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CORRELATION OF SOIL PHYSICO-CHEMICAL FACTORS WITH VAM FUNGI DISTRIBUTION UNDER DIFFERENT AGROECOLOGICAL CONDITIONS

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ABSTRACT

An attempt was made to analyze the correlation between the physical and chemical factors of soil with the distribution of VAM fungi under different Agroecological condition. Two fields from Chandur Bazar Tehsil of Amravati district were selected for the present investigation because the edaphic factors of the particular district show highly variable soil geography. The study revealed that all the physical and chemical factors viz; soil moisture, soil texture, soil pH and EC, nitrogen, Phosphorus, Potassium and Carbon are positively correlated with the distribution of VAM fungi

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KEY WORDS

Physical parameters, Chemical parameters, Correlation, VAM fungi, Agroecological conditions

INTRODUCTION

Arbuscular mycorrhiza fungi (AMF) are geographically ubiquitous and occur over a broad ecological range. They are commonly found in association with numerous plant species including agricultural crops. It is estimated that AM fungi occur over 90% of earth's plant species (Kendrick and Berch, 1985). Vesicular - arbuscular mycorrhizas are though widely distributed but, there is limited knowledge of occurrence of individual or dominant species in relation to soil and climate. Several biotic and abiotic factors influence the VAM in various ways. Arbuscular mycorrhizal fungi are most frequent in plants growing on mineral soils. The population of AM fungi is greatest in plant communities with high diversity where they have many potential host plants and can take advantage of their ability to colonize a broad host range. There is a lower incidence of mycorrhizal colonization in very arid or nutrient rich soils. Mycorrhizas have been observed in aquatic habitats; however, water logged soils have been shown to decrease colonization in some species (Smith and Read, 2002).Mycorrhiza is undoubtedly of extra ordinary importance in plant production, plant and soil ecology plays a key role in sustainable agriculture (Moawad, 1979; Gianianazzi *et al.,* 1994).

MATERIALS AND METHODS

For the present investigation, the Amravati District was selected because of its distinct soil geography and climatological conditions. The area represents plains with black clayey soil. Hence, two different sites were selected for collection (Site 1 and Site 2).

1. Physicochemical analysis of soil:

The soil mycorrhizal population in terms of percentage of root colonization and the number of resting spores produced was greatly affected by edaphic factors as well as soil nutrient conditions (Daniels Hetrick , 1984; Reena Singh and Alok Adholeya , 2002). Soil pH , type , moisture influenced the VA mycorrhizal population in natural ecosystem (Mohankumar and Mahadevan,1999). Therefore, it is necessary to understand the VAM fungal dynamics, their qualitative and quantitative association and impact of soil physicochemical factors on VAM distribution.

The following standard methodology was adopted to analyze the physical and chemical status of the soil samples.

A. Physical parameters:

a. Soil moisture:

Moisture content of the soil samples were calculated immediately by oven drying method (Jackson, 1967).

10 g of composite soil sample was kept in hot air oven for 24 hrs. at 105°C. Dry weight of the sample was taken till it showed its constant weight.

The percent moisture was expressed as follows.

$$\text{Moisture \%} = \frac{(W_1 - W_2)}{100} \times 100$$

Where , W_1 = Weight of soil before oven drying

W_2 = Weight of soil after oven drying.

b. Soil texture:

Relative proportion of different size soil particles is an important physical parameter to determine soil texture. The percentage of the soil particles were determined by Robinson's pipette method (Piper, 1964).

20g air dried soil sample was mixed with 50 ml distilled water. The mixture was heated for 5 minutes to break the large sized soil particles.10 ml hydrogen peroxide was added to the mixture. The solution was filtered through ordinary filter paper. The soil residue of filter paper was then treated with 250 ml of 5 N HCl and left it for overnight. The solution was filtered again and soil residue dried in air. The dried soil was passed through 0.2 mm pore sized scientific sieve. The coarse sand left on the sieve was weighed (W_1). The sieved soil was transferred into a flask and sufficient amount of 1 N sodium hydroxide (NaOH) was added to make the contents alkaline. After shaking the flask for six hours on the mechanical shaker, the content was transferred to 500ml measuring jar and the volume was made up to 500ml by distilled water. It was stoppered and was allowed to settle after shaking. The solution was pipetted out with Robinson's pipette into a dish. The required quantity was pipetted and evaporated in a dish and weighed which gave the quantity of clay content (W_4). Silt (W_3) was calculated by deducing clay from silt. The supernatant was poured into a separate beaker and soil was washed again and again till the suspension was clear. This fraction was transferred to another tarred dish, heated and weighted, which was the reading of fine sand (W_2).

