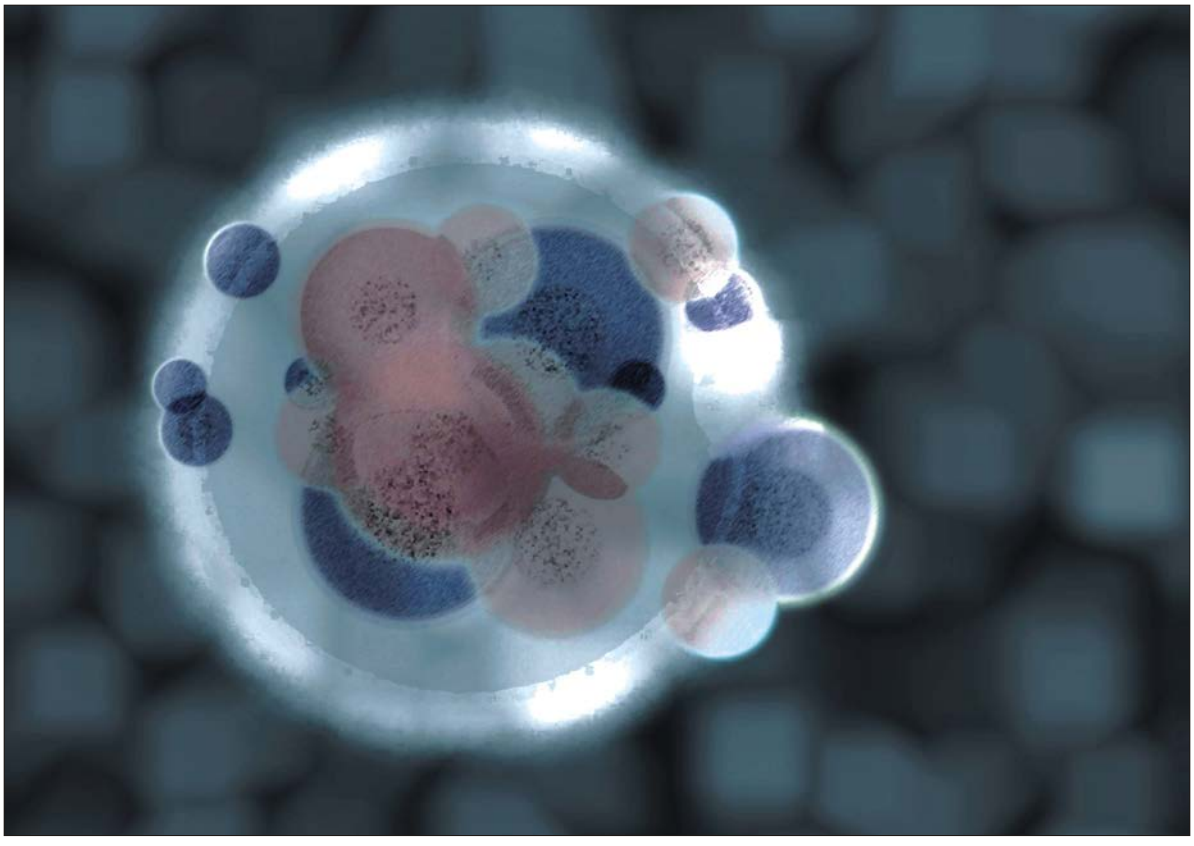

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Influence of Edaphic Factors on Distribution of Mycorrhiza Associated with Medicinal Plants in Indian Central Himalayas

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Abstract: This study analyzed the effect of various Edaphic factors on the Vesicular Arbuscular Mycorrhiza (VAM) associated with medicinal plants. Based on the richness of medicinal plants in Himalayan region, this study considered three plants i.e., *Catharanthus roseus* Linn., *Ocimum* spp. and *Asparagus racemosus* Willd. The study was conducted at five districts of Uttarakhand state in India viz. Pauri Garhwal, Haridwar, Dehradun, Udham Singh Nagar and Almora. This study has evaluated and analyzed the effect of edaphic factors like pH, organic carbon, potassium, phosphorous and soil type on the spore number. The correlation between the individual variations of different edaphic factors with spore number was also investigated. Change in edaphic factors greatly influence mycorrhizal diversity. Maximum number of spores were isolated from soils of pH 6.0-7.0 and with an organic carbon contents ranging from 0.82 to 0.85%. Generally, phosphorous content in the soil samples ranged from 27.11 to 37.21 k ha^{-1} and a feeble negative correlation was observed with respect to spore population. As the moisture content of the soil increased, it was observed that VAM fungal spore number decreased. Potassium content of soil showed significant positive correlation with spore numbers. *Glomus* species was observed as more versatile in comparison to others and therefore existed in even adverse soil and climatic conditions.

