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## Variability in growth and nutrition of *Solanum viarum* Dunal. as influenced by indigenous arbuscular mycorrhizal fungi

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A study was conducted under greenhouse nursery condition on the efficacy of seven indigenous arbuscular mycorrhizal (AM) fungi in the improvement of growth, biomass and nutrition in the roots and leaves of *Solanum viarum* Dunal. Seedlings were raised in polythene bags containing soil inoculated with isolates of seven different indigenous AM fungi, viz., *Acaulospora scrobiculata*, *Archaeospora trappei*, *Glomus aggregatum*, *Glomus fasciculatum*, *Glomus pakistanika*, *Gigapora margarita* and *Scutellospora persica*. The seedlings rose in the presence of AM fungi generally showed an increase in plant growth, biomass and nutritional status over those grown in the absence of AM fungi. The extent of growth, biomass and nutritional status enhanced by AM fungi varied with the species of AM fungi inhabiting the roots and leaves of *S. viarum* seedlings. Considering the various plant growth parameters and nutritional status in the roots and leaves of *S. viarum*, it was observed that *Glomus aggregatum* was the best AM symbiont for *S. viarum* used in this experiment.

**Key words:** *Solanum viarum*, AM fungi, biomass

### Introduction

The introduction of beneficial organisms into soil is a present crux of applied mycorrhizal research. Utilization of mycorrhizal bioinoculants in the cultivation of medicinal and aromatic plants is of recent interest. Arbuscular mycorrhizal (AM) fungi have been used to enhance the plant growth, nutrients and yield of medicinal crops and to help maintain good soil health and fertility that contributes to a greater extent to a sustainable yield and good quality of the products (Rajan *et al.*, 2004). The activity has gained momentum in recent years due to higher cost and hazardous effects of heavy doses of chemical fertilizers.

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The advent of innovative technologies and the importance are being given to sustainable agriculture. AM fungal association is of great economic significance on the growth and nutrition of agricultural and medicinal crops. Thus, it is essential to screen for an efficient AM fungus for a particular host in order to harness the maximum benefit from the fungus (Bagyaraj and Varma, 1995). Furthermore, since AM fungi cannot be grown on laboratory media, production of a large quantity of the inoculum for inoculation of the soil under field conditions is difficult. Nevertheless, since, the most of the commercially important medicinal crops are raised under nursery conditions before being transplanted to the main field, the inoculation of soil in the nursery would not only result in the saving of the inoculum needed but also help in better establishment of the transplanted seedlings. There are few published reports on the influence of AM fungi on the growth and nutrition of medicinal plants (Gupta and Janardhanan, 1991; Earanna *et al.*, 2002; Chandrika *et al.*, 2002; Rajeshkumar *et al.*, 2008). “Soda apple” or “Medicinal solanum” or “Marunthu Kathiri” (*Solanum viarum* Dunal) is an important medicinal plant largely used in ‘Indian Siddha Medicine’ and a member of the family solanaceae. The leaves and fruits are used for the treatment of cancer, Addison’s diseases, rheumatic arthritis, domestic contraceptive formulations and anabolic agents. The extracts of leaves possesses some nematicidal and bactericidal properties (Chandra and Srivastava, 1978). This plant concerned its cultivation as the richest source of ‘solasodine’ a nitrogenous analogue of diosgenin (Saini, 1966). Hence, the present investigation was done to screen for an efficient AM fungus for *S. viarum* and also to study the effects of the association on the growth, biomass and nutritional status in the roots and leaves of *S. viarum*.

