Journal of Ornamental Plants
Available online on: www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

The Effect of *Trichoderma* Isolates on Morpho-Physiological Changes of *Polianthes tuberose* Under Drought Stress Conditions

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Received: 13 April 2018 Accepted: 19 August 2018
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Abstract

In order to evaluate the effect of different Trichoderma isolates on reducing the effects of drought stress on *Polianthes* tuberose flower, two factorial experiments were carried out based on a completely randomized design with three replications. In the first and second experiments, the first factors were Bi and 65 isolates of *Trichoderma harzianum* at three levels (0, 10, 20% v/v) and the second factor in both experiments was three levels of drought stress (25, 50, 100% field capacity). The results showed that both isolates increased biomass fresh and dry weights and root dry weight of *Polianthes tuberosa* plants exposed to stress conditions. Biomass fresh weight was increased by 4 g at 25% stress level by the isolate Bi and 14 g by the isolate 65, indicating the growth of this flower in the presence of *Trichoderma* fungus. The amount of proline in the treatment of isolate 65 at 20% stress level was increased compared to other levels of the fungus at 25 and 50% stress levels. Relative water content and leaf green area were enhanced when the two fungal isolates were applied under increasing stress levels. In general, it seems that the isolates Bi and 65 of *Trichoderma harzianum* have different effects under drought stress conditions.

Keywords: : Leaf relative water content, Photosynthesis, Proline, Root dry weight.

INTRODUCTION

Water scarcity is considered as one of the most critical environmental factors reducing yields and agricultural products in the world, and this is particularly important in arid and semi-arid regions (Kumar *et al.*, 2012). The depletion of groundwater resources, the decline of rainfall, the gradual warming of air temperature, and the excessive extraction of water from groundwater tables have resulted in the limitation of water in most parts of Iran and have incurred a lot of costs on water supply for irrigation. Therefore, solutions need to be developed for this problem (Ansari *et al*., 2010). Studies have shown that the use of soil-borne biological factors, such as mycorrhiza and *Trichoderma* fungi, can play a role in mitigating the effects of environmental stresses (Kaew-chai *et al*., 2010).

Trichoderma is an opportunistic, non-pathogenic, and soil-borne fungus that established a symbiotic relationship with plant roots. Due to its high competitiveness and metabolic diversity, it is a dominant organism of soil in most regions and is one of the most commonly cultivated fungi that can be easily propagated (Kaewchai *et al*., 2010; Joshi *et al*., 2010). These fungi can increase plant growth and development by biologically controlling soil-borne pathogens, producing growth hormones, dissolving insoluble elements, increasing the absorption and transfer of nutrients, eliminating poisons, increasing the transfer of sugar and amino acids in the roots of plants, and creating inductive resistance against environmental stresses (Mazhabi *et al*., 2011; Cuevas, 2006). *Trichoderma* has been effective in escalating the growth of a wide range of ornamental and field crops such as tobacco, tomato, eggplant, pepper, chrysanthemum, and *Dianthus* (Chang *et al*., 1986).

Tuberose (*Polianthes tuberosa*), belonging to the family Amaryllidaceae, is a monocotyledonous, cormous and perennial plant species (Eidyan *et al*., 2014). Most of the tuberose varieties have single or double flowers, of which the former ones are cultivated to produce flower buds and the latter ones are cultivated to produce cut flowers. Tuberose is native to Mexico. Around 300 ha in Iran are under the cultivation of tuberose, and it has the fourth rank in terms of production after gladiolus, rose, and *Dianthus* (Taher *et al*., 2014).

Tuberose flower is one of the most important commercial and fragrant flowers (Bahrehmand *et al*., 2014). Recently, besides Iran, it has received the attention of some countries such as Kenya, India, and Mexico due to the popularity of its florets and fragrance, and has been traded commercially in flower and ornamental plant markets such as in the United States, Europe, and Japan (Waithaka *et al*., 2001). Considering the global phenomena of drought, high temperatures, and oxidative stresses and as a result of their negative effects on the production of food and ornamental plants, it is essential and inevitable to put forth some efficient planting patterns with high production capability and good quality and to management the use of sources and inputs effectively (Putra and Yuliando, 2015).

Given the growing demand of the community for cut flowers and the popularity of this flower among people, as well as the occurrence of abiotic stresses, especially drought, which limit the growth of plants, it seems necessary to explore the effect of biological agents on the growth and development of tuberose plants. Therefore, this study was carried out to evaluate the effect of *Trichoderma* isolates and drought stress on morpho-physiological changes of tuberoses.

MATERIALS AND METHODS

In order to investigate the effect of *Trichoderma* isolates on drought stress, two separate factorial experiments were carried out based on a completely randomized design in Ferdowsi University of Mashhad during 2016-2017. In the first and second experiments, the first factor was *Trichoderma harzianum* fungus isolate Bi and *Trichoderma harzianum* isolate 65, respectively, both at three levels (0, 10, and 20% v/v), and the second factor was assigned to three levels of stress (25, 50, 100% of FC) in both experiments. Pots with dimensions of 20×30 cm were filled with a uniform medium incorporated with the fungal treatments. After being prepared, the fungi were cultivated at two levels (10-20%) in a mixed medium (peat mass and perlite with the ratio of 1:1).

The method of applying drought stress treatment

Drought stress treatments were applied at three levels of 25, 50 and 100% of FC, and the irrigation was performed after the emergence of apical meristem until the end of plant growth. Weighting method was used to apply irrigation treatments.

In this experiment, some morpho-physiological traits, including photosynthesis, relative water content, proline content, electrolyte leakage, corm fresh and dry weight, root dry weight, biomass weight, biomass dry weight, and green leaf area were measured. Photosynthesis rate was measured on the youngest fully-developed leaf by using Portable Photosynthesis System LCD (Zomorodi, 2013). To measure the relative water content of the leaves, samples were taken from young leaves at the top of the plant (Cornic, 1994). To measure proline content, the method proposed by Bates *et al*. (1973) was used. Electrolyte leakage was estimated by Marcum (1998)'s method. After measuring the initial weights of corms, the dry weight of corms, root dry weight, biomass weight, biomass dry weight, and green leaf area were measured. Since it is difficult to prepare tuberose corms with the same primary weights, and there is high variation between them, this factor was considered as a covariate in the experiment, so the effect of the factors on the traits is completely determined; therefore, the factor was added in the variance analysis table. Data were analyzed using the Minitab 18 and SPSS software packages. Figures were drawn by using MS-Excel software, and means comparison was performed by using LSD test at the 5% probability level.

