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Review article

STUDY OF *JUNIPERUS PROCERA* AND ARBUSCULAR MYCORHIZAL FUNGI (AMF) IN SAUDI ARABIA

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ABSTRACT
The woodlands of *Juniperus procera* Hoechst. ex Endl. in the southern region of Kingdom of Saudi Arabia has declined tremendously due to several factors involving the human impact, weather conditions, physical and chemical characteristics of soil. In the surviving trees, Arbuscular mycorrhiza fungi (AMF) were observed to colonize the roots which conferred some ecological advantage to the plants. AMF are symbiotic relationship between mycorrhiza fungi and plants roots. AMF absorb and transport nutrients and water from soil to roots of plants and in return the plant provide shelter and carbohydrate to the fungi. AMF also increase resistance to disease and ecological stress. This review focuses on the possible relationship between AMF and *J. procera* and the benefits it confers to each other with the ultimate goal of understanding future restoration program of the damaged woodlands.

KEY WORDS: *Juniperus procurer*, arbuscular mycorrhiza fungi, Saudi Arabia

INTRODUCTION

In Saudi Arabia, *J. procera* is found in the southern Sarawat or the Asir. It was previously reported that it was distributed into the highlands of Yemen but now they have disappeared. Few large trees remain as they are cut down for fuelwood, timber and even for exportation. Besides that, the population of *J. procera* is declining with the change of land use patterns, grazing by buffalo and elephants as well as due to the increase in plantations of fast growing exotic species (Coppen, 1995). Their growth pattern has fluctuated time and again as a result of changes in climate, competition and human activities which including the clearing and abandonment of land, and changes in grazing (Schwartz *et al*., 2005).

Mycorrhizas can be classified into two main groups, endomycorrhiza and ectomycorrhiza (ECM) by inspecting for the different extraradical or intraradical hyphal structures that may be present (Bonfante and Perotto, 2000). More than 80% of land plant families are colonized by AMF. These plants are able to develop AMF associations with a wide range of AMF, thus AMF symbiosis is generally non-specific. Collectively, in both natural and agricultural ecosystems, AMF symbiosis is very crucial (Smith and Read, 2008). Both of these associations are of great economic and ecological importance (Habte, 2000). Endomycorrhizas are also known as AMF although initially the term vesicular-arbuscular mycorrhiza was used for many decades. However, it was changed when studies showed that not all fungi formed vesicles (Koide and Mosse, 2004; Smith and Read, 2008).

The Kingdom of Saudi Arabia is a large country. It is situated at latitudes between 15°45’N and 34° 35’N and longitudes between 34° 40’E and 55° 45’E (MOP, 1990). The topography of Saudi Arabia ranges from below sea level to 3000 m above sea level. It includes a major part of the Arabian Peninsula with an area of 2,250,000 square kilometers (Chaudhary and Al-Jowaid, 1999). The Arabian Peninsula is situated in the northern geographical belt of the great deserts which consist of the Great Nafud in the North and the vast Empty Quarter in the South. It is covered with approximately 30% of sand (Chaudhary and Le Houérou, 2006). It is an arid country with some sub humid regions on the south western highlands which encompasses a variety of terrestrial habitats (El-Juhany, 2009). Depending on altitude and season of the region, its climate differs greatly. The south western monsoon brings damp oceanic winds and causes the high lands to receive variable rainfall. Most rain falls occur in April/May and July/August and the annual average rainfall is 600-800 mm in the Escarpment Mountains, while in the wettest areas it can rise up to 1000 mm (Miller, 1994; El-Juhany, 2009). The Arabian Peninsula has low relative humidity and the summer temperatures can reach up to 50°C (Chaudhary and Le Houérou, 2006). In Saudi Arabia, the natural forests cover the mountainous region stretches from Taif in the north to northeast Jazan. Sarawat or Aseer or Hejaz Mountains or (Assarah) is the name of the mountain from Taif to Abha City (Chaudhary, 1997). The positions of the Sawarat Mountains are similar to the Red Sea and it spreads for approximately 1800 km (NCWCD and JICA, 2006). According to old records, the mountains of the Hijaz and Asir were once densely covered with woodlands. In the mountains of Saudi Arabia, especially in inaccessible areas, only an estimated 2.7 million hectares are still remaining forested (NCWCD, 2005). Species diversity is abundant throughout the southwestern region even though the areas were highly inhabited by humans since the ancient times (NCWCD, 2005). Eighty percent of the total forest area is distributed throughout the Sarawat mountain range situated in the southwestern region (NCWCD and JICA, 2006). In most parts of the Peninsula, there are specialized types of plants and plant communities. This is because of the low rainfall in the area and also the mostly irregular soil characteristics. There are various terrestrial habitats and they vary from mangrove forests to coastal and intermountain acacia woodlands to Juniperus forests to tall shrub, deep sand plant communities, dew-dependent shrub community to empty and highly saline lands (Chaudhary and Le Houérou, 2006).

