Effect of Physico-chemical Properties on Spore Density and Root Colonization of Mycorrhizal Fungi in Industrial Wastelands in Kota, Rajasthan

Abstract

This study was conducted in selected industrial waste dump sites in the Kota district of Rajasthan, India to investigate the impact of various edaphic factors on spore density and root colonizationof arbuscularmycorrhizal (AM) fungi.The current research shows that AMF root colonization rates were insignificantly negatively correlated with EC, soil temperature, P, K, Fe, Cu, Zn, and Mn but significantly positively correlated with soil pH, soil moisture, and insignificantly positively correlated with N and OC ($P < 0.05$). Spore density of mycorrhiza was insignificantand negatively correlated with soil moisture ($P < 0.05$), EC, soil temperature, P, K, Fe, Cu, Zn, and Mn but significantly positively correlated with soil pH and insignificantly positively correlated with N and OC.

Edaphic factors may influence the root colonization and spore density of mycorrhiza differentially. Except for pH and soil moisture, almost all other parameters have a very insignificant influence on mycorrhizal root colonization and spore density in industrial wastelands.

Keywords: Edaphic factors,Root colonization, Spore density. Industrial wastelands.

Introduction:

Mycorrhiza are obligate symbiotic soil fungi that colonize the roots of the majority of plants forming an intricate network in the root cortex, regulating community and ecosystem functioning. An arbuscularmycorrhizalfungi (AMF) is a type of mycorrhiza in which the symbiont fungus penetrates the cortical cells of the roots of a vascular plant forming arbuscules.About 80% of land plants develop mutual associations with arbuscularmycorrhiza(Zhuet al., 2012; Lehnert and Kessler, 2018). Many host plants are dependent on AMF (Gemma et al., 2002)for their nutrition as they help the plant to acquire mineral nutrients from the soil (Harleyand Smith, 1983) especially in nutrient-poor soil. Mycorrhizaplaysimportant role in increasing nutrient uptake, notably phosphorus and zinc (Bolan, 1991; Burkert and Robson, 1994).They also prevents soil erosion (Miller and Justrow, 1992) through the formation of soil aggregates, building up a macrocarpous structure of soil that allows the penetration of water and air in the soil. Mycorrhiza help host plants by enhancing their resistance to root pathogens (Borowicz, 2001) and abiotic stresses,

such as drought and metal toxicity (Meharg and Cairney, 2000;Smith and Gianinazzi-Pearson, 1988).They also improve plant growth by substantially increasing the absorption of water and nutrients in the surface area(Rouphael et al., 2015; Bowles et al., 2016). AM fungi facilitate the exchange of various macro and micronutrients, like nitrogen, phosphorus, potassium, sulfur, calcium, copper, and zinc, from the soil at the cost of precious photosynthateswhen they are associated with host plant roots (Porras-Soriano et al.2009, Bati et al., 2015; Wang et al.,2017).

The major contributor tothe dynamics of distribution and diversity of AM fungal species, root colonization, and spore population are soil physico-chemical parameters(Song et al., 2019;Smilauer and Smilauerov, 2020), especially the availability of mineral elements (Johnson et al., 2010),pH (Dumbrell et al., 2011), and electrical conductivity (Giri et al.2007; Sheng et al. 2008). Several edaphic factors viz, texture and pH of the soil, organic matter, soil moisture, and levels of macro and micro-nutrients have been shown to affect root colonization,spore germination, and efficacy of AM fungi (Khalil et al., 1992). Climatic, as well as edaphic factors can substantially influence AM fungi and their populations, thus changes in soil nutrients may affect AM association with root and spore number (Abbott and Robson, 1991). Owing to its role in stress endurance, stress tolerance, and pathogen resistance mycorrhiza are considered to play important role in the conservation of biodiversity. Hence knowledge of the various factors that influence the population biology of AM fungi is essential in any attempt to use them in environmental conservation (Allen, 1991).

Kota (24° 33’ and 25° 50’ N latitude and 75°37’ and 76° 31’E)is located along the banks of the Chambal River in the south-eastern part of Rajasthan, India. The district covers an area of 527sqkm andis known as an industrial city in Rajasthan, with Kota Thermal power plant, DCM industries, and stone mining industry as major industries. Kota has fertile land with black soil and is the trade centre for coriander and building limestone “Kota Stone”. DCM Shriram 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 situated on the banks of river Chambal whereas Limestone mines are present in the Ramganjmandi area of Kota. Non-industrial areas in the district are taken control.

Previous studies have shown that mycorrhizal association and spore formation potential of AMF was significantly lowered in soil disturbed due to industrial waste dumping (Rajpurohit and Jaiswal, 2021). Industrial wastelands have many times enriched nutrient status or nutrient stressed conditions that are different from native undisturbed soil**.** The objective of this study is the impact of various physico-chemical parameters of soil on mycorrhizalroot colonization, and spore density in 3 different industrial wastelands in the Kota district of Rajasthan, India.

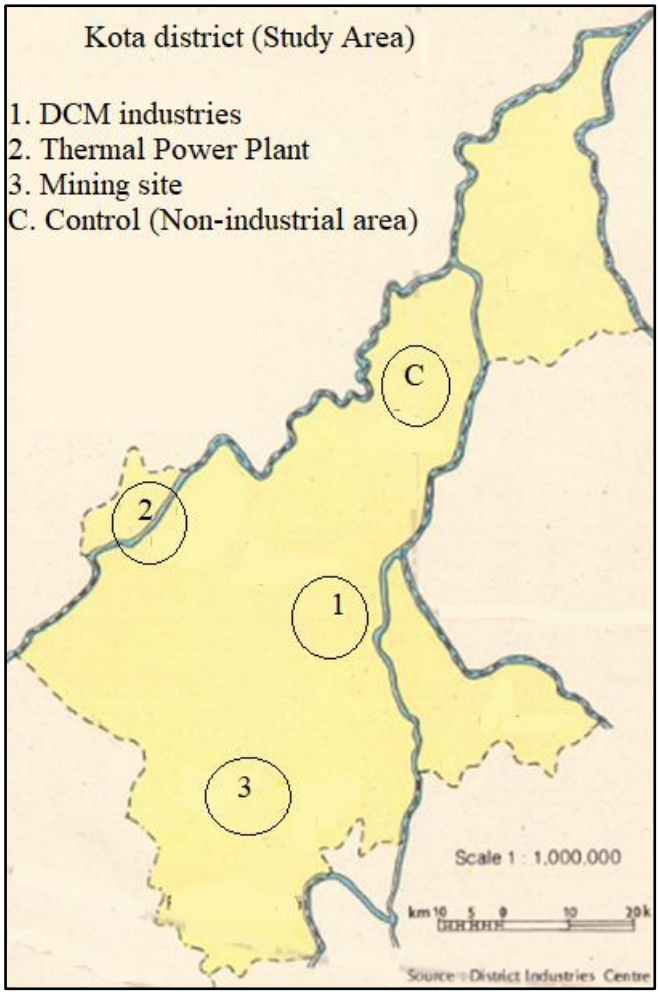


Figure 1: Map of the study area (Kota) showing control site and 3 experimental sites,