Key words: Mycorrhiza, vesicular arbuscular mycorrhiza, *Catharanthus roseus* Linn., *Ocimum* spp., *Asparagus racemosus*, edaphic factors

INTRODUCTION

The term Mycorrhiza refers to the symbiosis of the fungi with the roots of plants. The word symbiosis signifies mutually beneficial associations between two dissimilar organisms. Mycorrhizas are often considered an ideal illustration of mutuality where both plant and fungal symbionts benefit from the reciprocal exchange of mineral and organic resources (Samarbakhsh *et al*., 2009). Mycorrhizal fungi provide great absorptive surface than root hairs and therefore help in the uptake of relatively immobile ions in the soil. Mycorrhizal fungi in symbiotic association of roots have generated worldwide interest because of their constructive role in uptake of nutrients and plant growth. There has been a great deal of interest in their prospective utilization in agricultural, medicinal and horticultural crops (Mukerji *et al*., 2000).

Mycorrhizal fungi are a heterogeneous group of about six thousand species belonging to the Zygomycotina, Ascomycotina and Basidiomycotina. Out of 260,000 known plant species, 240,000 have been known to have the potential to form mycorrhizal association with about 6,000 fungal species. Almost, 90-95% of land plants maintain some type of mycorrhizal association (Mukerji *et al*., 2002). Mycorrhizas can be classified to seven different types based on involvement of different groups of fungi and host plants and distinct morphology patterns. These seven types are Arbutoid mycorrhiza, Ecto mycorrhiza, Ectendo mycorrhiza, Ericoid mycorrhiza, Monotropoid mycorrhiza, Orchid mycorrhiza and Vesicular Arbuscular Mycorrhiza (VAM). VAM is found in almost all agronomic, medicinal plants and vegetable crops. VAM can be identified by the presence of arbuscules in the region of the root cortex. Vesicles which are generally observed in plant root, function as reserve organs and a site for fungal multiplication (Gaur and Kaushik, 2011; Mathur and Vyas, 2007).

The existence of life on earth can be adequately attributed to the presence of medicinal plants. Almost one third of approximately 2,50,000 higher plant species on earth are medicinal (Joy *et al*., 2010). The Uttarakhand state of India in central Himalayas registers a huge presence of medicinal and aromatic plants. Generally these medicinal plants occur naturally and most of them propagate vegetatively by means of underground rhizomes, stems and bulbs or corms (Gaur and

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Kaushik, 2011). Due to enormous presence of medicinal plants in the region, this research work considered three medicinal plants viz. Sadabahar (*Catharanthus roseus* Linn.), Tulsi (*Ocimum* spp.) and Shatavar (*Asparagus racemosus* Willd.) These medicinal plants were chosen for this study because of their tremendous medicinal value. The Sadabahar roots are used as sedative, tranquillizer, tonic and stomachic. An infusion of Sadabahar leaves are administered for relief in menorrhagia and their juice is extremely effective for wasp-stings. It is also used as medication for diabetes and its extract has demonstrated growth inhibitory effect in certain human tumours. Tulsi has a proven Ayurvedic remedial record as antiseptic, diuretic, expectorant, stomachic and cardiac stimulant. The decoction of Tulsi is often used in catarrh, croup and bronchitis (Gaur and Kaushik, 2011). Shatavar roots are considered to have medicinal values as antibacterial, antidiarrhoeal, antidysentric, refrigerant, demulcent, diuretic, aphrodisiac and galactagogue (Kaushik and Dhiman, 2000). The medicinal value of these plants have been proved and employed since Vedic period (Tiwari, 2006). Due to their immense medicinal values, this research targeted study of VAM fungi associated with them and various factors affecting their growth.

A vast variety of microscopic and macroscopic forms of environmental condition constantly change the medium of soil (Bajwa *et al*., 2002). The distribution and occurrence of VAM fungi differ both qualitatively as well as quantitatively with the change in edaphic factors (Singh *et al*., 2010; Gorsi *et al*., 2002) and the type of vegetation. VAM fungal spores multiply only in association with plant roots, which act as suitable ecological niche for germination of spores (Mahmood and Rizvi, 2010). The spores are then released in the soil and could be easily isolated. The properties of soil such as texture, pH, organic matter, moisture content and phosphorous content can be measured or determined (Tufenkci *et al*., 2005). These properties are used to characterize the type of soil inhabited by a particular type of medicinal plant.

Despite of enormous research data available on microbiological aspects and their ecology in diversified habitats in India, there is practically no data on the taxonomy and ecology of vesicular arbuscular mycorrhizal fungi of medicinal plants of Uttarakhand state. However, some researchers have reported and analyzed the randomized information gathered from some areas of Uttarakhand state. In order to fill up this lacuna, efforts have been made to understand the existing knowledge of these fungi as well as the factors affecting their growth and distribution. This research work was carried out at five districts of Uttarakhand state in India viz. Pauri Garhwal, Haridwar, Dehradun, Udham Singh Nagar and Almora. Based on the collected data, this research evaluated and analyzed the effect of edaphic factors like pH, organic carbon, potassium, phosphorous and soil type on the spore number. The correlation between the individual variations of different edaphic factors with spore number was also investigated.

MATERIALS AND METHODS

The present research work investigated rhizosphere soil samples and plant samples of three medicinal plant species. The study was started in December 2005 and soil samples were initially collected during January-February 2006. After initial collection, the samples were collected regularly at an interval of 2-3 months.

