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RESEARCH ARTICLE

Impact of various edaphic factors on AMF spore population and diversity in *Catharanthus roseus* at Gwalior

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SUMMARY

Several edaphic factors like soil type, pH, Electrical conductivity, organic carbon, nitrogen, phosphorus and potassium brings impact on arbuscular mycorrhiza fungi (AMF). Maximum number of AMF species and population were isolated from the soils of natural site with moderate pH, electrical conductivity, high soil organic carbon, nitrogen and potassium, least available phosphorus content as compared to artificial site. Study of the effect of edaphic factors on AMF spore density and diversity by correlation analysis revealed a negative correlation of AMF spore density with pH and phosphorus and significant positive correlation with EC, Organic carbon, nitrogen and potassium. The present study would help to determine to what extent and which soil environment variables affects the density and abundance of AMF associations in *Catharanthus roseus* in the semi-arid environment encountered on the north east of Madhya Pradesh.

Key Words : AMF, *Catharanthus roseus*, Edaphic factors, Correlation

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Mycorrhiza could be defined as a symbiotic association, essential for one or both partners between a fungus (specialized for life in soils and plants) and a root (or other substrate-contacting organ) of a plant, that is primarily responsible for nutrient transfer. (Brundrett, 2002). Arbuscular mycorrhiza (AM), are a type of mycorrhiza which is characterized by inter and intra fungal growth in the root cortex, forming specific fungal structures referred to as arbuscles and vesicles.

AM fungi are commonly found as communities that vary in composition and diversity (Cuenca and Menedes, 1996). Approximately 37 different AM fungal taxa have been found at one site (Bever *et al*, 2001). These AMF species have a specific multidimensional niche that is regulated by the plant species present at a site and also by edaphic factors such as pH, moisture content, phosphorus (P) and nitrogen (N) availability, as a result there is large variation between and within site in the composition of AM fungal taxa (Burrows and Pfleger, 2002). AMF Spore populations are dynamic, being influenced by soil type, soil moisture, light intensity, nutrient availability, seasons and land usage.

AM Fungi forming associations include about 150 species belonging to the class Zygomycetes order Glomales, families Glomaceae (G*lomus* and *Sclerocystis*), Acaulosporaceae *(Acaulospora* and *Entrophosphora)*, Gigasporaceae (*Gigaspora* and *Scutellispora*) (Morton and Benny, 1990).

Catharanthus roseus (Sadabahar), belongs to the family Apocynaceae and is native and endemic to Madagascar. In Madhya Pradesh (India), it is found both wild as well as in artificial state, Vinblastine and Vincristine extracted from the plant are used in the treatment of leukemia, Hodgkin's lymphoma and many other diseases.

The plant *Catharanthus roseus* is the potential

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rekindling natural resource, has gained fame in medical sciences. Therefore, the present work was taken up with the objective to test the impact of certain edaphic factors in relation to quantitative assessment of AMF in *Catharanthus roseus* in natural and artificial soils.

MATERIAL AND METHODS

The work on status of AM fungi in *Catharanthus roseus* Linn. was performed during the year 2011-2012 at Gwalior.

Geographical and ecological conditions of the region:

The city of Gwalior is located at 26.22º North Latitude and 78.18º East longitude, in the state of Madhya Pradesh. The average altitude of Gwalior is about 197 meters above msl, The soil type of this area is deep loamy, alluvium, shallow to sandy and loamy grey brown.

The topographical conditions make the summer temperature going up to 48ºC or even more, while the winters are usually chilling with the temperatures ranging from 1ºC to 2ºC. During the study period the region experienced heavy rainfall in monsoons.

Study sites and sample collection:

Two different sites were chosen at two different localities of Gwalior, *i.e.*, natural site at Medicinal plant garden of Botany department Jiwaji University Gwalior, selected as site A and artificial site at Saraswati Nagar near Saraswati Shishu Mandir Gwalior, arbitrarily assigned as site B. Soil samples from 15 cm depth (approx. 500 g) were collected from both sites beneath healthy plants of *Catharanthus roseus* regularly at an interval of three months, during three different seasons *i.e.*, winter, summer and monsoon, respectively, the samples were air dried and were then subjected to experimental analysis.

