

Role of Bio Fertilizer in Plant Establishment and Growth of *T.Undulata*

K.K.Srivastava, Neelam Verma, Sangeeta Singh

Abstract— At AFRI, during survey of the ecologically different sites, dominance of *G. fasciculatum* was found in all the sites. Occurrence of other species was comparatively moderate to low. The spore population varied from site to site. Maximum spores (429 spores/100 gm soil) were found in agro-forestry area (Mogra) and minimum (130 spores/100 gm soil) from mining area (Chokha). Seasonal variations in the spore population were also observed. Maximum spores occurred in soil during the month of July and August and minimum during the month of summer i.e., May and June. 100 gm of rhizosphere soil having AMF propagules (pieces of colonised roots) with minimum 500 spores is the best for treatment of *T. undulata* seedlings. The best results were obtained when effective dose of inoculum was placed below 1" or 2" in pots or by side banding method. Selection of efficient isolates of VAM fungi showed that indigenous consortium of AMF isolate occurring in the rhizosphere soil of mature plants is the most suitable for *T. undulata* in terms of root colonization and biomass production. The similar studies were also carried out in irrigated and unirrigated areas at IGNP and agroforestry sites.

I. INTRODUCTION

Afforestation and reforestation on massive scale have been recognized as the most appropriate approach to improve living conditions in arid regions by stepping up food, fodder, fuel and timber production besides ameliorating soils and environment of the deserts. Among the different methods adopted for afforestation of these areas, application of micro-organisms for the establishment of higher plant species is well recognized. Inoculating seedlings with suitable and improved microbial fertilizers including Arbuscular mycorrhizal (AM) fungi/*Rhizobium* which are tolerant to extremes of temperature, high soil pH, drought, etc., is one of the important techniques for the rehabilitation of these areas. The AM fungi can bind soil particles into larger aggregates, necessary for a stable and porous physical structure of soil. This is particularly important in dry areas, where aggregation of particles permits more efficient use of available water. The fungi can therefore, play an important role in stabilizing sand dunes and establishment of shelterbelts (Tarafdar, 2005). The AM fungi increases the efficiency of nutrient acquisition by the fungal mycelia in soil, translocation within extraradical hyphae to intraradical fungal structures, and transfer to plant

cells. A little is known about the AM fungi of tree species in arid and semiarid regions (Mukerji and Kapoor, 1986; Tarafdar and Rao, 1990; Bohra *et al.*, (2007).

Being, a multipurpose indigenous tree species of western Rajasthan, study of mycorrhizal associations of *T. undulata* [commonly called as *rohida* or *marwar teak*], at nursery and plantation sites are crucial to access their dependence on mycorrhizal fungi as well as selecting out candidate species of endomycorrhizal fungi for mass multiplication. In turn, selected AM fungi may be used for fortifying the desired tree species in nursery before taking them for plantation at harsh sites like arid zone of Rajasthan.

II. MYCOBIONTS ASSOCIATED WITH *T.UNDULATA*

Periodical survey of *T. undulata* plantations were undertaken to collect root and rhizosphere soil samples from ecologically different sites *viz.*, Bhaktasani, Chopasni, Chokha, Churu, Jawadia, Jhalamand, Keirala (Gumti), Mogra, Osean and Phalaudi to isolate and identify the VAM fungi associated with *T. undulata* and also to estimate spore population and percentage of root colonization. The genera, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* were isolated and identified on the basis of the characteristic features of the resting spores in soil. In all, 2 species of *Gigaspora viz.*, *Gigaspora gigantea* (Nicol & Gerdman) Gerdman & Trappe (Plate 1 fig. a) and *Gigaspora margarita* Becker and Hall; 12 species of *Glomus viz.*, *Glomus aggregatum*: Schenck and Smith emend Koske ; *Glomus constrictum* Trappe ; *Glomus convolutus* Gerdman & Trappe; *Glomus deserticola* Trappe, Bloss & Menge; *Glomus fasciculatum* (Thaxter) : Gerdman & Trappe emend walker and koske; *Glomus fulvum* (Berk. & Broome) Trappe & Gerdman; *Glomus intraradices* Schenck and smith; *Glomus macrocarpum* Tul & Tul.; *Glomus magnicaule* Hall; *Glomus mosseae* (Nicol & Gerd.) Gerd & Trappe; *Glomus occultum* walker. ; *Glomus reticulatum* Bhattacharjee & Mukerji; *Glomus sp.*; one species of *Sclerocystis viz.*, *Sclerocystis indica* Bhattacharjee & Mukerji and 3 species of *Scutellospora bionarta* Spain, Sieverding & Toro.; *Scutellospora erythropha* (Koske & Walker) Walker & Sanders. ; *Scutellospora savannicola* (Harr & Ferr.) walker and Sander's. and *Scutellospora sp.* (Plate 2 fig. g) were frequently found in the rhizosphere soil of *T. undulata*.

