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Association of AM Fungal Spores with a Miracle Tree of Indian Thar Desert

Nazneen1 and Anil Vyas 2
Microbial Biotechnology and Biofertilizer Laboratory,
Department of Botany, J. N.V. University Jodhpur, Rajasthan, India.
nazneen.jdpr23@gmail.com

ABSTRACT

The present study described occurrence and colonization of different AM fungal spores with Thar desert plant Moringa oleifera. The 4 different area of Rajasthan's Thar Desert were covered for the study the occurrence and root colonization of AM fungi in Moringa Oleifera. The AM fungal spores found in their rhizospheric soil was belonging to 4 genera viz. Glomus, Acaulospora and Gigaspora, Sclerocystis. Glomus was found to be most dominant as compare to others.
Key words – *Moringa oleifera, Thar desert, Glomus, Acaulospora, AM fungi.*

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INTRODUCTION

In our ecosystem there is a vast diversity of microorganism found in soil. The microorganism may be saprophytic, symbiotic and pathogenic in nature. Mycorrhizal fungi is an example of symbiotic partner with many terrestrial plants. Help in survival of many plants in different climatic conditions. *Moringa oleifera* commonly grow in arid region gives nutrition and income security to the people of that area. Thar desert is comprises broad range of climate factors i.e. dry environment, low rain fall, high temperature, low water availability etc. besides these conditions, many plants survive normally there. In their survival AM fungi play a vital role. Deficiency of essential nutrients, scarcity of water, high temperature, high intense light are some conditions that make the climate of Thar desert much harsh and difficult for plant survival, but many plants combat and successfully established in this stressful environment by inhabiting their roots with Mycorrhiza. Mycorrhizal fungi are ancient and major component and generally account 50% biomass of soil microflora [1] that play an important role in maintaining soil fertility by nutrient recycling [2], bioremediation [3] help in uptake of water and minerals by increasing the nutrient absorbing zone of plant roots, aggregates soil particle, also help in nitrogen fixing activity of the microbes [4]. Other advantages that provide by the AM fungi to plants includes resistance to plant against drought and pathogens, increase plant growth and yield [5;6]. It has been recorded that almost all plant types like crops, cereals, legumes, grasses, forest trees, vegetable and medicinal plants [7-9] are associated with the mycorrhizal fungi.

Moringa oleifera is a multipurpose tree belonging to the moringaceae family. Having its socio economic importance. It called as "Tree of life" or "miracle tree" because it's high medicinal and nutritious values that make it's a gift from God for us. Every part of the plant is useable. It's a drought resistant and slow growing tree of Thar Desert commonly known as "horseradish and drumstick tree". In india it is also use as a vegetable, famous among people as sahjan. It can be grown in tropical and subtropical regions with temperature of 25-35◦C and slightly alkaline pH. It can grown with help of direct seeding and also can be propagated by cuttings. It contain high nutrients level of phosphorus, potassium, magnesium, zinc, iron, copper and also of vitamin A, vitamin B6, vitamin C, folic acid etc. As we know that Thar Desert soil is deficient for many important nutrients mainly N, P and K, these nutrient elements are important for plant growth and physiological activities but are present in soil as immobile form that can't be available for plants.

AM fungi belonging to Glomeromycota phylum [10], are ubiquitous and symbiotic biotrophs with 80% terrestrial plants [11]. AM fungi mutually benefit the host plant as helping in absorption of nutrients by convert their immobile form to mobile (available) form with help of some enzymes in exchange of sugar supplement by host plant. AM fungi not only a biotroph it also a bioprotectant, biosafe and a biofertilizer

microbe, that helps the plants to grow in adverse conditions. As different environmental conditions impact on plant growth and their establishment. *Moringa oleifera* commonly successfully grown in these adverse conditions of Thar desert. The AM fungal species are generally affected by different edaphic and climatic factors of different ecosystems, so it is important to see its spatial distribution and colonization of AM fungi, so the present work is attempted to investigate AM fungal spores associated with *Moringa oleifera* grown in Thar desert of Rajasthan that helps plant to combat with different climatic conditions of desert and their successful establishment.

