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Arbuscular Mycorrhizal Fungal Dynamics in the Rhizospheric Soil of Five Medicinal Plant Species

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Abstract: The seasonal variation of arbuscular mycorrhizal fungi was investigated in the rhizosphere of five medicinal plants species in Kurukshetra, India. Five host species (*Spilanthes acmella, Withania somnifera, Salvia officinalis, Mentha spicata* and *Melissa officinalis*) and twelve sampling months (January to December) were studied. The current study was undertaken in order to examine the seasonal dynamics of several soil variables (soil pH, soil temperature and moisture content of soil) with a specific interest to determine the rate of arbuscular mycorrhizal root colonization, vesicles and arbuscules formation in the root and AM fungal spore population in the rhizosphere of all five medicinal plants which showed a wide range of changes with in every month through out the year.

Key words: Seasonal dynamics · Arbuscular mycorrhizal fungi · Medicinal plants

INTRODUCTION

Many microorganisms form symbiosis with plants that range, on a continuous scale, from parasitic to mutualistic. Among these, arbuscular mycorrhizal (AM) fungi are ubiquitous plant root symbionts that can be considered as 'keystone mutualists' in terrestrial ecosystem, forming a link between biotic and abiotic ecosystem components via. carbon and nutrient fluxes that pass between plant and fungi in the soil [1]. Arbuscular mycorrhizal fungi have been observed to be associated with medicinal and aromatic plants [2, 3]. Distribution, diversity, abundance and functioning of AM fungi are primarily based upon the extent of root colonization and spore count which further depends upon many environmental factors [4]. In addition to the sensitivity of soil type, some other factors that affect behaviour of AM fungi are host plant, crop rotations, soil pH, moisture content of soil, soil temperature, nutrient levels and interaction with other soil biota. As, AM colonization is the result of interaction between soil, host and mycorrhizal fungi, the rate of germination and efficiency of these fungi depends upon the composition of AM species which change with the season [5-7].

Diversity and dynamics of AM fungi in different host plant species in a particular agro- climatical zone with seasonal changes are very important in order to evaluate the natural status of these fungi in that region. Keeping the above factors in view, the present investigation was undertaken to study the seasonal variation of AM fungi in rhizosphere of five selected medicinal plants namely *Spilanthes acmella, Withania somnifera, Salvia officinalis*, *Mentha spicata* and *Melissa officinalis*.

MATERIALS AND METHODS

Survey and Site Description: The survey was made during the year 2008. The plants to be investigated were grown in Herbal and Botanical gardens of Botany Department, Kurukshetra University, Kurukshetra. Kurukshetra is a small district falls in north- east part of Haryana state (India), bounded by North latitudes 29°53'00" and East longitudes 76°26'27" and is about 250 meters above sea level. The climate of area is tropical monsoonal type. The three seasons in the year are mainly summer season (March to May), which is hot and dry, rainy season (June to September) and winter season (October to February) are wet and cool dry respectively. The summer season raises the mercury to as high as 110°F, while the temperature dips to as 40°F in winter. The normal annual rainfall of Kurukshetra district is 582 mm. which is unevenly distributed over the year.

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Soil and Plant Root Sampling: For analysis of soil moisture, soil pH, spore numbers and AM fungal root colonization, the soil and root samples from the rhizosphere of the plants under study were collected at the beginning of each month. Three different plants of each type were randomly selected to perform the study. About 100 g. of the soil along with fine roots of plants were collected from approximately 15-30 cm. depth from each plant. The soil rhizosphere and plant root samples were transported to the laboratory in polythene bag and stored at 4°C until processed. These samples were mixed together to form a composite sample and three replicates were taken for further analysis from each composite sample.

Estimates of AMF root colonization and AM spores:

Mycorrhizal root colonization in all the plants was studied by 'Rapid Clearing and Staining Technique' [8]. Ten grams of soil from each replicate was utilized to determine AM fungal spore number. Extraction of AM fungal spores from soil was done by following 'Wet Sieving and Decanting Technique' [9]. The identification of AM spores was done by using different manuals of [10-14].

The analysis of moisture content in soil was performed every month by taking 50g. of soil. Soil sample was freshly weighed and dried at 105° C, then it allowed to cool and weighed again to note down the loss of weight on drying. The moisture percentage was calculated by the following formula:

$$\text{Moisture Percentage} = \frac{\text{Initial weight of sample}}{\text{Loss of weight of drying}} \times 100$$

The pH variation was also recorded for the samples studied by mixing soil and distilled water (1:2) using digital pH meter and the temperature of soil was also recorded by soil thermometer in the morning.

