Original Research Article

~~Effect of~~ Seasonal variation ~~on~~ of mycorrhizal association (Root colonization and Spore count) in selected industrial area sites ~~in~~ of Kota District of Rajasthan, India

Abstract

The ~~research paper assesses~~aim of this paper is to assess the impact of seasonal variation on mycorrhiza~~tio~~l association in selected industrial wastelands in 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 in 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 the root colonization in mycorrhiza. To determine 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 site maximum mean spore density was observed in summer (March-June) season, but in case of percentage root colonization, the value was found maximum in humid season in control site whereas in three experimental site, maximum root colonization was observed in summer season(March-June). Hence, it may be concluded that hot weather is favourable for mycorrhizal spore formation and root colonisation. ~~Thus it can be conclude that hot climate is favourable for spore production in mycorrhiza and root colonization was also favoured by hot climatic conditions.~~

Comment [K1]: Type month

Key Words: Seasonal variation, Arbuscular mycorrhizal fungi, Root colonization, Spore count

Introduction

Mycorrhiza are obligate symbiotic soil fungi having mutualistic relationship with large majority of terrestrial plants **[1]** having ability to form intimate association 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 enhances nutrient uptake by plants notably of immobile nutrients such as phosphorus (P) and zinc (Zn**) [4; 5].** Arbuscular Mycorrhizal

Fungi (AM fungi) forms an extensive hyphal network after biotropically colonizing the root cortex resulting in increased surface area for nutrient absorption thus helping to provides water and nutrients to living plants **[6]**and acquire mineral nutrients from soil **[7].** AMF can also enhance resistance to root pathogens **[8].** 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 prevention of soil erosion by the formation of soil aggregates and building up a macrocarpous structure of soil **[10].**

AMF form main component of soil mycoflora belonging to phylum Glomeromycota. ~~Varoius~~Various factors have been reported to affect mycorrhizal association. Seasonal variation and climatic variables was also found to play a vital role in determining AMF spore density and percentage root colonization. Studies on seasonal variation on mycorrhizal association in tropical soils are very few and that too are based on very few observations **[11].** 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 or AMF in restoration attempts on wastelands need to understand the diversity, distribution and association of AMF on native plants species in variable seasons of local habitats. The aim of the study is to conduct a detailed examination of the AMF parameters (AMF spore density and root colonization) and the influence of different seasons on AMF to understand seasonal dynamics in the study area. ~~The aim of the study was to examine the status of percentage mycoorhizal colonization and spore density in response to local seasonal variation (summer, rain and winter) in the area.~~

Material & Methods

Study Area

The study was conducted in industrial wastelands in Kota district of Rajasthan, which is considered as industrial city with DCM Shri Ram, Thermal Power Plant and Lime stone 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.

Kota lies in the south-eastern part of Rajasthan, India. Geographical coordinates of Kota is 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.

Comment [K2]: Soil sampling and 4 site details has to be given clearly

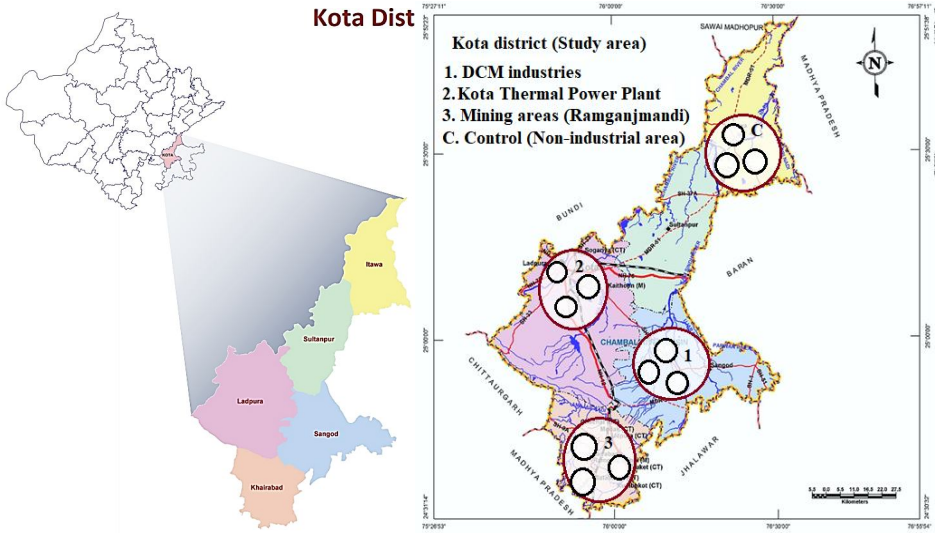

Figure 1: Map and sampling ~~design~~ location of the study site.

