Structural colonization of Arbuscular mycorrhizal fungi in three acacia species of different sizes in Riyadh, Saudi Arabia

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Abstract

In this study, we investigated the status of Arbuscular mycorrhiza fungi (AMF) living natural symbiotically with different sizes of the acacia. Root colonization and infection of different sizes of Acacia tortilis, A. ehrenbergiana and A. gerrardii with AMF varied irrespective of tree species and size. The overall highest infection was recorded in A. ehrenbergiana medium size at Raudhat Khuraim site (70%) followed by A. tortilis short size (60%), A. gerrardii medium size (58.7%) and short size (57.7%) at Washlah site. The lowest infection was found in A. gerrardii large size (6%). The maximum vesicles were found in A. tortilis large (95%) followed by A. ehrenbergiana medium (91%) and short (97.7%) at Khuraim. The lowest infection was found in large size A. tortilis medium (67.3) at Washlah, A. gerrardii and A. tortilis short size (51.7), (50.0) at Khuraim and Huraymila. The minimum was recorded with A. gerrardii medium (4%) in Khuraim. The highest arbuscular infection was recorded with short size A. tortilis medium (4%) in Khuraim. The highest number of spore was observed in medium size A. gerrardii at Washlah (230) and the lowest number (21) in short size A. gerrardii in Khuraim. In conclusion, our results indicate high infection of AMF in the rootsof short acacia sizes followed by medium size and least with large size trees.

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Introduction
Land degradation and low vegetation are increasing every day due to environmental factors, climate change and human activities. Among different factors, symbiosis relationships, low soil fertility and water scarcity. Arbuscular mycorrhiza fungi (AMF) are important soil microbial community currently for the trees by increasing supply of the water, increase drought tolerance and help in increasing uptake of slow release nutrients especially in phosphorus deficient soils and help plant establishment and growth in harsh environments.

The beneficial associations of microbes, studies examining are obligate symbiont and survive in exchange for carbon from plant hosts, the association is essential for plant ecosystem function (Compant et al., 2010) because the great majority of plant species depend on it for mineral nutrient uptake. This task is efficiently performed by the extensive extra radial mycelium of the fungal symbionts. Within root cells Arbuscular mycorrhizal (AM) fungi form typical tree-like structures, the arbuscules or hyphal coils. Some also produce storage organs, termed vesicles which form a monophyletic group in the true fungi, the phylum Glomeromycota (Redecker & Raab, 2006).

Colonization percentage naturally high infection in Saudi Arabia (SA) with Arbuscular in Petunia hybrida and Gaillardia pulchella shrubs roots (Al-Qarawi et al., 2012), low infection with old Acacia gerrardii tree (Alnohait, 2015) due to plant species and root physiology, less finer attacked roots, carbon sufficient and availability. Fungal inoculation in nursery was very low infection compared to the native infection (Manaut et al., 2015), low growth of Acacia gerrardii inoculated with AMF during seedling time under nursery conditions (Hashem et al., 2016) could be due to inability to adapt during short time and unfavorable conditions. Better AMF inoculation effects result when inocula are composed of native fungi instead of exotics.

No works have been done in Saudi Arabia in different aspect of AMF particularly in Acacia tortilis, Acacia ehrenbergiana and Acacia gerrardii with different sizes (heights) for each species. It is urgently needed to perform some studies in relation among studied Acacia species and their classes to the practical application of mycorrhizal fungi in Saudi Agriculture, Range land and Forestry.

Materials and methods
Screening and collection of soil and roots samples
Soils and roots were collected during the period from January to March 2016 from three sites namely, Washlah, Raudhat Khuraim and Wadi Huraymila at Riyadh, Saudi Arabia (Fig. 1). Soil samples and fine roots were collected from the rhizosphere of randomly selected Acacia tortilis, Acacia ehrenbergiana and Acacia gerrardii with heights of 1-1.5, 1.5-3 and more than 3 meters from each site. Samples were wrapped in polyethylene bags in icebox and brought to the laboratory, where they were incubated at 4°C until further processing. Soil samples were carefully removed from the plant roots and preserved in refrigerator at 4°C for further use and analysis for major soil properties. The roots were carefully cleaned and immersed in 50% ethanol for later use.

Fig. 1. Map of the study site and sampling location.

