



Growth and development of two cultivars of *Mentha piperita* and Shirazi mint under fungus *Trichoderma* treatment

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Abstract

Mentha piperita and Shirazi mint are two important cultivars of the Lamiaceae family, which attracted the attention of researchers because of their economic and pharmaceutical importance. *Trichoderma* is a saprophytic fungus that has a global spread and is seen in almost all agro-soils. This fungus belongs to the Deuteromycota fungus and has about 25 species, the most important of which are *T.harizianum*, *T.viride* and *T.virens*. Accordingly, the aim of this study was to investigate the effects of various concentrations of *Trichoderma* fungi Bi isolate on different characteristics of mint (*Piperita* and Shirazi). The present study was conducted as a pot experiment under non-soil culture conditions in a greenhouse based on a factorial experiment based on a completely randomized design with four concentrations of *Trichoderma harizianum* Bi: 0% (control), 5%, 10% and 15% vol. 50 liters Water and two cultivars of mint (*Piperita* and Shirazi) with 4 replications were established and implemented at the research greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. Based on the results of statistical analysis, the *Trichoderma* fungus concentration had a significant effect on leaf number and number of nodes. The highest amount of leaf was observed in the concentration of 15% of the fungus, which was observed to be 59.41% higher than the control treatment, where the concentration of fungus was zero (control). In the concentration of fungi, 15% was observed in 143 nodes, which increased 91% relative to control treatment. The concentration of *Trichoderma* fungus and the type of crop used could have a significant effect on plant height. The highest plant height was 88 cm in the treatment of fungi with a concentration of 15% and *Piperita* cultivar. The lowest height was observed in Shirazi cultivar and zero concentration of fungus was 22.5 cm. Different concentrations of *Trichoderma* fungi were not effective on all of the measured biochemical traits. But the type of cultivar used had a significant effect on the amount of chlorophyll a, b. The highest levels of chlorophyll a, b, total in *Piperita* cultivar were observed, which increased compared to Shirazi cultivar. None of the treatments had significant effect on carotenoid level.

Keywords: mint leaves, growth, fungi concentration,



Introduction

Today, the increasing trend of degradation of water, soil and environment due to the untapped use of chemicals in agriculture and the world-leading food production practices in the world have increased the attraction of researchers to the sustainable agriculture sector (Avis et al.,2008). In this regard, the use of organic materials such as agricultural, urban and industrial waste compost to reduce the use of chemical fertilizers and the application of useful soil microorganisms such as *Trichoderma* species (*Trichoderma.spp*) for the biological control of crops and better analysis of soil organic matter It is among the goals of sustainable agriculture (Tarango et al.,2009; Bennett and Whipps,2008). Few antagonistic fungi have the ability to produce plant growth enhancerous metabolites (Culter et al.,1986). Among the mechanisms that influence the growth of plants, various *Trichoderma* species, in addition to the ability to produce auxin, also have the ability to produce organic acids such as gluconic acid, citric acid and fumaric. This ability to produce organic acids reduces the soil pH, dissolves phosphate and some elements such as iron, magnesium and manganese to be easily accessible to the plant (Harman et al.,2004). *Trichoderma* fungi are a group of soil fungi whose main role is to convert organic residues and compost production (Baker,1998; Howell,2002).These fungi can quickly and easily ferment and decompose cellulose into cellulose and lignin and are very useful in the production of compost (Harman et al.,2004). The antagonistic mechanisms of this fungus include processes such as chemistry and strand connectivity, and twist and strain intrusion. These steps are associated with secretion of extracellular enzymes such as ketinase and beta-gluconase, and protease as secondary metabolites (De Marco et al.,203; Lorito et al.,1996). *Trichoderma* is a fungus that occurs in many soils where the plant is grown. This fungus is known to be a growth enhancer in a wide range of crops and ornamentals (Chang et al.,1986). Antagonistic fungi such as *Trichoderma*, by producing strong hydrolysis enzymes, have an inhibitory effect on the growth of a wide range of pathogenic fungi. *Trichoderma* kinase genes express kitolactiny enzymes with antifungal activity reported by chemical fungicides (Lorito et al.,1996).

Materials and Method

Accordingly, the aim of this study was to investigate the effects of various concentrations of *Trichoderma* fungi Bi isolate on two cultivars of Peppermint (Pepper and Shirazi). The present study was conducted as a pot experiment under non-soil culture conditions in a greenhouse based on a factorial experiment based on a completely randomized design with four concentrations of *Trichoderma hirsianum* Bi: 0% (control), 5%, 10% and 15% vol. 50 liters Water and two cultivars of mint (*Piperita* and Shirazi) with 4 replications were established and implemented at the research greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad.

