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Status and Need of Research on Growth Improvement of Olive (*Olea europaea L.*) with Microbial Inoculants in Saudi Arabia

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The Olive (*Olea europaea* L) was introduced in Kingdom of Saudi Arabia (KSA) in 1980. The cultivation of olive in KSA increased rapidly because of suitable climatic conditions, increasing demand for olive oil, domestic use in different diets and its importance in the social life and economy of the country. The present status of available literatures on the role of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth Promoting Rhizobacteria (PGPR) as Microbial Inoculants (MI), on growth improvement of olive plants were surveyed and presented here. It was found from the literatures that MI was used in many different olive growing countries of the World and it improved plant growth both in green house and field conditions. We also highlighted the need of research for olive plantations in KSA in relation to their availability in the soils, inoculum production, growth under green house and field conditions, role on improved water relation, tolerance to salinity and heavy metals, use of green manure and natural fertilizers under organic cultivation etc. Although AMF and PGPR are regarded as important MI, and are commonly associated with many terrestrial plants, but they have been utilized recently for the growth improvement of olive plants throughout the olive growing areas of the world and no research activities was found in KSA until now.

Key words: Microbial inoculants (AMF and PGPR) literatures; Olive growth improvement; potential research needed; Saudi Arabia.

The Olive (*Olea europaea L)* is an evergreen, woody species cultivated worldwide especially in Mediterranean climate. Olive is the second most important oil fruit crop worldwide after oil palm. It plays an important role in the social life and economy of many Mediterranean countries including Saudi Arabia where these trees are growing.

Olive cultivation is traditionally concentrated in the Mediterranean region. Southern European countries account for about 74.9% of the world production, with Spain being the main producer (38.7%), followed by Italy (21%) and Greece (12.9%). Other important olive oil producers are Turkey, Tunisia and Syria (17.1%) as well as Jordan, Morocco and Algeria [1]. They are also grown in the subtropical regions of Australia, Southern Africa, and North and South America [2]. Old statistic shows that Mediterranean countries have about 90% of the total acreage out of 8 million hectors of olive grown worldwide [3].

Considering the growing interest of the Agricultural development companies and private farm owners of KSA about cultivation of olive and to meet the demand of oil and lack of scientific knowledge in the country about growth improvement by using Microbial Inoculants (MI), present article was written to highlight the current status of research on use of MI for growth improvement of olive in Saudi Arabia and other olive growing countries of the World.

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MATERIALS AND METHODS

To prepare this article, most of the relevant literatures on the role of AMF and PGPR on growth improvement of Olive plants throughout the World were collected and after screening them, the cross references were also collected to provide a clear picture of the selected subject. The status of AMF research on growth improvement of Olive plant is presented under two different headings: one in nursery conditions and other in field conditions. We have highlighted the soil and environmental conditions needed for olive growth in Saudi Arabia. We have also surveyed some of the Olive garden and interviewed some of the manager and owner of the gardens to find out the existing problems to grow olive in Saudi conditions and how to overcome them. The paper also highlighted the need of research on microbial inoculants for the growth improvement of the introduced olive plantations in Saudi Arabia.

RESULTS AND DISCUSSION

Arbuscular Mycorrhizal Fungi (AMF)

