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ORIGINAL ARTICLE

Seasonal distribution of Arbuscular Fungi spores and their root colonization in Fruit plants of Thar Desert of Rajasthan

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ABSTRACT
Symbiosis with arbuscular mycorrhizal fungi (AMF) is an effective survival strategy for plants growing in stressful conditions. The present study is aimed to evaluate the association of arbuscular mycorrhizal fungi in some fruit plants of Indian Thar Desert along with arbuscular mycorrhizal (AM) fungal population density in the rhizosphere soils of the fruit plants to investigate for qualitative composition of AM fungal species and their per cent root colonization. The results showed that the number of AM fungal propagules in fruit plans collected from different localities varied from 42.3-87.6 spores per 100gm soil. Because of the widespread nature of AM fungi, they occurred in almost all the soil samples but with a some variation in both the number and type of spores. Altogether, 10 AM fungal species were isolated belonging to the genera of Glomus, Acaulospora, Scalerocystis and Gigaspora. Glomus was observed to be predominant followed by Acaulospora in the rhizosphere soils of all the four fruit plants. The spore distribution, density and the composition of AM fungi were observed to be changed by environmental and physico -chemical factors. The AM spore population, percentage of root colonization and distribution varied by the fluctuations in moisture, pH and soil mineral nutrients availability such as N, P, K etc. The data revealed that phosphorous deficient soils appeared to have more number of AM fungal propagules, while the soils having high levels of phosphorus content harboured least number of AM fungal spore population.
Keywords*: Mycorrhiza, VAM Fungi, Fruit plants, Glomus mossae, Acaulospora laevis, summer, winter.*

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INTRODUCTION

Mycorrhizal fungi are key components of soil microflora and also interrelate with other microorganisms in rhizosphere. Mycorrhizal association changes several aspects of plant physiology, nutrition and physical properties of the rhizospheric soil. In a wide range of land plants shows different types of mycorrhizal association. AM fungi differ widely in the level of colonization in root system with vary in plant and in their impact on nutrient uptake and plant growth. For the abundance and distribution of VAM fungi in several plants have been studied in various parts of the world. The present work was undertaken to study mycorrhizal association in some fruit plants present in Thar Desert area of Rajasthan on the light of distribution and colonization. Thar desert is comprises broad range of climate factors i.e. dry environment, low rain fall, high temperature, low water availability etc. Despite the fact that plants have a range of biochemical and physiological pathways to deal with undesirable environmental factors, these mechanisms are often insufficient to withstand extreme and consistent levels of environmental stress [1]. Besides these conditions, but many plants combat and successfully established in this stressful environment by inhabiting their roots with mycorrhizal fungi. The mycorrhizal symbiosis between plants and arbuscular mycorrhizal fungi (AMF) is thought to be an important factor in plant resistance to such conditions. The goal of this research is to see whether environmental factors affect the spatial and temporal distribution of AM fungi associated with Thar Desert Fruit Plants and whether there are seasonal variation on AMF distribution.

MATERIAL AND METHOD

Collection of the sample - The plant samples used for the present study were collected from different areas of four districts includes- Jodhpur (Balotra, Boranada, Piparcity), Bikaner (Kismidesar rural, Sujandeshar, Gangashahar) and Jaisalmer (Kishanghat, Ramgarh rd., Ramkund), Barmer (Gehoon, Daroora, Venasar Naadi) district of Thar Desert of Rajasthan.

Coordinates of district wise sampling areas

District name	Area	Coordinates	
		Latitude	Longitude
Jaiselmer	1. Kishanghat	26°55'32.6"N	70°54'07.0"E
	2. Ramgarh rd.	26°55'12.3"N	70°54'16.8"E
	3. Ramkund	26°54'57.8"N	70°54'01.8"E
Jodhpur	1. Balotra	25°49'54.5"N	72°15'09.0"E
	2. Boranada	26°10'20.1"N	72°56'02.5"E
	3. Piparcity	26°23'29.2"N	73°31'43.4"E
Bikaner	1. Kismidesar Rural	27°58'34.3"N	73°19'48.8"E
	2. Sujandeshar	27°59'46.0"N	73°17'32.3"E
	3. Gangashahar	27°59'20.2"N	73°17'45.7"E
Barmer	1. Venasar Naadi	25°44'40.4"N	71°23'03.3"E
	2. Daroora	25°45'08.9"N	71°22'51.7"E
	3. Gehoon	25°45'49.6"N	71°21'49.2"E

Following fruit plants were selected for present study-*Moringa oleifera* L. (Sahjan), *Capparis decidua* (Ker), *Aegle marmelos* (Bael), *Tamarindus indica* (Imli). Plant materials were collected in two seasons i.e., winter (December-March) and summer (April-June). Root and soil samples of respective plant species were collected and placed in plastic bags. These samples were quickly transported to the laboratory and moisture content was estimated immediately by Oven Drying method.

