma fungal species are of high importance. Based on different researches, it seems that this fungus with food and spatial competitive power, sporulation in soil environment and particularly around root of most agricultural and nonagricultural plants and resistance induction in plant not only decreased pathogenic factors in soil but also promoted growth of shoot and root of some of these plants [5], [12], [10], [17], [6]. 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 [10]. Lettuce has Vitamins A, B, C and other substances such as iodine, iron, phosphor, magnesium, zinc, manganese and copper in terms of nutritional value. Today, lettuce is cultivated to extract oil from its corn and consume fresh fruit [3]. 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 [4]. Lettuce is a leafy vegetable with a relatively high production and consumption in Iran and mostly is used in fresh consumption; thus, nitrate concentration rate in the product is of great concern. The nitrate amount is one of the most important factors in determining the quality of vegetables. Nitrate accumulation mostly occurred in the vessels, petioles and leaves can have undesirable impact on human health. Management of hydroponic systems, on the one hand, requires appropriate plant nitrogen nutriment to make achievement of high yield possible, and on the other hand, it should be tried to prevent the loss of the element, which may cause environmental pollution and the capital loss. One solution is the use of compounds capable of keeping the nutrients as reservoir and gradually providing them to the plant [10]. Today, due to excessive use of fertilizers containing nitrogen to

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accelerate the growth, most vegetables especially leafy vegetables contain a high percentage of nitrate, which in many cases is higher than certain specified standards. However, the nitrate (NO_3) is not considered itself toxic for man, but the nitrite (NO_2) resulted from its reduction can combine with amines and form Nitrosamine, which is a carcinogen for the body. Sometimes, when keeping vegetables in the stock or during performing treatment on them, the nitrate is converted to nitrite, and the people fed with foods containing nitrite would be at risk of Methemoglobinemia [4]. Also, in canned vegetables, high values of nitrates in a few months in storage can trigger the release of zinc in them [10].

II. MATERIAL AND METHODS

Preparation of isolate:

The mentioned isolate was prepared from Plant pathology department of Faculty of Agri-culture which included T_{Bi} and was kept in PDA and Petri dishes with diameter of 10 cm at 25˚C in oven for 5 days .

Growth of fungus in medium Davet:

To prepare extract of fungi from the Davet selective culture medium which included 1 gram 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 medi-um 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 dis-tilled water were prepared [15]. This culture medium was poured in 20-liter containers 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 .

Extraction:

After this process, solid phase was separated from liquid phase with a thin cloth which we poured at the bottom of the fiberglass and liquid phase was kept for being used in the next stages in refrigerator at 4˚C.

Design and construction of Soilless culture system:

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 Ferdoowsi University of Mashhad. This system was designed such that each row was fed only with one of the tanks .

Transplanting:

Seed of two cultivars of lettuce called Gretleke and siaho 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 .

Nutrition:

5 sources were used and no extract was added to source A and was regarded as control. Five volume percents of the source was added to source B, 10 volume percents of the source was added to source C and 15 volume percents of extract source was added to source D. the remaining volume of sources wad filled with Hoagland [8] nutrient solution. Source E was filled with iron and calcium and separated entered in the system. In early days, only pure water was given to the shrubs. After ensuring full placement of shrubs in 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. PH, KOH and HCL were set about 6.5. Feeling was controlled like commercial cultivation.

The measured traits:

Nitrate concentration (accumulation) was measured by Spectrophotometry. Thus, after drying the samples and powdering them (200 mesh), 0.2 g of each sample was weighed, and 20 ml of 2% acetic acid was added to the samples. After preparing the solution, the samples were shaken for 20 minutes to achieve the uniform solution of nitrate extract. To measure the nitrate concentration by the Spectrophotometer, the device was set at 540 nm, and the samples were first measured without the nitrate reagent, and at the second time after adding the nitrate reagent. Then, the difference between these two values was calculated and placed in an equation resulted from standard curve of light passage through the sodium nitrate solution at different concentrations (20, 40, 60, 80, 100 and 120 ppm), and thus, the nitrate concentration for each

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Table 2: Comparison of the mean effects of different levels of Bi *Trichoderma harzianum* isolates on chlorophyll and nitrate of two varieties of lettuce

Measured traits	Concentration levels (%)			
	١٥	١٠	٥	٠
Chl. a (µg/mg)	5.41[a]	5.48[a]	5.59[a]	5.08[b]
Chl. b (µg/mg)	2.37[a]	2.49[a]	2.57[a]	2.12[b]
Nitrate (mg/Kg)	1500[b]	1505[b]	1559[a]	1469[c]

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

vegetable was determined. The chlorophyll "a" and "b" types were extracted by Lichtenthaler and Wellburn method from the leaf tissue using methanol. For this purpose, 0.2 g of fresh leaves was grinding in a mortar, which had been put inside a container with ice to prevent the degradation of chlorophyll. During grinding, 25ml of methanol was added to it. The samples obtained were poured into plastic tubes and put into the fridge for one night. A volume of 10 ml of each sample was centrifuged at speed of 2000 rpm for 7 minutes at the temperature of 21 degrees Celsius. The, they were read at wavelengths of 666 and 653 nm using a Spectrophotometer, and the values of chlorophyll a and b were measured using the relevant formula [12]. 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 3: Comparison of the mean effects of cultivar type on the amount of chlorophyll and nitrate in two varieties of lettuce