Study area: The soil samples for the present investigation were collected from different parts of Uttarakhand state viz. Pauri Garhwal, Haridwar, Dehradun, Almora and Udham Singh Nagar. The collection sites were chosen such that the samples represented the complete state in terms of its major division of Garhwal and Kaman region and different heights of Himalayan ranges.

The collection sites chosen from district Pauri were Dhari, Srinagar, Srikot, Pauri, Ghurdauri, Khandusain, Kotdwar, Safdarkhal, Minthi, Doggada, Kaliasaur, Satpuli, Lansdown, Buvakhal, Jwalpadevi, Patisain, Gumkhal, Dandapani, Kyunkaleshwar, Kandolia, Nagdev, Dhumakot, Nainidanda, Kalagarh, Chilla and Binsar. The sites chosen from district Haridwar were Bhagwanpur, Laksar, Jwalapur, Khanpur, Roorkee, Manglaur, Bahadrabad, Narsan, Patanjali, Kankhal, Sultanpur, Pathri, Jhabreda, Landora and Pirankaliyar. The sites in Dehradun district were Tapovan, Mussourie, Rishikesh, Chakrata, Dakpathar, Sahastradhara, Jollygrant, Ballupur, Tigerfall, Khoonigarh, Lakhamandal, Rajpur, Raipur, Pathribagh, Lachhiwala, Kalsi, Anarwala, Sinola, Kisanpur, Doiwala and Forest Research Institute (FRI). The collection sites in Almora district were Bhanoli, Jainti, Someshwar, Chaukutia, Bhikiasain, Sult, Bhatraujkhan, Marchula, Manila, Dunagiri, Pandhuka, Majkhali, Binsar, Jalna, Sheraghat, Takula, Gananath, Kaparkhan, Binta, Gangolihat, Katpuriya, Sitlakhet, Upal, Gwalakhot, Kosi-Katarmal, Kausani, Jageshwar, Ramkhet and Dwarahat. The collection sites in district Udham Singh Nagar were Khatima, Rudrapur, Pantnagar, Sitarganj, Kichha, Gadarpur, Bazpur, Kashipur, Jaspur, Tanakpur, Nanakmatta, Doraha and Negigarhi.

Fine roots of plants along with soil samples were collected from these sites. The roots were preserved and later on stained for determination of percent mycorrhizal

colonization. Sterilized polythene bags were taken to the site for soil sample collection. Rhizosphere soil samples were collected at the depth of 4-16 cm. These samples were naturally air dried for further experimental analysis.

Isolation and Identification of VAM spores: In this study, wet sieving and decanting technique was used for extraction of spores. This technique is used to remove the clay and sand fractions of the soil while retaining spores and other similar sized soil and organic matter particles on sieves of various diameters.

The VAM fungal spores were analyzed qualitatively by identifying them for their genera and species. The VAM fungal spores collected on filter paper (Whatman filter paper No.1) after wet sieving and decanting technique were observed under Stereoscopic binocular. These spores were picked through needle and mounted in lactoglycerol on slide. As an alternative, Polyvinyl lactic acid was also used as mounting medium. All slides with spores on mounting medium were observed cautiously under high power research microscope for isolation into genera and followed by species identification.

VAM spores were identified using standard monographs given by Hall and Fish (1978), Hall (1984, 1987), Gerdemann and Trappe (1974) and Schenck and Perez (1989) and INVAM (http://www.invam.caf.wvu.edu).

Chemical analysis of soil samples: To enumerate the influence of edaphic factors on occurrence of VAM fungi, following methods for analysis of soil samples were employed.

Soil pH: One gram of soil was taken in 10 mL of distilled water (1:10 w/v). This suspension was thoroughly shaken and kept for 30 min. Later on, the pH of supernatant was determined by using pH meter.

Moisture content of soil: For determination of moisture content of soil, oven-drying method (Pandya *et al.*, 1968) was used. Five to ten gram of soil was immediately weighed and kept in the hot air oven at 105-110°C for 24 h. The amount of water lost, which is the water content of the sample is calculated as follows:

H_2O lost = Weight of moist soil-Weight of oven dry soil

The moisture in the soil (% w/w) was calculated.

Soil organic carbon: Soil organic carbon was calculated as per the method of Walkley and Black (1934). The following reagents were used:

- 1 N Potassium dichromate solution- This solution is made by dissolving 49.04 g of $K_2Cr_2O_7$ crystals in distilled water so that the final volume is made up to 1 L
- Concentrated sulfuric acid
- Orthophosphoric acid
- Ferrous amonium sulphate (N/2)- Dissolved 392 g of AR grade FAS in water added 15 mL sulfuric acid and diluted to 2 L
- Diphenylamine indicator solution-Dissolved 0.5 g diphenylamine in 100 mL sulfuric acid, added 20 mL of water and stored in a colored bottle

A 0.25 g of soil was taken in a 150 mL conical flask, 5 mL of 1 N $K_2Cr_2O_7$ was added to it and mixture was shaken gently. Ten millilitre of concentrated H_2SO_4 was added to it and was shaken for 1 to 2 min. Conical flask was then covered with watch glass, so those fumes of sulfuric acid do not escape. After 30 min, 100 mL of distilled water and 5 mL of Orthophosphoric acid was added. Before titration 0.5 mL of diphenylamine was added. Reaction mixture was then titrated with N/2 Ferrous amonium sulphate till the violet color changes to purple and finally to green.