The isolate was sterilized after transferring a part of the fungus to a dextrorugar potato culture medium and in a petri dish 10 cm in diameter (autoclaved at 120 ° C for 20 minutes) at 25 Centigrade in oven was kept for 5 days to allow fungi to grow properly for the next step. In order to prepare the fungal extract, a semi-selective culture medium containing one gram of calcium nitrate, 1 (gr) of calcium chloride, 250 (mg) of potassium nitrate, 250 (mg) of



monopotassium phosphate, 50 (mg) of citric acid, 2 grams of sucrose, 25 (gr) Agar, 30 (mg) of streptomycin sulfate per liter of distilled water and culture medium containing 0.2 (g) of magnesium sulfate, 0.9 (gr) D-potassium phosphate, 1.5 (gr) of potassium chloride, 3 (gr) of glucose, 20 (gr) of agar per each The liter of distilled water was used (Papavizas and Lumsden, 1982).

The medium was sterilized in 2-liter arranules that were sterilized by autoclave at a temperature of 120 ° C at atmospheric pressure of 10 atm. Then, the fungus colonies were transferred from the dextro agar culture medium to the Sweh culture medium.

Seeds of two cultivars of mint were planted in Peat Moss seedlings, and trays were kept in a greenhouse at 25 ° C for 17 days under the same conditions. Seedlings of identical and identical size were selected selectively and transferred to pots of 10 cm in diameter transported by cocopeat and perlite.

The studied traits were number of leaves, nodes and plant height. From the beginning, transplanting into the pot, the number of leaves and nodes was counted alternately until the flowering time of the plant, and the average counted was eventually used. The height of the plant was measured by meter in cm.

Chlorophyll a, b, total and carotenoid levels were analyzed according to the method in Dere et al (1998). So that 200 (mg) of fresh leaves are completely separated from the leaves and poured into Chinese moss, then crushed to be well crushed. 10 ml of 99% methanol was added to the samples, then placed in centrifuge at 6000 rpm for 10 minutes. The extracted extracts from the centrifuge were transferred to the glass balloon. The sample was sprayed into a balloon in a cuvette spectrophotometer and separately absorbed by absorption spectrophotometer at 663 nm for chlorophyll a and 653 nm for chlorophyll b and 470 nm for carotenoids. Finally, using the formula The following amounts of chlorophyll a, b, total and carotenoids were obtained in terms of mg / g fresh weight.

$$\text{Chl a} = 15.65 A_{666} - 7.340 A_{653} \quad (1)$$

$$\text{Chl b} = 27.05 A_{653} - 11.21 A_{666}$$

$$C_{x+c} = \frac{1000 A_{470} - 2.860 \text{ Chl a} - 129.2 \text{ Chl b}}{245} \quad (2)$$

$$\text{Chl T} = \text{Chl a} + \text{Chl b} + C_{x+c} \quad (3)$$

The data were analyzed using JMP8 software. The average of traits was measured using LSD test at 5% probability level. Draw tables using Excel-2013 software.



Result and Discution

Based on the results of statistical analysis, the *Trichoderma* fungus concentration had a significant effect on leaf number and number of nodes. The highest amount of leaves was observed in the concentration of 15% of the fungus, which was observed to increase by 59.41% as compared to the control treatment, where the concentration of fungus was zero (Fig1).

