Effect of Edaphic and Climatic Factors on Vesicular-Arbuscular Mycorrhizae under Neem based Agroforestry System

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Abstract

Influence of edaphic and climatic factors on build up of VAM population in crops and tree rhizosphere under arid and semi-arid regions was analyzed and a mathematical equation for prediction of number of VAM fungal propagules derived. The influence of zinc (Zn) content on VAM prediction (30%) under tree rhizosphere was followed by maximum temperature (15%). Prediction values were 70% when Zn, $CaCO_3$, and temperature were taken into consideration for neem tree and for crop rhizosphere (pearl millet and mung bean). Organic carbon gave the maximum prediction value (34%) followed by relative humidity (19%). Both climatological factors as well as edaphic factors were found to influence the prediction of VAM infection in crops under neem based agroforestry systems.

Key words: Agroforestry system, VAM population, prediction, edaphic and climatic factors

INTRODUCTION

Beneficial aspect of Vesicular-arbuscular mycorrhizal (VAM) fungus in agroforestry in terms of soil fertility, nutrient cycling, soil conservation, soil physical properties are well recognized to ensure a healthy soil plant system. This concept of sustenance of productivity is dependent on the unity and interdependence of a healthy plant-soil system in the face of natural and cultivable stresses, which depends on the soundness of the interface between plant and soil, the rhizosphere. It is well recognized that agriculture leads to greater diversity of VAM fungi (Abbott and Robson 1977); which is a pre-requisite to maintain a sustainable agro-ecosystem. The importance of VAM fungal diversity in a minimum input agriculture system is very well illustrated (Dodd et al. 1990).

Limited information is available on VAM association in agroforestry ecosystems. In general, there is not much

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host specificity in VAM fungi but, when a tree and agriculture crop are grown together, there is bound to be change in VAM species composition. Commercial point of view most effective VAM fungal species have to be screened out, but it required area specific research. Since the species incidence of VAM fungi changes with ecosystem and is affected by levels of soil variables (pH, salinity, moisture stress, soil fertility, rainfall gradient, soil types and other host effects) we need to be aware of the ability of a species to persist in any particular ecosystem or fertility level. In view of these facts, an extensive field investigation was carried out to ascertain VAM population in neem based agroforestry fields of semi-arid and arid parts of Rajasthan, India. Attempt has also been made to derive a mathematical equation which could predict the number of VAM fungal propagules just by monitoring the various physical and chemical properties of soil in question with the assumption that VAM fungal propagules in soil, is the resultant effect of various climatic and physical and chemical properties of soils.

MATERIALS AND METHODS

The field study, which was undertaken during 1998-99, pertains to extensive survey of soil of traditional neem based agroforestry systems in the region where rainfall varied from 140 mm to 1000 mm in arid and semi arid zones of Rajasthan covering six districts of different agro-ecological zones (Table 1). The rainfall, mean maximum temperature, mean minimum temperature and relative humidity data for the last 10 years (1990-99) was collected from Agro-meteorological section, CAZRI, Jodhpur. For minimizing error and variations to too many crops, crops studied were restricted to pearl millet and mung bean. The collection of roots and rhizosphere samples was done at the time of crop harvest, which synchronizes the time of maximum spore build up (Rosendahl et al. 1989). These data were further used to develop relationship between VAM fungi and soil parameters.

The tree sampled (under the canopy of which roots and rhizosphere soil samples were collected) was varying in age from 12 to 15 years. Trees and crops from each agro-ecosystem, both under rainfed and irrigated conditions were selected at random. Total 37 locations were identified for collection of rhizosphere soil samples and roots from neem based agroforestry fields. Field sampling involved the structural measurement of standing neem trees in selected agroforestry fields. At each site 7 trees having more or less uniform collar diameter, canopy diameter and height were selected. Information on the quantitative details of the crop grown in the selected fields were collected from the farmers. This was done to ascertain that

Table 1. Range of VAM fungal population and per cent root colonization in neem based agroforestry fields

