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Some Physiological and Growth Parameters of *Pistachio vera* L. under coinoculation with endomycorrhizae and Bacillus subtilis in response to salinity

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ABSTRACT
Coexist with vesicular carbuncular my corrival fungi improve plant growth by increasing nutrient uptake, improve the relationship of water and the protection of plants against diseases. In order to evaluate the effect of microbial treatment mycorrhizal fungi formed mycorrhizal G. Klarom and helpful bacteria Bacillus subtilis on the biochemical parameters of pistachio trees. Factorial experiment in a completely randomized design with three replications for potted inoculated with fungi and bacteria under controlled greenhouse and after a period of three months, was salinity with different concentrations of NaCl for 10 days. The samples were removed for laboratory examination of the pot. Duncan's multiple range test to compare the mean yield at 5% and SAS software was used to determine correlations among traits. Analysis of variance and mean comparison showed an increase in salinity caused a significant increase praline, proteins, enzymes, antioxidants, sodium, potassium and glucose and the percentage of co-existence in the plant so that the maximum praline, protein and antioxidants in control samples and least of them in mycorrhizal samples with bacteria were seen. Most of the sodium, potassium and glucose in the sample with bacteria and mycorrhizal lowest rate was observed in control samples.The high salinity caused a significant reduction factor of plant growth and chlorophyll content control. The highest mycorrhizal samples with bacteria in view of the increase in chlorophyll a, chlorophyll b was over and the lowest was observed in control samples and compared the number of leaves was the opposite. Overall, the survey results showed the effect of salinity on growth parameters of plants inoculated with mycorrhizal fungi and bacteria reduction is less than the control sample. Note that the parameters for samples inoculated with bacteria significantly higher than the other samples.
Keywords: endomycorrhize, salinity, G. Klarom, Bacillus subtitles, pistachios.

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INTRODUCTION

In most parts of the world as an abiotic soil salinity plant growth and crop production is affected. Among the different responses, reactions of ionic osmotic plants to maintain osmotic equilibrium and osmotic stress and prevent ion in the cell toxicity is of particular importance (1). Plant antioxidant system that includes enzymes such as peroxidase and catalase as well as flavonoids and carotenoids are non-enzymatic molecules as part of the plants' ability to withstand salinity (2). In addition protection systems are essential to plants to salinity stress.

Tqryba90 percent of the plants are mycorrhizal symbiosis. Six types, including arboscular mycorrhiza, Arbotovaid, Aktetedo, Arikvaid, Monotropid and Orchid with specific morphological classification criteria are very common and predominant among them arboscular mycorrhizal (3).

Five arboscular mycorrhizal fungi to soil microbial biomass, make up fifty percent. These fungi have positive effects exist that the most important of which can increase the absorption of nutrients such as phosphorus by the plant, improve plant nutrition, increase water efficiency as well as plants against environmental stresses such as drought and salinity resistance is noted (4).

Arboscular mycorrhizal fungi (AM) belongs to Gelomeromikata (5) with their symbiotic relationship with the most advanced plants (6).

By increasing the resistance of plants to environmental stresses, obtain nutrients and improve soil quality benefit (7). Arboscular mycorrhizal fungi inoculation on soybean fields, millet, composite, three leaf, rice and legumes 17 special tropics of improving economic impact on farming and gardening (8) through increased crop growth and has been shown (9). Arboscular mycorrhizal symbiosis within a range of chemical and biological parameters of affected plants, including to the pattern of secondary compounds in plants.

The accumulation of flavonoids (10), and cyclohexane derivatives, Apokartenoeidha (11), phytoalexins and phenolic compounds (12), more teriterpenoeds and glucosinolates (13) in plants inoculated with arboscular mycorrhizal fungi have been reported. After mycorrhizal symbiosis between host plants on different aspects of plant physiology and biochemistry, and improve its growth is affected.

Symbiosis with mycorrhizal fungi resistance to diseases and pests and stresses such as drought and salinity increases (14). They believe that the increase in resistance due to increased absorption of nutrients such as nitrogen, phosphorus absorption of micronutrients and water. Moreover mycorrhizal fungi play a significant role in maintaining the stability and strength of soil structure, improving water relations, improve soil structure and increases the imposition of the increased pH (15).

Mycorrhizal fungi generally interact with multiple types of organisms in the soil show that this can have a negative effect on mycorrhizal community, neutral or positive. For example, certain bacteria stimulate the growth of mycorrhizal fungi mycelia are formed, in other words in the mycorrhizal plants their strength and so helpful bacteria called mycorrhiza formation (16).

