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Effects of Arbuscular Mycorrhizal Fungi (AMF) on Heavy Metal and Salt Stress

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A R T I C L E I N F O

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A B S T R A C T

The Arbuscular mycorrhizal fungi (AMF) are microorganisms that live symbiotically with plant roots and have many benefits to soil and plants. In this study, some effects of AMF which are known to be soil and plant beneficial, have been evaluated and solution proposals have been put forward against heavy metal and salinity stress in the soil. Salt accumulation and high concentrations of heavy metal in the soil affects negatively the microbial diversity and activity. Removal of salt acumulation and heavy metal from contaminated soil by chemical and physical methods is both very expensive and ineffective. Therefore, AMF are important for alleviating the heavy metal and salt stress in plants. AMF can alter plant physiology and root morphology, increase the uptake of nutrients and water from the soil through an extensive hyphal network, decrease the use of chemical fertilizer, interact with other soil microorganisms plant growth promoting, induce of some resistance parameters in the plants and produce the glomalin which develops the properties and structure of soil. AMF are eco-friendly solutions according to traditional methods and the use of suitable plant-fungi combinations increases the chances of success of these applications.

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Arbüsküler Mikorizal Funguslar (AMF)’ın Ağır Metal ve Tuz Stresi Üzerine Etkileri

M A K A L E B İ L G İ S İ

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Ö Z

Arbüsküler mikorizal funguslar (AMF) bitki kökleri ile simbiyotik yaşayan, toprak ve bitkiye faydalı mikroorganizmalardır. Bu çalışmada toprak ve bitkiye yararı olduğu bilinen AMF’ın topraktaki ağır metal ve tuzluluk stresi üzerine bazı etkileri değerlendirilmiş ve çözüm önerileri ortaya konulmuştur. Toprakta yüksek konsantrasyonlardaki ağır metal ve tuz birikimi, mikrobiyal çeşitlilik ve mikrobiyal aktiviteyi olumsuz etkilerken, kontamine olmuş topraklardan ağır metal ve tuz birikiminin fiziksel ve kimyasal yöntemlerle uzaklaştırılması hem pahalı hem de etkisi düşük bir yoldur. Bu bağlamda, AMF bitkilerdeki ağır metal ve tuz stresinin en aza indirilmesi için büyük önem arz etmektedir. AMF, bitki fizyolojisi ve kök morfolojisini değiştirebilme, geniş bir hif ağı vasıtasıyla topraktan su ve besin maddelerinin alımını artırabilme, kimyasal gübre kullanımının azaltma, bitki gelişimini teşvik edici diğer toprak mikroorganizmalarıyla etkileşime girebilme, stres faktörüne bağlı olarak bitkideki bazı dayanıklılık parametrelerinin aktive edebilme ve ürettikleri glomalin ile toprağın yapısını ve özelliklerini iyileştirebilme gibi birçok özelliğe sahiptir. AMF, geleneksel mücadele yöntemlerine göre çevre dostu olup uygun bitki-fungus kombinasyonlarının kullanılması ile bu uygulamaların başarı şansı daha da artacaktır.

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Introduction

Mycorrhiza is a symbiotic form between fungi and plants. It was described by Frank as “fungus-root common life” in 1885 (Sieverding, 1991). In this symbiosis, the host plants provide the fungus carbon sources while the mycorrhizal fungi provide the host plants nutrients and water from the soil. This mutual cooperation between mycorrhizal fungi and plant roots constitutes the most common symbiotic life form in nature (Marschner, 1998). Mycorrhizal fungi colonize the plant roots, thus the nutrient uptake and plant growth is several times higher. In mycorrhiza inoculated plants, phosphorus (P), copper (Cu), zinc (Zn), nitrogen (N), potassium (K), calcium (Ca), iron (Fe), and magnesium (Mg) contents increased significantly.

