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Soil moisture fluctuation influences AMF root colonization and spore population in tree species planted in degraded entisol soil

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Abstract

Arbuscular Mycorrhizal fungi are ubiquitous in soil form symbiotic relation with majority of the plants and pays dividend to host during adverse conditions. As AMF development in soil and in plant roots varies greatly due to changing soil moisture, nutrient and temperature the benefits of symbiosis are also affected. In present investigation soil moisture fluctuation and its influence on AMF root colonization and spore population has been determined to establish the relationship between AMF and soil moisture and temperature. Monthly data on soil moisture AMF root colonization and spore population were recorded from eight tree species plantation of entisol soil, Bilaspur, India by assessing representative samples of root and soil during 2016-17. Result shows that the average annual AMF root colonization ranged 36.94 – 55.83% varied significantly seasonally and monthly at P <0.01. The colonization was highest in *T. arjuna* and lowest in *P. indica,* while in case of seasonal variation the highest colonization found during rainy season (July- August) and lowest during summer season (May month). Spore population recorded maximum during March – April and minimum in spring season with avg. annual range between 68.13 to 91.12 spore/100g soil. Correlation analysis demonstrated significantly positive relationship with AMF root colonization and soil moisture while negative correlation between spore population and soil moisture. Negative linear equation was also observed between root colonization × spore production, root colonization × temperature and soil moisture × temperature. Soil moisture a key element of rainfall identified as important limiting factor determining AMF development in entisol soil.

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Introduction

Now, AMF (*Arbuscular mycorrhizal fungi*) does not require any introduction as it has been an important component of rhizosp here, forming symbiotic association with the majority of plant species and soils (Smith and Read, 1997) necessarily requires for both the partners (Millner and Wright, 2002;Quilambo, 2003) belonging to agriculture, horticulture and in forest species. It promotes plant survival, growth and in improvement of quality planting stocks majorly by mitigating nutrient-deficient and water stresses (Thompson, 1996; Mohammad *et al.*, 1998; Auge, 2001; Chandra, 2013). The fungal mycelium emanating from the root system for beyond the rhizosp here and therefore can acquire nutrients from soil volume to which roots have no access through a complex mycelium network (Graves *et al.,* 1997; Robinson and Fitter, 1999). The mutual understanding of plant with AMF through root colonization pay number of dividends to partner plant such as higher uptake of nitrogen, phosphorus, zinc, copper and water from the soil beyond the rhizosphere (Ziang *et al.*, 2013). Besides better nutrient supply, mycorrhizal associated plants develop drought tolerance and disease resistance (Auge 2001) and thus higher plant growth (Chandra, 2015). Moreover, the time of inoculation of AMF is also not standardized so far due to very high rate of variation in AMF development inside and outside root of hosts, soils and seasons. To overcome these problems, a deep understanding of AMF distribution and function on host and site specific manners attempting season and monthly variation is urgently neede das these aspects of AMF in natural ecosystems are still poorly understood.

Although some studies have examined the seasonal fluctuation of AMF colonization in roots of many plant species and communities (Siguenza *et al.,* 1996; Ruotsalainen *et al.*, 2002; Muthukumarand Udaiyan, 2002; Lugo *et al.*, 2003) but most of them failed to observe consistent seasonal patterns of AMF development. The patterns and timing of AMF development in host depend on the edaphic conditions (Morammad *et al.* 2003) or climatic conditions (Saito and Kato, 1994; Carvalho *et al.*, 2001; Muthukumar and Udaiyan, 2002). However, Yang *et al.* (2012) state that the selectivity by host plants affects the distribution of AMF. Similarly, Oliveira and Oliveira (2010) have suggested that AMF colonization and spore numbers were positively influenced by the rainfall due to induces root growth, leading to enhanced germination of AMF spores and subsequent colonization. In other investigation (Guitton, 1996), only AMF spores were influenced by the seasonal precipitation. The influence of soil pH and micronutrients on AMF colonization and spore numbers has also been reported (Chandra 2013; Vinod Kumar *et al.*, 2016) while Guitton (1996) did not find any significant influence of soil characteristics on mycorrhizal variables. In addition, studies have attributed differences in rates of colonization in plants in range from mutually symbiotic to parasitic depending on the nutritional demands of the plant (Nouri *et al.*,2014). This signifies that plants are able to alter rates of mycorrhizal colonization in their roots and promote the symbiosis as long as they are limiting by one of the factor. Entisol soil commonly known as bhataland, extents in about 20% of Chhattisgarh, forms red colour hard spherical mass (Murum) of iron oxides and is categorized as wasteland due to its very low productivity (Planning Commission Report, 2013). Besides poor nutrients, poor soil moisture also is a major limiting factor in this soil, whose fluctuation triggers nutrients availability and AMF symbiosis in plants but such studies are sparse in literature. Only few studies have been conducted on the distribution of AMF in tree species of entisol soils (Bhardwaj and Chandra, 2017) needs further investigation. To understand the functions of AMF in natural ecosystems, as well as their basic biology, it is essential to document the monthly and seasonal variation of these fungi and different factors affecting the min order to initiate restoration program of such sites selecting best suitable species. The root colonization and fungal spore production are two important criteria for study fungal life history (Hart and Reader, 2002), therefore the influence of moisture content of soil on AMF colonization and spore population in different tree species plantations of entisol soil was conducted considering both seasonal and monthly variations of AMF development and soil moisture.

Materials and methods

Site description

The present study site located at Bilaspur district (C.G.), lies between 83° 46' 32" E longitudes and 22° 18' 51"N latitude. In order to collect soil and root samples for present study, a systematic survey was made in different tree plantations *Albizia lebbeck* (L.) Benth (Kala siris), *Azadirachta indica* (A. Juss) (Neem), *Dalbergia sissoo* (Roxb) (Shisham), *Eucalyptus globulus* (Labill) (Nilgiri), *Millettia pinnata* (L. Panigrahi) (Karanj), *Peltaphorum ferrugineum* (Decne) Benth (Peltophorum), *Phyllanthus indica* (L.) (Aonla) and *Terminalia arjuna* (Roxb) Wight and Am. (Arjun) species already planted before 25 years at 4 x 4m distance in blocks in entisol soil.

