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And COL RADIES	Isolation, Identification and Diversity of AMF Spores in Disturbed and Undisturbed Soils of Gwalior Region with Reference to Withania Somnifera.								
KEYWORDS	AM fungi, Withania somnifera, Diversity, disturbed and undisturbed sites.								
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ABSTRACT The study was conducted to isolate, identify the existing Arbuscular mycorrhiza (AM) and their diversity in Withania somnifera growing in two different site during three seasons in Gwalior region. Isolation and the identification was done by using Schenck and Perez1990, manual Glomus (07) species and Acaulospora (04) species were found in abundance the number was high in Undisturbed site (11) as compared to Disturbed site (08). Diversity was carried out by following Simpson's Diversity index. The index value of undisturbed site was (0.09) and of disturbed site was (0.14).

INTRODUCTION:

Arbuscular mycorrhiza (AM) fungi are the most ancient and widespread obligate symbionts, and they act as microscopic pipelines that can transport carbon and minerals to and away from the plant. They have also been reported to improve the growth and biomass of a wide host range and are efficient phosphate solubilizers and transporters. When soil is disturbed or is partially removed, a decrease in the number of mycorrhizal propagules occurs (Miller 1979). It was shown that current forest practice influenced significant changes in vesicular-arbuscular mycorrhizae propagules (Ahmad 1996). Species of Acaulospora and Glomus dominated the AM fungal spore population. Changes to soil properties occurring during succession or between sites with similar climates can be correlated with the predominance of different species or isolates of AM fungi (Bethenfalvay et al., 1982). Spores of AM fungi showed seasonal patterns for abundance in natural environments. Spore populations typically are greatest in autumn in areas where there are marked warm/cold seasons (Douds and Chaney, 1982; Gemma and Koske, 1988).

Soil Disturbance: Soil is disturbed by various ways like garbage, plastics, chemical, Animal waste dumping etc. The nature and texture of soil changes with the dumping of these waste material.

MATERIALS AND METHODS:

The work on isolation and identification of AM fungi in Withania somnifera (Ashwaganda) was performed during the year 2011-2012 at Jiwaji University Gwalior. The work is focused on Arbuscular Mycorrhiza Fungi (AMF) associated with Withania somnifera and influence of edapho-climatic factors on occurrence and distribution of AMF during three different seasons. The samples were collected from two different habitats of Jiwaji University Campus Gwalior. First from Undisturbed site i.e., from Medicinal plant garden named as (Site A) and other from Disturbed site near Bio-Medical technology department named as (Site B)

Samples were collected regularly during three different seasons, i.e., winter, summer and monsoon respectively. Rhizospheric soil samples from both sites A & B, weighing 500 gm, at depth of 15cm, were collected from healthy plants of Withania somnifera. These samples were taken in sterilized polythene bags and then air dried for two hours.

AM spores were isolated from the soil samples by using wet

sieving and decanting technique (Gerdemann and Nicolson's 1963 method). Spores were then analyzed qualitatively and quantitatively. These spores were picked with the help of a sharp and sterilized needle and mounted with polyvinyl lacto glycerol (PVLG) on a slide. All the spores on mounting medium were observed cautiously under high power research microscope for identifying them for their genera and species. AMF spore identification and their morphological characteristics like shape, size, colour, texture, wall layers, attached hyphae, sporocarps were determined by using Manual of (Schenck and Perez 1990).

Diversity index: it was computed by following formula (Simpson E. H 1949).

$$D = \frac{\sum n_i(n_i - 1)}{N(N - 1)}$$

D = Diversity index N = total number of species

ni = number of individuals of a

particular species.

RESULTS AND DISCUSSION:

The total of 151 AMF spores were found in site A during all the three seasons, comprising of 11 species i.e; 07 Glomus species and 04 Acaulospora species (Table 1) in which Glomus caledonium (22 spores) dominated followed by Glomus diamorphicum (20 spores). The total AMF spores in site B was 61 comprising of 8 species in which Glomus caledonium and Glomus clarum were dominant with (12 spores) each (Table 1). The presence of Glomus and Acaulospora species at both site A and B reflected that both are higly associated with Withania somnifera . As the site B soil was under anthropogenic disturbance therefore waste materials such as polythenes , leather etc reduced the AM spore count. Glomus intaradices, Glomus monosporum and Acaulospora bireticulata were sensitive to any disturbance and therefore were not found at site B, during all three seasons. (Table 1)

During summer season the number of spores of all species declined at both sites i.e., A and B, except Acaulospora bireticulata at site A, and Acaulospora collicula at site B.

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During monsoon season the spore number was high at both sites A and B, except Glomus clarum, Glomus intaradices at site A and Acaulospora collicula at site B. The decomposition of litter fall and other waste material add acidity to the soil with the result pH decreases and spore number also decrease (Sharief and Moawad 2006).

AM Diversity index :

The D values of the sites A was 0.09 and for site B was 0.14. As per simpson's index higher D value suggests lower diversity and lower D value suggests high diversity. The result reveals that site A has low D value than site B which means site A has high diversity.

Low species diversity suggests that the environment was quite stressful and any further stress would probably be harmful to the ecosystem i.e., site B.

High species diversity suggests that the site has less disturbances and good and healthy soil as well as environmental conditions i.e., site A

		Site A spore number/50 gm of soil.				Site B spore number/50 gm of soil.			
S No	Name of species	w	s	М	Т	w	S	М	Т
1	Glomus caledo- nium	8	5	9	22	4	2	6	12
2	Glomus diamor- phicum	5	4	11	20	2	3	3	08
3	Glomus clarum	9	4	3	16	4	3	5	12
4	Glomus ag- gregatum	4	3	5	12	3	-	7	10
5	Glomus etuni- catum	5	1	5	11	2	1	5	08
6	Glomus intaradi- ces	4	2	3	09	-	-	-	0
7	Glomus mono- sporum	4	4	5	13	-	-	-	0
8	Acaulospora bi- reticulata	3	6	3	12	-	-	-	0
9	Acaulospora laevis	5	4	5	14	2	-	2	04
10	Acaulospora col- licula	5	3	8	16	-	2	1	03
11	Acaulospora morrowae	2	1	3	06	2	-	2	04
	Total				151				61

Table 1 Occurance of species during three seasons at both sites:

W = Winter; S = summer; M = Monsoon; T = Total

Calculation of Diversity Index at site A Calculation of Diversity Index at site B

$$D = \frac{\sum n_i(n_i - 1)}{N(N - 1)} \quad D = \frac{\sum n_i(n_i - 1)}{N(N - 1)}$$
$$D = 0.09 \qquad D = 0.14$$

CONCLUSION:

Seasonal variation, disturbance of soil as well as texture of soil affect the AM diversity and number. The spore number was found high in Monsoon season followed by Winter but during summer season it was the least. Summer season shows low AM diversity as the soil loose moisture which indirectly affects the soil properties. The changing environmental conditions show greater affect on AM diversity.

Less disturbed soil had high species diversity and greater spore number Glomus species was in abundance at both the sites, whereas Acaulospora species were less in number and species.

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