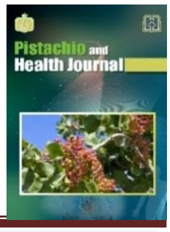

The Impact of Symbiosis with Beneficial Microbes in Soil on *Pistacia Vera*

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Abstract

Introduction: *Pistacia vera* (pistachio) is a strategic crop in Iran. Some stressors, such as pathogen invasion, drought and salinity negatively affect pistachio growth and production. Chemical methods which have been employed for a long time, can be a serious threat to the environment and human health. Establishing appropriate symbiosis between pistachios and beneficial microorganisms of soil has recently attracted a lot of attention, which could replace chemical methods.

Results: Mycorrhizal fungi are among the most common symbionts of pistachios. These fungi increase the root surface for the uptake of water and some immobile elements, especially phosphorous. *Trichoderma* is a free-living fungus which can establish symbiosis with pistachios to protect them against different soil pathogens, apart from having some nutritional benefits. Some soil-borne bacteria can also provide symbiosis with pistachio roots as plant growth-promoting rhizobacteria. They improve plant growth by increasing the uptake of some elements, such as phosphorous, iron, and zinc, in addition to producing some phytohormones.

Conclusion: Screening and selecting the most suitable symbionts is a major step in providing useful symbiosis between pistachios and soil microorganisms. Co-inoculation with several compatible microbes could also be a good solution for benefiting plants and overcoming environmental stresses. However, it needs more investigations.

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1. Introduction

Plant symbiotic microorganisms play various important roles in benefiting their host plants. It is believed that the initial appearance of plants on the earth has coexisted with their symbiotic interactions with microbes [1]. These microorganisms are equally effective in promoting crop productivity and plant growth under stressful and normal conditions through direct or indirect mechanisms [2- 4].

Crop plants are continuously exposed to varied stressful environmental conditions. Although some plants have adapted to such tensions, certain biotic and abiotic stresses negatively affect plant survival, growth, development, as well as crop productivity [5]. The detrimental effects of stress could be exerted to plants via one or more methods. For example, drought as the most devastating stress disrupts water relations, reduces water use efficiency, and decreases the leaf size, stem extension, and root proliferation [6, 7].

Plants have different physiological and biochemical responses at the cell and whole plant levels, which make this phenomenon more complex [7]. Salinity affects plant growth and development through ion toxicity, osmosis, and some specific ion effects [8, 9]. Heavy metals and pollutants can decrease crop productivity by affecting various plant processes [10, 11].

Since plants are sessile organisms not able to escape from sub-optimal environmental conditions, they need to develop multiple strategies to withstand or cope with stressful constrains. Due to climatic changes and human population growth, promoting plant tolerance to environmental stress is an essential need. The application of chemicals, such as inducers, fertilizers, and fungicides could improve plant tolerance against some adverse conditions. However, they could also be toxic to the environment and have side effects [1].

A suitable solution for this problem is the use of naturally occurring microorganisms, such as mycorrhizae, *Trichoderma*, and PGPR[1], which can provide symbiotic interactions with plant roots [12, 13]. Past research confirms the effectiveness of these symbiotic interactions in promoting plant growth and development [12, 14].

A summary of beneficial plant-microbe relationships and near-term applications has been presented in Table 1 [15]. Implementing a plan for the use of environment-friendly microorganisms is an alternative for minimizing chemical applications with direct and indirect detrimental effects on human health [3].

Pistachios (*Pistacia vera* L.), as subtropical plants, belong to the family Anacardiaceae and are mostly planted in some Asian (Iran and Syria), European (Italy, Turkey, and Greece), and American (USA) countries [16]. Pistachios cover an area of about 5×10^5 hectares in the world, with an average production of 1.3 tons/hectare [17]. Kerman province and especially Rafsanjan County are recognized as the main producers of pistachios in Iran [18].

In these regions, pistachio trees are often exposed to biotic and abiotic stress conditions, such as soil-borne pathogens, drought, salinity, high temperature, as well as infertile and salty

[1] Plant Growth Promoting Rhizobacteria (PGPR)

soils [19- 22]. It is well established that pistachio trees have a high capacity for withstanding some environmental stresses, such as drought and salinity, due to their deep root system and leaf structure. However, they require to be in favorable conditions for optimal growth and production, [23]. In this study, some of the major symbionts of pistachios and their effects on ameliorating different environmental stresses were examined.

