

Studies on the Occurrence and Distribution of Mycorrhiza in Different Sites of Kota, Rajasthan, India

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Abstract Arbuscular mycorrhizal fungi (AMF) are ecologically important for most vascular plants because they benefit plant growth and survival. The present study provide the inventory and comparative assessment of AMF diversity in disturbed and undisturbed soil in Kota, Rajasthan and their role in ecological restoration of industrial waste disposal sites and degraded land. Rhizosphere soil samples from four different sites; one natural soil (undisturbed soil) and three industrial waste disposal sites (disturbed sites) were collected, AMF were identified and spore density was calculated. Decrease in overall spore density in industrial waste disposal sites as compared to undisturbed site shows that degraded soil properties have negative impact on mycorrhizal association, whereas increase in spore density of some mycorrhiza species in disturbed sites indicates the possibilities of selection of host plant for revegetation in restoration efforts.

Keywords: *Arbuscular mycorrhizal fungi, Degradation, Ecological restoration, Revegetation*

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1. Introduction

Large number of microorganisms inhabits the rhizosphere layer of soil. These microorganisms carry out various types of activities, which are very helpful to plants as well as to the organisms, hence they live in symbiotic association with plants. The most prevalent and widespread type of association is 'Mycorrhizal Association' [1]. Arbuscular mycorrhizal fungi (AMF) are found in many soils around the world, and they form association with 80% of all terrestrial plants. Maximum Mycorrhizal propagules are concentrated in the rhizosphere [2,3], hence when soil is disturbed, a decrease in the number of mycorrhizal propagules occurs.

Arbuscular mycorrhizal fungi have shown to help plants to acquire macronutrients [4] and micronutrients [5] thus enhancing soil fertility. AMF help in the binding of soil particles and improve soil aggregation and soil conservation [6]. Colonization with AMF can cause a beneficial physiological effect on host plant in increasing uptake of soil phosphorous [7,8]. Arbuscular Mycorrhizal Fungi have also been found to increase water uptake and/or otherwise alter the plant physiology to reduce stress response to drought and salinity. The improved nutrient uptake and better water utilization in endomycorrhizal plants reduce the transplant shock, quick recovery after temporary wilting, survival after transplanting and improves drought stress tolerance [9]. An interesting effect is the increase in resistance and tolerance of

arbuscular mycorrhizal plants to soil pathogens and some of the pests. In addition to the beneficial nutritional effect of the symbiosis on plants and the positive impacts on hydric status, soil aggregation and mineral enrichment, AMF act as bio-fertilizer and can reduce the incidence and importance of root diseases through biological process [10]. Besides all these they also have been successfully used to remove heavy metals from soils in phytoremediation technology.

Lots of plant species are completely dependent on microbial symbionts for growth and survival [11]. Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The protection and enhanced capability of uptake of minerals results in greater biomass production, a prerequisite for successful remediation and restoration. The importance of mycorrhizae in the nutrition of most vascular plants and the health of forest ecosystem has been overwhelmingly demonstrated in recent decades [12]. On degraded lands, they enhance the uptake of nutrients from low nutrient environment and hence contribute to the success of plant establishment and survival.

Disturbances in ecosystem whether natural or anthropogenic affect the physical, chemical and biological process in the soil. Mycorrhiza may play an important role in the reclamation and restoration of degraded ecosystems caused due to disposal of industrial wastes. Reclamation and restoration could be accelerated either by inoculation of mycorrhiza or manipulation of their population [13,14,15]. Generally, the plants which invade disturbed areas are non-mycotrophic *i.e.* without mycorrhizal

association [13,14,16]. Thus, if disturbance causes a loss of mycorrhizal propagules, recovery of the degraded areas is only possible if these propagules are reintroduced by natural processes or by human intervention. Nicolson (1967) [17] suggested that plant growth in wastelands could be effectively improved by incorporating AMF. Inoculation with AMF can improve the growth and survival of desirable plant species selected for revegetation. As mycorrhiza has its own importance in balancing an ecosystem, the proper knowledge of this microflora may be a sensitive way to reconstruct the disturbed or degraded ecosystems. For this purpose a thorough assessment and understanding of the dynamics of AM fungal communities in sites with different regimes of disturbances is a prerequisite to identify and select specific beneficial fungi for use in re-establishment of degraded forests and maintenance of sustainable forestry.

