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Seasonal Variation of Mycorrhizal Association (Root Colonization and Spore Density) in Selected Industrial Sites in Kota District of Rajasthan, India

Suresh Singh Rajpurohit [a] and Poonam Jaiswal [b++*]

[a] Janki Devi Bajaj Government Girls College, Kota, India.
[b] Department of Botany, Janki Devi Bajaj Government Girls College, Kota, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This paper aims to assess the impact of seasonal variation on mycorrhizal association in selected industrial wastelands in the Kota district of Rajasthan. In the study mycorrhizal association was quantified in terms of percentage root colonization and spore density in three different seasons. The study was conducted during 2019-2021 and the data was collected for 3 seasons; summer (March-June), Rainy (July–October), and winter (November-February) to study the response to variable climatic factors to the root colonization and spore density of mycorrhiza. To determine the significance of variations in AMF spore density and percentage root colonization during different seasons, one-way ANOVA was performed. Results showed that in all the sites maximum mean

[++] *Associate Professor;*
**Corresponding author: E-mail: poonamjaisjdbkot@gmail.com, poonamjaiskota@gmail.com;*

spore density was observed in the summer (March-June) season but in the case of percentage root colonization, the value was found maximum in the humid season (July-October) in the control site whereas, in the three experimental sites, the maximum root colonization was observed in summer season (march-June). Hence, it may be concluded that hot climatic condition is favourable for mycorrhizal spore formation and root colonization was also favoured by hot climate.

Keywords: Seasonal variation; arbuscular mycorrhizal fungi; spore density; root colonization.

1. INTRODUCTION

Mycorrhiza are obligate symbiotic soil fungi having a mutualistic relationships with the large majority of terrestrial plants [1] having the ability to form an intimate associations with 70 to 90% of plant species [2]. In this association, the fungus takes over the role of the plant's root hairs and acts as an extension of the root system [3]. Being obligatory symbionts, they are dependent upon the host plant for carbon nutrition; in return enhance nutrient uptake by plants notably of immobile nutrients such as phosphorus (P) and zinc (Zn) [4,5]. Arbuscular Mycorrhizal Fungi (AM fungi) form an extensive hyphal network after bio-tropically colonizing the root cortex resulting in increased surface area for nutrient absorption thus helping to provide water and nutrients to living plants [6] and acquire mineral nutrients from soil [7]. AMF can also enhance resistance to root pathogens [8]. The ecological importance of AMF can't be underestimated as be as provide resistance to abiotic stresses, such as increased metal toxicity and drought conditions [9], play a role in the prevention of soil erosion by the formation of soil aggregates and building up a macrocarpous structure of soil [10].

AMF forms the main component of soil mycoflora belonging to phylum Glomeromycota. Various factors have been reported to affect the mycorrhizal association. Seasonal variation and climatic variables were also found to play a vital role in determining AMF spore density and percentage root colonization. Studies on effect of seasonal variation on mycorrhizal association in tropical soils are very few and that too are based on very few observations [11]. The diversity of AMF in response to seasonal variation has been studied mainly in sand dune systems and not much studied on other habitats [12].

The potential of AMF in restoration attempts on wastelands needs to understand the diversity, distribution, and association of AMF on native plant species in variable seasons of local habitats. The study aims to conduct a detailed examination of the influence of different seasons on AMF parameters (AMF spore density and root colonization) and to understand the seasonal dynamics of mycorrhiza in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

Kota lies in the southeastern part of Rajasthan, India. The geographical coordinates of Kota are 24° 33' and 25° 50' N latitude and 75°37' and 76° 31'E longitude and is located along the banks of the river Chambal River. The district covers an area of 527sqkm and has fertile land with black soil.

The study was conducted in industrial wastelands in the Kota district of Rajasthan, which is considered an industrial city with DCM Shri Ram, Thermal Power Plant, and Limestone mining as major industries. DCM Shri Ram Industries is a chemical industry that manufactures Caustic soda, Sodium Hypochlorite, Chlorine, Hydrogen, and Hydrochloric Acid. Kota Thermal Power Plant is a coal-based electricity generation plant whereas Limestone mines are present in the Ramganjmandi area of Kota. Non-industrial areas with natural vegetation are taken as control site.

2.2. Soil Sampling

The soil samplings were done from 2019 to 2021 in three seasons; winter (November to February), summer (March to June), and Monsoon or rainy (July to October). Soil samples for isolation and identification of mycorrhizal species were collected from all three industrial wastelands (experimental sites) and control site.

