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# Status and Need of Research on Arbuscular Mycorrhizal Fungi and *Rhizobium* for Growth of Acacias

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**Acacias are widely grown plantation trees that are extensively planted in China, India, Indonesia, Malaysia, the Philippines, Thailand and Vietnam; there are approximately two million hectares planted worldwide. The plants are very useful for many different purposes. Research on tripartite association of arbuscular mycorrhizal fungi, *Rhizobium* and Acacias for restoration of ecosystems is very limited throughout the world particularly in Saudi Arabia. There is a growing need to find out ways and means to reduce the problem of desertification in order to rehabilitate and increase plant diversity of degraded rangelands in Saudi Arabia. Arbuscular Mycorrhizal Fungi (AMF) is widespread throughout the world and is found in the majority of terrestrial ecosystems including plants in arid and semiarid regions. They can improve plant growth by up taking P, N, K, Ca, S, Cu, Mn and improving water absorption; enhance the salt tolerance; improve soil aggregates and plant diversity along with other benefits. *Rhizobium,* beneficial soil bacteria, is useful for N fixation and also other benefits to the Acacias. Although AM fungi and *Rhizobium* are important to the persistence of vegetation in harsh environment, little is known about the diversity of these beneficial symbioses in rangelands ecosystems and their beneficial role for the sustainable management of arid and semi-arid ecosystems. Here we have explored the present status of research on AMF and *Rhizobium* with Acacias and their need for rehabilitation of rangeland ecosystems to reduce the desertification in Saudi Arabia. The interactive effects of AMF and *Rhizobium* under both saline and drought stress conditions on growth of Acacias and their out planting performance in association with green manure mycotrophic plants under field condition are some of the innovative ideas which are urgently needed for successful restoration of rangeland ecosystems in Saudi Arabia to reduce the desertification.**

**Key words**: Research Status; Arbuscular Mycorrhizal Fungi; *Rhizobium;* Acacias.

The Kingdom of Saudi Arabia, with a total area of about 2.25 million km is by far the largest country in the Arabian Peninsula. Because of the aridity, the country is facing extremes of temperature and wide variations between the seasons and regions. Most of the areas of the country are arid and semiarid. Millions of people throughout the world are living in arid lands characterized as too dry for conventional rain fed agriculture. The arid regions of the world are often very extensive[1]. In arid, semi-arid and sub humid areas of the world, desertification process is claiming several million hectares annually[2,3] and having a negative impact on the environment[4,5,6,7]. The disturbance of the vegetation cover, loss of available nutrients, organic matter and microbial activities which affects proper nutrient cycling and increase in soil erosion, are the characteristics of desertification[2,3,4]. The establishment of a suitable plant cover is needed for improving the chemical, physical, and biological properties of the soil[2,3,4] however; the non-availability of compatible

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microbial population in the disturbed soil[8.9,10,11,12] can cause a problem to plant establishment.

Tree legumes are capable of producing symbiotic associations with both arbuscular mycorrhizal fungi and *Rhizobium* and they are very important for revegetation in ecosystems where there is insufficient water and low availability of N, P and other nutrients[3, 13, 14]. The associations of *Rhizobium* or *Bradyrhizobium* spp. have been reported from root nodules of different woody legumes, but little information is available concerning the potential of woody legume-*Rhizobium* combination to maximize biological $N_2$-fixation[3, 14].

The Acacias are globally distributed and widely grown plantations trees and they are extensively planted in China, India, Indonesia, Malaysia, the Philippines, Thailand, Vietnam[15, 16] and South Africa[17]. There are approximately two million hectares planted worldwide e.g.[18]. The benefits and utilization of Acacias are well documented [19, 20, 21, 22, 23]. Acacias are reported to be salt tolerant (5 to 10dS/$m^{-1}$), because these plants enrich soil nitrogen in symbiotic association with *Rhizobium* and form associations with arbuscular mycorrhizal fungi [24, 25]. The application of bio-inoculants (Arbuscular mycorrhizal fungi (AMF) and *Rhizobium*) for improving of salt-tolerant plants is one of great importance because it minimizes the production costs and environmental hazards [26].