Depending on places, the flora and fauna found can be very rich to very poor, common to rare and specialized at places. In Saudi Arabia, the only renewable resource is its vegetation. However, agricultural practice is very limited and erratic as the deserts have low productivity and low carrying capacity (Chaudhary and Le Houérou, 2006).*Juniperus procera* Hoechst. ex Endl. is one of the three gymnosperms of the Cupressaceae family which is native to East Africa and is the only indigenous African Juniper (Melville, 1960). It is also known as the African Pencil Cedar, African Juniper or East African Juniper (Coppen, 1995; Adams, 2004; Couralet, 2004). There are reportedly 60 species of *Juniperus* L. with hundreds of cultivars (Adams and Turner, 1970; Adams, 1987; Galderen and Smith, 1989). *J. procera* grows relatively fast (Teel, 1984) to a height of 40 m (Teel, 1984; Pohjonen and Pukkal, 1992; Berhe and Negash, 1998; Couralet, 2004) and 3 m in diameter at breast height (Pohjonen and Pukkal, 1992), making it the largest juniper in the world (Negash, 1995; Berhe and Negash, 1998; Couralet, 2004). It has a thin gray bark with vertical fissures and it is similar to *J. virginiana* which is commonly found in eastern North America because of the nature of foliage and its appearance (Noad and Birnie, 1999). According to Searle (1999), these characteristics enable easy identification of *J. procera*.

J. procera is a highland species which prefers cold high ridges and has been found to grow well between the altitude 2000 m and 3000 m (Coppen, 1995; Couralet, 2004). *J. procera* distributes from Arabia and extends southwards through Sudan, Ethiopia, Djibouti, Somalia, Kenya, Uganda, Tanzania, Zaire, Malawi and Zimbabwe (Lind and Morrison, 1974; Friis, 1992). Friis (1992) and WCMC (1998) noted that this species can be commonly seen growing in high and on rock-strewn grounds. *J. procera* was also found to occur in dry forests or dispersed in pure stands on rocky well-drained soils (Coppen, 1995). In the Asir Highlands of Saudi Arabia, *J. procera* forests have been reported to be showing an extensive decline (Fisher, 1997). Junipers were once found to dominate a large part of woodlands but because of ongoing and persistent human influences, these trees have been reduced to isolated patches (IUCN, 2010). The present population of *J. procera* in Saudi Arabia only represents a small fragment of the woodlands that once existed (Coppen, 1995; Negash, 2002; Couralet, 2004). *J. procera* has been included in the IUCN Red List of Threatened Species (WCMC, 1998).

Other factors that cause deterioration of the forest land include the extensive forest clearing for urban development cultivation, over-grazing, and exploitation of forests for fuelwood and construction materials without any replantation program (Hajar *et al.*, 1991). In areas where the roots of junipers are at risk of being damaged or trampled by humans and animals, the absorption of nutrients and water taken up by junipers can affect its survival (Hajar *et al.*, 1991).

The situation becomes worse when some species of junipers do not propagate well via seeds (Ortiz, 1998; Helmersson and von Arnold, 2009). Change of land use patterns and the gradual replacement of indigenous species to fast growing exotics in plantation forestry (Hall, 1981) have caused severe genetic depletion of junipers (WCMC, 1998).

The depletion of juniper forests has occurred all over the Middle East. Among them are the secondary destructions of forests in Lebanon, complete elimination of *J. procera* subsp. *polycarpos* forests in the highlands of Yemen, elimination of *J. procera* from the Erkwat Mountains of Sudan, death of *J. procera* forests in Saudi Arabia highlands, Djiboutim in Oman, Baluchistan and Pakistan (Chaudhary and Le Houérou, 2006).