The composite soil sample was collected from three sample plots in five replicates for the isolation of spores. 100 gm of rhizosphere soil samples were collected from the vicinity of the root region of the plant and collected from a depth of 15 cm stored in polythene bags, labeled then refrigerated at 4° C until they were further processed.

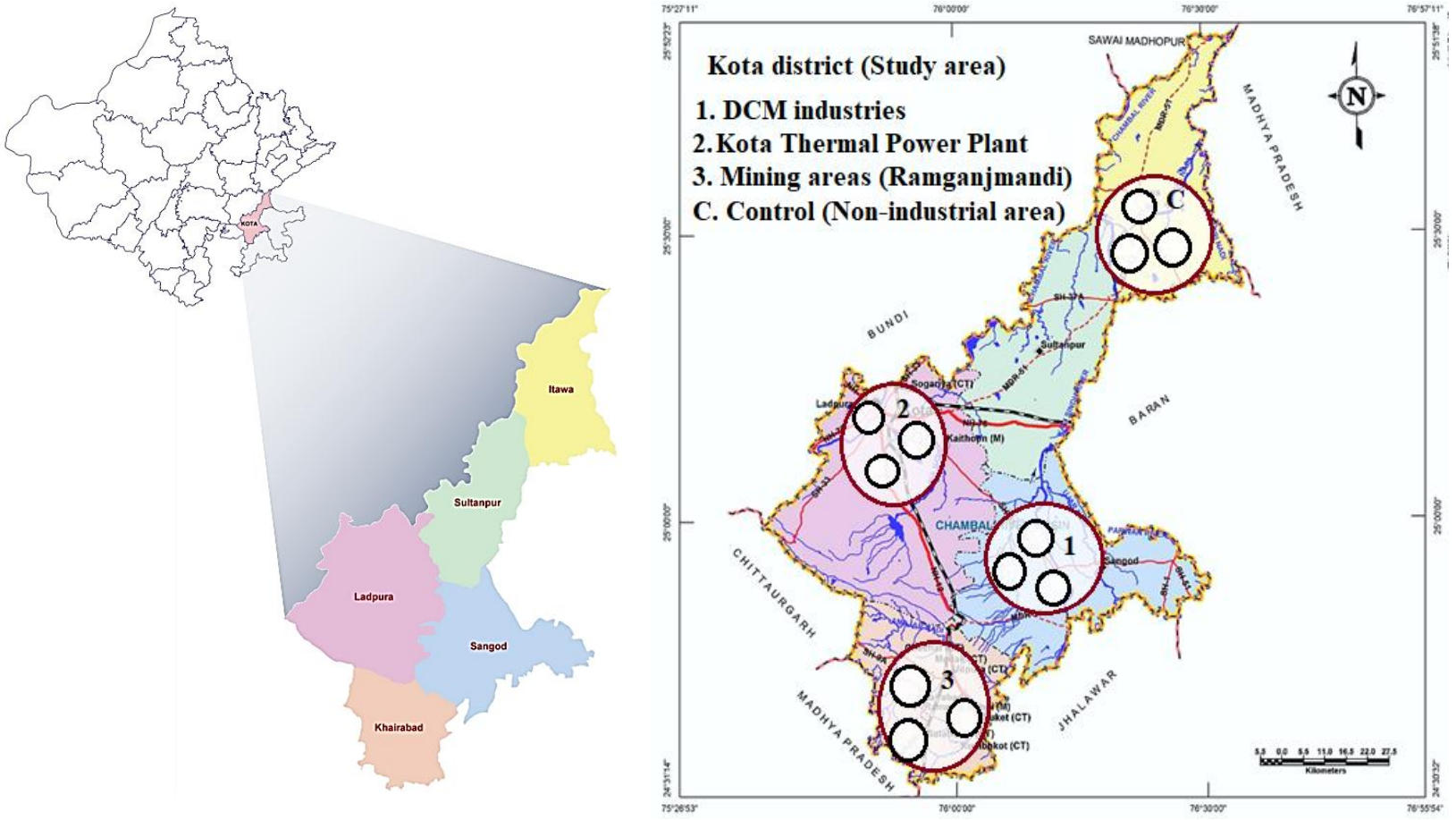

Fig. 1. Map and sampling design of the study site

2.3 Estimation of Root Colonization and AMF Spore Density

The AM fungal spore density was analyzed by using the wet sieving and decanting method [13]. About 100 g of soil was taken from each replicate, mixed thoroughly in 1,000 ml of water, and after some time a supernatant was poured through the stacked sieves. Different-sized sieves were used in a stack of 250, 210, 150, and 75 µm from top to bottom. The spores were recovered on Whatman filter paper No. 1 and quantification was carried out using Leica EZ4 stereo-microscope under x400 magnification. The AM fungal spores were identified using the Manual for Identification of vesicular-arbuscular mycorrhizal fungi [14]. The identification up to species was based on their morphology using taxonomic keys, such as color, size, shape, hyphal attachment, bulbous suspensor, wall structure, number of wall layers, the thickness of walls, etc.