Estimation of root colonization and AMF Spore Density:

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 the three industrial wastelands (experimental sites) whereas non-industrial areas having natural vegetation in the district are taken as control.

Comment [K3]: Name the dominant native plant species found in that area

The soil samples were collected in sterile condition at stored at 4~~0~~°C until they were processed. The AM fungal spore density was analyzed from 100 g of rhizosphere soil by using wet sieving and decanting method **[13].** The composite soil sample was collected from three sample plots in five replicates were used for ~~for~~isolation of spores. About 100 g 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. The total spore count was carried out using Leica EZ4 stereomicroscope. The AM fungal spores were identified using the Manual for Identification of vesicular arbuscularmycorrhizal fungi **[14].** The identification was based on their morphology using taxonomic keys, such as color, size, shape, hyphal attachment, bulbous suspensor, wall structure, number of wall layers, 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 destained with lactic acid and observed under a compound microscope. The percentage root colonization was determined by slide count and gridline intersect method **[16]** using the following formula:

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \text{x}100$$

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.

~~Observation and~~ Results

When overall mean root colonization (%) in a specific site was observed, in control site (non-industrial area) maximum percentage root colonization was observed in rainy season (58.16%) and lowest in winter season (41.5%). In DCM industries wastelands, maximum root colonization was found in summer season (31%) and lowest in winter season (13.83%). In thermal power plant waste dump site, maximum root colonization was found in summer season (15.58%) and lowest in winter season (7.91%). In mining wastelands, maximum root colonization was found in summer season (17.33%) and lowest in winter season (7.91%) **(Table 1).** When overall mean spore density in a specific site was observed, in all the sites, control site maximum spore density was observed in rainy season (33.5) and lowest in winter

season (18.41). In all the experimental sites, maximum spore density was found in summer season and lowest in winter season **(Figure 2).**

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 case of percentage root colonization, the calculated value is more than 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 season 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 case of spore density, the calculated value is more than 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 season 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=9).

	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

Formatted Table

Table 2: F values (Variance ratio) ofANOVA for seasonal variation in Percentage root colonization and spore density in control site and three experimental sites.

Parameter	**Study sites**	**F value**
Percentage root colonization (samples from 3 seasons, 3 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, 3 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