RESULTS AND DISCUSSION

The results of analysis of variance showed that the interaction of stress and Bi fungus levels was significant for biomass fresh and dry weights, proline, relative water content of leaves, and leaf green area, and the interaction of stress and fungus 65 levels was significant for photosynthesis, proline, relative water content of leaves, and leaf green area (Tables 1 and 2).

Photosynthesis

The results related to the effect of different levels of Bi fungus isolate (first experiment) showed that photosynthesis was significantly affected by drought stress ($P < 0.01$) (Table 1). The maximum and minimum photosynthesis values were obtained from control and stress level of 25% FC, respectively. The amount of photosynthesis at the stress level of 50% FC was decreased by 10% compared to the control (Fig. 1). In the second experiment, the amount of photosynthesis was significantly affected by drought stress ($P < 0.01$) (Table 2) and the interaction of drought stress and fungus had a significant effect on the trait (Tables 1 and 2). The highest photosynthesis was observed at 50% stress level when no isolate of fungus 65 was used, and no significant difference was observed between different levels of fungus at each individual stress level (Fig. 2). The results showed that photosynthesis was decreased when drought stress was intensified. It seems that the plant's ability to preserve its photosynthesis capacity can play a vital role in its drought resistance (Masoomi *et al*., 2012). Photosynthesis, together with cell growth, is among the primary processes to be affected by drought (Chaves, 1991). The effects can be direct such as the loss of CO_2 availability due to limitations of diffusion through stomata and mesophyll (Flexas *et al*., 2007) or the alterations of photosynthetic metabolism (Lawlor and Cornic, 2002), or they can arise as secondary effects, namely oxidative stress.

Table 1. Results of analysis of variance related to the effects of different levels of drought stress and *Trichoderma* isolate Bi on some morpho-physiological traits in *Polianthes tuberose*.

Bi	df	Leaf area	RWC	Proline	Biomass dry weight	Biomass weight	Electrolyte leakage	Root dry weight	Corm fresh weight	Corm dry weight	Photosynthesis
Covarios	1	3650^{ns}	0.045ns	0.0001^{ns}	0.55^{ns}	93.3^{ns}	161^{ns}	0.020^{ns}	4.47^{ns}	5.310^{ns}	0.004^{ns}
Stress (S)	2	1913^{**}	124^{**}	0.021^{**}	3.00^{**}	478^{**}	234^{*}	0.331^{**}	4.00^{ns}	0.469^{ns}	0.126^{**}
Trichoderma (T)	2	1727^{**}	106^{**}	0.015^{*}	3.68^{**}	854^{**}	16.4^{ns}	1.030^{**}	19.19^{ns}	16.87^{**}	0.006^{ns}
S×T	4	2848^{**}	12.6^{**}	0.011^{*}	1.49^{**}	178^{*}	54.0^{ns}	0.010^{ns}	38.0^{ns}	0.391^{ns}	0.013^{ns}
Error	17	6528	0.130	0.003	0.35	45.6	51.6	0.010	20.9	1.48	0.021
CV (%)		13.2	8.56	5.4	13.5	13.5	6.6	14.6	15	14.3	8.3

*, ** and ^{ns} show significance at the 5% and 1% probability levels and non-significant difference according to LSD test, respectively.

Table 2. Continued. Results of analysis of variance related to the effects of different levels of drought stress and *Trichoderma* isolate 65 on some morpho-physiological traits in *Polianthes tuberose*.

65	df	Leaf area	RWC	Proline	Biomass dry weight	Biomass weight	Electrolyte leakage	Root dry weight	Corm fresh weight	Corm dry weight	Photosynthesis
Covarios	1	2178^{ns}	0.04^{ns}	0.0001^{ns}	0.094^{ns}	0.05^{ns}	131^{ns}	0.057^{ns}	1.18^{ns}	0.086^{ns}	0.019^{ns}
Stress (S)	2	2883^{**}	150^{**}	0.0340^{**}	6.470^{**}	105^{**}	191^{*}	1.00^{**}	10.19^{ns}	5.220^{ns}	0.469^{**}
Trichoderma (T)	2	8381^{*}	201^{**}	0.0080^{ns}	4.050^{**}	505^{**}	16.6^{ns}	0.861^{**}	15.7^{ns}	6.100^{ns}	0.309^{**}
S×T	4	5394^{*}	4.23^{**}	0.0210^{**}	0.599^{ns}	113^{ns}	51.4^{ns}	0.024^{ns}	19.8^{ns}	0.465^{ns}	0.226^{**}
Error	17	1705	0.35	0.004	0.505	55.2	45.1	0.038	36.0	2.23	0.021
CV (%)		14.3	11.1	8	10.1	13.2	7.5	15	14.3	12.4	9

*, ** and ^{ns} show significance at the 5% and 1% probability levels and non-significant difference according to LSD test, respectively.

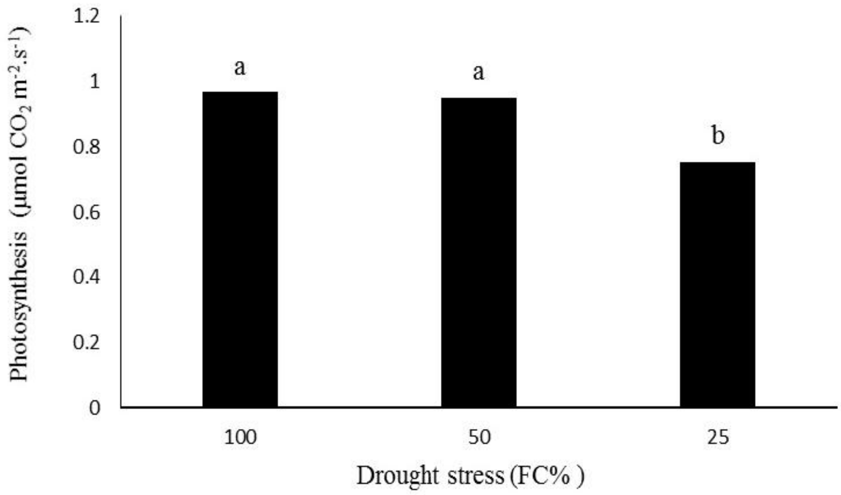

Fig. 1. Effect of drought stress on photosynthesis rate in tuberose (isolate Bi).

