

**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.jjbpas.com

**PHYTOCHEMICAL SCREENING OF SELECTED MEDICINAL HERBS
INOCULATED WITH ARBUSCULAR MYCORRHIZAL FUNGI**

TEJAVATHI DH* AND JAYASHREE DR

Department of Botany, Jnanabhathi, Bangalore University, Bangalore-560056, India

Department of Biotechnology, M.S.Ramaiah College of Arts, Science and Commerce,

MSRIT Post, MSR Nagar, Bangalore-560054

*Corresponding Author: E Mail: tejavathi_hanu@yahoo.com; Mobile: +91 9448924734

ABSTRACT

Qualitative and Quantitative Phytonutrient analysis of *Euphorbia hirta*, *Eclipta alba*, *Leucas aspera* and *Achyranthus aspera*, inoculated with AM fungi was carried out. The AM fungi selected were *Aculospora bireticulata*, *A.laevis*, *A.lacunosa*, *Glomus aggregatum*, *G.geosporum*, *G.fasciculatum* and *G.mosseae*. The effect of the AM fungi on the contents of the primary and secondary metabolites of the selected medicinal herbs was analyzed. Quantitative analysis has revealed an increase in primary metabolites like proteins, carbohydrates and amino acids in AMF treated plants compared to control. While secondary metabolites like phenols, flavonoids alkaloids and terpenoids were found to be more in *E.hirta* inoculated with *G.mosseae*. Flavonoid contents have enhanced in all the selected medicinal plants inoculated with *G.aggregatum*, *G.mosseae* and *G.fasciculatum*. While the contents of total alkaloids have increased in *E.hirta* and *L.aspera* inoculated with *G.mosseae* and total terpenoids was high in *E.hirta* and *E.alba* inoculated with *G.mosseae* and *G.aggregatum*. Thus, the present study confirmed the host specificity by AM fungi. The significance of the distribution of their chemical constituents is discussed with respect to their yield.

Keywords: Medicinal Herbs, AMF, Phytochemicals, Yie

INTRODUCTION

Herbs are known as sources of phytochemicals or active compounds that have medicinal properties and are widely sought after. Nowadays, the growing interest in herbal medicines stems from a greater number of people seeking natural or alternative medicine, considering those medicines as “healthier” than the conventional ones [1, 2, 3]. O’ Hara *et al.*, [4], suggested that one of the best explanation for the enthusiasm towards natural plant products is that they are commonly perceived to be “healthier” than synthetically manufactured medicines.

The market for herbal medicine was considered as one of the most vital and high growth industries [5]. Hence there is need to develop herb varieties with better growth and increased concentrations of phytochemicals [6], with new techniques and new perspectives. Phytochemicals are often criticized for their lack of standardized concentrations from the natural plants [7]. Thus large scale and sustainable cultivation of medicinal herbs offering consistent phytochemical concentrations is highly desirable.

An alternative way of producing medicinal herbs could reside in the exploitation of the arbuscular mycorrhizal symbiosis. Once AMF

colonizes the host roots, the production of secondary plant compound has been shown to be altered. Despite numerous papers published in the last two decades on secondary metabolism in AM plants, very little work has been done on the accumulation of phytochemicals in AM medicinal herbs. There have been reports on the enhanced activity of antioxidants in AM plants, but mainly in response to abiotic stresses [8, 9, 10]. Other reports on medicinal herbs and AMF are aimed to understand the effects of inoculation on growth responses of these plants. The present study aims to provide an understanding of how the AM symbiosis affects host plants in a way that could be related to the production of higher concentrations of phytochemicals in medicinal herbs. Effect of various AM fungal species (*Acaulospora birecticulata*, *A.lacunosa*, *A.laevis*, *Glomus aggregatum*, *G.fasciculatum*, *G.geosporum* and *G.mosseae*) on the growth performance of commonly grown selected medicinal weeds such as *Achyranthes aspera*, *Eclipta alba*, *Euphorbia hirta* and *Leucas aspera* was studied.

MATERIALS AND METHODS

The experiment was designed with 8 treatments in 3 replicates. Totally 96

uniformly sized plastic pots (7x7') with capacity of holding 4 kg of sterilized sandy loam soil (autoclaved twice at 108 kpa for 90 min) was set up at green house in the department of Botany, Bangalore University. pH of the soil was adjust to 6.8 to 7. AMF inoculums of 0.8g (1gm = 1 lakh propogules) were placed at the depth of 2cm from the soil surface. The presoaked mature seeds collected from the selected plants were sterilized with 1% sodium hypochlorite were placed over the surface of the sterilized soil, pushed down to 1.5 cms deep in the soil and covered by the surrounding soil. Regular watering with shower was done on every alternate day and maintained for 90 days under green house conditions. The seedlings were thinned after 12 days of emergence to maintain 2 seedlings per pot. Pots without AMF inoculums served as control. After 90 days of treatment, the selective medicinal plant materials were harvested and used for phytochemical screening.