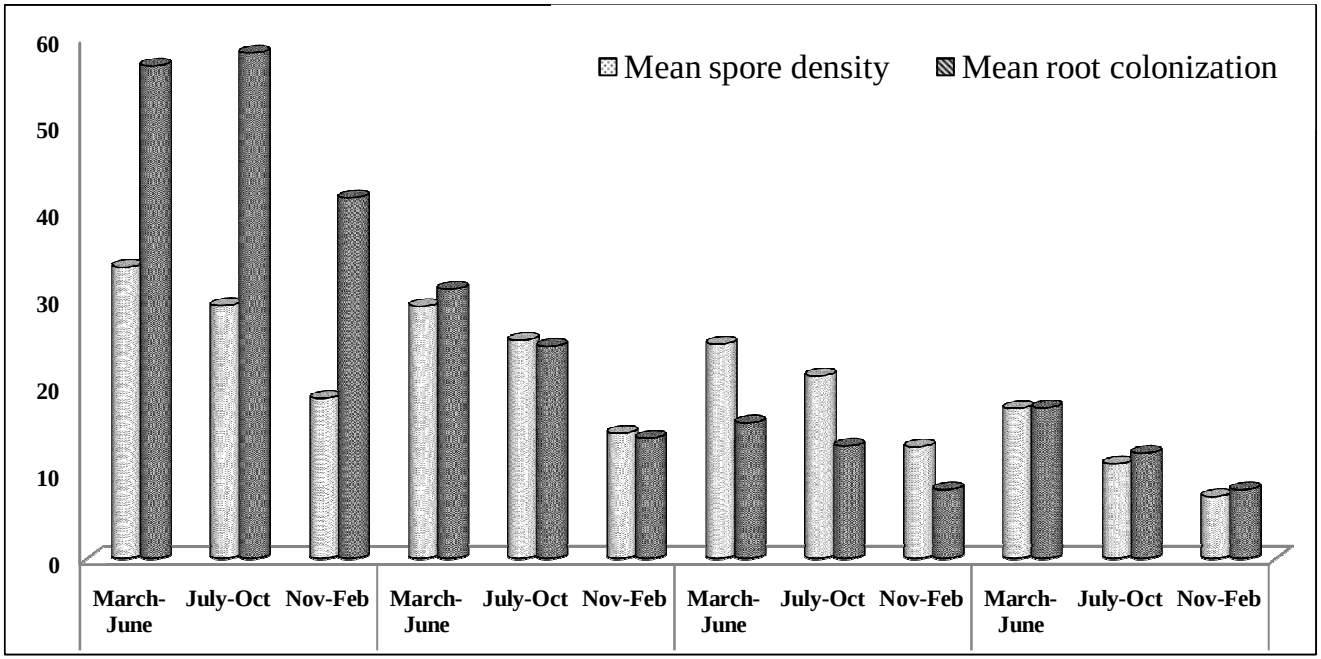

Figure 2 : Seasonal Variation of Mean spore Density and Mean Root colonization in different sites in study area.

Discussion

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 aeration, soil moisture and soil physico-chemical properties in that particular field **[19].** The decrease of spore density can slow down the asexual reproduction of fungi, reduce the energy consumption, and help AMF survive adversity.

Table 1 presents variation in percentage root colonization in response to the season. The seasonal variations are the combined effect of local climatic conditions at a particular area. Temperature and precipitation are the main factors which define 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 **[20].** Soil moisture is also directly related to soil temperature. There are various studies that show that 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. Soil temperature influences soil moisture, aeration and availability of plant nutrients which are necessary for plant growth **[20].** Increase in soil temperature improves root growth because of the increase in metabolic activity of root cells and the development of lateral roots **[21].**

Effect of seasonal variation on AMF spore density

Seasonal variation plays important role on the occurrence of AM fungi **[22].** Studies on salt marsh soil show that spore density was highest during the summer (dry season) and lowest during the wet season **[23].** This pattern also occurs intemperate grasslands **[24].** In xeric Mediterranean grasslands, dry and wet periods control the variation in total spore density **[12].** In plants of family Lamiaceae root colonization was highest during summer **[25].** Results of the present study also exhibit same pattern i.e. highest spore density in summer. Many other interacting factors such as plant communities, soil characteristics, sporulating nature of fungi, growing season of host plant, and climate also causes variation in spore density. There may be many factors responsible for low spore density during wet season viz. spore germination, dispersal, leaching, predation, mortality, and other factors.

Effect of seasonal variation on AMF root colonization

Root colonization was highest recorded in wet season (July-Oct) in control site only whereas in other 3 experimental sites, root colonization was highest in dry season (March-June). Several researches explain reason for seasonal variation in root colonization, including production of easily oxidizable compounds **[26]**orexudation of toxic metabolites **[27].** There are several edaphic and climatic factors may also influence the process of root colonization **[28].** Community of AM fungi may also determine host plant association and production **[29].** 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 that the infection ratio differed with the species of AM fungi **[30].**Physico-chemical properties results due to seasonal changes which affects presence of AM inoculums in the soil at a particular time **[31].**

As indicated in the present research, in control site colonization increases in hot and humid season (July-October) in accordance with the study reported by Chandra and Jamaluddin **[32].** Whereas in all three experimental sites colonization tend to increase in dry season (March- June) showing higher mycorrhizal activity in dry seasons when compared to the rainy season. The adaptability of plants to water stress conditions are due to mycorrhiza because plants received water from fungi present in the soil to increase the absorption rates of watered nutrients **[33;34].** Mycorrhizal glycoprotein Glomalin produced by AMF and released into the soil were higher dry season than in the rainy season **[35].** This glycoprotein is produced by AMF in response to environmental stresses, such as drought and salinity **[36]**and acts on soil aggregation and structuring **[36].** Decrease in the glomalin values during rainy season indicates a decrease in the activity of the fungus.