The bacteria also Actinomyces and Endomyces and in that *Bacillus subtilis* bacteria are an example of this (17). Bacillus subtilis is due to the widespread use of phosphate solubilizing bio-fertilizers to improve the nutritional status of the soil.

Such a facility to mycorrhizal plants has led to discussion on various aspects of sustainable agriculture, genetic research and mass production is considerable interest mycorrhizal (18). This study combined inoculation of mycorrhizal fungi Endomyces has helpful bacteria in response to salinity stress was evaluated pistachios.

MATERIALS AND METHODS

Akbari Pistachio seeds Zarand 10% sodium hypochlorite disinfectant for 20 minutes, and then in order to speed up the germination of seeds kept in distilled water for 48 hours. Then for 7 days on Cloths were placed in a petri dish and sterilized seeds were sterile wet day with some water to germination took place.

To evaluate the effects of mycorrhizal inoculation mycorrhizal fungi and bacteria of the Bacillus subtilis help on biochemical parameters of Pistachio, a factorial experiment in a randomized complete block design with three replications in controlled conditions. There are two randomized block inoculation and inoculation with mycorrhizal fungus G. Klarom and for each block, and no inoculation two control treatments without inoculation of bacteria Bacillus subtilis and control will be considered.

The experimental data collected were stored after the averaging in Excel. When the data normality test and pilot errors, homogeneity of variance test experiment was performed using SAS software. In order to analysis of simple variance based on completely randomized factorial design to compare the averages of the traits Duncan's multiple range test was performed at 5%.

RESULTS AND DISCUSSION

The results showed that the control plants and plants inoculated with PGPR and mycorrhizal arboscular mycorrhizal fungi, by increasing salt concentration and root dry weight, shoot dry weight and leaf number was reduced. The inoculation of bacteria and fungi and their interaction in control plants under salt stress leads to increased root and shoot dry weight of the plant. But the results showed inoculated with fungi and bacteria on the number of leaves in control plants under salt stress had no significant effect. (Figure 1, 2, 3, 4, 5).

Analysis of variance showed that increasing salinity levels, stem length and inoculated control plants showed no change (Figure 6).

But root length and inoculated control plants was significantly reduced (Fig. 7).

The results showed that the length of the stem and roots inoculated with bacteria in control plants under salt stress increased significantly. Inoculation with mixed mushrooms and fungi and bacteria in control plants under salt stress on shoot and root length was significantly reduced. Several studies on the effect of salinity on plant growth indicates that the sensitivity of plants to salinity, depending on genotype, age and species of plant resistance to salinity (26). Salinity in the beans is also reduced stem length and shoot weight (27). Salinity plant stem diameter is reduced due to the reduction in vascular tissue.

But what about the effect of salinity on the tomato plant roots has been reported that salinity has decreased root length and root surface area. In the flax plant also reduced root length and root to shoot ratio also increased (28).

In bean root length decreased in salinity (29). It is reported that salinity plant growth due to water stress in the root zone (toxic ions in plant tissues) reduces. Salinity with declining growth rates, reducing the length and diameter of stems and small leaves will respond. Salinity with declining growth rates, reducing the length and diameter of stems and small leaves will respond. The effect of salinity on leaf can be noted that the immediate response of plants to increasing salinity reduced leaf growth.

Because it reduces cell division leaves, stomata closure and reduce the sugar is produced, as well as salinity caused by cuticle cell wall fragmentation is crumpled and wrinkled and leading to irregularities in the openings, causing the disintegration of chloroplasts and mitochondria (30). Sodium chloride, as well as salinity increases photosynthesis in the chloroplasts of higher plants and by reducing the impact on growth (31).

Another report on the effect of salinity flax, Atriplex and Bean show, salinity increases the thickness of the epidermis, mesophyll thickness, the palisade parenchyma cells, palisade parenchyma diameter is the diameter spongy parenchyma cells. But halophytes salinity in mangroves thickness of the epidermis cells, mesophilic and reduced intercellular space (32).

Analysis of variance showed that increasing salinity levels of potassium and sodium concentrations increased significantly. Inoculation of bacteria and fungi on potassium concentration control plants and plants under salt stress had no significant effect (Figure 8), but significantly reduced the sodium concentration (Fig. 9).

Potassium cations for plants is important that it's accumulation during osmotic stress in the regulation of stomata control osmotic pressure and plays a role.