2. Study Area

Kota is located along the banks of the Chambal River in the south-eastern part of Rajasthan. The cartographic coordinates are 24°33' and 25°50' N latitude and 75°37' and 76°31' E longitude. It covers an area of 527sqkm and has an average elevation of 271 metres. Kota has a semi-arid climate with minimum and maximum temperature range 13°C-45°C. The monsoon season follows with comparatively lower temperature but higher humidity. The brief mild winter starts in late November and lasts until the last week of February.

Kota has fertile land with black soil and greenery with good irrigation facilities through canals that makes the land fertile. Two main canals; called as left main canal (towards Bundi) and right main canal (towards Baran) originated from the reservoir created by Kota Barrage. The comparatively rocky, barren and elevated land in the southern part of the city descends towards a plain agricultural land in the north. The city is the trade centre for corianders and building lime stone "Kota Stone". Kota district is a major industrial city in Rajasthan and is surrounded by five power stations with its 50 km radius.

3. Methodology

The study was conducted at four different sites of Kota, Rajasthan. Undisturbed land covering land area under Department of Forest is taken as control and 3 industrial waste disposal sites; near DCM industries (Experimental site A), Thermal Power Plant (Experimental site B), and lime stone mining area of Kota (Experimental site C) are taken as experimental sites. The soil sampling was done from November 2018 to October 2019. Rhizospheric soil samples were collected at 20-80 cm depths. Before sampling, the soils from the upper layer were scrapped off to remove foreign particles and litter. The soil samples were brought to the laboratory in sterile conditions and stored in a refrigerator at 4°C for further processing.

Host plants for mycorrhizal association were selected on the basis of common plant species which occur in any

of the disturbed sites along with control site. The AMF spores in the soil were assessed by wet-sieving and decanting method by Gerdemann and Nicolson (1963) [18]. Total spore number of mycorrhizal fungi in the soil samples were estimated and spore densities were expressed as the number of spores per 10 gram of soil. The isolated spores were picked up with a needle under a dissecting microscope and were mounted in polyvinyl-lactoglycerol (PVLG). All the spores were examined using a stereo binocular microscope. Taxonomic identification of spores up to species level was based on spore size, spore colour, wall layers and hyphal attachment using the identification manual [19].

4. Observation and Result

The present experimental findings revealed the relationship of mycorrhizal spores with various physico-chemical properties of undisturbed and disturbed soil. All the plants growing under natural conditions had possessed AMF spores as a regular component of a soil microflora. The soil samples of different locations showed different types of spores. All the recovered spores are represented by four genera namely *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis*. There are 4 species of *Glomus*; *Glomus intraradiaceus*, *Glomus fasciculatum*, *Glomus mossae* and *Glomus macrocarpum*: 2 species of *Gigaspora*; *Gigaspora margarita*, *Gigaspora albida*: 3 species of *Acaulospora*; *Acaulospora foveata*, *Acaulospora niger*, *Acaulospora levis* and 2 species of *Sclerocystis*; *Sclerocystis microcarpum*, *Sclerocystis celvispora*.

Jatropha curcus L., *Acacia nilotica* (L.) Willd. ex Del.ssp. *Indica* (Benth.), *Cassia tora* L., *Ziziphium nummularia* (Burm.f.) Wight. & Arn., *Calotropis procera* (Ait.) Ait. f. ssp. *Hamiltonii* (Wight.) Ali, *Solanum xanthocarpum* L. and *Cynodon dactylon* (L.) Pers. are found in all the four sites taken under study. In terms of availability, *Acaulospora levis* is found associated with the largest number of host plants (12) followed by *Gigaspora margarita* and *Glomus fasciculatum* which were found associated with 4 and 2 plants respectively. In terms of spore density *Acaulospora foveata* and *Gigaspora margarita* are found with the highest spore densities; 40 spores/10gm of soil.