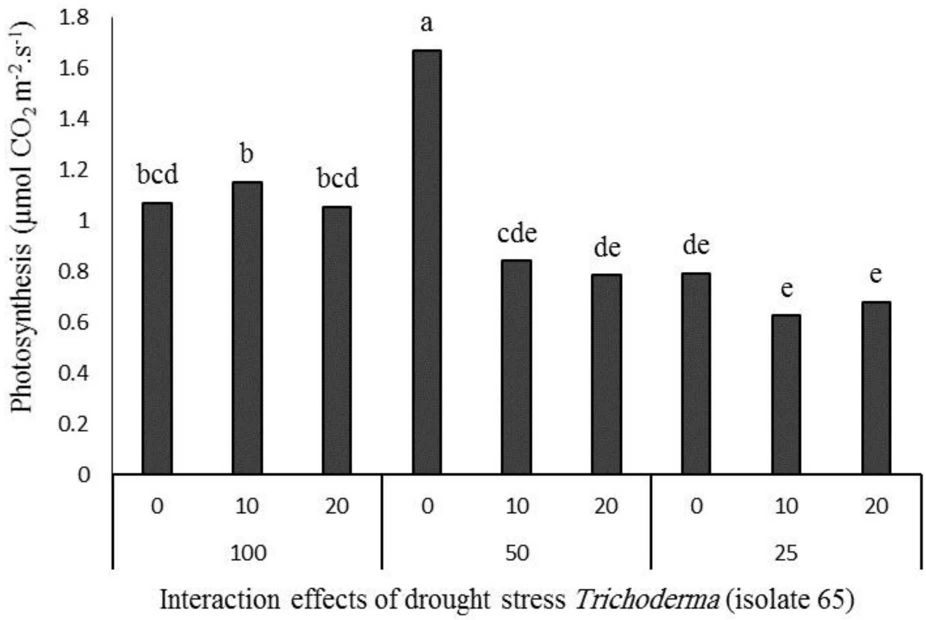

Fig. 2. Interaction of drought stress and *Trichoderma* (isolate 65) for photosynthesis rate in tuberose.

Corm dry weight

In both first and second experiments, the treatments did not have a significant effect on corm fresh weight (Tables 1 and 2). The results of analysis of variance showed that in the first experiment, different levels of Bi fungi had a significant effect ($P < 0.01$) on corm dry weight, but other treatments in this experiment and the second experiment did not have a significant effect on the trait (Tables 1 and 2). The application of different levels of fungi in the first experiment had a negative effect on corm dry weight so that at different fungus levels of 0, 10, and 20%, the trait was measured to be 7.2, 5 and 4.8 g, respectively (Fig. 3). The lack of effect of *Trichoderma* on growth traits has also been reported in some plants. For example, in a study on onion, Altintas and Bal (2008) reported that *Trichoderma* could not significantly increase the quality and yield of onion. It seems that one of the reasons for the modest effect of *Trichoderma* on corm is the low number of lateral shoots and the lack of capillary roots or the low ratio of root to stem compared to other crops (Altintas and Bal, 2008).

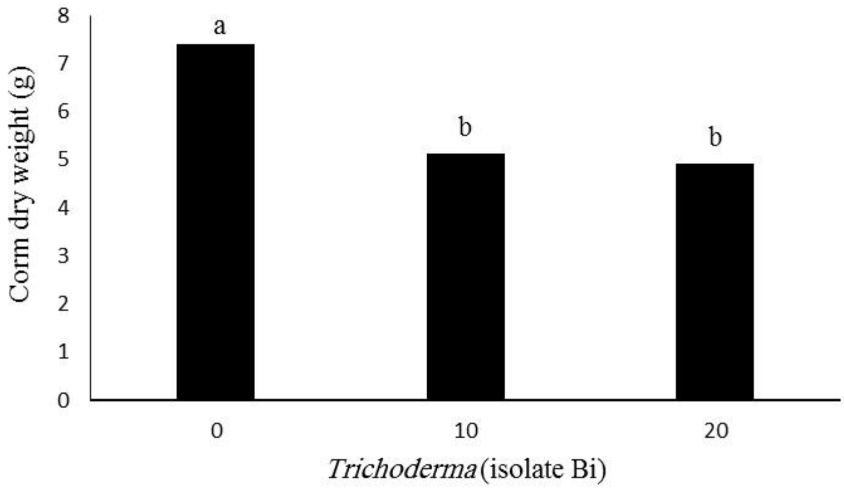

Fig. 3. Effect of *Trichoderma* (isolate Bi) on corm dry weight.

Root dry weight

In the first and second experiments, the effects of different levels of fungi and stress were significant ($P < 0.01$) on root dry weight while the interaction of stress and fungi was not significant (Tables 1 and 2). As the level of fungi was increased, root dry weight was increased significantly. The highest root dry weight was observed at 50% irrigation level and the lowest was found at 100% irrigation level, which showed a significant difference with one another. Increasing stress level up to 50% improved root dry weight while decreasing stress level to 25% reduced root dry weight (Fig. 4 and 5). Yeidia *et al*. (2001) showed that root dry weight was enhanced as stress level was intensified, which is consistent with our results, but this is inconsistent with a study on cucumber seedlings (Taghinasab, 2012). In addition to producing ABA by *Trichoderma* which can probably increase root volume and subsequently, increase the absorption of nutrients, these fungi directly improve root growth and ultimately, increase plant growth by controlling non-pathogenic destructive microbial population and by digesting the toxic metabolites produced by this microflora by a series of enzymes (Harman *et al*., 2004).

Electrolyte leakage

In both experiments, different levels of stress had significant effects on electrolyte leakage ($P < 0.05$). However, the effect of different levels of fungi and their interaction with stress levels were not significant (Tables 1 and 2). Higher stress level was related to higher electrolyte leakage (EL) by about 10% (Fig. 6 and 7). Vannozi and Larner (2007) showed that drought stress prevented cell wall evolution and induced electrolyte leakage from cell walls. Due to the vulnerability of the cytoplasmic membrane, cell contents leak out. It has been also documented that electrolyte leakage increases under drought stress conditions (Beltrano and Ronco, 2008; SabetTeimouri *et al*., 2010).