Statistical Analysis: The data was statistically interpreted by using analysis of variance (ANOVA) followed by post hoc test through computer software SPSS 16.0. Means were then ranked at P=0.05 level of significance using Duncan's Multiple Range Test for comparison.

RESULTS AND DISCUSSION

Arbuscular mycorrhiza fungi (AMF) have been described as 'keystone mutualists' in ecosystems due to their unique position at the root- soil interface. In order to more fully understand the basic ecology of arbuscular mycorrhizal fungi and their role in natural ecosystems, it is necessary to document seasonal changes of various aspects of the life history of these fungi. Despite the importance of these soil fungi in various ecosystems, few studies exist that examine the seasonality of different variables of AM fungi which are directly related to ecosystem functions. The current study was undertaken in order to examine the seasonal dynamics of several soil variables (soil pH, soil temperature and moisture content of soil) with a specific interest to determine the rate of AM root colonization, vesicles and arbuscules formation in the root and AM fungal spore population in the rhizosphere of *Spilanthes acmella*, *Withania somnifera*, *Salvia officinalis*, *Mentha spicata* and *Melissa officinalis*, which showed a wide range of changes with in every month through out the year.

In *Spilanthes acmella*, it is evident from Table 1.1 and Fig 1.1 that AM spore number and root colonization varies with different months of the year. Spore morphotypes steadily increased in different intervals of the year i.e. from September (14.33±1.52) to January (63.0± 4.58), February (23.66± 3.05) to April (63.0± 5.00) and May (11.66±1.52) to August (35.00±2.64). The maximum number of AM spores were observed in the month of January ((63.0± 4.58) and April (63.0± 5.00) and minimum in May (11.66±1.52). Thus, the abundance of AM spores in *S.acmella* was observed in late winter season followed by the start of summer season and least towards the end of summer season. AM root colonization was recorded highest in November (85.81±4.05) and lowest colonization was observed in June (22.47±0.91). This showed that the percentage AM root colonization was maximum in winter season and lowest in early rainy season. The results showed that AM root colonization decrease gradually from February (70.08±1.30) to April (39.75±2.69) and then increase from June (22.47±0.91) to August (73.28±1.79). Once it declined in September (53.82±3.59) and again increases towards November (85.81±4.05). Variation in pH, temperature and moisture content were also observed in different intervals of month. The maximum AM spore count was observed in alkaline soil pH (8.09) having temperature 13.2°C and soil moisture content 19.646%. The least number of spore population was recorded in slightly acidic soil (pH 6.9) having temperature 31.4°C and soil moisture content 16.319%. The mycorrhizal root colonization was observed maximum in alkaline soil (pH 8.03), 22°C temperature and low moist soil (6.449%) and minimum in the soil having pH 7.21, temperature 35.3°C and soil moisture content 14.385% respectively.

Table 1.1: Seasonal Dynamics of Arbuscular Mycorrhizal Fungi in plants of *Spilanthes acmella*

Months	Mycorrhizal Spore Count / 10g. of soil	Percentage mycorrhizal root colonization	Type of Infection *M	*V	*A	pH of soil	Temperature of soil (°C)	Moisture Content of soil (%)
January	**63.0±4.58^{a}	67.90±5.14^{b}	+	+	+	8.09±0.11^{a}	13.20±0.44^{g}	19.646±0.65^{a}
February	23.66±3.05^{d}	70.08±1.30^{b}	+	+	+	7.42±0.12^{bcd}	19.46±1.12^{f}	15.246±1.04^{def}
March	51.00±3.00^{b}	48.35±1.98^{de}	+	+	-	7.51±0.20^{bc}	22.60±0.56^{e}	17.228±0.89^{bc}
April	63.00±5.00^{a}	39.75±2.69^{f}	+	+	-	7.29±0.11^{cde}	24.53±0.75^{d}	10.461±1.13^{g}
May	11.66±1.52^{g}	85.45±3.29^{a}	+	+	+	6.99±0.22^{e}	31.43±0.83^{b}	16.319±0.80^{bcd}
June	23.00±1.00^{de}	22.47±0.91^{g}	+	-	-	7.21±0.14^{cde}	35.33±1.40^{a}	14.385±1.11^{ef}
July	17.66±1.52^{ef}	47.83±6.45^{e}	+	+	+	7.12±0.11^{de}	34.33±0.85^{a}	17.350±1.20^{b}
August	35.00±2.64^{c}	73.28±1.79^{b}	+	+	+	7.47±0.30^{bc}	32.50±0.70^{b}	15.481±0.84^{de}
September	14.33±1.52^{fg}	53.82±3.59^{d}	+	-	-	7.76±0.24^{ab}	28.33±1.05^{c}	13.607±0.40^{f}
October	20.33±2.08^{de}	61.65±1.83^{c}	+	+	+	7.84±0.24^{a}	25.80±0.95^{d}	10.511±1.15^{g}
November	17.33±5.50^{ef}	85.81±4.05^{a}	+	+	+	8.03±0.18^{a}	22.00±0.61^{e}	6.449±0.54^{h}
December	36.00±3.00^{c}	34.63±1.55^{f}	+	+	-	7.96±0.16^{a}	18.06±0.85^{f}	15.583±1.09^{cde}