Soil analysis and assessment for spores and root colonization - The soil pH was determined by an electric pH meter using the suspension with soil water of 1:2.5 (w/v) ratio. Oven dried soil used further for analysis of edaphic factors. Available nitrogen by micro-kjeldahl method, available phosphorus (P) and potassium by Jackson (1973) method was analysed. Mycorrhizal spores were separated from soil by wet sieving and decanting method of Gerdemann and Nicolson (1963) and eventually were recovered by sieve filtered onto whatsmann filter paper. The intact spores on filter paper were counted under a dissecting microscope. The spores were identified by the manual of Trappe (1962), Schenck and Perez (1987). The roots were separated from soil by sieving washed several times in water. Then the roots were cut into 1.0 cm pieces which were cleared (for removing the pigmentation) with 10% KOH and stained with 0.05% Trypan Blue, root staining method given by Phillips and Hayman (1970). Percentage of root length containing fungal hyphae, vesicles and arbuscules were determined under the stereomicroscope by Gridlineline intersect method of Giovannetti and Mosse (1980) using the formula given below-

$$\textbf{\% root colonization -} \quad \frac{\textbf{No. of mycorrhizal positive root segments}}{\textbf{Total no. of root segments observed}} \textbf{ X 100}$$

Data analysis - The relationship between AMF percent root colonization and spore density was examined using Pearson's correlation co-efficient by using XLSTAT software.

Table 1. – Location wise distribution of AM fungal species.

AM mycorrhizal species	Location			
	S1	S2	S3	S4
Acaulospora leavis	+++	-	+++	++
Acaulospora mellea	+	-	+	+
Sclerocystis rubiformis	+++	+	++	++
Sclerocystis microcarpus	+	-	-	+
Glomus fasciculatum	++	++	+	++
Glomus mossae	+++	+	++	+++
Glomus aggregatum	+	-	+	-
Glomus geosporum	-	-	+	+
Gigaspora species	++	-	++	+

S1-Jodhpur, S2-Jaiselmer, S3-Bikaner, S4-Barmer

+ shows presence and – shows absence

Table 2. - Association of different VAM fungal spores with different host plant.

S.No.	Host Plants	VAM fungi
1.	*Tamarindus indica*	*Acaulospora leavis, Gigaspora sp., Glomus mossae, Sclerocystis rubiformis, Glomus aggregatum.*
2.	*Aegle marmelos*	*Acaulospora sp, Gigaspora sp., Glomus mossae, Sclerocystis microcarpus.*
3.	*Capparis decidua*	*Acaulospora mellea, Sclerocystis rubiformis, Glomus sp.*
4.	*Moringa oleifera*	*Acaulospora leavis, Sclerocystis microcarpus, Gigaspora sp. Glomus fasciculatum, Glomus geosporum.*

Table 3. - physio-chemical factors in the rhizosphere soils of different district in summer season.

S. No.	Host plants	pH	Moisture (%)	Phosphorus (mg/100g)	Nitrogen (mg/100g)	E.C. mmho/cm
1.	*Tamarindus indica*	8.0	20	20.8	.31	.28
2.	*Aegle marmelos*	7.0	23	27.4	.25	.26
3.	*Capparis decidua*	7.2	18	30.5	.30	.24
4.	*Moringa oleifera*	8.3	30	20.5	.33	.35

Table 4 -physio-chemical factors in the rhizosphere soils of different district in winter season.

S. No.	Host plants	pH	Moisture (%)	Phosphorus (mg/100g)	Nitrogen (mg/100g)	E.C. mmho/cm
1.	*Tamarindus indica*	8.0	25	18.9	.31	.27
2.	*Aegle marmelos*	7.0	28	25.1	.23	.24
3.	*Capparis decidua*	7.2	21	26.7	.24	.22
4.	*Moringa oleifera*	8.3	35	18.2	.28	.31

RESULTS

Distribution of AMF - The study shows that VAM fungal species is closely related to host plant, soil parameter and plant distribution area. All of the sample sites had a wide range of species, but *Glomus mossae* and *Sclerocystis rubiformis* were present in nearly all of the soils. The significant variations in AM fungi composition found in the study sites may be attributed to edaphic influences and seasonal differences. Climate change has an effect on AMF distribution because it controls the occurrence of particular fungal strains in the soil. Cultural practices and vegetation in the study sites can also play a role in deciding a specific species' dominance. The parameter that were calculated, such as AMF species distribution, spore density, and percentage root colonization, differed by area and also by host plant. As in table 2, association of AMF species is also found different in different host plants.