Extraction

The shoot of selected herbs were thoroughly washed and cut into pieces. The samples were dried in an oven at 60°C for 24 hrs [11] and powdered using mortar and pestle, preserved in labeled polytene packets. The samples were subjected to extraction with aqueous, ethanol

(90%), methanol (80%), chloroform (AR) and petroleum ether (AR).

Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures as described by Sofowara [12], Trease and Evans [13] and Harborne [14].

Qualitative Analysis

Test for Proteins (Biuret Test)

To 0.5 ml aqueous extract of samples, 0.5ml of 1% NaOH solution and 1 to 2 drops of 1% CuSO₄ were added. A violet color indicated the presence of peptide linkage of the molecules.

Test for Carbohydrates (Molish's Test)

To 0.5 ml of aqueous sample extract, 1 drop of Molish's reagent was added and shaken well. 0.5 ml of concentrated H₂SO₄ was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates. .

Test for Amino Acids (Ninhydrin Test)

To 0.5 ml of aqueous sample, 0.5ml of Ninhydrin reagent was added, kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Reducing Sugars (Fehlings Test)

To 1ml of aqueous extract, 0.5ml of Fehlings solution I & II were added. The mixture was boiled in water bath for 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

Test for Phenols (Folin Ciocalteu Test)

0.5 ml of the extract was mixed with 1ml of FC reagent and 0.5 ml of sodium carbonate. The tubes were vortexed for 15 seconds and allowed to stand for 30 minutes at 40°C for color development. An appearance of blue color indicates the presence of phenols.

Test for Tannins (Ferric chloride Test)

About 0.5ml of plant extract was dissolved in 10ml of boiling distilled water and filtered; to this one ml of 6% ferric chloride reagent was added. A deep green color precipitate which confirms the presence of tannins.

Test for Flavonoids (Aluminium Chloride Test)

0.5ml methanolic extract of the sample solution was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% NaNO₂ solution. After 6 minutes, 0.15ml of a 10% AlCl₃ solution was added and allowed to stand for 6 minutes, then 2ml of 4% NaOH solution was mixed along with distilled water to make up the volume to 5 ml. It was mixed thoroughly and allowed to stand for 15

minutes. Appearance of pink color indicated the presence of flavonoids.

Test for Alkaloids (Mayer's and Dragendorff's Test)

0.5gm of aqueous extract was stirred with 4ml of 1% diluted HCl. It was boiled and filtered.

Mayer's Test

1ml of the filtrate was treated with few drops of Mayer's reagent. Turbidity or precipitation indicated the presence of alkaloids.

Dragendorff's Test

1ml of the filtrate was treated with few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloids.

Test for Terpenoids (Salkowski Test)

1 ml of methanolic extract, 0.5ml of chloroform and 0.5ml of conc.H₂SO₄ were mixed to form a monolayer of reddish brown coloration at the interface in between the extract and chloroform which was shown to form a positive result for the terpenoids.

Quantitative determination of the Primary and Secondary Plant metabolites by Spectrophometric Method**Estimation of Total Protein - Lowry's Method [15]**

1ml of sample extraction in crude aqueous distilled water was estimated with 0.6 ml alkaline CuSO₄ and 0.5 ml Folin-Ciocalteu Reagent and Bovine Serum Albumin as

standard measured at 660 nm. From the standard graph x-y axis concentrations, total protein present in the sample was calculated.

Estimation of Total Carbohydrates- Anthrone Method [16]

1ml of crude 80% ethanolic sample extract with 4ml anthrone reagent was boiled in water bath for 10 minutes, cooled and read at 630nm. Glucose was used as standard and a graph was plotted. Total carbohydrates in the sample were calculated.

Estimation of Amino acid - Ninhydrin Method [17]

1ml of 80% methanolic sample extract, along with 1 ml of 0.25% Ninhydrin reagent was boiled for 15 minutes in water bath. After cooling it was read at 570nm. Tyrosine was standardized and a graph plot was drawn to calculate the amount of Amino acids.

Estimation of Total Phenolics - Folic-Ciocalteu Method [18]

1ml of 70% methanolic extract with 4ml of 1M Na₂CO₃ and 5ml Folin-Ciocalteu reagent (1:10 dilution) was mixed thoroughly and allowed to stand at room temperature for 15 minutes and read at 765nm. Standard graph against catechol was plotted and total phenols were calculated. All standards were carried out in triplicates and results were recorded as mean \pm SD.