Arbuscular mycorrhizal fungi are by far the most widespread mycorrhizae in nature, which forms symbioses with majority of plants, influence plant community development, nutrient uptake, water relations, salt tolerance and above-ground productivity[27]. AMF association is commonly found in nodulated, $N_2$-fixing legumes and increase the performance of legume growth[28]. Moreover, to overcome the stressed conditions, the woody legumes showed a considerable degree of dependence on mycorrhizae [8, 9, 10, 12, 29, 30]. Also they enhance the nutrient uptake and help in establishing and coping with stress situations under desertification situations [11,31, 32]. The mycorrhizal associations in nodulated tree legumes (Acacias) plants may accelerate the natural succession by providing mycorrhizal inoculum for the new seedlings coming under natural conditions. The introduction of selected plant species inoculated with AMF and *Rhizobium* could be successful biotechnological methods for recovery of degraded ecosystems to reduce the desertification.

## MATERIALS AND METHODS

The present manuscript was prepared after collecting most of the relevant literatures on arbuscular mycorrhizal fungi and *Rhizobium* on growth improvement of Acacias. The cross references were also collected to have most of the literatures. The status of AMF research on growth improvement of Acacias is presented under three different headings: 1) Present status of research on AMF and *Rhizobium* with Acacias; 2) Alleviation of environmental stresses (Salinity and Drought) and 3) Responses to arbuscular mycorrhizal fungi and *Rhizobium* on Acacias. The paper also highlighted the Need of research for rehabilitation of ecosystem for the growth improvement of Acacias in Saudi Arabia.

## RESULTS AND DISCUSSION

### Present status of research on AMF and *Rhizobium* with Acacias

Although the importance of tripartite association of AMF, *Rhizobium* and tree legumes is widely recognized in maintaining fertility, in soil conservation and alleviation of abiotic stress, there have been limited studies on these symbiotic associations to explore the beneficial effects on desert ecosystems and their potential and constraints and management strategies. The related references and distantly related references may be found in some of the reviews and publications e.g. [18, 33, 34, 35, 36, 37, 38, 39] etc and most relevant references are mentioned here.

The legume genus Acacia has some 1350 species and is distributed throughout the world, particularly in Africa, Asia and Australia. The genus is exploited in natural habitats and plantations for many purposes. It forms a symbiotic association with strains of at least six genera of root-nodule bacteria (rhizobia) that are also widely distributed e.g.[18]. Although Acacias represent 6–7% of more than 20,000 known species of legumes and make a very substantial contribution to the total amount of nitrogen (as high as 100 million tonnes annually that is fixed on earth), yet the potential for exploiting Acacia nitrogen fixation has been almost completely overlooked e.g.[18]. There is usually a diversity of strains of rhizobia in soils where Acacias grow naturally. Many of these strains do not nodulate Acacia spp. at all and many others that do form nodules have little or no capacity to fix nitrogen. There appears to be scope to use inoculation with effective strains of rhizobia to improve the vigour and nitrogen fixation of seedlings grown in plant nurseries for out planting into the field. Where out plantings are made, inoculated well-nodulated seedlings survive better and grow faster than their uninoculated counterpart e.g.[18]. The information on the distribution of nodulation and $N_2$ fixation in the Leguminosae can also be found in Sprent and Parsons[40] and Sprent[35]. Barnet and Catt,[41] reported the distribution and characteristics of root-nodule bacteria isolated from Australian *Acacia* species and also reported the biological nitrogen fixation and root-nodule bacteria (*Rhizobium* sp. and *Bradyrhizobium* sp.) in two rehabilitating sand dune areas planted with *Acacia* spp in an earlier paper. Acacias are very useful for many different purposes, including re-vegetation, tanning, fodder, protein-rich seeds and fruits, firewood, agroforestry, windbreak, control of soil erosion, enhancement of bio-productivity and overcoming salt stress problems e.g.[38]. Dhar and Mridha[42] reported the different levels on infection of arbuscular mycorrhizal associations in *A. auriculiformis* A. Cunn. ex Benth. growing in different locations of Bangladesh. The inoculation of Acacias in the nursery and out planting them in the fields has been studied by several investigators [43] and elaborately by Giliana and his colleagues [44, 45, 46, 47].