Calculations for % organic matter:

- Weight of soil taken =W g
- Volume of FAS required for reducing =V_A mL
- Volume of FAS required for reducing the
- Excess of dichromate (experimental reading) =V_B mL
- 1 mL of $K_2Cr_2O_7$ = 0.003 g carbon
- Percent of organic carbon of soil:

$$=\frac{V_A - V_B}{W} \times 0.003 \times 100$$

- Percent of organic matter = Percent of organic carbon of soil×1.724, where 1.724 is a Van Bammelian factor

Soil phosphorus: For the estimation of available phosphorus in the soil the procedure given by Olsen *et al.* (1954) was used. The extracting reagent for Olsen's P is molar sodium bicarbonate prepared by dissolving 42.0 g of $NaHCO_3$ reagent in distilled water to form 1 L of the solution. The pH is adjusted to 8.5 with 10% NaOH.

A 2.5 g of soil was taken in 100 mL conical flask, then a small quantity of Darco G 60 or equivalent grade of activated carbon is added, followed by 50 mL of Olsen's reagent. The flasks are shaken for 30 min and filtered through dry filter paper (Whatman No.10) into clean and

dry beakers or vials. In the filtrate, phosphorus is estimated colorimetrically by Olsen's method.

Standard curve for phosphorus: For preparation of the standard curve, different concentration of P (1, 2, 3, 4, 5 and 10 mL of 2 ppm P solution) was taken in 25 mL volumetric flask. These 5 mL of the extracting reagent (Bray's/ Olsen's) Olsen *et al.* (1954) is added and this 400 mL of 10 N HCl is added and made upto 1 L with adding Stannous chloride. The colorimeter reading is taken against 660 mm (red) filter just after 10 min. The curve is plotted taking the colorimeter reading on the vertical axis and the amount of P μg in the horizontal one.

Calculation: Available P (kg ha^{-1}) =

$$R \times \frac{\text{Total volume of the extractant}}{\text{Volume of aliquot}} \times \frac{1}{\text{Wt. of soil taken}} \times 2.24E+6/10^{6}$$

where, R = μg P in the aliquot:

Olsen's (kg ha^{-1}) P = R×(50/5)×(1/2.5)×2.24 = μg of P X8.96

Soil potassium: The procedure introduced by Hanway and Haidal (1952) was employed for the determination of available potassium in the soil. According to this method, 5 g of soil was vigorously shaken with 25 mL of neutral (i.e., pH = 7) normal ammonium acetate for 5 min and filtered immediately through a dry Whatman filter paper. Potassium concentration in the extract was evaluated using flame photometer after necessary setting and calibration of the apparatus.

The standard curve for potassium is prepared from the stock solution. The measured aliquots were diluted with ammonium acetate solution (CH_3COONH_4) in volume flasks of 100 mL, which resulted in 10-40 ppm of potassium. Subsequent to attaching the suitable filter and adjusting the gas and air pressure, the reading of flame photometer was set at zero for blank (CH_3COONH_4) and at 100 for 40 ppm potassium. The curve is obtained by plotting the readings against different concentrations of potassium such as 5, 10, 15, 20, 25, 30, 35 and 40 ppm. It was assured that the gas and air pressure does not fluctuate. Later the available potassium content in soil was calculated by using following formula:

$$\text{Available K (kg ha}^{-1}) = RVx\frac{\text{Volume of extract}}{\text{Weight of the soil taken}} \times 2.24E+6/10^{6}$$

where, R = ppm of K in the extract = ppm of K×11.2 (Obtained from standard curve).

Statistical analysis: Duncan's Multiple Range Test (DMRT) is employed for carrying out the statistical analysis (Duncan, 1955). Statistical analysis is done for obtaining statistics related to pH, organic carbon, potassium and phosphorus.

Data gathered are subjected to statistical analysis using ANOVA (Analysis of Variance) to determine the significance of the results and DMRT to confirm the test of significance. Correlation analysis is also conducted to determine the trend of the parameters tested. ANOVA is also worked out and the mean values are also compared by DMRT of phosphorous and pH values.

The data regarding distribution of vesicular arbuscular mycorrhizal fungi of medicinal plants in Uttarakhand State is almost unavailable till date. Furthermore, the effect of various edaphic factors on VAM have also not been evaluated. However, some unsystematic information gathered in some areas of Uttarakhand State had been analyzed by some researchers in past. Thus efforts have been made to fill up the gap. Using statistical methods the mean, standard deviation, least significant difference for pH, organic carbon, phosphorous and potassium is obtained for various sites spread over five districts. Further, the correlation factor for spore count with respect to different edaphic factors is also evaluated.