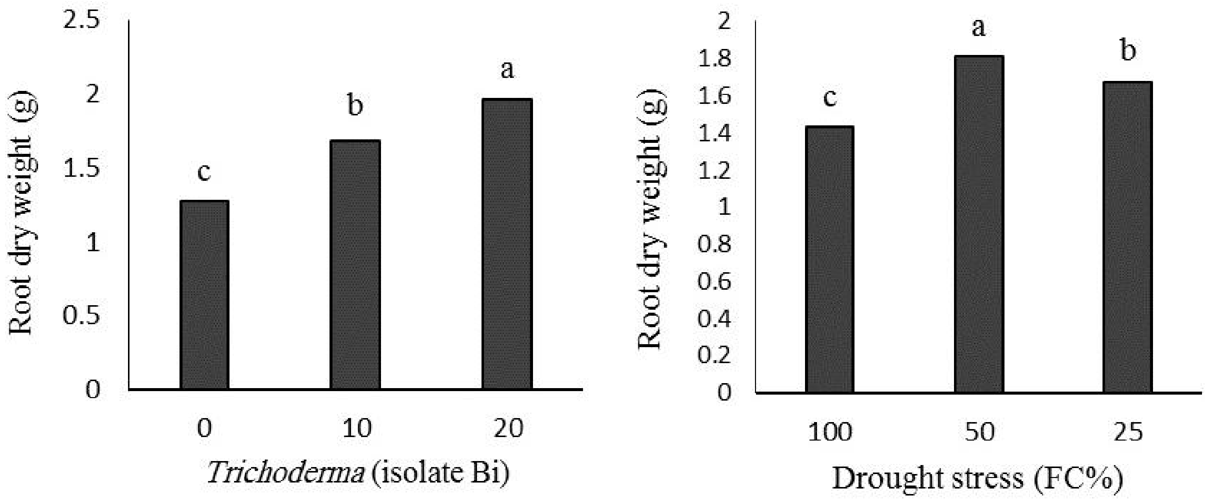

Fig. 4. Effect of *Trichoderma* [isolate Bi (A) and drought stress (B)] on root dry weight in tuberose.

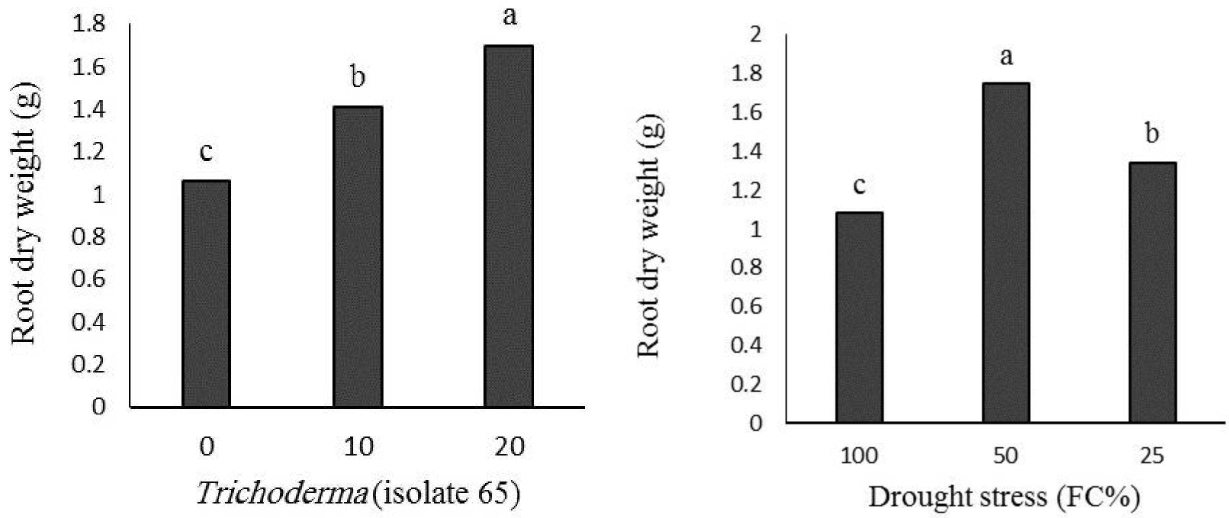

Fig. 5. Effect of *Trichoderma* [isolate 65 (A) and drought stress (B)] on root dry weight in tuberose.

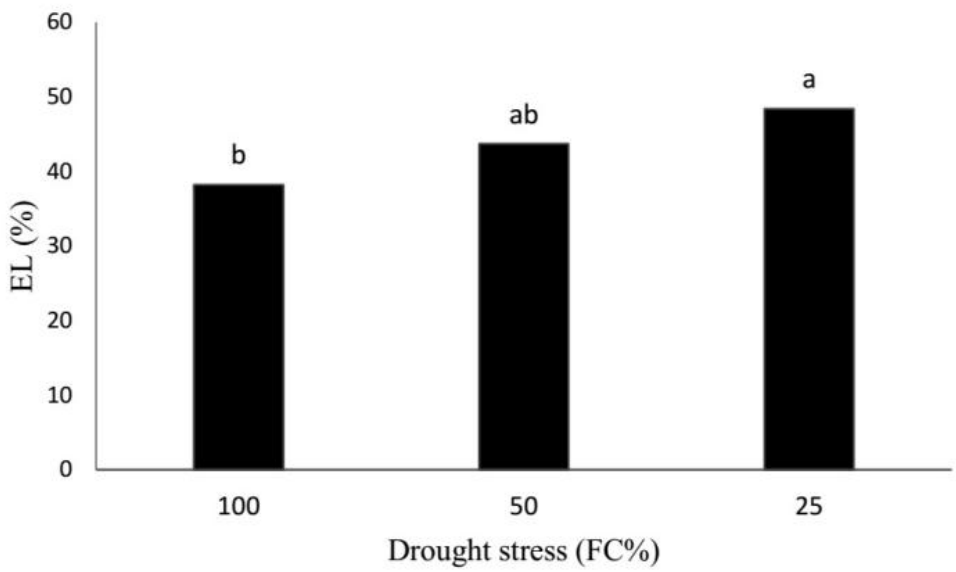

Fig. 6. Effect of drought stress on EL% in tuberose (isolate Bi).