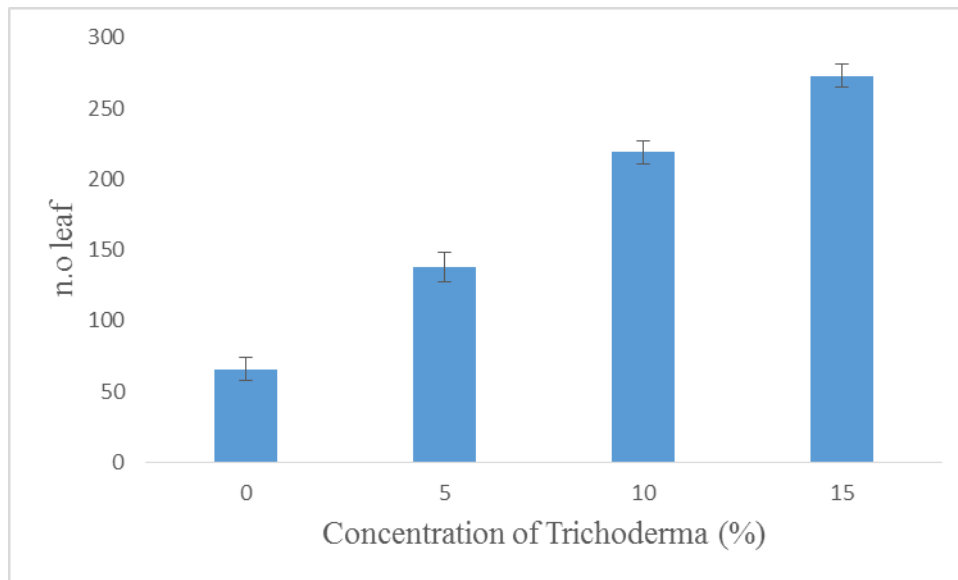


Figure 1. Effect of fungal concentration on leaf number

In the concentration of fungi, 15% was observed in 143 nodes, which increased 91% as compared to control (Fig2).

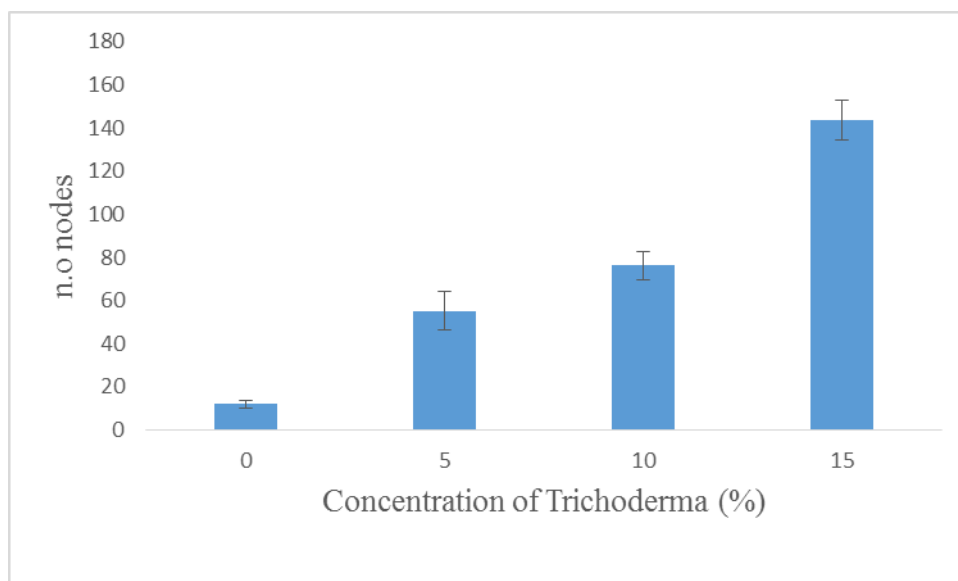


Figure2. Effect of fungal concentration on nodes number



The concentration of Trichoderma fungus and the type of crop used could have a significant effect on plant height. The highest plant height was 88 cm in the treatment of fungi with a concentration of 15% and Piperita cultivar. The lowest altitude was 22.5 cm in Shirazi cultivar and zero concentrations (Fig3).