## **Materials and methods**

### ***Pot culture experiment***

This investigation was carried out under nursery condition in a green house. The soil used in this study was collected from an uncultivated field at a depth of 0-30 cm and was classified as fine, entisol, isohyperthermic kanhaplustalfs. The soil pH was 7.2 (1:10 soil to water extract ratio), and it contained 2.7 ppm available phosphorus (extractable with  $\text{NH}_4\text{F} + \text{HCL}$ ) and an indigenous AM fungal population of 60 spores/50 g of soil. Nursery was raised by sterilizing the seeds of *S. viarum* with 5% chloramine T solution for 30 min, then washing and sowing in polybags (10 x 15 cm) containing sterilized soil: vermiculite mix (1:1v/v). Ruakura nutrient solution at 50 ml per polybag was applied once in 10 days. After

30 days seedlings were transplanted to PVC pots of size 18 x 24 cm containing 3 kg of stabilized soil : sand (1:1 v/v).

The AM fungal species used in this study (Table 1) were isolated from the rhizosphere soil of soda apple cultivated at the herbal garden of Tamil University, Tamilnadu, India. These AM fungal species were isolated by using wet - sieving and decanting technique as modified from Gerdemann and Nicolson, 1963. The species level identification of different AM fungal species was done following the keys provided by Trappe (1982) and Schenck and Perez (1990). These fungi were multiplied using sterilized sand : soil mix (1:1 v/v) as the substrate and onion as the host. After 90 days of growth, shoots of onion was severed and the substrate containing hyphae, spores and root bits was air dried and used as inoculum. The inoculum potential (IP) of each culture was estimated adopting the Most Probable Number (MPN) method as outlined by Porter (1979). The soil in each pot was mixed with this inoculum at different rates so as to maintain an initial IP of 12,500 per pot. Each pot containing the potting mixture, with or without AM inoculum as the treatment may be, was planted with one seedling of *S. viarum*. One set of plants without inoculation was served as the control. Each treatment with 5 replications was maintained in a greenhouse and water regularly so as to maintain the field capacity of the soil. Ruakura plant nutrient solution without phosphate was added to the pots at the rate of 50 ml per pot once in 15 days.

### ***Experimental analysis***

Ninety days after transplanting, the plants were harvested for determination of the mycorrhizal status, growth response, biomass and nutritional status. Plant height was measured from soil surface to the growing tip of the plant. Dry biomass was determined after drying the plant sample at 60°C to a constant weight in a hot air oven. Soil sample (100 g) was collected from each pot and subjected to wet sieving and decantation method of Gerdemann and Nicolson (1963) to estimate the population of spores. The root system was removed and assessed for AM fungal infection by the grid-line intersect method of Giovannetti and Mosse (1980) after cleaning the roots with 10% KOH and staining with trypan blue (0.02%) as described by Phillips and Hayman (1970). Shoot and root P, K and N concentrations were determined by employing the vanado molybdate phosphoric yellow colour, flame photometric and microkjeldahl methods respectively which modified from Jackson (1973). Atomic absorption spectrophotometry was employed to estimate zinc, copper and iron content of the plant samples, using respective hollow cathode lamps. The data thus generated was subjected to statistical analysis of completely

randomized block design with five replicates and the means were separated by Duncan's Multiple Range Test (DMRT) for significant difference  $P < 0.05$ ).

## Results and discussion

### *Screening, growth, biomass and mycorrhizal development*

In the field survey, soda apple plants growing in uncultivated p- deficient sandy loam soils was almost the same as in cultivated soils. Microscopic examination of their roots revealed extensive colonization by AM fungi with 96.5% level of infection. A large number of inter and intra - matrical vesicles were noticed between 120  $\mu\text{m}$  and 140  $\mu\text{m}$  in size. The vesicles were globose to subglobose and the subtending hyphae were simple. Based on the morphological characters, the AM fungal isolate was identified as *Glomus* species. Altogether seven AM fungi were isolated from rhizosphere soils and identified (Table 1). Among them, *Glomus aggregatum* and *Glomus fasciculatum* were predominant. However, *Acaulospora*, *Archaeospora*, *Gigaspora* and *Scutellospora* rarely occurred.