Attempts in natural regeneration of this species have not been successful because of human interference and therefore, modifications of soil and moisture conditions (Berhe and Negash, 1998). However, the destruction of natural forests has stopped further exploitation of these trees (Pohjonen and Pukkala, 1992). This review is carried out to understand the habit of *J. procera* and possible importance of mycorrhizal relationship.

Characteristics of *J. procera*

J. procera is a dioecious plant that and pollinates through wind (Negash, 1995). It has scale-like leaves and firm branches (Couralet, 2004). The male cones are small, round, develop singly and are yellowish in colour. The plant has small branches and terminal. The berry-like and rounded female cones are fleshy and soft once ripened. Besides that, four pyramidal seeds can be produced (Negash, 1995). The juvenile and adult stages are the two developmental phases in *J. procera*. This tree has a unique trunk and is crown-shaped. When young, it has a pyramidal shape and as it grows older it has a more spreading appearance (Berhe and Negash, 1998). *J. procera* consist of two types of leaves, namely the needle-like but spreading and scale-like but imbricate leaves (Migahid, 1974).

Importance of *J. procera*

Forests and trees play important roles in social, economic and natural environment (El-Juhany, 2009). The renewable natural resources of Saudi Arabia are the forests and they are vital to the ecosystem of the Kingdom considering the vast area of the country and its diverse environment. The forests help to distribute water and control its flow, thus increasing the moisture in the soil. They also protect the soil from water and wind erosion. These forests also have economic and recreational values. Moreover, they are of scenic, touristic and weather regulating importance (Summit, 2002). The junipers not only benefit the local population, but also to those in the world. Juniper woodlands house a vast diversity of living organisms and as part of the global forest plant life. They play an important role in collecting carbons in its biomass and soils (El-Juhany, 2009).

The wood of *J. procera* is of medium hardness (Bekele *et al.*, 1993; Negash, 1995) and resistant to termite and rot (Negash, 1995). This tree can be used for construction and lining of buildings, transmission and other poles, fence posts, furniture, floors, and pencils (Negash, 1995). Moreover, these trees are cleared by the locals and made into fuel wood and timber (Couralet and Bakamwesiga, 2007; El-Juhany, 2009).

It is used in the manufacturing of lead-pencil and pen-holders, the construction and lining of buildings, joinery, strips and parquet flooring, and outdoor works, such as roofing-shingles, fence posts, plant trays, water-flumes and telegraph poles (Bekele *et al.*, 1993; Negash, 1995; Couralet and Bakamwesiga, 2007). In Ethiopia, timber from *J. procera* was highly valued to construct Orthodox churches and houses of early nobility. It can also be made into veneer and plywood, hardboard and particle board, and as pulpwood (Couralet and Bakamwesiga, 2007). Junipers also serve as shade or ornamental trees as well as a windbreaker (Coppen, 1995; Couralet and Bakamwesiga, 2007). In Eritrea, Ethiopia and Kenya, *J. procera* is replanted in deforested areas for soil conservation or improvement and for erosion control (Couralet and Bakamwesiga, 2007).

Since ancient times, the extracts of juniper berries have been used as medicines (Chaudhary and Al-Jowaid, 1999). In traditional medicine, *J. procera* is used locally to treat tuberculosis and jaundice. Juniper berry oil is well known for its spectrum of pharmacological activities (Samoylenko *et al.*, 2008). The fruits are used to treat headaches and skin diseases. It acts as a stimulant and also a medicine against ulcers as well as against liver diseases if the resin is combined with honey (Hvoslef-Eide and Preil, 2005, Couralet and Bakamwesiga, 2007). *J. procera* is also used for the preparation of traditional eye medications used in the treatment of eye infections such as conjunctivitis, blepharitis and other eye-related disorders (Klaus and Adala, 1994). The people of the Kikuyu tribe of East Africa drink the young twigs and buds which have been grounded and soaked in water for the treatment of intestinal worms (Kokwaro, 1976).

Mycorrhiza

More than a decade ago, a scientist in German named Frank created the term "mycorrhiza" which means fungus-root (Habte, 2000). It is the most common association between microorganisms and higher plants (Habte, 2000) with 80% of plant species recorded to be colonized with arbuscular mycorrhizal fungi, AMF (Wang and Qiu, 2006; Koltai, 2010). Mycorrhizas have been reported by Wilcox (1991) to occur in 83% of dicotyledonous and 79% of monocotyledonous plants and it has also been reported to occur in Gymnosperms.