Methodology:

Waste dumping sites nearby stone mines, Kota thermal power station and DCM Industries are selected for the sampling required for the study and non-industrial area with natural vegetation is taken as control site.Each site was at equidistance within the periphery of 5-6 km. In each site 3 sample plots (five replicates from each sample plot)were selected randomly from where soil and plant specimen were collected by random sampling method.For physico-chemical characterization of soil, approximately

1,000 g of soil samples were collected from rhizospheresof randomly selected plants in each sample plot of selected site.Soils were collected from 15 to 30 cm depth, and were filled in sterile polythene bags and were brought to the laboratory and stored at 5°C–10°C. Each replicate of the soil sample was analyzed forphysico-chemical characteristics, like pH, EC, soil moisture, soil temperature, macro-nutrients (N, P, K), and micronutrients (Fe, Zn, Cu, Mn). The pH of the soil was measured using a pH meter. The Electrical conductivity was determined using a conductivity meter in 1:5 (W/V) soil water suspensions at 25°C. Organic Carbon (OC) was estimated using the chromic acid titration method (Walkely and Black, 1934). The Kjeldahl method was used to estimate the available N content using alkaline permanganate (Subbiah and Asija 1956). Available P in the soil was determined by Olsen's method by extraction with sodium bicarbonate using a spectrophotometer (Olsen et al., 1954). Total exchangeable K was determined by the ammonium acetate method (Hanway and Heidel, 1952) using a flame photometer. Fe, Mn, Zn, and Cu were estimated by acid digestion of the soil method (Jackson, 1967).

The soil samples were also used for the isolation, quantification, and identification of AM fungal spores. The fine roots of plants having mycorrhizal association were collected, rinsed with tap water, and used to investigate the percentage of root colonization. To prepare roots for the assessment of percentage root colonization Philips and Hayman's root staining and clearing method was used Philips and Hayman (1970). The percentage root colonization was determined by slide count and gridline intersect method (Giovannetti and Mosse, 1980) using the following formula:

Root colonization (%) = (Number of AM positive segments /Total number of segments observed) × 100

The AM fungal spore density was analyzed from 100 g of rhizosphere soil by using wet sieving and decanting method (Gerdemann and Nicolson,1963). 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. The total spore count was carried out using Leica EZ4 stereomicroscope.

Statistical Analysiswas done for Pearson's correlation coefficients of the different physico-chemical parameters of soil versus AM fungal spore density and root colonization associated with host plants was calculated.Significance of the correlation coefficient value is validated with student's *t* test.

Observation and Results:

Physico-chemical properties of soil

Table 1: Physical and chemical properties of control and threeexperimental sites (industrial waste dump sites).

S. N.	Parameter				
		Control	**DCM**	**Thermal**	**Mining**
1	pH	7.19±0.21	7.51±0.33	7.66±0.16	7.86±0.24
2	EC (ds/m)	0.22±0.02	0.28±0.24	0.26±0.20	0.24±0.21
3	Average Soil temp. (C°)	29.9±0.76	31.43±0.32	32.87±0.24	34.1±0.41
4	Moisture Content (%)	14.60±0.91	10.92±0.46	9.92±0.85	8.02±0.46
5	N (Kg/hect.)	363.2±4.05	395.7±3.96	318.16±4.21	291.43±3.62
6	P (Kg/hect.)	32.43±1.07	27.13±0.29	23.16±1.01	20.03±0.86
7	K (Kg/hect.)	168.66±2.33	183.75±3.42	206.6±5.03	223.7±4.25
8	OC (%)	0.48±0.02	0.44±0.24	0.44±0.31	0.35±0.26
9	Fe(ppm)	2.59±0.56	3.8±0.28	5.2±0.41	6.3±0.59
10	Mn(ppm)	2.3±0.39	2.45±0.08	2.66±0.51	2.82±0.09
11	Cu (ppm)	0.97±0.62	1.31 ±0.53	0.85±0.08	0.76±0.24
12	Zn (ppm)	0 .72±0.05	0.85±0.09	0.69±0.11	1.68±0.14
13	Mean Root colonization (%)	53.51±1.33	23.10±1.02	12.15±1.08	12.45±2.46
14	Mean spore density (spores/10 gm of soil)	26.5±1.15	19.17±0.09	9.75±0.31	7.83±0.33

Characterization of the soil samples collected from 4 different sites show variation in almost all parameters. The alkalinity of the soil showsan increasing trend in wastelands of DCM industries, thermal power plant sites, and mining waste dump sites whereas control site have slightly alkaline soil pH (7.19 ±0.21). DCM industrial area has maximum electrical conductivity whereas it is least in the control site. Average moisture content is found to be highest in control are whereas it is least in waste dump sites in the mining area. Likewise,the mean soil temperature is highest in mining waste dump sites and lowest in control areas. Lack of vegetation cover may be the reason for low moisture content and high soil temperature in mining waste dump sites as well as in thermal waste dump sites (Table 1).

The organic carbon content of the DCM industrial wasteland shows increased values, whereas the mining waste dump site shows decreased organic carbon. The thermal power plant waste dump site has organic carbon values almost similar to the undisturbed (control) site. Lack of vegetation may be the reason for low organic carbon content in mining waste dump sites. Available Nitrogen was highest in DCM waste dump sites and lowest in mining waste lands. Potassium increase in all the three experimental sites as compared to the control site whereas Phosphorus decreases from the control area to DCM industries to thermal to mining waste dump sites. This proves that among macro-nutrients, Potassium increase with increasing disturbances whereas available nitrogen and Phosphorus decreases. Amongst micro-nutrients, Iron and Manganese increase in all three experimental sites whereas Cu shows the highest value in DCM industrial waste dump site and Zinc shows the highest value in mining waste dump sites (Table 1).

