Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH THE RHIZOSPHERE OF *PHOENIX DACTYLIFERA* L. IN SEMI-ARID SOILS.

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ABSTRACT

In order to survey biodiversity and root colonization of arbuscular mycorrhizal fungi (AMF) on date palm (*Phoenix dactylifera* L.) tree, the present study was undertaken in semi-arid areas of Jaipur district at fifteen sites. The genera identified were *Gigaspora, Glomus, Scutellospora, Entrophosphora* and *Sclerocystis*. The root colonization by AM fungi varied significantly, ranging from 78% to 93% in date palms rhizosphere. *Gigaspora* species were found in abundance and their occurrence frequency ranged from 60-70%, *Glomus* species from 40-50%, *Sclerocystis* upto 30%, *Scutellospora* from 10-20% and *Entrophosphora* upto 10%. Overall evaluation revealed that the root colonization and the spore population varied to different extend. The AM colonization and number of spores in date palm roots were found to be negatively correlated with soil phosphorus content and electric conductivity of soil samples and positive correlation between number of spores and root colonization.

KEYWORDS: **Diversity, AM fungi, date palms, physico- chemical parameters.**

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INTRODUCTION

Mycorrhizae, the mutual symbiotic relationship between soil fungi and plant roots are found associated with more than 90% of terrestrial plants, distributed in all climates types and ecosystems regardless of soil type, vegetation and environmental conditions[1]. Among the different types of mycorrhizae, arbuscular mycorrhiza is one of the most common and frequently occurring endomycorrhiza all over the world which are non-pathogenic obligate symbionts. AM has an important role in greater soil exploration, increasing the absorption of nutrients P,N, K, Zn, Cu, S, Fe, Mg, Ca and Mn and the supplying them to host roots from the soil of all ecosystems. AM fungi belong to phylum Glomeromycota which include more than 10 genera viz.: *Acaulospora, Archaeospora, Diversispora, Enthrophospora, Glomus A, Glomus B, Gigaspora, Sclerocystis, Scutellospora, Paraglomus*, *Pacispora*[2]. Identification of individual Glomeromycotan fungi can be done by studying characteristic root morphology[3] and important morphological features of spores and vesicles that include variation in size, shape, thickness of wall, Number of layers, their position and presence, branching patterns of hyphae, the diameter and makeup of hyphae near entrance points.Presence of arbuscular mycorrhizal fungi (AMF) is very eco-friendly as fungi receive carbohydrates from plants and plants in turn get benefited through various ways like increase in nutrient absorption capacity and combating various cereal pests and pathogens, thus decreasing the use of synthetic pesticides and fertilizers. The AMF found globally establishes a symbiotic association with the majority of land plants including those of the arid areas[4]. AMF enhances root mineral nutrition, especially phosphorus, and support plant growth, defend plants against environmental stress such as metal toxicity, soil salinity[5] and drought[6] and amplify plants tolerance to pathogens thereby acting as a bio-control agent. The fungal hyphae extend into the soil from host roots and improve the efficiency of nutrient uptake, such as immobile phosphate ions[7]. Colonization is restricted to root cortex and does not enter the vascular cylinder. Mycorrhizal plants show resistance to all types of environmental stresses. Spore quantification has been extremely useful for evaluating the level and diversity of mycorrhizae because spores are resistant to unfavorable conditions[8].

Fungi has a wide vital function in several ecological and microbiological processes, effecting soil fertility, cycling and decomposition of minerals and organic matter, as well as plant health and nutrition status[9]. Moreover, variation in the population of these fungi and their symbiosis with plant roots is related to host plant as well as soil properties. The occurrence of AMF in diverse ecological regions and their relations to soil properties and local plants have been investigated. There is restricted knowledge about mycorrhizal status of palms in arid soils. Thus this study was carried out assessing VAMF associated with date palms. In the arid and semi-arid regions of Rajasthan, date palm trees are considered vital to the ecosystem as they protect the surrounding vegetation against desert influences and provide an adequate microclimate to the under storey crops. The association of VAM with rhizosphere of date palms was found to promote growth especially on nutrient deprived soil and was absolutely important for their establishment and survival[10]. Keeping this in mind, for developing better inoculants of AM fungi as well as requirement of better understanding of its occurrence and characterization in economic crops, the present investigation was undertaken to survey and evaluate VA mycorrhizal status of date palm roots at Jaipur district, Rajasthan, India.