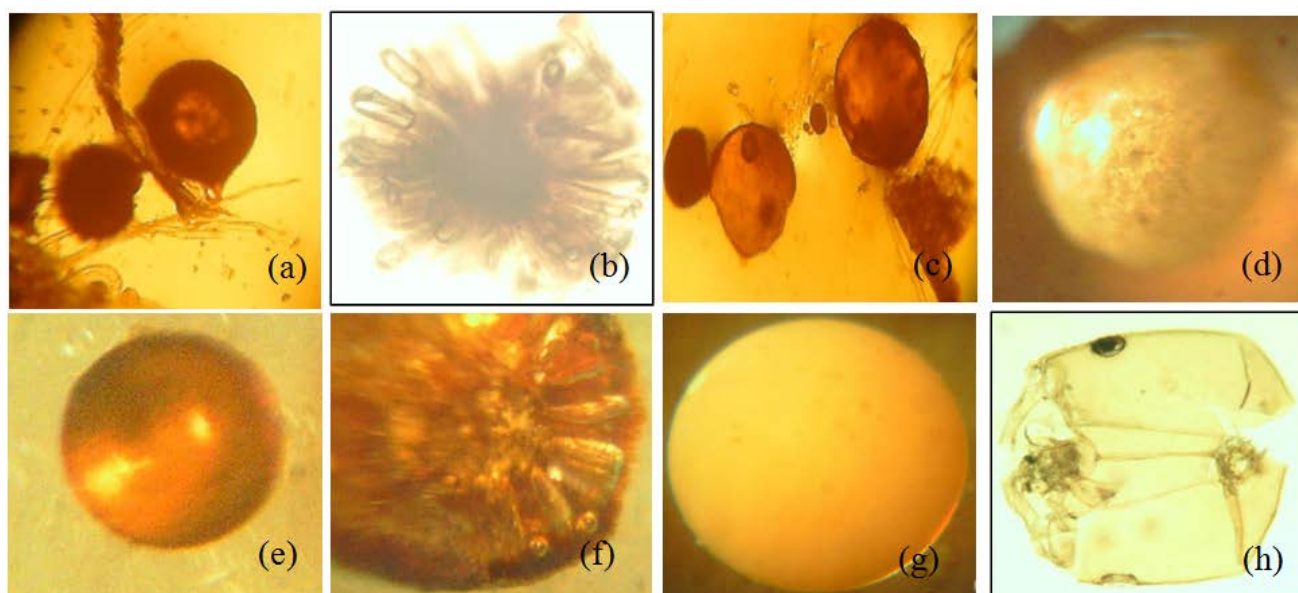
The soil samples collected from all the four sites exhibited the presence of a varied range of spore population in the soil. Spore density and number of mycorrhizal species are found decreasing as Control > DCM industry waste disposal site > Kota Thermal Power Plant waste disposal site > mining industries waste disposal site. The study shows that in site A spore density of *Glomus macrocarpum* increased by 33.3% and in site B, *Glomus macrocarpum* and *Acaulospora levis* increased by 16.66% and 25% respectively, whereas in site C spore densities of all the mycorrhizal species decreased. There were abundant AMF spores and the largest number was noticed in the rhizosphere soil of *Acacia nilotica* (L.) Willd. ex Del.ssp. *Indica* (Benth.) in undisturbed land and *Cassia tora* L. in disturbed land while the least number of spores was noticed in *Tridax procumbens* L. in both undisturbed and disturbed land.

