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EFFECT OF MYCORRHIZAL FUNGI ON MACRONUTRIENTS AND MICRONUTRIENTS IN THE WHITE SEEDLESS GRAPE ROOTS UNDER THE DROUGHT CONDITIONS

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ABSTRACT

To determine effect of infected roots of seedless white currant grape with three species of Mycorrhizal fungi (Glomus fasiculatu, intraradices Glomus, and Glomus mosseae) and macro and micronutrients in leaves and roots under the water stress conditions, this factorial experiment in the randomized complete block design with four treatments. The results indicated that increase in drought stress led to reduction in the factors including shoot growth, number of leaves, leaf level, root dry weight, and shoot dry weight. Inoculation with mycorrhizal fungi had a positive effect on the above-mentioned traits compared with control group; in this case, the highest positive effect was on the root phosphorus uptake, root dry weights and root zinc uptake among the fungal treatment traits. Intraradices Glomus fungi had the highest positive effect on the interaction between the fungi and water stress for copper in the root and the least amount of manganese. There was not any different treatment between irrigation levels of 25% and 50% under the water stress conditions.

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1. INTRODUCTION

Grape is one of the main horticultural products in Iran with the first rank among fruit trees in terms of cultivation area and ranked after pistachio and date palm economically [1]. Herbal archeological studies suggest that grape domestication has begun since the second half of the fourth millennium BC in two neighboring areas, Mezopotamia (southern Anatolia, Syria, northern Lebanon, Kurdistan, and western Iran) and south of Caspian Sea. Water scarcity is a factor, which limits the function of fruit trees in arid and semi-arid areas. Sustainable agriculture has been at the center of

attention in recent years and the objective of this subject is to emphasize on the sustainable management of the soil and water resources. The term of Mycorrhizalin indicates the coexistence relation between fungi and plant roots, which is the most common coexistence. Although there are several groups of mycorrhizae, Vesicular Arbuscular Mycorrhizae (VAM) end mycorrhizae are the most popular type of them. These funguses are significant microorganisms in the soil, which can coexist with 90% of roots of all types of plants [2]. The most prominent feature of Arbuscular Mycorrhizae symbiosis is material transfer between cortex cells of the roots of the colonized plant with the fungi and arbuscules. In such symbiosis, the fungus receives carbohydrates from the plant in form of sucrose and sends nutrients (mostly phosphorous) to the plant. In this process, nutrients are transferred from the arbuscule membrane into the plant throughout the membrane carriers, which acts with proton slope so that the existing carbohydrates are converted to the glucose and fructose in the floodplain then adsorbed by the carriers. Mycorrhizal fungi, in particular, Arbuscular Mycorrhizae, play a vital role in providing water and nutrients for plants so that symbiotic mycorrhizal plants can tolerate higher concentrations of heavy metals, salinity and soil dryness with resistance against various pathogens and high soil heat.

2. THEORETICAL LITERATURES

2.1 WHITE SEEDLESS GRAPE

Grapevine is from the Ampelidaceae family called Saramantaceae or Vitacae. This family belongs to the Rhamnales species, which is Dialypetalae belonged to the angiosperms from the Spermatophytes. Such species include shrubs with Knitted shoot sand grows upward due to their ivies. This cultivar, which is also called Sultana, can be found in abundance around the grapevine areas in Iran. There are two types of white to yellow and red seedless grape and these two types are the best table grapes. Drought is defined as an environmental condition of the soil or air and or both of them, which prevents the plant from getting enough water requiring for life leading to water loss in plant's tissues [3]. Drought is one of the most important factor limiting the production of agricultural products and harming these products due to such conditions. Average rainfall in Iran is less than the third of global average [4]. About 65% of the Iran's area consists of the arid and semi-arid regions with rainfall level of <150mm. Under the water deficit conditions, herbal cells lose their turgor mode and transpiration rate is higher than the uptake speed [5]. Reduction in water level of the soil and lack of water replacement leads to water potential in roots and the plant; on the other hand, the higher the water stress rate, the lower the photosynthesis speed will be. Furthermore, water stress disrupts physiological processes and plant functions leading to plant drying.

2.2 DROUGHT RESISTANCE

Drought resistance in agriculture is the economic ability to produce a product under the water deficit conditions. A drought-resistant plant is a plant, which can ensure survival under conditions of water-limited availability [6].