Mycorrhizal colonization not only increases the uptake of nutrients but also increases the water uptake of the plants (Ortaş, 2000). In addition to these benefits to plants, AMF also have important roles in plant defence by activating the morphological and biochemical resistance mechanisms of plants against pests and diseases. Palta et al. (2010) found that AMF, protect plants against root pathogens. AMF play an important role in the prevention of soil erosion by providing a good soil structure by extending the surface area of plant roots through extrametrical hyphae (Ortaş, 2000; Palta et al., 2010).

Another effect of AMF are to increase the resistance of the plant against drought, heavy metal stress and salinity resulting from environmental factors and to stimulate the growth of the plant hormones (Smith and Read, 2008; Palta et al., 2010). Unconscious fertilization and spraying cause some undesirable conditions and pollution in the soil. As a result of conducted studies, mycorrhizal fungi are shown as the most effective natural application in soil improvement and soil fertility. For mycorrhizal fungi to be effective, they must interact directly with the roots. Expanded of the root surface area can be a serious solution to the problem of drought by increasing the water and nutrient intake of the roots 5-7 times depending on the conditions. The activities of AMF can show themselves better, especially in adverse environments (drought, coldness etc.) and soil conditions (aridity, desertification, heavy metal accumulation, salting, etc.) (Akça et al., 2009).

Soil has become a very important and indispensable production tool in order to ensure the sustainability of agricultural production from past to present day. Consequences of unconscious practices the soil has faced the threat of pollution and extinction. This situation affects directly and indirectly the amount of agricultural production negatively. It is necessary to elaborate the interaction of AMF, plant and soil, which is used in agricultural production activities, which has no negative effect on human and environmental health and which forms symbiotic life with plant roots. In this revive, it was aimed to reveal the roles of reducing the stress factor of AMF in soil under heavy metal and salinity stress.

Effects of AMF on Heavy Metal Pollution

At the present time, the heavy metals contamination of the environment has shown a serious rising around the world (Sheetal et al., 2016). The result of unconscious or excessive fertilization of agricultural soil is polluted by heavy metals rapidly (Ghosh and Singh, 2005) and poses a serious threat due to the adverse effects on agricultural production (Singh and Agrawal, 2010).

Heavy metal accumulation at high concentrations in the soil affects microbial diversity and activity negatively. AMF, microorganisms that provide direct interaction between soil and plant roots and live symbiotically with plant roots, has an important role on mobile and immobile metal cations in the soil. Removing of heavy metals from contaminated soils with the traditional physical and chemical methods is a very expensive method and at the same time the chances of success are very low (Benavides et al., 2005; Shrama and Dubey, 2005). Therefore, it is necessary to develop effective and cheap technologies against heavy metal pollution. In this context, it was stated that mycorrhizal fungi are very important for remediation soil contaminated with heavy metals (Meier et al., 2015). AMF can assist the survival of plants growing on heavy metal contaminated soils by facilitating nutrient uptake, protecting plants from metal toxicity, absorbing metals and also improving Phyto stabilization and phytoextraction (Gaur and Adholeya, 2004; Jahromi et al., 2008; Javaid, 2009).

AMF decrease concentration of heavy metal by binding in the chitin cell wall (Hildebrandt et al., 2007), the secretion of glomalin (Gonzalez-Chavezet al., 2004) and accumulating non-toxic forms of these pollutants in plant roots (Joner and Leyval 1997). The increase in mycorrhizal colonization in the soil causes increment in the amount of plant biomass and shoots as well as the decrease in concentrations of heavy metals such as cadmium (Cd), Cu, Zn, and Mn in roots and shoots. Accordingly, the root density of AMF is effective on heavy metal tolerance depending on mycorrhizal inoculum (Weissenhorn et al., 1995). The *Trigonella foenum graceum*- Funneliformis mosseae (*Glomus mosseae*) interaction, which is grown in soil contaminated with heavy metal arsenic, shows an increase in heavy metal tolerance in mycorrhizae compared to non-mycorrhizae. Previous studies have also reported that AMF are effective in reducing the transport of heavy metals from the root to the shoots in plants and plant-fungus combination plays an important role at the beginning of factors affecting heavy metal uptake (Tonin et al., 2001; Shetty et al., 1995). Cadmium accumulation causes pollution in agricultural soils. The fact that plants are not affected by this situation is based on the symbiotic interaction between plant roots and soil microorganisms. The amount of heavy metals such as Cd, which is included in the soil with chemical fertilizers, can be reduced considerably by using mycorrhiza.