Isolation, identification and quantification of AMF spores from the soil samples:

In this study, wet sieving and decanting technique (Gerdemann and Nicolson's 1963 method) was used for extraction of spores. AMF spore identification and their morphological characteristics were determined and analyzed qualitatively by using manual of Schenck and Perez (1990). AMF spore density was estimated as the mean number of spores per 100gm soil (Stahl and Christensen, 1982).Quantification was carried out in 9cm Petri dishes with a gridline of 1cm per slide under stereo microscope at 50x (Lugo and Cabello, 2002)

Analysis of physico chemical characteristics of soil:

Soil pH:

The soil pH was determined by taking 10gm of soil sample in 100ml distilled water at 1:10.(w/v) soil water suspension it was then thoroughly shaken, later on pH of the supernatent was determined with the help of digital pH meter (Jackson, 1967).

Electrical conductivity(EC):

Electrical conductivity of the soil sample was determined in the same 1:2 (w/v) soil water suspension used for measuring pH with the help of a conductivity meter (Jackson, 1967).

Soil organic carbon (SOC):

Soil organic carbon was calculated as per the method of Walkley and Black (1934). Calculations for % organic matter.

$$\text{Organic carbon (\%)} = 1 + \frac{10\,(BR - SR)}{BR} \times 0.003 \times \frac{100}{\text{wt of sample (g)}} \quad (1)$$

where, B R-blank reading, S R-sample reading and wt. weight of sample

Available nutrients (N) nitrogen, (P) phosphorus, and (K) potassium:

Mineralizable Nitrogen by Kjeldahl method (Subbiah and Asija, 1956).

Calculation:

$$\text{Available N}\left(\frac{Kg}{Ha}\right) = \frac{(SR - BR) \times 0.00028}{20} \times (10)^6 \times 2.24n$$

SR Sample Reading, BR Blank Reading

Available phosphorus in soil:

Olsen *et al*. (1954) method was used.

Calculation:

$$\text{Available N}\left(\frac{Kg}{Ha}\right) = \frac{R \times \text{volume make up} \times 25 \text{ (last vol.)} \times 2.24 \times (10)^6}{\text{Volume of aliquot} \times \text{wt. of soil} \times (10)^6}$$

where, R means reading of colorimeter

Available potassium content:

Was determined by Hanway and Heidel method (1952).

Calculation:

Available K (kg/ha) = Reading × 25/5 × 2.24

Data analysis:

Pearson's correlation co-efficient was used to assess the relationship between spore density, and edaphic factors.

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Soil analysis: pH and Electrical conductivity (EC):

The study revealed slightly alkaline to alkaline soils (pH range 7.46 – 8.25) at site A and 7.82 to 8.27) at site B. Low to

moderate electrical conductivity (EC) was recorded ranging from 0.31 to 0.73 dSm^{-1} at site A and 0.16 to 0.29 dSm^{-1}at site B, respectively (Table1 and 2).

Available nutrients (Organic carbon, nitrogen, phosphorus and potassium);

Relatively higher organic content (1.15% was observed from site A in winter season which decreased to 0.63 % in monsoon season, At site B The maximum SOC was found during winter and which decreased to 081% during monsoon (Table 1 and 2).

Nitrogen and Potassium content was found in abundance at both the sites in winter and summer seasons. Highest nitrogen content 392.23 kg/ha was found at site A during winter season, and comparatively minimum nitrogen content 313.61 kg/ha, was found at site B in summer (Table 1 and 2).

Relatively lower phosphorus content was recorded in both the sites, the maximum phosphorus was recorded at site A during monsoons (17.39 kg/ha), and minimum Phosphorus content observed was (13.45) kg/ha at site B during winter. (Table1 and 2)

Highest value of potassium content in the soil was observed from site A (404.11kg/ha) during winter followed by lowest (309.29kg/ha) at site B during monsoon season (Table 1 and 2).

(V) AM spore density:

Sitewise results of variation in spore density of AMF in the rhizosphere of plant species undertaken for the study are depicted in Table 3. The number of spores varied significantly between the sites with the variations in edaphic environment, The natural soil was rich in both spore number and species, maximum number of spores 151spores /100g soil) were recorded at site A, during winter season, and minimum of 24 spores/ 100g soil at site B during monsoon season.