District	Rainfall (mm)	Mean Max. temperature* (°C)	Mean Min. temperature* (°C)	Relative humidity* (%)	VAM fungal propagules (100 g⁻¹ soil)		Root colonization (%)	
					Tree	Crop	Tree	Crop
Jaisalmer	100-200	46	2	66	144-284	74-180	47-66	47-58
Barmer	210-300	49	0	56	210-342	157-219	54-66	49-62
Jodhpur	305-400	45	3	53	310-520	158-264	68-87	61-82
Jaipur	408-600	45	3	54	237-448	230-418	57-63	60-68
Swai Madhopur	605- 850	40	3	62	256-433	205-215	64-72	52-57
Kota	855-1000	45	9	48	111-299	193-213	41-52	40-54

*average of ten years (1990-1999)

Table 2. Physico-chemical properties of neem based agroforestry fields in different districts

Parameters	Jaisalmer		Barmer		Jodhpur		Jaipur		Swai-Madhopur		Kota	
	Tree	Crop	Tree	Crop	Tree	Crop	Tree	Crop	Tree	Crop	Tree	Crop
pH	7.64-9.61	8.19-8.91	7.89-9.67	8.06-9.73	7.90-8.49	8.17-8.73	7.91-8.85	8.21-9.18	8.28-8.50	8.41-8.76	7.99-8.75	7.95-8.52
EC (mmhos cm⁻¹)	0.07-0.64	0.07-0.15	0.29-2.56	0.15-0.77	0.15-0.65	0.10-1.04	0.48-1.76	0.61-1.49	0.36-1.17	0.28-1.07	0.72-1.49	0.46-0.93
OC (%)	0.01-0.90	0.06-0.22	0.04-0.44	0.03-0.08	0.24-1.45	0.18-0.95	0.94-1.97	1.37-1.88	1.07-1.59	1.00-2.12	1.26-1.70	1.30-2.20
CaCO$_3$ (%)	1.34-2.16	0.79-1.98	1.19-1.82	0.54-0.99	0.90-1.80	0.30-1.00	0.29-0.41	0.24-0.40	0.48-0.59	0.34-0.69	0.41-0.89	0.32-0.41
P (ppm)	3.9-7.8	20.8-39.3	3.8-6.2	10.2-25.0	2.4-8.2	9.4-31.3	1.0-2.6	14.4-20.2	0.6-16.7	10.6-14.6	0.2-2.7	5.3-11.5
Zn (ppm)	1.3-1.8	0.9-1.5	1.3-1.7	1.2-2.4	2.1-2.6	1.4-5.2	1.1-1.4	1.1-2.2	0.5-1.4	1.0-1.4	1.5-2.4	1.4-2.1
Fe (ppm)	0.4-1.0	0.3-0.7	1.4-2.2	0.7-1.4	2.1-3.4	0.4-2.3	2.5-2.8	0.9-1.5	0.8-1.6	6.0-8.7	0.1-1.2	1.4-3.8
Cu (ppm)	0.2-1.2	0.3-0.6	0.8-1.2	0.2-1.1	0.6-1.0	0.2-0.4	0.2-0.6	0.1-0.3	0.6-0.9	0.1-0.3	0.1-0.8	0.1-0.3
Mn (ppm)	14.5-18.7	4.7-8.4	6.7-16.8	5.3-6.7	16.2-18.5	3.4-6.2	8.3-12.0	5.2-9.7	6.6-8.4	2.7-4.4	3.8-16.7	1.4-5.1
Co (ppm)	0.2-0.6	0.3-0.6	0.3-0.6	0.3-0.6	0.5-0.7	0.2-0.3	0.3-0.5	0.1-0.3	0.3-1.0	0.1-1.6	0.5-1.1	0.1-0.3

degree of crop diversification and crop grown is almost similar in selected sites. This was followed by the collection of soil samples from each selected site.

In each selected site the rhizosphere soil samples were taken form two situations. The soil from the top was scraped off to remove foreign particles and litter. In the first case, the soil sampling was done at a depth of 5 – 15 cm under the canopy of the standing neem trees. In case of the other situation, the soil samples were taken at a similar depth but from 25 meters away from the canopy periphery of the tree. The samples were homogenized; all stones, plant material, coarser roots (> 1 cm diameter) were removed by passing the sample through 0.5 cm diameter sieve.