Soil analysis - The data of physio-chemical factors of rhizosphereic soil of selected 4 fruit plants were collected from four different regions of Thar Desert of Rajasthan in relation to number of propagules. In the present study all the soils investigated were of **sandy soil** type. The soil pH range between 7.0 to 8.3. According to Bainard [8] the pH tolerance of mycorrhizal fungi varies; some prefer low pH soils (e.g., some Acaulosporaceae), while others prefer alkaline and neutral substrates (e.g., some Glomeraceae). High alkaline soil harboured more number of propagules. The moisture content ranged from 18 – 30%. Nitrogen ranged from .24 to .33 mg/100g while the Electrical conductivity was .22 to .35 mmho/cm. Light textured sandy soil with neutral to slightly alkaline pH shows the positive correlation with pH and low phosphorus content favoured extensive mycorrhizal root association [9]. In our study, the soil pH ranged from 7.0 to 8.3, which was closer to neutral to slightly alkaline and has more number of AM fungal propagules.

AM fungi Spore density - Table 5-8 shows the site-by-site effects of seasonal differences in spore density of AM fungi in the four fruit plant species studied. The density of spores differed significantly in aspect of season, different studied site and plant species. The spore density found more in summer season as compare to winter season. Maximum spore density was observed in *Moringa oleifera* as 87.6 (spores $100g^{-1}$) in summer at Bikaner district while for other like *Tamarindus indica* maximum spore density recorded 78.1 (spores $100g^{-1}$) at Jodhpur district, *Aegle marmelos* max. spore density as 69.0 (spores $100g^{-1}$) at both Bikaner and Jodhpur site, *Capparis decidua* max. spore density ranges upto 66.1 (spores per 100g) at Bikaner district. While in winter season for all the plants shows low AM fungal spore density

with very minimum spore density among all of them, that were recorded as 43.1 (spores per 100g) in *Capparis decidua* at Bikaner site in winter season.

Percent root colonization – The findings showed that the 4 fruit plants studied were mycorrhizal, but the degree of root colonization varied depending on the species and season. The max. percent root colonization was depicted in *Tamarindus indica* as 70.6% (in winter) while min. recorded in *Moringa oleifera* as 21.3 (in summer).

Fungal structures – From the observation of all Four plant's roots we found three fungal structures within the plant roots like arbuscules (A), hyphae (H) and vesicles (V), that confirm the mycorrhizal infection in host plant roots.

Table 5. - Vesicular Arbuscular Mycorrhizal fungus status in the roots of fruit plants of the Bikaner district.

S. No.	Host plants	Fungal structure		Colonization rate (%)		Spore density (spore per 100g of soil)	
		Summer	Winter	Summer	Winter	Summer	Winter
1.	*Tamarindus indica*	A,H,V	H	29.6	31.2	74.3	56.5
2.	*Aegle marmelos*	H,V	H,V	36.8	42.1	69.0	50.4
3.	*Capparis decidua*	H,V	H,	33.5	45.2	66.1	43.1
4.	*Moringa oleifera*	A,H,V	H,	24.2	30.5	87.6	66.4

Table 6. - Vesicular Arbuscular Mycorrhizal fungus status in the roots of fruit plants of the Barmer district.

S. No.	Host plants	Fungal structure		Colonization rate (%)		Spore density (spore per 100g of soil)	
		Summer	Winter	Summer	Winter	Summer	Winter
1.	*Tamarindus indica*	A,H,V	H	28.9	30.4	60.2	52.3
2.	*Aegle marmelos*	H,V	H,V	34.2	36.9	54.8	50.5
3.	*Capparis decidua*	H,V	H,	31.2	35.6	51.1	49.7
4.	*Moringa oleifera*	A,H,V	H,	21.3	25.7	78.6	64.6

Table 7. - Vesicular Arbuscular Mycorrhizal fungus status in the roots of fruit plants of the Jaisalmer district.