Table 1. Distribution of mycorrhiza species in different sites of Kota

Plant species	Control site	Experimental site (A)	Experimental site (B)	Experimental site (C)	Mycorrhiza species
<i>Anogeissus pendula</i> Edgew	+	+	-	-	<i>Glomus intraradiaces</i> <i>Acaulospora niger</i>
<i>Jatropha curcus</i> L.	+	+	+	+	<i>Gigaspora albida</i> <i>Acaulospora levis</i>
<i>Acacia nilotica</i> (L.)Willd. ex Del.ssp. Indica (Benth.)	+	+	+	+	<i>Acaulospora levis</i>
<i>Achyranthes aspara</i> L. var. aspara	+	-	-	+	<i>Acaulospora levis</i> <i>Sclerocystis microcarpum</i>
<i>Cassia tora</i> L.	+	+	+	-	<i>Acaulospora levis</i> <i>Glomus marcocarpum</i>
<i>Ziziphus nummularia</i> (Burm.f.) Wight. & Arn	+	+	+	+	<i>Acaulospora foveata</i>
<i>Lantana camara</i> L.	+	-	-	+	<i>Gigaspora margarita</i>
<i>Prosopis cineraria</i> (L.) Druce	+	-	-	+	<i>Gigaspora margarita</i> <i>Glomus fasciculatum</i>
<i>Tridax procumbens</i> L.	+	-	+	-	<i>Gigaspora margarita</i> <i>Acaulospora levis</i>
<i>Eucalyptus globulus</i> Labil	+	+	-	-	<i>Acaulospora levis</i>
<i>Ricinus communis</i> L.	+	+	-	-	<i>Acaulospora levis</i> <i>Glomus fasciculatum</i>
<i>Cynodon dictyolon</i> (L.) Pers.	+	+	+	-	<i>Acaulospora levis</i> <i>Gigaspora margarita</i>
<i>Phoenix sylvestris</i> (L.) Roxb.	+	+	-	-	<i>Acaulospora levis</i>
<i>Calotropis procera</i> (Ait.) Ait. f.ssp. <i>Hamiltonii</i> (Wight.) Ali	+	+	+	+	<i>Sclerocystis calvispora</i> <i>Acaulospora levis</i>
<i>Solanum xanthocarpum</i> L.	+	+	+	+	<i>Acaulospora levis</i>
<i>Indigofera cordifolia</i> Heyne. ex Roth.	+	+	-	+	<i>Glomus mossae</i>

Table 2. Number of spores per 10 gm samples

S. No.	Species	No. of plants with association	Control site	Exp. Site (A)	Exp. site (B)	Exp. Site (C)
1	<i>Acaulospora foveata</i>	1	40	35	0	10
2	<i>Gigaspora margarita</i>	4	40	0	10	15
3	<i>Acaulospora niger</i>	1	35	30	13	0
4	<i>Glomus fasciculatum</i>	2	30	20	22	0
5	<i>Glomus macrocarpum</i>	1	30	40	35	10
6	<i>Glomus intraradiaces</i>	1	26	20	0	5
7	<i>Gigaspora albida</i>	1	25	25	0	0
8	<i>Acaulospora levis</i>	12	20	18	25	20
9	<i>Sclerocystis microcarpum</i>	1	20	15	0	0
10	<i>Sclerocystis calvispora</i>	1	10	0	0	6
11	<i>Glomus mossae</i>	1	10	7	0	10
	Total; Average		286; 26	210; 19.09	105; 9.55	76; 6.91

Figure 1. Photomicrograph of mycorrhizae spores isolated from 4 study sites; (a) *G. margarita* (b) *S. microcarpum* (c) *A. foveata* (d) *G. fasciculatum* (e) *A. levis* (f) *S. microcarpum* (g) *G. mossae* (h) *G. marcocarpum*

