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Effect of arbuscular mycorrhizal fungi on growth and nutrition of *Wedilia chinensis* (Osbeck) Merril.

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Abstract
A greenhouse experiment was conducted to select an efficient Arbuscular mycorrhizal fungal (AMF) strain to improve the growth of *Wedilia chinensis* (Osbeck) Merril. Seedlings were infested with seven different indigenous AM fungi viz., *Acaulospora deligata, Glomus aggregatum, G. feugianum, G. fasciculatum, G. rubiforme, Gigaspora margarita* and *Scutellospora heterogama.* In general, all the strains of fungal inoculations resulted in increase of the plant growth, biomass and nutrition. Among the seven AM fungal treatments, pre-inoculation with *Glomus fasciculatum* improved the total seedling biomass and nutrition uptake better in this medicinal plant.

Keywords: *Wedilia chinensis*, AMF, growth, biomass, nutrition, mycorrhizae, medicinal plant.

Introduction
Mycorrhizal associations are symbiotic relationships between nearly all higher plants and soil fungi. Arbuscular mycorrhizae are the most widespread symbiotic associations known to occur between the roots of more than 80% of higher plants and belong to the phylum Glomeromycota (Schußler *et al*., 2001). The major benefits of this mycorrhizal association include enhanced plant growth through improved access to the plants of mineral nutrients and water (Jakobsen *et al*., 2003). A few scientists have observed wide variation among and different species on Arbuscular mycorrhizal fungi (AMF) in their ability to promote plant growth (Sreenivasa, 1992).

Different AMF species induce differential growth responses, in terms of biomass production and clonal growth patterns of coexisting plant species (van der Heijden, 1998). Furthermore, the biomass produced by most of the plant species varied significantly among treatments with different single AMF taxa, indicating that different plant species benefited to different extents from different AMF taxa. This led to the concept of host preference by AM fungi (Mosse, 1973). Hence, the present study was initiated to select an efficient AM fungus for a particular host-soil-climate combination to harness maximum benefits.

Wedilia chinensis (Osbeck) Merril. (Asteraceae) are traditionally used to treat skin, digestive and respiratory diseases (Alasbahi *et al*., 1999). In India, *W. chinensis* is commonly known as 'Manjal karisalankanni' in Tamil and 'Pilabhamgara' in Hindi and largely used in Indian siddha medicine. The plant is astringent, bitter, acrid, thermogenic, anti-inflammatory, vulnerary ophthalmic, cardiotpnic, anthelmintic, diuretic and aphrodisiac and is useful in vitiated conditions of Kapha and Vata, inflammation, elephantiasis, otalgia, cephalagia, wounds, ulcers, dysopia, colic, helminthiasis, anaemia, seminal weakness, fever, baldness and greyness of hair. The plant is very specific for viral hepatitis (Anonymous, 1983). Hence, an attempt was made to study the growth response of this medicinal plant to different indigenous AMF inoculation and to select an efficient AMF strain for this particular host in order to harness the maximum benefit from the fungus.

Materials and methods
A green house experiment was conducted to evaluate the effect of indigenous AMF on growth, biomass and nutrition of the important medicinal plant *W. chinensis*. The AMF spores (*Acaulospora deligata, Glomus aggregatum, G. feugianum, G. fasciculatum, G. rubiforme, Gigaspora margarita* and *Scutellospora heterogama*) used in this study were isolated from the rhizosphere soil of *W. chinensis* cultivated at the herbal garden of Kongunadu Arts and Science College, Coimbatore, Tamil Nadu. These AM fungal species were isolated by using wet sieving and decanting method (Gerdemann & Nicolson, 1963). The species level identification of different AMF species was done following the keys provided by Schenck and Perez (1990). These fungi were multiplied using sterilized sand: soil mix (1:1 v/v) as a 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 inoculums. The soil in each polythene bag was mixed with these inoculums. The soil used in this study was sterilized sand-soil mixture (1:1), having pH 7.2, and available phosphorus 2.7 ppm, total nitrogen 2.3 ppm and organic carbon 0.13%. Each bag containing the potting mixture with or without inoculums was planted with one seedling of *W. chinensis*. One set of the plant without inoculation was the control. Each treatment with 5 replication was maintained in green house and watered regularly. All the bags were irrigated with Ruakura plant nutrient solution.

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Ninety days after transplanting, the plants were harvested for determination of the growth, 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 samples at 60°C to a constant weight in a hot air oven. Phosphorus and potassium content of the plant tissue (plant powder) were determined by employing the vanadomolybdate phosphoric yellow colour and flame photometric method (Jackson, 1973; Jones & Isaac, 1969) respectively. Atomic absorption spectrophotometry was employed to estimate zinc, copper, manganese and iron content of the plant samples, using respective hollow cathode lamps (Clarson, 2002). The data thus generated were subjected to statistical analysis of completely randomized block design and the means were separated by Duncan's multiple range test (DMRT).

Table 1. Different native AM fungi and their influence on growth of W. chinensis (mean of 5 replicates).