Correlation between soil physico-chemical properties and spore density and root colonization

When parameters related to mycorrhizaare analyzed, mean root colonization (%) and spore density (Spore/ 10 gm of soil) show a decrease from the control site in all three experimental sites. Thermal power plan waste dump site show least root colonization whereas least spore density was recorded in mining waste dump sites.

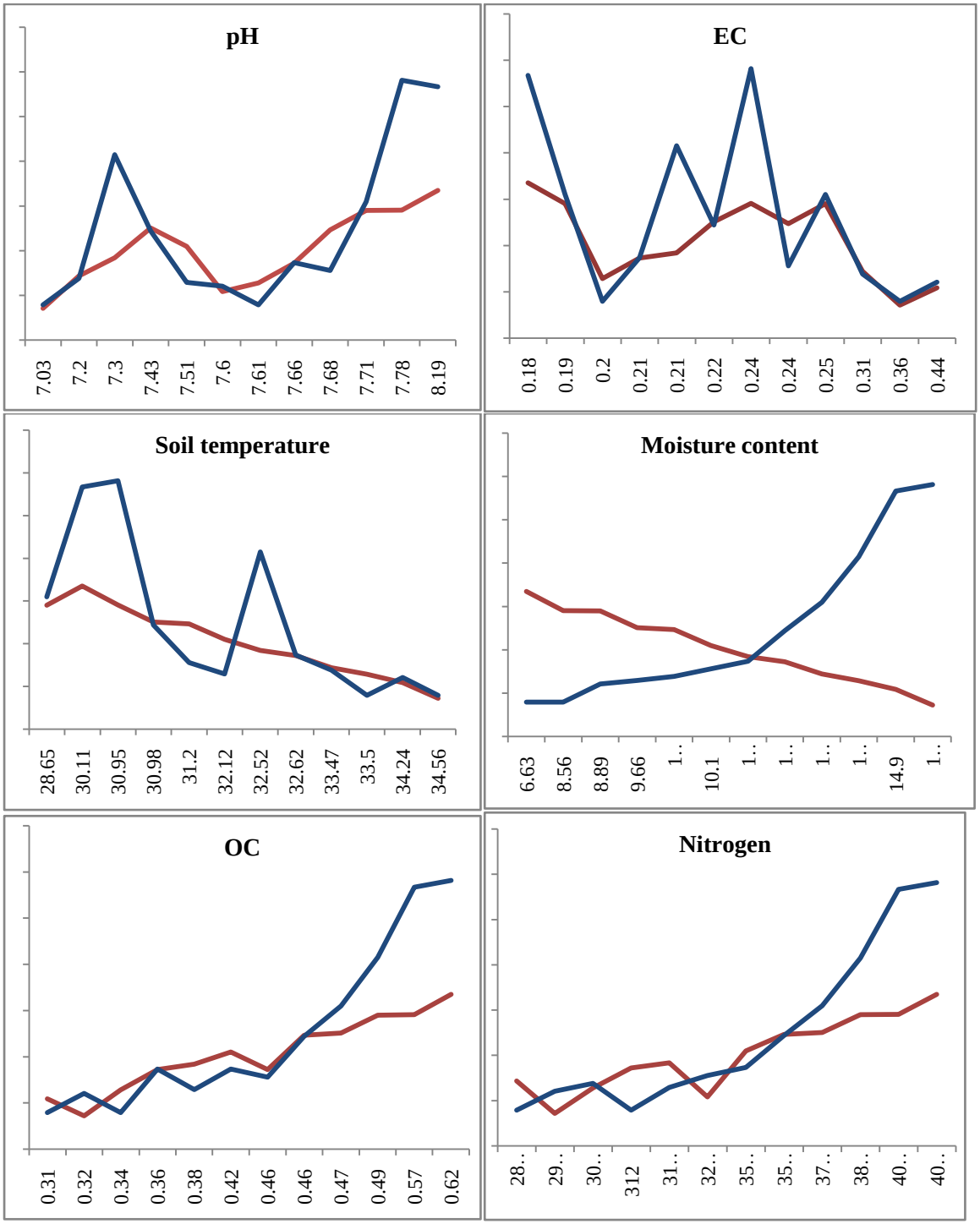
A correlation study was carried out to study the effect of physico-chemical characteristics of the soil on spore density and mean root colonization. Pearson's Correlation coefficient was estimated between rootzone soil parameters, such as pH, EC, soil moisture, soil temperature, OC, available N, P, K, Cu, Zn, Mn, and Fe, and % root colonization and spore density of mycorrhiza (Table 2).

Table 2: Pearson's Correlation coefficient between physico-chemical properties of soil with percentage root colonization and spore density of mycorrhiza.

Variable	Spore Density	Root colonization
Root colonization	0.540^{S}	
pH	0.738^{S}	0.575^{S}
EC	-0.087^{NS}	-0.093^{NS}
Soil moisture	-0.506^{NS}	0.589^{S}
Soil temperature	-0.063^{NS}	-0.043^{NS}
OC	0.071^{NS}	0.216^{NS}
N	0.114^{NS}	0.112^{NS}

P	-0.227 ^{NS}	-0.193 ^{NS}
K	-0.106 ^{NS}	-0.077 ^{NS}
Fe	-0.557 ^{NS}	-0.615 ^{NS}
Zn	-0.068 ^{NS}	-0.050 ^{NS}
Cu	-0.025 ^{NS}	-0.018 ^{NS}
Mn	-0.040 ^{NS}	-0.027 ^{NS}

^{S}Correlation is significant at ($P < 0.05$) level, ^{NS}Correlation is significant at ($P < 0.05$) level