Status of AMF research on growth improvement of Olive under nursery conditions

Although Arbuscular Mycorrhizal Fungi (AMF), regarded as one of the important Microbial Inoculants (MI), are obligate and commonest symbionts associated with most terrestrial plants including a wide range of economically important crops (see [4, 5]), but the influence of AMF was not studied until recently for the growth improvement of Olive by using them in emerging technologies such as bioprotectors, phytostimulators or biofertilizers [6]. AM fungi help to uptake P and also absorb other nutrients like N, K, Ca, S, Cu, and Zn from the soil and translocate them to associated plants, improve water relation; alleviate salinity in the soils, play a regulatory role in the growth dynamics of the symbiosis in the increasing temperature, facilitate direct and indirect transfer of biologically fixed N to non-legume crops, involve in soil aggregation and improvement of soil conditions that reduce the soil erosion, act as agent of plant diseases control etc. (see [5]). Many agricultural development companies and private farms of Saudi Arabia are interested about olive cultivation and now the country has a large number of olive plantations covering more than twelve thousand hectares [7] of lands and many companies and private farms have established oil processing plants, but until now no initiative has been taken by the researchers in Saudi Arabia for using beneficial soil organisms like AMF and PGPR. Many other Olive growing countries like Spain, Turkey, Brazil, France etc started using microbial inoculants as a useful material for growth improvement of olive along with organic farming. The present status of available literatures on the role of Arbuscular Mycorrhizal Fungi (AMF) as Microbial Inoculants (MI), on growth improvement of olive plants were surveyed and presented here.

Influence of arbuscular mycorrhiza on plant growth and root system morphology of *Olea europaea* L. rooted cutting was reported by Citernesi *et al.*, [8]. Differences were seen in the degree of colonisation of roots and similarly, the same cultivar can respond differently to different AM fungi. In addition, these fungi can change the morphology of the root system, favouring the establishment of young plants.

Under drought and stressed conditions, mycorrhizal olive seedlings (inoculated with *Glomus intraradices)* showed significantly higher photosynthetic and transpiration rates, stomatal conductance and foliar P concentrations than non mycorrhizal [9, 10].

Nursery and field response of olive trees inoculated with arbuscular mycorrhizal fungi was studied by Estaún *et al.,* [11] and they found that the early inoculation of olive seedlings increased early plant development and crop productivity. Inoculation in the nursery with selected AM fungi prior to transplanting in the field improved plant growth and crop yield in the three years following inoculation. The growth was higher with *Glomus intraradices* inoculated plantlets than with *G. mosseae* or other native endophytes [11] present in the field.

The early inoculation of mistpropagated olive plantlets (cv. Cornicabra), with arbuscular mycorrhizal fungi showed a strongly positive response with the three fungal species of AM fungi both in nursery phase plantlets and in older plants grown in pots [12]. Soriano-Martin *et al.* [13] reported that olive root colonization with *Glomus* species reduced the juvenile period of olive trees. Therefore, inoculation of olive plantlets with

Glomus species is recommended as an olivicultural practice.

Porras-Soriano *et al.* [14] found that plantlets inoculated with *Glomus* species (*G. mosseae*, *G. intraradices* and *G. claroideum*) grew taller, had longer shoots, and showed higher plant N, P and K concentrations. AM fungi are plant root symbionts that provide many benefits to crop production and agroecosystem function.

Binet *et al.,* [15] reported the higher plant survival and subsequent plant development and growth of *Glomus mosseae* inoculated micro plantlets with micropropagated three French varieties of olive (*Olea europaea* L.).

Dag *et al.,* [16] studied the nursery and post-transplant field response of olive trees to arbuscular mycorrhizal fungi in an arid region. They mentioned that AM fungi inoculation technology can benefit olive cultivation, particularly in arid regions where native AMF levels are low.

Inoculating olive plantlets under nursery conditions with the different species of arbuscular mycorrhizal fungi (AMF) increased plant growth and the ability to acquire nitrogen, phosphorus, and potassium from non-saline as well as saline media [17] and also increased survival rate after transplant. *G. mosseae* was the most efficient fungus in reducing the detrimental effects of salinity and the highest salinity tolerance of *G. mosseae*-colonized olive trees was concomitant with an enhanced K concentration in olive plants, as Potassium plays a significant role in the osmoregulation processes.

Status of AMF research on growth improvement of Olive under field conditions

Limited research has also been done throughout the olive growing areas of the world under field conditions and no research activities are available in Saudi Arabia with microbial inoculants for growth improvement of Olive plantation. As early as 1985, Roldan-Fajardo and Barea [18] studied the mycorrhizal dependency of the olive tree (*Olea europaea* L.) and they reported that the Olive trees grown under natural conditions are highly mycotrophic, as the establishment of mycorrhizal associations favors their development. They also mentioned that early mycorrhizal establishment in olive plants by inoculation of planting stocks during nursery propagation will be beneficial.