MATERIAL AND METHODS

Collection site - The rhizospheric soil and root samples were collected from Jaisalmer, Barmer, Bikaner and Jodhpur of rajasthan Thar desert areas. A total of 12 samples were collected from rhizoshperic zone of plant species from three different sites of each area.

Sample storage - the soil samples collected from rhizosphere by digging out approximately 20cm beneath from the upper soil surface. The fine roots were collected from feeder roots of trunk that were spreaded in soil under the tree canopy. Soil was air dried. Both the samples of soil and root were collected in individual labeled polythene bags and stored at 4°C in laboratory. Roots were gently washed and stored in FAA (formalin, acetic acid and alcohol) and some times in 70% alcohol.

Soil properties analysis – For assessment of soil properties 5gm soil were used from collection sample of each sites of area was take and tested in laboratory.

Isolation of AM fungal spores from soil samples – Isolation of spores from rhizoshperic soil was done by wet sieving and decanting method [12]. Spores were observed and collected from sieved sample on whatsman filter paper. The fine roots found on upper sieve plate were collected, washed and stained by tryphan blue **(**13) with the other sample roots.

AM spores identification – identification of AM fungal spores were done by using key proposed by Schenck and Perez (14) or Gerdemann and Trappe (15).

Observation of infection in roots by AM fungi – AM fungi do not change in the morphology of host roots. They make arbuscules and some vesicles in cortical cells. Root colonization observed by the presence of these fungal structures like hyphae, vesicles, arbuscules and intraradical spores. For better visualization, cortical cells were cleared using clearing agent for removal of pigments and phenolic compounds that hide, them along with differentially stain for fungal structures from plant tissues were used.

Method – (i) Roots cleaning – prior to cleaning root samples could be preserved in 70% ethanol (16) and in FAA at **4**°C. Gently wash the roots with tap water then fill the glass beaker with 10% KOH containing root samples and autoclave at 15 psi for 10 min. or heat at 90 degree C for one hour in waterbath. This procedure's duration depends on roots pigmentation, their age (young and old) and their type (monocot or dicot). Again wash with water and acidify with 2%HCl for 2 min. to neutralize the effect of KOH.

(ii) Root staining – roots were stained by lactophenol containing Tryphan blue. Philips and Hayman (13).

Estimation of Root Colonization - For estimate the percent root colonization we used Gridline intersect method proposed by Giovannetti and Mosse, (17). The sample roots were stained and cut into 1mm pieces. These pieces evenly spread with in petriplates containing lactoglycerol. Observed under stereomicroscope. Along with horizontal and vertical intersect section count the total parts of root pieces cross the lines and in them how much parts of root pieces containing mycorrhizal structures (crosses the lines). Then count the percent root colonization using this formula given below –

$$\text{\% Root colonization} = \frac{\text{No. of mycorrhizal positive root segments}}{\text{Total no. of root segments observed}} \times 100$$