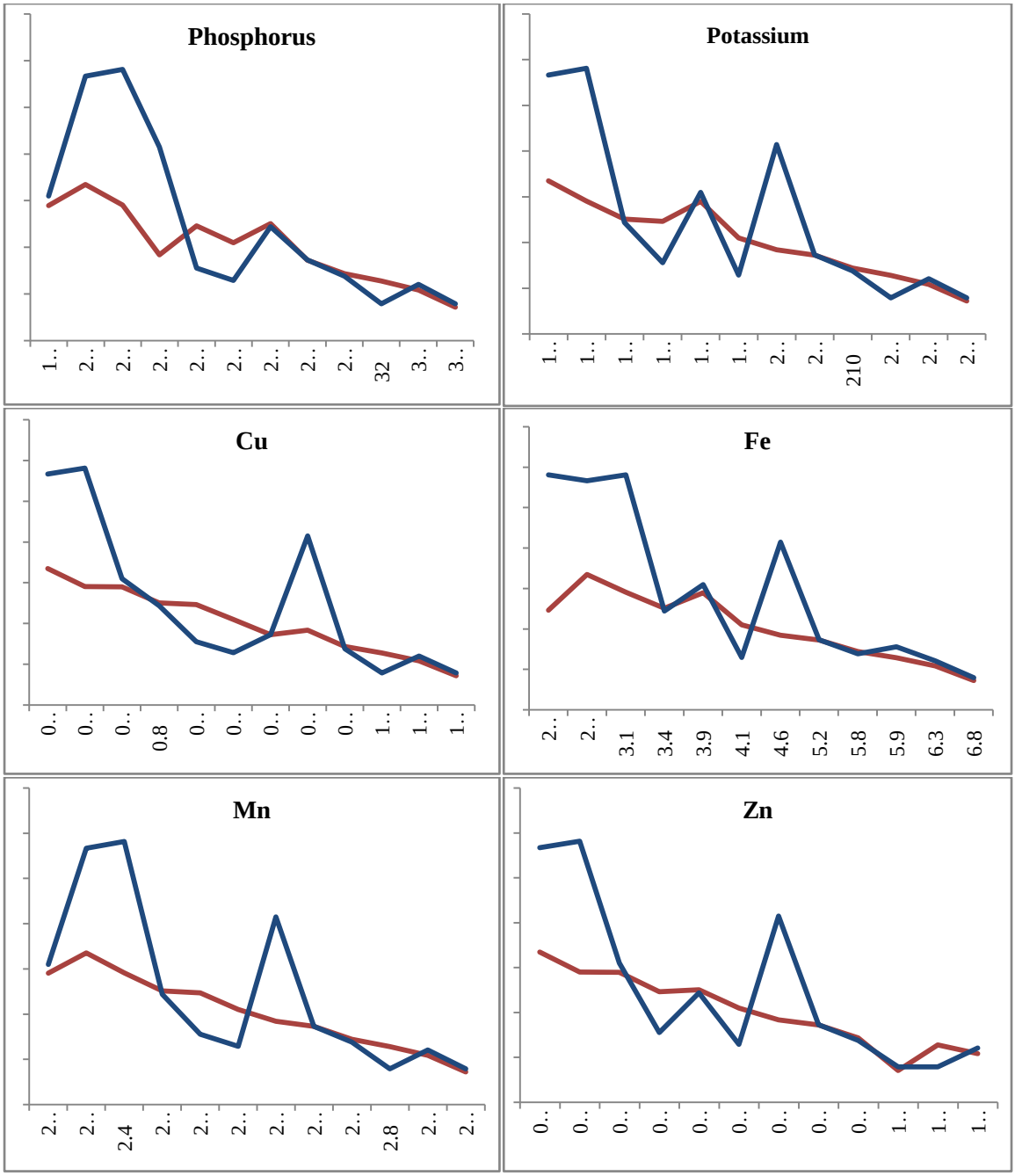


Figure 2: Correlation between physico-chemical parameters of soil and percentage mycorrhizal association and spore density (spore density is shown by the red line and root colonization by blue line. Physico-chemical parameters are taken on X-axis and spore density and % root colonization on Y-axis).

The results show that mean spore density and mean root colonization both have a significant positive correlation with soil pH. Spore density and root colonization of mycorrhizaare negatively correlated to soil Electrical conductivity (EC) which is not significant. Soil moisture is negatively correlated (non-significant) with spore density but positively correlated

with percentage root colonization (significant). Soil temperature is negatively correlated (not significant) with both the spore density and root colonization. All the macronutrients (P, K) except N and micronutrients (Fe, Zn, Cu, and Mn) exhibited no significant negative correlation with spore density and root colonization.

Table 2 show that the correlation between physico-chemical parameters of the soil; pH, EC, and soil temperature show correlation with spore density and root colonization but they do not show a clear-cut trend of their correlation (Figures 2). The same is the trend in the case of macro and micro-nutrients in soil.

Discussion: The change in soil properties and physiological function of plants will directly affect colonization, growth, N metabolism, and P uptake of AMF (Lin et al. 2015).VAM fungi associated with plants occur across a broad range of pH, both acid and alkaline, with more species occurring in the alkaline range. Results of the present study show that all the soil samples including the control show slightly alkaline pH.Mycorrhizal association and spore number were positively influenced by soil pH but their response to soil pH may doesn't show the same trend. Though effect of soil pH on root colonization was statistically significant but in graphical presentation it not found to be consistent (Figure 2).Mycorrhizal colonization and community structure can vary with soil pH (Coughlan et al., 2000; Van Aarle et al., 2002; Melo et al., 2017). Acidic soils have been reported to reduce the diversity of AM fungi (Coughlan et al., 2000; Wang et al., 1993). Variations in soil pH alter the concentration of many nutrients and toxic ions and hydrogen ions in a soil solution hence may affect the development and functioning of arbuscularmycorrhiza (Hayman and Tavares, 1985). The variation may also be attributed to the host's mediated changes in pH of rhizosphere. The response of AM fungi to soil pH may depend on the species and strains constituting the indigenous AM flora (Robson and Abbott, 1989). Soil electrical conductivity (EC) is a measure of the amount of salts in the soil. A higher conductivity value indicates that there are more chemicals dissolved in the water.Most of the industrial effluents carry dissolved and solids salts that may increase the EC of the soil. Though EC of the soil is insignificantly and negatively correlated with both the root colonization and spore density it doesn't show a consistent correlation with root colonization (Figure 2).