The climatological data used in present study was obtained from TCB College of Agriculture and Research Centre, Bilaspur, C.G., India. Atmospheric temperature observed minimum 12.8°C during December while the maximum 42.5°C in May month with mean annual temperature of 26.8°C (Fig. 1). The area has low in altitude (267 m from sea level) and mean annual precipitation of about 1259 mm. Most rainfall occurs from July to September (Fig. 2).

Soil Scanting and Root sampling staining for AMF examination

For the seasonality analysis, root and samples were collected on monthly basis during 2016 -17. Each tree species planation was demarcated in 50 x 50 m quadrate area and then three points were further marked randomly within quadrate in each plantation. Representative soil along with root samples were collected from each of the point marked within plantation by digging 3 pits of 30 cm wide, 30 cm deep and 30 cm in length and assured that roots were connected to the sampled tree species. Scanting and decanting of AMF spores was done after passing soil sample following the wet sieving and decanting method (Gerdemann and Nicolson, 1963) and examined and counted under Stereo microscope Leica DM250A on 100g soil sample basis. Young roots were washed and chopped into 1 cm size, cleaned with tape water, heated in 10 % KOH solution for 20 Minutes at 90°C and were treated in 3% hydrogen per-oxide at room temperature for 5 minutes then acidified with in1% HCL solution for overnight and stained with 0.05% trypanblue solution (1:1:1; water:glycerol:lactic acid) further heated at 80-85°C for 10 minutes (Phillips and Hayman,1970). Over stained roots were kept with 50% glycerol solution to remove excess stain and finally examined under compound microscopic Leica DM 2500. A total of 5 segments of each root sample were mounted on the microscopic slide with50% glycerol and smashed softly after placing a cover glass on the root pieces. From each tree species plantation 20 slides were prepared for examination. Root samples were observed by a compound microscope at 200 magnifications. The percentage root colonization was calculated by following the method described by Mc Gonigle *et al.* (1990). The presence of mycelium was recorded as AM positive and total Mycelia colonization was treated as percentage root colonization using following formula:-

$$\text{AMF root colonization (\%)} = \frac{\text{Number of AMF possitive roots}}{\text{Total number of roots observed}} \text{X } 100$$

Soil moisture (%) was determined from soil sample collected from rhizosp here of different tree plantations of entisol soil by using formula:-

$$\text{Soil Moisture (\%)} = \frac{\text{Wet weight of Soil} - \text{Dry weight of soil}}{\text{Dry weight of soil}} \text{X } 100$$

Statistical analysis

All statistical analyses were done using SPSS 16.0 version for calculation and interpretation of the data. Pearson's correlation coefficients were employed to determine the relationships between fungal colonization parameters and environmental factors. Standard errors of means were calculated for all the parameter studied and for Duncan Multiple Range test was used for mean comparison at 5% P level.

Results

Seasonality of AMF root colonization

The forest species studied were colonized by AMF throughout year (Table 1, Figs 3, 6) showed intra- and intercellular hyphae, vesicles and arbuscules in the root tissues. The average annual root colonization ranged 36.94 – 55.83% and was quite present in all surveyed species. The highest average colonization occurred 55.83% in *T. arjuna* followed in *A. lebbeck* (49.29%) and lowest 36.94% in *P. indica*. Root colonization in each species differed but showed similar pattern of seasonal variation (Table 1, Figs3, 6). Mean root colonization found highest 57.91% during rainy season followed by spring (49%) and the lowest 28.88% during summer season. It was an indicative of poor root colonization due to adverse effect of summer while rainy season found to have growing season for both host and microbe as it rendered the maximum rate of colonization (Table 1).

Table 1. AMF root colonization (%) in different tree species of entisol soil, Bilaspur, India, as influenced by season. Data represent ± indicates standard error while groups that are statistically similar with respect to root colonization share the same letter row-wise only, whereas different letters indicate a significant difference (P <0.001).

S.No.	Species	Winter	Spring	Rainy	Summer	Average
1	*A. lebbeck*	52.00±2.34^{b}	53.33±2.23^{ab}	60.33±3.14^{a}	31.50±3.09^{c}	49.29±1.93
2	*A. indica*	38.17±3.00^{ab}	35.50±2.69^{bc}	44.83±2.82^{a}	29.73±1.37^{c}	37.06±1.43
3	*D. sissoo*	48.33±1.93^{c}	48.67±2.52^{b}	62.00±2.77^{a}	26.17±1.29^{c}	46.29±1.99
4	*E. globulus*	41.50±2.46^{b}	41.83±2.00^{b}	52.33±5.24^{a}	26.67±1.88^{c}	40.58±1.96
5	*M. pinnata*	49.33±2.54^{b}	54.67±2.47^{b}	65.00±3.50^{a}	27.33±1.49^{c}	49.08±2.19
6	*P. ferrugeinum*	36.33±2.01^{c}	56.50±2.68^{b}	66.83±4.11^{a}	25.17±1.63^{d}	46.21±2.52
7	*P. indica*	39.25±3.55^{a}	44.83±2.11^{a}	43.17±2.92^{a}	20.50±2.38^{b}	36.94±1.86
8	*T. arjuna*	53.83±2.82^{b}	56.67±2.97^{b}	68.83±2.41^{a}	44.00±2.29^{c}	55.83±1.73

Monthly variation also showed significant difference in AMF colonization rate in species (Figs 3, 6) throughout the growing season. In general, clear trend obtained in root colonization pattern which showed often low in May month there after start recovery at the onset of monsoon and reached peak in July or August months in all species except for *P. indica* which exhibited its peak in December. Root colonization showed decline trend after September continued till May identified as the minimum root colonization month for 50% of the species. However, *E. globulus, P. ferrugeinum, P. indica* and *T. arjuna* showed poorest root colonization in one or two month early than May month as other species of present investigation shown (Fig. 3).

