

**DIVERSITY OF ARBUSCULAR MYCORRHIZAL (AM) SPECIES IN ACACIA NILOTICA SUBSP. CUPRESSIFORMIS (J.L. STEWART) ALI & FARUQI UNDER ARID AGROECOSYSTEMS OF WESTERN RAJASTHAN**

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ABSTRACT: Arbuscular mycorrhizal (AM) fungi associated with *Acacia nilotica* subsp. *cupressiformis* (kabulikikar, ramkanta and ramkati babul) were assessed for their qualitative and quantitative distribution from five districts of Rajasthan. A total of two species of *Acaulospora*, two species of *Gigaspora*, fourteen species of *Glomus*, three species of *Sclerocystis* and two species of *Scutellospora* were recorded. A high diversity of AM fungi was observed and it varied at different study sites. Among these five genera, *Glomus* occurred most frequently. *Glomus fasciculatum*, *G. aggregatum*, and *G. mosseae* were found to be the most predominant AM fungi in infecting *A. nilotica* subsp. *cupressiformis*. *G. fasciculatum*, *Sclerocystis* was found in all the fields studied, while *Gigaspora* species and *Scutellospora* species were found only in few sites. The more number (22) of AM fungal species were isolated and identified from Pali whereas, only thirteen species were found from Nagaur. The total three species of *Sclerocystis* were identified, *Sclerocystis sinuosa* reported from Jodhpur, Nagaur, Sikar, Pali and Sirohi. The spore density was varied between 178 to 500 propagules (100 g⁻¹) soil. The per cent root colonization was varied (48 to 79 %) from place to place. The pH of study area was ranged between 8.01 to 8.65; EC was recorded from 0.10 to 0.44 (dSm⁻¹); per cent OC ranged from 0.27 to 0.39 and available P content varied from 3.44 to 5.48 mg kg⁻¹ for *A. nilotica* subsp. *cupressiformis*. A significant correlation of AM population was observed with root colonization and per cent organic carbon while other variables studied had a non-significant correlation with total AM population.

Key words: Arbuscular mycorrhizae; arid agroecosystems; diversity; root colonization; correlation; *A. nilotica* subsp. *Cupressiformis*

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INTRODUCTION

Acacia nilotica (Mimosaceae) is one of the most useful tree species occurring throughout arid & semi-arid regions of the country and well adapted to the harsh bio-physical & climatic conditions. The species is abundant in northern Rajasthan, where rainfall is comparatively better. *A. nilotica* subsp. *indica* (Benth.) Brenan and *A. nilotica* subsp. *cupressiformis* (J.L. Stewart) Ali & Faruqi are common. *A. nilotica* subsp. *cupressiformis* is a perennial tree native to India and Pakistan. Its previous name was "*Vachellia nilotica*" subsp. "*cupressiformis*". Common names for it include kabulikikar, ramkanta and ramkati babul. This sub-species bears a characteristic crown which make it conspicuous and identifiable from other sub-species of *A. nilotica*. The tree has a narrow erect cypress like crown or cupressiform in appearance. The branches tend to go upwards and make a narrow angle with the main stem. Pods generally narrowly and regularly constricted between the seeds. Flowers are perennial in nature and flowering time is March-November. This sub-species is preferred on agricultural cropland because of its narrow crown and widely used in agroforestry, social forestry, and reclamation of wastelands & rehabilitation of degraded forests. In traditional agroforestry systems, it provides fuel, fodder, gum, tannin, and timber [1,2,3]. Root nodulations in this species help in biological nitrogen fixation [4] and enhance soil fertility [2].

