

Original Research Article

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

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 colonization of arbuscular mycorrhizal (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 insignificantly and 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. About 80% of land plants, develop mutual associations with arbuscular mycorrhiza (Zhu et 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 (Harley and Smith, 1983) especially in nutrient-poor soil. Mycorrhiza plays an important role in increasing nutrient uptake, notably phosphorus and zinc (Bolan, 1991; Burkert and Robson, 1994). They also prevent 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 photosynthates when they are associated with host plant roots (Porras-Soriano et al. 2009, Bati et al., 2015; Wang et al., 2017).

The major contributor to the 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; 20 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 (Abott 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).

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 mycorrhizal root colonization, and spore density in 3 different industrial wastelands in the Kota district of Rajasthan, India.

Methodology:

Comment [PR1]: characterize these sites in the methodology section, indicating distance between sites

Comment [PR2]: At the beginning of this section, it is necessary to indicate the criteria for selecting the study sites, and where the control area was located.

For physico-chemical characterization of soil, soil samples were collected from rhizospheres. From three fields of each selected site (three replicates), approximately 1,000 g of soil sample was collected from the rhizosphere of randomly selected plants in each field. Soils were collected from 15 to 30 cm depth, and were filled in polythene bags and were brought to the laboratory and stored at 5°C–10°C. Each replicate of the soil sample was analyzed for physico-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 stereomicroscope. The total spore count was carried out using Leica EZ4 stereomicroscope.

Statistical Analysis was 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.

Observation and Results:

Physico-chemical properties of soil

Table 1: Physical and chemical properties of control and four experimental 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

Comment [PR3]: above at the beginning of the methodology three sites are indicated, where does the fourth appear from?
The Control should be an isolated site to the industrial zone, please clarify this

Characterization of the soil samples collected from 4 different sites show variation in almost all parameters. The alkalinity of the soil shows an 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 area 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).

Comment [PR4]: for these statements to be relevant, it is suggested to include a comparison test between zones like anova or Kruskal-Wallis

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 mycorrhiza are 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.

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}

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

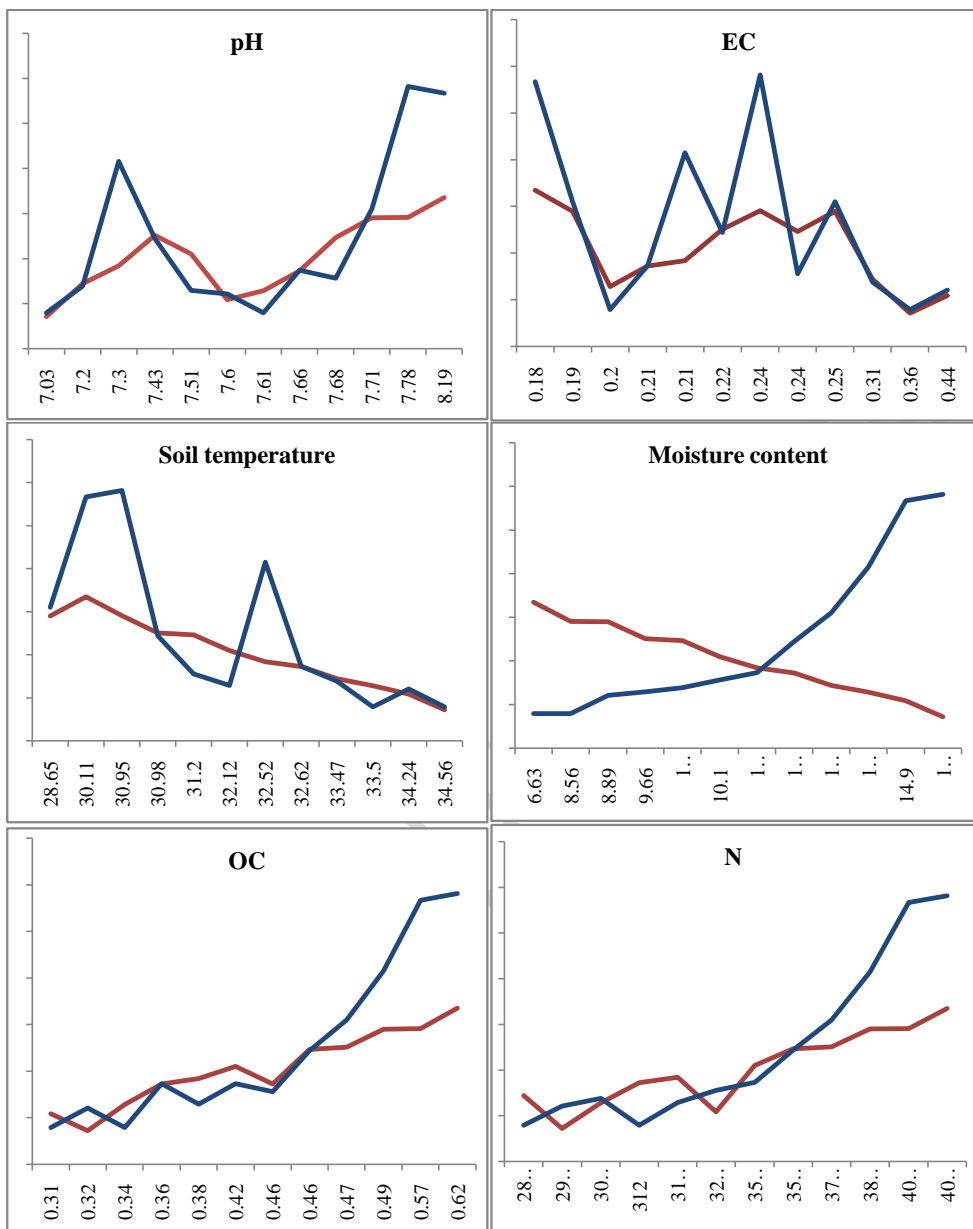


Figure 1: Correlation between physico-chemical parameters of soil and percentage mycorrhizal association and spore density (spore density is shown by the red line and root colonization by blue line. Physico-chemical parameters are taken on X-axis and spore density and % root colonization on Y-axis).