Various soil properties that depend on fluctuation in environmental factors and climatic variables viz. soil temperature and moisture also affect mycorrhiza communities. The effects of temperature on root colonization are complex and the responses of mycorrhiza vary with

both host plant and fungus (Smith and Read, 2008). VAM colonization is repressed at low temperatures (15°C) (Zhang et al., 1995). The maximum temperature for mycorrhizal association ranged between 26.2 and 29.3°C (Jerbi et al., 2020).Higher temperature favours root colonization because root elongation rate is enhanced under increased temperature leading to a better AMF root colonization (Frater et al., 2018). Whereas at a low temperature, reduced nutrient acquisition by AMF leads to a decrease in mycorrhizal colonization (Hetrick and Bloom, 1984). Growth and colonization of mycorrhiza occur at an optimum medium temperature. In the present study 30°C is found to be the optimum temperature for the mycorrhizal association but the correlation between soil temperature and root colonization is not significant.When the temperature exceeded an optimum it had a negative effect on root colonization (Soudzilovskaia et al., 2015). The responses of AMF to an increase or a decrease in temperature seem to vary according to the host plant species (Heinemeyer and Fitter, 2004).

Soil moisture content and seasonal fluctuations also influence AM fungal communities (Deepika and Kothamasi, 2015; Shinde and Singh, 2017). Mycorrhizal colonization was directly correlated with precipitation (Zhang et al., 2016), on a contrary, Augé et al., (2015) reported that AMF colonization increased under water-limiting conditions.Results of the present study go with Allen, (1984) and Khanam et al., (2006) who reported that soil moisture and AM fungal colonization were positively correlated but contradict Dickman et al., (1984) who reported that spore population was positively correlated with soil moisture. Generally, AM fungi are sensitive to soil moisture and optimum moisture for plant growth is suitable for AM colonization and sporulation (Redhead, 1975).

In the present study, organic carbon showed a non-significant positive correlation with both mycorrhizal spore density and root colonization, thereby corroborating previous reports (Boddington and Dodd 2000; Khanam et al. 2006). These results are also in line with the findings of Liu et al. (2000) and Sivakumar (2013) who also found a high correlation between OC and spore production. Organic matter also affects the mycorrhizal community in soil (Torrecillas et al., 2014; Wang et al., 2015). There are a few contradictory reports that OC and spore density were negatively related (Hindumathi and Reddy, 2011). Colonization rates affect the capacity of AMF to confer its associated host plant with soil nutrients in return for Carbon that is required for the growth of mycorrhiza, which directly affects spore germination and the growth of fungal hyphae (Cai, 2017). Organic matter enhances the water holding capacity of soil which may increase the sporulation of AM fungi thus showing a

positive correlation with organic carbon content in the soil (Mohammad et al. 2003; Mathur et al. 2007). AMF is thought to positively influence soil Carbon pool (Wilson et al. 2009), and in the long-term may increase carbon storage (Iversen et al. 2012).

The amount of available N positively influences the AM fungi spore population and colonization (Egerton-Warburton et al., 2007; Silvana et al., 2020) and positively correlated with root colonization and spore number (Khanam, et al., 2006). The results of the present study also corroborate with these findings. Aziz and Hebte (1989) reported the stimulation of root colonization by soil N. There are reports that N can either stimulate or suppress root colonization and spore production through modifications of soil pH (Sylvia and Neal, 1990). Nitrogen plays an important role in influencing the formation of mycorrhizal association and functions mainly through changes in soil pH. However, the effect of Nitrogen and spore abundance is related to other soil factors and to the host with which they are associated.

AM fungi are often important in root colonization, especially in soil with limited phosphorus (Allen 1991). Soil P was negatively correlated with the abundance of root colonization (Khanam et al., 2006; Morita and Konishi, 1989; Bainard et al., 2014; Nguyen et al., 2019) but there are reports that a high soil P supply does not always have a negative impact on AM fungi colonization (Gosling et al., 2013) and spore density also (Khade and Rodrigues, 2009). On the contrary Khanam et al., (2006); Bhardwaj et al., (1997) reported that soil P was positively correlated with spore density. Variation in response to root colonization and spore number to the soil P could be attributed to several factors. It is also reported that higher soil P can reduce AM formation and the inhibition may be due to a direct effect on the external hyphal growth or be indirectly associated with host P status (Sander, 1975). Other factors which attribute to the variation in response of root colonization and spore density are sensitivity of mycorrhiza species and strain to phosphorus (Trouvelot et al., 1987). The varied host root growth response to changes in P levels (Smith, 1982), or change in the cell membrane permeability to varying cellular P concentrationsalso affect the degree of AM colonization and sporulation (Daniels Hetrick, 1984).

Soil Potassium was found to be insignificantly negatively correlated with root colonization as well as with spore density. The findings were in harmony with those of Khade and Rodrigues (2009) but contradicted Gaur and Kaushik (2011), and Abubacker et al. (2014) who observed a positive relationship between Available Potassium and spore density, and Khanam et al. (2006) who reported a positive correlation with root colonization. A negative correlation

between Available Potassium and root colonization was also reported by Ardestani et al. (2011). An inverse relationship between AMF root colonization and Available Potassium could be due to the fact that AMF tends to lose its potential to develop its structural components such as arbuscules as the levels of Potassium concentration in soil increase (Vogel-Mikuš et al. 2005). Soil micronutrients (Fe, Cu, Zn, and Mn) are insignificantly negatively correlated with root colonization and spore density.

Environmental factors that influence mycorrhizal root colonization are chemical soil characteristics and climatic factors, whereas physical soil properties had no significant influence on AMF root colonization (Jerbi et al., 2020). Studies indicate that soil pH had a greater influence on AM fungal communities than host plant species (Dumbrell et al., 2010).Lin et al., (2020) reported that except, for soil moisture, there was no significant correlation with the other soil physico-chemical factors with root colonization and spore density. But relative content of N and P in the soil affects AMF colonization and spore density. Some studies indicate that the host plant favours mycorrhizal association. On industrial wastelands plants of family Fabaceae have higher root colonization (Rajpurohit and Jaiswal, 2020).

Conclusion

In the present research,it can be concluded that most of the edaphic factors had a varied influence on AM fungal colonization and spore number. The mycorrhizal root colonization and spore density respond differentiallyto physical, chemical, and climatic factors of the soil.Various soil factors have a combined influence on the mycorrhiza whose response may be specific to mycorrhiza species. The research also shows that in response to physico-chemical properties, root colonization shows no perfect correlation whereas spore density follows a more or less perfect correlation (Figure 2).There are possibilities that the availability of host plants has a greater role to play in mycorrhizal colonization and spore density than soil physico-chemical properties of soil.