The fungus is supplied with soluble carbon sources by the host plants, whereas the fungus provides the host plant with a better ability to take up water and nutrients from the soil (Entry *et al.*, 2002). It is recognized that the association between mycorrhiza and the growth of plants yields positive outcomes (Smith and Read, 2008).

Classification of arbuscular mycorrhiza fungi (AMF)

Smith and Read (2008) described arbuscular mycorrhizal fungi (AMF) as the most common group of mycorrhizal fungi. Previously, Gerdemann and Trappe (1974) classified AMF into four different genera, namely *Glomus*, *Sclerocystis*, *Gigaspora*, *Acaulospora in the order Endogonales. Nevertheless, in 1990,* AMF were then classified under the division of Zygomycota and in the Order Glomales. It included 6 genera with the most researched species being those from the genus *Glomus* (Morton and Benny, 1990). However, further studies found that AMF do not form zygospores and thus were not monophyletic with any part of the Zygomycota. Since then the Order Glomales has been reclassified and placed in the phylum Glomeromycota. In addition, along with the establishment of other new orders, the Order Glomales was rewritten as Glomerales (Schüßler *et al.*, 2001). Redecker and Raab (2006) stated that there are currently 10 genera of the Glomeromycota (*Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, *Entrophospora*, *Pacispora*, *Diversispora*, *Archaeospora*, *Geosiphon*, *Paraglomus*).

Morphological characteristics of arbuscular mycorrhiza fungi (AMF)

According to Mosse (1981), AMF form fan-shaped mycelia. These mycelia branches out from the runner or trunk hyphae in the form of dichotomously branched hyphae. The mycelia consist of mainly coarse hyphae measuring 8-20um in diameter and thin-walled absorbing hyphae which are repeatedly branched.

AMF have two distinctive characteristics; the arbuscules and vesicles. Arbuscules form 2-5 days after fungal penetration and only live for a short period (Sieverding, 1991). After the fungus penetrates into the host cell wall, the arbuscules develop into highly branched hypha. On the other hand, vesicles are located at the hyphal tip in the form of sack-like swellings. They play a role as storage organs and are mostly filled with fat-like droplets (Mosse, 1981). Sieverding (1991) stated that the shape, wall structure, content and number of fungi depends on the fungi species. Among the various morphological characteristics, spores are principally used to identify AMF to genus level. This is because the majority of the structural diversity maintained in glomalean fungi is found in the spores. These features include the mode of creation of spores, the sub cellular structure of spores, and the mode of spore germination (INVAM, 2004). Additional information such as germination structure and spore wall structure, namely the composition of the layers, their thickness, colour and ornamentation permits the detailed identification of AMF (Morton and Bentivenga, 1994).

Type of arbuscular mycorrhiza fungi (AMF)

Colonization with AMF is only visible with the help of the microscope because there are no morphological changes in the root, mycelia mantle or large fungal fruit bodies, which when removed, stained and examined, can be observed (Kendrick, 1992). Arbuscles may be hard to identify under normal conditions even though they have distinct features (Hawley and Dames, 2004).

Based on the morphological aspects, two AMF types have been identified and are differentiated by the occurrence or absence of intercellular hyphae in the intercellular spaces of the root cortex (Smith and Smith, 1996; Smith and Smith, 1997). In the past, the step to colonization with

AMF formation was largely divided into two types: Arum-type and Paris-type (Brundrett *et al*., 1996; Smith and Smith, 1997; Brundrett, 2004).

In the *Arum*-mycorrhizal type, highly ramified structures called arbuscules, are characteristically formed inside the cortical cells of the roots which are produced by the intraradical mycelium. On the other hand, in the Paris-type, hyphal coils are formed. There is no definite distinction between these two types, as many intermediary types can be seen (Dickson, 2004). The ecological, functional and taxonomic importance of these differences in Arum-Paris range has not been clearly elucidated (Dickson *et al*., 2007). Large storage cells known as vesicles are formed by various species of Glomeromycota. It is because of this that glomeromycotan fungi are at times called vesicular-arbuscular mycorrhizal fungi (VAM) (Santos-González, 2007).