RESULTS

The effect of diverse edaphoclimatic parameters is observed on the occurrence and distribution of VAM fungal spores through various ecological studies. VAM fungal spores were found to be distributed well over different soil samples, however, their number and class varied significantly. In the present study, it was established that 99.5% of the sites accounted for VAM fungal spores. In *Catharanthus roseus*, presence of *Glomus fasciculatum* and *Glomus mosseae* were found to be dominant. However, *Glomus aggregatum* and *Glomus fasciculatum* were predominantly present and associated with all the *Ocimum* species. *Glomus coronatum*, *Glomus mosseae* and *Sclerocystis* species were found to be abundantly associated with *Asparagus racemosus*. The number of spores ranged from 52 to 197 per 10 g of soil considering all medicinal plants individually under study (Table 1 to 3). The average number of spores from 24 sites contained more than 120 spores per 10 g of soil, whereas 38 and 25 sites contained 100-120 and 80-100 spores per 10 g of soil, respectively, while 17 sites contained less than 80 spores per 10 g of soil (Fig. 1-4).

Distribution of VAM fungi affected by various properties of soil: Soil properties like pH, organic carbon,

phosphorous, potassium and moisture content affects the spore population of VAM fungi. The mean, standard deviation and least significant difference obtained through statistical analysis carried out for all sites has been shown in Table 1, whereas the correlation of these soil properties with respect to number of VAM fungal spores is shown in Table 3. The variation of spore number with these soil properties for some specific sites (named as A to O in Table 2) selected from each districts of Pauri, Hardwar, Dehradun, Almora and Udham Singh Nagar have been shown in Fig. 1 (pH), 2 (Organic matter), 3 (Phosphorous) and 4 (Potassium). The description of the effect of pH, Organic Matter, Phosphorous and Potassium related to abovementioned figures is also provided.

As seen in Fig. 1, maximum number of spores were isolated from soils with pH range 6.0-7.0. However, with increase in pH from 7.0-7.5, no distinct variation in spore number was observed. In some areas where phosphorus content is less, the statistically significant negative

Table 1: Statistical analysis of soil chemical properties of different district in Uttarakhand state

Property/Parameters	Pauri	Haridwar	Dehradun	Almora	U.S. Nagar
pH					
Mean	6.205	6.91	6.87	6.21	7.09
Standard Deviation	0.172	0.124	0.128	0.169	0.378
Least significant difference (0.01)	0.599	0.599	0.599	0.599	0.599
Organic carbon					
Mean	0.725	0.829	0.805	0.719	0.828
Standard Deviation	0.064	0.061	0.06	0.139	0.017
Least significant difference (0.01)	0.162	0.162	0.162	0.162	0.162
Phosphorous					
Mean	27.42	34.92	37.21	27.11	36.41
Standard Deviation	8.401	10.31	7.48	11.98	13.64
Least significant difference (0.01)	24.12	24.12	24.12	24.12	24.12
Potassium					
Mean	258.10	302.21	304.12	255.8	302.4
Standard Deviation	73.61	34.98	23.10	52.7	41.24
Least significant difference (0.01)	103.99	103.99	103.99	103.99	103.99

Table 2: District wise localities chosen for statistical analysis and graphical representation of pH, Organic Carbon, Phosphorous and Potassium

Identification	Locality	District
A	Lansdown	Pauri
B	Patisain	Pauri
C	Kyunkaleshwar	Pauri
D	Manglaur	Haridwar
E	Narsan	Haridwar
F	Patanjali	Haridwar
G	Rishikesh	Dehradun
H	Dakpathar	Dehradun
I	Tigerfall	Dehradun
J	Someshwar	Almora
K	Jalna	Almora
L	Gangolihat	Almora
M	Pantnagar	Udham Singh Nagar
N	Gadarpur	Udham Singh Nagar
O	Nanakmatta	Udham Singh Nagar

Table 3: Relationship between spore populations of Uttarakhand region with the soil chemical properties

Property/Parameter array (α)	Property/Parameter array (β)	Correlation (r)
Spore count	pH	0.087898733
Spore count	Organic carbon	0.261011252
Spore count	Phosphorous	-0.103347728
Spore count	Potassium	0.682053741