Bibliography:

1. Abbott L, Robson A. 1991. Factors influencing the occurrence of vesicular—arbuscularmycorrhizas. *AgricEcosyst Environ* 35:121–150
2. Abubacker M., Visvanathan M, Srinivasan S. 2014. Impact of Pesticides on AMF Spore Population and Diversity in Banana (*Musa* Spp.). *Soils.*2: 1279–1286.

3. Allen EB. 1984. The role of mycorrhiza in mined land diversity. In: Proceedings of the third biennial Symposium surface mine reclamation on the great plains, *Montana*, 19–21 March 1984, 273–295
4. Allen, M.F., 1991. The Ecology of Mychorrhizae, Cambridge University Press. Cambridge.
5. Ardestani NK, Zare-Maivan H, Ghanati F. 2011. Effect of different concentrations of potassium and magnesium on mycorrhizal colonization of maize in pot culture. *African Journal of Biotechnology* 10: 16548-16550.
6. Augé RM, Toler HD, Saxton AM. 2015. Arbuscularmycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*., 13–24. https://doi.org/10.1007/s00572-014-0585-4 pmid:24831020
7. Aziz, T and Habte, M 1989. Influence of inorganic N on mycorrhizal activity, nodulation, and growth of *Leucaenaleucocephala* in an oxisol subjected to simulated erosion. *Commun. Soil Sci. Plant Anal.* 20, 239–251.
8. Bainard LD, Bainard JD, Hamel C, Gan Y. 2014. Spatial and temporal structuring of arbuscularmycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. *FEMS Microbiol Ecol*., 88:333–44; doi:10.1111/1574-6941.12300
9. Bati CB, Santilli E, Lombardo L. 2015. Effect of arbuscularmycorrhizal fungi on growth and on micronutrient and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels. *Mycorrhiza,* 25:97–108; doi:10.1007/s00572-014-0589-0
10. Bhardwaj S., S.S Dudeja, A. L. Khurana 1997 Distribution of Vesicular-ArbuscularMycorrhizal Fungi in the Natural Ecosystem, *Folia Microbiologica.* 42(6):589-594, DOI: 10.1007/BF02815471
11. Boddington CL, Dodd JC 2000. The effect of agricultural practices on the development of indigenous arbuscularmycorrhizal fungi. *Plant Soil* 218:145–157
12. Bolan NS. 1991. A critical review of the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil* 134:189–208
13. Borowicz VA 2001. Do arbuscularmycorrhizal fungi alter plantpathogen reactions? *Ecology* 82:3057–3068
14. Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE. 2016. Effects of arbuscularmycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. *Sci Total Environ*, 566:1223–34; doi:10.1016/j. scitotenv.2016.05.178
15. Burkert B, Robson A. 1994. Zn uptake in subterranean clover (Trifoliumsubterraneum L.) by three vesicular-arbuscularmycorrhizal fungi in a root-free sandy soil. *Soil BiolBiochem* 26:1117– 1124
16. Cai, X. Z.2017. Effects of nitrogen addition on arbuscularmycorrhizal fungi and nitrogen uptake of Chinese fir seedlings. PhD Thesis, Fujian Normal University.
17. Coughlan A, Dalpe Y, Lapointe L, Piche Y. 2000. Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscularmycorrhizal fungi

from healthy and declining maple forests. *Canadian Journal of Forest Research* 30: 1543–1554.

18. Daniels Hetrick BA 1984. Ecology of VA mycorrhizal fungi. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. *CRC Press, Boca Raton, Fla*, 35–55
19. Deepika S, Kothamasi D. 2015. Soil moisture - a regulator of arbuscularmycorrhizal fungal community assembly and symbiotic phosphorus uptake. *Mycorrhiza*, 25:67–75; doi:10.1007/s00572-014- 0596-1
20. Dickman L A, Libera A E, Anderson R C. 1984. Ecological interation of little blue stem and vesiculararbuscularmycorrhizal fungi, *Can J Bot*, 62: 2272-2277
21. Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T. Distinct seasonal assemblages of arbuscularmycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol. 2011 May;190(3):794-804. doi: 10.1111/j.1469-8137.2010.03636.x.
22. Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *The ISME Journal* 4: 337–345.
23. Egerton-Warburton LM, Johnson NC, Allen EB. 2007. Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. *EcolMonogr*, 77:527–44.
24. Frater, P. N., Borer, E., Fay, P. A., Jin, V., Knaeble, B., Seabloom, E., Sullivan, L. L., Wedin, D. A., &Harpole, W. S. (2018). Nutrients and environment influence arbuscularmycorrhizal colonization both independently and interactively in Schizachyriumscoparium. *Plant and Soil*, *425*(1-2), 493-506. https://doi.org/10.1007/s11104-018-3597-6
25. Gaur S, Kaushik P. 2011. Analysis of vesicular arbuscularmycorrhiza associated with medicinal plants in Uttarakhand state of India.*World. Appl. Sci. J*. 14: 645-653
26. Gemma JN, Koske RE,Habte M. 2002.Mycorrhizal dependency of some endemic and endangered Hawaiian plant species. *Am J Bot* 89:337–345
27. Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizalendogone species extracted from soil by wet sieving and decanting. *Trans Br MycolSoc* 1963;46:235–44.
28. Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytol*., 84:489–500.
29. Giri B, Kapoor R, Mukerji KG. 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscularmycorrhiza*Glomusfasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. *MicrobEcol*, 54:753–60.
30. Gosling P, Andrew M, Maude P, Hammond JP, Bending, GD. 2013. Contrasting arbuscularmycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. *New Phytol* 2013;198:546–56; doi:10.1111/nph.12169
31. Hanway JJ, Heidel H. 1952. Soil analysis methods as used in Iowa state college soil testing laboratory. *Iowa State CollAgric Bull*, 57:1– 31.
32. Harley, J.L. and Smith, S.E., 1983. Mycorrhizal Symbiosis. *Academic Press. London*, pp 483.