The roots were separated from collected soil samples. VAM fungal association was measured after stained in Trypan blue following the method of Phillips and Hayman (1970). One hundred root segments were examined for each sample. VAM fungal propagules were isolated from the rhizosphere soil samples using wet sieving and decanting technique by following Gerdemann and Nicolson (1963) followed by sucrose centrifugation technique (Jenkins 1964). The spore density was expressed in terms of number of spores per 100 g of soil. Important physico-chemical properties of the soils such as pH, electrical conductivity, organic

C, available P, $CaCO_3$ content, were analyzed as per Jackson (1967). The micronutrients content such as Cu, Fe, Mn, Zn and Co were analyzed using atomic absorption spectrophotometer (Perkin Elmer Model 3110). Using SPAR software to work out the relationship between dependent and independent variables did multiple linear regression analysis.

RESULTS

The physico-chemical characteristics of the soils under neem based agroforestry fields in different districts are presented (Table 2). The matrix of the correlation between VAM fungal population (dependent variable) and various soil parameters (independent variables) in agroforestry fields of tree rhizosphere is presented in table 3. Significant and positive correlations between VAM spore population and HCl – extractable zinc and iron were observed (r = 0.46** and r = 0.53**, respectively). In case of climatic parameters, maximum temperature was found to be negatively correlated (r = -0.39*). In case of crop rhizosphere (Table 4), a high degree of positive correlation was observed between VAM fungal population and soil properties such as electrical conductivity (r = 0.60**), organic carbon (r = 0.58**), iron (r = 0.40*) where as negative correlation was observed with phosphorus (r = - 0.45**) and $CaCO_3$ content (r = -0.64**). Along with the

Table 3. Matrix of simple correlations (r) between VAM fungi and edapho-climate factors in case of tree rhizosphere soil

	VAM Fungi	EC	OC	Fe	pH	Zn	Cu	Mn	Co	P	CaCO$_3$	Temp. (Max.)	Temp. (Min.)	Rainfall	RH
VAM	1.00														
EC	0.02	1.00													
OC	0.21	0.46**	1.00												
Fe	0.53**	0.18	0.42	1.00											
pH	-0.31	0.04	-0.14	-0.09	1.00										
Zn	0.46**	-0.25	-0.03	0.13	-0.35*	1.00									
Cu	0.23	-0.17	-0.49**	0.11	-0.01	0.08	1.00								
Mn	0.23	-0.33*	-0.43**	-0.03	-0.17	0.42**	0.29	1.00							
Co	0.25	0.10	0.16	0.10	-0.21	0.29	-0.10	-0.02	1.00						
P	0.15	-0.17	-0.34*	-0.05	0.06	-0.02	0.41**	0.04	0.03	1.00					
CaCO$_3$	-0.24	-0.45**	-0.74**	-0.43**	0.07	0.23	0.35*	0.30	-0.06	0.29	1.00				
Temp. (Max.)	-0.39*	-0.28	-0.63**	-0.31	0.33*	0.09	0.40*	0.07	-0.27	0.09	0.61**	1.00			
Temp. (Min.)	-0.18	0.24	0.53**	-0.05	-0.15	0.22	-0.60**	-0.12	0.28	-0.41**	-0.41**	-0.25	1.00		
Rainfall	-0.01	0.48**	0.77**	0.26	-0.10	-0.10	-0.58**	-0.41**	0.33*	0.36*	-0.75**	-0.57**	0.79**	1.00	
RH	-0.24	-0.33*	-0.46**	-0.53**	0.06	-0.39*	0.19	0.20	-0.23	0.34*	0.43	0.02	-0.56**	-0.59**	1.00

* Significant at 0.05 probability level; ** Significant at 0.01 probability level

Table 4. Matrix of simple correlations (r) between VAM fungal population and edapho-climatic factors in crop rhizosphere soils