In terms of genetics, drought resistance mechanisms are classified into two categories of drought avoidance and drought tolerance [7]. However, plants develop more than one mechanism for drought resistance. The ability of the plants for growth and functioning with minimum loss after the end of stress period is defined as the drought compensation [3].

The plant growth is influenced by mutual effects of several internal processes such as photosynthesis, respiration, transmission, water relations, and nutrients balance. Growth is a process of increase in the dry matter, volume, length or level of cell. Water stress effect on the cellular development is more obvious than the cell division because growing cell occurs due to turgor pressure. Hence, any water deficit leads to growth pause [8].

Severe water deficit at grape growth step wilts the leaves and diminishes moisture in shoots. This wilting can be seen in grapes, which are cultivated in the pot with the soil, which is wilting. Such condition is observable in hot weather and sand low-deep soils, which all parts of them reach to the wilting point under the farm conditions. This situation is rarely seen in deep soils, as the soil around the growth area does not wilt within short time [9].

2.3 MYCORRHIZAL FUNGI IN GRAPE

The term "mycorrhizal", introduced by Frank in 1885, is composed of two words "Myco", which means fungus and "Rhiza", which means roots indicating symbiosis between the fungus and plant roots. In this system, the fungus forms broad cover of the filamentous called hyphae around the host plant's root. Many of plants can form mycorrhizal system; 83% of Dicotyledon and 79% of Monocotyledon plants can develop my corrhizal system [2]. Fungal hyphae, which grow from the soil spurs or roots of adjacent plant contact with the root surface during the VAMs formation and then divided in this place to form an Appressorium and start the initial colonization; this is the first difference factor between the fungi and plant. Appressoriums are not formed on the non-host plants' roots. Penetration into the roots occurs throughout the Appressoriums and distances between two epidermis cells in the outer skin [10]. The symbiotic relationships of mycorrhizal play a vital role in decomposition of soil organic materials, mineralization of plants' nutrients and nutrients cycle [11]. Increased absorption of water and nutrients by the mycorrhizal can be stem from growth fungus hyphae up to 20mm from the root surface compared to 1.5mm growth in hairy roots (HRs) as well as the low penetration power of root compared with the penetration potential of hyphae into the soil pores. Hyphae of these funguses penetrates into the areas of soil, which are not penetrable by roots; hence, this conditions increases the transmission level between mineral nutrients, water and soil soluble compounds [12]. Various studies indicate that Phosphorous, Nitrogen, Potassium, Zinc, Copper, Sulfur, Magnesium, Manganese, Calcium, and Iron are absorbed and transferred into the plant by the mycorrhiza system. In general, adsorption mechanism is done through increasing available soil volume by the fungus hyphae. Mycorrhiza uptakes high amount of phosphorous among other nutrients. Mycorrhiza plays a minor role in nitrogen uptake owing to its high emission rate. If there is low amount of phosphorous in the soil, mycorrhiza considerably increase phosphorous absorption and therefore plant growth. Hyphae can receive phosphate from the 15cm distance from the root surface to the several meters in depth of the soil under the roots. Moreover, hyphae can penetrate into the soil pores, which are not penetrable by root hairs (diameter of root hairs is 20µm at least, while maximum diameter of hyphae is 1-2µm). In addition, hyphae would increase nutrient uptake considerably by increasing contact level or effective root length. There are 2-4cm roots, 1-2m root hairs and greater than 50m hyphae at each 1cm³ soil [13].

The major portion of phosphorous in the soil is insoluble and unusable directly for the plant.