33. Hayman D S, Tavares M. 1985. Plant growth responses to vasiculararbuscularmycorrhiza.XV. Influence of soil pH on the symbiotic efficiency of different endophytes. *New Phytol*. 100:567-577
34. Heinemeyer A, Fitter AH. 2004. Impact of temperature on the arbuscularmycorrhizal (AM) symbiosis: growth responses of the host plant and its AM fungal partner. *J Exp Bot*. 55(396): 525–534. https://doi.org/10.1093/jxb/erh049 pmid:14739273
35. Hetrick BAD, Bloom J. 1984. The Influence of Temperature on Colonization of Winter Wheat by Vesicular-ArbuscularMycorrhizal Fungi. *Mycologia*. 76(5): 953–956. https://doi.org/10.1080/00275514.1984.12023937.
36. Hindumathi A and Reddy BN. 2011. Occurrence anddistribution of arbuscularmycorrhizal fungi andmicrobial flora in the rhizosphere soils of mungbean [*Vignaradiata* (L.)] and soybean (*Glycine max* (L.)Merr.] fromAdilabad, Nizamabad and Karimnagardistricts of Andhra Pradesh state, India. *AdvBiosciandBiotechnoogy*. 2: 275-286
37. Iversen CM, Keller JK, Garten CT, Norby RJ. 2012. Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of CO_2-enrichment. *Global Change Biology* 18: 1684-1697.
38. Jackson ML. 1967. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi, India, 36–82.
39. Jerbi M., Labidi S., Lounès-HadjSahraoui A., Chaar, H., & Ben Jeddi, F. 2020. Higher temperatures and lower annual rainfall do not restrict, directly or indirectly, the mycorrhizal colonization of barley (*Hordeumvulgare* L.) under rainfed conditions. *PLoS ONE, 15*.
40. Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *ProcNatlAcadSci USA*, 107:2093–8.
41. Khade SW, Rodrigues BF. 2009. Arbuscularmycorrhizal fungi associated with varieties of *Carica papaya* L. in tropical agro-based ecosystem of Goa, India. *Tropical and Subtropical Agroecosystems*10: 369-381.
42. Khalil S, LoynachanT E, McNabb H S. 1992. Colonization of soybean by mycorrhizal fungi and spore population in Iowa soil. *Agron J*. 84:832-836
43. Khanam D, Mridha M. A. U., Solaiman A. R. M. and Hossain T. 2006 Effect of edaphic factore on root colonization and spore population of arbuscularmycorrhizal fungi, Bull. *Inst. Trop. Agr. Kyushu Univ.* 29: 97-104
44. Lehnert M, Kessler M. 2018. Mycorrhizal relationships in lycophytes and ferns. *Fern Gazette, British Pteridological Society, The Natural History Museum, Cromwell Road, London, UK*, 20(3):101–16
45. Lin C., Wang Y., Liu M. Li Q., Xiao W., & Song X. 2020. Effects of nitrogen deposition and phosphorus addition on arbuscularmycorrhizal fungi of Chinese fir (*Cunninghamialanceolata*). *Sci Rep*. 10, 12260. https://doi.org/10.1038/s41598-020-69213-6
46. Lin JX.2015. Research progress on effects of nitrogen deposition on symbiont of plant-arbuscularmycorrhizal. *Grassl. Turf.* **35**:88–94.

47. Liu Y, Su MY, Yan XH, Liu WT. 2000. The mean-square slope of ocean surface waves and its effects on radar backscatter. *Journal of atmospheric and oceanic technology* 17: 1092-1105.
48. Mathur N, J Singh, AfshanQuaizi, AVyas. 2007. ArbuscularMycorrhizal Fungi: A Potential Tool for Phytoremediation, *J Plant Science*. 2(2):127-140 DOI: 10.3923/jps.2007.127.140
49. Meharg AA, Cairney JWG. 2000. Co-evolution of mycorrhizalsymbionts and their hosts to metal-contaminated environments. *AdvEcol Res.* 30:69–112
50. Melo CD, Luna S, Krüger C., Walker C., Mendonça, D., Fonseca, H M A C., Jaizme-Vega, M., Machado, A da C.2017. Arbuscularmycorrhizal fungal community composition associated with *Juniperusbrevifolia* in native Azorean forest. *ActaOecol*., 79:48–61.
51. Miller RM, Justrow JD. 1992. The application of VA mycorrhizae to ecosystem restoration and reclamation. In: Allen MF (ed) Mycorrhizal functioning. *Chapman and Hill, London,*438–467
52. Mohammad MJ, Hamad SR, Malkawi HI 2003. Population of arbuscularmycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. *Journal of Arid Environments* 53: 409-417.
53. Morita A, Konishi S 1989. Relationship between vesicular arbuscularmycorrhizal infection and soil phosphorus concentration in tea fields. *Soil Sci Plant Nutr*. 35: 139-143
54. Nguyen TD, Cavagnaro TR, Watts-Williams SJ. 2019. The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi: a physiological and molecular assessment. *Sci Rep*., 9:14880; doi:10.1038/s41598-019-51369-5
55. Olsen SR, Cole CV, Watanabe FS, La D. 1954. Estimation of available phosphorus by extraction with sodium bicarbonate (Circular 39). USDA, Washington, DC.
56. Philips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscularmycorrhizal fungi for rapid assessment of infection. *Transact Br Mycol Soc*., 55(1):158–61.
57. Porras-Soriano A, Soriano-Martín ML, Porras-Piedra A, Azcón R. 2009. Arbuscularmycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J Plant Physiol*, 166:1350–9; doi:10.1016/j.jplph.2009.02.010.
58. Rajpurohit S. S and PoonamJaiswal 2020. Studies on the Occurrence and Distribution of Mycorrhiza in Different Sites of Kota, Rajasthan, India, *Applied Ecology and Environmental Sciences,* 8(6):408-413, DOI:10.12691/aees-8-6-12
59. Rajpurohit S. S and PoonamJaiswal 2021.Mycorrhizal Association in Industrial Wastelands in Kota, Rajasthan, India, *International Journal for Research in Applied Sciences and Biotechnology,* 8(3):231-236, https://doi.org/10.31033/ijrasb.8.3.28
60. Redhead, J F. 1975 .Endtrophicmycorrhiza in Nigeria Some aspects of the ecology of the endotrophicmycorrhizal association of *Khayagrandiflora* C DC In Sanders, F E , Mosse, B and Tinker, P B (Eds), Endomycorrhizas*Academic Press, Ney York*pp 447-460