Comment [PR5]: Include titles in x and y-axis

Comment [PR6]: it is recommended to separate these graphs by 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,

Comment [PR7]: three replicates is a small sample, therefore it is likely that the normality condition for Pearson's coefficient is not satisfied

OC, available N, P, K, Cu, Zn, Mn, and Fe, and % root colonization and spore density of mycorrhiza (Table 2).

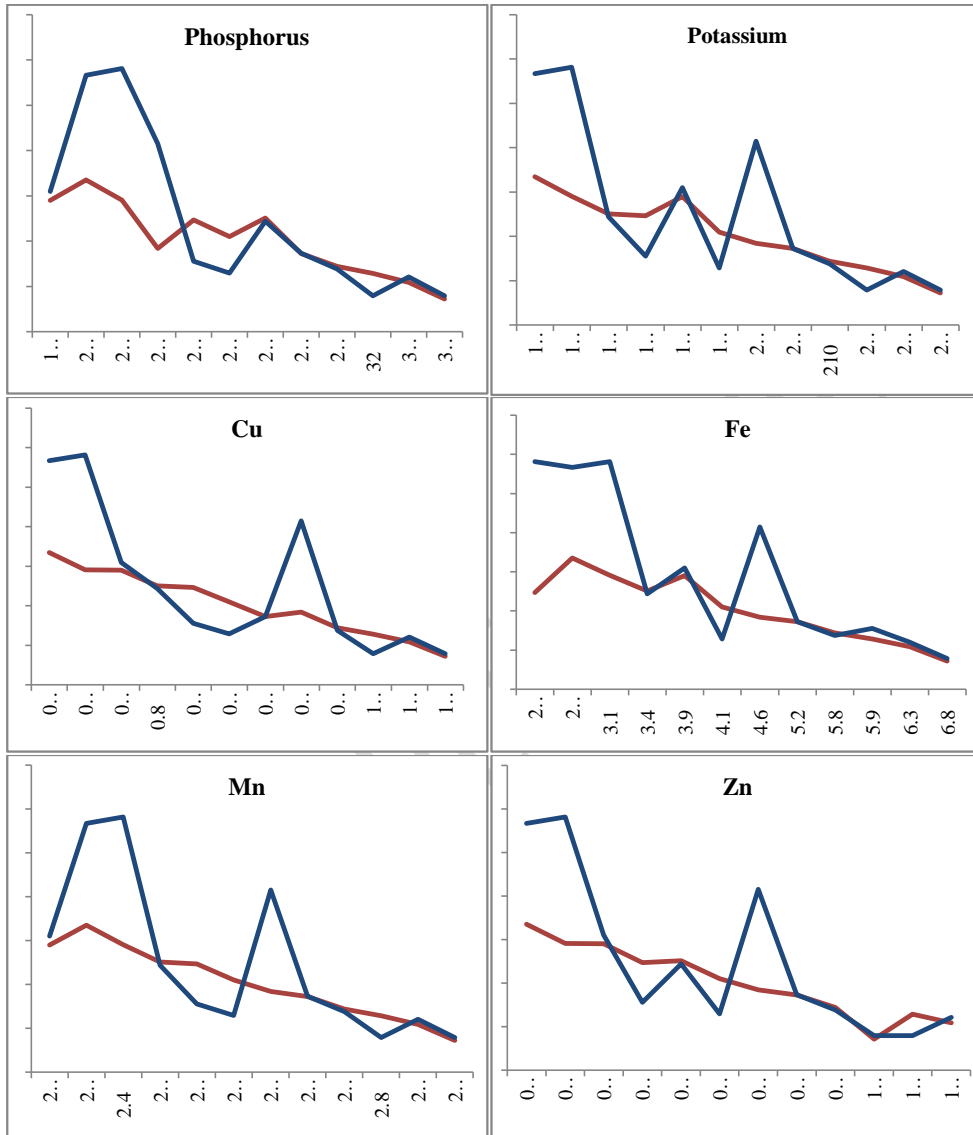


Figure 2: Correlation between physico-chemical parameters of soil and percentage mycorrhizal association and spore density (spore density is shown by the red line and root colonization by blue line. Physico-chemical parameters are taken on X-axis and spore density and % root colonization on Y-axis).

Comment [PR8]: observation similar to Fig. 1

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 mycorrhiza are

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.

Figures 1 and 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 they do not show a clear-cut trend of their correlation. 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. The effect of soil pH on root colonization was not found to be consistent (Figure 1). 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 arbuscular mycorrhiza (Heyman 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 1).

Comment [PR9]: The correlation must be interpreted from the table, since the significance is included there

Comment [PR10]: However in table 2, it was significant, explain what could be causing this

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 concentrations also 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 is weproved found~~ 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 differentially to 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 (Figures 1 and 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.

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