** Each value is mean of three replicates

*M: Mycelium, V: Vesicles, A: Arbuscules

± : Standard deviation

Mean value followed by different alphabet/s are significant over one another at P=0.05.

Table 1.2: Seasonal Dynamics of Arbuscular Mycorrhizal Fungi in plants of *Withania somnifera*

Months	Mycorrhizal Spore Count / 10g. of soil	Percentage mycorrhizal root colonization	Type of Infection *M	*V	*A	pH of soil	Temperature of soil (°C)	Moisture Content of soil (%)
January	**112.0±4.58^{a}	37.46±7.42^{f}	+	+	+	7.89±0.45^{abc}	11.66±0.57^{i}	14.193±0.49^{e}
February	28.00±1.00^{e}	52.18±1.97^{bcd}	+	+	+	7.52±0.43^{bcd}	14.40±1.40^{h}	18.806±0.58^{a}
March	35.33±3.51^{b}	49.76±5.56^{cde}	+	-	-	7.53±0.63^{bcd}	20.50±0.79^{f}	10.426±0.31^{fg}
April	33.33±2.51^{b}	43.44±1.90^{e}	+	+	+	7.34±0.15^{bcde}	25.26±0.86^{e}	12.489±0.67^{de}
May	8.00±3.60^{f}	48.89±5.57^{cde}	+	+	+	6.82±0.36^{de}	29.33±1.45^{c}	14.504±0.62^{bc}
June	21.33±2.08^{d}	26.92±1.80^{g}	+	-	-	6.68±0.45^{e}	35.23±1.42^{a}	13.467±1.00^{cd}
July	34.33±2.51^{b}	100.0±0.00^{a}	+	+	+	6.63±0.57^{e}	34.20±0.82^{ab}	11.138±0.57^{f}
August	26.33±1.52^{c}	56.43±2.32^{b}	+	+	+	7.19±0.14^{cde}	32.46±0.95^{b}	13.224±0.99^{cd}
September	35.33±1.52^{b}	47.54±2.29^{de}	+	+	-	7.51±0.21^{bcd}	28.36±1.11^{cd}	11.729±0.58^{ef}
October	13.00±4.00^{ef}	57.56±2.25^{b}	+	+	-	7.68±0.26^{bc}	26.66±1.19^{de}	9.240±1.09^{g}
November	21.33±2.51^{d}	54.42±1.18^{bc}	+	+	-	8.02±0.15^{ab}	21.40±0.75^{f}	12.674±0.95^{de}
December	17.00±1.00^{de}	52.21±2.27^{bcd}	+	+	-	8.44±0.55^{a}	18.50±0.72^{g}	15.634±0.72^{b}

** Each value is mean of three replicates

*M: Mycelium, V: Vesicles, A: Arbuscules

± : Standard deviation

Mean value followed by different alphabet/s are significant over one another at P=0.05