Arbuscular mycorrhizal (AM) fungi are major component of rhizosphere micro-flora in natural ecosystems. Accumulating evidence indicate that mycorrhizal association plays a significant role in decomposition of soil organic matter, mineralization of plant nutrients and nutrient recycling [5,6]. Mycorrhizal plants have greater ability to absorb nutrients, soil water increased plant fitness, which may lead to better survival under stressed environmental conditions [7]. The population pattern of AM fungi varies greatly and their diversity is affected by various factors including soil, environmental condition, host plant and agricultural practices [8]. Plants infected with AM fungi get more easily established on disturbed sites through improved mineral nutrition [9] and provide a primary mechanism for phosphorous uptake from the soil [10]. The geographic distribution of AM fungal species influenced by edaphic factors plays an important role for their distribution. Although a large number of factors affect in predicting levels of indigenous AM population but to understand mycorrhizal dynamics, identification and quantification are necessary. Keeping this objective in view the present study was undertaken to analyse the mycorrhizal status in *A. nilotica* subsp. *cupressiformis* and to study the number of species and population of mycorrhizal fungi present at different sites and their ability of infection on *A. nilotica*.

MATERIALS AND METHODS

The study was conducted in natural and planted stands of *A. nilotica* subsp. *cupressiformis* located in different parts of western Rajasthan of India. Periodical survey for *A. nilotica* subsp. *cupressiformis* plantations were undertaken to collect rhizosphere soil samples and roots from ecologically different sites viz. Jodhpur, Ganganagar, Nagaur, Pali and Sirohi district of western Rajasthan state. The samples were taken from Jhalamand (Jodhpur); 7 MD, 11 P (Patroda) (Ganganagar); Jhareli (Nagaur) Khivandi, Pomava, Sumerpur (site-1, site-2 & site-3) (Pali); Bapunagar (site-1 & site-2), Paldi, Posalia, Viravillpur (Sirohi) district. Fifteen samples were taken from each place. The samples were processed for isolation, identification of Arbuscular mycorrhizal (AM) fungi associated with *A. nilotica* subsp. *cupressiformis*. These data were further used to develop relationships between AM fungi and soil parameters. The collection of rhizosphere soil samples and roots were done at the time (July to September) when the spore built up is the maximum [11]. Tree with average girth diameter at breast height $21.25 + 1.55$ cm were taken for study. Samples were collected at the base of five trees, which were selected, at random. Fifteen rhizosphere soil samples were taken from each site in sealed polythene bags. The soil sampling was done at a depth of 30 cm under the canopy of the standing trees and were analysed for soil pH, Electrical conductivity (EC), organic carbon (OC) and phosphorous (P) contents.

Roots were separated from collected soil samples and assayed for AM fungal association after staining in Trypan blue as described by Phillips and Hayman [12]. A total of 100 root segments were examined for each replicate and percentage of segments with colonization was calculated. The AM fungal infection was examined by using Optiphot-2 "Nikon" compound microscope. The Percentage of root infection was determined by Giovannetti and Mosse, [13]. AM spores were isolated by wet sieving and decanting technique [14]. Semi-permanent slides were prepared by mounting the spores in lactophenol or polyvinyl lactophenol. The photographs were taken by Nikon Optiphot-2 compound microscope. The spore density was expressed in terms of the number of spores per 100 g of soil. The spores were identified on the basis of colour, shape, size, surface, nature of spore cell wall and hyphal attachment with the help of synoptic keys of the Schenck and Perez [15] and Raman and Mohankumar [16]. The soil samples were analysed for Physico-chemical properties viz., pH, EC, organic carbon by Walkley-Black method [17] and phosphorous by Olsen's method [18]. The relationship between AM propagules and nutrient status of soil under different sites were also worked out.

RESULTS AND DISCUSSION

The main purpose of this study was to isolate and identify the arbuscular mycorrhizal (AM) fungi associated with *A. nilotica* subsp. *cupressiformis*. The infection and spread of endophytes in root tissues, and percentage of root colonization is influenced by as climatic and edaphic factors. The important climatological features of the *A. nilotica* growing areas in western Rajasthan were presented (Table 1). The results of the present investigations pertain to influence of varying soil properties and climatic variations on the AM associations in *A. nilotica* subsp. *Cupressiformis* in agrclimatic zones/based systems of the arid regions of Rajasthan. A high diversity of AM fungi was observed and it varied at different study sites (Table 2). The important genera identified were *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora*. Among these five genera, *Glomus* occurred most frequently. The species of *Gigaspora* and *Scutellospora* were distinguished from the genera *Sclerocystis* by the presence of bulbous suspensor in the former.