Response of young mycorrhizal and nonmycorrhizal plants of olive trees (*Olea europaea* L.) to saline conditions was reported by Rinaldelli and Mancuso [19]. Inoculation of olive planting stocks with AMF revealed significant increases in plant growth, precocity, production and tolerance to salinity [18, 19, 20, 6] even in long-term field experiments [11].

It has been shown that mycorrhizal fungi reduce the stress suffered by olive plantlets at transplant and increase the latter's resistance to disease, improve their water relations and nutrient uptake, increase their rate of photosynthesis and generally improve their vigor – aspects of great importance in plant propagation systems [21] see [12].

Calvente *et al.,* [22] in 2002 reported the mycorrhizal effect on performance, biotic stress response and defence-related gene expression of olive plantlets. The presence of a mycorrhizal system in 'Picual' induces enzyme activity that might be involved in the plant's defence against certain soil pathogens [22]. However, different cultivars have shown different responses to the same fungus. The inoculation of 'Moraiolo' and 'Frantoio' with *Glomus mosseae* leads to increased plant development, but 'Leccino' plantlets experience no benefit over controls lacking mycorrhizal systems.

Caravaca *et al.,* [23] carried out a field experiment in a semiarid area to assess the influence of mycorrhizal inoculation and soil compost addition on establishment of *Olea europaea* seedlings. The growth of *O. europaea* was significantly enhanced by both composted organic residue addition and mycorrhizal inoculation treatment. They also reported the increased soil aggregate stability with inoculation of mycorrhizal fungi and decreased soil bulk density because of addition of composted residue and also mentioned that the soil biological agents play an important role in improving soil structure [23].

Mycorrhizal colonization and its effect on growth, P uptake and tissue phenolic content in the European olive (*Olea europaea* L.) was studied by Ganz *et al.,* [24] and they reported that the inoculation of the roots leads to improved development and an increase in root and leaf phenolic contents.

Arbuscular mycorrhizal fungal inoculation and composted residue application was studied [25] to help in restoration of desertified areas under Mediterranean climate with *Olea europaea* subsp. *sylvestris* along with others test plants. It was reported that mycorrhizal inoculation helped in increasing the ability of transplanted plants under field conditions for acquisition of nutrient by plants and the organic amendment significantly increased propagule production in the rhizosphere of mycorrhiza inoculated plants.

Arbuscular mycorrhizal fungi can contribute to plant growth and survival during acclimatization of micropropagated plants. AMF are naturally occurring microbial components of soils that enter a mutualistic symbiosis with the roots of most vascular plant species, including the majority of those currently being micropropagated [26].

Inoculation of olive plants (*Olea europaea* L. ssp. *sylvestris)* with a mixture of native AM fungi increased antioxidant enzyme like catalase, ascorbate peroxidase and dehydroascorbate reductase activities, but not monodehydroascorbate reductase and glutathione reductase activities under semi-arid Mediterranean conditions [27].

A field experiment was conducted by Querejeta *et al.,* [28] to evaluate the role of infection with *Glomus intraradices* on the water use efficiency of *Olea europaea* ssp *sylvestris*, for revegetation in Southeast Spain. They reported that water use efficiency was significantly enhanced during drought in *Olea europaea* ssp. *sylvestris* and also mentioned that the differences in water use efficiency for arid land plant communities may be due to different physiological responses to mycorrhization.