In *Withania somnifera*, it is envisaged from Table 1.2 and Fig. 1.2 that the density of AM fungal spores was maximum in the month of January (112.0±4.58) and minimum in the month of May (8.00±3.60) and October (13.00±4.00). Variation in spore population was recorded in different intervals of the year. Here also the maximum sporulation was observed in late winter season and minimum in late summer season followed by early winter. The rate of AM root colonization was registered highest in the month of July (100.00±0.0) and minimum in June (26.92±1.80). The intensity of root colonization was declined in different spells i.e. from August (56.43±2.32) to September (47.54±2.29), October (57.56±2.25) to January (37.46±7.42) and February (52.18±1.97) to June (26.92±1.80) with little fluctuations. So the maximum root colonization was observed in late rainy season and minimum by the start of rainy season. The highest spore count was noticed at 11.6°C, alkaline pH (7.8) and 14.193% moisture content of soil. The minimum spore number was observed at pH 6.8 (acidic soil), 29.3°C temperature and 14.509% soil moisture content. Similarly, highest rate of AM root colonization was recorded at acidic soil (pH 6.6), 34.2°C temperature and 11.138% soil moisture content, while the minimum intensity of root infection was registered at 6.6 pH, 35.2°C temperature and 13.467% moistened soil. Here, pH of the soil does not show any correlation with root colonization and spore number.

Table 1.3: Seasonal Dynamics of Arbuscular Mycorrhizal Fungi in plants of *Salvia officinalis*

Months	Mycorrhizal Spore Count / 10g. of soil	Percentage mycorrhizal root colonization	Type of Infection *M	*V	*A	Temperature pH of soil	Moisture of soil (°C)	Content of soil (%)
January	**68.66±4.72^{a}	44.44±2.31^{d}	+	+	-	7.76±0.48^{abc}	13.53±0.85^{g}	19.558±1.32^{a}
February	16.66±2.51^{e}	89.84±4.05^{a}	+	+	+	7.68±0.37^{abcd}	15.20±0.75^{g}	14.247±1.05^{bc}
March	36.33±2.08^{c}	66.49±2.77^{c}	+	-	-	7.01±0.76^{cde}	22.36±1.38^{e}	18.836±0.48^{a}
April	36.66±3.21^{c}	36.15±5.85^{e}	+	-	+	6.80±0.48^{e}	25.33±0.97^{d}	13.067±1.00^{cd}
May	11.66±1.52^{f}	75.80±5.55^{b}	+	+	+	6.96±0.23^{de}	31.50±1.17^{b}	11.873±0.89^{de}
June	24.00±2.00^{d}	21.50±3.41^{g}	+	+	-	7.06±0.23^{cde}	35.90±1.70^{a}	14.854±1.12^{b}
July	18.33±1.52^{e}	65.82±1.91^{c}	+	+	-	7.30±0.16^{bcde}	33.60±0.75^{b}	17.974±1.15^{a}
August	23.00±4.00^{d}	43.94±6.25^{d}	+	+	+	7.74±0.46^{abc}	32.56±1.76^{b}	15.386±0.60^{b}
September	24.33±1.52^{d}	26.50±6.67^{fg}	+	+	-	7.91±0.40^{ab}	28.23±1.38^{c}	11.210±0.60^{ef}
October	17.00±2.00^{e}	31.81±1.68^{ef}	+	+	-	7.28±0.29^{bcde}	25.60±0.89^{d}	9.737±0.80^{f}
November	43.00±3.00^{b}	38.75±2.68^{de}	+	+	-	7.62±0.31^{abcd}	21.36±1.41^{e}	15.758±0.89^{b}
December	14.00±2.64^{ef}	38.54±1.29^{de}	+	+	+	8.16±0.20^{a}	17.40±0.87^{f}	5.463±1.09^{g}

** Each value is mean of three replicates

*M: Mycelium, V: Vesicles, A: Arbuscules

± : Standard deviation

Mean value followed by different alphabet/s are significant over one another at P=0.05

Table 1.4: Seasonal Dynamics of Arbuscular Mycorrhizal Fungi in plants of *Mentha spicata*