The similar studies were also carried from IGNP command area in various tree species such as (i). *A. nilotica*, *A. tortilis*, *D. sissoo*, *E. camaldulensis* & *T. undulata* and (ii). *A. nilotica*, *A. tortilis*, *E. camaldulensis*, *P. cineraria* and *T. undulata* from irrigated and un-irrigated plantation of agroforestry sites revealed the presence of VAM genera *viz.*, *Acaulospora*, *Glomus*, *Scutellospora* and *Sclerocystis* were isolated and identified (Srivastava *et al.*2014). In irrigated plantations of IGNP command area, the total number of 17 species of *Glomus*; 2 species of *Acaulospora*; 2 species of *Sclerocystis* and (1) species of *Scutellospora* were isolated

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K.K.Srivastava, Forest Protection Division, Arid Forest Research Institute-Jodhpur-342005

Neelam Verma, Forest Protection Division, Arid Forest Research Institute-Jodhpur-342005

Sangeeta Singh, Forest Protection Division, Arid Forest Research Institute-Jodhpur-342005

and identified on screened tree species. Among the different species *G. fasciculatum* was found to be the most dominant species followed by *G. aggregatum*, *G. constrictum* and *G. microcarpum*. In agroforestry sites, 16 species of *Glomus*; 2 species of *Acaulospora*, (1) species of *Sclerocystis* and (1) one species of *Scutellospora* were isolated and identified on all the tree species screened. Among the different VAM fungal species *G. aggregatum* was found to be the most predominant fungus followed by *G. fasciculatum*, *G. microcarpum* and *S. sinuosa*.

III. DISTRIBUTION ,FREQUENCY AND SPORE POPULATION

Dominancy of *G. fasciculatum* was found in all the sites studied, while occurrence of other species were comparatively moderate to low. The spore population varied from site to site . Maximum spores (429 spores/100 gm soil) were found in agro-forestry area (Mogra) and minimum (130 spores/100 gm soil) from mining area (Chokha) . Seasonal variations in the spore population were also observed. Maximum spores occurred in soil during the month of July and August and minimum during the month of summer i.e., May and June . This clearly indicates that extreme dry conditions invariably adversely effect the endomycorrhizal formation as well as sporulation by the mycobionts. The variation in spore population was negatively correlated with soil moisture (Table-1). Under extreme dry soil conditions, the sporulation was reduced to minimum, however, after the rainy season decline in soil moisture upto certain level favoured more sporulation in all the sites. Due to non availability of the data regarding soil reaction, result could not obtained regarding the role of pH on the spore population

Table 1 - Variation in spore population and root colonization

Factors	Sites										
	1	2	3	4	5	6	7	8	9	10	11
1. pH	7.5	8.2	7.7	7.4	7.6	7.8	7.4	8.2	7.8	8.0	8.0
2. Moisture	4.9	5.5	6.5	5.8	6.0	9.0	8.0	7.5	5.2	6.0	6.0
3. % of Colonization	8.9	7.0	6.5	7.2	6.0	5.5	4.0	5.0	4.5	3.0	3.0
4. Spore population	429	306	338	378	310	130	130	237	333	233	233

Sites: 1.Mogra; 2.Phalaudi; 3.Mathania; 4.Osean; 5.Chopasni; 6.Chokha; 7. Bhaktashni; Jhalamand; 9. Churu; 10.Jawadia

Influence of season on VAM colonization & VAM propagules population under irrigated and unirrigated conditions in IGNP area and agroforestry system