AMF diversity:

Over all eight different AMF species were isolated from the rhizosphere soils of plant species considered at both the sites A and B during three seasons, belonging to three genera *Acaulospora, Glomus* and *Gigaspora.*

Glomus was found to be the predominant genus in the rhizosphere of both the sites, as its five species followed by with *Gigaspora* having two and *Acaulospora* with single species (Table 4)

Table 1 : Physico chemical characteristics of soil of *Catharanthus roseus* at site A during three different seasons in Gwalior

Season	Site A					
	Physico-chemical characteristics of soil					
		dSm^{-1}	%		kg/ha	
	pH	EC	SOC	N	P	K
Winter	7.46 ± 0.01	0.31 ± 0.02	1.15 ± 0.03	392.23 ± 0.10	15.53 ± 0.02	404.11 ± 0.75
Summer	8.09 ± 0.01	0.16 ± 0.02	0.69 ± 0.01	359.81 ± 0.24	17.33 ± 0.15	342.72 ± 1.03
Monsoon	8.25 ± 0.02	0.73 ± 0.07	0.63 ± 0.11	334.6 ± 0.21	17.39 ± 0.33	342.78 ± 2.20

Table 2 : Physico chemical characteristics of soil of *Catharanthus roseus* at site B during three different seasons in Gwalior

Season	Site B					
	Physico-chemical characteristics of soil					
		dSm^{-1}	%		kg/ha	
	pH	EC	SOC	N	P	K
Winter	7.82 ± 0.14	0.16 ± 0.02	1.07 ± 0.01	361.73 ± 0.5	13.45 ± 0.21	402.76 ± 3.21
Summer	8.23 ± 0.03	0.20 ± 0.01	0.84 ±0.02	313.61 ± 0.68	17.46 ± 0.23	331.17 ± 0.74
Monsoon	8.27 ± 0.02	0.29 ± 0.03	0.81 ± 0.01	353.66 ± 0.11	17.98 ± 0.51	309.29 ± 0.54

EC; Electrical conductivity, SOC; Soil Organic carbon, N: nitrogen, P: phosphorus, K: potassium, Kg/ha: kilogram per hectare, dSm^{-1}: deci Siemens per meter.

Table 3 : AMF Spore density with associated species in *Catharanthus roseus* at site A and site B during three different seasons at Gwalior

Study site	Total number of AMF spores per 100 g soil			Associated AM fungal species*		
	Winter	Summer	Monsoon	Winter	Summer	Monsoon
Site A	151**	114**	53**	ALVS, LFSC, LGSP, LMSS, LINR, LHTS, GGNT,	ALVS, LFSC, LGSP, LMSS, LINR, LHTS, GGNT	ALVS, LFSC, LMSS, LINR, LHTS, GGNT
Site B	69**	58**	24**	ALVS, LFSC, LMSS, LINR, GGNT, GMAR.	ALVS, LFSC, LMSS, LINR, GGNT,	ALVS, LFSC, LMSS, LINR, GMAR

*Unique code of AMF species (Schenck and Perez, 1990)

**denotes mean of three replicas., ALVS ; *Acauospora laevis*, LFSC ; *Glomus fasciculatum*, LGSP ; *Glomus geosporum*, LMSS ; *Glomus mossae*, LINR ; *Glomus intraradices*, LHTS ; *Glomus heterosporus*, GGNT ; *Gigaspora gigantia*, GMAR ; *Gigaspora marginata*

Correlation analysis of soil parameters with respect to AMF spore count:

The statistically significant correlation (r = -0.890968 and - 0.745008931) was observed between spore number and pH, at site A and B, respectively, at pH 7.45 more AMF spores were isolated but with the much increase in the pH (8.25) less spores were isolated.

It has been widely speculated that OC, N, P and K and other factors in the soil enhances AMF development, a well defined relation was observed between these parameters and AMF spore number (Table 5).

The present investigation concludes the effect of various soil parameters like pH, EC, soil organic carbon, soil phosphorus, nitrogen and potassium content on AMF population and diversity, the study reflected a decreased trend in the abundance and diversity of AM fungi with the variation of edaphic factors.

Occurrence of many AMF are influenced by soil pH, many species like *Glomus mossae and Glomus intraradices* occur frequently in neutral to alkaline soil whereas species of *Acaulospora* are usually found in acidic soil. pH alone may not be responsible for germination of AMF spores, as with the change in the soil pH, chemical properties of the soil also changes. The relationship between soil pH and mycorrhization is complex and depends on the plant species and also the soil type, forms of phosphorus and fungal species involved. The optimum pH for different AMF endophytes can vary in different soils and specific endophytes have an optimum pH at which they execute best (Gaur and Kaushik, 2011 and Wang *et al.,* 1985).