In all, two species of *Acaulospora*, viz., *A. bireticulata*, *A. mellea*, two species of *Gigaspora* viz., *G. albida*, *Gigaspora* (1), fourteen species of *Glomus* viz., *G. aggregatum*, *G. citricolum*, *G. constrictum*, *G. deserticola*, *G. fasciculatum*, *G. macrocarpum*, *G. microcarpum*, *G. mosseae*, *G. multisubstensum*, *G. occultum*, *G. pachycaulis*, *G. pallidum*, *G. pubescens*, *G. reticulatum*, three species of *Sclerocystis* viz., *S. dussii*, *S. indica*, *S. sinuosa*, and two species of *Scutellospora* viz., *S. bionarta*, *S. erythropha* were frequently found in the rhizosphere soil of *A. nilotica* subsp. *cupressiformis*.

It is evident that the occurrence of various species of AM fungi varied considerably from place to place. *G. aggregatum*, *G. fasciculatum* and *G. mosseae* were found to be the most predominant AM fungi in infecting tree species. *G. fasciculatum* and *Sclerocystis* was found in all the fields studied, while *Scutellospora* species were found only in few sites. The maximum number twenty two (22) of AM fungal species were isolated and identified from Pali whereas, only thirteen (13) species were found from Nagaur (Table 2).

Table-1: Important climatological feature of various districts of Western Rajasthan growing *A. nilotica* subsp.cupressiformis plantation (Source: Raj. Govt. Official website)

District	Latitude and longitude	Area Sq. Km.	Rainfall (mm)	Mean maximum temperature (°C)	Mean minimum temperature (°C)	No. of rainy days
Barmer	25° 45' N, 71° 25' E	28,309	270	49	3	11
Bikaner	28° 01' N, 73° 22' E	30,356	260	47	2	16
Jodhpur	26° 17' N, 73° 1' E	22,892	330	45	3	18
Ganganagar	29° 49' N, 73° 50' E	11,003	200	41	6	16
Hanumangarh	29° 35' N, 74° 21' E	9,672	250	40	5	15
Sikar	27° 36' N, 75° 15' E	7,739	460	46	4	30
Nagaur	27° 00' N, 73° 40' E	17,696	388	47	3	22
Pali	25° 46' N, 73° 25' E	12,355	490	41	10	22
Sirohi	24° .61' N, 72° .52' E	5,135	562	47	23	29

Table-2: Distribution of Genera and species of the Glomeromycota in various districts of western Rajasthan of India

Genus	No.	AMF species	Jodhpur	Ganganagar	Nagaur	Pali	Sirohi
			3	4	7	8	9
<i>Acaulospora</i>	1	<i>Acaulospora bireticulata</i>	✓	-	✓	✓	✓
	2	<i>Acaulospora mellea</i>	✓	✓	-	✓	✓
<i>Gigaspora</i>	3	<i>Gigaspora albida</i>	✓	✓	-	✓	✓
	4	<i>Gigaspora sp.</i>	-	-	-	✓	-
<i>Glomus</i>	5	<i>Glomus aggregatum</i>	✓	✓	✓	✓	✓
	6	<i>Glomus citricolum</i>	✓	-	-	✓	✓
	7	<i>Glomus constrictum</i>	✓	-	✓	✓	✓
	8	<i>Glomus deserticola</i>	✓	-	✓	✓	✓
	9	<i>Glomus fasciculatum</i>	✓	✓	✓	✓	✓
	10	<i>Glomus macrocarpum</i>	✓	✓	-	✓	✓
	11	<i>Glomus microcarpum</i>	✓	✓	-	✓	✓
	12	<i>Glomus mosseae</i>	✓	✓	✓	✓	✓
	13	<i>Glomus multisubstansum</i>	-	✓	✓	✓	✓
	14	<i>Glomus occultum</i>	✓	✓	✓	✓	✓
	15	<i>Glomus pachycaulis</i>	✓	-	✓	✓	✓
	16	<i>Glomus pallidum</i>	✓	✓	-	✓	✓
	17	<i>Glomus pubescens</i>	✓	-	✓	✓	✓
	18	<i>Glomus reticulatum</i>	✓	✓	✓	✓	-
<i>Sclerocystis</i>	19	<i>Sclerocystis dussii</i>	✓	✓	-	✓	-
	20	<i>Sclerocystis indica</i>	-	-	✓	✓	✓
	21	<i>Sclerocystis sinuosa</i>	✓	✓	✓	✓	✓
<i>Scutellospora</i>	22	<i>Scutellospora bionarta</i>	✓	✓	-	-	✓
	23	<i>Scutellospora erythroa</i>	✓	-	-	✓	-