Studies on influence of season on VAM colonization & VAM propagules population on various tree species namely *Acacia nilotica*, *Acacia tortilis*, *D. sissoo*, *E. camaludensis* and *Tecomella undulata* under irrigated and unirrigated conditions in IGNP area and agroforestry system showed typical vesicular, arbuscular or both and hyphal structure. Some of the root samples had extrametrical hyphae with *Glomus* spores. The root segments of *D. sissoo*, *E. camaludensis* and *A. tortilis* had broader fungal hyphae with large number of vesicular structures. Arbuscular structures were pre-dominant in the younger age plantation. It was observed that both VAM colonization (%) an propagule number were increases as the age of the plantation increased. The maximum root colonization was noticed in the 2nd quarter (July to September) and 3rd quarter (October to December). The minimum percent of root colonization was observed in late winter months (January to March) and in summer months (April to June) in all the tree species. The higher VAM spore number was recorded in 3rd quarter (October to December). Moderate number of spores was observed in 2nd quarter (July to September) The minimum number of spores recorded n late winter months (January to March)and in summer months (April to June) .Seasonal variation in spore population frequently observed by several workers (Sylvia, 1986). Effect of season on population of AMF spores is a natural phenomenon and have been studied by several workers (Bakshi, 1974; Hayman, 1970; Harni Kumar and Bagyaraj, 1988; Mohan and Mahadevan, 1988; Gemma and Koske, 1988; Shankar *et al.*, 1990; Thapar *et al.*, 1991). The present observations resembles with the study of Hayman (1970), who has reported an increase in the number of spores during July and a decrease in population during winter. Bakshi (1974) has also reported an increase in the population of *Endogone* spores and *Gl. macrocarpum* after rains during June-October due to high soil moisture and their population falling to low levels between November and May. These results were correlated with soil nutrient data. It was observed that increased soil pH directly influences the percentages of root colonization in different species of irrigated plantations in IGNP command area. Similarly soil phosphorous and organic carbon were associated with maximum spore population in rhizosphere of various tree species screened.but in arid areas of western Rajasthan due to limited rains and sandy soil problems of water logging is less. Therefore, in arid areas maximum sporulation occurs after first rain in the month of July since it favours sporulation. Similar observations were also made by Gemma & Koske (1988) in sand dune soils of Rhode island. **VAM inoculation studies on arid zone tree species**
The experiment was laid out in the year 1996-97 at AFRI Nursery, Jodhpur (Raj.). There were four species viz., *Azadirachta indica*, *Ailanthus excelsa* *Dalbergia sissoo* and *Tecomella undulata*.The experiment was consisting of two treatments 1. Inoculated (I) and 2. Uninoculated (UI) with twenty replications in each treatment. The consortium inoculums (with the dominance of *G. fasciculatum*) was used for the experiment. Inoculum containing about 400-500 spores/plant was given. The inoculation was done by side

banding method. The observations on shoot height root length and root colonization (%) were recorded bimonthly. **Percentage of root colonization.** It is evident from Table 2, that VAM inoculated seedlings of all tree species performed better than un-inoculated in term of shoot height, root length and percentage of VAM colonization. The seedlings showed positive effect after 60 DAI, 120 DAI and 180 DAI (Table -2).

Table 2 - Effect of VAM inoculation on various tree species of arid zones

Tree Species	Treatments	Mean Shoot height (in cms)			Mean Root Length (in cms)			% VAM inoculation		
		60 DAI	120 DAI	180 DAI	60 DAI	120 DAI	180 DAI	60 DAI	120 DAI	180 DAI
<i>Azadirachta indica</i>	(I)	21.6	30.2	40.2	6.2	7.2	9.3	92	92	94
	(UI)	18.2	24.9	29.2	5.1	6.4	8.2	36	42	45
<i>Tecomella undulata</i>	(I)	26.8	36.2	42.8	6.0	6.9	10.2	88	89	90
	(UI)	24.2	30.4	34.6	4.9	5.8	7.7	34	35	35
<i>Ailanthus excelsa</i>	(I)	34.1	42.9	49.2	7.1	9.4	12.4	74	74	80
	(UI)	31.2	37.3	41.4	6.2	8.2	10.1	30	30	32
<i>Dalbergia sissoo</i>	(I)	29.2	36.8	42.1	5.9	7.8	10.4	77	78	86
	(UI)	26.3	31.6	36.4	5.8	6.6	8.1	33	34	36

IV. SELECTION OF MOST EFFICIENT ISOLATE

The pot experiment indicated that out of four different treatments, Indigenous mixed inocula, Indigenous *G. fasciculatum*; Mixed inocula (Dehradun) and *G. monosporum* (Bangalore), The indigenous consortium of AMF isolate occurring in the rhizosphere soil of mature plants is the most suitable for *T. undulata* in terms of root colonization and biomass production. The efficacy pattern showed - mixed inocula (indigenous) > *Glomus fasciculatum* > mixed inocula (Dehradun) > *Glomus monosporum* (Bangalore).