Available soil organic carbon also plays a major role in affecting the number of AM fungal spores, Our present analysis largely denotes that AMF spore population increases with the increase in organic carbon. Table 5 shows organic carbon has significant positive correlation with spore number. Perhaps the reason may be that it buffers the soil from strong changes in pH holds a great proportion of nutrients, cations and trace elements that are of importance to AMF and plant growth. Leu (2007). It is well said that soils having elevated levels of organic carbon correspond to the more spore population, this view is supported by other workers like (Lei Cheng *et al*., 2012), (Gaur and Kaushik, 2011)

Phosphorus is also one of the major plant nutrient and an essential component of the soil for plant growth and development of the AMF. In present investigation phosphorus content of the soil is in low levels in natural and artificial soils (Table 2). In contrast with other seasons of the year, phosphorus levels are high and spore population is low, which depicts that higher levels of the phosphorus levels in the soils results in the decrease in the AMF spores multiplication. From the correlation analysis between phosphorus and spore population in relation to the seasons in and a significant

Table 4 : Species diversity of AMF in rhizospheric soils of *Catharantus roseus* at undisturbed (site A)and disturbed sites (site B) at Gwalior

Study site	Sr. No.	List of (V)AM fungi identified	Code*
Site A	1	*Acaulospora laevis*	ALVS
	2	*Glomus heterosporum*	LHTS
	3	*Glomus fasciculatum*	LFSC
	4	*Glomus geosporum*	LGSP
	5	*Glomus mossae*	LMSS
	6	*Glomus intraradices*	LINR
	7	*Gigaspora gigantia*	GGNT
Site B	1	*Acaulospora laevis*	ALVS
	2	*Glomus fasciculatum*	LFSC
	3	*Glomus mossae*	LMSS
	4	*Glomus intraradices*	LINR
	5	*Gigaspora gigantia*	GGNT
	6	*Gigaspora marginata*	GMAR

*Unique code for AMF species (Schenck and Perez, 1990)

Table 5 : Pearson's correlation values between spore population and various soil parameters

	Site A		Site B	
AMF parameter	Soil parameter	Correl. factor (r).	Soil parameter	Correl. factor(r)
Spore count.	pH	- 0.89968994	pH	-0.745008931
Spore count.	OC	0.84809354	OC	0.761678972
Spore count.	N	0.77492577	N	0.41310344
Spore count.	P	- 0.804672334	P	-0.073986863
Spore count.	K	0.88727009	K	0.833789664

negative correlation was observed, in monsoon higher content (17.98 kg/ha) of phosphorus was observed from undisturbed site resulting in decrease of spore count.

Our findings shows specificity with findings of Gaur and Kaushik (2011), and Javadi *et al.* (1991), they also found that spore population and phosphorus content in the soil is negatively correlated, this view is also supported by other researchers (Timmer and Den,1980) they further elaborated that when phosphorus deficiency occurs in the soil, plants may release large amounts of amino acids and sugars into the rhizosphere which are utilized by AM fungi for their growth. Such a relationship was verified previously in phosphorus deficient sorghum by Grahm *et al*. (1981). Elevated phosphorus levels in the plants may reduce the substrate leakage, thus suppressing fungal infection in the roots, this however, did not limit the uptake and concentration of phosphorus in the leaf tissue. The abundance of phosphorus in the soil was adequate to compensate for the reduced number of AMF spores and root phosphorus uptake was adequate (Javadi *et al*., 1991).

Based on the statistical observations obtained potassium content of the soil is positively correlated with the spore number (Table 5) this can be clearly observed from the table that higher the levels of available potassium more is the spore density and diversity as well. Our results are in agreement with the results of Gaur and Kaushik (2011), but were in contradiction with Khanam *et al.* (2006), they observed negative correlation between soil potassium and spore number.

A significant positive correlation was found between spore count and available soil nitrogen content, maximum spores were isolated from the soils having higher nitrogen content, it is generally said that at moderate nitrogen content in the soil spore density is maximum, but at further increase in nitrogen content in soil results in decline of spore population In contrast no direct correlation was found between the two (Sastry *et al.*, 1999).

The potential reason for maximum number of spore availability in undisturbed soils is that the spores keep on multiplying in association with the plants and remain in the soil for isolation later on, whereas in disturbed site top soil layer is disturbed at regular bases, supported by (Bhardwaj *et al.*, 1997).

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