Influence of Arbuscular Mycorrhizal Fungi on Biochemical Changes in *Wedilla Chinensis* (Osbeck) Merril.

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ABSTRACT

A greenhouse experiment was conducted in order to evaluate the efficiency of seven Arbuscular Mycorrhizal Fungi (AMF) namely, Acaulospora deligata, Glomus aggregatum, G. feugianum, G. fasciculatum, G. rubiforme, Gigaspora margarita, and Scutellospora heterogama collected from the rhizosphere soils of Wedilia chinensis (Osbeck) Merril. They were evaluated on the same host for improvement of phytochemical constituents, namely total phenols, ortho dihydroxy phenols, flavonoids, alkaloids, tannins and saponins and enhanced levels of acid and alkaline phosphatase, nitrate reductase, peroxidase and polyphenol oxidase activities. The extent of phytochemical constituents and levels of enzymes varied with the species of AM fungi inhabiting the W. chinensis seedlings. Analyses performed 90 days after inoculation showed that maximum beneficial effect in terms of increased phytochemical constituents and defense related enzyme levels was with Glomus fasciculatum.

Key words: *Wedilia chinensis, AM fungi, phytochemical constituents, phosphatase, nitrate reductase, peroxidase, polyphenol oxidase.*

INTRODUCTION

More than a century, observations have revealed that the roots of majority of land plants are associated with fungi. However, it is only in recent years that the significance of this association has emerged. Fungi forming an Arbuscular Mycorrhizae (AM) are most ubiquitous. AM fungi of the phylum Glomeromycota (Schüßler et al., 2001) are symbiotic organisms that colonize plant roots. AMF have been used to enhance the plant growth and yield of medicinal crops and to help maintain good health and fertility that contributes to a greater extent to a sustainable yield and good quality of the products (Rajan et al., 2004). This includes enhanced resistance to soil- borne pests and disease, improved resistance to drought and tolerance of heavy metals (Gosling *et al*., 2006). AM fungi are well known to bring about biochemical changes in plants by increasing various enzymatic activities (Mathur and Vyas, 1996). Phytochemical constituents, namely total phenols, ortho dihydroxy phenols, flavonoids, alkaloids, tannins and saponins are important in plant disease resistance. Acid and alkaline phosphatase and nitrate reductase are important enzymes of Phosphorous and nitrogen metaboilism respectively. Peroxidase andpolyphenol oxidase are important components of the defense mechanism of plants against pathogens.

Wedilia chinensis (Osbeck) Merril. (Asteraceae) are traditionally used to treat skin, digestive and respiratory diseases (Alsabahi *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 used in 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 evaluate possible benefits of AM fungal inoculation on phytochemical constituents and related enzyme levels of *W. Chinensis*.

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 and Nicolson, 1963).

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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 five replication was maintained in green house and watered regularly. All the bags were irrigated with Ruakura plant nutrient solution

Ninety days after transplanting, the plants were harvested for the determination of phytochemical constituents and enzyme level of the seedlings. The content of secondary metabolites, viz. total phenols, ortho dihydroxy phenols, flavonoids, tannins, saponins and alkaloids of the plants, were assayed (Farkas and Kiraly, 1962; Mahadevan and Sridhar, 1996; Sadasivam and Manickam, 1996 and Zakaria, 1991) respectively. Acid and alkaline phosphatase, Nitrate reductase, Peroxidase and polyphenol oxidase activity was estimated (Ikawa *et al*., 1964; Torriani, 1967; Pulter, 1974; Mahadevan and Sridhar, 1996) respectively.

RESULTS AND DISCUSSION

Mycorrhizal inoculated 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 were found to be varied. The phytochemical constituents of *W. chinensis* seedlings were found to be significantly higher in plants raised in soil inoculated with AM fungi (Table 1). The seedlings raised in the presence of *Glomus fasciculatum* showed the most increase of all phytochemical constituents in the plant tissues. Such a variation in the phytochemical constituents in relation to the fungal species for other medicinal plant species *Acacia holosericea* and *Adhathoda vasica* was also well documented (Rajan *et al*., 2004 and Rao *et al*., 2004) respectively.