The total three species of *Sclerocystis* were identified, *Sclerocystis sinuosa* reported from Jodhpur, Nagaur, Sikar, Pali and Sirohi. AM fungal species identified in *A. nilotica* subsp. *cupressiformis* in various districts of western Rajasthan varied from site to site is presented in Table 2. As far as the distribution of AM fungal species in *A. nilotica* subsp. *cupressiformis* in various districts of western Rajasthan concerned it varied from site to site (Table 2). In general, *G. fasciculatum* was found to be most abundant species. The different AM spores identified under *A. nilotica* subsp. *cupressiformis* of different sites were presented (Plate 1).

The results of the study of AM population (Table 3) showed that maximum spore density was recorded in tree rhizosphere from (Pomava) Pali (519 spores 100 g⁻¹ soil) and minimum (178 spores 100 g⁻¹ soil) from (Jhareli) Nagaur in *A. nilotica* subsp. *cupressiformis*. The maximum per cent root colonization (79 %) was recorded in (Pomava) Pali whereas, the minimum colonization of (48 %) was recorded from (Jhareli) Nagaur in *A. nilotica* subsp. *cupressiformis* (Table 3).

Table-3: Physico-chemical properties, phosphorous (P) content, AM population and root colonization (%) in plantation of *A. nilotica* subsp. *cupressiformis* in different Districts of western Rajasthan of India.

Zone	Districts	pH (1:2.5)	EC(dSm ⁻¹)	OC (%)	Available P (mg kg ⁻¹)	AM Population (100 g ⁻¹)	Root Colonization (%)
Zone I A	Jodhpur						
	Jhalamand	8.01	0.20	0.39	5.12	313	62
	Mean	8.01	0.20	0.39	5.12	313	62
Zone I B	Ganganagar						
	7 MD	8.17	0.42	0.29	4.84	264	51
	11 P, Patroda	8.27	0.44	0.33	4.98	278	63
	Mean	8.22	0.43	0.31	4.91	271	57
Zone II A	Nagaur						
	Jhareli	8.65	0.18	0.27	4.62	178	48
	Mean	8.65	0.18	0.27	4.62	178	48
Zone II B	Pali						
	Khivandi	8.25	0.11	0.36	4.23	511	79
	Pomava	8.22	0.16	0.39	4.23	519	79
	Sumerpur, site-1	8.25	0.14	0.35	3.99	488	70
	Sumerpur, site-2	8.27	0.14	0.37	4.14	490	75
	Sumerpur, site-3	8.31	0.1	0.38	4.16	492	77
	Mean	8.26	0.13	0.37	4.15	500	76
	Sirohi						
Zone II B	Bapunagar, site-1	8.1	0.24	0.34	3.84	352	61
	Bapunagar, site-2	8.06	0.21	0.31	3.81	347	59
	Paldi	8.12	0.23	0.32	3.92	374	69
	Posalia	8.15	0.25	0.36	4.02	378	70
	Viravillpur	8.17	0.27	0.37	4.01	384	79
	Mean	8.12	0.24	0.34	3.92	367	69

Table-4: Correlation Coefficient (r) with number of AM spores and other edapho-climatic factors