Phytoremediation Technology

High concentrations of heavy metals in the soil have harmful effects on the ecosystem and pose risks to human health as they can be added to the food chain via agricultural products or contaminated water. “Phytoremediation”, which is a cheap technology based on the removal of pollutants through plants, has become an increasingly important concept. Phytoremediation is a very slow process. Therefore, it takes considerable amount of time removal heavy metals from the soil and increasing yield. The phytoremediation is based on AMF-plant interaction and this method is an attractive system. During the symbiotic interaction, the fungus extends the hyphae structures to the root system of the host plants. Thus, it has the potential to remove heavy metals from more soil volume through the expanded root surface area. (Gohre and Paszkowski, 2006). Another role of the AMF is to prevent the plant from absorbing these pollutants in the soil by forming a chelate of heavy metals. AMF can also provide heavy metal tolerance indirectly. Reduction of heavy metals in the plant by increasing uptake of P or reducing the number of bacteria that reduce Mn in the rhizosphere is the indirect mechanism of action of these fungi. The most important point in this interaction is that the amount of heavy metals in the soil is not at a level that limits the development of AMF (Dixon and Buschena, 1988).

Mycorrhiza Assisted Remediation (MAR) Method

MAR is a sustainable remediation method that uses natural organisms for the treatment of polluted soils. This method refers to the use of AMF in the treatment of contaminated soils, while creating a different direction for biological remediation. MAR is not only a way of reducing or eliminating soil pollutants, but also a method of helping the plant to uptake nutrients by improving the structure of the soil. AMF can detoxify toxic substances, therefore they have been utilized for the remediation of organic and inorganic pollutants on heavy metal contaminated soils. Remediation of contaminated soils can be done with ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM). But AMF is commonly used for remediation process because they colonize almost all plant roots unlike ECM. The basic mechanism of MAR in the cleaning of inorganic contaminants in the soil is phytoextraction and Phyto stabilization (Chibuike, 2013). Conducted studies have shown that contaminated soil with heavy metals (including Cu, Zn, Pb, U, and Cd) can be cleaned by MAR (Chen et al., 2005; Chen et al., 2008); Marques et al., 2006; Janouskova et al., 2006; Wang et al., 2007; Trotta et al., 2006).

The plant species in which the fungus is colonized is associated with MAR's ability to effectively remove heavy metals from the soil. Chen et al. (2007) announced that MAR was more effective when plants such as *Trifolium repens*, *Coreopsis drummondii* and *Pteris vittata* were grown together in the high Cu concentration. Moreover, using *Lolium perenne* did not produce significant results. The amount of heavy metal removed from the soil is determined by the species of mycorrhizal fungus. Mycorrhizal fungi isolated from soil contaminated with heavy metals provide a more effective remediation of soil than other mycorrhizal fungi. (Orłowska et al., 2012). This situation can be explained by the fact that the fungus has gained the ability of high adaptation in contaminated soil.

The basic mechanism for clearing organic pollutants is biodegradation by supporting microorganism activities of mycorrhizal fungi (Gao et al., 2011; Binet et al., 2000). Many microorganisms have the ability to biodegrade organic pollutants. When these microorganisms are used in combination with mycorrhizal fungi, both the activity is more and the process is running faster.