Table 1. Changes of phytochemical constituents in *W. chinensis* inoculated with AM fungi.

Treatments	Total Phenol	Ortho dihydroxy-phenol	Flavonoids	Alkaloids	Tannins	Saponins
Control	94.5[a]	63.4[a]	3.46[a]	4.58[a]	0.272[a]	0.160[a]
Acaulospora deligata	123.8[c]	75.2[c]	3.85[b]	4.68[b]	0.285[b]	0.182[c]
Glomus aggregatum	135.5[d]	85.6[d]	3.92[c]	4.72[c]	0.290[c]	0.192[d]
Glomus feugianum	98.4[b]	68.2[b]	3.52[a]	4.62[a]	0.274[a]	0.172[b]
Glomus fasiculatum	128.5[c]	78.4[c]	3.86[a]	4.69[b]	0.286[b]	0.186[c]
Glomus rubiforme	99.5[b]	68.5[b]	3.54[a]	4.62[a]	0.276[a]	0.178[b]
Gigaspora margarita	126.2[c]	78.2[c]	3.85[b]	4.68[b]	0.285[b]	0.185[c]
Scutellospora heterogama	95.4[b]	67.5[b]	3.54[a]	4.61[a]	0.275[a]	0.174[b]

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

AM fungi inoculated plants showed higher acid and alkaline phosphatase than the uninoculated plants (Table 2). Out of seven AM fungi tested *Glomus fasciculatum* was found to increase the activity most efficiently. Phosphatase activity was much related to AM fungal activity. Mathur, *et al*. (2007) reported the increase in phosphatase activity with AM fungi inoculated *Tecomella undulate* and similar result was also obtained by Chethan kumar, *et al*. (2008) in *Sida cardifolia*. The nitrate reductase activity was also increased (Table 2). AM inoculation also increased peroxidase and polyphenol oxidase activity in *W. chinensis* seedlings (Table 2). Peroxidase and polyphenol oxidase are involved in the oxidation of phenolic compounds to quinones, which are presumably toxic to pathogens. The increased peroxidase activity may be due to an increased P-uptake, whereas the increased polyphenol oxidase activity may be due to increased accumulation of phenol content in plants. The result of this study was similar to that of Mathur, *et al*., (2007) in *Tecomella undulata* with eight AM fungal species. Among the seven AM fungi studied *Glomus fasciculatum* showed increase in all defense related enzyme levels followed by *Glomus aggregatum*.

It is concluded that *W. chinensis* seedlings show varied responses to different AM fungi, with *Glomus fasciculatum* conferring greater benefits compared to all other fungi used in this study. This fungal species caused elevation of phytochemical constituents and assimilating enzymes most efficiently. The results clearly indicate that AM fungal inoculation can substantially reduce fertilizer requirement in seedling production and in obtaining healthy seedlings.

Table 2. Changes of enzymes in *W. chinensis* inoculated with AM fungi.

Treatments	Acid phosphatase (µmp – nitrophenol / mg fresh wt. / min)	Alkaline phosphatase (µmp – nitrophenol / mg fresh wt. / min.)	Nitrate reductase (µmol – n^{-1} g^{-1} fresh wt.)	Peroxidase (OD/Min/g/ fresh wt.)	Polyphenol-oxidase (Catechol)
Control	242.0	172.0	0.284	2.65	0.36
Acaulospora deligata	285.0	264.0	0.292	2.94	0.38
Glomus aggregatum	298.0	282.0	0.298	2.98	0.42
G. feugianum	256.0	186.0	0.289	2.74	0.39
G. fasciculatum	294.0	275.0	0.294	2.96	0.39
G. rubiforme	258.0	188.0	0.292	2.74	0.38
Gigaspora margarita	292.0	272.0	0.296	2.94	0.38
Scutellospora heterogama	255.0	185.0	0.288	2.72	0.38

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