Four AM species, namely, *G. intraradices* (BEG 123), *G. mosseae* (BEG 124), *G. clarum* (BEG 125) and *G. viscosum* (BEG 126), were identified from an established olive tree plantation by Calvente et al., [6]. The effectiveness of native AM fungi was assessed on two target varieties of olive (Arbequina and Leccino) and was found the most effective fungi to improve the development of both olive varieties. Current emphasis in low input-based agrotechnology for crop production systems has stimulated the study and management of microbial interactions in the rhizosphere [29]. In the particular case of modern olive production in the Mediterranean Basin, emerging technologies include the use of inoculants of arbuscular mycorrhizal fungi as bioprotectors, phytostimulators or biofertilizers during the nursery production of quality olive planting stocks, among other approaches [6].

Porras-Soriano *et al.,* [14] reported the growth, nutrition, tolerance to transplanting stress, and resistance to *Verticillium dahliae* of olive plantlets inoculated with different arbuscular mycorrhizal fungi. Inoculated plants showed better tolerance to the stress of transplanting than non-inoculated plants and they grew taller, had more and longer shoots, and higher plant N, P and K in comparison to control.

Castillo *et al.,* [2] studied the influence of arbuscular mycorrhizal fungi (AMF) and the root-knot nematodes on plant performance and nematode infection of olive planting stocks. The AMF inoculation significantly increased growth of olive plants, irrespective of cultivar, plant age and infection by nematode and it was also concluded that pre inoculation of olive plants with AMF in the nursery conditions may improve the health status and vigour of planting stocks, which contribute to the resistance of the plantlets against Nematode.

Meddad-Hamza, *et al.,* [30] reported the influence of inoculation of two native AM fungi on the growth of micropropagated olive tree. They also compared the *G. mosseae* inoculation with chemical fertilization. The significantly higher growth was recorded with inoculated plants than uninoculated ones and with *G. mosseae* the root growth of the inoculated plantlets was doubled in comparison to that of the fertilized plants. The higher root growth in relation to shoot growth permitted greater utilization of soil resources and strengthened the plant's capacity to resist transplantation shock and water stress.

Soil and roots samples of seven olive tree cultivars were assessed to determine the length of active extraradical and total mycelium, and the rate and intensity of mycorrhizal colonization and spore density and diversity[31] in Brazil. Nine AMF species (*Acaulospora denticulata, Acaulospora scrobiculata, Acaulospora sp1, Acaulospora sp2, Entrophospora sp1, Gigaspora sp1, Glomus mosseae, Scutellospora pellucida* and *Scutellospora* sp1) were reported. The results confirmed the mycotrophism of the species.

However, no effect of cultivars was observed on the distribution of AMF propagules, which were evenly distributed in the different rhizospheres.

Plant Growth Promoting Rhizobacteria (PGPR)

Free-living root inhabiting soil bacteria that provide benefits to plants are regarded as PGPR[32]. Because of their beneficial role in nutrient uptake by the plants, they are extensively used for plant growth. The rhizosphere is an active area of high biodiversity. The PGPR promote plant growth by increasing supply of phosphorus, sulphur, iron and copper and nitrogen fixation in legumes. They are promoting free-living nitrogen-fixing bacteria, producing plant hormones and enhancing other beneficial bacteria or fungi. They are also helpful in controlling fungal and bacterial diseases as well as insect pests[33,34]. The ePGPR includes *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas* and *Serratia* [35], see [36] and the iPGPR includes the endophytes and Frankia species both of which can symbiotically fix atmospheric N 2 with the higher plants[37], see [36]. The soil bacterial genera such as *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium* of the family Rhizobiaceae are regarded as endophytes and they stimulate growth either directly or indirectly by forming nodules after invading the root systems in crop plants [38]. We will be discussing here only the PGPR used for growth improvement of Olive plant growth in both nursery and field conditions. Knowledge on the interactions between beneficial microbial inoculants and woody plants is still very limited and very scanty research activities were found in the literatures with growth improvement of olive plantations with PGPR. The present status of available literatures on the role of Plant Growth Promoting Rhizobacteria (PGPR) as Microbial Inoculants (MI), on growth improvement of olive plants were surveyed and presented here.