Zokarini also reported that inoculation with mycorrhizal salt stress was increased absorption of phosphorus and potassium in lettuce and maximum efficiency at high levels of salinity were obtained mycorrhizal fungi (34). These findings correspond with the findings. With increasing salinity levels of sugar and praline content increased significantly. Inoculation of fungi and bacteria in concentrations of 240 mill Molar glucose control plants increased (Figure 10). Bacteria and mycorrhizal inoculation at a concentration of 60 mill Molar significantly increased the amount of sugar, but praline content in plants inoculated with fungi and bacteria showed a significant reduction (Figure 11).

Inoculated with bacteria, mycorrhizal and praline combined reduction was not significantly different. Dubai Racing has reported the Cacia plant, the plant proteins increased in salinity, which is corresponding to an increase in the synthesis of proteins such as HSP, LEAS (35). Overexpression of the gene LSP5CS, LSNCED in non-mycorrhizal plants to mycorrhizal plants shows that mycorrhizal fungi have a high tolerance to salinity stress suffer less. It was also reported that the leaves of tomatoes, the amount of soluble sugars and polysaccharides of the salinity increased remarkably, but no significant change in the amount of starch (36). It also showed that increased praline to increase plant resistance to salinity, the changes depends on the plant species. Praline accumulation during stress, the result of genetic manipulation praline metabolism in plants.

Sometimes it amounts to 100 times the plant under control plants is because its accumulation in plants and adaptive role as a carbon and nitrogen storage Smoltz suggested. Sometimes it amounts to 100 times the plant under control plants is because its accumulation in plants and adaptive role as a carbon and nitrogen storage Osmolytes suggested. Factor for breathing and a source of energy for healing stress on the plants used (38).

It has been reported that non-excretory-secretory leaves of mangrove, salt and praline increased remarkably (39). Several reports by the increased praline under salt stress on plant barley and soybeans and corn, lentils and rice cultivars come (40).

With increasing salinity content of chlorophyll a, b and total chlorophyll decreased shoots. The amount of chlorophyll in plants and fungi and bacteria-inoculated control plants under stress significantly increased. The increase because of improved mineral nutrition of plants in mycorrhizal (Figure 12, 13 and 14). With increasing salinity levels of catalase activity, peroxidase and the significant increase in plant protein. Inoculated control plants and fungi and bacteria enzyme activity in stressed plants significantly reduced (Figures 15, 16 and 17).

Mycorrhizal inoculation bacterial enzyme activity in bacteria and mycorrhizal inoculation than alone decreased significantly. Activity of SOD (superoxide dismutase) and peroxidase in soybean were observed in mycorrhizal plants.

Call antioxidant plants is different depending on the type of plant and fungi. This variation may be related to micronutrients. The presence of iron can increase the activity of catalase and peroxidase (41). Iron, copper, zinc and manganese in the air organs in inoculated with both SOD activity increases. Salinity

stress caused a significant increase in the percentage of co-existence. The highest percentage of mycorrhizal symbiosis at all levels of salinity alone and the lowest percentage of symbiosis of fungi and bacteria inoculation (Figure 18).

The coexistence and root colonization by fungi in addition to the type of plant and fungal species, largely a function of soil physical and chemical properties as well.

Environmental factors such as temperature, light intensity, soil moisture content, nutrient levels and its interaction with other organisms can affect the symbiotic relationship between fungi affect the pattern of colonization (42). Soil texture, salinity and pH as well as the factors that are important in this symbiotic system. Mycorrhizal fungi can live in a wide range of pH and reduce some of the tensions arising from it (43). Amerian and colleagues investigated the effect of G. Mosses and intra radices on maize to the conclusion that the highest percentage of colonization of G. Mosses is as much as 93%. Intra radices least 78% of species colonization, this ability depends on the characteristics of the root cortex and epidermis (44).

CONCLUSION

Salinity is one of the important environmental stresses, leading to production cuts and plant cell by creating oxidative stress can be macromolecules such as proteins, lipids, nucleic acids and other cellular constituents attack.

The effect of different salt concentrations on the growth of bacteria and fungi inoculation pistachio plant was studied by the results of other researchers on the physiological effects of bacterial and fungal inoculation corresponded to the growth and development of plants. The results showed that salt stress decreased growth rate and physiological parameters studied. Bacterial and fungal inoculation parameters listed in the salinity had a healing effect.

The results were presented according to the following recommendations:

1- Due to the positive effects of mycorrhizal and bacteria and mycorrhizal interactions on improving growth and physiological parameters pistachio plant under salt stress conditions using mycorrhizal fungi spores in the formulation of bio-fertilizers is recommended.