Seasonality of AMF spore population

Results of AMF spore population in different species are summarized in Table 2, Figs4, 6; depicts that the population differ significantly at P<0.05 in all tree species in different seasons and months. The mean value of sporulation observed highest (134.34 spores /100g soil) during summer and the lowest in spring seasons (58.93 spores/ 100g soil) (Table 2).

Table 2. Seasonal variation of AMF Spore population represents in 100 g soil samples taken from rhizosp here soil under different tree species planted in entisol soil of study site. Data represent ± indicates standard error while groups that are statistically similar with respect to root colonization share the same letter row-wise only, whereas different letters indicate a significant difference (P <0.001).

S.No	Species	Winter	Spring	Rainy	Summer	Average
1	*A. lebbeck*	55.30±6.83^{b}	54.93±7.54^{b}	64.53±6.27^{b}	124.17±9.80^{b}	74.73±5.31
2	*A. indica*	65.77±7.57^{b}	45.80±4.25^{b}	48.83±5.21^{b}	112.13±9.87^{a}	68.13±4.88
3	*D. sissoo*	68.37±6.70^{b}	56.67±5.16^{b}	71.77±4.27^{b}	117.37±11.39^{a}	78.54±4.70
4	*E. globulus*	56.63±6.42^{b}	57.77±6.45^{b}	55.20±5.12^{b}	124.17±9.80^{a}	73.44±5.17
5	*M. pinnata*	67.60±6.62b	56.67±5.26b	72.00±6.09b	125.40±11.36a	80.42±5.11
6	*P. ferrugeinum*	75.53±7.85	64.43±5.32^{b}	62.13±4.89^{b}	133.50±9.59^{a}	83.90±5.15
7	*P. indica*	78.03±9.31^{b}	68.60±6.63^{b}	68.03±4.08^{b}	118.53±9.44^{a}	83.30±4.62
8	*T. arjuna*	77.50±8.18^{b}	66.53±4.53^{b}	70.93±6.05^{b}	149.50±10.19^{a}	91.12±5.75

The average spore population in species ranged 68.13 - 91.12 spores in 100 g soil sample. *A. indica* rendered lowest spore population and *T. arjuna* gave the highest spore in sampled soil collected from rhizo sp here of tree species. In contrast to root colonization, spore population showed completely opposite trend as it was lowest during summer while former attribute was highest in this season. Fig. 4 and 6depict significant monthly variation with species and the highest sporulation observed either in March or April and the lowest in December month. Species such as *A. lebbeck, A. indica, D. sissoo, E. globulus,* and *P. indica* reached its peak in March while remaining species showed peak sporulation period in April months.

Table 3. Soil moisture (%) status in soil under different tree species plantations of entisol soil of Chhattisgarh. Data represent ± indicates standard error while groups that are statistically similar with respect to root colonization share the same letter row-wise only, whereas different letters indicate a significant difference (P <0.001).

S.No.	Species	Winter	Spring	Rainy	Summer	Average
1	*A. lebbeck*	11.51±0.17^{c}	12.74±0.09^{b}	13.64±0.17^{a}	10.12±0.12^{d}	12.00±0.19
2	*A. indica*	11.45±0.17^{c}	12.54±0.08^{b}	13.00±0.23^{a}	9.65±0.23^{d}	11.66±0.19
3	*D. sissoo*	11.52±0.17^{c}	12.79±0.09^{b}	13.79±0.15^{a}	10.23±0.11^{d}	12.08±0.19
4	*E. globulus*	11.51±0.17^{c}	12.76±0.10^{b}	13.68±0.17^{a}	10.15±0.14^{d}	12.03±0.19
5	*M. pinnata*	11.46±0.17^{c}	12.59±0.08^{b}	13.13±0.20^{a}	9.74±0.16^{d}	11.73±0.19
6	*P. ferrugeinum*	11.50±0.17^{c}	12.70±0.09^{b}	13.49±0.18^{a}	10.01±0.13^{d}	11.92±0.19
7	*P. indica*	11.52±0.17^{c}	12.76±0.09^{b}	13.71±0.16^{d}	10.17±0.12^{a}	12.04±0.19
8	*T. arjuna*	11.54±0.17^{c}	12.81±0.10^{b}	13.83±0.15^{a}	10.26±0.13^{d}	12.11±0.19

The trends of spore population also indicated maximum during March – April then start declining continuously till July month for majority of the species and showed short recovery during July - August and eventually declined till December month. Again after January with the slight increment in temperature (Fig. 1 and 2), the spore population turned accelerating mode more or less in all the species.

Seasonality of soil moisture content

Moisture content in entisol recorded very low compared to other soils due to presence of large quantity of hard nodules and also due to poor water holding capacity. Seasonal and monthly variations in soil moisture under different tree plantations exhibited significant difference both seasonally and with species (Table 3, Figs 5, 6).

Results revealed that season played important role in fluctuation of soil moisture irrespective of the species. The mean value of soil moisture noticed highest 13.53% during rainy season dropped up to 10.04% during summer season. Soil moisture in winter and spring season recorded 11.50% and 12.71% respectively.

The highest soil moisture retained by*T. Arjuna*(12.11%) followed by *D. sissoo* and lowest by*A. indica* (11.66%). Monthly fluctuation in soil moisture furnished in Fig. 5 reveals that majority of rhizo sp here soil under different tree species were able to hold the maximum quantity of moisture during July and in August with the highest rainfalls in these months (388 and 369 mm respectively) (Fig. 2).