Physico-chemical properties of soils
Physico-chemical analyses were conducted on air-dried soil samples which were cleaned from plant residues, roots fragments and rocks larger than 2mm.
Selected physico-chemical properties were explored by following the methods: Soil particle size distribution methods of (Sharpley et al., 2004), pH and Electrical conductivity (EC) in 1:1 (W/V) in soil: water suspension using pH meter and (Rowell, 2014), CaCO$_3$ content by volumetric method (Kassim, 2013), available K$^+$, P, soluble ions Cl$^-$, CO$_3^{2-}$, SO$_4^{2-}$, soluble cations Ca$^{2+}$, Mg$^{2+}$, Na$^{+}$, K$^+$, (Page et al., 1982). O. M estimated by method of (Salehi et al., 2011) and total nitrogen by the Kjeldahl method were measured as described by (Wi, 2005).

Assessment of AMF root colonization

Fine and feeder roots along with rhizosphere soils of A. tortilis, A. ehrenbergiana and A. gerrardii from all three sizes (1-1.5, 1.5-3 and more than 3m) were collected. Roots were washed and cleaned from soil and debris and preserved in 50% alcohol. Root segments of a length of approximately 1cm were chopped for AM fungal structural analysis. The washed and cleaned root segments were placed in 10% KOH and heated to 90°C for 30-60 minutes depending on the color and thickness of the roots. They were then washed in distilled water and immersed in 3% H$_2$O$_2$ for 5-10 minutes. After that, they were washed in distilled water and acidified with 5N HCl for 2-3 minutes.

The root segments were stained with 0.05% trypan blue in lacto glycerol (1:1:1 lactic acid, glycerol and water) for 30 minutes at 90°C in a water bath. Excess stain was removed with clear acidic glycerol (Phillips & Hayman, 1970); (Koske & Gemma,1989). Fungal colonization was mounted in lacto glycerol solution on glass slides. Ten segments were mounted on each slide. After segments mounting, the cover slip was gently pressed to facilitate the observation of different types of structures present in the whole root segment under compound microscope. A minimum of 50 segments from each sample were observed for the assessment of structural colonization of AM fungi associated with roots.

Presence of mycelium and coiled hyphae, vesicles, as well as Arbuscular was recorded and analyzed to determine the structural colonization.

To estimate the percentage of mycorrhizal colonization, intensity of infection (mycelium, vesicles and Arbuscular development) in the infected region of the roots was estimated in root samples stained for total infection as described previously (Giovannetti & Mosse, 1980).

A root segment was considered to be infected if it showed mycelium, coiled hyphae, vesicles, Arbuscular, or any other combination of these structural characteristics of AM fungi. The intensity of colonization was classified as poor, moderate and abundant type of colonization with each of the individual structure (Dhar & Mridha, 2012).

Depending on the presence of mycelium, coiled hyphae, vesicles, Arbuscular in a sample, the intensity of infection of AM fungi was estimated as poor if only mycelia were present, moderate if mycelium and vesicles or Arbuscular were present and abundant if mycelium, vesicles and Arbuscular were present. Mycelial colonization was regarded as total AM colonization. Percent colonization was calculated by following formula:

\[
\% \text{ Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segemets studied}} \times 100
\]

Spore extraction and identification

Spores were separated by wet sieving and decanting method (Gerdemann & Nicolson, 1963). Soil samples were air-dried before extraction, counting and identification of AM fungal spores. From each sample, 100g soil was taken in a bucket of 1-litre capacity and 800ml of water was added to the soil. The soil was mixed well with water to make a soil-water suspension. Depending on soil physical structure (clay or sandy), the suspension was left for 3 (for sandy soil) to 5 minutes (for clay soil) for settling down of insoluble and heavy soil particles.

The suspension was passed through the ASTM-60, ASTM-100, ASTM-240 and ASTM-400 sieves gradually to extract the spores. The residues of the individual sieves were washed with water jet and collected individually in a small beaker by backwashing.
Fifty percent of the washed solution were used for isolation of the spores through centrifugation at 2000rpm (Gerdemann & Nicolson, 1963) for 2 minutes. The other 50% of the solution were used for filtration method to have intact spores with morphological structures.

The individual collection of spore suspension was filtered through gridded Whatman filter paper No.1 (to facilitate easy counting of the spore) placed in a funnel fitted with conical flask (vacuum). The supernatants of the sieves were examined under stereo-binocular microscope to observe the presence of sporocarps and larger spores. After water filtration, the paper was examined under the stereo-binocular microscope at 2.5x10 magnification and the number was counted. The total number of spore population in each individual sample was calculated per 100g dry soil basis. Spores were identified on the basis of morphological characters under compound microscope mounting in water, lacto glycerol, PVLG and PVLG + Meltzer’s reagent (Morton, 1988); Morton and Benny (1990). The identification was based on spore color, size, surface ornamentation and wall structure with online references of species description (Schüßler & Walker, 2010) Brzostowicz, 2011; Mirzai & Noorbakhsh, 2014) as well as originally published species descriptions, wherever possible.