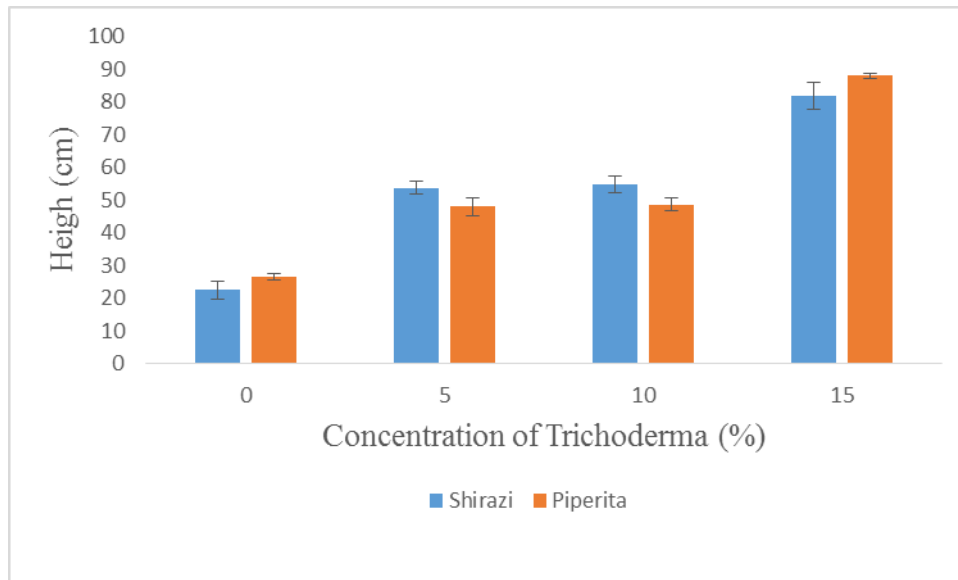


Figure3. Effect of fungal concentration and cultivar on height

Different concentrations of Trichoderma fungi were not effective on all of the measured biochemical traits. But the type of cultivar used had a significant effect on the amount of chlorophyll a, b and total. The highest amount of chlorophyll a, b, total was observed in Piperita cultivars, which increased in comparison to Shirazi variety (Fig4a,b,c). None of the treatments used had significant effect on the amount of carotenoids.