Because of this reason, the fungus, *Cunninghamella echinulata* and the bacterium, *Sphingomonas paucimobilis* are used together with mycorrhizal fungi to degrade contaminants and to clean soils. (Alarcón et al., 2008). Another bacterium that allows pollutants to break down in contaminated soil is *Bacillus subtilis*. It does this by producing biosurfactants which are capable of enhancing biodegradation of organic pollutants (Cameotra and Bollag, 2003; Xiao et al., 2012). *B. subtilis* also increases AMF colonization in the plant roots by developing of fungi hyphae. The combined use of mycorrhizal fungi with other bacteria and fungi allows more rapid removal of more contaminants in the soil (Xiao et al., 2012).

MAR has some advantages and disadvantages that are used to remove pollutants in the soil.

Advantages of MAR;

- After performing the soil cleaning process, it accelerates the vegetation.
- It is an eco-friendly method because it is a natural process.
- It can be applied in the removal of pollutants include heavy metal, salt etc.
- The remediation process continues as long as the plant remains in the soil, since fungal spores can remain in the soil for a long time.
- Because it does not require complex technologies, it is a relatively cheaper and easier method than chemical and thermal treatment processes.

Disadvantages of MAR;

- MAR is a very slow method, since it takes a long time to completely remediation the soil.
- Despite the fact that some species of mycorrhizal fungi are pollutant-specific, the use of the wrong mycorrhizal fungus species cannot obtain the desired results.
- The effectiveness of MAR depends also on the variety of plant. Some plants may not form association with AMF. If these plants are used, the remediation of the soil through MAR can fail. (Chibuike, 2013).

Effects of AMF on Soil Salinity

Soil salinity is an important problem and it's increasing worldwide (Giri et al., 2003; Al-Karaki and Al-Raddad, 1997). About 77 million ha of 1.5 billion ha cultivated land has excessive salt content (Sheng et al., 2008). This situation adversely affects nutrient uptake. Plants have different mechanisms to alleviate the negative effects of soil salinity. Regulation of plant water potential and accumulation of α-amino acids such as proline can be given as an example of these mechanisms (Sajedi et al., 2010).

AMF has also an important role on salt stress in plants. There are different opinions on the mechanism of AMF's reduction of salt stress in plants. The increase in the uptake of plant P and other minerals, the K/Na ratio and the improvements in the physiological processes of some plants, are related to the salt tolerance of AMF in plants grown in saline conditions (Asghari, 2004). AMF have the potential to alleviate salt stress with the ability to change plant root morphology and form an extensive hyphal network, AMF also can increase the uptake of water and nutrients by the host plants, alleviating both biotic and abiotic stresses. AMF can improve the properties and structure of soil by producing glomalin, change plant physiology, increase plant growth and nutrient uptake, and decrease the use of chemical fertilizers (Estrada et al., 2013).

AMF can increase the resistance of plants to salt stress by: storing Na^{+} and Cl^{-} ions in vacuoles (Smith and Read, 2008; Smith et al., 2010), increasing the uptake of water and nutrients from the soil through hyphal network (Bharti et al., 2014), by regulating plant physiology and morphology so that the host plant is less affected by the stress factor (Miransari et al., 2008), producing plant hormone and interacting with other soil microorganism such as (PGPR) plant growth promoting rhizobacteria (Mardukhi et al., 2015).

The effects of AMF on salt stress in the plant have been demonstrated by many researchers. Inoculated plants with AMF show better development than non-inoculated. (Zuccarini and Okurowska, 2008; Sannazzaro et al., 2007: Giri et al., 2003). It has been reported that after the Rhizophagus intraradices (*Glomus intraradices*) inoculation of *Cucurbita pepo* plants under salt stress, the nutrient content and quality increase due to the amount of nutrients uptake from the soil (Sharifi et al., 2007).