Zone	Districts	AM Population (100 g ⁻¹)	Root Colonization (%)	pH (1:2.5)	E.C. (dSm ⁻¹)	OC (%)	Available P (mg kg ⁻¹)	Rainfall (mm)	Mean max. temperature(°C)	Mean mini. temperature(°C)	No. of rainy days
Zone I A	Jodhpur	313	62	8.01	0.20	0.39	5.12	330	45	3	18
Zone I B	Ganganagar	271	57	8.22	0.43	0.31	4.91	200	41	6	16
Zone II A	Nagaur	178	48	8.65	0.18	0.27	4.62	388	47	3	22
Zone II B	Pali	500	76	8.26	0.13	0.37	4.15	490	41	10	22
	Sirohi	367	69	8.12	0.24	0.34	3.92	562	47	23	29
	Correlation (r)		0.984**	0.501 NS	0.366 NS	0.727*	0.552 NS	0.535 NS	0.477 NS	0.472 NS	0.281NS

*Significant at 5% level, **Significant at 1% level, NS-non significant.

The soil samples were analysed for soil pH and it varied from 8.01 to 8.65, minimum being at (Jhalamand) Jodhpur and maximum at (Jhareli) Nagaur (Table 3). Minimum EC 0.10 (dSm⁻¹) was recorded at (Sumerpur, site-3) Pali and maximum EC 0.44 (dSm⁻¹) at (11 P, Patroda) Ganganagar. Per cent organic carbon ranged from 0.27 at (Jhareli) Nagaur to 0.39 at (Pomava) Pali. Available P content varied from 3.81 (mg kg⁻¹) to 5.12 (mg kg⁻¹) (Table 3).

Linear regression equation for AM population with their characteristics (*A. nilotica* subsp. *cupressiformis*)

$$Y_{i(am)} = -225.84133 + 9.05496 X_{i(RC)} \\ (r = 0.88567 \text{ P Value for } a = 0.03212 \text{ P Value for } b = 2.51E-05) \dots\dots 1.1$$

$$Y_{i(am)} = 920.89737 - 125.59778 X_{i(AP)} \\ (r = 0.51673 \text{ P Value for } a = 0.00388 \text{ P Value for } b = 0.058493) \dots\dots 1.2$$

$$Y_{i(am)} = -335.53199 + 2083.94366 X_{i(OC)} \\ (r = 0.73239 \text{ P Value for } a = 0.10927 \text{ P Value for } b = 0.00289) \dots\dots 1.3$$

$$Y_{i(am)} = -528.16918 - 655.78273 X_{i(EC)} \\ (r = 0.64097 \text{ P Value for } a = 5.37E-07 \text{ P Value for } b = 0.01350) \dots\dots 1.4$$

$$Y_{i(am)} = 1463.50230 - 131.48723 X_{i(pH)} \\ (r = 0.19103 \text{ P Value for } a = 0.37903 \text{ P Value for } b = 0.51297) \dots\dots 1.5$$

Where, $Y_{i(am)}$ = AM population,

$X_{i(RC)}$ = Root colonization (%)

$X_{i(AP)}$ = Available P

$X_{i(OC)}$ = Organic carbon (%)

$X_{i(EC)}$ = Electrical conductivity

$X_{i(pH)}$ = value of pH



Plate-1: Overall picture of identified AM spores under *A. nilotica* subsp. *cupressiformis*

The linear regression equations were worked out considering AM population of *A. nilotica* subsp. *cupressiformis* (plantation) with other variables viz., per cent root colonization, available P, per cent organic carbon (%OC), electrical conductivity (EC) and value of pH. The regression equations no. 1.1 to 1.5 is written as above for the *A. nilotica* subsp. *cupressiformis* along with the estimated parameters intercept and slope. Also, the value of correlation coefficient and the P values of estimated parameter are given in parenthesis. A perusal of above regression equations shows that there is good relationship between AM populations with per cent root colonization followed by with per cent Organic Carbon. However, it can be seen that there is no significant relationship of AM population with available P and pH (Table 4).