Reading:

W_1 – Coarse sand.
W_2 – Fine sand.
W_3 – Silt.
W_4 – Clay.

c. Determination of soil pH:

pH value as a measure of the hydrogen ion activity of the soil water system and expresses the acidity and alkalinity of the soil. It is a very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of soil. The pH of soil water suspension was determined using Equiptronics pH meter as described by Jackson (1967).

20 g soil sample was mixed with 40 ml distilled water in 1: 2 ratio. The suspension was stirred intermittently with glass rod for 30 minutes and left for one hour. The electrode was inserted into supernatant and pH was recorded .The electrode was washed with distilled water every time to record the other new reading of the soil sample.

d. Determination of electrical conductivity of soil:

Electrical conductivity (EC) expresses ion contents of solution. Conductivity as the measure of current carrying capacity, gives a clear idea of the soluble salts present in the soil. The electrical conductivity of a soil samples was determined on an Equiptronic's digital electrical conductivity bridge.

In a 20g soil, 40 ml of distilled water was added. The suspension was stirred intermittently for half an hour and kept it for 30 minutes without any disturbances. Conductivity cell was inserted in solution and EC was recorded.

B. Chemical parameters:

a. Estimation of available nitrogen (N):

Nitrogen of soil mainly present in organic form together with small quantities of ammonium and nitrate forms. The nitrogen supplying ability of the soil was determined by distilling soil with alkaline potassium permagnate solution. During the distillation easily utilizable and amino- N hydrolyzed nitrogen liberated as ammonia is measured. This serves as an index of nitrogen status of soil. Alkaline potassium permagnate method (Subhaiah and Asija, 1956) was followed to estimate available N of soil samples.

In 1000 ml round bottom distillation flask (Kjeldahl flask), 20g soil was taken. To this 20ml distilled water was added. Then 100ml each of 0.32 % potassium permanganate and 100 ml 25% NaOH solution were mixed and immediately connected it to keelhaul assembly. The froth during boiling was prevented by adding liquid paraffin (1ml) and bumping by adding a few glass beads. The contents were distilled in a kjeldahl at a steady rate and liberated ammonia collected in an Erlenmeyer flask (250 ml), containing 20 ml of 2 % boric acid solution with methyl red and bromocresol green indicator. With the absorption of ammonia, the pinkish colour turns to green. After 30 minutes it was titrated with 0.02 N H2SO4 till the colour changed from green to original shade (pink). Blank (without soil) was run simultaneously.

Available nitrogen was calculated from the following formula,

$$\% \text{ Available N} = (A\text{-}B) \times (\text{N. of acid}) \times 0.014 \times \frac{100}{\text{Wt. of soil (g)}}$$

$$\text{Available nitrogen (Kg/hec)} = \% \text{ N} \times \frac{2240000}{100}$$

Where,

1. Wt. of soil sample - Wt.
2. Volume of std. acid required for soil - A ml
3. Volume of std. acid required for blank - B ml.
4. Normality of Sulphuric acid. - N

b. Estimation of available phosphorus (P) :

Soil available phosphorus found as orthophosphate in several forms and combinations, but only a small fraction of it may be available to plants. Available phosphorus was estimated by Olsen's method (Olsen, *et al*., 1954) modified by Watanbe (1965).

The reagent for Olsen's P was 0.5 M $NaHCO_3$ (pH 8.5) prepared by dissolving 42 g $NaHCO_3$ in distilled water and made up to 1 lit. The pH was adjusted at 8.5 with 20 % NaOH solution. 2.5 g of air dried soil was weighed into 150 ml Erlenmeyer flask, 50 ml of Olsen's reagent (0.5 M $NaHCO_3$ Solution , pH 8.5) and one teaspoonful of activate charcoal were added. The flasks were shaken for 30 minutes on the electrical shaker and contents filtered immediately through Whatman filter paper (No. 41). 5 ml of the filtrate was pipetted out into 25 ml of volumetric flask and was neutralized with 1: 4 H_2SO_4 using paranitrophenol as indicator. The volume was made up by adding distilled water. Colour developed when few crystals of stannous oxalate were added. The solution was shaken well and intensity of blue colour was read in photoelectric calorimeter within 10 min. at wavelength of 730 to 840 µm. A blank was run without soil.