2- Recommended by other studies, other species of mycorrhizal fungi and bacteria growing donor also be examined.

3- Parameters also been studied in other pistachio and the results are compared with each other.

4- All procedures and methods of work in the farm environment and natural conditions of soil, climate and weather study, and the results were compared with each other.

Table 1. Analysis of variance squares of salinity, microbial treatment and the effect of microbial treatment of salinity on growth factors

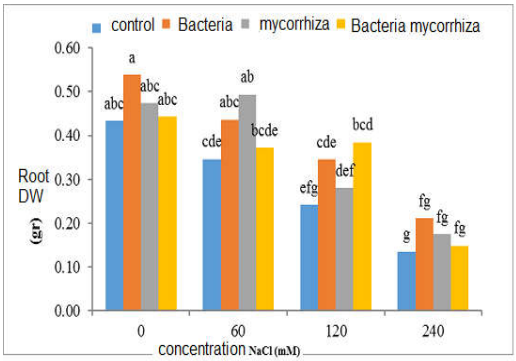
Root length	Shoot length	Percent coexistence	Leaves	Shoot dry weight	Shoot fresh weight	Root dry weight	Root fresh weight	Degrees of freedom	Sources of changes
9.437500 ns	25.9843750 ns	20.153262 ns	5.0625000 ns	0.02952890 ns	0.00577544 ns	0.00121902 ns	0.15518527 ns	2	Block
568.305556 *	19.4305556 ns	729.939604 ns	233.1666667 *	0.03637650 ns	0.08381247 ns	0.21339480 *	0.71423072 *	3	Salinity
397.250000 *	56.0833333 ns	80.483438 ns	44.6666667 ns	0.01793383 ns	0.02535180 ns	0.01851819 ns	0.07511072 ns	3	Microbial treatment
13.175926 ns	22.6064815 ns	5.272426 ns	3.7222222 ns	0.00555393 ns	0.03144882 ns	0.00569447 ns	0.01711537 ns	9	Microbial treatment effect on salinity
26.237500	6.2843750	48.943401	8.662500	0.00412418	0.02249566	0.00547398	0.04289718	30	Error
								47	Total
									Coefficient of variation

* NS is 0.05 respectively significant and non-significant.

Table 2. Analysis of variance squares of salinity, microbial treatment and the effect of salinity on microbial treatment on biochemical parameters

Proline	Total Chlorophyll	Chlorophyll b	Chlorophyll a	Peroxi-dase	Catalase	potas-sium	sodium	Protein	sugar	DF	Sources of changes
0.00036662 ns	0.02751396 ns	0.00030580 ns	0.02390703 ns	0.0403517 0 ns	2.8942616 ns	0.1321807 7 ns	0.4722828 3 ns	0.0031320 4 ns	0.7305708 2 ns	2	Block
0.05533607 *	0.99316146 *	0.03998624 *	0.65662304 *	0.1088466 1 ns	33.179054 7 ns	15.834331 58 *	16.495612 94 *	0.1723978 4 *	2.4055930 1*	3	Salinity
0.00594588 *	0.13574831 *	0.02378622 *	0.06469312 *	0.2046975 4 *	56.345669 1 ns	0.8847534 7 ns	4.9563834 2 *	0.1970279 6 *	0.4851056 9 ns	3	Microbial treatment
0.00044208 ns	0.04914109 *	0.01533878 *	0.01997446 *	0.0034856 7 ns	1.9574080 ns	0.5852414 7 ns	0.3550347 8 ns	0.0001461 3 ns	1.7161086 3 *	9	Microbial treatment effect on salinity
0.00053798	0.00340662	0.00014252	0.00325434	0.0202432 1	7.3815605	0.2992173 1	0.1031960 7	0.0026729 1	0.1182801 6	30	Error
										47	Total
											Coefficient of variation

* NS is 0.05 respectively significant and non-significant.