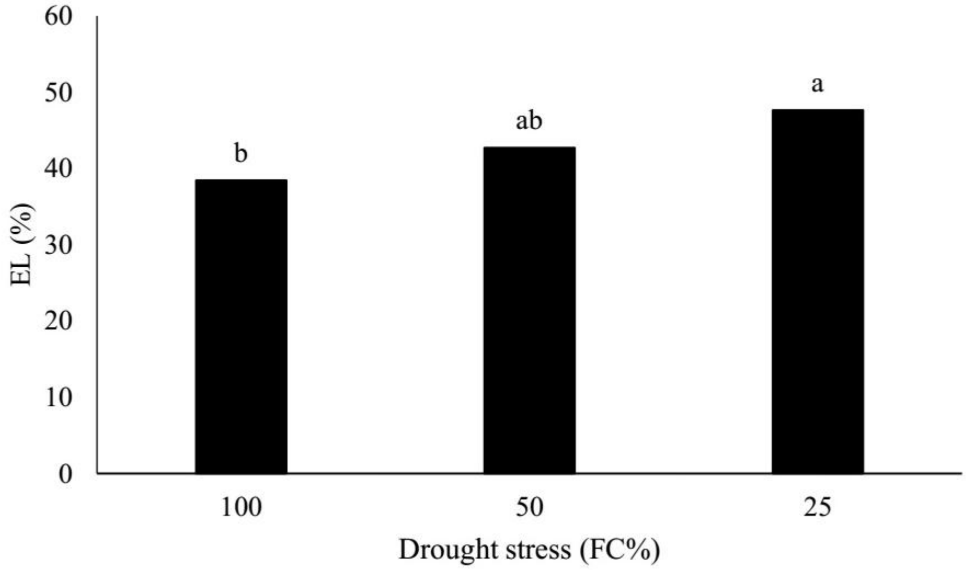

Fig. 7. Effect of drought stress on EL% in tuberose (isolate 65).

Fresh and dry weight of biomass

In the first experiment, biomass fresh and dry weight was significantly affected by drought stress (Table 1), and the interaction between drought stress and fungus isolate Bi was significant for biomass weight so that the highest and lowest weights were observed in 10% Bi fungus × 0% stress and 0% fungus × 50% stress, respectively. When stress level was increased, biomass fresh and dry weights were increased by 3 and 0.5 g in non-fungal samples, respectively. The levels of fungi at each stress level did not differ significantly, but they had higher biomass weight than 0% fungi (Fig. 8 and 9). In the second experiment, the level of isolate 65 and stress levels had a significant effect on biomass fresh and dry weight ($P < 0.01$). The highest fresh and dry weights of biomass were 45.60 and 4.21 g, respectively observed at 100% irrigation level, and biomass weight was decreased when the stress level was increased. The fungus isolate 65 caused an increase in biomass weight as compared to non-fungal samples, but no significant difference was found between 10% and 20% fungus levels (Fig. 10 and 11). In a study, the use of *Trichoderma* increased the growth parameters of tomato in aerial parts (Araghi *et al*., 2011). Many researchers believe that *Trichoderma* isolates stimulate plant growth by producing biochemical materials or mitigate the effects of some growth inhibiting compounds in soil, which can lead to weight gain (Culter *et al*., 1986; Vinale *et al*., 2004). In general, *Trichoderma* species stimulate the growth of underground or aerial organs of these plants by the establishment and production of large amounts of spores in soil, especially around the root of most crops, vegetables and ornamental plants (Araghi *et al*., 2011).

Proline

In both first and second experiments, the interactions of the treatments were significant for proline content (Tables 1 and 2). In both experiments, the highest proline content (0.3 μmol/g dry weight) was related to 10% fungus level in the control plants. No significant difference was found among the treatments in the first experiment. In the second experiment, the maximum amount of proline was related to the stress level of 50% (Fig. 12). Proline amino acid is an osmotic adjusting compound that increases in plants exposed to environmental stress conditions such as salinity and drought (Peng *et al*., 2008). However, in the present study, by increasing the levels of stress, not much difference was observed in the amount of proline in control plants, which could be due to the different mechanism of tuberose plant's response to stress. The isolate 65 of *Trichoderma* fungus resulted in an increase in proline content at 50% stress level compared to the control. Zhung *et al*. (2016) found that the use of *Trichoderma* fungus increased proline content in wheat under salt stress. It has been already shown that increasing proline levels in plants under stress conditions may be due to the activation of proline biosynthesis, which increases protein turnover. In fact, proline plays an important role in protecting plants under stress conditions (Khan *et al*., 2010).

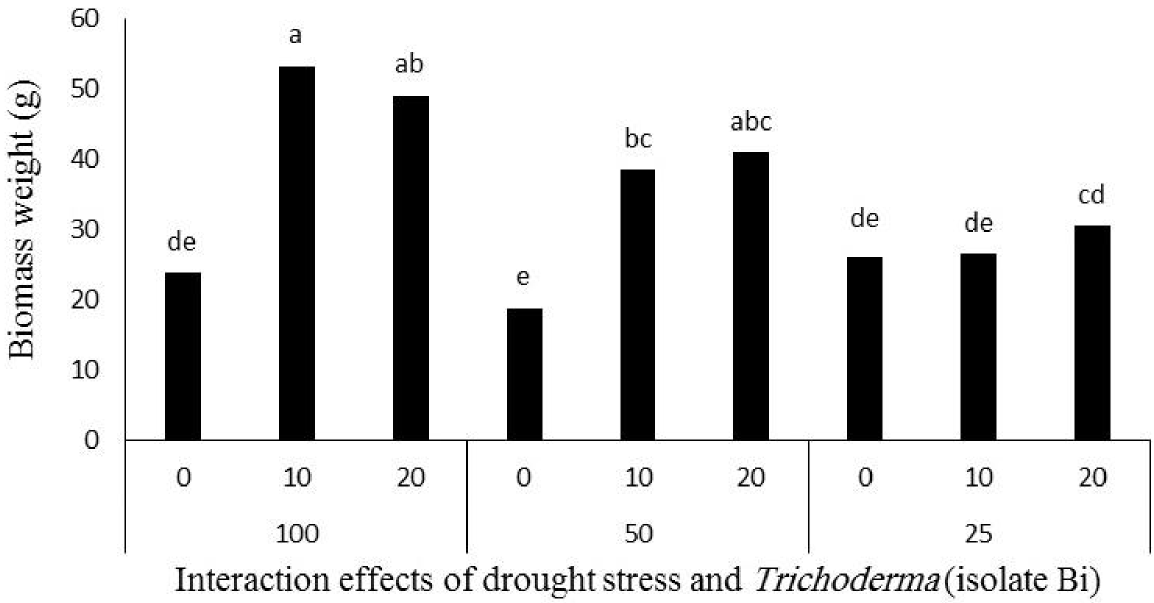

Fig. 8. Interaction of drought stress and *Trichoderma* (isolate Bi) for biomass weight in tuberose.