	VAM Fungi	EC	OC	Fe	pH	Zn	Cu	Mn	Co	P	CaCO$_3$	Temp. (Max.)	Temp. (Min.)	Rainfall	RH
VAM	1.00														
EC	0.60**	1.00													
OC	0.58**	0.67**	1.00												
Fe	0.40*	0.01	-0.06	1.00											
PH	0.14	0.18	0.00	0.11	1.00										
Zn	-0.14	-0.13	-0.20	0.06	-0.00	1.00									
Cu	-0.01	-0.08	-0.12	0.16	-0.18	0.59**	1.00								
Mn	-0.01	-0.09	-0.39*	-0.02	-0.18	-0.17	-0.31	1.00							
Co	-0.11	0.08	0.40*	-0.46**	-0.00	-0.28	-0.33*	-0.24	1.00						
P	-0.45**	-0.38*	-0.49**	-0.18	0.10	0.07	-0.07	0.47**	-0.20	1.00					
CaCO$_3$	-0.64**	-0.57**	-0.64**	-0.29	0.03	0.01	-0.14	0.35*	0.01	0.64**	1.00				
Temp. (Max.)	-0.33*	-0.37*	-0.66**	-0.13	0.13	0.02	-0.21	0.33*	-0.15	0.17	0.39*	1.00			
Temp. (Min.)	0.08	0.19	0.60**	-0.30	-0.29	-0.09	-0.08	-0.56**	0.44**	-0.44**	-0.42**	0.25	1.00		
Rainfall	0.42**	0.56**	0.87**	-0.07	-0.13	-0.16	-0.10	-0.58**	0.45**	-0.58**	-0.67**	-0.57**	0.79**	1.00	
RH	-0.44**	-0.36*	-0.43**	-0.25	0.08	-0.31	-0.23	0.50**	0.01	0.60**	0.68**	0.03	-0.56**	-0.59**	1.00

* Significant at 0.05 probability level; ** Significant at 0.01 probability level

edaphic parameters, climatic factor such as rainfall was positively correlated (r = 0.42**). However, maximum temperature and relative humidity were negatively correlated with VAM fungal population (r = - 0.33* and r = -0.44**, respectively).

Multiple linear regression analysis was done to work out the relationship between dependent and independent variables by taking all the independent parameters together. An attempt was also made to examine the significance of inclusion of all the independent factors, irrespective of their mutual correlation in improving the overall prediction. Further, step-down regression was done so as to eliminate step by step the factors, which were least significant and obtain variables, which were most significant.

Tree Rhizosphere

The step-down regression analysis of soil of tree rhizosphere exhibited that Zn, $CaCO_3$, maximum and minimum temperature significantly affect the VAM fungal population. These factors when individually analyzed showed that zinc content in tree rhizosphere soil was the most important factor influencing the VAM fungal population. The prediction value was in order of 30% and the following equation derived as follows:

$$Y = 165.18 + 63.39 \, X_1$$

Where, Y is the VAM population and X_1 is the zinc content

The second best prediction value obtained was that in case of, maximum temperature. It gave prediction value in order of 15%. The equation obtained is as under

$$Y = 1026.61 - 15.90 \, X_1$$

Where, Y = VAM population and X_1 = maximum temperature

The other factors such as calcium carbonate (X_1) and minimum temperature (X_2) though contributing significantly in controlling VAM population, showed poor prediction value of 6 and 3% respectively, when regressed with VAM fungal population. The equation obtained was in the following form:

$$Y = 349.8 - 43.66 \, X_1 \text{ and}$$

$$Y = 333.3 - 9.70 \, X_2$$

To enhance the prediction value, the above four parameters (Zn, $CaCO_3$, minimum and maximum temperature) were pooled together, resulted in the prediction value of 70% and the equation was as follows:

$$Y = 868.79 + 15.66 \, X_1 - 83.16 \, X_2 - 13.55 \, X_3 - 32.94 \, X_4$$

Where, X_1 = Zn; X_2 = $CaCO_3$; X_3 = maximum temperature; X_4 = minimum temperature

The step down regression analysis with only edaphic factors i.e. soil parameters, yielded zinc and iron to be factors

influencing the VAM fungal population. The prediction value of 44% was obtained. The equation obtained was as under

$$Y = 32.08 + 8.45 X_1 + 58.18 X_2$$

Where X_1 = zinc and X_2 = iron

The regression analysis with significant climatological factors was carried out to predict VAM fungal population with the variables such as rainfall, temperature and humidity. The following equation was obtained having prediction value of 63%.