RESULT AND DISCUSSION

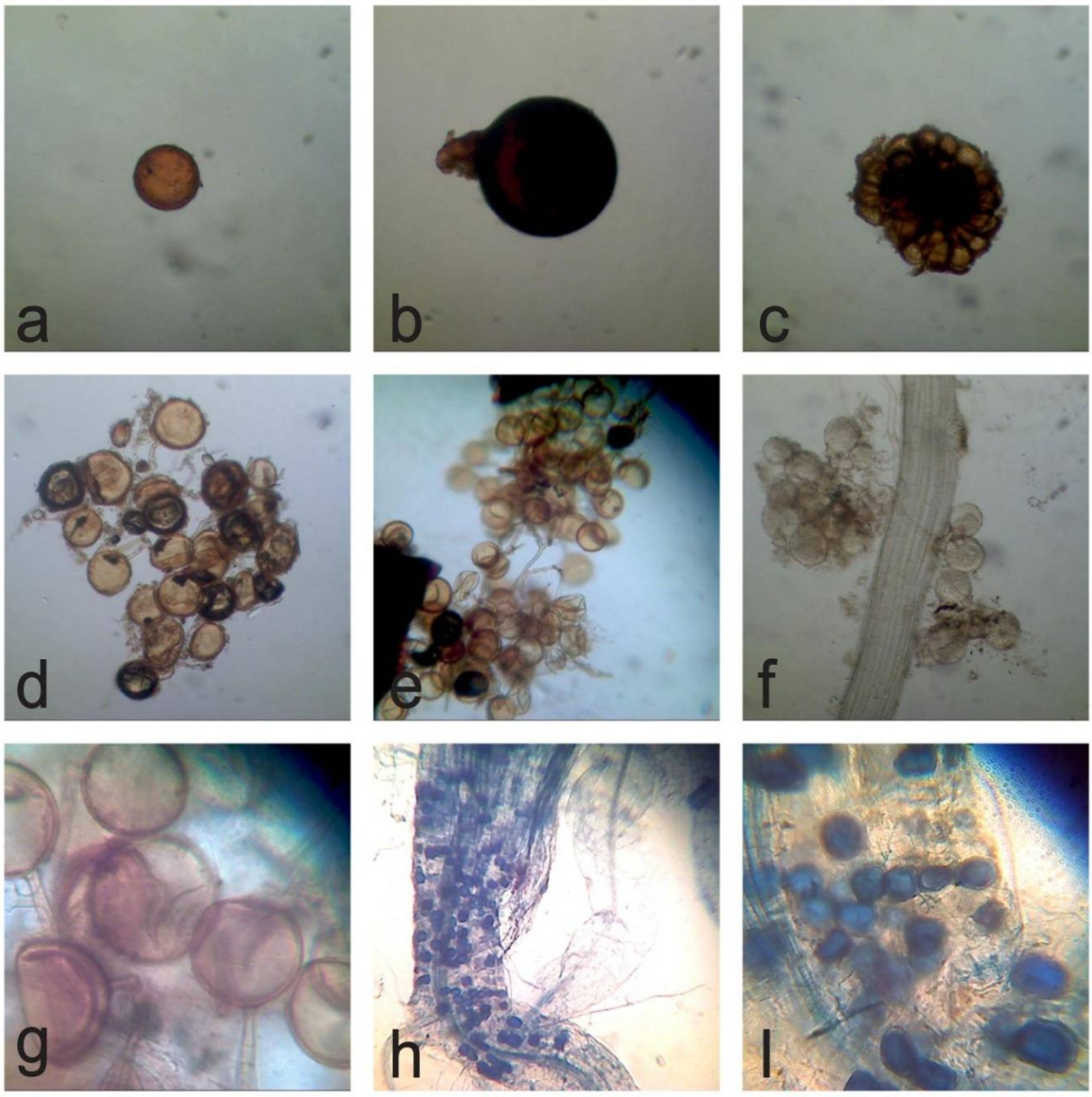
Analysis of soil properties – The edaphic factors founds varied as show in table 1 like-

(i) pH varied from 7.3-8.2.
(ii) available P content varied from 15.9-20 (mg/100g)
(iii) soil moisture varied from 19-30 %

Assessment of mycorrhizal spores in rhizospheric soil – it is seems that mycorrhizal relationship is found to be associated with almost terrestrial plants regardless the soil type, climate, plant growth and age. *Moringa oleifera* also a mycotrophic plant, shows the mycorrhizal association by means the presence

of AM fungal structures within their roots. Figure 1 (a to i) shows the different AM spores and structures observed within roots of *Moringa oleifera*.

Percent root colonization – The high percent root colonization ranges from 60 to 80 %, and the vesicles were clearly shown in the root that show the AM infectivity in the roots of *Moringa oleifera*.



a. *Acaulospora leavis*
b. *Gigaspora* sp.
c. *Sclerocystis* sp.
d. *Glomus* sp.
e. Sporocarp of *Glomus* sp.
f. Extraradicular spores
g. 100x view of *Glomus* spores
h &I shows vesicles in the root section of plant

Figure 1 (a to i) : Shows the different AM spores and structures observed within roots of *Moringa oleifera*.