Root staining and clearing method was used to prepare roots for the assessment of percentage root colonization [15]. Roots were washed thoroughly to remove soil particles and treated with 10% potassium hydroxide solution for 1 hour in a hot water bath. Then, they were washed with tap water and further treated with 2% HCl solution for 5 minutes. The acidified roots were stained with 0.05% trypan blue in lactic acid for 10–15 minutes in a hot water bath. Afterward, the roots were de-stained with lactic acid and observed under a compound microscope. The root segment having blue-coloured mycorrhizal hyphae was counted as a segment having root colonization [15]. The percentage root colonization was determined by slide count and gridline intersect method [16] using the following formula:

Root colonization (%)

$$= \frac{\text{Number of AM}-\text{positive segments}}{\text{Total number of segments observed}} \text{x}100\%$$

2.4 Statistical Analysis

To determine significant variations in AMF spore density and percentage root colonization during different seasons, analysis of variance (ANOVA) was performed by one-way ANOVA.

3. OBSERVATION AND RESULTS

When overall mean root colonization (%) in a specific site was observed, in the control site (non-industrial area) maximum percentage root colonization was observed in the rainy season (58.16%) and lowest in the winter season (41.5%). In DCM industries wastelands, maximum root colonization was found in the summer season (31%) and lowest in the winter season (13.83%). In thermal power plant waste dump site, maximum root colonization was found in the summer season (15.58%) and lowest in the winter season (7.91%). In mining wastelands, maximum root colonization was found in the summer season (17.33%) and lowest in the winter season (7.91%) (Table1). When overall mean spore density in a specific site was observed, in all the sites, the control site maximum spore density was observed in the rainy season (33.5) and lowest in the winter season (18.41). In all the experimental sites, maximum spore density was found in the summer season and lowest in the winter season (Fig. 2). In the study are only 17 plant species were recorded having mycorrhizal association and only 12 mycorrhiza species were identified (Table 3).

Statistical analysis of the percentage root colonization and spore density in response to seasonal variation ANOVA was performed between mycorrhizal parameters (percentage root colonization and spore density) separately in each study site. In the case of percentage root colonization, the calculated value is more than the table value at 5% level of significance (3.29 with *df* 2 and 33) except in thermal waste dump sites, this clearly indicates that the percentage root colonization in three different seasons differs significantly in control site, DCM waste dump site and mining waste dump site but it doesn't vary significantly in Thermal power station waste dump site (Table 2).

In the case of spore density, the calculated value is more than the table value at 5% level of significance (3.29 with *df* 2 and 33) except in thermal waste dump sites, this clearly indicates that the spore density in three different seasons differs significantly in control site, DCM waste dump site and mining waste dump site but it doesn't vary significantly in Thermal power station waste dump site (Table 2).

Table 1. Seasonal variation in root colonization (%) and spore density in control and three experimental sites (N=15)

	Control site			DCM industries waste dump site			Thermal Power plant waste dump site			Mining wasteland		
	March- June	**July-Oct**	**Nov-Feb**	**March- June**	**July-Oct**	**Nov-Feb**	**March- June**	**July-Oct**	**Nov-Feb**	**March- June**	**July-Oct**	**Nov-Feb**
Spore density ($\pm SE_M$)	33.5 ±0.8	29.08 ±1.1	18.41 ±1.3	29 ±1.45	25.1 ±0.86	14.4 ±1.03	24.66 ±0.28	21 ±0.62	12.83 ±0.51	17.25 ±0.64	10.87 ±0.71	7.12 ±0.33
Root colonization % ($\pm SE_M$)	56.69 ±1.42	58.16 ±2.68	41.5 ±0.88	31 ±0.96	24.41 ±2.37	13.83 ±0.99	15.58 ±0.91	12.91 ±0.18	7.91 ±0.39	17.33 ±0.33	12.08 ±0.44	7.91 ±0.21
Soil Temperature ($\pm SE_M$)	39.8 ±0.33	32.32 ±0.91	17.62 ±0.74	40.21 ±0.31	34.5 ±0.62	19.6 ±0.32	41.7 ±0.63	36.4 ±0.65	20.5 ±0.55	43.2 ±1.08	37.6 ±0.52	21.5 ±1.34
Soil moisture ($\pm SE_M$)	12.83 ±0.41	16.09 ±0.63	14.9 ±0.63	8.56 ±0.66	14.6 ±0.87	9.6 ±0.64	9.66 ±0.81	11.31 ±0.60	8.8 ±0.69	7.4 ±0.76	9.1 ±0.61	7.6 ±0.12
pH($\pm SE_M$)	8.35 ±0.9	8.21 ±0.92	7.9 ±0.49	6.96 ±0.71	6.43 ±0.67	7.13 ±0.71	7.45 ±0.52	7.83 ±0.43	7.5 ±0.82	7.61 ±0.88	7.72 ±0.43	7.82 ±0.95