$$Y = 3300 - 40.64 X_1 - 4.82 X_2 - 16.79 X_3$$

Where, X_1: maximum temperature; X_2: rainfall and X_3: humidity

When all the edapho- climatic factors were regressed together on VAM fungal population the prediction value as high as 76% was obtained. The equation was as under:

$$Y = 1809.59 + 4.86 X_1 + 23.28 X_2 + 0.32 X_3 + 28.04 X_4 + 26.68 X_5 + 38.51 X_6 + 5.85 X_7 + 6.52 X_8 + 4.41 X_9 + 38.15 X_{10} + 10.19 X_{11} + 19.82 X_{12} + 2.49 X_{13} + 5.72 X_{14}$$

Where, X_1: Zn; X_2: Fe; X_3: Mn; X_4: pH; X_5: EC; X_6: OC; X_7: Cu; X_8: Co; X_9: P; X_{10}: CaCO$_3$; X_{11}: Maximum temperature; X_{12}: Minimum temperature; X_{13}: Rainfall. X_{14}: Relative humidity

Crop rhizosphere

The regression analysis showed that edaphic and climatological factors played an important role in influencing the VAM fungal population. It was observed that OC, Co and Zn content affect VAM fungal population, whereas, minimum temperature and relative humidity were the climatological factors, which significantly influenced the VAM population. Organic carbon (X_1) was the most important parameter giving the highest prediction value (34%) as a single factor. The regression equation was obtained as under

$$Y = 162.04 + 58.54 X_1$$

Next factor in order of importance was relative humidity (X_1), regression of which on VAM fungal population gave a prediction value of 19% and the regression equation was as under

$$Y = 535.7 - 5.92 X_1$$

The factors, Zn (X_1) and Co (X_2) content showed little significance in predicting the VAM fungal population being 2 and 3 per cent respectively. The regression equation obtained was as follows:

$$Y = 225.54 - 6.22 X_1$$

$$Y = 228.62 - 6.67 X_2$$

The least prediction value of 0.7% was obtained in case of minimum temperature when regressed against VAM fungal population and the equation was as under

$$Y = 190.05 + 3.32 X_1$$

(X_1 = minimum temperature)

The collective regression of all the above factors accounts for 70 per cent of the prediction value and the equation was as follows:

$$Y = 709.10 + 74.98 \, X_1 - 12.13 \, X_2 - 9.94 \, X_3 - 21.35 \, X_4 - 7.03 \, X_5$$

Where, Y: VAM fungal population; X_1: Organic carbon; X_2: Zn; X_3: Co; X_4: minimum temperature; X_5: relative humidity

The step down regression analysis of only edaphic factors i.e. crop rhizosphere soil parameters revealed that organic carbon, iron and manganese were important factors influencing the VAM fungal population. The prediction value of 59% was obtained with the equation as under:

$$Y = 30.39 + 71.62 \, X_1 + 60.5 \, X_2 + 1.12 \, X_3$$

Where, X_1: Zinc; X_2: Iron and X_3: Manganese

The regression analysis with significant climatological factors was carried out to predict fungal population with environmental variables such as rainfall, temperature and humidity. The equation obtained, with the prediction value being 63% was as under:

$$Y = 518.49 - 28.88X_1 + 2.42X_2 - 5.46X_3$$

Where, X_1: minimum temperature; X_2: rainfall and X_3: relative humidity

All the factors irrespective of their statistical significance when pooled together, it was observed that there was a hike in the prediction value from 70 to 74%. The equation was obtained as follows:

$$Y = 807.81 - 9.82X_1 + 4.20X_2 + 0.94X_3 + 10.23X_4 + 0.72X_5 + 67.09X_6 - 2.00X_7 - 7.87X_8 - 1.56X_9 - 7.28X_{10} - 4.59X_{11} - 12.80X_{12} - 0.73 \, X_{13} - 7.18 \, X_{14}$$

Where, X_1: Zn; X_2: Fe; X_3: Mn; X_4: pH; X_5: EC; X_6: OC; X_7: Cu; X_8: Co; X_9: P; X_{10}: $CaCO_3$; X_{11}: maximum temperature; X_{12}: minimum temperature; X_{13}: rainfall; X_{14}: relative humidity

DISCUSSION

Multiple correlation and regressions of soil parameters on VAM population in neem based agroforestry system revealed that both VAM spore population and percentage of root colonization are affected by soil EC, OC and available Fe, Zn, P and $CaCO_3$. This demonstrates the importance of soil fertility in influencing the population of VAM fungi (Hayman and Tavares 1985; Abbott and Robson 1985).