Months	Mycorrhizal Spore Count / 10g. of soil	Percentage mycorrhizal root colonization	Type of Infection *M	*V	*A	pH of soil	Temperature of soil (°C)	Moisture Content of soil (%)
January	*40.33±2.51^{e}	44.38±1.66^{g}	+	+	-	7.98±0.19^{abc}	12.6±1.08^{g}	13.755±0.66^{b}
February	68.66±3.05^{b}	33.16±3.18^{i}	+	+	-	7.54±0.35^{cde}	19.4±0.79^{f}	11.742±0.90^{cd}
March	55.33±2.08^{c}	29.15±4.16^{i}	+	-	-	8.13±0.15^{ab}	23.3±1.01^{e}	6.932±1.08^{f}
April	45.66±1.52^{d}	63.46±3.33^{e}	+	-	-	7.34±0.20^{def}	24.46±0.81^{d}	7.220±0.22^{f}
May	18.66±1.52^{i}	100.0±0.00^{a}	+	+	-	6.93±0.23^{fg}	30.96±1.35^{c}	13.343±0.90^{b}
June	27.33±2.08^{g}	38.64±1.26^{h}	+	+	-	7.28±0.12^{ef}	36.76±1.55^{a}	10.754±1.10^{de}
July	32.66±1.52^{f}	87.63±2.17^{b}	+	+	+	6.78±0.42^{g}	33.60±0.82^{b}	11.760±0.55^{cd}
August	22.33±1.52^{hi}	73.86±1.14^{c}	+	+	+	6.99±0.16^{fg}	35.60±1.25^{ab}	12.515±0.41^{bc}
September	73.33±4.04^{a}	68.62±1.74^{d}	+	+	-	7.58±0.17^{cde}	29.13±1.10^{c}	11.398±0.66^{cd}
October.	25.00±2.064^{gh}	26.77±1.78^{i}	+	-	-	7.75±0.15^{bcd}	26.63±1.70^{d}	9.634±1.09^{e}
November	36.33±2.51^{f}	53.66±4.25^{f}	+	-	-	7.98±0.18^{abc}	22.70±1.55^{e}	13.977±1.00^{b}
December	19.33±1.52^{i}	45.82±0.35^{g}	+	+	-	8.36±0.33^{a}	18.76±1.16^{f}	15.899±0.76^{a}

** Each value is mean of three replicates

*M: Mycelium, V: Vesicles, A: Arbuscules

± : Standard deviation

Mean value followed by different alphabet/s are significant over one another at P=0.05

With reference to the third selected plant, *Salvia officinalis*, spore number and extent of AM root colonization was found to be varying with months of year, soil temperature, soil pH and soil moisture. It is perceptible from Table 1.3 and Fig 1.3 that spore abundance was recorded highest in late winter season (January: 68.66±4.72) and minimum in late summer season (May: 11.66±1.52). Maximum spore population was noticed at 13.5°C temperature, 7.7 pH (alkaline soil) and 19.558% soil moisture, while the lowest spore count was registered at pH 6.9 (slightly acidic soil), 31.5°C temperature and having 11.873% content of moisture in soil. The intensity of AM root colonization in *S.officinalis* was found to be maximum in February (89.84±4.05), then it declined up to April (36.15±5.85) and again increase in May (75.80±5.50). After attaining the minimum root infection in June (21.50±3.41: early rainy season), the AM root colonization again increased in July (65.82±1.91) and then declined up to September (26.50±6.67). It further increased from October (31.81±1.68) onwards to attain its maximum value in the month of February (89.84±4.05) i.e. the end of winter season. Here also the extent of root colonization was influenced by edaphic factors. With reference to pH, it was observed that AM root colonization was highest at soil pH level 7.6 (alkaline soil) and lowest at pH 7.0 (neutral soil). When the effect of temperature and moisture content in soil was studied, it was found that the maximum AM root colonization was observed at 15.2°C and 14.247% soil moisture and minimum was observed at 35.9°C and 14.854% soil moisture respectively.

In *Mentha spicata*, results depicted in Table 1.4 and Fig. 1.4 showed that the maximum count of AM spores was recorded in the month of September (73.33±4.04) and

Table 1.5: Seasonal Dynamics of Arbuscular Mycorrhizal Fungi in plants of *Mellisa officinalis*