Table 2. F values (Variance ratio) of ANOVA for seasonal variation in Percentage root colonization and spore density in the control site (non-industrial site) and three experimental sites (industrial wasteland sites)

Parameter	**Study sites**	**F value**
Percentage root colonization (samples from 3 seasons,5 replicates/season in each site)	Control site	4.65
	DCM industrial waste dump site	4.90
	Thermal waste dump sites	2.57
	Mining waste dump site	7.53
Spore density (samples from 3 seasons, 5 replicates/season in each site)	Control site	5.95
	DCM industrial waste dump site	5.38
	Thermal waste dump sites	2.28
	Mining waste dump site	5.48

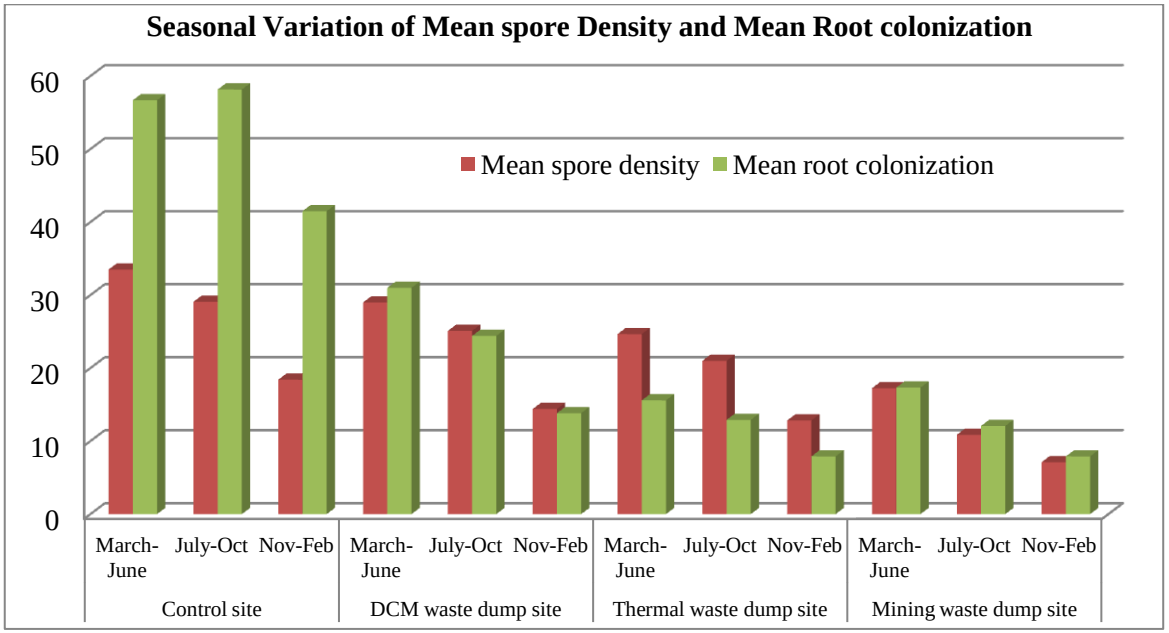

Fig. 2. Effect of seasonal variation of mean spore density and mean root colonization in different sites in the study area

Table 3. Plant species having mycorrhizal association and mycorrhiza species identified in the study area