Standard curve*:*

Analytical grade potassium dihydrogen orthophosphate (KH_2PO_4) was dried in hot air oven at 60 C for 1 hr and allowed to cool. Exactly 0.439 g of KH2PO4 was dissolved in 500 ml of distilled water.25 ml of 7N H_2SO_4 was added and made up to 1 lit. with distilled water . This gives 100 ppm standard stock solution of KH_2PO_4.. From this diluting it 5 times and made a 2 ppm P solution.

For the preparation of standard curve different concentrations of P 0,2,4,6,8 and 10 ml of 2 ppm P solution were taken in 25 ml volumetric flask separately, which corresponds to 0, 0.16, 0.32, 0.48, 0.64 and 0.80 ppm P respectively. To these 5ml of the extracting reagent 0.5 ($NaHCO_3$) was added to each flask and pH was adjusted as above. The content was diluted with 20 ml water and 4ml reagent (Dickman and Brays reagent). Volume was made up and intensity of blue colour was read in photoelectric calorimeter using 730-840 m filter or using red filter (660nm). Graph was constructed by plotting reading on X-axis and concentrations of P on Y-axis.

$$\text{Factor (F)} = \frac{\text{Concentration of P}}{\text{Corresponding reading of above concentration}} = \frac{0.32}{30} = 0.01$$

= 1 Calorimeter reading = 0.01 ppm (P) phosphorus.

Calculations:

The amount of phosphorus was estimated by using formula,

$$\text{P(ppm in soil)} = \text{ppm P in aliquot} \times \frac{\text{Total volume of extract}}{\text{Aliquot taken (ml)}} \times \frac{1}{\text{Wt. of soil (g)}} \times (R \times F)$$

P (Kg/ ha) = ppm P in soil × 2.24
P_2O_5(Kg/ ha) = P(Kg/ ha) × 2.29
Conversion factors = P ×2.29 = P_2O_5
P = P_2O_5 × 0.437

c. Determination of available potassium (K) :

Only small fraction of total K is held in exchangeable form, while the rest remains in fixed or non-exchangeable form. When the crop exhausts the supply of exchangeable K, more K is released from the fixed reserve. Exchangeable K, is therefore, also referred to as 'available K'. The flame photometric method (Jackson, 1958) was employed to estimate available K of samples.

5g of air dried sample was taken in 150 ml Erlenmeyer flask and 25 ml of 1 N ammonium acetate was added to the flask. The contents were shaken for 5 minutes on a mechanical shaker and filtered immediately through a dry filter paper (Whatman No.1).

The filtrate was collected in a beaker. 5 ml of filtrate diluted with 25 ml with distilled water. Atomized the above diluted extract to flame photometer to note the reading.

The amount of potassium was estimated by formula:

Calculations:

$$\text{Available K (Kg/ha)} = (R \times F) \times \text{Vol. of extract} \times DF \times \frac{2.24 \times 10^6}{\text{Soil wt.} \times 10^6}$$

$$\text{Available } K_2O \text{ (Kg/ha)} = \text{Available K (Kg/ha)} \times 1.20$$

Where,

R = reading.
F = Conc. of K/ corresponding reading.
DF = dilution factor.

d. Determination of organic carbon (C) :

Organic matter plays an important role in supplying nutrients and water and provides good physical conditions to the plants. The quantity of organic carbon of the soil was estimated by the method of Walkey and black (1934) described by Jackson (1967).

1g finely ground soil sample passed through 0.5 mm sieve without loss was taken into 500 ml conical flask, to which 10ml of 1 N potassium dichromate and 20 ml Conc. H_2SO_4 were added with measuring cylinder. The content was shaken for a minute and allowed to set aside for exactly half an hour. Then 200 ml distilled water 10 ml ortho-phosphoric acid and 1 ml diphenylamine indicator was added. The solution was titrated against std. ferrous ammonium sulphate (FAS) or ferrous sulphate, till colour flashes from blue violet to brilliant green. The blank titration was carried at the beginning without soil.