**Alleviation of environmental stresses (Salinity and Drought)**

Salt and drought stresses are major constraints in the arid and semi-arid tropics and have become a major threat on growth and productivity of plants. Salinization of soil is a serious problem and is increasing steadily in many parts of the world, particularly in arid and semi-arid areas [48,49]. Saline soils occupy 7 % of the earth's land surface[50] and increased salinization of arable land will result in 50 % land loss by the middle of the 21st century[51].

AMF symbiosis can protect host plants against detrimental effects caused by drought stress[52, 53]. Quilambo[54] in a review on the vesicular-arbuscular mycorrhizal symbiosis mentioned several references related to the mechanisms of drought stresses in general. Sprent[55] reported that drought has a considerable negative impact on nodule function. It inhibits photosynthesis and disturbs the delicate mechanism of oxygen control in nodules[56] and improved acquisition of phosphorus, nitrogen and other growth promoting nutrients[57]. AMF can also reduce the impact of environmental stresses such as saline[58]. The role of arbuscular mycorrhizal fungi in alleviating salt stress are well documented e.g.[35]. They reviewed the significance of arbuscular mycorrhiza in alleviation of salt stress and their beneficial effects on plant growth and productivity in general and also focused the progress in research on biochemical, physiological and molecular mechanisms in mycorrhizal plants to alleviate salt stress.

AMF have an important role in promotion of biological and chemical properties of plants under stressed environment. AM help plants to adapt to and resist a wide range of biotic and abiotic stresses they encounter in the environment. The arbuscular mycorrhizal symbiosis may alleviate plant responses to moderate moisture deficit by several mechanisms including increased water uptake from the soil by hyphae, altered hormonal levels, causing changes in stomatal conductance, increased turgor by lowering leaf osmotic potential, improved nutrition of the host, and improved plant recovery after drought by maintaining the soil-root continuum see [59]. AM fungi can enhance plant growth under saline stress, especially in soils with low level of P and are able to enhance plant tolerance under salinity through altering plant physiology and increasing water and nutrient uptake.

Giri *et al*.,[48, 60] studied the influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *A. auriculiformis*. They have also examined the effect of arbuscular mycorrhizal fungus, *Glomus fasciculatum*, and salinity on the growth of *A. nilotica*. Mycorrhizal plants maintained greater root and shoot biomass at all salinity levels compared to nonmycorrhizal plants. AM-inoculated plants had higher P, Zn, and Cu concentrations than uninoculated plants. In mycorrhizal plants, nutrient concentrations decreased with the increasing levels of salinity, but were higher than those of the nonmycorrhizal plants. They also indicated that mycorrhizal fungus alleviates deleterious effects of saline soils on plant growth that could be primarily related to improved P nutrition. The degree of tolerance of different species of *Acacia*. tested in saline soils under field conditions showed that *A. ampliceps, A. nilotica, A. redolens, A. saligna* and *A. stenophylla* are more or less tolerant[61,62, 63, 64]. In glasshouse experiments, Zou *et al*.,[65] reported that under increased concentration, the salt tolerance strains produced better performance than salt sensitive strains and Bala *et al*.,[66] suggested to use effective, salt-tolerant strains of rhizobia to inoculate *Acacia* species for out planting on salt lands. Singh *et al*.,[67] mentioned the performance of *A. nilotica* on salt affected soils.