Host Plant species	Mycorrhiza species
*Acacia nilotica (L.)*Willd.*ex*Del.ssp. *Indica*(Benth.)	*Acaulospora foveata*
Achyranthes aspara L.var.*aspara*	*Glomus mossae*
Anogeissus pendula Edgew	*Sclerosystis clavispora*
Calotropis procera(Ait.) Ait. f.ssp. *Hamiltonii* (Wight.) Ali	*Acaulospora laevis Acaulospora nigra*
Cassia tora L.	*Gigaspora albida*
Cynodon dactylon (L.) Pers.	*Gigaspora margartia*
Datura metal L.	*Glomus fasciculatum*
Eucalyptus globulus Labil	*Glomus intraradiaces*
Indigofera cordifolia Heyne. Ex Roth.	*Glomus macrocarpum*
Jatropha curcusL.	*Sclerocystis microcarpum*
Lantana camara L.	*Sclerocystis sinuosum*
Phoenix sylvestris(L.) Roxb.	
Prosopis cineraria (L.) Druc	
Ricinus communis L.	
Solanum xanthocarpum L.	
Tridax procumbens L.	
Ziziphus nummularia (Burm.f.)Wight. &Arn	

4. DISCUSSION

Climatic as well as edaphic factors play important role in mycorrhizal colonization and spore population. Seasonal fungal patterns are closely related to host phenology and climatic variations [17,18]. It was however not clear why AM colonization and sporulation were favoured by the root habitats of those plant species and what environmental or host factors influenced their dominance. The diversity of root infection might be due to soil pH, soil moisture, nutrient level, and soil physico-chemical properties [19]. These factors differentially affect root colonization and spore density [20].

Seasonal variations are the combined effect of local climatic conditions in a particular area. Temperature and precipitation are the main factors that define the climatic conditions of a site. Soil temperature is directly responsible for the processes that take place in the soil which are necessary for plant growth [21]. Various studies show that the soil moisture and availability of macro and micronutrients to the plant and soil microbe is dependent on soil temperature. The soil temperature is a catalyst for many biological processes. Table 1 presents the variation in percentage root colonization in response to the season. Improved root growth due to the increase in the metabolic activity of root cells and the development of lateral roots at increased temperature [22] may be the reason for increased root colonization and spore density in hot summer months.

4.1 Effect of Seasonal Variation on AMF Spore Density

Seasonal variation plays an important role in the occurrence of AM fungi [23]. Studies on salt marsh soil show that spore density was highest during the summer (dry season) and lowest during the wet season [24]. This pattern also occurs in temperate grasslands [25]. In xeric Mediterranean grasslands, dry and wet periods control the variation in total spore density [12]. Results of the present study also exhibit the highest spore density in summer. Many other interacting factors such as plant communities, soil characteristics, the sporulating nature of fungi, the growing season of the host plant, and climate also causes variation in the population of mycorrhizal fungi [26]. There may be many factors responsible for low spore density during the wet season viz. spore germination, dispersal, leaching, predation, mortality, and other factors.

4.2 Effect of Seasonal Variation on AMF Root Colonization

Root colonization was highest recorded in the wet season (July-Oct) in the control site in accordance with the study reported by Chandra and Jamaluddin [27] whereas in the other 3 experimental sites, root colonization was highest in the dry season (March-June) showing higher mycorrhizal activity in dry seasons when compared to the rainy season. Several researches explain the reason for seasonal variation in root colonization, including the production of easily oxidizable compounds [28] or the exudation of toxic metabolites [29]. There are several edaphic and climatic factors may also influence the process of root colonization [30]. The community of AM fungi may also determine host plant association and production [31]. Variations in climate also influence the selection of AMF as climate regulates the incidence of specific fungal strains in the soil. Host plant also plays a decisive role in colonization because each endophyte multiplied quite differently on different host plants and the infection ratio differed with the species of AM fungi [32]. Alteration in physico-chemical properties of soil resulting due to seasonal changes affects the presence of AM inoculums in the soil at a particular time [33].

The adaptability of plants to water stress conditions is due to mycorrhiza because plants received water from fungi present in the soil to increase the absorption rates of watered nutrients [34,35]. Mycorrhizal glycoprotein; Glomalin produced by AMF and released into the soil was higher during dry season than in the rainy season [36]. This glycoprotein is produced by AMF in response to environmental stresses, such as drought and salinity [37], and acts on soil aggregation and structuring [37]. A decrease in the glomalin values during the rainy season indicates a decrease in the activity of the fungus.

5. CONCLUSION

Mycorrhizal association has a great affinity with climatic factors and season in terms of spore density and root colonization. Based on statistical analysis of the results of the present investigation, it can be concluded that mycorrhizal association and spore density of mycorrhiza are differently affected by the seasonal variation and the variation is site specific. Industrial wastelands in the study area are usually water scarce which exhibit higher

spore density and root colonization in the hot climates. Exceptionally, root colonization was higher in humid season in non-industrial areas. Overall root colonization and spore density were higher in summer months except for root colonization in non-industrial areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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