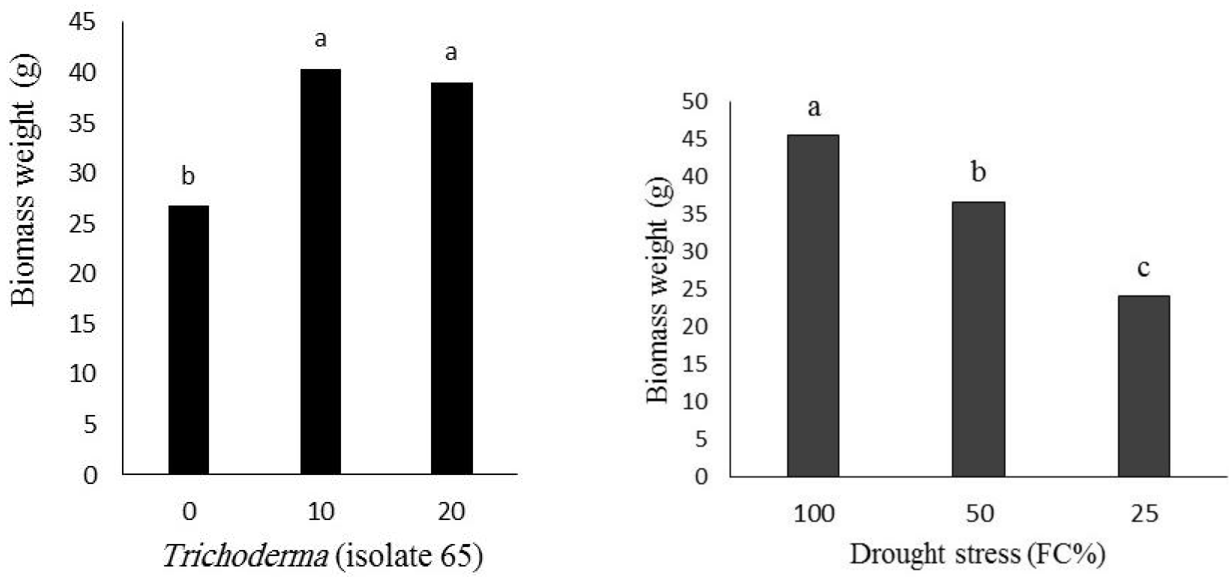

Fig. 9. Effect of *Trichoderma* [isolate 65 (A) and drought stress (B)] on biomass weight in tuberose.

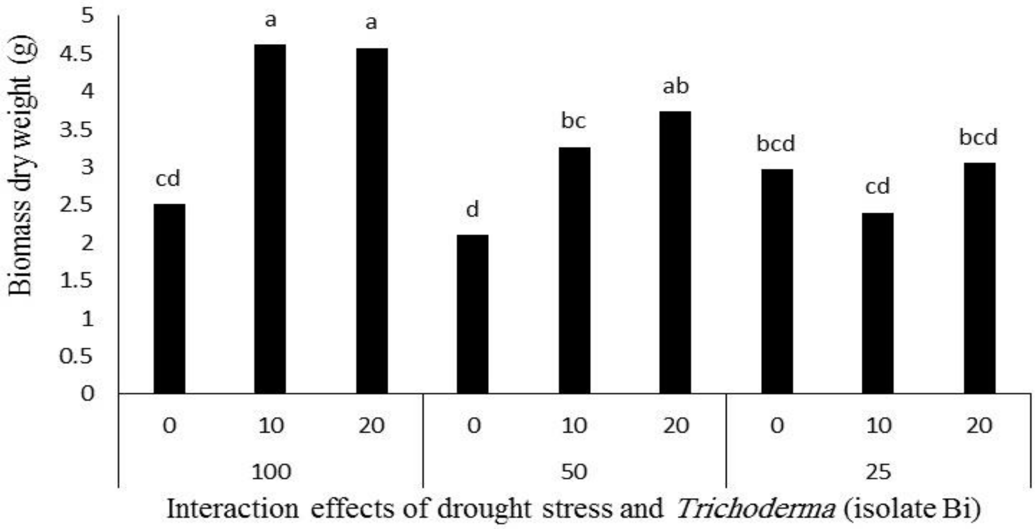

Fig. 10. Interaction of drought stress and *Trichoderma* (isolate Bi) for biomass dry weight in tuberose.

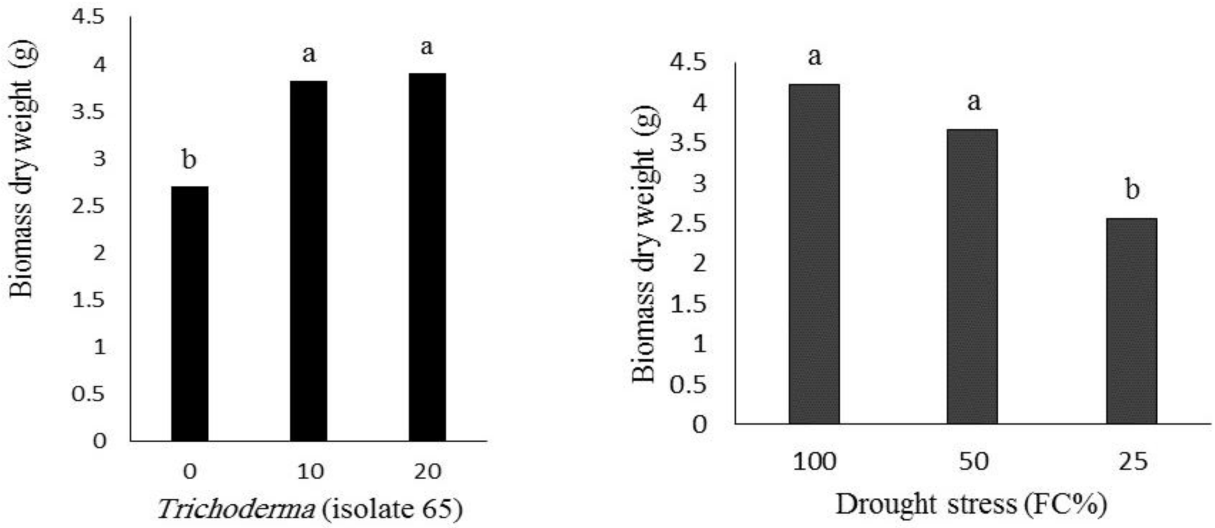

Fig. 11. Effect of *Trichoderma* (isolate 65), A) and drought stress B) on biomass dry weight in tuberose.

