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Studies on Isolation and Identification of VAM Fungi in *Solanum viarum* Dunal of Medicinal Plants

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ABSTRACT

Medicinal plants are nature's gift to cure a number of diseases of living organisms. The plant Solanum viarum is the potential renewable natural resources. The plant is cultivated under the agro climate soil conditions and the field of biotechnology as most of the pharmaceutical industries depends Largely on the utilization of plants for the production of compounds to study the variability in natural infection rates, spore density and species richness of AM fungi associated with and medicinal plant Solanum viarum which was culture from herbal garden at poondi, Thanjavur. The efficient of AM fungi and their mass production of Sorghum bicolor roots by pot culturing techniques were assessed. The physico chemical characteristics of the soil at the study site were, analysed. The observation recorded on the influnce on growth are also described.

INTRODUCTION

The symbiotic arbuscular mycorrhizal (AM) fungi develop on extensive hyphal network and provide water and nutrients to plants. Soil microorganism can influence the soil structure and play important role for AM fungi colonization in roots. The medicinal plant *Solanum viarum* is potencial renewal natural resources. *Solanum viarum* (Family – Solanaceae), a drug plant commonly called as 'Marunthu Kathiri' (Soda apple). A drug plant is potential in Ayurvedic and siddha systems of medicine in gaining prominence recently. Standardization of this plant is helpful for effective quality control. Therefore, this medicinal plant is selected for the present investigation as influenced by arbuscular mycorrhizal fungi (AMF). The efficient in AM fungi and their mass production of *Sorghum bicolor* roots by pot culturing techniques were assessed. The observation recorded growth and biomass of AM fungi for techniques *S.viarum.*

MATERIALS AND METHODS

Study Site:
To assess the native AM fungi from roots and rhizosphere soils of *Solanum viarum* which were collected from Herbal garden at Tamil University, Wagaiyur of Thanjavur district, Tamilnadu, India.

Sample Collection:
From each study site, 3-5 healthy plants were selected. The roots of *Solanum viarum* and their rhizosphere soil samples were collected at o-40cm soil depth [1]. Roots of *Solanum viarum* was washed thoroughly free of attached soil particles and cut into 1cm bits and fixed in formalin acetic alcohol (FAA) in the field itself [2]

Analysis of soil Physico – chemical Properties:-
Test plant rhizosphere soil samples were collected separately from each study site, and a portion of soil was for analyzed soil texture, PH, EC, OM, N, P,K,Zn, Cu, Mn and Fe at the soil testing Laboratory. The following standard methods [3,4,5]. Form the remaining soil sample, 100g was used to estimate AM fungal spore number per sample bag.

Root colonization by AM Fungi;
Root segments of ***S.viarum*** were first washed thoroughly in distilled water and then placed in 10% KOH and heated to 90^{o}C for 15-30 minutes. They were then washed in distilled water and immersed in alkaline 3% H_2O_2 for 5-10 minutes. They were then washed in distilled water and acidified with 5N HCL for 2-3 minutes. The root segments were stained with 0.05% trypan blue in lacto phenol for 15-30 minutes and the excess stain was removed with clear lacto phenol [2].

Root segment were mounted on glass slide with lactophenol and observed under compound microscope. A minimum of 100 segments for each samples were observed for the assessment of percentage colonizatiation of AM fungi using the following formula.

$$\% \text{ AM colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of root segments observed}} \text{ x100}$$

Isolation and identification:-
Spores of AM fungi were isolated form rhizosphere soil by the wet sieving and decanting technique [6]. Approximately 100 g of individual air-dried rhizosphere soil samle was dispersed in 500 ml of water in a beaker and the suspension was left undisturbed for 15-20 min. The suspension was then decanted through the stack of sieves 180 and 38 µm (arranged in decreasing order of mesh size from top to bottom). Same process was repeated 2-3times and the residue from each sieve was collected into Petri plates with little distilled water. Intact AM fungal spores were examined and counted under stereomicroscope (Olympus OIC 1629) and identifications were made by observing diagnostic characteristics such as spore wall, colour, size and type of hyphal attachment accordeing to Schenck and Prez 1990 [7] under compound microscope (Nikon-Optiphot-2).