Amira *et al*.,[38] investigated the alleviation of salt stress on growth and development of *A. saligna*, by using arbuscular mycorrhizal fungi and *Sinorhizobium terangae* (R), individually or in combination (AMF+R). Salt stress increases the percentage of sodium (Na) and calcium (Ca) contents as well as proline; meanwhile, it reduces the leaf osmotic potential, growth parameters, nodulation parameters, nitrogen, phosphorus, potassium (N. P. K.) contents, total carbohydrates percentages and chlorophyll contents. co-inoculated (AMF+R) stressed plants were able to maintain a higher osmotic potential of cells leading to the significantly rapid growth, enhanced nodulation parameters, N, P, K, Ca, total carbohydrates percentages and chlorophyll contents as well as proline in leaves, and significantly reduced the Na percentage. In conclusion, co-inoculated (AMF+R) enabled the plants to maintain osmotic adjustments and enhanced the plants tolerance against salinity. The plants enrich soil nitrogen in symbiotic association with rhizobia and form associations with arbuscular mycorrhizal fungi [24, 25]. The application of arbuscular mycorrhizal fungi and *Rhizobium* for improvement of salt-tolerant plants is one of great importance because it minimizes the production costs and environmental hazards [26]. The effects of salinity on growth of four strains of *Rhizobium* and their infectivity on two species of *Acacia* were studied by Craig *et al.*, [68]. Birhane *et al*.,[69] mentioned the impacts of arbuscular mycorrhizal fungi on competitive interactions among *A. etbaica* and *Boswellia papyrifera* seedlings under drought stress. Ndiaye *et al*.,[70] reported improved growth of *A. senegal* after inoculation with arbuscular mycorrhizal fungi under water deficiency conditions.

**Responses to arbuscular mycorrhizal fungi and *Rhizobium* on Acacias**

It is believed that the N-fixing capability of *Rhizobium* may enhance if the host plant is also in symbiosis with AM. Under such situation and with regard to enhancing the colonization rate, uptake of inorganic nutrients and plant growth, *Rhizobium* and AM are synergistic. This can be very advantageous under the conditions that nutrients are not available at high amounts. Different AM species are able to increase nodulation and N fixation differently. The structure, functioning, and nutritional demand of nodules are different with plant roots. Nodules are produced by cortical cell division, in which rhizobia with high energy and P requirements reside and fix N. Barea and Azcon-Aguilar [71] have demonstrated that AMF are known to be one of the most efficient ecological factors in improving growth and N content in legumes.

The integration of $N_2$ fixing trees into stable agroforestry systems in the tropics is being tested because of their ability to produce higher biomass N and P yields, when symbiotically associated with rhizobia and AMF. The tripartite symbiosis among AM, bacteria and legumes is of great significance both for agriculture and for ecology, and scientists have been trying to find the most efficient combination of AM and bacteria. In agroforestry, mycorrhizal associations contribute much to the growth of *Acacia* species in unfertilized fields [72]. With pot experiments, Jasper *et al*., [10] reported the positive growth of *A. concurrens* to additions of phosphorus and to inoculation with VA mycorrhizal fungi in soils stockpiled during mineral sand mining. Reddell and Warren [73] listed nearly 50 species of *Acacia* with mycorrhizal associations. Ba *et al*.,[74] studied the effect of time of inoculation on in vitro ectomycorrhizal colonization and nodule initiation in *A. holosericea* seedlings. Ba *et al*., [75] also reported Glomales from *A. holosericea* and *A.*