Lowest soil moisture found in April month for most of the species except in *A. indica* and *M. pinnata* which had lowest moisture in May.

In the area monsoon comes in middle of the June and continued till 15th September and this period is usually observed as growing season for all the biological organisms.

Table 4. Regression equations relating AMF variables with soil moisture and average temperature in different tree species of entisl soil, Bilaspur, Chhattisgarh, India.

Species name	Root colonization × Spore population		Root colonization × Soil moisture	
	Linear Equation	R^2 value	Linear Equation	R^2 value
A. lebbeck	Y = -1.0985x+ 129.23	-0.1712	Y = 0.0858x+7.7473	0.6062
A. indica	Y = -1.3213x+ 117.62	-0.1716	Y = 0.1071x+7.6684	0.4871
D. sissoo	Y = -1.2471x+ 136.74	-0.3938	Y = 0.0807x+8.1657	0.7698
E. globulus	Y = -1.7461x+ 144.89	-0.4488	Y = 0.0846x+8. 5725	0.6306
M. pinnata	Y = -1.4172x+ 150.48	-0.4415	Y = 0.0927x+7.1619	0.9847
P. ferrugeinum	Y = -1.372x+ 147.81	-0.5529	Y = 0.0753x+8.248	0.9012
P. indica	Y = -1.5567x+ 141.36	-0.7424	Y = 0.0854x+8.8681	0.6026
T. arjuna	Y = -2.4428x+ 228.42	-0.4814	Y = 0.1143x+5.6975	0.767
Species name	Root colonization × Avg. Temperature		Spore population × Soil Moisture	
	Linear Equation	R^2 value	Linear Equation	R^2 value
A. lebbeck	Y = -0.1799x + 35.703	-0.2432	Y = -0.0269x + 14.012	-0.4198
A. indica	Y = -0.0912x + 30.19	-0.0327	Y = -0.0382x + 14.269	-0.6330
D. sissoo	Y = -0.1334x + 33.005	-0.1610	Y = 0.0317x + 14.416	-0.4696
E. globulus	Y = -0.1369x + 32.385	-0.1490	Y = -0.0304x + 14.257	-0.5520
M. pinnata	Y = -0.1217x + 32.798	-0.1466	Y = -0.029x + 14.068	-0.4148
P. ferrugeinum	Y = -0.0096x + 27.245	-0.0013	Y = -0.0374x + 15.07	-0.7554
P. indica	Y = -0.1406x + 32.022	-0.1491	Y = -0.0488x + 16.117	-0.643
T. arjuna	Y = -0.0272x + 28.325	-0.004	Y = -0.0298x + 14.834	-0.6464
Species name	Spore population × Avg. Temperature		Soil Moisture × Avg. Temperature	
	Linear Equation	R^2 value	Linear Equation	R^2 value
A. lebbeck	Y = 0.0653x + 21.909	0.2260	Y = -0.6326x + 34.38	-0.0366
A. indica	Y = 0.0622x + 22.545	0.1560	Y = -1.2803x + 41.728	-0.152
D. sissoo	Y = 0.0711x + 21.204	0.1803	Y = -0.4829x + 32.555	-0.0178
E. globulus	Y = 0.0521x + 22.958	0.1468	Y = -0.5805x + 33.778	-0.0303
M. pinnata	Y = 0.0682x + 21.30	0.2095	Y = -1.1633x + 40.448	-0.1233
P. ferrugeinum	Y = 0.0692x + 20.973	0.2326	Y = -0.7865x + 36.178	-0.0555
P. indica	Y = 0.0954x + 18.828	0.224	Y = -0.5409x + 33.311	-0.0267
T. arjuna	Y = 0.0582x + 21.477	0.229	Y = -0.4155x + 31.832	-0.016

The trends exhibited by soil moisture was related to rainfall pattern of the area as it was at peak during July declined continuously till May month due to increasing aridity and soil again started gaining moisture with the onset of monsoon (Fig. 5).

Correlation analysis

The relationship between soil moisture, root colonization and spore population are shown through radar and markers graphic (Fig. 6), gives idea about how well these attributes are interrelated. In addition Pearson correlation coefficient analyses (Table 4) further proves the relationship of AMF root colonization with spore population, soil moisture and temperature. It observed that the root colonization negatively correlated with spore population (r^2 =-0.1712 – 0.7424) but the root colonization exhibited significant positive correlation with soil moisture. Species *viz. M. pinnata* and *P. ferrugeinum* showed strong correlation with AMF root colonization and soil moisture while *A. indica* showed comparatively poorest r^2 value (0.4871). Root colonization was negatively correlated with the increasing temperature while the population of spore improved with increasing temperature.

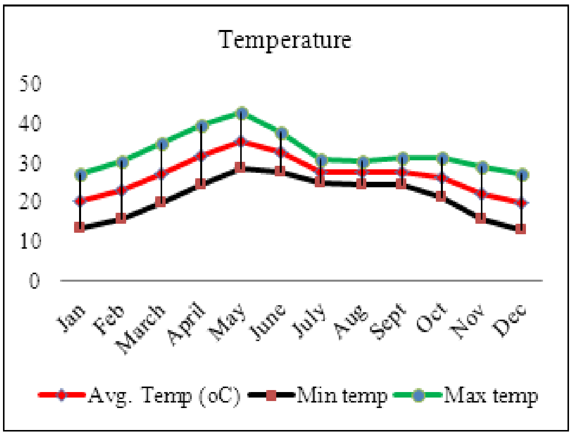

Fig. 1. Average min temp, max temp and average temperature of Bilaspur district, Chhattisgarh, india.