Estimation of AM fungi
The term frequency was used to assess the establishment and survivability of AM fungi in the rhizosphere of the host. Frequency denotes the number of samplings in which spores of a particular AM fungus present during the study period and expressed as percentage[8]..

$$\text{Percentage of frequency (\%)} = \frac{\text{Number of Sampling in which a particular AM Fungus was recorded}}{\text{Total number of sampling}} \times 100$$

The density and distribution of AM fungi in the rhizophere soil samples were expressed in term of percentage occurrence.

$$\text{Percentage of occurrence} = \frac{\text{Total number of spores of individual AM fungus}}{\text{Total number of spores al AM fungi}} \times 100$$

Data Analysis:-

The relationship between percent root colonization and spore density were analysed by Karl Pearson's correlation [9]. From the data obtained the spore density and richness, for each sampled site was worked out according to the standard derivation. The diversity of AM fungi in all the two study site were assessed based on diversity indices [10].

AM fungal inoculum preparation

Sorghum bicolor L. plants were used as host plant for AM fungal inoculum preparation. Five dominant indigenous AM fungi such as *Acaulospora bireticulata, Glomus fasciculatum, Glomus aggregatum,Gigaspora margarita* and *Scutellospora heterogama* used for inoculum production. Glass funnels of 5 cm diameter were filled with strerilized soil sand (1:1) mixture. Spores were surface disinfected with Chloramine–T (2%) and 50–200 spores each were layered on the soil: sand mixture using funnel technique. Five seeds were sown on each funnel. *Sorghum bicolor* plants were transplanted from funnel to pots after 20 days of germination. Small pots of 18 cm diameter x 15 cm height were filled with sterilized soil:sand(1:1) mixture.Test plants from the funnel were transplanted to pots. The pots were kept in green house (30 ± 1ºC) and watered regularly. The infectivity of *S. bicolor* roots by the AM fungi were checked at interval of 15 days. After 3 months, the pot cultures were harvested by pruning *Sorhaum bicolor* plants to the soil level. The soil mass was removed from the pot and the mycorrhizal roots were chopped into small pieces. Inoculum potential of three AM fungal species was done by using MPN (Most Probable Number) method.

Effect of native AM fungi

Glomus aggregatum, Gigaspora margarita and *Scutellospora heterogama* on growth, nutrition of *S. viarum* by pot culture was conducted at Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autonomous) , Poondi, Tamilnadu, The experiment was undertaken in brown sandy loam soil with 168.38 kg ha^{-1} available nitrogen, 18.68 kg ha^{-1} phosphorus and 205.60 kg ha^{-1} pottassium. Soil was neutral to alkaline with pH7.4. PVC pots of 18 cm diameter were filled with sterile brown sandy loam soil of 3 kg/pot. Three species of AM fungi, *G. aggregatum, Gigaspora margarita* and *Scutellospora heterogama* were screened for their efficacy. Pure culture of AM fungal species were maintained *Sorghum bicolor.* The experiment was laid out in randomized complete block design (RCBD) with four replications. The treatments were (i)Control (without AM fungi), (ii)Inoculated with *Glomus aggregatum* alone, (iii) Inoculated with *Gigaspora* margarita alone, (iv)Inoculated with *Scutellospora heterogama* alone. (Plate -1)

Glomus aggregatum,Gigaspora margarita and *Scutellospora heterogama* maintained in *Sorghum bicolor* as the host plant was inoculated to the raised nursery beds measuring 1 x 2 m at

the rate of 1.5 kg/m^2. Uniform 5 cm *S. viarum* whenever necessary. Seedlings raised in uninoculated beds served as control plants. The seedlings were maintained in the nursery beds for three weeks. Then they were transplanted to the pots. 60 g of dry soil inoculum containing 400 – 500 spores / 50 g soil, was mixed in the top of 6 cm of the soil of each treatment pot.

Control plants received 6 g of soil containing non – mycorrhizal root pieces of onion. The amount of AM inoculum for each treatment was so adjusted that equal quantity of soil inoculum could be added to each pot. Pots were maintained under green house conditions. Plants were harvested after 75 days. Growth parameters were observed such as observations like plant height , shoot and root biomass of *S.viarum* were examined 75 days after transplanting. Mycorrhizal root colonization and spore numbers in the root-zone soils were assessed by grid line intersection method [11] and Wet-sieving and decantation method [12].