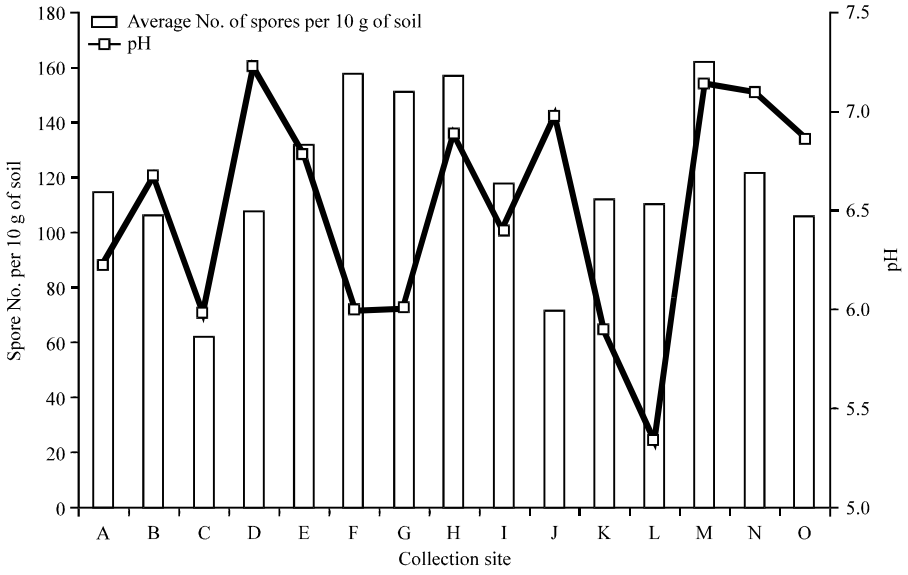

Fig. 1: Distribution of average number of VAM fungal spore population in soil samples collected from different sites of Uttarakhand with respect to pH value

Fig. 2: Distribution of average number of VAM fungal spore population in soil samples collected from different sites of Uttarakhand with respect to organic carbon level

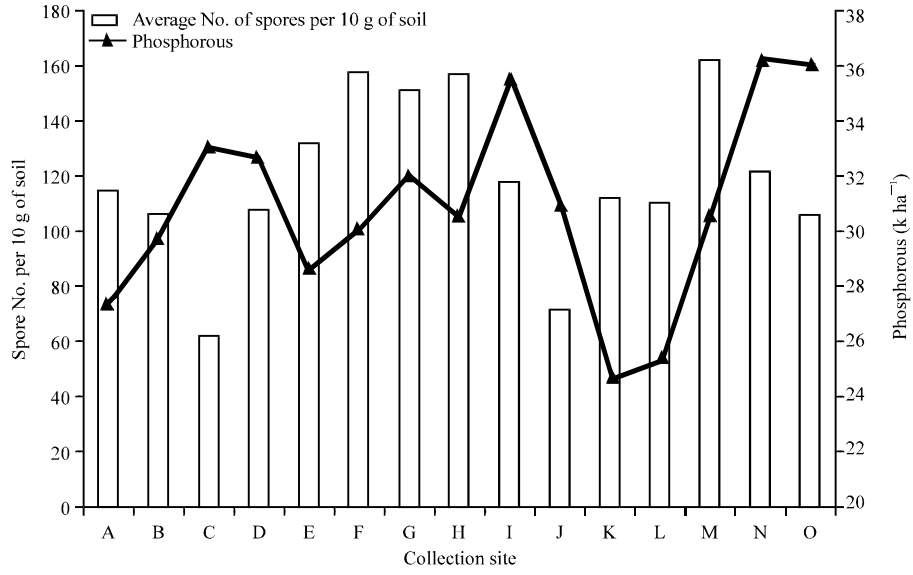

Fig. 3: Distribution of average number of VAM fungal spore population in soil samples collected from different sites of Uttarakhand with respect to phosphorous level

correlation was observed between spore number and pH. At pH value of 6.0 and 7.5, few spores were isolated but in soil samples with pH range 6.2 to 7.0 more spores were isolated.

An undefined relation was observed in Fig. 2 between soil organic carbon level and VAM fungal spore number. Maximum number of spores were observed in soil with an organic carbon content that ranged from 0.82 to 0.89%. Similar relationship was observed between spore number and percent organic matter. For few exceptional cases, spore numbers were higher with high organic matter content of the soil.

At lower levels of phosphorous, maximum number of spores were observed (Fig. 3). Generally, phosphorous content in the soil samples ranged from 27.11 to 37.21 k ha^{-1} and a feeble negative correlation is observed

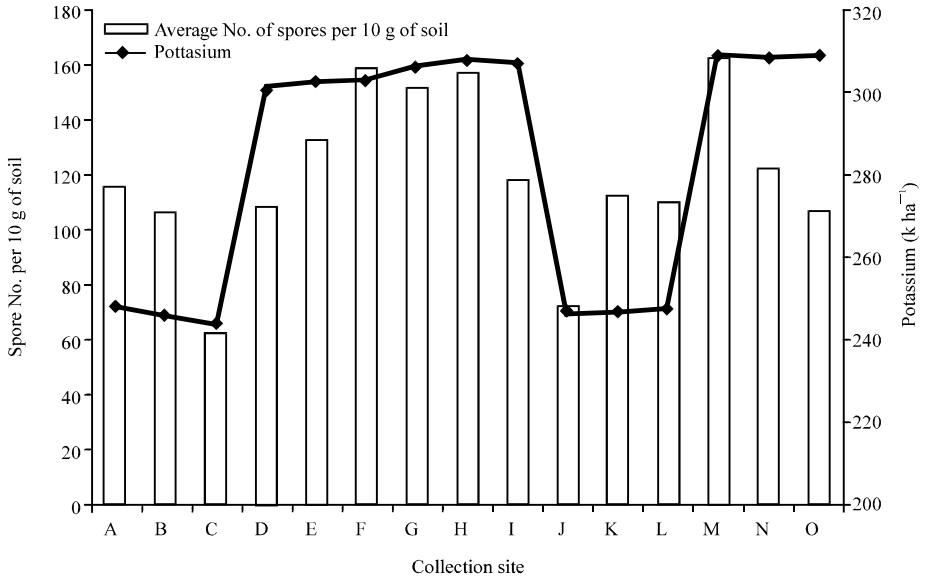

Fig. 4: Distribution of average number of VAM fungal spore population in soil samples collected from different sites of Uttarakhand with respect to potassium level