S. No.	Host plants	Fungal structure		Colonization rate (%)		Spore density (spore per 100g of soil)	
		Summer	Winter	Summer	Winter	Summer	Winter
1.	*Tamarindus indica*	A,H,V	H	25.3	30.5	55.1	52.0
2.	*Aegle marmelos*	H,V	H,V	33.1	34.5	50.0	49.9
3.	*Capparis deciduas*	H,V	H,	30.0	35.2	46.7	42.3
4.	*Moringa oleifera*	A,H,V	H,	22.3	26.1	65.5	63.6

Table 8. - Vesicular Arbuscular Mycorrhizal fungus status in the roots of fruit plants of the Jodhpur district.

S. No.	Host plants	Fungal structure		Colonization rate (%)		Spore density (spore per 100g of soil)	
		Summer	Winter	Summer	Winter	Summer	Winter
1.	*Tamarindus indica*	A,H,V	H	56.5	70.6	78.1	69.9
2.	*Aegle marmelos*	H,V	H,V	66.4	67.8	69.0	54.8
3.	*Capparis decidua*	H,V	H,	35.3	60.3	55.2	47.5
4.	*Moringa oleifera*	A,H,V	H,	40.4	41.2	83.4	76.5

DISCUSSION

The presence or absence of a host plant, nutrient supply, soil aeration, soil moisture content, and altitude are the most significant factors that cause variations between locations and seasons [10]. Sivakumar [11] observed similar findings in sugarcane in his research. D'Souza and Rodrigues [12] investigated the seasonal diversity of AMF in the mangroves of Goa, India. Soil analyses shows that in all the studied site's there was a deficiency of usable P, it has been confirmed that 80-85% P is made inaccessible to plants due to immobilization and fixation. pH was found neutral to slightly alkaline. It is reported that

endomycorrhizal infection occurs more around pH 8 (8). Some studies reveals (By Mathur N. and Vyas A., [9]) that spore density of AMF is positively correlate with soil pH while negatively correlation shows with soil P content. In this study, AMF spores were found in both neutral and moderate to slightly alkaline soils. Some AMF species can grow in both acidic and alkaline soils, while others can grow in both [13, 14]. Change in soil pH can influence the accumulation of certain nutrients and harmful ions in the soil, which can affect AM fungi colonization and spore germination indirectly AMF development and function. As a result, it's thought that the density of AMF spores differs with change in soil pH. However, since the pH range in the soils measured was between (7.0–8.3), a favourable association between soil pH and AM spore density was found in this study. There was also a spatial and seasonal variations were showed by AMF species difference, spore density and % root colonization.

Seasonal variations have a significant impact on the occurrence of AM fungi [15]. Summer (dry season) had the highest spore density, while winter had the lowest (wet season), Many overlapping factors influence spore density, including plant populations, soil characteristics, fungi's sporulating behaviour, host plant's growing season, and atmosphere. It's critical to comprehend the relationship between spore development and plant type. Exudation of toxic metabolites [16] and the development of readily oxidizable compounds are two possible reasons for the seasonal difference in root colonization [17]. Although these factors are important in colonization, other edaphic and climatic factors may also have an impact [7]. It's also been suggested that the population of AM fungi influences the interaction and development of AMF in host plants [18]. Bever [19] found that each mycorrhizal spores multiplied differently on different host plants and also that the infection ratio varied depending on the AM fungi species. In the current research all four Thar Desert fruit plants obtained from four separate districts of Rajasthan reported more than 65 percent colonization in both seasons. This high rate of colonization may be attributed to the fact that the plants in the study sites were largely P-deficient. Results also shows that percent root colonization and spore density is not correlated to each other as it seems in table 5 the max. spore density was found in *Moringa oleifera* as 87.6/100g soil with 42.1% root colonization which is lower than *Tamarindus indica* (45.2%) along with max. spore density (74.3/100g) that is lower than *Moringa oleifera*, so it shows that spore density can't affected to each other.

Correlation between spore density and root colonization – The coefficient of determination is a metric that describes how much variability in one factor can be related to its relationship with another factor. Here we found zero value of correlation of determination that indicate the dependent variable can't be predicted by other independent variable and the linear line in scattered plot depicted that there is no relationship between percent root colonization and spore density. The spore count is independent from percent root colonization or root infection.