The number of AM propagules present in the soil, may be the resultant effect of various climatic, physical and chemical properties of soils. In case of tree rhizosphere a significant correlation of AM population was observed with Root colonization ($r = 0.984$) and % organic carbon ($r = 0.727$) while other variables under study had a non-significant correlation with total AM population. Large variation occurred in the spore population within the same plant species were found in present study, which may be attributed to the variation in edaphic [19] and climatic factors [20]. The present study revealed that the rhizosphere soils of *A. nilotica* subsp. *cupressiformis* in Pali have high AM diversity (Table 2), as compared to other districts *i.e.*, Jodhpur, Ganganagar, Nagaur and Sirohi.

The study revealed that the *Glomus* has been the most dominant genus in arid regions (Table 2). The predominance of *Glomus* species varying edaphic conditions may be due to the fact that it is highly adaptable to varied soil and temperature conditions, and can survive in acidic as well as alkaline soil [21]. The present study revealed that *G. fasciculatum* was the most dominant AM fungal species under *A. nilotica* subsp. *cupressiformis*. The similar observations were also made by Pande, [3]; Verma *et al.*, [22]. Perhaps, it may be due to the ability of fungus to produce excellent inoculum under *A. nilotica* subsp. *cupressiformis* in this environment. The pH of our study area was very narrow *i.e.* from 8.01 to 8.65 and we got no significant relationship of AM population with available pH. Effects of pH are particularly difficult to evaluate since many chemical properties of the soil vary with changes in pH.

The Maximum spore population was recorded from (Pomava) Pali and minimum from (Jhareli) Nagaur in *A. nilotica* subsp. *cupressiformis*. This can be attributed to increase in moisture regime beyond an optimum level at Barmer and Nagaur. The main reason for lower spore count in Nagaur might be due to low rainfall (app. 388 mm) and high temperature (upto 47°C) than Pali (460-490 mm rainfall) and mean maximum temperature 41-46°C). Aridity hampers the spore germination and thus results in the decline of spore population. The increase in moisture leads to decrease in the spore built up [23]. These findings are in consonance with the similar observations made by several workers [24,25]. In Pali, highest AM population was recorded which may be due to its location, which experiences optimum rainfall and temperature that are conducive for AM sporulation. The higher number of AM fungi in Pali and lower in Nagaur as indicated from the study may be due to a difference in moisture and thermal regimes, because an optimum level of soil and environmental conditions are required for the AM fungi to sporulate for its development and infectiveness [26].

In Pali, highest AM population was recorded which may be due to its location, which experiences optimum rainfall and temperature that are conducive for AM sporulation. Higher infection in *A. nilotica* subsp. *cupressiformis* trees growing in this area might be because of the adaptability of AM fungi to the native soils. Under optimum conditions, as in Pali, climate provides favourable conditions for colonization, and therefore nearly the entire length of roots was found to be colonized by these myco-symbiont [27].

The present study clearly demonstrated for the first time that at least 22 species from five genera are associated with *A. nilotica* subsp. *cupressiformis* and revealed that both AM fungal population and percentage of root colonization are affected by organic carbon (OC). This demonstrates the importance of soil fertility in influencing the population of AM fungi [28, 29]. It has been observed that in tree rhizosphere soil, phosphorous had no significant relationship with AM population (lack of relationships with P) may be due to relatively low levels of P, since no fertilizer application in the vicinity of the tree roots in crop fields is practiced generally. This perhaps may be due to low level of phosphorous, as no fertilizer application in the vicinity of the tree roots in crop fields is generally practiced. Similar observations were also reported from abroad by Harley and Smith, [30] and Pande, [31]. The intensity of AM colonization in the roots and spores in the rhizosphere of *P. cineraria* plantation sites varied according to the plant and site factors including soil nutrients, moisture and other environmental conditions [22]. Mycorrhizae are an important consideration in maximizing land productivity, which can be managed by using appropriate AM and a complete understanding of profile of AM associated with plant can be useful in finding AM symbiosis in particular host species.

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