Treatments*	Plant height (cm)		Plant dry weight (g/plant)	
	Shoot	Root	Shoot	Root
Control (without AM fungi)	52.5^{a}	20.4^{a}	14.5^{a}	8.5^{a}
Acaulospora delegata	62.0^{b}	26.2^{ab}	18.0^{b}	10.2^{b}
Gigaspora margarita	64.5^{c}	28.4^{c}	19.0^{ab}	10.4^{b}
Glomus aggregatum	66.2^{c}	29.2^{c}	19.4^{c}	10.6^{c}
Glomus fasciculatum	68.5^{d}	29.8^{d}	21.2^{d}	11.2^{d}
Glomus feugianum	64.2^{c}	26.4^{c}	19.1^{c}	10.2^{b}
Glomus rubiforme	63.4^{b}	24.2^{ab}	18.4^{ab}	10.6^{c}
Scutellospora heterogama	63.2^{b}	23.2^{b}	18.2^{b}	10.2^{b}

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

Results and discussion

Mycorrhizal seedlings showed distinct variations than the non-mycorrhizal seedlings for most of the growth parameters. The response of *W. chinensis*, pre-inoculated with different AM fungal species was found to be varied. Mycorrhizal inoculation resulted in a significant increase in height, biomass and nutrient content of *W. chinensis* seedlings. This report supports earlier investigations in medicinal plants (Earanna *et al*., 2002; Rajan *et al*., 2004) and the previous studies also indicated the host preference to the AM fungi. It is well known that enhanced nutritional status of a plant is manifested in improved growth (Jeffries, 1987). *W. chinensis* plants inoculated with AM fungi showed a general increase in growth parameters such as plant height, total dry weight than those of the uninoculated plants. However, the difference in shoot length and root length was significant between the treatments (Table 1).

Seedlings raised in the presence of *G. fasciculatum* showed an increase in shoot and root length as well as in dry weights, followed by those grown in the presence of *G. aggregatum* than the other treatments. Earlier studies also showed such a trend for medicinal plants subjected to AM inoculation (Bukhari & Rodrigues, 2008; Rajeshkumar *et al*., 2008; Ndiaye *et al*., 2009). Thus, AM fungi used in inoculations are efficient in colonization as reported earlier in *Ablemoschus esculentus* (Krishna & Bagyaraj, 1982). The nutritional status of *W. chinensis* seedlings, viz., phosphorus, potassium, zinc, copper, magnesium and iron content, was also significantly higher in plants raised in soil inoculated with AM fungi (Table 2). The extent of increase in plant nutrient content varied among the fungi studied. Seedlings grown in the presence of *G. fasciculatum* had significantly higher content of nutrient followed by *G. aggregatum*. Such variations in plant nutrient status in relation to the fungal species for other medicinal plant species were well documented (Rajan *et al*., 2004; Rajeshkumar *et al*., 2008; Ndiaye *et al*., 2009). Differences on N, P, K uptakes recorded with *Glomus* species, confirmed that genetic factors play a role in translocation of mineral elements (Diop *et al*., 2003). The enhancement in growth and nutritional status is also related to the percent root colonization apart from several soil and environmental factors.

This study clearly shows an efficient biological response of *W. chinensis* seedlings towards different AM fungi, with *G. fasciculatum* conferring greater benefits compared to all other fungi used. The results clearly indicate that AM fungal inoculation can substantially reduce fertilizer requirement in seedling production. Application of efficient AM fungal symbionts can be

Table 2. Influence of native AM fungi on P, K, Zn, Cu, Mg & Fe content of W. chinensis (Means of 5 replicate)

Treatments*	P (mg/plant)	K (mg/plant)	Zn conc. (µg/plant)	Cu conc. (µg/plant)	Mn conc. (µg/plant)	Fe conc. (µg/plant)
Control (without AM fungi)	3.14^{a}	3.0^{a}	145.8^{a}	59.4^{a}	24.8^{a}	62.4^{a}
Acaulospora delegata	4.28^{b}	3.6^{b}	154.2^{b}	65.4^{b}	33.2^{b}	64.2^{b}
Gigaspora margarita	5.01^{c}	4.1^{c}	160.8^{c}	66.2^{c}	34.5^{c}	68.8^{c}
Glomus aggregatum	5.12^{c}	4.2^{c}	162.6^{c}	68.8^{c}	45.6^{d}	72.4^{c}
Glomus fasciculatum	5.84^{d}	4.4^{d}	168.4^{d}	72.4^{d}	48.2^{d}	78.5^{d}
Glomus feugianum	4.42^{b}	3.4^{b}	156.4^{b}	65.2^{b}	33.4^{b}	66.4^{b}
Glomus rubiforme	4.38^{b}	3.3^{b}	155.6^{b}	64.5^{ab}	33.8^{b}	64.2^{b}
Scutellospora heterogama	4.45^{b}	3.5^{b}	158.6^{b}	63.2^{cb}	34.2^{c}	65.8^{b}

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

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effective in obtaining healthy seedlings which could be used commercially. Further research need to be focused on finding ecologically adapted and efficient AM fungal species for the growth and yield of economically important plants. There is another area of interest is to examine whether such AM fungal treatment enhances the biochemical content of our interest in those medicinal plants.

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