In contrast to root colonization, spore population showed negative relationship with soil moisture. From the present study it is strongly established that species differ in root colonization and sporulation depending upon the soil moisture and temperature. Soil moisture favour the root colonization while at the same time it plays negative role in sporulation. Rainfall also found to have significantly positive correlation with soil moisture and as soon as temperature increases the soil moisture decreases and has negative correlation between each other.

Discussion

The influence of edaphic factors on AMF development and root colonization in different hosts has been widely and regularly reported (Carrenho *et al.*, 2007; Antunes *et al.*, 2012; Nouri *et al.*, 2014; Vinodkumar *et al.*, 2016) which strongly supports the role of soil nutrients for the symbiotic and parasitic functions of AMF. As per these studies, plants rely on balance nutrition and for any deficiencies plant interact with AMF symbiosis and when all the conditions particularly nutrition's required by plants are in favorable, AMF role became parasitic. But these factors have minimal chances of introducing fluctuations in the natural community. Similarly variation in AMF colonization and spore population exists probably due to selectivity of host plants, host specificity and host preferential (Smith and Read, 2008;Yang*et al.*, 2012).

Effect of soil moisture on AMF root colonization and spore population

The impact of soil moisture on AMF root colonization and spore population found to influence greatly, as reflected from the results of present study. In entisol, soil moisture recorded 9.73 and 13.79% in summer and rainy season respectively identified as one of the limiting factors for symbiotic relationship in tree species planted in entisol soil.

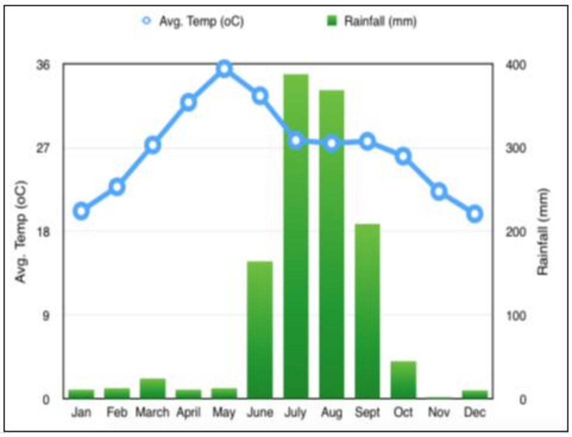

Fig. 2. Mean rainfall pattern and average temperature of Bilaspur, Chhattisgarh, India.

The rate of root colonization differ significantly in different tree species in entisol soil due to fluctuation in soil moisture in different months and seasons supported through significant positive correlations(r^2 = 0.4871 to 0.9847) in all the species under study. Result reveals that even slight increment in soil moisture lead to higher change in root colonization while lower moisture inhibit root colonization in tree species inentisol soil.

The higher colonization during rainy season and low in summer months, illustrate the need of moisture in maintaining symbiotic relationship with plants. It has been reported that AMF colonization could be coordinated with growth stages of plants during the long stress of drought and rain (Kennedy *et al.*, 2002). Our results also supports their findings that in rainy season plant growth insist due to heavily root colonization in newly emerged root compared to summer months. In contrast to this other researchers has reported the peak root colonization in spring season (Mohammad *et al*., 1998; Lugo *et al*., 2003; Bohrer *et al.,* 2004) and low in summer (Siguenza *et al*., 1996). Similarly, soil moisture has been reported to be positively correlated with AMF colonization (He *et al*., 2002; Bohrer *et al*., 2004; Lingfei *et al*., 2005; Oliveira and Oliveira, 2010).