Various studies have shown that mycorrhiza can synthesize Phosphatase enzyme in order to expand the phosphorus availability. Some types of mycorrhiza produce chelating acids to increase phosphorous solubility for absorption [14]. Arbuscular Mycorrhizae funguses create association between geochemical and living parts of the soil ecosystem by phosphorous uptake and transferring it to the plants and therefore affect the speed and patterns of phosphorous cycle in both of agriculture and natural ecosystems. This process protects the plant health against environmental stresses and improves the soil structure by forming aggregates requiring for a good soil [10]. Drought leads to some changes in the root structure, which may affect the colonization percent and frequency of different fungal structures of mycorrhiza. Nevertheless, different types of funguses show different behaviors under the drought stress conditions. For instance, some experiments on the citrus show that drought stress decreases colonization of G. vermiform and G. mosseae funguses, which have root and reduces volume of hyphae in the soil considerably [15]. Changing moisture rate of the soil leads to change in the root form, pores size and penetration angle of roots. Hairy roots are highly sensitive to the water deficit and root system function, as well as nutrient adsorption, will be reduced under such conditions [3]. Phosphorous ion can be highly adsorbed by clay soil under the drought conditions and therefore a small part phosphate ion is soluble. Under the drought conditions, phosphate ion will be reduced not only due to its low solubility but also for reducing uptake potential of roots [16]. [17] evaluated the response of six grapevines to the nutrient uptake under drought conditions. Their results showed a significant difference between grapevines in terms of nutrients uptake including nitrogen, phosphorus, potassium, calcium, and magnesium. Grafted cultivar had a significant effect on the phosphorous adsorption. A study inoculated micro propagated pomegranate with four types of mycorrhiza fungus examining some parameters such as shoots length, root length, fresh and dry weight of root and seedling survival percentage. It was observed that the mentioned parameters in symbiotic plants were significantly different with control plants (absence of fungus) 60-90 days after inoculation; however, there was not any significant difference between mycorrhiza treatments. This research suggested the improved nutrients uptake and water associations as well as the increased photosynthesis as causes for enhanced plant biomass. Symbiosis between mycorrhiza fungus and grape roots expands the nutrients uptake by the fungus and the vine sends photosynthesis materials to the fungus in exchange. Mycorrhiza contribution can be expressed as follows: 1- increasing nutrients uptake 2- protecting against pathogens living in the soil 3- protecting and stabilizing the soil structure by connecting soil particles to the hyphae network 4- improving drought resistance by absorbing higher water and phosphorous amounts 5- increasing soil moisture [18]. In [19], the study carried out to observe on the effect of mycorrhiza fungus on the minerals uptake by the grapevine root in poor soils and reported that besides the highest effect of fungus on the phosphorous uptake, other minerals such as Zinc, Copper, Nitrogen, Potassium, Calcium, and Iron can be influenced under different conditions.

The work of [20] carried out a study on effect of nitrogenous fertilizers like urea, calcium nitrate, ammonium sulfate and ammonium nitrate on the activity of fungal type of Glomus Mosseae, grapevine nutrition, and its aggregation variations and it was determined that nitrogen fertilizer could vary rootstock colonization by mycorrhiza funguses and sporulation of these funguses, grapevine growth, nutrition, and aggregation structure. On the other hand, urea stops the rootstock colonization and fugal sporulation. Grapevine rootstock inoculation by mycorrhiza and adding calcium nitrate as

the nitrogen source increase the weight of dry shoots and number of leaves compared with the control trees. In addition, there was a high density of micronutrients like iron, molybdenum, zinc, and copper in non-mycorrhiza plants compared to mycorrhiza ones. [18] conducted a study on the role of mycorrhiza in grapevine nutrition and reported that external hyphae on the grapevine root surface transfer the nitrogen between the grapevine and resources of organic nitrogen in the soil; therefore, symbiosis between mycorrhiza and grapevine is a prominent case for suitable nutrition in improper soils. There is lack of a comprehensive study on identification of Arbuscular Mycorrhizae funguses in the grape rhizosphere in Iran.

3. MATERIALS AND METHODS

This study was conducted to improve nutritional situation, soil moisture and optimal growth of the white seedless grape under drought stress conditions. In this research, effects of annual grape seedling roots inoculated with several mycorrhiza funguses was examined on the water and nutrition relations under low irrigation conditions in the pot compared with the control group (without inoculation). This study was done during two years (2013-2014) in form of a factorial experiment in the randomized complete block design with four treatments. The factors included inoculation with three mycorrhiza fungus species (Glomus mosseae, G. fasciculatum, and G. intraradices) and without inoculation (four levels), and irrigation at three levels (stress levels). The soil bed of the pot composed of wind sand and crop soil in equal amount. The white seedless grape cuttings were prepared then rooted in the wind and using Mamarov method. Half of the seedlings were inoculated in the Arbuscular Mycorrhizal (AM) fungi suspension at the same bed and rest of them were used as the control samples.

3.1 PREPARATION OF MYCORRHIZA PLANTS

Mycorrhiza fungus inoculums (spore, mycelium, mycorrhizal roots, and soil) were taken from the Turan Biotechnology Company of Shahrood and propagated on Sorghum roots.