Table 1. Summary of beneficial plant-microbe relationship and near-term applications [15]

Activity	Application
Phytohormone production	Plant growth promotion
Biological nitrogen fixation/phosphate solubilization	Biofertilizer
Plant protection	Biocontrol
Abiotic stress tolerance	Boost plant biomass on marginal land
phytoremediation	Remediation of contaminated land
Endophytic specialization	Novel pathways and reduced genomes for synthetic applications

2. Mycorrhizal fungi

Mycorrhizal symbiosis is the relationship between mycorrhizal fungi and host plant roots, which is recognized as a mutualistic association. It is well established that plants benefit mycorrhizae by meeting their carbohydrate requirements. It is also proved that fungal mycelium, which grows on plant roots, provides a tridimensional network that links plant roots to the soil environment. Fungal mycelium can access areas beyond the reach of plant roots, thereby improving the uptake of water and nutrients, especially P, Zn, Cu, and Mo in lowly fertilized soil [24]. Chen et al reported in their study that mycorrhizal symbiosis could increase plant height, dry weight, stem diameter, and the root-to-shoot ratio in cucumber seedlings [25]. In fact, mycorrhizal fungi help plants grow under semi-arid and marginal conditions [26]. Since pistachio trees normally suffer from water and nutrient deficiencies under alkaline and saline conditions [27], it is better to focus on the environment-friendly pistachio-mycorrhizae symbiosis which has already been recognized. The biochemical and physiological aspects of drought tolerance were studied in AMF[2] and non-AMF treated pistachio seedlings [28]. According to the results, plant growth, the leaf area, and pigments increased by symbiotic interactions under both drought stress and well-irrigated conditions. Better plant growth could be attributed to the improvement in water and nutrient uptake [29].

In fact, the concentration of some soil nutrients (P, N, Ca, Zn, Fe, and Cu) was significantly higher in symbiotic pistachios than in non-symbiotic ones, which could be due to the higher absorption surface provided by extensive fungal mycelium [28, 29]. It was also found out that the concentration of low

[2] Arbuscular Maycorrhizal Fungi (AMF)

molecular mass solutes, like amino acids and soluble sugars, increased more in AM-treated pistachios than in non-AM treaded plants. These solutes could regulate the osmotic potential of cells, thereby improving water absorption under drought stress conditions [28, 30]. Mycorrhizal symbiosis could also increase soluble proteins in pistachio leaves and roots to alleviate oxidative stress resulted from drought conditions [28].

Salinity is another serious problem in arid and semi-arid regions of Kerman province. It is expected that the mycorrhizal colonization of pistachio roots decrease under saline stress conditions. This stems from the fact that salinity could prevent mycorrhizal colonization capacity, spore germination, as well as hyphae growth [31]. However, these fungi are mainly found in saline soils, with many studies indicating that their symbiosis with plant roots increases their host resistance to salinity [32]. Mycorrhizal symbiosis improves nutrient absorption [32] and ionic balance [31], maintains the function of enzymes [33], facilitates water uptake and water-use efficiency [34], increases the accumulation of osmotic regulators [35], and finally elevates the photosynthesis rate in plants [32]. An experiment showed that mycorrhizal pistachio plants had greater root and shoot growth than non-symbiotic plants at all salinity levels (0.5, 3, 6, and 9 dSm^{-1}) [36]. In another study, it was reported that mycorrhizal fungi increased the total dry weight and electron transfer in photosystem II (PSII); it also reduced energy loss and increased the total performance of photosynthesis under saline and normal conditions [37].