Table 1- Analysis of soil properties

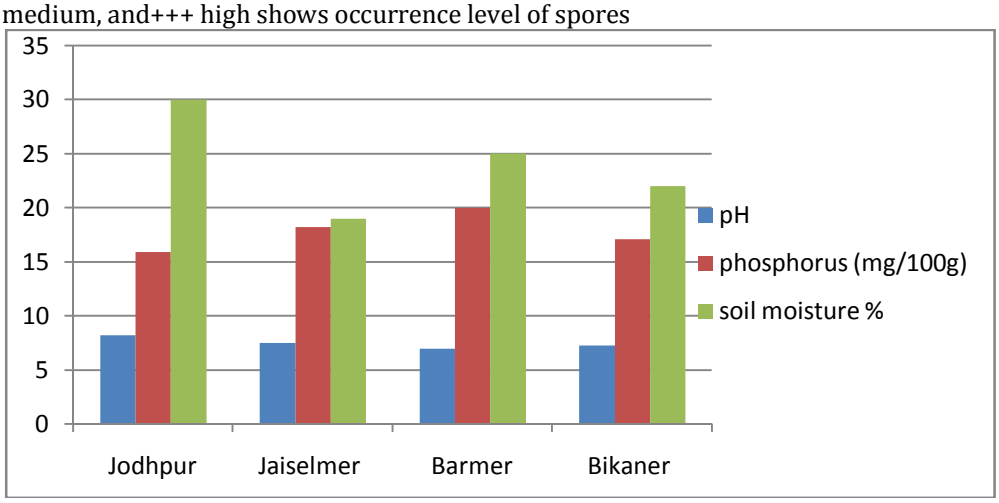
Sample collection site	pH	Phosp-horus (mg/100g)	Soil moisture (%)	Spore no. per 100gm of soil	% root colonization	Characteristic of infection
Jodhpur	8.2	15.9	30	59	80	H,V,A
Jaiselmer	7.5	18.2	19	55	75	H,V,A
Barmer	7.0	20	25	52	60	H,A
Bikaner	7.3	17.1	22	57	65	H,V

H = hyphae, V= vesicle, A= arbuscular type of infection.

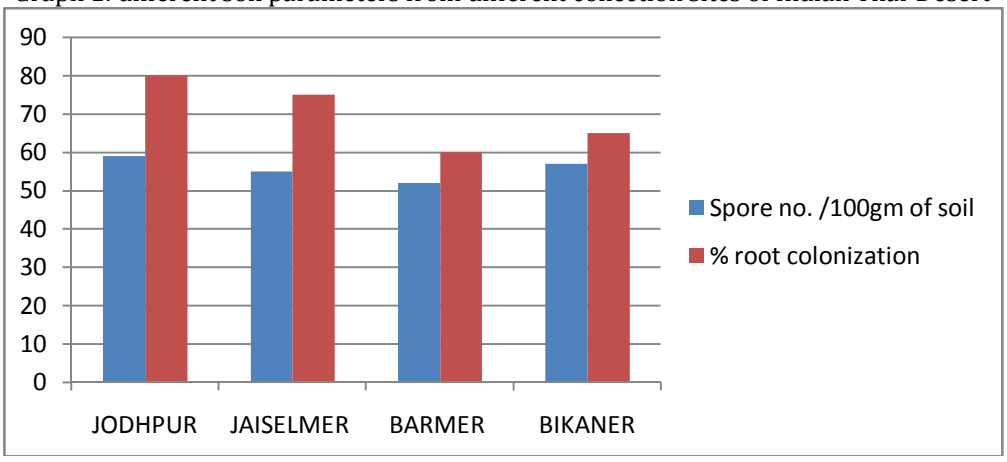
Table 2 - Absence and Presence Spores of AM mycorrhizal species in various Site

AM mycorrhizal species	Location			
	S1	**S2**	**S3**	**S4**
Acaulospora leavis	**+++**	**+**	**+**	**++**
Sclerocystis rubiformis	**++**	**-**	**+**	**++**
Glomus fasciculatum	**+++**	**+**	**++**	**++**
Glomus mossae	**+++**	**++**	**++**	**+++**
Gigaspora species	**++**	**-**	**-**	**+**

S1-Jodhpur, S2- Jaiselmer, S3- Barmer, S4- Bikaner
+ shows presence and – shows absence
+ low,++ medium, and+++ high shows occurrence level of spores



Graph 1: different soil parameters from different collection sites of Indian Thar Desert



Graph 2: spore no. per 100gm of soil and percent root colonization in *Moringa oleifera.*

CONCLUSION

A total of 5 different AM fungal spores were found from rhizospheric root of *Moringa oleifera* belonging to four genera of Glomeromycota. The determination of AM fungi population was done in terms of mycorrhizal spores and sporocarps in rhizospheric soil. By study of mycorrhizal spores associated with *Moringa oleifera* as shown in table 2 mostly present AM fungi species was from Glomus genera and total four genera were found. All four were mostly present in Jodhpur as compare to other areas.