with respect to spore population. However, a weak positive correlation was observed between spore number and phosphorous level, for the places where phosphorous content was extremely low. Most of the areas studied contained high level of soil phosphorous, however, some areas contained medium level of soil phosphorous.

As observed in Fig. 4, the potassium content of soil ranged from 255.8 to 302.21 k ha^{-1} in soil samples analyzed. Potassium content of soil showed significant positive correlation with spore numbers. The high potassium content of soil, results in increase of spore population.

As the moisture content of the soil increases, it was observed that VAM fungal spore number decreases. Maximum numbers of spores were isolated from soil samples with 2% of moisture contents. However, for the places where phosphorous level was low, a negative correlation was observed between VAM spore number and moisture content of the soil.

Statistical analysis was carried out and the mean values of pH, organic carbon, potassium and phosphorous were compared by Duncan's Multiple Range Test (DMRT). The pH values from different districts were statistically analyzed. The analysis was also subjected to ANOVA. The data from the Table 1 revealed that significantly higher pH values were recorded from the Udham Singh Nagar District.

As per Table 3, there was no significant correlation (r = 0.087898733) of spore number with pH. However, organic matter content revealed positive correlation (r = 0.261011252) with spore number. The statistical analysis showed negative correlation (r = -0.103347728) between spore number and phosphorous content. The potassium content of soil showed significant positive correlation (r = 0.682053741) with spore numbers.

ANOVA was also worked out and the mean value was also compared by DMRT.

DISCUSSION

Changes in edaphic factors greatly influence mycorrhizal diversity. The present research also included the effect of soil pH, soil phosphorous, organic matter content of soil, soil potassium and moisture content of soil on the number of VAM spores. The correlation between the individual variations of different edaphic factors with spore number was also investigated. The statistical results corresponding to the effect of soil pH, soil phosphorous, organic matter content of soil, soil potassium and moisture content of soil on the number of VAM spores for some specific sites chosen from each districts have been shown in Table 1 and 3.

Effect of soil pH: Distribution of various VAM fungi is slightly influenced by soil pH. Some *Glomus* species are very common in neutral or alkaline soil but few in acid soil, whereas species of *Acaulospora* are usually found in acid soil. The pH alone may not be responsible for germination of VAM fungal spores, since with change in soil pH,

chemical constitution of soil also changes. The relationship between soil pH and mycorrhization is complex and depends on the plant species and also soil type, forms of phosphorous and fungal species involved. The optimum pH for different endophytes can vary in different soils and specific endophytes have an optimum pH at which they perform best.

The soil samples analyzed during the present study had the pH range 6.0-7.2 and the results did not reveal any significant correlation (Table 1, 3). However, the relationship cannot be even categorized in non-significant relationship. The reason behind this may be due to widely distributed geographical locations covered and the different types of medicinal plants studied. Maximum number of spores were isolated from the pH range of 6.2-7.1 (Fig. 1). On the basis of results obtained during the present study, it can be said that almost neutral condition or slightly acidic conditions are more favourable for survival of spores and their sporulation process. It was observed that extreme alkaline and acidic conditions are not favourable for endurance of spores. However, in some locations it was observed that pH had negative correlation to spore number (at sites D and L), whereas in sharp contrast to this situation pH had positive correlation with spore number at other few locations (site M). During the analysis carried out, it was noted that the occurrence of spores of *Glomus* species was maximum in versatile and adverse soil and climatic conditions. This clearly indicates that the genus of *Glomus* species has high tolerance to the harsh and unfavourable conditions.

Wang *et al*. (1993) also studied the effects of pH on Arbuscular Mycorrhiza. Similar to our results, they observed small effects of pH in the range 5.5-7.5, however, colonization was greatest at pH 6.5, at places Rothamsted and Woburn. Furthermore, they also observed that only fine endophyte was infected at pH 4.5 and was present as a diminishing fraction of total colonized root at pH 5.5 and 6.5, whereas being absent at 7.5 (Wang *et al*., 1985). This complete shift in species with pH (apart from possible changes in the coarse endophytes) had remarkably little effect on the total fraction of the root length colonized.

Effect of organic matter content: Organic matter content is an essential factor for soil fertility. It was established that organic matter content plays an important role in affecting the number of spores. The increased mycorrhizal colonization with the addition of organic matter in the form of manure was reported by Johnston (1949). But in sharp contrast, Nicolson (1960) reported a decrease in mycorrhizal colonization with increased soil organic matter.