Months	Mycorrhizal Spore Count / 10g. of soil	Percentage mycorrhizal root colonization	Type of Infection *M	*V	*A	pH of soil	Temperature of soil (°C)	Moisture Content of soil (%)
January	**69.33±2.51^{a}	47.64±2.27^{b}	+	+	-	8.17±0.21^{ab}	11.53±0.87^{i}	17.163±0.83^{ab}
February	19.33±1.52^{de}	43.10±1.39^{cd}	+	+	+	7.57±0.14^{cd}	14.60±0.75^{h}	12.963±0.75^{f}
March	55.66±1.52^{b}	33.75±0.73^{f}	+	+	-	8.13±0.29^{ab}	22.10±0.95^{f}	18.057±0.21^{a}
April	55.66±2.30^{b}	46.24±0.84^{bc}	+	-	-	7.36±0.35^{de}	25.60±0.92^{e}	12.663±0.33^{f}
May	15.66±1.52^{ef}	39.34±2.71^{de}	+	+	-	7.29±0.20^{de}	29.96±0.70^{c}	15.961±0.45^{bcd}
June	21.00±2.00^{d}	20.46±1.57^{g}	+	+	-	7.16±0.26^{def}	35.43±1.23^{a}	15.808±0.81^{cd}
July	21.00±3.00^{d}	76.76±3.64^{a}	+	+	-	6.74±0.37^{f}	33.23±0.47^{b}	16.917±0.39^{abc}
August	18.66±1.52^{de}	75.61±1.13^{a}	+	+	+	7.01±0.30^{ef}	33.36±0.72^{b}	14.764±1.10^{de}
September	27.00±3.00^{c}	40.59±0.92^{de}	+	+	-	7.54±0.26^{cde}	28.30±0.92^{d}	13.747±0.77^{ef}
October.	15.66±1.52^{ef}	37.48±4.78^{e}	+	+		7.91±0.23^{bc}	24.80±0.90^{e}	13.377±0.64^{f}
November	10.66±1.52^{g}	18.44±1.44^{g}	+	-	-	8.11±0.43^{ab}	21.50±1.15^{f}	11.487±0.64^{g}
December	13.66±3.78^{fg}	38.54±1.30^{e}	+	+	-	8.45±0.31^{a}	18.46±1.22^{g}	10.536±0.86^{g}

** Each value is mean of three replicates

*M: Mycelium, V: Vesicles, A: Arbuscules

± : Standard deviation

Mean value followed by different alphabet/s are significant over one another at P=0.05.

minimum in the month of May (18.66±1.52) and December (19.33±1.52). The extent of AM root colonization declined from July (87.63±2.17) onwards and was sparse during the month of October (26.77±1.78). It further declined from November (53.66±4.25) to March (29.15±4.16) and increased during the month of May (100.00±0.0). Though maximum mycorrhizal colonization was observed during late summer season and minimum during early winter season. The AM spore density was attained maximum at the end of rainy season, while the minimum was occurred during late summer and mid winter season. The maximum mycorrhizal colonization was recorded at pH 6.9, temperature 30.9°C, 13.343% soil moisture and minimum at pH 7.7, 26.6°C temperature and 9.634% soil moisture respectively. Similarly, 7.5 pH of soil favoured spore abundance while the least spores registered at 6.9pH of soil. Temperature and moisture level in soil also have adverse effect on sporulation as high number was recorded at 29.1°C and 11.398% soil moisture while the least number was observed at 30.9°C and 13.343% soil moisture respectively.

In case of *Melissa officinalis*, it is clear from the Table 1.5 and Fig. 1.5, the spore morphotypes was highest in the month of January (69.33±2.51) and lowest in the month of November (10.66±1.52). Mycorrhizal root colonization was found to be highest in the month of July (76.76±3.64), then declined to a minimum in the month of November (18.44±1.44), it once increased in December (38.54±1.30) and further decreased gradually from January (47.64±2.27) to March (33.75±0.73) and Arpil (46.24±0.84) to June (20.46±1.57). Hence, the maximum endospore formation and percent root colonization was noticed in late winter and mid rainy season respectively while the minimum was found during early winter season. With regard to pH, temperature and moisture content in soil, the highest spore population was occurred at pH 8.17, 11.5°C temperature and having 17.163% moisture content and maximum root colonization was observed at 6.7 pH, 33.2°C temperature and 16.917% moisture content. The minimum spore abundance and mycorrhizal colonization were registered at pH 8.11, 21.5°C temperature and 11.487% soil moisture content.

The data was analyzed by applying Duncan's Multiple Range Test (DMRT). Mean value followed by different alphabets/ letters are significant over one another at 0.05% level.