RESULTS AND DISCUSSION

Soil physico-chemical parameters of study site

The physico-chemical characteristics of the soil of the two study areas Herbal garden at Tamil University and Herbal garden at Poondi in soil temperature and moisture (8.45-9.12%) were in accordance with the climatic changes during different seasons. Edaphic characteristics of samples collected from areas indicated that the soil PH was neutral to alkaline (PH 7.2 and 7.4) in herbal garden at Tamil University and Poondi garden respectively, with low to moderate electrical conductivity (ECse 1.2 – 1.6). The physico-chemical properties of the soils varied considerably among the samples particularly with low organic carbon (1.21-1.92%), less P level (18.2-20.kg/acre), moderate to high level of K (121 – 135.2kg/acre) and also with other micronutrient contents such as zinc (1.8 0- 2.2ppm), copper (1.6-2.3ppm), manganese (3.2 – 5.6 ppm) and iron (1.4-2.6 ppm). The available N content of the soils irrespective of the study localities were invariably high (77.4 – 98.8 kg/acre). Results on chemical analysis and PH of soil are presented in (Table-1) by the following standard method.[3,4,5].

AM STATUS OF *S.viarum*

The Significance of AM in plant ecology is based on its widespread occurrence in natural ecosystems [13]. The present study was undertaken to make a detailed examination of AM status of *Solanum viarum* collected from the cultivated fields of two different localities of Thanjavur district, Tamil Nadu, South India in relation to soil physico chemical characteristics. The selection of sampling area and *Solanum* plants were based on soil characteristics as suggested by [14].

The aim of the present work was to determiner the influence of medicinal plant S.v with association of AM fungi. The association of AM fungal work have been done in the medicinal plants [15]. the garlic , carrot and gram [16]ginger [17] turmeric[18].

In the present study the medicinal plants S.v was positive for AM fungai colonization in the roots of both the study sites. Also, were reported by[19]. The mycorrhizal treatement were colonized, sporte density and species richness by the AMF. (Table -2) This shows that identify to the AMF can determine the species of AM colonizing the roots varied from high as 98.2% and 82.5% similar observation were reported by [20].

Mass Inoculum Production

Bagyaraj 1992[21] . studied that several host plants including as Sudan grass (S.bicolor var Sudanense). have been used for there suitability to multiply AM fungal inoculum. Also was reported by [22].

Incidence of AM fungal colonization in the roots of *S.viarum*

In ourstydy *Sorghum bicolor L* plants were used as host for AM fungal inoculum preparation. Totally eleven AM fungai species were isulated from rhizophare soils of testplant (Table -3) The Five AM fungal species were isolated and identified. Such *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora.* The effect of AM fungal species and their mass production of plants were inoculated into S.bicolor roots by using pot culturing technique.(Plate-1).The AM fungal spores and sporocarps isolated from rootzone soil of *S.viarum* Plate-II.

Beneficial effect of AM fungi for *S. viarum*

It is now largely agreed that infection with AM fungi can cause growth increase in medicinal plants mainly due to increase in the supply of phosphours [23]. The AM fungi have been noticed in several palnts species and are widely distributed in the soils. [24] observed two-fold increase in the growth of barley by inoculation of *G.fasciculatum* . In the present study, individual AM fungal inoculation shows increased plant height and dry biomass was increased. The beneficial effect of AM fungi has also been oberseved in horticultural crops [25] medicinal plants [26,27]. observed that *Leucaena leucocephala* seedilings inoculated with *G.fasciculatum* had significantly greated shoot and root dry biomass, leaf area and nutrition than non-mycorrhizal plants. They also revealed that *G.aggregatum* alone inoculated plants showed greated shoot and root biomass, P,K,Mn,Zn,Fe and Cu contents in the leaves and roots .[28 , 29]studied the effect of 13 different AM fungi to the seedling of *Acacia nilotia and Cathiandra calothrus*. The Occurrence of Vesicular arbuscular nugcorrhizal fungi in *S. viarum* medicine plants. In the presents VAM as uses of Biofertilizer in the medicine plant of *S. viarum.*

The observation recorded *G.aggregatum* and *G.margarita* was found to be colonized in the root zone of *S.viarum*.[Table 4,5.] The result indicated that also in *Solanum viarum* plants inoculated with *G.aggregatum* alone showed increased mycorrhizal colonization, spore number, greater plant height, root and shoot biomass, rich chlorophyll content and photochemical constituents. The Present study have been used for there suitability to multiply AM fungal inoculum. The Occurance of Vesicular arbusculer mycorrhizal fungi in *S.viarum* medicinal plant VAM fungi may be attribute to biofertilizer for the plant. The medicinal plant *S.viarum* to cure a number of diseases in living organisms.