Peyvandi *et al.*, [39] studied the root formation and root architecture of olive microshoots after inoculated with different concentrations of *Pseudomonas fluorescent* P19 or P21. The density of 10^{8} CFUmgl $^{-1}$ induced almost two times increase in number and length of roots per explants, in contrast to that achieved by 10^{5} CFUml $^{-1}$. They also reported that by application of L. Tryptophan up to 10mgl -1 in the media, the length and numbers of adventitious and lateral roots were increased. Bacterial treatments were more efficient than IBA. Moreover different inoculation methods revealed that co-inoculation of the soil and microshoots increased the numbers and length of the roots.

Because of organic agriculture systems growing exponentially in Spain, Montero-Calasanz *et al.*, [40] worked to develop new alternative systems to produce organic olive cuttings. Several bacterial isolates, namely *Pantoea* sp. AG9, *Chryseobacterium* sp. AG13, *Chryseobacterium* sp. CT348, *Pseudomonas* sp. CT364 and *Azospirillum brasilense* Cd (ATCC 29729), were used to induce rooting in olive semi-hardwood cuttings of Arbequina, Hojiblanca and Picual cultivars of olive (*Olea europaea* L). They inoculated the olive cuttings in two different ways: (i) by dipping cuttings in a liquid bacterial culture or (ii) by immersing them in a paste made of solid bacterial inoculant and sterile water. It was reported that under nursery conditions all of the tested bacterial strains were able to induce the rooting of olive cuttings to a similar or greater extent than the control cuttings treated with indole-3-butyric acid (IBA). They also reported that the olive cultivars responded differently depending on the bacterial strain and the inoculation method. The strain that consistently gave the best results was *Pantoea* sp. AG9, the only one of the tested bacterial strains to express the enzyme 1-aminocyclopropane-1-carboxylate ACC) deaminase.

Need of research on growth improvement of olive in Saudi Arabia

The cultivation of olive trees is as old as 4,000 years [41]. With the introduction of olive in KSA in 1980 with young trees collected from Jordan, Turkey and Syria [7] and availability of suitable soil, plenty of water and favorable climatic conditions and also due to the increasing demand for olive oil by national and international markets and domestic use in many diets and its economic importance, the plantation of olives has increased rapidly and steadily across the Northern region of KSA and the plantation occupied large areas in Sakaka, Quarait, Macon, Bousaita and Tabuk. Because of long dry season in arid and semi arid condition in KSA, low water availability has become a problem for crop production and plays a significant role in transport and the uptake of the solutes needed for growth of the plants [42]. The plants growing under these conditions are also suffering from draught, high salinity, high temperatures, low organic matter in the soils, low nutrient availability and the calcareous and sandy soils which has low water holding capacities, high infiltration rates, high evaporation rates, low fertility level etc. Country needs urgent research on MI to use them as biofertilizers, biocontrol agents, and bioregulators covering the following areas of research:

a) The present status of MI (both AMF and PGPR) from olive plantations may be determined by isolating and identifying them and also inoculum production may be developed with organisms to use them as inoculants.
b) Growth of MI inoculated plantlets under green house conditions and post-transplant survival and growth under field conditions.
c) The role of MI on improved water relation, tolerance to salinity and heavy metals.
d) The role of MI and green manure in an organic farming system under field conditions on growth, nutrient uptake and fruit quality of olive.
e) Use of Natural fertilizers like bone meal, fish meal, rock phosphate etc may be used to provide some slow releasing nutrients that will also activate AMF and PGPR in the soils.
f) The long term management techniques for growth of olive plantation under field conditions may be formulated.

The agricultural development companies and many private farms investing in this sector and now the country have a large number of olive plantations covering more than twelve thousand hectares [7] of land. Because of good harvest, many companies and private farms have established oil processing plants in the country. The rapid increase of olive plantation and oil processing plants indicated that olive plantation is a profitable business and country will be self sufficient soon.

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