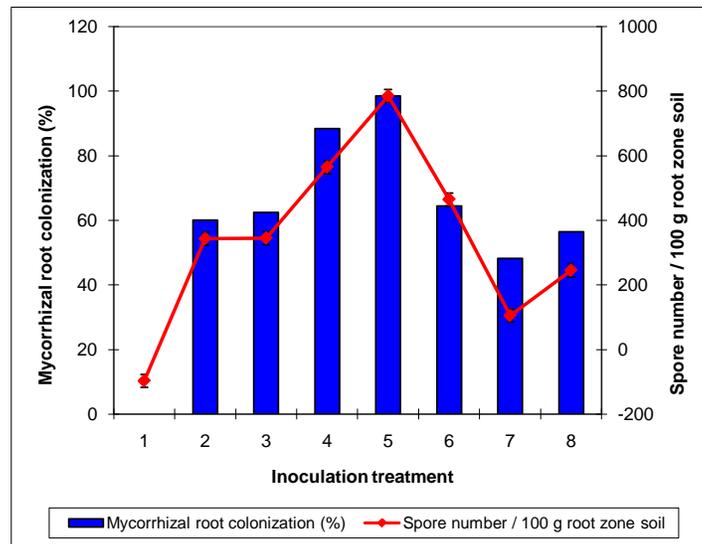
The growth response, biomass, nutritional status and mycorrhizal development of plants raised in sandy loam soils were assessed for the impact of inoculation with different native AM fungi. The responses of the soda apple plants to inoculation with different AM fungi were found to be varied. Mycorrhizal inoculation resulted in a significant increase in height, biomass and nutrient content of soda apple seedlings. However, there was no positive correlation between plant growth parameters and mycorrhizal colonization. Earlier studies also showed the same trend for medicinal plants subjected to AM inoculation (Earanna *et al.*, 2002; Reena and Bagyaraj, 1990; Chandrika *et al.*, 2002; Chiramel *et al.*, 2006) and these studies also indicated the need for selecting efficient native AM fungi for plant species. The present study conducted with an objective of screening for an efficient indigenous AM fungi for *S. viarum* seedlings has also resulted in varied plant growth responses to different AM fungi. Among the seven different AM fungi tested, the plants inoculated with *Glomus aggregatum* showed maximum plant height, root and shoot biomass which differed significantly from all other treatments (Table 1). More number of spores were encountered in the root zone of inoculated plants compared to uninoculated plants. Maximum number of spores occurred in the root zone soils of soda apple plants inoculated with *G. aggregatum* followed by *G. fasciculatum*, both being statistically on par with each other (Fig. 1). The lowest number of spores was noticed in the root zone of *G. pakistanika* inoculated plants. Mycorrhizal root colonization was also maximum in plants inoculated with *G. aggregatum*, followed by plants inoculated with *G. fasciculatum* (Fig. 1). These two treatments did not differ significantly from each

other. It is well known that enhanced nutritional status of a plant is manifested in its improved growth. Soda apple plants grown in the presence of AM fungi showed a general increase in such growth parameters as plant height and total dry weight as against those grown in soils uninoculated with AM fungi (Table 1).