It is affirmed from the results that AM sporulation and root colonization has seasonal dynamics, which is directly or indirectly influenced by different intervals of year, soil temperature, soil pH and soil moisture. The seasonal changes and different range of spore density and root colonization are due to a wide range of hosts. Sporulation and root colonization of AM fungi have been found to be host dependent and this is probable one of the reasons that the plants under study have shown different results when compared. de Oliveira and de Oliveira [15] have reported that the AMF sporulation and colonization are seasonal and dependent on host plant species and maximum sporulation and percent colonization of AMF was registered in rainy season in case of *Theobroma grandiflorum* and *Paullinia cupana*. Similar were the findings in our study where maximum AM sporulation in rainy season was observed in *M. spicata* and maximum root colonization was reported in *W. somnifera* and *M. officinalis*.

The maximum sporulation in this season could be correlated with the fact that during this period most photosynthate is allocated to roots and rhizomes, which helps fungal symbionts to produce more spores [16]. The level of AM fungal association depends upon root morphology, metabolism rate of plant growth [17] as well as on specific soil plant system in terms of chemical nature of root exudates [18]. Bohrer *et al.* [19] observed that seasonal fluctuations of mycorrhizal association were closely related to plant phenology. Similar were the findings in our investigation with regards to *S.acmella* and *S.officinalis*. When Lugo and Cabello [20] and Lugo *et al.* [3] studied seasonal variation of AM fungi in a mountain grassland, they reported the higher number of vesicles, arbuscules and maximum root colonization during summer season. Similar was the finding in case of *M.spicata* where maximum root colonization was registered in summer season. The higher root colonization may be attributed to the capacity of AM fungi to obtain higher profits and growth at higher temperature and abundant rainfall. In the present investigation, the higher pH resulted in increase in AM sporulation and percentage of root colonization. Extraradical mycelium formed at higher pH in present study is supported by earlier findings [21]. Intensity of AM root colonization and spore density has shown to depend on soil pH [22]. Daniels and Trappe [23] suggested that pH induced differences in nutrient availability in soil are responsible for stimulation or inhibition of VAM fungal spore germination. The response of AM fungi to soil pH may depend upon the species and strains constituting the indigenous AM flora [24]. Maximum number of AM spores and root colonization was reported at pH 7.2- 7.4 by [25] whereas pH 6- 7 was reported to be best for mycorrhizal development [26]. Similarly a wide range of variation in soil pH with different plant species and with different sampling months has been recorded in present study. This might be one of the reason of distribution and diversity of AM fungi in all studied medicinal plants and these variations could be attributed to host mediated changes in the rhizosphere of plants.

Temperature fluctuations with different seasons influence the AM spore population and root colonization directly or indirectly. The AM spore count has showed a significant correlation with the temperature in all the studied medicinal plants while the rate of root colonization showed correlation with temperature in case of *M.officinalis*. Staddon *et al.* [27] studied that the intensity of mycorrhizal fungi alter with increase or decrease in temperature, which supports the findings of our investigation. Schenck and Schroder [28] studied the effect of temperature on VAM establishment and registered the greatest root colonization between 28-34°C. A significant correlation of AM root colonization with temperature has been observed in *Plantago lanceolata*, while there was no significant effect of temperature on *Holcus lanatus* [29]. All these reports confirm the results obtained in the present investigation.

Soil moisture had profound influence on VAM spore population as well as colonization of host plants. Allen and Allen [30] suggested that mycorrhizal status and succession could vary depending upon moisture and nutrient conditions of soil. In case of *S.officinalis* AMF spore count and root colonization showed significant correlation with soil moisture content while in case of *M.spicata* and *M.officinalis*, AM spore count has positive correlation with moisture content. Percent infection and number of resting spores in the rhizosphere increased in sunflower plants grown under water stressed condition of 10 percent soil moisture level [31]. Better AMF development at reduced soil water could be attributed to the better soil aeration. It is possible that the low moisture observed in present study did not cause significant reduction in aeration to the point of compromising the mycorrhizal association in these varying environmental conditions. The relationship between AMF colonization and soil moisture may be also associated with the development of the plant root system due to increase in the water content of soil and with the formation of new roots and there will be a simultaneous increase in nutrient absorption and liberation of root exudates, stimulating mycorrhizal spore germination and subsequent infection [32].

However, the studies regarding the seasonal dynamics of AM fungi are useful as we may predict the season and edaphic conditions crucial for development of AM fungi and further recommend that particular period or season for propagation of medicinal plants. Finally, it is clear that more refined and targeted approaches focusing on ecology and intricacies of the association may provide an accurate picture of AM fungal formation and function under prevailing edaphic- climatic conditions.

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