MATERIALS AND METHODS

(i) Study site and sampling

An intensive survey on occurrence of AM in date palm rhizosphere was done in arid zone of Tordi region located in the Jaipur district. Fifteen different sites were surveyed in January 2013 and the collected samples were preserved at 4^{o}C until further use. For each tree, three rhizospheric Soil and plant root samples were collected at single site from

surface to 30 cm depth, and mixed together. Root samples were fixed in FAA and fine roots from each sample were selected, washed with tap water and then root colonization was determined. Then the soil samples were air dried at room temperature for spore counting.

(ii) Physical and chemical analysis of soil

Soil samples from each location were analyzed for their physical and chemical properties. Soil properties measured were the pH in 1:1 water, available phosphorus[11], nitrogen (N- NH4 N- NO3) [12], soil texture and organic matter. This later soil property was indirectly measured by comparing the dry weight after 6 hrs at 105°C with the dry weight after 4 hrs at 550°C, and soil texture.

(iii)Spore extraction, Root colonization and characterization of VAMF

AM Spores were isolated using wet sieving-decanting and sucrose gradient techniques[13]. For this100 g of rhizospheric soil of each sample was rinsed in through a sequence of 850, 500, 250, 100 and 50 um sieves; soil material was recovered from each sieve, suspended in water, and centrifuged at 3000 RPM for 3 minutes, followed by removal of the supernatant and the soil material was re-suspended in a sucrose solution (60%) and centrifuged at 1000 RPM for 2 minutes. The supernatant containing spores was filtered through filter paper. The spores were recovered under the dissecting microscope, separated according their morphology and evaluated for their respective abundance. The number of spores was expressed as the mean of three replicates. Roots were rinsed with distilled water to remove adhering soil and debris. Improved procedure [14] for clearing root and staining AM fungi for was used. For this the root system was cut into 0.5 to 1cm segments, heated at 90°C for 10 min in 10% KOH, Then cooled and KOH was neutralized by addition of 1% HCl. After washing the roots with tap water, roots were stained with 0.5% trypan blue in lacto glycerol (lactic acid: glycerol: distilled water 2:2:1) solution for 30 min. After decanting the dye, roots were distained by the addition of lacto glycerol solution. Later root segments were mounted on slides for microscopic examination and then percentage of root colonization was calculated. Based on morphology and colouration, Species identification was made using the taxonomic keys of http://invam.caf.wvu.edu and Schenck and Perez[15]. The diversity of VAMF was found by studying the species frequency and richness (total number of species per site). All statistical analysis was done using SPSS statistics software. A correlation analysis was done to study the relationships between different soil properties and mycorrhizal colonization.

RESULTS

An intensive survey done in Jaipur district to investigate the distribution, population and occurrence of AM fungi in association with *Phoenix sylvestris* L. revealed that the occurrence of different AM species varied up to a certain extent. The study of the presence and the abundance of mycorrhizal symbionts in the rhizosphere of the cultivated date palm trees was an important step to assess the diversity and richness of the community of AM fungi. Mycorrhizal richness was observed at all sites studied. Arbuscules, vesicle and sporocarp were detected in almost all date palm root sampling surveyed. Sustained detection of AMF structures inside date palm roots harvested confirms the arbuscular mycorhizal status of date palm. Total 11 species were isolated from 15 localities and regarding species richness, they occurred in the following descending order (Table-1) –