3.2 MACRO AND MICRO-MINERALS IN ROOT AND LEAF

Amounts of nitrogen, phosphorus, potassium, magnesium, iron, and zinc in the leaf and amounts of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc and manganese in root were measured. Leaf samples, which had been selected for growth parameters measurement, were prepared at the final growth steps. The prepared samples were washed, dried in the oven (under the 72°C for 48 hours), then powdered with the mill and finally, the extract was prepared using digestion with dry burning. Amount of phosphorous was measured using Colorimetric method (Vanadate molybdate yellow) and spectrophotometer within 470nm wavelength then phosphorous rate was calculated as

P= concentration in the regression chart \times given dilution (10) \times 1.33 \times 100/ (3g) sample \times 10000 (1).

Potassium amount was determined using flame emission and Flame Photometer (Jenway PFP10, England); zero, 20, 30, 40 and 50mg standard solutions in potassium chloride (KCl) were prepared and their standard chart was plotted using flame photometer. Then, 10ml plant extract was diluted using distilled water and reached to 100ml then the sample potassium was calculated based on ml/l

using flame photometer. Finally, equation 3-6 was used to estimate the amount of potassium based on mg/100g herbal dry leaves [10] and then one gram of the milled sample of each treatment was weighted and nitrogen percentage of samples was determined within different experiment steps using Kjeldahl flask:

$$(a-b) \times \frac{1}{1000} \times \frac{V}{W} \times \frac{100}{D.M}$$
 (2),

where

a: potassium concentration in diluted sample (mg/l)

b: potassium concentration in control (mg/l)

v: volume of the extract obtained from digestion (ml)

w: weight of plant sample (g)

D.M: percent of dry plant.

3.3 STATISTICAL ANALYSIS OF DATA AND APPLIED SOFTWARE

Before data analysis, normal distribution of data was examined using Kolmogorov-Smirnov test (K-S) through SPSS® Software. Variables with non-normal distribution were standardized using suitable conversions. SPSS® software was employed for analysis of variance (ANOVA) and comparison of the measured traits. Means were compared using Duncan's multi-domain test.

4. RESULTS AND DISCUSSION

ANOVA Results of effects of mycorrhizal fungus treatments and water stress on the macro and micronutrients in plant roots

Change df Mean square Copper source Nitrogen Phosphorous Potassium Calcium Magnesium Iron Zinc Manganese Fungus 3 08.0^{ns} 01.0 * 02.0^{ns} 00.0^{ns} 10.0^{ns} 21.0ns 69.11ns 70.1^{ns} 57.24ns 01.0^{ns} $01.\overline{0^{ns}}$ 32.2^{ns} $76.2\overline{2^{ns}}$ 00.0^{ns} 31.0ns Irrigation 2 00.0^{ns} 05.0ns 95.1ns $00.\overline{0^{\text{ns}}}$ 6 03.0ns 00.0ns 00.0ns 43.0ns 08.0^{ns} 40.15* 80.7* 46.86** Fungus × irrigation 80.23 Error 36 06.0 05.0 01.0 0.0003.0 04.0 47.5 76.2 Change 12 15 11 8 9 13 16 15 percentage

Table1. ANOVA of macro and micronutrients in the plant root

ns: lack of significant difference, ** and * indicate significant difference at 1% and 5% levels, respectively

4.1 PHOSPHOROUS IN ROOTS

ANOVA results indicated significant fungal and water treatments for phosphorous amount in roots of the white seedless grapevine at 1% level (Table 1). According to comparison between means, the highest amount of phosphorous was observed in Glomus fasiculatum, which was significantly different with the control treatment (Figure 1).

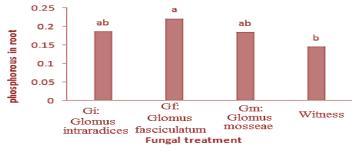


Figure1. Comparing average amounts of phosphorous in root under the effect of fungus treatments. GM: Glomus mosseae, GF: Glomus fasciculatum, GI: Glomus intraradices, Witness (control).