61. Robson AD, Abbott LK.1989. The effect of soil acidity on microbial activity in soils. In: Robson AD (ed) Soil acidity and plant growth. *Academic Press, Sydney*, 139–165
62. Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M. 2015. Arbuscularmycorrhizal fungi act as bio-stimulants in horticultural crops. *SciHort*, 196:91–108; doi:10.1016/j. scienta.2015.09.002
63. Sheng M, Tang M, Chan H, Yang B, Zhang F, Huang Y. 2008. Influence of arbuscularmycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza*, 18:287–96.
64. Shinde BP, Singh N. 2017. Effect of arbuscularmycorrhizal fungi on growth parameters of sweet corn under NaCl salinity. *Int J CurrMicrobiolAppl Sci*., 6(2):1317–25; doi:10.20546/ijcmas.2017.602.149
65. Silvana VM, Carlos FJ, Lucía AC, Natalia A, Marta C. 2020. Colonization dynamics of arbuscularmycorrhizal fungi (AMF) in Ilexparaguariensis crops: Seasonality and influence of management practices. *J King Saud Univ Sci*., 32(1):183–8; doi:10.1016/j.jksus.2018.03.017
66. Sivakumar N, 2013. Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscularmycorrhizal fungi in sugarcane fields. *Annals of microbiology* 63: 151-160.
67. Smilauer P, Smilauerov M. 2020. Contrasting effects of host identity, plant community, and local species pool on the composition and colonization levels of arbuscularmycorrhizal fungal community in a temperate grassland. *New Phytol*, 225(1):461–73 doi:10.1111/nph.16112
68. Smith S E 1982. Inflow of phosphate into mycorrhizal and nonmycorrhizal plants of*Trifoliumsubterraneum* at different levels of soil phosphate. *New Phytol*. 90, 293–303.
69. Smith SE, Read DJ. 2008. Mycorrhizal Symbiosis, 3rd Ed. *Academic Press (Elsevier), New York.* 787 pp.
70. Smith, S.E. & V. Gianinazzi-Pearson. 1988. Physiological interaction between symbionts in vesicular - arbuscularmycorrhizal plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 39: 221-224.
71. Song J, Han Y, Bai B, Jin S, He Q, Ren J. 2019. Diversity of arbuscularmycorrhizal fungi in rhizosphere soils of the Chinese medicinal herb *Sophoraflavescens*Ait. *Soil Tillage Res*, 195:104423. doi: 10.1016/j.still.2019.104423
72. Soudzilovskaia NA, Douma JC, Akhmetzhanova AA, Van Bodegom PM, Cornwell WK, Moens EJ, et al. 2015. Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. *Global EcolBiogeogr*. 24(3): 371–382. https://doi.org/10.1111/geb.12272.
73. Subbiah BV, Asija GL. 1956. A rapid procedure for determination of available nitrogen in soils. *CurrSci*, 25:259–60.
74. Sylvia DM, Neal LH. 1990. Nitrogen affects the phosphate response of VA mycorrhiza. *New Phytol* 115:303–310
75. Torrecillas E, Alguacil MM, Roldán A, Díaz G, Montesinos-Navarro A, Torres P. 2014. Modularity reveals the tendency of arbuscularmycorrhizal fungi to interact

differently with generalist and specialist plant species in gypsum soils. *Appl Environ Microbiol*, 80:5457–760; doi:10.1128/AEM.01358-14

76. Trouvelot A., Gianinazzi, S., and Gianinazzi-Pearson, V., 1987, Screening of VAM fungi for phosphate tolerance under simulated field conditions. In :Mycorrhizae in the New Decade. Proc. 7th North Am. Conf. on Mycorrhiza. DM. Sylvia; L. L. Hung and J. H. Graham (eds.) Univ. Florida. Gainesville, Florida, IFAS, p39
77. Van Aarle IM, Olsson PA, Söderström B. 2002. Arbuscularmycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytologist* 155: 173–182.
78. Vogel-Mikuš K, Drobne D, Regvar M. 2005. Zn, Cd and Pb accumulation and arbuscularmycorrhizalcolonisation of pennycress Thlaspi praecox Wulf.(Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environmental Pollution* 133: 233-242.
79. Walkely AJ, Black IA. 1934. Estimation of soil organic carbon by the chromic acid titration method. *Soil Sci.,* 37:29–38.
80. Wang C, Gu Z, Cui H, Zhu H, Fu S, Yao Q. 2015. Differences in arbuscularmycorrhizal fungal community composition in soils of three land use types in subtropical hilly area of Southern China. *PLoS One, 10(6):1–16; doi:10.1371/journal.pone.0130983*
81. Wang GM, Stribley DP, Tinker PB, Walker C. 1993. Effects of pH on arbuscularmycorrhiza I. Field observations on the long-term liming experiments at Rothamsted and Woburn. *New Phytologist* 124: 465–472.
82. Wang W, Shi J, Xie Q, Jiang Y, Yu N, Wang E. 2017. Nutrient exchange and regulation in arbuscularmycorrhizal symbiosis. *Mol Plant*, 10(9):1147–58 doi:10.1016/j.molp.2017.07.012
83. Wilson GW, Rice CW, Rillig MC, Springer A, Hartnett DC. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscularmycorrhizal fungi: results from long-term field experiments. *Ecology Letters* 12: 452-461.
84. Zhang F, Hamel C, Kianmehr H, Smith DL. 1995. Root-zone temperature and soybean [*Glycine max*. (L.) Merr.] vesicular-arbuscularmycorrhizae: Development and interactions with the nitrogen fixing symbiosis. *Environmental and Experimental Botany* 35: 287-298.
85. Zhang J, Wang F, Che R, Wang P, Liu H, Ji B. 2016. Precipitation shapes communities of arbuscularmycorrhizal fungi in Tibetan alpine steppe. *Sci Rep*., 6: 23488. https://doi.org/10.1038/srep24556 pmid:27002188
86. Zhu XC, Song FB, Liu SQ, Liu TD, Zhou X. 2012. Arbuscularmycorrhizae improve photosynthesis and water status of *Zea mays* L. under drought stress. *Plant Soil Environ*, 58(4):186–91; doi:10.17221/23/2011-pse.