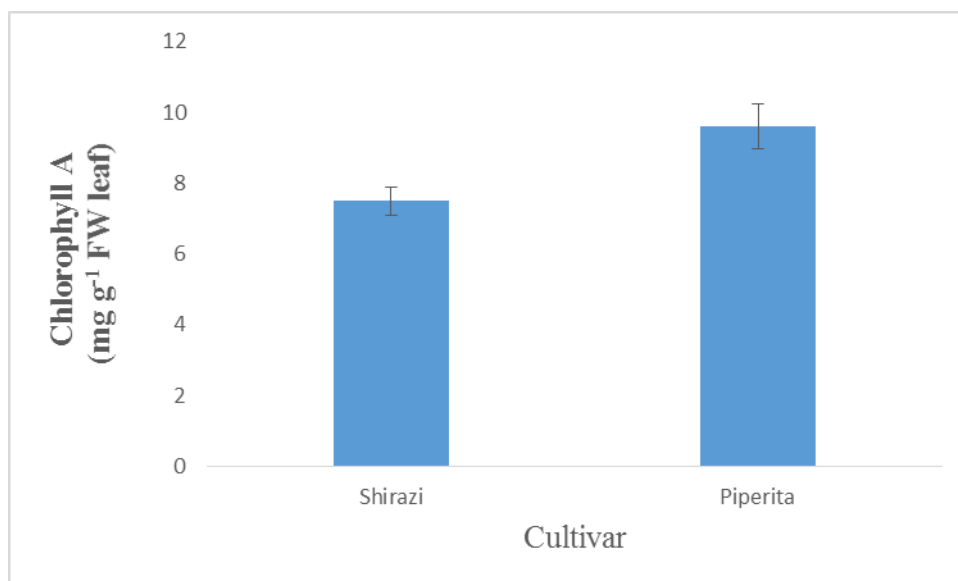


Figure4a. Effect of cultivar on Chlorophyll A

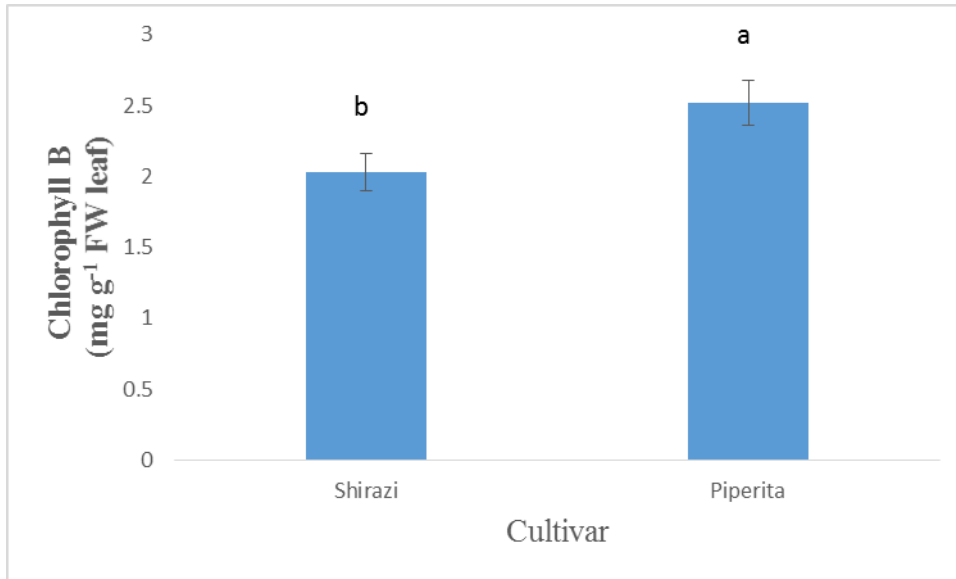


Figure4b. Effect of cultivar on Chlorophyll B

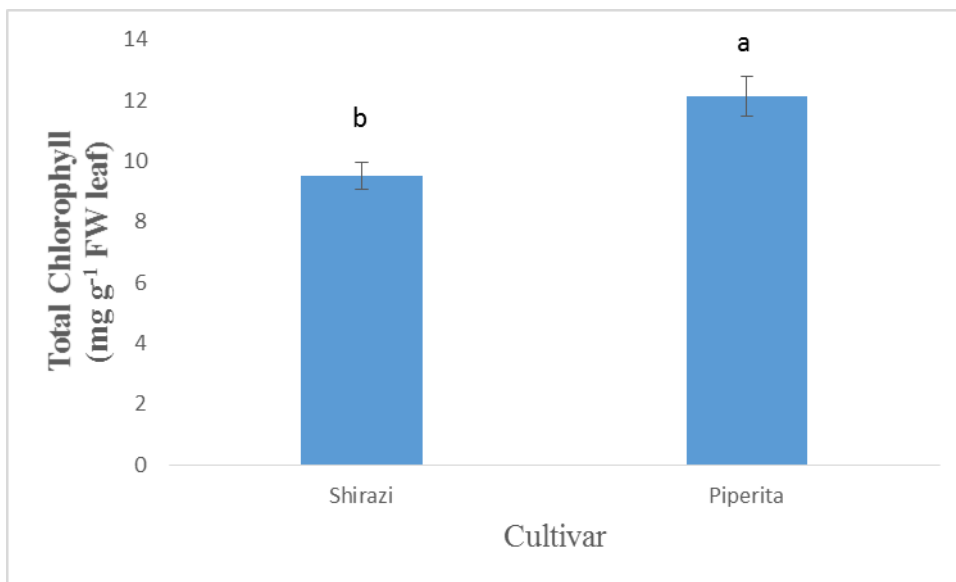


Figure4c. Effect of cultivar on total Chlorophyll



The ability to secrete biochemical compounds and various extracellular enzymes in the soil, the high ability of colonization of the rhizosphere, the ability to coexist in the root, high spore ability, high tolerance to soil heavy elements, salinity and other compounds in the soil and root environment, high nutritional competition. The most important characteristics of the various species of *Trichoderma* are the factors that cause fecundity in the rhizosphere and, most importantly, the ability to create and induce resistance by stimulating the plant to produce phytoalexin compounds (Samuels,1996). Thus, it can be concluded that the *Trichoderma* fungus contributes to the growth of the leaf and knots in the peppermint, by helping the plant to synthesize chlorophyll, as well as suppressing the pathogenic pathogens. In his research on a large number of plants, Yedidia, et al (2001) stated that the increase of lateral branches and number of leaves is a general part of the effects of this type of fungus. The complex mechanisms of this growth increase are probably due to the production and release of growth stimulating compounds into the extract of this fungus, which increases the efficiency of manure (Gravel et al.,2007). In other studies, plant height, leaf number and area, dry weight were significantly increased due to inoculation of *Trichoderma* fungi and its extract (Kleifeld and Chat, 1992). *Trichoderma* fungi, by controlling the non-pathogenic microbial destructive population and also by digesting the toxic metabolites produced by these microflora by a series of enzymes, directly increase root growth and ultimately increase plant growth (Harman et al., 2004).

The results of the study indicate that the type of plant and the type and amount of secondary metabolites secreted in the extract by different species and isolates of *Trichoderma* can influence the level of their growth effects in the plant-*Trichoderma* interaction (Vinale et al.,2008). Apparently, some species and even isolates from *Trichoderma* have better effects on some species of plants and there is a kind of compatibility between the isolates used by plants. The soil type and the interaction of soil and plant can also be effective in the success of *Trichoderma* species (Bal and Altintas ., 2008). According to these studies, it can be concluded that the effect of no fungus on any of the biochemical traits may be due to the different type and function of the isolates of *Trichoderma*, the interaction between the plant and soil and the type of soil (Buyer et la.,2002; Altintas and Bal,2005).

In general, it can be concluded that the *Trichoderma* fungus inoculum and its extract significantly affect plant growth.



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