Measured traits	Cultivar	
	Siaho	Gretlake
Chl. a (µg/mg)	5.84[a]	5.15[b]
Chl. b (µg/mg)	2.67[a]	2.04[b]
Nitrate (mg/Kg)	1595[a]	1419[b]

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

III. DISCUSSION OF RESULTS

Analysis of data obtained from the test showed that the extract of Trichoderma fungus has different growth effects on the rate of chlorophyll and nitrate accumulation in lettuce leaves. Based on the analysis of variance (Table 1), the effects of concentration and cultivar and the mutual effect of concentration×cultivar on nitrate accumulation was significant at the 1% probability level. The effect of cultivar on the amounts of chlorophyll a and b was significant at the 5% probability level. The effect of concentration and the mutual effect of concentration×cultivar were not significant.

The results of comparing the means showed that the 5% concentration of T_{Bi} isolate with an increased concentrations of 6.13% in nitrate accumulation compared to the control and increased 3.56% and 3.96% compared to 10% and 15% treatments, respectively, showed the highest increased levels of nitrate, and was significant at 1% probability level (Table 2).

The 5% treatment of fungus extract showed the maximum increased nitrate rate on the Siahoo cultivar, and the mutual effect of cultivar × concentration on nitrate accumulation at this concentration and cultivar was significant at 1% probability level. Based on the results in Table 3, the Siaho cultivar showed increase in the nitrate concentration as well as in the amounts of chlorophyll a and b in comparison with Gretlake cultivar, and was significant at the 5% probability level.

The results indicated that the T_{Bi} isolates of *Trichoderma harzianum* make some changes in nitrate concentration rate on lettuce. Direct and indirect causes can be mentioned for such changes in nitrate concentration. Low light, high temperature and moisture stresses lead to reduced enzyme activity reducing the nitrate and more accumulation of nitrate. The density in cultivation also has a considerable impact on nitrate accumulation. For example, by considering higher cultivation density, due to low light intensity, higher levels of nitrate will accumulate in the organs. In a research, the use of extracts of two strains of *T. harzianum* T_{22} and *T. atroide* P1 led to significant increase in fresh and dry weights of shoots and roots, plant height, number of leaves and fruits of the tomato [13]. According to the conducted study, the growth effects of isolates of two *T. harzianum* and T. koningii species on shoot and root dry weight of tobacco and tomato were evaluated positive. Also, the two T_{56} and T_8 isolates belonging to two mentioned species, while making a significant increase in Horseradish dry weight, led to 1-3 days decrease during the germination time of tomato, corn and the tobacco [18]. It has been proved that one of the

Table 1. Analysis of variance of Trichoderma fungus effect of chlorophyll and nitrate accumulation in lettuce cultivars.

Sources of changes	DF	Mean squares		
		Chl. a	Chl. b	NO_3
Rep	5	5.85*	0.99^{ns}	0.82^{ns}
Concentration	3	0.15^{ns}	0.51^{ns}	15377**
Cultivar	1	4.32*	0.37*	370657*
Concentration * Cultivar	3	2.35^{ns}	2.12^{ns}	3896**
Experiment error	35	1.73	0.52	310
Total error	47			

ns: Non-significant difference; *: Significant at 5% probability level; **: Significant at 1% probability level

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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 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 [3]. In a research, application of 0.1% of commercial material of *T. harzianum* T_{Bi} (TRIANUM-P)(®) for each tomato , Carnac cultivar increased yield by 33.34% and significantly reduced blossom end rot due to shortage and no intake of calcium. Calcium intake had direct relationship with volume of root and water intake and because Trichoderma settles in root and volume of root increases, calcium intake increases and finally blossom end rot is reduced [9].

It was mentioned in another report that using the Trichoderma could increase the concentrations of N, No_3 and K^+ in the papaya leaf juice respectively as 20%, 25% and 30% [10]. The Trichoderma strains can probably affect the solubility of plant nutrients, such as phosphorus, iron, copper, manganese and zinc. The elements have low solubility in some soils and despite their presence in the soil, the plant shows signs of their shortage. Also, it is proved that the Trichoderma T-22 reduces the oxidative characteristic of iron by increasing the solubility and production of materials with high- affinity such as iron chelate [6], [16]. In an experiment conducted in hydroponic culture conditions, it was reported that the Trichoderma spp. increases the absorption and concentrations of elements such as copper, phosphorus, iron, manganese and sodium in the roots of the plants [6], [14]. The Trichoderma strains can probably affect the solubility of plant nutrients, such as phosphorus, iron, copper, manganese and zinc. The elements have low solubility in some soils and despite their presence in the soil, the plant shows signs of their shortage. Also, it is proved that the Trichoderma T-22 reduces the oxidative characteristic of iron by increasing the solubility and production of materials with high- affinity such as iron chelate. In a research by, it was reported the solubility of phosphorus is correlated with the stimulation of plant growth and development. This fungus can play a positive role in increasing the absorption of phosphorus and other elements by increasing the root surface and enhancing the solubility of elements such as phosphorus and iron [6]. The extract of Trichoderma had no significant impact on the chlorophyll rate effect.

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