The *Arum*-type has a significant intracellular stage of hyphal development and creation of terminal arbuscles on intracellular hyphal branches, while the *Paris*-type has a broad intracellular hyphal development giving coils of hyphae (Smith and Smith, 2008). In the Arum-type, arbuscules are formed when intercellular hyphae run beside longitudinal grooves between cortical cells in a straight line before penetrating the cortical cells. In the Paris-type, the intracellular hyphae develop inside cortical cells as coils. These morphological types of AMF structures have been proposed to vary in the areas where metabolic activity is performed, even though they have the same degree of root infection. Nutrients are principally released at the arbuscules in the Arum-type, while it is not yet determined whether this occurs in the hyphal coils and arbuscules in the Paris-type (Van Aarle *et al*., 2005).

Paris-type colonisation strategies have been found to be more common than the Arum-type possibly because the Paris-type lives longer than the Arum-type (Smith and Read, 2008). This may mean that Paris-type hyphae had a longer life span and are more resistant to stressful environmental situations such as dryness (Hawley and Dames, 2004). At present, only Ranunculus is acknowledged to have varied species within the same genus, forming either Arum or Paris type. In the majority of cases, plants form Arum-type mycorrhizal colonization, while a lot of trees and forest herbs form the Paris-type. A large proportion of experimental studies use crop plants, and because of this, the Arum-type has been largely considered to be the most frequent (Dickson *et al*., 2003). It is suggested that the Arum-type grows in roots that have uninterrupted longitudinal air spaces in their cortices. The presence of these air spaces most likely augments the easy growth and extension of intracellular hyphae and causes fast chronological penetration of cortical cells and development of arbuscules. Consequently, the intermediary AMF morphology observed between Arum- and Paris-types might be due to the presence of restricted or interrupted intercellular spaces in the roots, or dissimilarities between inner and outer cortices (Smith and Smith, 2008).

Arbuscular mycorrhiza fungi (AMF) and plants

Infective propagules of AMF include the resting spore of the fungus, germinating or extra radical hyphae in the soil or hyphae that are associated with the root fragments. These propagules are the sources where fungal growth can commence (Brundrett *et al*., 1996). Both the root and the adjacent soil are colonized by fungi which help to increase the reach of the plants root system (Smith and Gianinazzi-Pearson, 1988; Habte, 2000). In the soil, AMF are found as spores or vegetative propagules in the root fragments. Specific fungal propagules obtained from an annual plant can easily propagate on a perennial plant because of the reported lack of host specificity demonstrated by species of AMF. In the same way, a different group of AMF species can colonize a particular plant root system (Wilson, 1984). The roots of most plants in the terrestrial ecosystem establish a symbiotic association with mycorrhiza fungi.

Among all the mycorrhizal associations, AMF is most commonly associated with plants (Wang and Qiu, 2006). In natural ecosystems, plant competition and succession is found to be influenced by AMF which can affect the functions and stability of an ecosystem (Wilson and Hartnett, 1997; Van der Heijden *et al*., 1998a; Van der Heijden *et al*., 1998b; Wilson and Hartnett, 1998; Smith *et al*., 1999).

The competitive balance between obligately-mycorrhizal, facultatively-dependent and non-dependent plant species can be affected by the presence or absence of AMF in soils. This will in turn influence the species composition and productivity of the ecosystem (Entry *et al*., 2002).

J. procera **and arbuscular mycorrhiza fungi (AMF)**

Little study was done on *J. procera* and its relationships with AMF in Saudi Arabia. However, in the dry Afromontane forest of Ethiopia *J. procera* has been infected by AMF (Wubet *et al.* 2003, Wubet *et al*., 2006). The presence of oval to elongated vesicles in *J. procera* was also observed (Dodd *et al.,* 2000).

It is important to carry out preliminary study on the ecological aspects of AMF in the woodlands of *J. procera,* to understand the types inoculums density of AMF in the soil and the presence of AMF in the roots of *J. procera* in this region.

CONCLUSION

The widespread destruction of *J. procera* throughout the Middle East is worrisome as only isolated patches of the forest are left behind. Several efforts to regenerate the forest have failed due to lack of understanding on the habit of forest plantation. Basic knowledge on the soil, plant, climate, microorganism and interactions effect of these factors are essential to ensure survival and the success in replantation of the forest in the future. In the future, utilization of suitable mycorrhizal fungi should be considered when carrying out efforts to preserve these threatened species and re-establish the degraded ecosystem.

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