Increased phosphorous uptake is facilitated by transferring phosphorous from the soil to the plant roots and phosphatase solubility, expressed that increase in plants' function is one of the most important effects of mycorrhizal funguses, in particular in low-moisture soils. Such functional improvement occurs due to increases adsorption rate of roots through penetration of fungus hyphae in the soil as well as the higher volume soil availability for the plant. Increased phosphorus absorption by the host plant associated with numerous internal hyphae branches of mycorrhiza inside of the epidermis cells of the plant roots, which provide a wide surface to transfer nutrients, in particular, phosphorous into the host plant [21]. Phosphorous uptake rate in mycorrhizal plant is 3-6 times greater than non-mycorrhiza plants [22]. [23] introduced the phosphorous uptake for plant's root as the main role of mycorrhizal funguses as the phosphorous is a nutrient with highly low mobility in the soil. If phosphorous solution is added to the soil, it will be stabilized in form of calcium phosphate or other forms becoming non-movable. In addition, production and secretion of enzyme phosphatase by mycorrhizal hyphae converts the insoluble and stabilized phosphate in the soil as a soluble nutrient, which can be absorbed by roots. Therefore, mycorrhizal funguses play an effective role in expanding minerals (in particular, phosphorous) and accumulating biomass of many of products in the soils, which contain low amount of phosphorous. To uptake this nutrient, the root should be in direct contact with phosphorous resource. Physically, mycorrhizal plant's use of soil phosphorous can be facilitated by hyphae with small diameter. It is estimated that about 80% of phosphorus uptake by the plant is done throughout the mycorrhizal funguses [24]. [25] carried out a study on effect of mycorrhizal fungus on minerals uptake by grapevine roots in poor soils and reported that besides the highest effect of fungus on phosphorous uptake, the other minerals such as Zinc, Copper, Nitrogen, Potassium, Calcium and Iron can be also effective; this result is not in line with the findings obtained in present paper except for the phosphorous case.

4.2 ZINC IN ROOTS

ANOVA results showed that fungal and water treatments had significant effect on the zinc amounts in roots of the white seedless grapevine at confidence level of 5% (Table 1). Mutual effects of the fungus and different moisture levels on the amounts of zinc on the roots are shown in Figure 2. The amount of zinc in the roots was influenced by the stress and fungus so that these comparisons indicated that the highest photosynthesis rate of zinc in roots occurred in GF fungus at each stress level. The highest amount of zinc in rootstocks of grapevines inoculated with Glomus fasiculatum was seen at stress levels of 75% and 25%; while this treatment obtained the lowest amounts at 50% level.

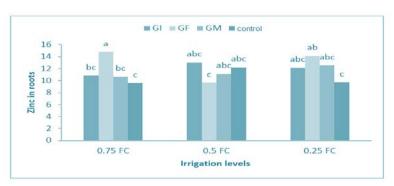


Figure2. Comparing average amounts of zinc in root under the mutual effects of fungus treatments and different water stress levels.

In [26], it was found that mycorrhiza could expand the concentration of zinc in aerial organs of almond up to 25-35% and underground organs up to 26-37%. Such expansion may occur due to more soil draining from the zinc owing to penetration of thin fungal hyphae into the fine soil pores.

Mycorrhiza symbiosis can expand non-movable nutrients such as zinc by increasing roots length and adsorption rate by fungal hyphae, while zinc expansion mechanisms are not similar to phosphorous uptake mechanisms. Increasing uptake rate, which is done by fungal hyphae in mycorrhizal corn plants leads to 22% increase in zinc concentration [27].

AVM funguses have higher potential to uptake zinc from the soil compared to Octavirucid Mycorrhiza. Symbiosis between plants and AVM funguses can provide about 25% of the zinc-requiring for the host plant [24]. This process has been observed in many plants such as corn [28], sorghum, soybeans [29], and field bean [30]. Figure 2 shows mutual effects of mycorrhizal fungus of Glomus fasiculatum and irrigation on expansion of zinc uptake in roots, which is in line with the results of conducted studies.

4.3 COPPER IN ROOTS

ANOVA results showed that fungal and water treatments had significant effect on the amount of copper in roots of the white seedless grapevine at 5% level (Table 1). Copper showed a behavior like zinc so that the amount of this nutrient in roots of grapevine inoculated by Glomus fasiculatum was higher than the control and other funguses at irrigation levels of 75% water need and stress of 25% water requirement. Although this difference between funguses was not significant at stress level of 25%, fugal Glomus fasiculatum species could uptake the copper at average water stress (50% water requirement) compared with other fungus types or even the control group and indicated the lowest amount of copper in rootstocks; nevertheless, intraradices Glomushad higher efficiency compared to other funguses. In other words, there was not any significant difference between mycorrhizal fungus and other grapevine inoculated by three fungus species under normal water conditions (75% water requirement), while funguses except for Glomus fasiculatum showed a better performance in acquiring copper from the soil compared to the soil under the 50% water requirement stress (Figure 3).