The present study substantially indicates that VAM fungal spore population increases with an increase in organic matter. However, it is extremely difficult to establish that a single factor influences the mycorrhizal spore population in a natural ecosystem. This is due to the reason that other environmental and climatic factors also influence soil microfauna and microflora as also the mycorrhizal spore population. Percent organic carbon level of soil that ranges from 0.725 to 0.829%, showed significant positive correlation with spore number (Table 1, 3). More number of VAM fungal spores were isolated from the soils having elevated level of organic matter content (Fig. 2).

Effect of soil phosphorous: Phosphorous is one of the major plant nutrients and an important content of the soil for the growth and development of VAM fungi. The high level of phosphorous in soil results in decrease in spore population which further reduces the multiplication of mycorrhizal spores.

In the present study phosphorous content ranged from 27.11 to 37.21 k ha^{-1} in the collected soil samples (Table 1). The soil samples were collected from a wide range of habitat randomly with mixed population of different mycorrhizal spores. In the present research work statistical analysis shows negative correlation between spore number and phosphorous content (Table 3). Maximum number of VAM fungal spores were present in the soil with phosphorous content around 30.3 k ha^{-1} (Fig. 3).

Soil is a complex system with all the edaphic factors inter related to each other. When pH increases phosphorous solubility decreases. Due to this reason a drastic change is found in the chemical property of the soil consequently affecting the spore population.

In almost agreement to our observations Javadi *et al*. (1991) found that spore concentration and phosphorous content in soil is negatively correlated. The reduced number of VAM fungal spores resulting from soil applications of phosphorous also agrees with the findings of other researchers (Hepper and Smith, 1976; Timmer and Den, 1980). These researchers further elaborated that when phosphorus deficiency occurs, plants may release large amounts of sugar and amino acids into the rhizosphere which are utilized by VAM fungi for growth. Such a relationship was demonstrated previously in phosphorus deficient sorghum by Graham *et al*. (1981). The amount of substrate leakage in phosphorus deficient plants has been negatively correlated to phospholipid levels in root cell membranes (Ratanayake *et al*., 1978). Therefore, elevated phosphorus concentrations in plants may reduce the substrate leakage rate, thus suppressing

fungal infection of the roots. This, however, did not limit the uptake and concentration of phosphorus in the leaf tissue. An abundance of phosphorus in the soil was adequate to compensate for the reduced number of VAM fungal spores and root uptake of phosphorus was adequate (Javadi *et al*., 1991).

Effect of soil potassium: Potassium is also considered to be an important factor for mycorrhizal development in soil. The potassium content of soil ranged from 255.8 to 302.21 k ha^{-1} (Table 1). Based on statistical results obtained, potassium content of soil showed significant positive correlation with spore numbers (Table 3). The high potassium content of soil results in the increase of spore population. This can also be clearly observed from Fig. 4. However, in contradiction to our observations Khanam *et al*. (2006) found a negative and non-significant correlation between soil potassium and spore count.

Effect of moisture content of soil: Mycorrhizal fungi are known to improve plant growth under water stress conditions. Low and high moisture content of soil directly effects root colonization by mycorrhizal fungi. In current study, the high moisture content in soil showed very low VAM fungal spore population. The moisture content of the soil samples collected ranged from 1 to 38%. The places where moisture content in soil is high, the VAM spore population is low. However, Udaiyan *et al*. (1996) observed that spore populations are positively correlated with soil moisture in *A. planifrons*. Dickman *et al*. (1984) also reported similar relationship between soil moisture and spore abundance in little blue stem [*Schizachyrium scoparium* (Michz.) Nash.]. In contrast to such observations, Udaiyan *et al*. (1996) found that spore abundance in *A. farnesiana* was negatively correlated to soil moisture. Anderson *et al*. (1983) also found a significant negative correlation between VAM spore numbers and soil moisture. Therefore, it can be inferred that spore production by VAM fungi is influenced by plant species to a considerable extent, in addition to edaphic factors, which may account for the contrasting response of spore abundance to soil moisture (Struble and Skipper, 1988).

CONCLUSION

The distribution and occurrence of VAM fungi differ both qualitatively as well as quantitatively with the change in edaphic factors and the type of vegetation. This study evaluated and analyzed the effect of edaphic factors like pH, organic carbon, potassium and phosphorous on the spore population. The statistical analysis revealed that the pH was almost uncorrelated with spore number. Percent organic carbon level of soil ranged from 0.725 to 0.829% in Uttarakhand. Percent organic carbon level showed significant positive correlation with spore number. More number of VAM fungal spores were isolated from the soil with higher level of organic matter content. The addition of organic matter in the form of manure increases mycorrhizal colonization. The high level of phosphorous in soil results in decrease in spore population which reduces the multiplication of mycorrhizal spores. The statistical analysis shows a negative correlation between spore number and phosphorous content of soil. Maximum number of VAM fungal spores were present in the soil with phosphorous content around 30.3 k ha^{-1}. The spore count showed a positive correlation with respect to potassium content in soil. With an increase in potassium content the spore count was observed to be higher.

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