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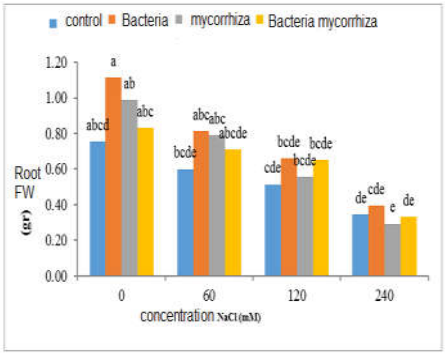

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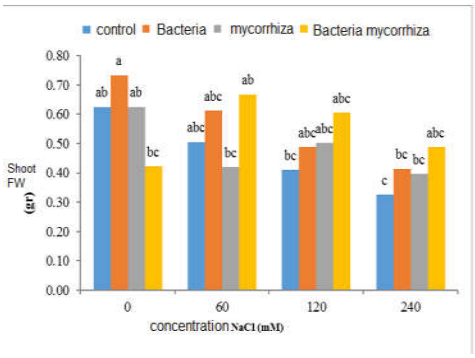

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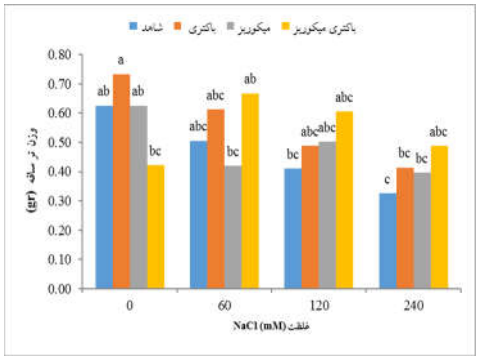

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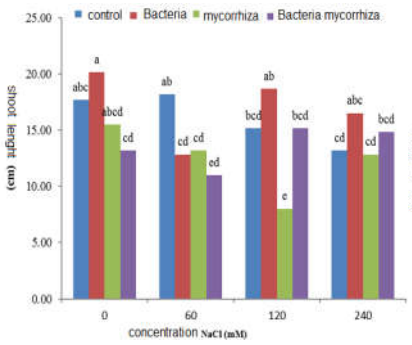

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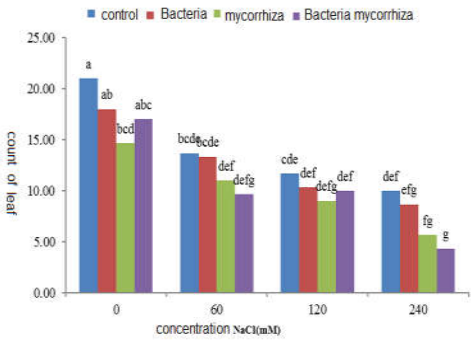
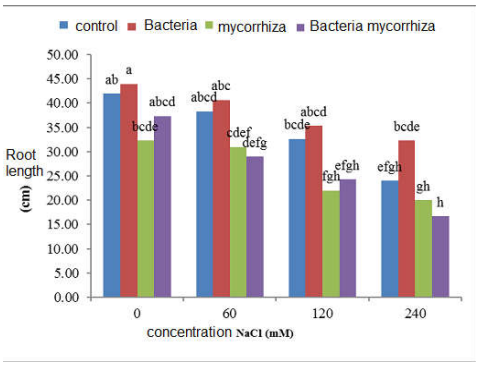


Figure 5



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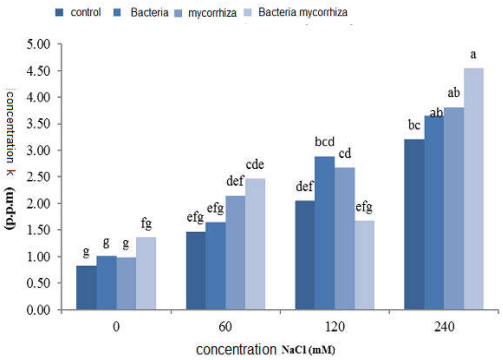

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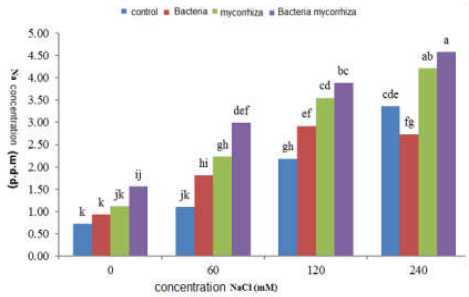

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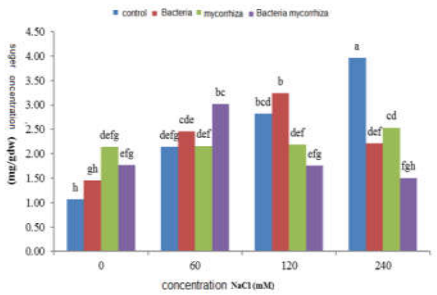