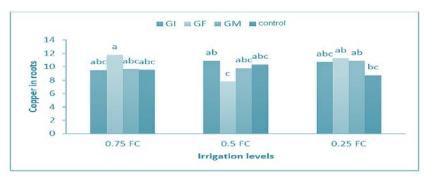


Figure 3. Comparing average amounts of copper in root under the mutual effects of fungus treatments and different water stress levels

Although concentration of copper in almond plant was not affected by the mycorrhizal symbiosis, 150kg increase in amount of phosphorous in each hectare of the soil led to 40-44% expansion in copper concentration in aerial and underground organs of the almond plant [26]. An increase in soil phosphorous amount reduces longitudinal growth of roots and as the copper is a non-movable

nutrients in the soil and plant, increased concentration of phosphorus in plant owing to its simple uptake in fertilized soils may lead to relative reduction in copper concentration in plant. As mycorrhizal phosphorous acquisition has not led to any significant difference in copper concentration, it can be found that despite to lack of significant of mycorrhiza on copper concentration in symbiotic plant but it can increase copper uptake compared to those plants, which do not provide the required phosphorous through mycorrhizal symbiosis.

Copper acquisition is not a similar process in different mycorrhiza plants; for instance, presence of mycorrhizal fungus in some plants such as field beans [30], white clover [31], and soybean [29] leads to expanded copper uptake. Mycorrhizal fungus in mycorrhiza symbiotic corn plant could expand copper concentration in aerial organs, while it had no significant effect in underground organs [27]. In present paper, increased copper concentration in roots was significant when mycorrhizal fungus of Glomus intraradices was used in fugal and irrigation treatments; it seems that such observed positive effect was associated with the hyphae expansion and presence of the penetrable soil.

4.4 MANGANESE IN ROOTS

ANOVA results showed that fugal and water treatments had significant effect on amount of manganese in roots of the white seedless grapevine at 5% level (Table 1). The highest amount of manganese in root of grapevine, which was inoculated by Glomus fasiculatum obtained within treatment of 75% water requirement. There was not any significant difference between the control sample and other funguses under normal water conditions. The first stress level reduced the performance of Glomus fasiculatumin manganese uptake while Glomus intraradices species could uptake the highest amount of manganese. By intensifying the stress condition, three funguses could perform better than control samples in case of manganese uptake (Figure 4).

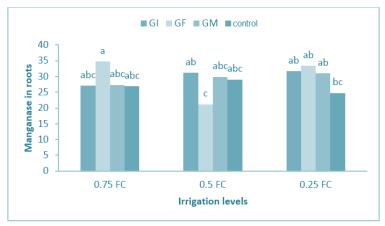


Figure4. Comparing average amounts of manganese in root under the mutual effects of fungus treatments and different water stress levels

The study [32] found significant increase in amounts of nitrogen, potassium, manganese, magnesium, and zinc in corn grains under the moisture stress conditions for the corn, which is inoculated by mycorrhizal funguses.

The work [26] conducted a study on almond plant and found significant concentration of manganese within mycorrhizal symbiosis at confidence level of 5%; this amount experienced about 15% increase in roots about 40% reduction in aerial organs. Increased 150kg phosphorous in each

hectare of soil led to 50% expansion in manganese concentration in plant roots as well as 30% reduction in aerial organs. According to the mentioned points, increase in phosphorous concentration in plant blocks the absorbed manganese preventing it from transferring to young tissues and lower organs. The opposite process has been observed in mycorrhizal soybean plant; manganese concentration was increased in leaves and reduced in roots [29].

Although mycorrhizal inoculation of corn reduces manganese concentration in aerial organs and plant roots [27], mycorrhizal funguses of Glomus intraradices and Glomus mosseae have reduced manganese in roots compared to control samples, have prevented from manganese transfer to the leaf, and have removed its toxic effect on leaves. According to Figure 4, mycorrhizal fungus of Glomus fasiculatum has the lowest amount with lower uptake compared to the control sample; this indicates mycorrhizal effect on reducing toxic effect of some nutrients such as manganese in the plant.

5. CONCLUSION

The reported work was designed to increase knowledge about the role of arbuscular mycorrhizal fungi on the phyto availability and allocation of some of the principal macroelements and microelements in white seedless grape roots growing in the drought conditions. The results obtained from ANOVA analysis suggest that fungal and water treatments had significant effect on the zinc and phosphorous amounts in roots of the white seedless grapevine. In conclusion, the results of this paper show that the use of mycorrhizal fungi in terms of drought stress has had a positive effect on the improvement of plant characteristics.

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