The observation was:

1. Weight of soil taken = Wg
2. Vol. of 1 N Potassium Dichromate added = 10 ml
3. Vol . of 0.5 N FAS required to neutralize 10ml of 1 N Pot. Dichromate solution (blank without Soil) = B ml
4. Vol of 0.5 N FAS required for soil = T ml
5. Vol. of 1 N $H_2Cr_2O_7$ solution used for the oxidation of organic carbon present in the sample. = 10(B-T)

The organic carbon content (in %) of the soil was calculated as follows.

$$\text{Organic Carbon \%} = \frac{10(B-T)}{B} \times 0.003 \times \frac{100}{\text{wt. of Soil (g)}}$$

RESULTS AND DISCUSSION

Physico-chemical analysis of soil:

Physical Parameters:

**** Soil moisture:***

If water content becomes too low, a plant becomes stressed. The amount of soil water available to plant is governed by depth of soil that roots and AM fungi can explore.

The average percent soil moisture of both the sites were recorded in between 7.886 to 8.85%, which clearly indicates that soil moisture percentage in site 1 is higher than site 2 as shown in the (**Table-1**).

Table-1
Physico-chemical characteristics of soil

Site	Soil type	Soil pH	EC (dsm/m)	% Soil moisture	Available nutrients (mg/kg)			
					N	P	K	C
S1	Clayed	8.02	0.41	8.85	179.2	51.80	123.2	0.54
S2	Clayed	7.28	1.75	7.886	153.0	43.90	3365.6	0.29

The soil moisture is found to be correlated with spore density. The increase in spore population may be due to increase in soil moisture. Similarly reports were reported by Mohankumar and Mahadevan (1999) and Bakshi (1974). Khan (1974) reported low spore density in the month of May. It indicates that spore density is correlated with moisture content.

** **Soil pH and EC**:*

pH is a measure of the hydrogen ion concentration i.e. acidity or alkalinity of the soil. pH can affect the availability of nutrients and activity of many essential micro-organisms. The pH of a soil may influence crops grown in the field and the types of soil microbiota.

The pH of all soil samples were found to be ranged in between 7.28 to 8.02 which indicated the slight alkalinity of soils (**Table-1**).
The unit of electrical conductivity is dsm m^{-1}. Electrical conductivity of two composite soil samples ranging between 0.41 to 1.75 as shown in (**Table-1**). There was no specific correlation found between EC and spore density.

The response of VAM fungi is variable according to the strains present in that soil. VA mycorrhiza is positively correlated with high pH value according to Sharief and Moawad (2006).

Chemical Parameters:
**** Available Nitrogen:***

Chemical analysis was carried out for the elements like N, P, K and C. It is presented in (**Table-1**).

In site 1, it was greater i.e. 179.2 and in site 2 comparatively less i.e. 153.0 mg/kg. Nitrogen content of soil directly affects soil pH which may be responsible for variation in spore density. Some other reports states that high nitrogen did not leads significant reduction in AMF colonization (Hartwig *et al*., 2001).

**** Available Phosphorus:***

Phosphorus is one of the key macronutrient required for plant growth and metabolism. Inorganic phosphate supplied to the soil as a fertilizer is rapidly converted into unavailable form. Soluble P converted into insoluble phosphate involves microorganisms. Mycorrhizal plants can take up more phosphorus than non mycorrhizal plants (Kapoor and Mishra, 1931).

Comparatively higher P- availability is recorded in site 1, 51.80 and in site 2 it is 43.90 mg/kg as shown in the (**Table-1**)
Percent colonization and spore density is greater in the soil having low P- values. This view is supported by Janaki Rani and Manoharchary (1994).

**** Available Potassium:***
Estimated K was recorded in the (**Table-1**)

The values were ranging between 123.2 to 3365.6mg/kg. The positive correlation was found by Joshi and Singh (1995).

Available Carbon:

The value of organic carbon was tabulated in (**Table-1**) ranging between 0.54 - 0.29 mg/kg.

Higher C percentage was record in fertile soil due to addition of higher inputs. Kliromonos *et al,*. (1998) observed that *Glomus* Sp. production were significant higher in response to high CO_2 elevation.

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