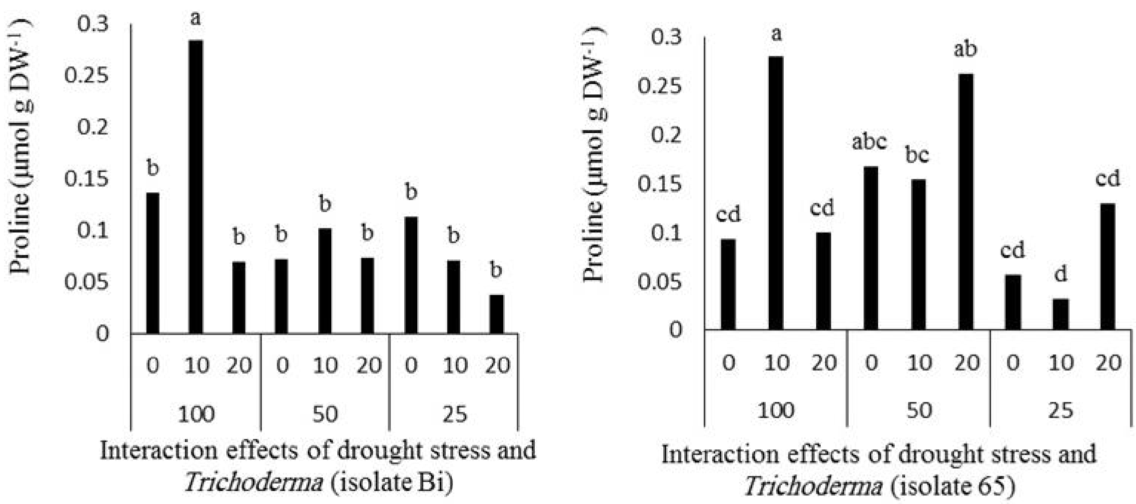

Fig. 12. Interaction of drought stress and *Trichoderma* isolate 65 (A) and isolate Bi (B) for proline content in tuberose.

Leaf relative water content

In both experiments, leaf relative water content was significantly affected by the interaction of the treatments (P < 0.01) (Tables 1 and 2). The highest leaf relative water content (80.4 and 80.7%, respectively) was observed in non-stress and 20% of both isolates Bi and 65 in both experiments. The lowest leaf relative water content was related to 0% fungus under stress level of 25% in both experiments. In isolates Bi, an increase in stress level led to a decrease in the relative water content of leaves while an increase in the level of fungus caused an increase in their relative water content (Fig. 13). In their studies on wheat, Gupta *et al*. (2001) and Zou *et al*. (2007) observed a reduction in the amount of relative water content in low irrigation stress, which is in agreement with our results. The large reduction of stomatal conductance with minor variations of relative water content indicates that signals sent from the root under drought stress conditions are the cause of stomatal closure and photosynthesis reduction; this chemical signal is ABA (Taiz and Zeiger, 2007). *Trichoderma* can increase the growth of the root system (Harman *et al*., 2004). High levels of relative water content under water stress conditions can be related to the behavior of the stomata and root system of the plant because maintaining the internal moisture content of a plant needs deep roots for water uptake (Hirayama *et al*., 2006).

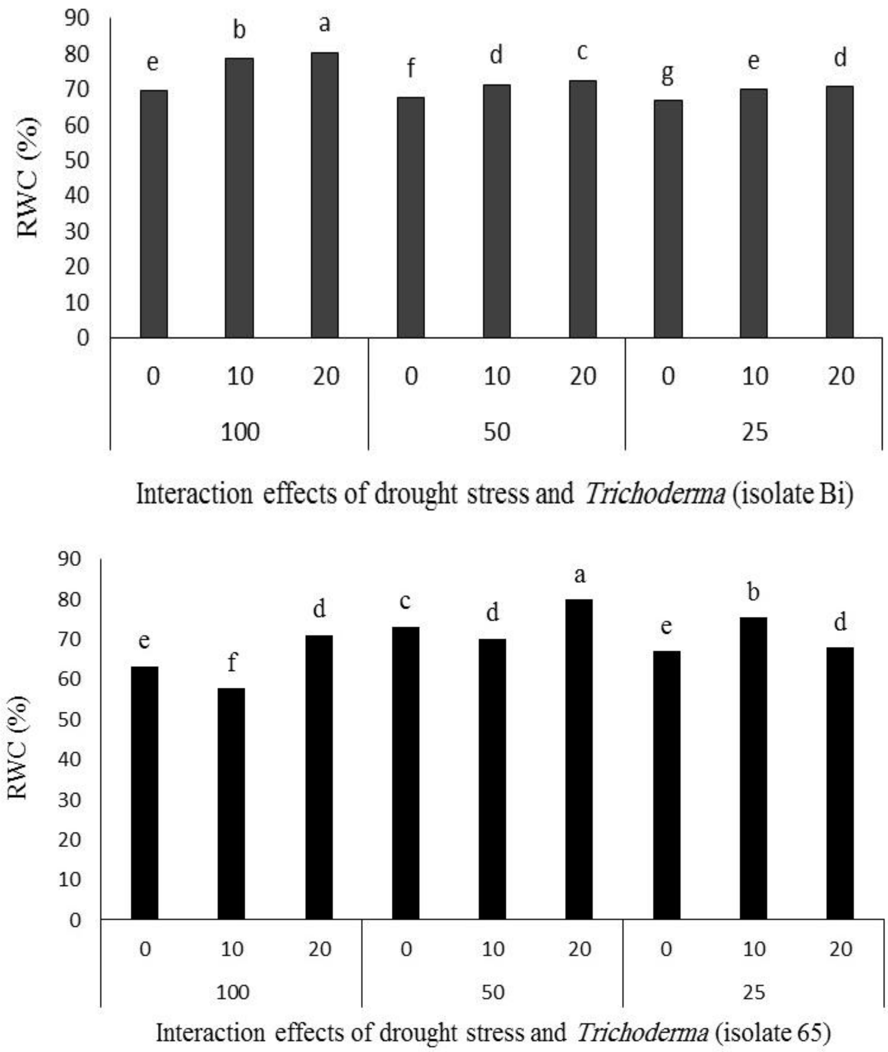

Fig. 13. Interaction of drought stress and Trichoderma isolate Bi (A) and isolate 65 (B) for leaf relative water content (%) in tuberose.