AMF species and plant genotype affect the ability of plants to tolerate high saltiness. The effects of AMF species on growth and yield of various wheat genotypes are also different. Claroideoglomus etunicatum (*Glomus etunicatum*) was the most effective fungus in wheat plants under salt stress, followed by Funneliformis mosseae (*Glomus mosseae*) and Rhizophagus intraradices (*Glomus intraradices*), respectively. Daei et al. (2009) concluded that the adverse effects of salt stress on wheat plants could be reduced if the correct combination of AMF and plant genotype is applied. Mardukhi et al. (2015) investigated the effects of different AMF species on nutrient uptake of different wheat genotypes under salt stress. Similar to the findings of Daei et al. (2009), the researchers concluded that both the host plant and the AMF strain were effective in reducing salt stress. When mixed inoculum (including *R. intraradices, F. mosseae* and *C. etunicatum*) was applied, the most effective result was obtained by promoting plant growth depending on the increase in nutrient uptake.

Reducing the uptake of Na + and Cl- ions and increasing the uptake of other nutrients is one of the basic mechanisms for overcoming the salinity stress of AMF. Hajiboland et al. (2010) determined the effects of *R. intraradices* on the growth of tomato plants under low, medium and high salinity stress using salt-sensitive and salt-tolerant genotypes. Investigators have found that *R. intraradices* plays an important role in alleviating salt stress by increasing P, Ca and K uptake and Ca/Na and K/Na ratios, while also promoting carbon assimilation by increasing the stomal conductance.

The effects of AMF on the development of tomato plant under salinity stress were investigated. Although salt stress significantly reduces plant development, AMF were able to alleviate the stress by increasing plant dry matter and leaf area (Huang et al., 2010). At the same time, AMF increased the activity of several antioxidants such as peroxidase, ascorbate peroxidase, catalase and superoxide dismutase in the early period of salt treatment. Consequently, it was stated that under high salt stress, AMF were able to alleviate oxidative stress by increasing production of antioxidants (Hajiboland et al., 2010; Huang et al., 2010; Miransari, 2017). Although most studies of the effects of AMF on salt tolerance emphasize the results for host plant physiology and growth, Hammer and Rillig (2011) have identified the effects of salt stress on glomalin production, a glycoprotein produced by AMF, which has an important role in soil aggregation. These researchers used NaCl as a salinity factor and glycerol as an osmotic stress factor on the growth of AMF *in vitro* culture. While the salinity stress induced by NaCl significantly increased the production of glomalin of *Rhizophagus intraradices* (*R. intraradices*), osmotic stress induced by glycerol was not found to be effective on glomalin production.

Conclusion

As a result of unconscious applications in conventional agricultural practices, heavy metals and salt are accumulating intensively in soil. These substances, which accumulate over time, cause both the deterioration of the fauna and flora of our soil and the negative effects on the plants. AMF are regarded as a source of hope for eco-friendly production systems that do not have adverse effects on the environment and human health, with beneficial properties such as increasing the uptake of nutrients in the soil that the plant needs, especially making the plant more resistant to diseases and improving the soil structure. Plant, soil and mycorrhizal fungus triangles are ecologically quite useful. If the conditions are favourable, colonization of the roots of these beneficial microorganisms occurs at a very high rate and this symbiosis reaches a maximum level. Mycorrhizal fungus colonization naturally occurs at low rates because agricultural ecosystems are under the influence of many factors. No doubt, the most important of these factors are some agricultural practices that cause soil pollution. These unconscious applications not only reduce the proportion of natural mycorrhiza species, but also affect other useful microorganisms in the soil in the negative. However, it is known that some AMF species may exist in the soil despite these negativities. As a result of the widespread use of eco-friendly AMF in agricultural production; the soil will be protected with protection of glomalin, some resistance mechanisms in the plant can be activated and the use of chemical fertilizers may be reduced. In this context, AMF should be used effectively in modern agricultural practices within the sustainable agriculture system and studies of identification of native AMF species should be accelerated.

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