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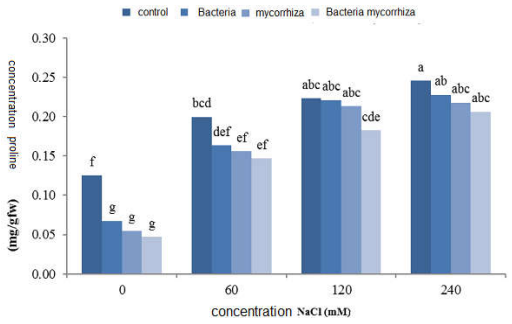

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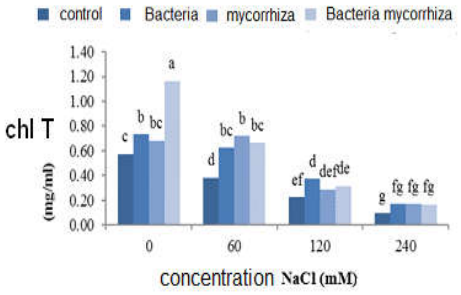

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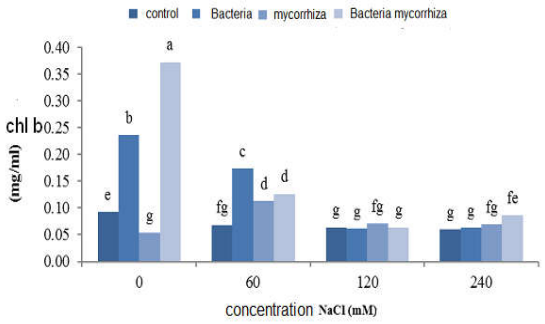

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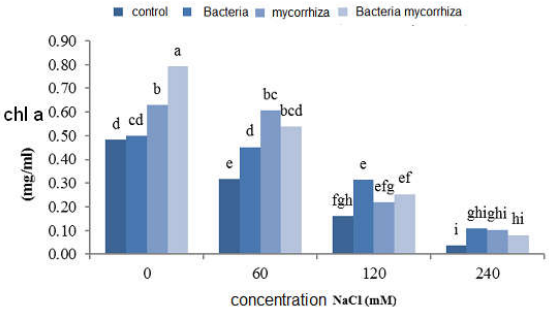

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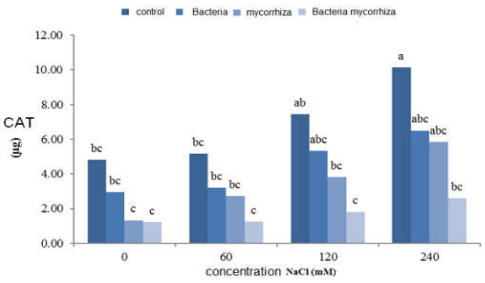

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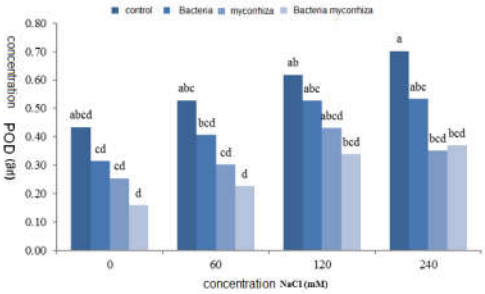

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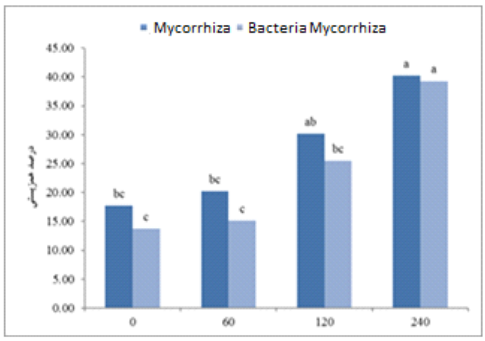

Figure18

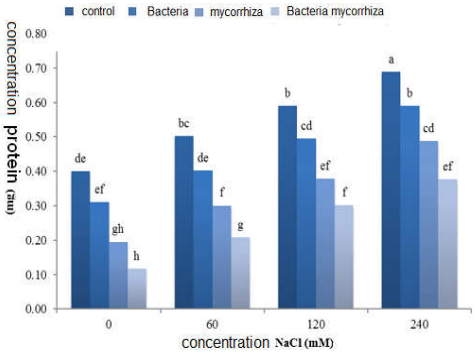

Figure17

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