*mangium*. Some aspects of the management of mycorrhizas in forestry have been dealt with by Grove and Malajczuk [76]and Jasper[77]. Reddell and Warren [73] drew attention to the potential for using inoculants of mycorrhizal fungi to improve the survival, establishment and growth of tropical *Acacia* plantations. Acacias form associations with both endomycorrhiza and ectomycorrhiza[73, 78]. The diversity and abundance of arbuscular mycorrhizal fungi associated with *Acacia* trees was reported from Ethiopia[39, 79]; Bangladesh[42]; Senegal [80, 81, 82]; Malaysia[83] and India [84]. Udaiyan *et al*., [85] studied the influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular mycorrhizal fungi in *A. farnesiana* Willd. and *A. planifrons* W.et.A. Diversity of endomycorrhizal fungi and their synergistic effect on the growth of *A. catechu* Willd was reported by Parkash and Aggarwal [86]. Raddad *et al*., [87] studied the nitrogen fixation in eight (*A. senegal*) provenances in dry land. Nodulation pattern and acetylene reduction (nitrogen fixation) activity of some highland and lowland *Acacia* species of Ethiopia was reported by Assefa *et al*., [88]. Renuka *et al*., [89] studied the arbuscular mycorrhizal dependency of *A. melanoxylon*. Acacias respond to inoculation with either type was reported by several authors e.g. [30, 82, 90, 91, 92.] see also [93]. Positive growth response of *A. mangium* Willd. and *A. saligna* Labillto inoculation of seedlings with mycorrhizal fungi was reported by Aggangan e*t al*., [94] and El-Khateeb *et al*., [95] respectively. Satter *et al*., [96] reported the performance of arbuscular mycorrhiza inoculated *A. mangium* seedlings on degraded land with different rates of phosphorus. Growth and mycorrhizal dependency of *A. mangium* Willd. inoculated with three vesicular arbuscular mycorrhizal fungi in lateritic soil were reported by Ghosh and Verma[97]. Habte, and Soedarjo[98] found positive response of *A. mangium* to vesicular – arbuscular mycorrhizal inoculation, soil pH, and soil P concentration in an oxisol. Jayakumar and Tan[99] studied the growth performance and nodulation response of *A. mangium* co-inoculated with *Bradyrhizobium* sp. and *Pisolithus tinctorius.* Jeyanny *et al*., [93] reported the effects of arbuscular mycorrhizal inoculation and fertilization on the growth of *A. mangium*. Seedlings of some Australian Acacias associate with both types of mycorrhiza and form root nodules[100] as well. While endomycorrhiza occur frequently in soils growing Acacias, ectomycorrhiza are less common and may be absent from some soils[101]. Duponnois *et al*., [102] reported that Australian Acacias like *A. holosericea* are excellent candidates for the revegetation of arid zones in Africa. Their high ability to develop multiple symbioses with soil microorganisms is crucial to their rapid development in adverse climatic and edaphic conditions. These symbioses include nitrogen fixation with rhizobia, vesicular arbuscular mycorrhization and ectomycorrhization. André *et al*., [103] mentioned that the Ectomycorrhizal symbiosis enhanced the efficiency of two *Bradyrhizobium* inoculated on *A. holosericea* plant growth. Combined mycorrhiza and root-nodule bacteria cf. [104] may synergistically stimulate N fixation in legumes growing in soil that is deficient in plant-available P. Dela Cruz and Yantasath[90] noted enhanced growth of *A. mangium* following inoculation with both mycorrhiza and rhizobia. Beniwal *et al*., [105] and Mandal *et al*., [106] demonstrated growth responses in *A. nilotica* to co-inoculation with rhizobia and mycorrhizal fungi. Lal and Khanna [107] noted that the growth of *A. nilotica* after joint inoculation with one rhizobial strain and *Glomus fasciculatum* was better than after inoculation with either organism individually. Michelsen[79] reported the growth improvement of Ethiopian Acacias by addition of vesicular-arbuscular mycorrhizal fungi or roots of native plants to non-sterile nursery soil. Using the natural abundance technique, Michelsen and Sprent[108] found that some vesicular arbuscular mycorrhiza improved $N_2$- fixation by four *Acacia* species growing in a nursery, although there was no corresponding increase in shoot N concentration. Sharma *et al*., [109] studied the growth responses and dependence of *A. nilotica* var. *cupriciformis* on the indigenous arbuscular mycorrhizal consortium of a marginal wasteland soil. Franco *et al*., [110] considered that joint inoculation of tree legumes with rhizobia and mycorrhiza held promise as an aid to land reclamation in the humid Amazon. Rhizobia isolated from some Acacias showed positive growth on *A. nilotica* under some stress conditions in North Africa[111]. Chung *et al*.,[112], on the other hand, found no benefit from co-inoculation. *A. confusa* and *A. mangium* in pot experiments responded to dual inoculation with vesicular-arbuscular mycorrhizal fungi and phosphorus-solubilising bacteria[113]; the *Acacia* species may not have been nodulated.

Bargali and Bargali [114] mentioned that *A. nilotica* is a multipurpose leguminous plant and the effect of VA mycorrhizal fungi and phosphorus on growth and nodulation of *A. nilotica* [105]; the role of mycorrhiza in street management for seedling growth of *A. nilotica*[115] and interaction between *Rhizobium* inoculation and nitrogen fertilizer application on growth and nodulation of *A. nilotica* was studied by Toky *et al*.,[116]. Chaer *et al*.,[117] in their article described several successful results in Brazil using $N_2$-fixing legume tree species for reclamation of areas degraded by soil erosion, construction and mining activities, emphasizing the potential of the technique to recover soil organic matter levels and restore ecosystem biodiversity and other environmental functions.