Under present investigation in case of crop rhizosphere, soil properties viz., electrical conductivity (EC), organic C and extractable Fe, P and $CaCO_3$ were found to be significantly correlated with VAM fungal population. Of these EC, OC and Fe were positively correlated, whereas, P and $CaCO_3$ had negative correlations. The

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soil with high P level was poor in VAM spore population and percent colonization. The decrease in the VAM population with high soil P levels may be due to decrease in VAM colonization of the roots or decrease in hyphal length and its activity (Tarafdar and Marschner 1994). The decrease in VAM population may also be due to decrease in the permeability of the root membrane (Graham et al. 1981). Increased P supply in the crop fields has shown to limit carbon export to the roots thereby, decreasing carbon availability to VAM fungi. However, in case of trees rhizosphere P was positively correlated with VAM spore population and percent colonization. This perhaps may be due to low level of P, as no fertilizer was generally applied in the vicinity of the tree roots in crop fields. The present study demonstrated that the VAM fungal population in the crop rhizosphere soils was negatively correlated to $CaCO_3$. This seems to be due to the ability of calcium carbonate to alter the root morphology and differentiation, by enhancing lignifications and suberinization of endodermis; thereby adversely affecting VAM infection (Dehne and Schonbeck 1997). It has been observed in case of cropping system, at high $CaCO_3$ levels, the roots are affected by solubilization of $CaCO_3$ in rhizosphere and its precipitation of cortex (Jailard et al. 1991). VAM fungal propagules and manganese were found to be negatively correlated may be due to the changes induced on VAM fungi by manganese reducer microorganisms have inhibitory effects on VAM population (Arines et al. 1992).

The study indicated that organic C had positive correlation with VAM population in crop rhizosphere. However, in case of tree rhizosphere significant correlation were not observed. This may be due to the fact that returning plant residual matter to soil after harvest increase organic C, which in turn increases microbial population. Despite of high correlation observed between VAM spore count and OC, the low VAM population may be due to the high P levels in cropping areas, a common feature emerged as a consequence of fertilizer application. In general the crop rhizosphere showed negative correlation of VAM population with Zn, Cu, Mn and Co, which may be due to the depressing effect of P fertilization on VAM population (Marschner 1997).

In case of tree rhizosphere, the simple correlation revealed that Zn and Fe showed highly significant correlation with VAM fungal population. It was also observed that trees had higher concentration of Zn and Fe as compared to arable crops. The positive correlation of available Zn and Fe with VAM fungi may be due to high nutrient content of Zn and Fe under the tree rhizosphere, which helps to tree to allocate photosynthate to the roots which is a source of nutrition in the VAM fungi.

It has been observed from the correlation analysis that maximum temperature was negatively correlated with fungal population. It may be due to the fact that temperatures rises to a level above 40°C which reduces the VAM fungal development and plant production (Parke et al. 1983). This may result in lower allocation of carbohydrate to VAM fungus, which may in turn result, in lower population.

The negative correlation of temperature to VAM fungal population may perhaps also be due to its influence on moisture availability to plants which in turn affect physiological activities of VAM fungi. Arid soil temperature exceeds air temperature and similarly exhibits wide variations diurnally as well as annually (Schenck and Schroder 1974). Temperature may affect infection through the direct effect on fungal metabolism or indirectly by influencing root metabolites necessary for fungal activity (Grahm et al. 1982).

The lower coefficients of determination in regression equation between VAM fungal spore and different soil parameters may be the consequence of complex interaction among plant nutrients, which may lead to little correlation between the nutritional status of the soil and root colonization of VAM fungi (Jefferies et al. 1988).

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