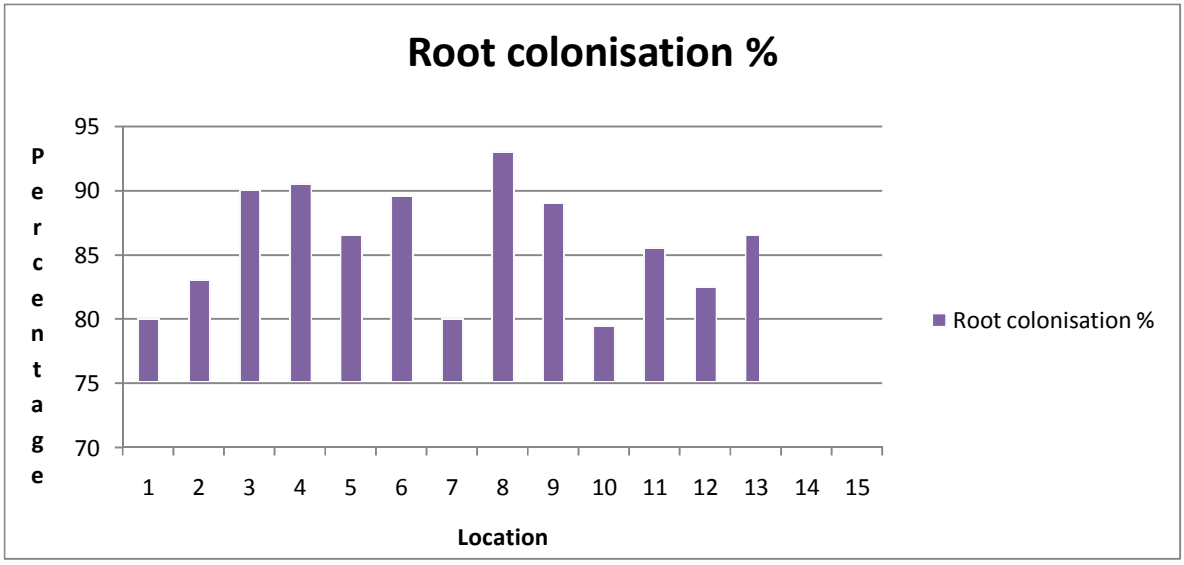
Gigaspora dicipiens > *Gigaspora gigantea* = *Gigaspora albidia* > *Glomus fasciculatum* = *Glomus mosseae* > *Glomus microcarpum* > *Scutellospora erythropa* > *Sclerocystis coreimoides* = *Scutellospora nigra* = *Scutellospora heterogama= Entrophosphora infrequens. Gigaspora* species were found in abundance sequenced by *Glomus*, *Scutellospora, Sclerocystis and Entrophosphora.*

Table1
Following Genera of VAM fungi was identified in different areas

Site no.	Site under study	VAM Species identified
1.	Tordi	*Scutellospora nigra, Glomus fasciculatum*
2.	Bhagwanpura	*Gigaspora dicipiens, Gigaspora albidia, Gigaspora gigantea*
3.	Chandsen	*Glomus mosseae*
4.	Diggi	*Glomus microcarpum, Glomus fasciculatum, Scutellospora erythropa*
5.	Chaprana	*Entrophosphora infrequens, Glomus microcarpum*
6.	Ambapura	*Sclerocystis coremioides, Gigaspora dicipiens*
7.	More	*Gigaspora gigantea, Glomus mosseae, Glomus fasciculatum*
8.	Kukad	*Glomus fasciclatum, Gigaspora gigantea*
9..	Rupayali	*Gigaspora dicipiens*
10	Pagdi	*Scutellospora erythropa, Scutellospora heterogama*
11	Ganwar	*Entrophosphora infrequens, Glomus fasciculatum, Glomus mosseae*
12	Pachewar	*Scutellospora hetrogama, Scutellospora nigra, Gigaspora dicipiens*
13.	Parli	*Sclerocystis coremioides, Glomus mosseae, Gigaspora albidia*
14	Sewa	*Gigaspora dicipiens*
15	Avikanagar	*Entrophosphora infrequens, Glomus mosseae, Gigaspora decepiens.*

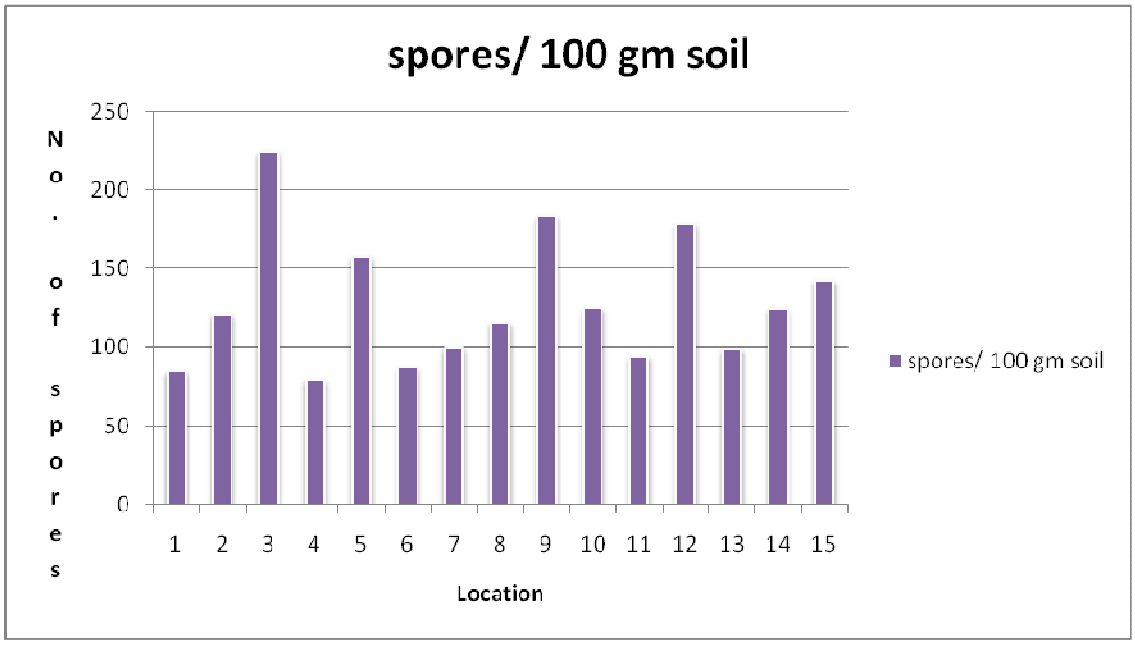
Physico-chemical analysis of collected soil samples was done. After the application of ANOVA on the Organic Carbon content of soil sample collected from different sites it was found that the locality1, 3, 4, 5,6, 7, 8, 9, 10 are showing significant variation and the rest of the location did not show any significant variation. On organic matter content the same results was found. Electrical conductivity did not show any significant variation with any of the localities.