3. Trichoderma

Trichoderma is a free-living and soil-borne genus of fungi, which colonizes the roots of numerous plants and establishes symbiotic interactions [38]. Several studies have reported that this genus of fungi induces plant resistance to a variety of fungal pathogens through some active and passive mechanisms [39]. The success of *Trichoderma* is because of its high reproduction and sporulation ability, survival under marginal conditions, tolerance of salinity and heavy metals, ability to change the rhizosphere environment, capability for colonizing plant roots and establishing symbiotic interactions, high nutritional competition, and high invasive power against soil pathogens [38]. It has been demonstrated that *Trichoderma*-treated plants have higher biomass production [39], higher induced systemic resistance to diseases [40], higher nutrient uptake [41], as well as higher efficiency of fertilizer utility and the seed germination rate [42] than non-treated plants.

Crown and root rot (gummosis) has for long been recognized as one of the major diseases of pistachio gardens, especially in Kerman province. Given the presence of various economic and environmental concerns over the application of chemical pesticides and the lack of the mass production of disease resistant root-stocks, using an appropriate biological control method could be effective in managing this disease. The results of greenhouse experiments indicate that pistachio co-inoculation with *T. harzianum* and pathogenic *Phytophthora melonis* (causing gummosis) increases root length and plant height as against plants inoculated with the pathogen alone [43].

Verticillium dahlia is another major plant pathogen, which causes vascular wilts in many plant species, including pistachios [44]. In some countries like Iran, verticillium wilt is considered a serious problem in pistachio gardens. Using *Trichoderma* as a bio-control agent could be an appropriate solution for replacing fungicides for plant protection purposes. According to past research, inoculating pistachio seedlings with *Trichoderma* increases the induction of defensive enzymes, total protein, and phenol contents to prevent verticillium wilt from invading pistachio seedlings [45]. One of the major practical aspects of the symbiotic relationship between microorganisms and pistachios is the possibility of their application in pistachio orchards, which has not yet been achieved. In this respect, further research is required to determine the beneficial aspects of *Trichoderma*. However, various species of these fungi are used to control diseases in greenhouse plants.

4. Plant Growth Promoting Rhizobacteria (PGPR)

PGPR is a group of free-living bacteria, which exists in the rhizosphere and can increase plant growth and development directly or indirectly [18, 46]. Some species of PGPR can synthesize ACC[3]-deaminase, thereby converting the ethylene precursor of ACC into ammonia and α-ketobutyrate so as to reduce ethylene synthesis and related damage under stress conditions [47]. They can also improve plant growth by producing siderophores (Fe-III-chelating agents), phytohormones (such as IAA[4], gibberellin, and cytokinin), and hydrogen cyanide, as well as enhancing the solubility of some immobile nutrients, like P, Fe, and Zn [48]. It has also been reported that shoot weight, plant height, and the total leaf number and area are increased in pistachio seedlings inoculated with ACC deaminase-producing bacteria under drought stress conditions [46]. Two isolates of IAA and ACC-deaminase-producing bacteria increased the uptake and concentration of P in pistachios. In general, the symbiosis of pistachios with IAA and/or ACC deaminase-producing bacteria improves plant growth under drought stress conditions [46].

The application of fluorescent pseudomonads, as a group of PGPR, increased the amounts of P, K, Ca, Mg, Zn, Ca/Na, and K/Na but reduced the accumulation of Na and Cl in pistachios under salinity conditions [18]. They also enhanced sucrose, phenolic compounds, the membrane stability index, and relative water contents but reduced malondialdehyde (MDA)[5] concentration under saline conditions [18]. The biofertilizer formulation of novel *Azotobacter vinelandii* isolates was studied in rice (*Oriza sativa* L.) fields [49]. According to the results, they were efficient enough to improve the growth and production of rice.

Some researchers investigated the effects of *Pseudomonas* spp. on the root-knot nematodes of *Meloidogyne incognita* in *P. vera* [21]. According to their results, inoculating bacteria could prevent *M. incognita* from forming gall in pistachio roots.