Leaf area

Drought stress, the fungus and the interaction between them had a significant effect on leaf area of tuberose in both first and second experiments. The highest leaf green area in both experiments was related to non-stress conditions and 20% fungus level. When stress levels were increased, leaf green area was decreased while an increase in the levels of fungus resulted in an increase in leaf green area in tuberose plants (Fig. 14). In a study on barley and rapeseed, an increase in leaf area was reported because of the application of *Trichoderma* (Taghavi Ghasemkheyli *et al*., 2014). *Trichoderma* alleviated the effects of salinity stress on some sugarcane growth indices, including increasing leaf green area versus control (Nasrabadi *et al*., 2015). The effect of drought and *Trichoderma* on some growth indices showed that the fungus had the highest means of relative growth rate and green area compared to no fungus application (Salarimiri *et al*., 2016). These results indicate the positive effect of *Trichoderma* on increasing leaf area. Complex mechanisms of this growth increase may be attributed to the production of growth stimulants by *Trichoderma* fungus (Gravel *et al*., 2007).

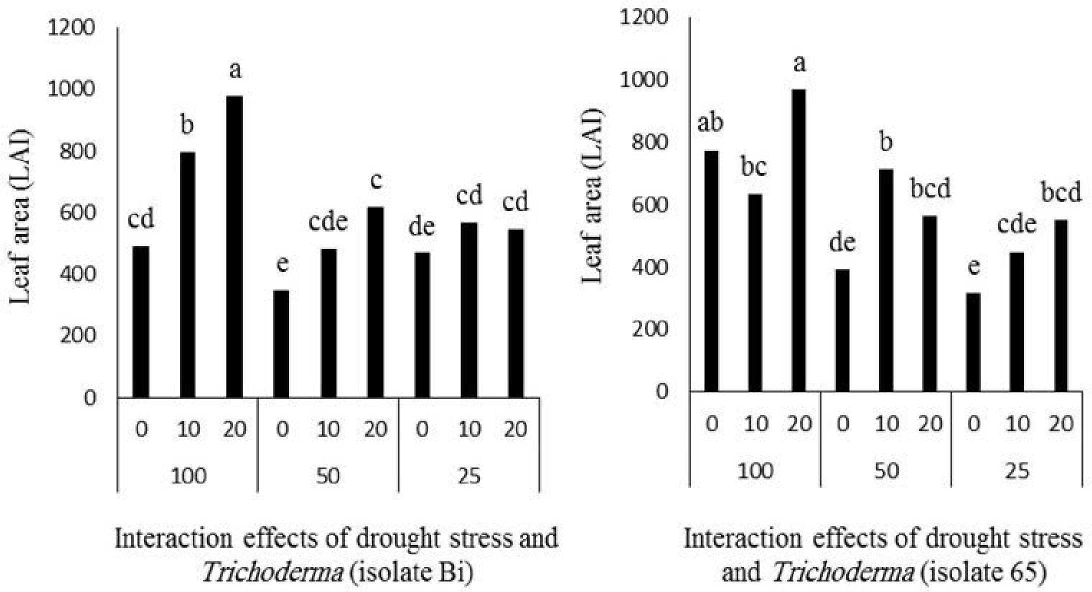

Fig. 14. Interaction of drought stress and *Trichoderma* isolate 65 (A) and isolate Bi (B) for leaf area in tuberose.

It has been shown that the effect of *Trichoderma* was statistically significant on growth traits of cucumbers in soils suffering from salinity stress and the deficiency of potassium and phosphorus. The highest and lowest means of the fungal population were found in acidic soil and very salty soil (Mazlumi *et al*., 2016). Finally, it can be said that one of the reasons for the low density of *Trichoderma* populations in some soils is the lack of proper soil conditions and, if the population is restored, plant growth under stress can be improved by adding successful fungal strains to the soil (Mazlumi *et al*., 2016).

Biological agents are significantly affected by environmental conditions (Buyer *et al*., 2002). The type of *Trichoderma* function can play an important role in inducing growth and plant yield (Bal and Altinas, 2008). It seems that some species and isolates of *Trichoderma* have better effects on some species of plants and there is a kind of compatibility between the isolates used and the plant. Soil type and the interaction of soil and plant can also be effective in the success of *Trichoderma* species (Bal and Altinas, 2008). The significant increase in growth indices of tuberose may be due to the secretion of phytohormones by *Trichoderma* by developing the relationship between fungus and plant and increasing the intake of foods similar to those of mycorrhiza (Blanchard and Bjorkman, 1996). *Trichoderma* may be effective in solubility of plant nutrients such as phosphate, iron, copper, manganese, and zinc (Harman *et al*., 2004). The production of growth regulators by microorganisms is one of the mechanisms that are often correlated with the stimulation of plant growth (Vessey, 2003). In a study, *Trichoderma* was able to synthesize IAA, which could be a reason for the growth and development of tomatoes (Gravel *et al*., 2007).

CONCLUSION

According to the results of the present study, the isolates of *Trichoderma* at different levels had different effects on morpho-physiological traits of tuberose plants exposed to drought stress. The application of *Trichoderma* isolates (Bi and 65) resulted in an increase in biomass fresh and dry weights, root dry weight, leaf relative water content, and leaf green area in tuberose plants compared to the control sample. It seems that the isolate Bi outperformed the insolate 65 in controlling the negative effects of drought stress on tuberose plants. In general, identifying the positive effect of *Trichoderma* fungus on improving the growth of tuberose plants under drought stress conditions requires further research to understand the mechanisms involved in these processes.

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How to cite this article:

Zekavati, H.R., Shoor, M., Rohani, H., Fazeli Kakhki, S.F. and Ganji Moghadam, E. 2018. The Effect of *Trichoderma* Isolates on Morpho-Physiological Changes of *Polianthes tuberose* Under Drought Stress Conditions. *Journal of Ornamental Plants, 8(4), 285-299.*

URL: http://jornamental.iaurasht.ac.ir/article_544858.html