Figure 1
Graph for Root colonization percentage of AM fungi



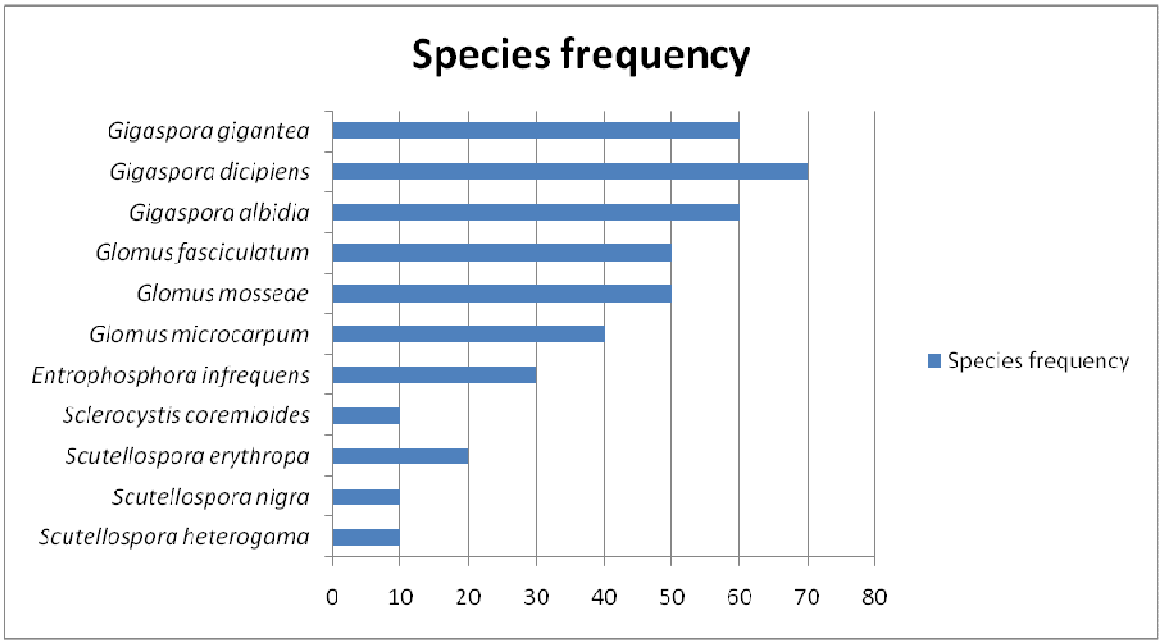
The results of VAM root colonization showed quite variation at different sites under study. The root colonization percentage was found higher at site no 3, 4, 6, 8 and 9. However the sites 2, 5, 11, 12 and 13, 14, 15 did not show much variation among them. The least root colonization was reported at site 1, 7 and 10. After overall evaluation we can say that there was significant variation (Figure 1).

Figure 2
Graph for no. of spores per gram of soil sample collected from different sites:



The results showed quite variation at different sites under study. The highest numbers of spore were found at the site No 3, 5, 9 and 12. However the sites 2, 6, 7, 8, 10, 11, 12, 13, 14 and 15 did not show much variation among them. The least no. of spore was reported at sites 1 and 4. After overall evaluation we can say that there was significant variation ranging from 70 to 230 (Figure 2).