Conclusion: On the basis of statistical analysis of the results of present investigation it can be concluded that mycorrhizal association and spore density of mycorrhiza are differently affected by the seasonal variation and the variation may be site specific. Mycorrhizal association has great affinity with climatic factors and season in terms of spore density and root colonization. Industrial wastelands in the study area are usually water scarce which exhibit higher spore density in hot climate. Overall root colonization and spore density were higher in summer months except root colonization in non-industrial area.

References:

1. Gadkar V, David-Schwarz R, Kunik T and Kapulnik Y. (2001). Arbuscular mycorrhizal fungal colonization. Factors involved in host recognition. *Plant Physiology*, 127: 1493–1499
2. Smith SE, Read DJ (2008). "Mycorrhizal Symbiosis" 17 3rd edition, Academic Press, London.
3. Kaushik P, Sandhu OS, Brar NS, Kumar V, Malhi GS, Kesh H & Saini I (2020). Soil metagenomics: prospects and challenges. In: mycorrhizal fungi-utilization in agriculture and forestry (Ed; R Radhakrishnan) *IntechOpen*, pp. 1-18
4. Bolan NS. (1991). A critical review of the role of mycorrhizae fungi in the uptake of phosphorus by plants, *Plant and Soil*, 134: 189-207.
5. Bürkert B & Robson A (1994). 65Zn uptake in subterranean clover (*Trifoliumsubterraneum* L.) by three vesicular-arbuscularmycorrhizal fungi in a root-free sandy soil. *Soil Biology and Biochemistry*, 26(9): 1117-1124.
6. Manimegalai V, Selvaraj T, &Ambikapathy V (2011). Studies on isolation and identification of VAM fungi in *Solanumviarum*Dunal of medicinal plants. *AdvApplSci Res*, *2*(4), 621-628.
7. Harley JL and Smith SE (1983). Mycorrhizal Symbiosis. Academic Press .London, 483.
8. Borowicz VA (2001). Do arbuscularmycorrhizal fungi alter plant pathogen relations.*Ecology*, 82:3057–3068.
9. Meharg AA, Cairney JW (2000). Ectomycorrhizas—extending the capabilities of rhizosphere remediation? *Soil Biology and Biochemistry*, 32(11-12): 1475-1484.
10. Miller RM, Jastrow JD (1990). Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biol. Biochem*. 22(5):579-584.
11. Muthukumar T, Senthilkumar M, Rajangam M, Udayan K (2006). Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. *Mycorrhiza*, 17(1):11–24.
12. Lugo MA, Cabello MN (2002) Native arbuscularmycorrhizal fungi (AMF) from mountain grassland (*Cordoba, Argentina*) I. Seasonal variation of fungal spore diversity. *Mycologia* 94(4):579–586