**Statistical analysis**

Field data of this study were analyzed as factorial experiment in CRBD using SPSS software (Version 22). Means were separated by using Less Significant Differences (LSD) and Tukey’s test at (p ≤ 0.05).

**Results and discussion**

**Physico-chemical properties of soils**

Physico-chemical properties of soils are given in (Table 1A & B). The results showed that texture classes of soil samples generally, ranged from sandy loam to clay loam and sand with slightly alkaline to alkaline pH (7.5–8.27). Soils collected from the rhizosp here of medium-size A. ehrenbergiana at Huraymila recorded the highest pH value (8.27) and lowest soil pH value (7.51) was found at the rhizosp here of large size (>3m) trees of A. gerrardii at Washlah site. The similarity of the pH values of soils in from different sites indicate limited leaching and slow rates of weathering and soil development in of the arid region accompanied by high soil content of calcium carbonate. These values are within the range reported by McCauley et al. (2009) for macronutrient availability for plant growth (between 6.5 and 8.5). The EC ranged between (0.13 –1.7) dS.m⁻¹. These EC values indicate that the soil is suitable for normal plant growth. The studied soils were calcareous in nature (32.5 % in average). In general, it was high in Was hlah and Huray mila sites and low in Raudhat Khuraim site. On the other hand, the soils were poor in their organic matter content. These findings agree with that of Yasir et al. (2015). However, soils organic matter content of tree rhizosp here was relatively higher (from two to four times compared to bare soils) possibly resulting from root exudates and microbial activities. Soil Na⁺, Ca²⁺, Mg²⁺ and K¹⁺ ions were the most dominant cations, while Cl⁻ and SO₄²⁻ ions were the most dominant inions. This means that dry conditions and limited rainfall has led to an increase in the concentration of salts and carbonate in the surfaces layers. Moreover, low amount of rainfall also led to reduced vegetation cover in the bare soils reflecting to the low of soil organic matter. On other hand, the soil was characterized by high levels of available P, which ranged from 0.35 to 22mg/kg and K (41.43 to 278mg/kg). Studied soils were characterized as poor in total nitrogen percentage, which ranged between 0.01 to 0.31%. In general, the arid conditions and the low vegetation cover affected soil properties.
The colonization of fine Acacia roots by AMF is shown in Fig. 5. The percentage infection in the roots of different species with the mycorrhizal fungi varied significantly (P < 0.05) (Fig. 2, 3 & 4).

Assessment of AMF root colonization

A wide and independent variation was recorded irrespective of acacia species and their sizes. The mycelium infection was regarded as the total infection of each of the three tree species and their heights in all sites.
Fig. 2. Graphs showing percent root colonization of (A) Mycelium (B) Vesicle and (C) Arbuscular in different size class of three Acacia species in Washlah. The range of variation of total infection was 6%-70%. The overall highest infection was recorded with A. ehrenbergiana medium size at Raudhat Khuraim site (70%) which was followed by A. tortilis short size (60%), A. gerrardii medium size (58.7%) and short size (57.7%) at Washlah site, and the lowest infection was found with A. gerrardii tall size at Raudhat Khuraim site (6%). The range of infection with vesicles was 4% with A. gerrardii medium size at Raudhat Khuraim and 95% with A. tortilis tall size.

Fig. 3. Graphs showing percent root colonization of (A) Mycelium (B) Vesicle and (C) Arbuscular in different size class of three Acacia species in Raudhat Khurain.

Fig. 4. Graphs showing percent root colonization of (A) Mycelium (B) Vesicle and (C) Arbuscular in different size class of three Acacia species in Huraymila.
The maximum vesicles were found with *A. tortilis* large size at Huraymila 95%, followed by *A. ehrenbergiana* medium size (91%) at Khuraim, *A. tortilis* medium size (67.3) at Washlah, *A. gerrardii* short size (51.7) at Raudhat Khuraim, *A. tortilis* short size (50.0) at Huraymila, *A. gerrardii* and *A. tortilis* tall size (48%) at Washlah and Huraymila respectively, and the minimum was with *A. gerrardii* medium size (4%) at Khuraim. In the case of total infection with Arbuscular, the highest percentage of infection was recorded with *A. tortilis* short size (97.7%) and the second highest infection with Arbuscular was recorded with *A. tortilis* medium size (51.7) at Huraymila, *A. tortilis* short size (50%), *A. ehrenbergiana* large size (49%) at Huraymila and *A. gerrardii* short size (46.7%) at Washlah, and the lowest infection was found with *A. tortilis* tall size (4.7%) at Washlah site.