There is no significant effect on AMF spore number and root colonization with changes in pH, but there is a negetive effect of the "P" availability in soil on spore numbers. the observation shows that the "P" availability is very low in Jodhpur and high in Barmer. With the change in "P" availability the spore count also changed. As in table 1. There are strong effect of "P" on the spore numbers and percent root colonization. As shown in table 2. The present investigation state that the *Glomus mossae* found to be more frequently as compare to other genera.

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REFERENCES

1. Olsson PA. (1999). Signature fatty acids provide tools for determination of distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29: 303– 310.
2. Tarafdar J C, Rao A V. (1997). Response of arid legumes to VAMfungal inoculation. Symbiosis, 22, 265-274.
3. Nishi Mathur , Joginder Singh , Sachendra Bohra and Anil Vyas , (2007) Arbuscular Mycorrhizal Status of Medicinal Halophytes in Saline Areas of Indian Thar Desert . International Journal of Soil Science, 2: 119-127.
4. Brundrett MC. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320: 37– 77.
5. Verma, R.K. and Jamaluddin (1994) Effect of VAM fungi on growth and survival of Acacia nilotica seedlings under different moisture regime. Proc. Nat. Acad. Sci. India, 64B, 205-210.
6. Mukerji, K.G. and Sharma, M. (1996), Mycorrhizal relationships in forest ecosystes, in: Forest-A Global Perspective, S.K. Mazumdar, E.W. Miller and F.J. Brenner, eds., The Penn. Acad. Sei., USA, pp. 95–125.
7. Bever J D, Morton J B, Antonovics J and Schultz P A (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J. Ecol. 84, 71-82.
8. Jalander, V. and Mamatha, M., (2015). Rhizosphere Mycoflora of Some Leguminous Plants. Int. J. Pure App. BioSci. 3(3): 262-266.
9. Poonam Verma, Sagar Barle, Mridul Shakya, , Sardul Singh Sandhu (2019). Assessment of root colonization by VAM fungi in vegetable plants in central India. G.J.B.B., VOL.8 (1) : 60-66.
10. Dessai SA, Rodrigues BF. (2012). Diversity studies on arbuscular mycorrhizal fungi in vegetable crop plants of Goa, India. Plant Pathology & Quarantine 2, 87–101.
11. Ezawa T, Cavagnaro TR, Smith SE, Smith AF, Ohtomo Ryo. (2003). Rapid accumulation of polyphosphate in extraradical hyphae of an arbuscular mycorrhizal fungus as revealed by histochemistry and a polyphosphate kinase/luciferase system. New Phytologist 161, 387–392.
12. Gerdemann J W and Nicolson T H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet-sieving and decanting. Trans British Mycol Soc 46: 235-44.
13. Phillips J H and Hayman D S (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans British Mycol Soc 55: 158-61.
14. Schenck N C and Perez Y (1990). Manual for the identification of VA mycorrhizal (VAM) fungi. University of Florida, Synergistic Publ., Florida, USA, 241p.
15. Gerdemann J.W. and Trappe J.M. (1974). The Endogonaceae in the Pacific Northwest. Mycol. Memoir 5: 1-76.
16. Kormanik, P. P. & McGraw, A,-C. (1982). Quantification of vesicular-arbuscular m~corrhizae in plant roots. In Methods and Principles of Mycorrhizal Research (ed. N. C. Schenck), pp. 37-45. St Paul: American Phytopathological Society.
17. Giovannetti, M. and Mosse, B., (1980). An evaluation of techniques for measuring vesicular--arbuscular mycorrhizal infection in roots. New Phytol., 84: 489--500.

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