**Table 1.** Influence of native AM fungi on plant growth response of *Scutellospora viarum*.

Inoculation treatment	Root Volume/Plant (cm <sup>2</sup> )	Root Length (cm/plant)	Shoot length (cm/plant)	Plant dry biomass		
				Shoot	Root	Total
Uninoculated control	12.0 <sup>d</sup>	15.8 <sup>d</sup>	30.5 <sup>d</sup>	3.550 <sup>d</sup>	1.030 <sup>d</sup>	4.580 <sup>d</sup>
<i>Acaulospora scrobiculata</i>	12.8 <sup>c</sup>	16.9 <sup>c</sup>	32.2 <sup>b</sup>	3.865 <sup>d</sup>	1.214 <sup>c</sup>	5.079 <sup>b</sup>
<i>Archaeospora trappei</i>	13.4 <sup>c</sup>	16.9 <sup>c</sup>	32.5 <sup>b</sup>	3.862 <sup>b</sup>	1.215 <sup>c</sup>	5.07 <sup>d</sup>
<i>Gigaspora margarita</i>	13.4 <sup>d</sup>	17.2 <sup>b</sup>	33.4 <sup>b</sup>	3.924 <sup>b</sup>	1.265	5.189 <sup>b</sup>
<i>Glomus aggregatum</i>	14.2 <sup>a</sup>	18.5 <sup>a</sup>	36.2 <sup>a</sup>	4.362 <sup>a</sup>	1.652 <sup>a</sup>	6.014 <sup>a</sup>
<i>Glomus fasciculatum</i>	13.8 <sup>b</sup>	17.4 <sup>b</sup>	32.8 <sup>c</sup>	3.878 <sup>c</sup>	1.042 <sup>d</sup>	5.220 <sup>b</sup>
<i>Glomus pakistanika</i>	13.0 <sup>c</sup>	16.8 <sup>c</sup>	32.0 <sup>c</sup>	3.624 <sup>d</sup>	1.045 <sup>d</sup>	4.669 <sup>c</sup>
<i>Scutellospora persica</i>	13.1 <sup>c</sup>	16.6 <sup>c</sup>	32.5 <sup>c</sup>	3.870 <sup>c</sup>	1.068 <sup>c</sup>	4.938 <sup>c</sup>

Means in each column followed by the same letter are not significantly different (P<0.05) from each other according to DMRT.



**Fig.1.** Influence of native AM fungi on percent mycorrhizal root colonization and spore numbers in the root-zone soils of *Solanum viarum*. 1. uninoculated control, 2. *Archaeospora trappei*, 3. *Acaulospora scrobiculata*, 4. *Gigaspora margarita*, 5. *Glomus aggregatum*, 6. *Glomus fasciculatum*, 7. *Glomus pakistanika*, 8. *Scutellospora persica*.