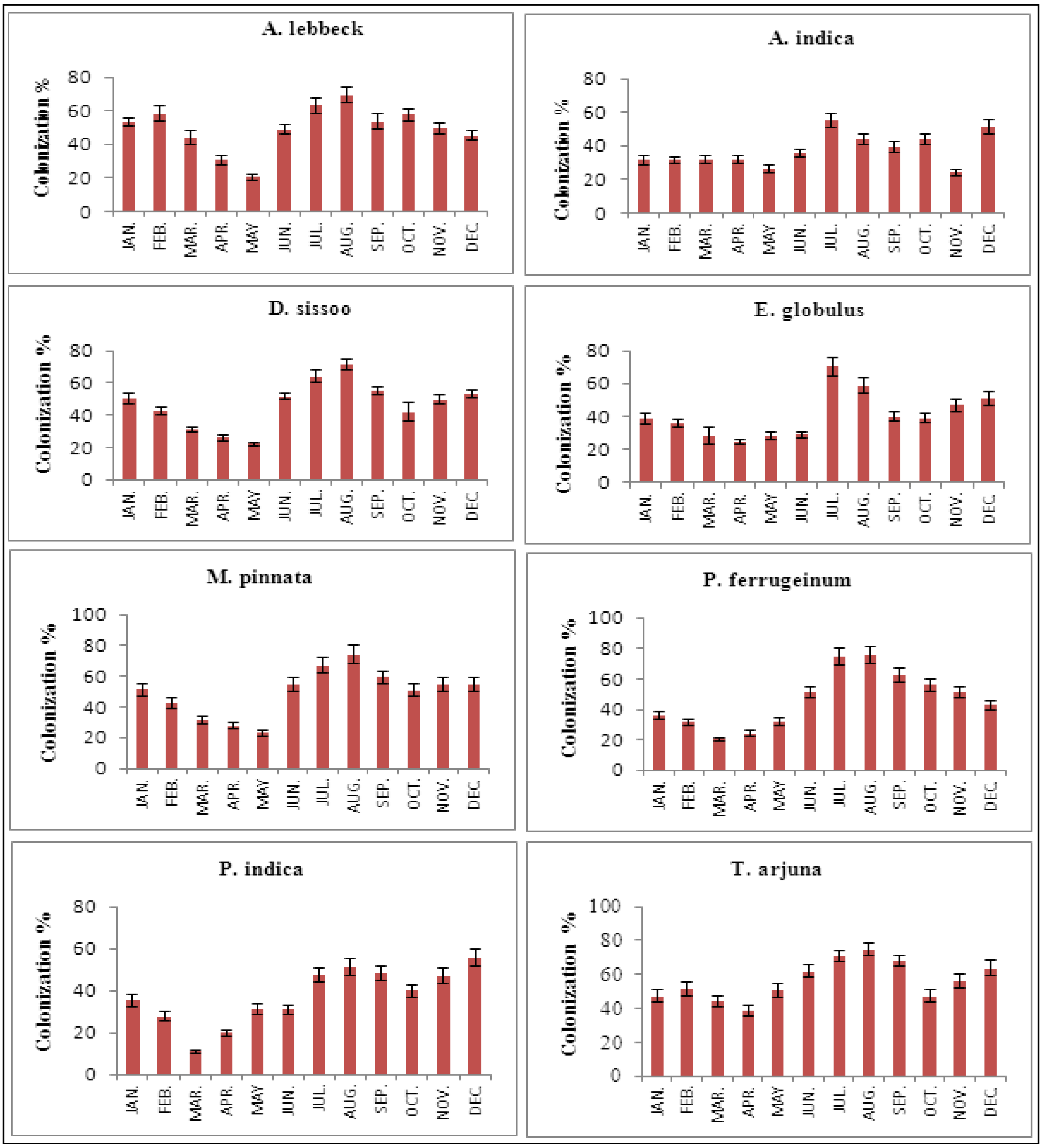

Fig. 3. AMF root colonization (%) in different tree species planted in entisl soil, Bilaspur, Chhattisgarh, India.

The fluctuation in AMF colonization for all plant species found significant throughout the year with almost similar pattern in all species indicates the importance of seasons in root colonization. However the coefficient of correlation differed among species. The correlation between soil moisture and root colonization was strongest in *M. pinnata* and *P. ferrugeinum* while the weak correlation in *A. indica* was in accordance with the results of Yang *et al.* (2012), while Bohrer *et al.* (2004) concluded that AMF seasonal dynamics is in response to plant phenology. Our results demonstrate that correlations coefficient was significant between soil moisture and temperature and with AMF colonization strongly support the opinion that climatic factors could influence the AMF colonization (Muthukumar and Udaiyan, 2002; Staddon *et al*., 2003). Similarly, soil moisture has been reported to be positively correlated with AMF colonization (He *et al*., 2002) might be a strong argument supporting our results, as rainfall was an important element of soil moisture.

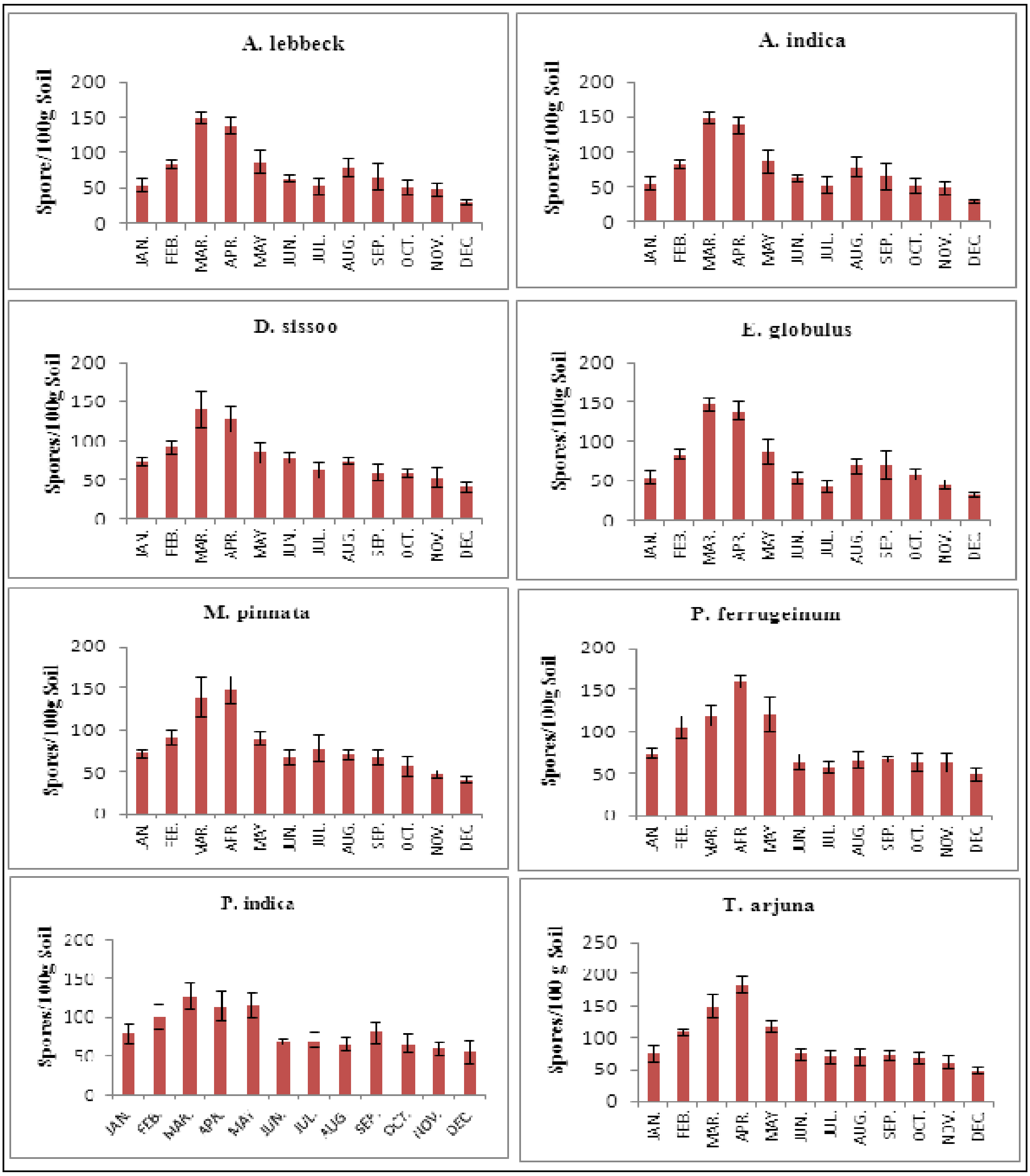

Fig. 4. Spore population in soil under different tree species planted in entisl soil, Bilaspur, Chhattisgarh, India. Standard error showed on bar significant different at P< 0.05%.