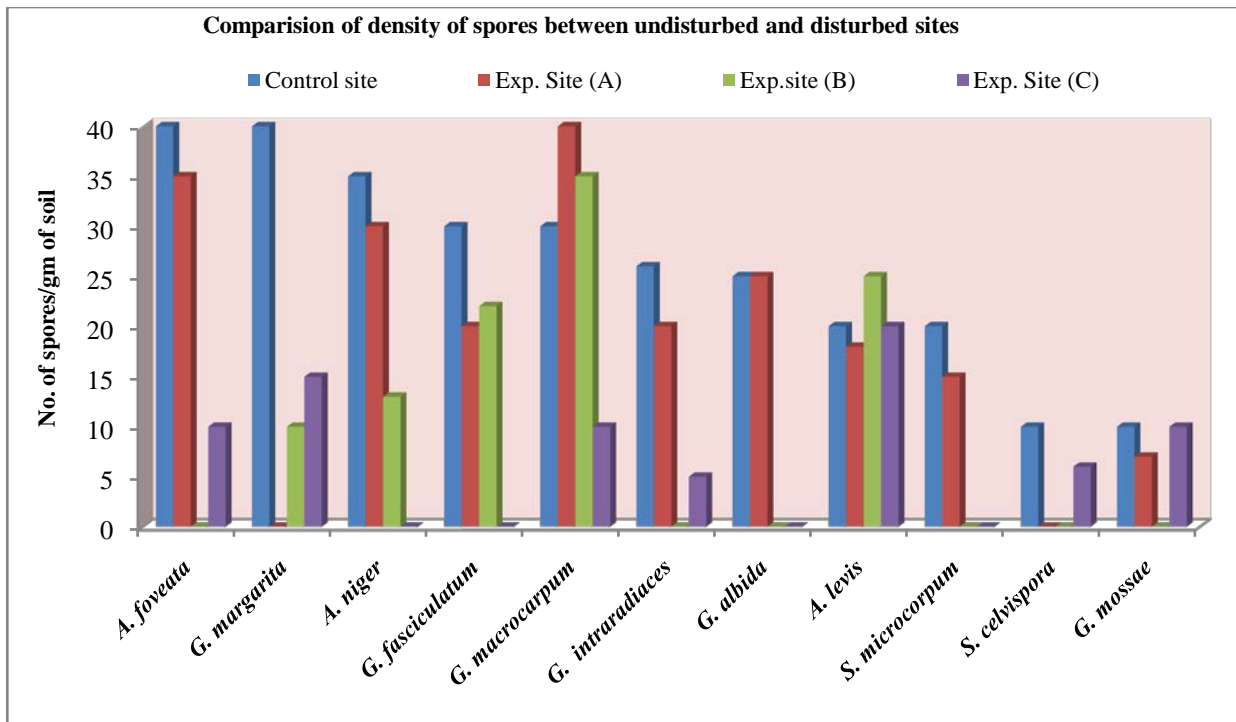


Figure 2. Comparison of spore density of mycorrhizae species in 4 different sites.

5. Conclusion and Discussion

Table 3. ANOVA Table

Source of variance	Degree of freedom	Sum of Squares (S.S.)	Mean Square (M.S.)	Variance Ratio (F)
Between sample	3	1063.36	35.45	3.88
Within sample variance	28	2556.85	91.31	
Total	31	3620.21		

Table value of 'F' is 2.95 at 5% level of significance is less than the calculated value (3.88 for the data collected) with degree of freedom 3 and 28 respectively for between sample and within sample (residual) source of variance. This indicates that the density of mycorrhiza

spores at different sites varies significantly from each other.

Among the isolated genera of AMF, *Acaulospora*, *Glomus* and *Gigaspora* were the most dominant AMF genus isolated during the present investigation while all other species shows decrease in spore count in all the three experimental sites. The dominance of the *Acaulospora* species in the polluted soil is due to its higher metal tolerance capacity. AMF communities of disturbed sites are characteristically dominated by disturbance tolerant species of the family Glomeraceae and more specifically the genus *Glomus* [20]. Gigasporaceae and Acaulosporaceae are competitors and stress tolerators respectively [20]. Generally, the presence of trace metals in the polluted soil may be responsible for the less number of mycorrhizal spores in the soil. The high alkalinity, pH and higher soil temperature in the polluted soil is also responsible for decrease in the number of mycorrhizal spores.