[3] Amino Cyclopropane-1-Carboxylate (ACC)

[4] Indole-3-Acetic Acid (IAA)

[5] Malondialdehyde (MDA)

5. Discussion

Pistachios, considered among the most important agricultural products in Iran, have been cultured widely in Kerman province, especially in Rafsanjan County [50]. They normally suffer from different biotic and abiotic environmental stresses, such as drought, salinity, high temperature, nutritional deficiency or excess, as well as soil-borne plant pathogens [19, 20, 22]. The application of some chemical compounds, including fertilizers, fungicides, pesticides, and the like could be subject to future restrictions by governments for their health and environmental concerns (Fig. 1) [1, 51]. The use of biological and eco-friendly methods, such as plant symbiosis with beneficial soil microorganisms to cope with environmental stresses, has recently attracted a lot of attention, which could be used in an integrated management plan to reduce chemical applications (Fig. 1) [1, 24, 48]. According to some field and laboratory studies, symbiosis with *Curvularia* species gives thermo-tolerance to *Dichanthelium lanuginosum*, with this symbiotic relationship being responsible for the survival of both partners in geothermal soils [52].

In the current study, some major symbiotic interactions with pistachios were reviewed. Symbiosis with mycorrhizal fungi is considered one of the major interactions with pistachios, particularly under arid and semi-arid conditions [53, 54]. This type of symbiosis improves the absorption of water and some immobile elements, especially phosphorous, under normal and stressful conditions [24]. The prominent feature of *Trichoderma* fungi in symbiosis with pistachios is plant protection against several fungal diseases, such as gummosis and verticillium wilt [43, 45]. However, they could also improve the uptake of nutrients and fertilizers by improving efficiency in plants [41, 42]. Another important interaction with pistachios occurs through PGPR, which is effective in improving plant growth by providing some phytohormones, thereby increasing the uptake and concentration of essential elements (P, Fe, and Zn), which could protect the plant against nematodes [48].

Fig. 1. Comparing the effects of chemical compounds with PGPR on the sustainability of the agriculture system and the environment [1]

The effectiveness of symbiotic interactions and the extent that plants benefit from symbiotic relationships with soil microorganisms depend mostly on the species and origins of plants and microbes as well as environmental conditions [25]. Some abiotic factors in soil, such as texture, structure, pH, temperature, moisture, and nutrient status affect the survival of symbionts and their effectiveness. For example, it was reported that fluorescent pseudomonas survived better in heavier soils (silt loam) than in lighter textured ones (loamy sand) [55, 56]. Microbial inoculants could be applied directly to soil, or inoculated on plant tissues and seeds [57, 58]. Nevertheless, the time of plant inoculation is effective in producing good results with soil microbes. The application of microbial symbionts to dry soil long before or after the irrigation time is practically impossible, because few microorganisms can survive [59].

Screening compatible and effective microbial partners is effective in improving pistachio growth under specific environmental conditions. The mixed inoculation of plants with several compatible microorganisms (two or more microbial partners) could be another appropriate method for improving symbiosis benefits. The multipartite interaction of many plants has been studied by many authors. For example, the effects of AM fungi alone or together with PGPR (*P. fluorescens*) and *Trichoderma* were examined on the growth and defense enzymes of pepper [60]. According to the results, triple treatment (AM+Tri+Pse) increased plant growth most among other microbial interactions. The activities of defense enzymes were also induced most in the combination of three microbial symbionts. However, few studies of this nature have been done on pistachios. In a study, the effect of co-inoculation with *Glomus mosseae* (AM) and *P. fluorescens* (PGPR) was investigated in *P. vera* cv. Qazvini under drought stress conditions [61]. Accordingly, it was shown that combined inoculation could increase the quantum yield of PSII photochemistry as well as the leaf area in pistachio seedlings under water stress conditions. However, achieving definite results in this respect needs further studies on pistachios.

6. Conclusions

Pistachios are regarded as the most important agricultural product in Iran, which normally encounter some environmental stresses. Using chemical compounds, in addition to being costly, could be followed by environmental and health concerns. Pistachio symbiosis with some beneficial microorganisms as eco-friendly agents could be a good method for replacing chemical applications. The selection of appropriate microbe species for co-inoculating plants with compatible microorganisms could be an effective solution for improving pistachio growth and development under different environmental stress conditions.

Conflict of interest

Environmental stresses detrimentally affect plant growth and production. Although chemical methods have long been used to overcome this problem, they may also be dangerous to the environment and human health. Some biological manners as using symbiotic microbes recently have been attracted much attention and can be good alternatives to the chemical methods.

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