Effect of season on AMF colonization and spore population

The influence of climatic variables on spore population corroborated by other researchers (Muthukumar and Udaiyan, 2002; Staddon *et al*., 2003; Lingfei *et al*., 2005) illustrating variation of AMF development in natural ecosystems but fail to provide consistent results.

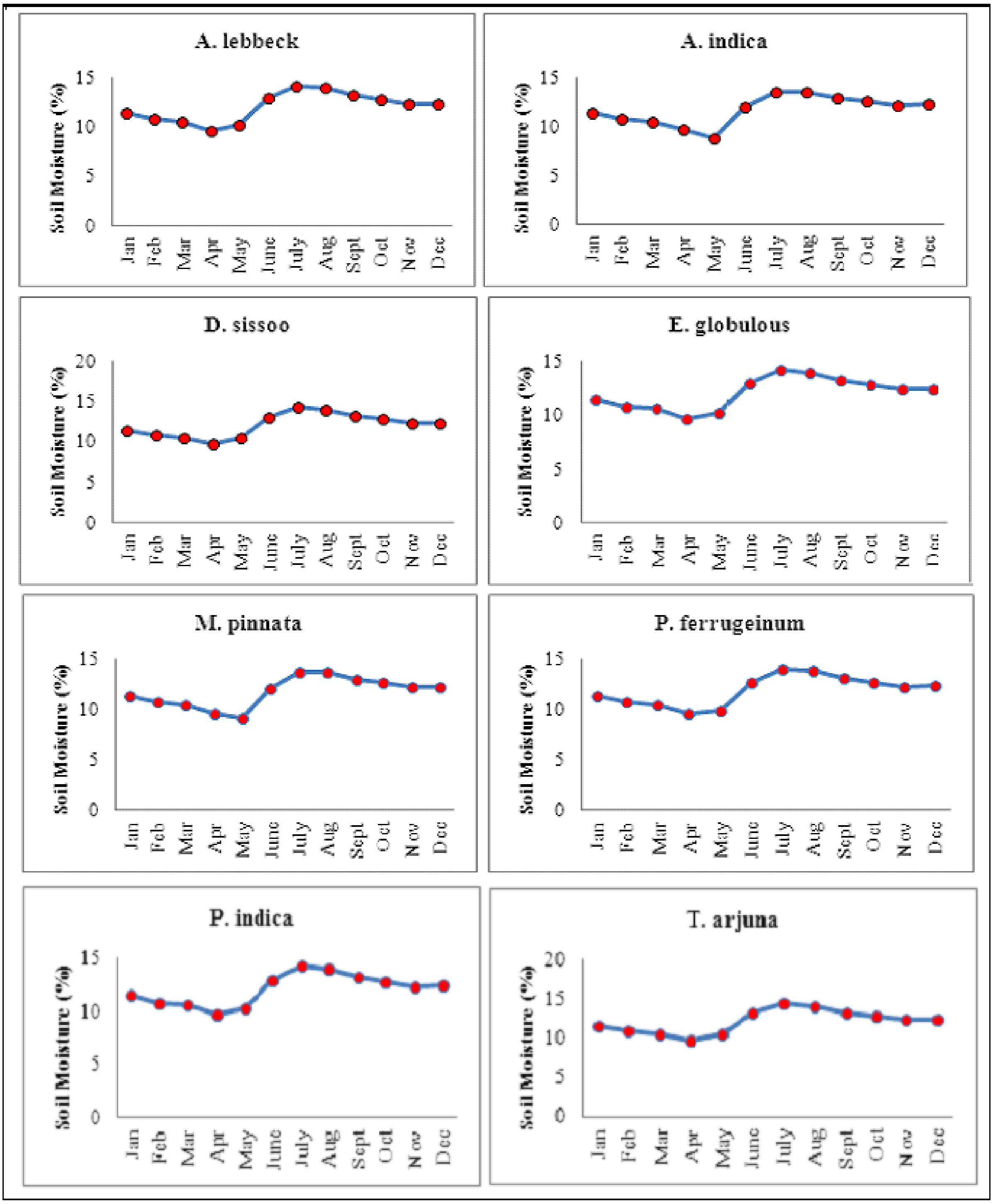

Fig. 5. Soil moisture fluctuations in different months in soil under different tree species plantations of entisl soil of studied site.

In present study spore population negatively correlated with AMF colonization, it means higher root colonization does not reflect the number of spore in soil exactly. In general, the root colonization is a results of the degree of symbiosis indicates how well both the partners interacting with each other for drawing benefits but for that the presence of effective AMF propagules are necessary rather than presence of high number of non-effective AMF in soil. However, the effectiveness of AMF varies with the species usually determined upon the understanding of both microbe and host on the ground to fulfill the demand of most limiting factor (Yang *et al.*, 2012; Nouri *et al.* 2014).