Figure 3
For AM species richness of the soil collected from different sites



DISCUSSION

Survey, study and analysis of the date palm rhizosphere have confirmed the occurrence and association of AM fungi with the roots harvested from site under study. It was found that AMF naturally occurred uniformly through out in the date palm rhizosphere at all the fifteen sites surveyed with almost relatively similar frequency and standard root colonization intensity. Such findings are in collaboration with previous studies[16]. The negative correlation was found between the available P and percentage of AMF root colonization, so adaptation of AM to low phosphorus soil was confirmed[17]. The variations in the spore density was found but that may be attributed to the difference in the microclimate of the rhizosphere[18] to microbiological and physico-chemical properties[19] and also to season in which sample was collected[20]. A significant relationship was found between soil's chemical and physical parameters and number of spores, as well as between these factors and percentage root colonization.

Gigaspora species are found in abundance followed by *Glomus* species, *Scutellospora* varies from 26.26 % and *Entrophosphora infrequens. Gigaspora* species were found in abundance and their occurrence frequency ranged from 60-70%, in *Glomus* species from 30-50%, in *Scutellospora* from 10-20% and in *Sclerocystis and Entrophosphora* it was around 0.09 % (Figure 3.). Overall evaluation revealed that the root colonization and the spore population varied to different extend. The consistent distribution of various species of mycorrhizae in rhizosphere of date palms throughout the 15 collecting sites, with relatively constant frequency and root colonization intensity revealed that arbuscular mycorrhizael association is naturally established and this result is similar to the results reported earlier[12, 21]. The relatively important root colonization levels analyzed in date palm roots harvested in February were associated to high spore density and reasonable species diversity. Similarly in semi-arid zones, AMF populations usually reached their maximum during the rainy season i.e., the period between October and May[22]. As also shown in the present study, the AM fungi differs in their infection potential, colonization and number which as it depends on the host and the inter species competition. A previous study on the cultivated date palm trees concealed the presence of arbuscular mycorrhizal fungi and their association in all root samples analyzed also reflected the mycotropic nature of this tree. The intensity of colonization varies from one locality to another. Colonization of the rhizosphere of date palm tree by AM fungi can be improved by the organic matter content of the soil. According to the previous study performed by various researchers[23] it was concluded that the content of phosphorus in the soil is the determining factor for the development and presence of AM fungi. The intensity of AM root colonization was found higher at sites Tordi, Bhagwanpura, Chandsen, More and Rupayali, contrary the phosphorus content was found low at these localities, which reflects the adaptability of AM fungi. Spore densities of AM fungi collected from the rhizosphere of date palm trees was highly variable, the highest value recorded was at the sites 6 and 23 and the lowest value was recorded at localities 1, 7 and 22 i.e. Tordi, Diggi and Ganwar. In general, fluctuation in AM spores number would be related to the process of spore formation, their germination and degradation[24]. They also depend on microclimatic variation[18] and the soil's physical and chemical properties[19]. In general the results have shown that there is no as such relationship between the number of spores and intensity of root infection as reported by various authors[16.]

Out of the eleven species isolated and identified, three belonged to *Glomus*, three to *Gigaspora*, three to *Scutellospora,* one to *Entrophosphora* and one to *Sclerocystis*. *Gigaspora* species were predominantly ranging in frequency from 60 to 70%. Because of their dominance under those ecosystems, species of *Gigaspora* have been considered as the best adapted genus for habitats subjected to drought and soil salinity stresses[25]. From this study 11 AM species were isolated and identified viz., *Scutellospora nigra, Scutellospora erythropa, Scutellospora heterog*am*a, Sclerocystis coremioides,*

*Glomus mosseae, Glomus microcarpum, Glomus fasciculatum, Gigaspora gigantea, Gigaspora dicipiens, Gigaspora, albidi*a and *Entrophosphora infrequens* from rhizosphere of date palm. This species diversity was quite comparable to the one found with in several other arid and semi-arid adapted plant varieties as reported earlier[17]. If fungal species distribution is concerned, various AM species have been frequently detected in arid and semi-arid zones of Africa, America, and India[17, 24].

CONCLUSION

The present study indicated wide natural AM fungal diversity in the rhizosphere of date palms. Selection of those genera, which were naturally found to be predominant and present in all rhizosphere of date palms could be a better inoculant for mass multiplication which could be further used to enhance commercial date palm production. Because of their natural occurrence these inoculants will certainly prove better then usually selected strains of AM fungi.

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