Table 3 - Selection of efficient strain of AMF for *T. undulata*

Mycorrhizal Isolates	Shoot Height (cm)	Root Length (cm)	Shoot Weight (gm)	Root Weight (gms)	Collar diameter (m)	No. of branching	% of colonization

	m)						
Uninoculated	34.8	17.5	1.98	8.58	8.0	3	0
Mixed inocula (Indigenous)	60.0	48.6	6.93	14.68	15.0	13	80.0
<i>G. fasciculatum</i> (Indigenous)	56.1	33.5	5.21	12.87	13.2	11	76.0
<i>G. monosporum</i> (Bangalore)	45.0	27.0	4.30	10.58	11.0	6	55.0
Mixed Inocula (Dehradun)	45.3	32.0	5.14	11.46	12.6	8	60.0

The experiment on selection of efficient strains of VAM fungi was laid out on various tree species namely *Prosopis cineraria*, *Eucalyptus camaludensis*, *Acacia nilotica* and *Tecomella undulata* in nursery. There were four treatments viz., *G. fasciculatum*; *G. microcarpum*; *G. aggregatum* and mixed inocula (*G. fasciculatum* + *G. aggregatum*). The experiment was laid out with four replications and thirteen plants/treatments. The observations were recorded bimonthly intervals. The final observations on shoot height and biomass revealed that all the treatments were found effective against uninoculated plants, whereas among the treatments *G. fasciculatum* was found best followed by *G. aggregatum* > *G. microcarpum* > mixed inocula (*G. fasciculatum* + *G. microcarpum*) in the case of *T. undulata* and the pattern of various strains was *G. microcarpum* > *G. aggregatum* > *G. fasciculatum* > mixed inocula (*G. fasciculatum* + *G. microcarpum*) in *P. cineraria*, *Eucalyptus camaludensis* and *Acacia nilotica* respectively (Table-4).

The similar pattern was also noticed with regards to other parameters i.e., root length, number of branching, collar diameter, root and shoot weight etc. (Srivastava and Srivastava, 2006) Similar observations regarding the efficiency of the native isolates have also been observed and reported by a number of workers including Vyas (1988), Chandel (1991), Srivastava (1992).

Table -4 - Selection of efficient strains of VAM (Mean Shoot height in cms)

Tree Species	<i>Prosopis cineraria</i>	<i>Eucalyptus camaludensis</i>	<i>Acacia nilotica</i>	<i>Tecomella undulata</i>

VAM isolates	90 D AI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
<i>G. fasciculatum</i>	26.72	29.94	27.15	32.69	39.03	42.24	24.77	26.26
<i>G. microcarpum</i>	31.34	32.03	28.88	33.76	45.34	48.70	23.02	24.19
<i>G. aggregatum</i>	26.98	31.89	28.08	33.27	42.85	45.25	24.72	25.15
<i>G. fasciculatum</i> + <i>G. aggregatum</i>	23.92	24.78	26.35	31.21	36.01	38.90	22.35	23.25

V. QUANTIFICATION OF EFFECTIVE INOCULUM DOSE

The experiments were arranged to evolve an effective technology with regards to method of inoculum, its application and quantification of the effective dose required for inoculation. The observations revealed that 100 gm of rhizosphere soil having AMF propagules (pieces of colonised roots) with minimum 500 spores is the best for treatment of *T. undulata* seedlings (Table-11) (Srivastava *et al.*, (2004). The seeds pelleted with AMF did not show encouraging results. The best results were obtained when effective dose of inoculum was placed below 1" or 2" in pots or by side banding method. These findings resemble with Sieverding (1991) in *Cassava* crop; and Verma *et al.*, (2009) in *Prosopis cineraria*. Ferguson and Woodhead (1982) have recommended inoculum density of approx. 300-500 spores (100 g soil) and 10,000 spores per m² of planting area to ensure rapid colonization in treated plants

VI. FUTURE STRATEGIES

In current practices we are using the chemical fertilizer to fulfill the plant nutrients requirement which have a serious impact on the soil and water bodies. The salinity of soil is one of the major threats by using utilizing excessive chemical fertilizer. The chemical fertilizers have a serious impact as loss of soil fertility, ground water and surface water pollution, salinity of soil whereas, application of biofertilizers has been reported to be efficient enough in restoration of the soil. Inoculated seedlings with suitable and improved microbial fertilizers including Arbuscular mycorrhizal (AM) fungi/*Rhizobium* which are tolerant to extremes of temperature, high soil pH, drought, etc., is one of the important techniques for the rehabilitation of these areas. AM fungi occur in all types of horticultural, agricultural crops and grasses in nature. The population of these fungi differ due to temperature, moisture, salinity and soil characters. Scattered

research is available on beneficial role of AM fungi in plants. Suitable indigenous strain of biofertilizers (AM fungi, *Rhizobium*, PSBs) and their availability to the users are need of today for afforestation of new planting areas under rehabilitation of mining and wasteland.

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