The intensity of infection in individual tree species with mycelium along with coiled hyphae, vesicles, and Arbuscular was estimated as poor, moderate and abundant in each case. The intensity of infection varied significantly in each individual tree species (Table 2). The samples, which had high total infection percent, may not show the high intensity of infection with vesicles and Arbuscular. It varied from tree species to another and their sizes. Intensity of infection with mycelium, the maximum infection as poor, moderate and abundant types was recorded with *A. tortilis* large size (41%) and (50%) at Huraymila, *A. ehrenbergiana* tall size (47%) at Raudhat Khuraim and the minimum was recorded with both *A. gerrardii* short size (2%) (1.5%) at Raudhat Khuraim, *A. gerrardii* short size (4.7%) at Washlah respectively.

**Fig. 5.** Photomicrographs of structural colonization of AMF in the roots (a) subtending hypha (SH), and vesicles (V); (b) (M) mycelium and Arbuscular (AR); (c) running hyphae (RH) and (d) vesicles.
In the same way, the intensity of infection with vesicles, the highest percentage of poor, moderate and abundant types was found with A. gerrardii short size (46.7%) at Huraymila, A. tortilis short size (47.3%) at Washlah. Both of A. tortilis short size at Washlah and A. ehrenbergiana medium size at Raudhat Khuraim were recorded as (25%). A. gerrardii tall size (3%) at Huraymila, A. ehrenbergiana short size (1.5%) at Raudhat Khuraim and A. gerrardii tall size (1%) at Huraymila showed minimum intensity of infection. While these density percentages of infection by Arbuscular were very weak, highest percentage of moderate type was recorded with A. tortilis short size (27.3%) at Washlah and lowest density percentage found with A. ehrenbergiana tall size and A. gerrardii tall size (1%) at Huraymila.

The mycorrhizal colonization for the selected acacia species with different size (heights) growing at Riyadh was studied for the first time for their structural colonization with AMF. (Al-Qarawi et al., 2012) studied soil and root infection of some plants such as Calendula officinalis, Catharanthus roseus, Convolvulus arvensis, Cynodondactylon, Petunia hybridra, Gaillardia pulchella, Oeimum sanctum, Phoenix dactylifera, Sesuvium portulacastrum and Tagetes patula in Riyadh, Saudi Arabia. Infection percentage and density varied significantly and independent variation was recorded in some plant species. Similarly, (Khalil, 1989) isolated Glomus fasciculatum and G. mosseae from the soil.
Also, (Hashem et al., 2016) reported that Claroideoglomus tunicatum; Rhizophaga intraradices and Funneliformis mosseae occurred at cultivated soil of Acacia gerrardii under greenhouse conditions.

**AMF spore population**

Spore population varied from 21- 230/100g in dry soils irrespective of plant species and their sizes (height). The highest spore population was recorded with A. gerrardii medium size at Washlah (230) and the lowest of 21 spores was observed under A. gerrardii short size at Khuraim site. Out of the different species of AMF recorded during the present study, *G. mosseae, G. intraradices, F. mosseae and G. etunicatum* were found and a few spores were unidentified.

Photomicrographs in (Fig. 6 A-F) show some spore morphology of AMF (*F. mosseae, G. intraradices, G. etunicatum*).

Al-Qarawi et al. (2012) reported that *G. fasciculatum* and *G. etunicatum* were dominated soil samples similar to those studied in the present investigation. However, (Dai et al., 2013) found that wide variation among samples for spore populations and AMF in soils from the different land use types and ecozones in Canada. Different mycorrhizal fungal structures like mycelium, coiled hyphae, vesicles, Arbuscular, and spores inside the root segments were found in most of the studied plant species. The present work is considered the first to study different acacia species with different heights at Riyadh, Saudi Arabia.

**Fig. 6.** (A-F) AMF Spores were isolated from soil samples A (10X): Funneliformis mosseae, (B - C) (10X): *G. intraradices*, D (10X): *G. etunicatum* and (E-F) (40X) Unknown spores.

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**References**