Table 9. - Statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
spore density	32	0	32	42.300	87.600	60.459	12.173
root colonization	32	0	32	21.300	70.600	37.275	13.210

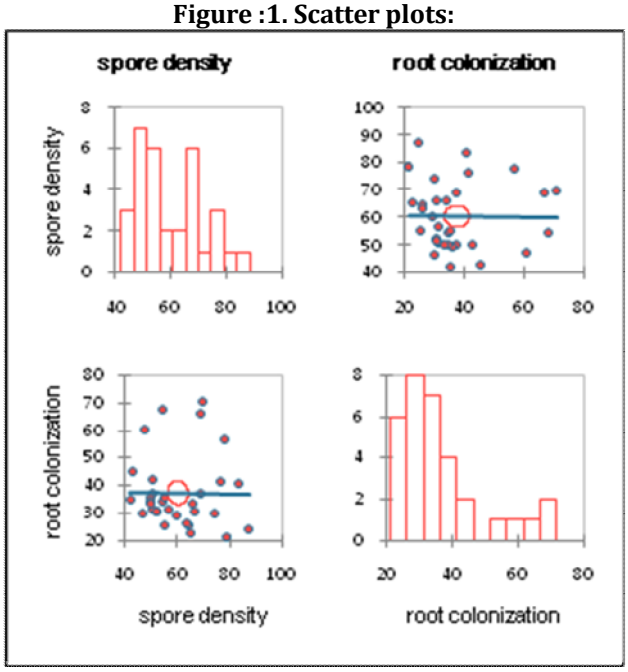
Table 10. - Correlation matrix (Pearson):

Variables	spore density	root colonization
spore density	**1**	-0.015
root colonization	-0.015	**1**

Table 11. - Coefficients of determination (Pearson):

Variables	spore density	root colonization
spore density	**1**	0.000
root colonization	0.000	**1**

Figure :1. Scatter plots:



In the present study, Glomus sp. were found dominant in all the selected plants rhizospheric soil. This is found similar to other study reports related to VAM association with other Thar Desert plants [20]. Similarily results were observed in medicinal plants grows in saline area of Indian Thar Desert [21] and also glomus were occurs more frequently detected in Chilli plant of Western Rajasthan reported by Vyas M. and Vyas A. [22]. Panwar J. and Tarafdar J.C. [23] also reported that in *Mitragyna parvifolia* Thar Desert medicinal endangered plant, this plant soil was also found dominating by *Glomus sps*. So the *Glomus sps*. are highly dominated spores among all other VAM spores. The difference in AMF flora could affect the infection ratio of AMF. Species diversity was apparent altogether study sites, but *G. mosseae* and *Sclerocystis* sp. were found in most of the soils. The marked difference observed in composition of AM fungi within the study sites could also be due to the influence of edaphic factors and seasonal differences. Variations in climate also influence the selection of AMF as climate regulates the incidence of specific fungal strains within the soil. Factors like cultural practices and vegetation within the study sites can also contribute to determining the dominance of a specific species. Therefore, the spore density of each AMF species, as well as the root infection with Thar Desert fruit plants, must be determined in order to promote the host plant's growth and development. Guadarrama and Alvarez-Sanchez [24] suggested that plant phenology is linked to spore abundance in dry seasons, when plants are less photosynthetically active due to leaf fall or stomata closure, resulting in reduced carbon flow to the roots [25]. As a result of low carbon availability in roots, the development of spores can be stimulated in dry soils. According to Buenos [26], Aridity hindered root colonization, who found that in extremely dry environments, available water recedes to smaller pores, resulting in reduced interaction between available spores and water films in the soil. Lack of soil nutrients also inhibited the development and separation of AMF spore for root infection and colonization, according to Van der Heidjen [27].

CONCLUSION

In this research, we discovered no any important association between the PRC (percent root colonization) and the spore density. Some other researchers [7,13] have recorded a lack of strong correlation between the number of spores and PRC, owing to the fact that some AMF species take a long time to germinate [28], whereas others seem to be incapable of germination. The pH of the soil influences AMF action [29]. The reaction of AM fungi to soil pH is complex and variable.

The present study reveals that seasonal variation in AMF varies with plant species and edaphic factors. Desert vegetation infections by AMF represent a survival mechanism for water and nutrient s in plant species.

SIGNIFICANCE STATEMENT

So far, no research on Arbuscular Mycorrhizal association in fruit tree species from Rajasthan's Thar Desert area has been reported. The relationship of Arbuscular Mycorrhizal fungi to root colonization and spore density in some dominant fruit tree species from Thar Desert areas is examined in this research. A extensive knowledge of the diversity and relationship of AMF with Desert plants in this ecologically stressed environment will be a crucial first step toward knowing more about its functional profile and agricultural significance. The study of AM fungi seasonal dynamics is useful in predicting the season and soil conditions that are favourable to maximum AM fungi growth, as well as also prefer the mycorrhizal fungi inoculation season for maximum symbiosis that help when mycorrhizal spores use as biofertilizer.

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