13. Gerdemann JW, Nicolson TH (1963). Spores of arbuscularmycorrhizalEndogone extracted from soil by wet sieving and decanting. *Trans Br MycolSoc*, 46:235-244.
14. Schenck NC. Perez Y (1990). Manual for the identification of vesicular arbuscularmycorrhizal fungi. Synergistic Publications: Gainesville, FL. U.S.A., pp.1-286.
15. Phillips JM, Hayman DS (1970). Improved procedures for clearing root and staining parasitic and vesicular arbuscularmycorrhizal fungi for rapid assessment of infection. *Tans. Bri. Mycol. Soc*.,55(1),158-161.
16. Giovannetti M and Mosse B (1980). An evaluation of techniques of measuring vesicular arbuscularmycorrhizal infection in roots. *New Phytol*., 84:489-500.
17. Rosendahl S, Rosendahl CN (1992). Seasonal variation in occurrence of VA mycorrhizal infection types in a Danish Grassland community. In: *Mycorrhizas in ecosystems*, Read DJ, Lewis DH, Fitter AH, Alexander IJ (*eds*.). CABI, Cambridge, 400.
18. Allen MF (1996). The ecology of arbuscularmycorrhizas: a look back into the 20th century and a peak into the 21st. *Mycol Res*, 100(7):769–782.
19. Hamel C (1996). Prospects and problems pertaining to the management of arbuscularmycorrhizae in agriculture. *Agriculture, Ecosystems & Environment*, 60(2-3), 197-210.
20. onwuka B, Mang B. (2018). Effects of soil temperature on some soil properties and plant growth. *Adv Plants Agric Res*. 8(1):34-37. DOI: 10.15406/apar.2018.08.00288.
21. Repo TI, Leinonen AR, Finer L (2004). The effect of soil temperature on bid phenology, chlorophyll fluorescence, carbohydrate content and cold bardiness of Norway spruce seedlings. *Physio Plant*. 121:93–100.
22. Mallesha BC, Bagyaraj DJ (1991) Season favouring sporulation of VA-mycorrhizal fungi in cardamom plantations. *J Soil Boil Ecol*, 11:75–78
23. Carvalho LM, Cacados I, Martiris-Loucao MA (2001). Temporal and spatial variation of arbuscularmycorrhizas in salt marsh plants of Tagus estuary (Portugal). *Mycorrhiza*, 11:303–309
24. Escudero V, Mendoza R (2005) .Seasonal variation of arbuscularmycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. *Mycorrhiza* 15:291–299
25. Mago P, Mukerji KG (1994). Vesicular arbuscularmycorrhizae in Lamiaceae: I. Seasonal variation in some members. *Phytomorphology* 44(1-2):83–88

Formatted: Default Paragraph Font, Font: (Default) +Body, 11 pt, English (United States), Pattern: Clear

26. St. John TV, Coleman DC (1983). The role of mycorrhizae in plant ecology. *Can J Bot*, 61:1005–1014
27. Iqbal SH, Queorshi KS (1986). The influence of mixed showing (cereals and crucifers) and crop rotation on the development of mycorrhiza and subsequent growth of crops under field conditions. *Biologia* 22:287–298
28. Giovannetti M (1985). Seasonal variations of vesicular arbuscularmycorrhizas and endogonaceous spores in a maritime and sand dune. *Trans Br MycolSoc*, 84:679–684
29. Van Der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396:69–72
30. Bever J (2002). Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant Soil*. 244:281–290
31. Sharma C, Gupta RK, Pathak RK, &Choudhary KK (2013). Seasonal colonization of arbuscular mycorrhiza fungi in the roots of *Camellia sinensis* (Tea) in different tea gardens of India. *International Scholarly Research Notices*.
32. Chandra K.K. and A. Jamaluddin (1998). Seasonal variation of VAM fungi in tree species planted in coalmine overbunden of Kusmunda (MP), *Journal of Tropical Forest*, 14(2):118–123.
33. Morte A, Lovisolo C, and Schubert A (2000). Effect of drought stress on growth and water relations of the mycorrhizal association *Helianthemumalmeriense-Terfeziaclaveryi*. *Mycorrhiza* 10, 115–119. doi: 10.1007/s005720000066
34. Al-Karaki G, McMichael B, and Zak J (2004). Field response of wheat to arbuscularmycorrhizal fungi and drought stress. *Mycorrhiza*, 14, 263–269. doi: 10.1007/s00572-003-0265-2
35. Vieira Junior WG, Moura JBD, Souza RFD, Braga APM, Matos DJDC, Brito, GHM, Santos JMD, Moreira RM and Dutra e Silva S (2020). Seasonal variation in mycorrhizal community of different cerradophytophysiomies. *Frontiers in Microbiology*, 11:576764.
36. Hammer EC, and Rillig MC (2011). The influence of different stresses on glomalin levels in an arbuscularmycorrhizal fungus—salinity increases glomalin content. *PLoS One* 6, 1–5. doi: 10.1371/journal.pone.0028426