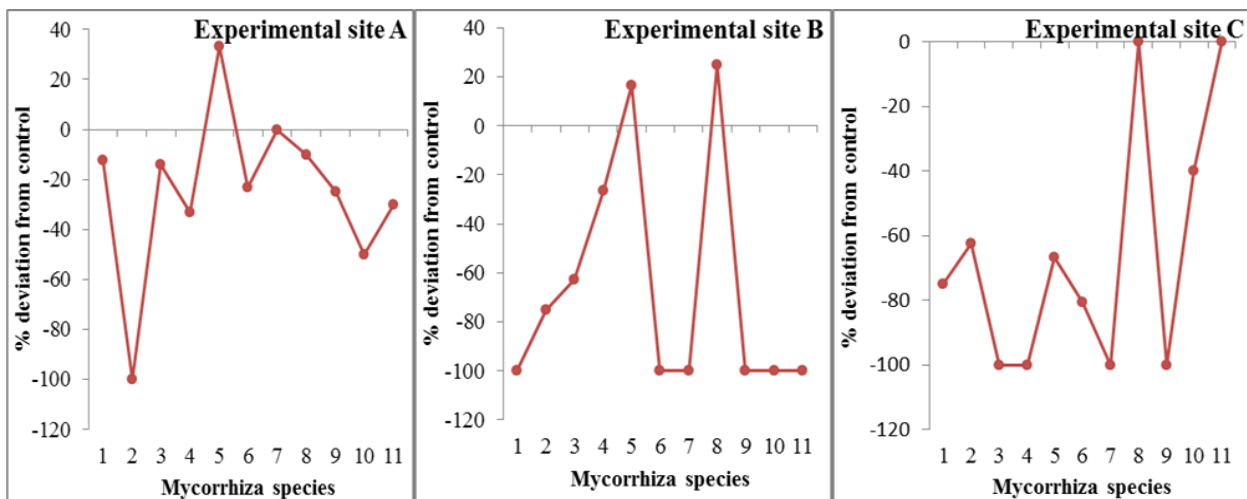


Figure 3. % deviation from control in spore density by mycorrhiza species in 3 experimental sites (0 line is control). Numbers on x-axis represents sr. no. of the mycorrhiza species as in Table 2

Generally, in an undisturbed forest ecosystem, great diversity of VAM fungi exists. However the alteration of a hitherto undisturbed ecosystem causes great changes in the structure and function of VAM fungi [21,22,23,24,25,26]. Mycorrhizae and most of the VAM fungal spore generally occur in the top 20 cm of soil profile [27,28]. Any disturbance in surface soil decreases markedly both the number of propagules of VAM fungi and the extent of mycorrhizae formation [29,30,31,32,33,34]. The species richness of AM fungal is also influenced largely by the intensity of disturbances [35,36].

In general, plants from mature ecosystems require the presence of mycorrhizae for their development (obligatory mycotrophs) [14,37]. AM fungi modify the root system of the host plant and play a critical role in the nutrient cycling in the ecosystem. Thus, restoration success depends on the augmentation of biological activity of the surface soil horizons [38]. Successional process is generally retarded or slow if a non-mycotrophic plant community is established on degraded land. AMF benefit plant establishment and survival in many ways in degraded lands [37,39,40,41]. Thus success of restoration of wastelands through revegetation can be achieved if the selected plant species have good mycorrhizal association.

The successful restoration depends on the capacity of the plants to capture resources at an early stage. On degraded lands, which may be droughty, nutritionally poor or otherwise stressed, there exists only a brief period favourable period for plant growth and the plants which do not establish within that time window fail to survive. In such conditions Mycorrhizae help the plant-soil-plant system by inter-bridging between the roots of different plants [42,43,44]. McGonigle and Fitter (1990) [45] demonstrated in the field that although AMF do not show specificity, they exhibit a preference for some host plants, thus by creating so-called "fertility islands" [46] with small groups of inoculated and fertilized plants may lead to the establishment of a network of hyphae that would then permit an accelerated colonization by native species.

In the present study out of 17 plant species sampled, 5 were leguminous plant having good association of mycorrhiza. The legumes with high to very level of VAM colonization can be used in restoration of degraded lands. Established mycorrhizal vegetation can facilitate the probability or extent of mycorrhizal infection of seedlings and thus mycorrhizal interaction among distantly related plants used for revegetation which may permit early succession of plants [47].

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