The literature reviews showed that the importance of AMF and *Rhizobium* for the survival of out planting of inoculated seedlings in general for Acacias and importance of management techniques with Green manure plants and role of existing life inocula for surrounding new plants and utilization of residual value of nutrients generated by the annual plants is largely lacking. Only recently Mridha and Al-Qarawi[118] proposed a methodology by using a fertilizer called Bio-organic and mycotrophic green manure plants for growth improvement of Date Palm. The same ideas may be introduced here for rehabilitation of rangelands with Acacias inoculated with AMF and *Rhizobium*.

**Need of research for rehabilitation of ecosystem:**

Most legume tree species are also able to form symbioses with arbuscular mycorrhizal fungi (AMF), which are known to improve the capacity of the plant to take up phosphorus and other macro- and micro-nutrients in sub-optimal situations [119]. Arbuscular mycorrhizal fungi are also known to mitigate water and salt stresses[34, 120] and to interact synergistically with *Rhizobium*, resulting in better plant performance [119, 121, 122]. However, as for free-living *Rhizobium* bacteria, propagules (spores) of AMF are almost totally absent in sub soils [123]. This led to the development of technologies to produce legume tree seedlings inoculated with selected rhizobial strains and AMF species suitable for the recovery of severely degraded areas. Resende *et al*.,[124] mentioned the use of nitrogen-fixing legume trees to revegetate degraded lands. The occurrence of *Rhizobium* from woody (tree) legumes is widely documented all over the world e.g. [37], but very limited research was done in Saudi Arabia.

The tripartite symbiosis among leguminous plants, *Rhizobium* species and AM fungi has been the subject of intensive research in recent years. A synergistic beneficial effect of dual inoculation with AM fungi and *Rhizobium* in growth and nutrition in legumes has been demonstrated by many workers. Generally AM fungi are known to improve phosphate nutrition, which in turn enhances plant growth and $N_2$-fixation.

Because of paucity of literatures and limited information about the biodiversity and proper identification of AMF and *Rhizobium* from Saudi rangelands; the inoculum production of obligate symbiont like AMF under Saudi conditions; the interactive effects of AMF and *Rhizobium* under both saline and drought stress conditions on growth of Acacias and their out planting performance in association with green manure mycotrophic plants under field condition are some of the innovative ideas which needs to be addressed for successful restoration of rangelands ecosystem in Saudi Arabia.

Acacias are globally distributed and widely grown plantation trees. Because of the aridity, Saudi Arabia is facing extremes of temperature and wide variations between the seasons and regions. Desertification process is claiming several million hectares and having a negative im-pact on the environment. The establishment of a suitable plant cover is needed for improving the chemical, physical, and biological properties of the soil. Acacias are very useful for many different purposes, including re-vegetation, tanning, fodder, protein-rich seeds and fruits, firewood, agroforestry, windbreak, control of soil erosion, enhancement of bio-productivity and overcoming salt stress problems. The introduction of selected plant species inoculated with AMF and *Rhizobium*, could be a successful biotechnological method for successful recovery of degraded ecosystems to reduce the desertification and will be providing benefits in improving the vegetation, providing fodder for grazing animals, improving soil aggregates, physical and chemical properties of the soils, persistence inoculum of AMF and *Rhizobium* for associated plants and also to reduce soil erosion hazard under climate change conditions in Saudi Arabia, that will directly or indirectly benefit the society, the environment and the economy. Research on tripartite association of arbuscular mycorrhizal fungi, *Rhizobium* and Acacias for restoration of ecosystems is very limited throughout the world particularly in Saudi Arabia. The plants growing under Saudi conditions, are suffering from different environmental constraints and adverse soils conditions. We need urgent research on AMF and *Rhizobium* to use them as biofertilizers, biocontrol agents and bioregulators.

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