Table1. Physico-chemical properties of study sites

Study site	Soil			Macronutrients Kg/acre*			Micronutrients in ppm			
	pH	Moisture (%)	Organic carbon (%)	N	P	K	Zn	Cu	Mn	Fe
S1 –Herbal garden at Tamil University	7.2	9.12	1.21	92.8	20.2	121.0	2.2	2.3	5.6	2.6
S2-Herbal garden at Poondi	7.4	8.10	1.92	89.4	19.5	135.2	1.9	1.6	4.6	1.9

General nutrient status of the soil (Anonymous, 1988).

Table 2. Colonization %, Spore density and Species richness of AM fungi associated with *S. viarum*
(Mean of five replicates)

Study sites	Root colonization	Vesicles %	Arbuscules %	Total number of AM fungal spores per 100 gsoil	Associated AM fungal species *	Positive for AM fungi in the roots
S1 –Herbal garden at Tamil University	98.2	60.2	38.5	685 ± 12.2	LMRC, LMCM, ABNRT, LAGR, LFSC, GMRG, LABS, SSNS	***Glomus fasciculatum***
S2-Herbal garden at Poondi	82.5	52.5	-	372 ± 8.2	ABNRT, LAGR, ASCB, SSNS, LMCM	***Glomus fasciculatum***

Unique code for AM fungal species (Schenck and Perez, 1990)

ABRT- ***Acaulospora birecticulata*** LAGR-***Glomus aggregatum*** LMRC-***Glomus microcarpum***
ASCB-***Acaulospora scrobiculata*** LDST-***Glomus deserticola*** CHTG-***Scutellospora heterogama***
GMRG-***Gigaspora margarita*** LFSC-***Glomus fasciculatum*** SSNS-***Sclerocystis sinuosa***

Table 3. Percentage of frequency occurrence of AM fungi in the rhizosphere soils of *S. viarum* at two different localities of Thanjavur district.

S. No	List of AM fungi identified	Code*	Study localities * *		Frequency %
			S1	S2	
1	***Acaulospora***				
	A. bireticulata	ABRT	+	+	100
	A. scrobiculata	ASCB	-	+	66.6
2	***Gigaspora***				
	G. margarita	GMRG	+	+	100
3	***Glomus***				
	G. ambosporum	LASM	+	-	33.3
	G. aggregatum	LAGR	+	+	100
	G. deserticola	LDST	+	-	33.3
	G. fasciculatum	LFSC	+	+	100
	G. macrocarpum	LMCM	+	-	66.6
	G. microcarpum	LMRC	+	-	33.3
4	***Scutellospora***				
	S. heterogama	CHTG	+	+	100
5	***Sclerocystis***				
	S. sinuosa	SSNS	+	+	66.6

* *Unique code for AM fungal species (Schenck and Perez, 1990)*
*** S1 – Herbal garden at Tamil University*
S2 – Herbal garden at Poondi.

Table 4. Influence of three different native AM fungi on plant growth and biomass production of *S. viarum*

Inoculation treatment	Plant height (cm)			Plant dry weight (g/plant)			Mycorrhizal effect (%)
	Shoot	Root	Total	Shoot	Root	Total	
T1 – Control (Without AM fungi)	38.5^{d}	$^{24.5d}$	63.0	4.85^{d}	3.62^{d}	8.47	--
T2 – Glomus aggregatum alone	54.5^{d}	38.2^{a}	92.7^{a}	6.42^{d}	4.82^{a}	11.24^{a}	132
T3 – Gigaspora margarita alone	250.2^{b}	36.4^{b}	86.6^{b}	6.14^{b}	4.33^{b}	10.47^{b}	122
T4 – Scutellospora heterogama alone	42.4^{c}	30.4^{c}	72.8^{c}	6.05^{c}	4.13^{c}	1018^{c}	121

Means in each column followed by the same letter do not significantly differ (P<0.05) from each other.

Table 5. Percent root colonization and AM fungal spores in the roots and root-zone soils of *S.viarum*

Inoculation treatment	% AM fungal root colonization	AM fungal spores /100 g of soil
T1-Control (without AM fungi)	--	--
T2-***Glomus aggregatum*** alone	92.5 ± 4.4	432 ± 6.2
T3-***Gigapora margarita*** alone	84.2 ± 3.2	386 ± 1.4
T4-***Scutellospora heterogama*** alone	65.5± 2.8	125± 1.2

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