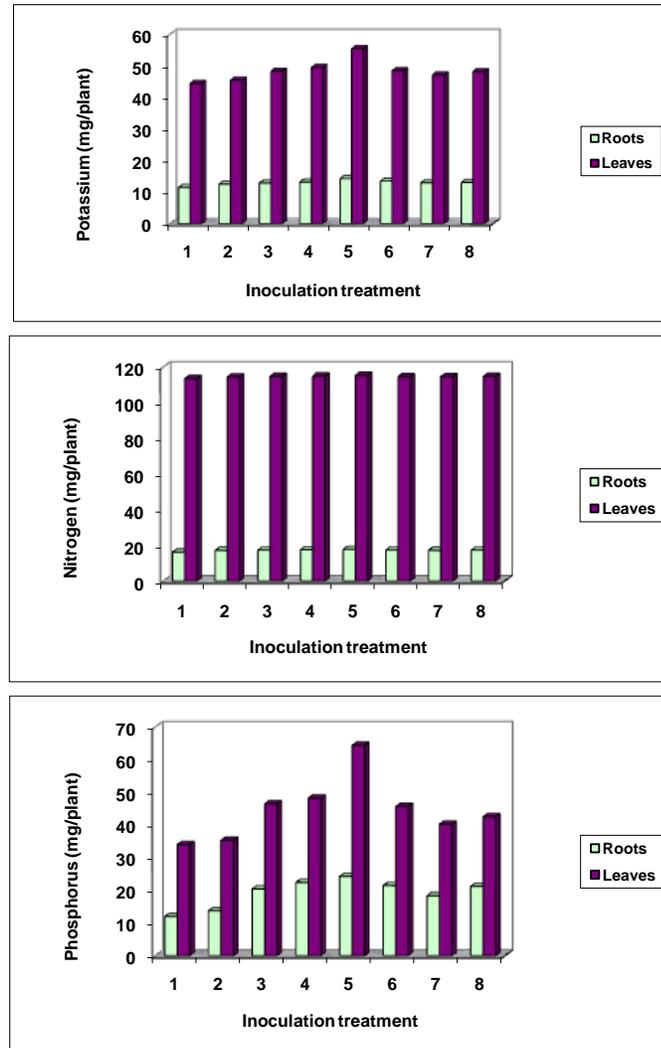
### *Nutritional status*

Mycorrhizal inoculation resulted in significant increase in shoot and root N, P, K, Zn, Cu, and Fe, content (Fig. 2, Table 2). The highest N, P, K, Zn, Cu, and Fe content was recorded in plants inoculated with *Glomus aggregatum* which differed significantly from other treatments (Fig. 2 and Table 2). Such a variation in the plant nutrient status in relation to the fungal species for other medicinal plant species is well documented (Reena and Bagyaraj, 1990; Chiramel *et al.*, 2006; Rajeshkumar *et al.*, 2008). The enhancement in growth, biomass and nutritional status is also related to the present root colonization apart from several soil and environmental factors. AM fungi differ greatly in their symbiotic effectiveness which depends on their preference for particular soils or host plant specificity (Dhillon, 1992), direct ability to stimulate plant growth, rate of infection, competitive ability, and tolerance to applied chemicals. Giving growth, biomass and nutritional status, *G. aggregatum* was found to be the best AM fungus for inoculating *S. viarum* in the nursery in order to obtain healthy, vigorously growing seedlings that could establish and perform better when planted in sandy loam soils.

**Table 2.** Influence of native AM fungi on Zn, Cu and Fe contents in shoot and root of *Scutellospora viarum*.

Treatment	Zinc content (µg/Plant)		Copper content (µg/Plant)		Iron content (µg/Plant)	
	Root	Shoot	Root	Shoot	Root	Shoot
Control (without AM fungi)	94.5 <sup>a</sup>	163.8 <sup>a</sup>	49.5 <sup>a</sup>	61.8 <sup>a</sup>	52.8 <sup>a</sup>	59.5 <sup>a</sup>
<i>Acaulospora scrobiculata</i>	105.6 <sup>b</sup>	194.2 <sup>b</sup>	56.8 <sup>b</sup>	74.8 <sup>b</sup>	68.4 <sup>b</sup>	64.2 <sup>b</sup>
<i>Archaeospora trappei</i>	106.4 <sup>b</sup>	198.2 <sup>b</sup>	58.2 <sup>b</sup>	76.2 <sup>b</sup>	66.2 <sup>b</sup>	68.4 <sup>b</sup>
<i>Gigaspora margarita</i>	128.4 <sup>c</sup>	212.4 <sup>c</sup>	64.2 <sup>c</sup>	112.3 <sup>c</sup>	70.4 <sup>c</sup>	72.8 <sup>c</sup>
<i>Glomus aggregatum</i>	192.5 <sup>d</sup>	290.5 <sup>d</sup>	68.5 <sup>d</sup>	116.6 <sup>d</sup>	92.4 <sup>d</sup>	95.8 <sup>d</sup>
<i>Glomus fasciculatum</i>	184.6 <sup>d</sup>	282.4 <sup>d</sup>	64.8 <sup>c</sup>	114.2 <sup>c</sup>	90.5 <sup>d</sup>	92.2 <sup>d</sup>
<i>Glomus pakistanika</i>	112.5 <sup>b</sup>	199.4 <sup>b</sup>	58.4 <sup>b</sup>	91.2 <sup>b</sup>	70.2 <sup>c</sup>	69.5 <sup>b</sup>
<i>Scutellospora persica</i>	124.6 <sup>c</sup>	212.8 <sup>c</sup>	58.6 <sup>b</sup>	98.4 <sup>b</sup>	70.6 <sup>c</sup>	72.4 <sup>c</sup>

Means (n=5) in each column followed by the same letter are not significantly different (P<0.05) from each other according to DMRT.



**Fig. 2.** Macronutrient content in the roots and leaves of *Scutellopora viarum* as influenced by native AM fungi. 1. uninoculated control, 2. *Arachaeospora trappei*, 3. *Acaulospora scrobiculata*, 4. *Gigaspora margarita*, 5. *Glomus aggregatum*, 6. *Glomus fasciculatum*, 7. *Glomus pakistanika*, 8. *Scutellopora persica*.

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