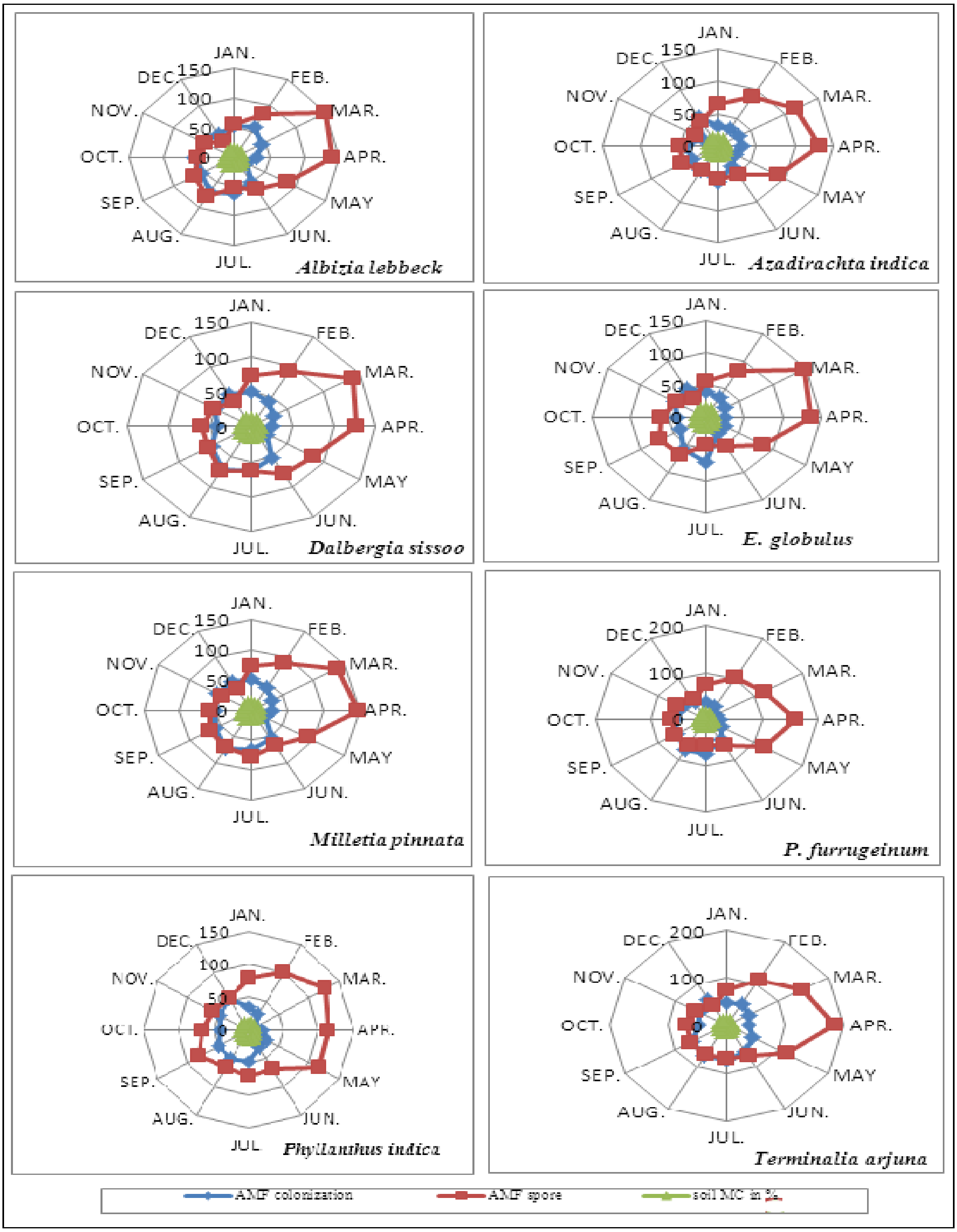

Fig. 6. Radar and marker showing relationship of AMF root colonization, spore population and in soil moisture under different tree species planted in entisl soil, Bilaspur, Chhattisgarh, India.

Therefore one species may be most effective for particular host and at the same time it may be non-effective for other host is a basic source of variation between root colonization and spore population in hosts. Our results showed variation in spore population with changing season, as the highest population reported during March-April, it means in the starting of summer and the lowest spore population in December or mid of winter (spring season). The influence of soil moisture noticed deleterious effect on spore population also confirmed by regression correlation equation ranged r^2 =-0.4148 to -0.7554 in different forest species plantations in entisol soil. Similar observation was made by Muthukumar and Udaiyan (2002) also supports our statement that mycorrhizal variables and rainfall had a negative correlation. In present investigation we found significant positive correlation between rainfall and soil moisture (r^2 = 0.6241). The temperature of spring season fall below 20°C during December might affects spore population adversely results lower spore population than other months of the year. Moreover, winter season is known for its dormant for number of plants and microbes and when the temperature raises since February onward loss in moisture began from soil due to increasing evapotranspiration results increase in spore population consistently during March – May month. We observe stimulatory effects of soil temperature on spore population but Yang *et al.* (2016) has reported decreased spore density and diameter due to temperature elevation but increase in the hyphal density. However, in several other studies, increased temperature had no effect on the length of roots colonized, spore density or the hyphal density of AMF (Heinemeyer and Fitter, 2004). These inconsistent results may indicate different responses of AMF communities in different ecosystems because the AMF species composition is often determined by environment and plant community composition (Antoninka *et al.*, 2011; Sun *et al.*, 2013).

Conclusion

The present study stressed the need to understand the variation pattern of AMF root colonization and AMF populations in relation to soil moisture fluctuations. Soil moisture identified as one of the most important limiting factor required for maintaining root colonization in tree species in entisol soil. Rainy season was best for higher root colonization while during summer season the rate of root colonization declines due to water stress. While for spore population the favourable season was March – April and the winter low temperature affects adversely. So results findings would enable to determination the time schedule of AMF inoculation in nursery and plantations so that the greater survival of inoculants of AMF for higher symbiosis may be promoted. This study could also be used in understanding of the basic biology of AMF with different tree species and one can save money through selection of appropriate time for AMF inoculations.

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