Estimation of Total Flavonoids: Aluminium Chloride Method [19]

0.5 ml 80% methanolic sample extract, 0.1 ml of 10% of aluminium chloride, 0.1 ml of potassium acetate and 2.8ml of 80% ethanol after incubation at room temperature for 40 minutes was measured at 415nm. The calibration curve was prepared by using quercetine solution as standard. The total flavonoid contents were determined against the standard.

Estimation of Total Alkaloids - Bromo Cresol Green Method [20]

1ml of 80% methanolic sample extract was washed in chloroform, pH was adjusted to neutral with 0.1N NaOH. To this, 5ml BCG solution and 5ml of Phosphate buffer was added, the mixture was shaken vigorously with 3ml of chloroform and the extracts collected was made up to 10ml with chloroform. The absorbance of the complex in chloroform was measured at 470nm against the Atropine as standard.

Estimation of total Terpenoids - Ferguson's method [21]

500mg of plant powder were taken separately and soaked in alcohol for 24 hours, filtered. The filtrate was extracted with petroleum ether and the ether extract was treated as total terpenoids. The residue obtained was dried and weighed.

$$\text{Terpenoid Content (\%)} = \frac{\text{weight of terpenoid extract (gms)}}{\text{weight of sample (0.5gms)}} \times 100$$

RESULT AND DISCUSSION

During the establishment of arbuscular mycorrhizal symbiosis, a range of chemical and biological parameters are affected in plants including the pattern of primary and secondary metabolites [22]. Studies have shown that AMF symbiotic association with higher plants can influence the levels of amino acids [23], phytohormones [24], reduced sugars [22], carotenoids [25], terpenoids [26] and phenols [27]. Further quality and quantity of essential oils in several aromatic plants have altered due to this symbiotic association [28].

The quantitative analysis of proteins and amino acids in the present investigation revealed the enhancement in the shoot samples of all the four selected medicinal herbs inoculated with *A. birecticulata*, *G. aggregatum*, *G. fasciculatum* and *G. mosseae* compared to other species of AM fungi tested in the present investigation. As AM fungi increase the uptake of phosphorus, nitrogen and water, the nutritional status of the mycorrhizal plants is naturally improves than the non-mycorrhizal plants [29]. France and Reid [30] had attributed the increase in amino acids and protein to reverse

translocation of the carbon compounds to the host. Fattah and Mohamedin [31] correlated the level of increase in amino acids in the percent of colonization of AMF in sorghum. However, Matsubara *et al.*, [23] are of the opinion that the reason for the increase in the pool of amino acids to mycorrhizal strawberry plants needs further investigation. In the present studies, highest amino acid content was observed in *Leucas aspera* inoculated with *Glomus mosseae* (Figure 1). However, Selvaraj, [32] had reported an increase in amino acids contents in *Prosopis juliflora* inoculated with *G. fasciculatum*. Since free amino acids in diseased plants exhibit significant change in its composition, Sadasivan and Manickam, [33] have suggested that the physiological and health status of plants can be measured by the estimation of total amino acids. Protein content also increased in arbuscular mycorrhizal fungus inoculated plants. On the dry weight basis, Figure 2, shows the relatively high percentage of proteins content in *E. alba* (9.44±0.01%), *L. aspera* (9.45±0.01%) and *A. aspera* ((9.04±0.01%) inoculated with *G. aggregatum*, *G. mosseae* and *G. fasciculatum* respectively, while *E. hirta* (8.83±0.01%) inoculated with *G. mosseae* are of moderate. However, non mycorrhizal controls were comparatively poor

(< 3.30± 0.01%). It was revealed that *G.fasciculatum* inoculated, tannery effluent treated *P.juliflora* showed an increase of protein content in both leaves and roots than the control, whereas plants treated with tannery effluent alone showed least protein content due to the absence of mycorrhizal influence [32]. Higher protein content in mycorrhizal roots than non-mycorrhizal root extract was also observed by Arines *et al.*, [34] in red clover. The presence of higher protein level in the mycorrhizal plants indicate towards their possible increased food value or a protein bioactive compound could also be isolated in future [35].

The carbohydrate status of mycorrhizal plants ought to change since they harbor the AM fungi in their root system. The primary roots of young seedlings compete for soluble sugars with hyphae of AM fungi, which lead to the lower soluble sugar levels in mycorrhizal roots and these mycorrhizal complexes can be called the “gully” of carbohydrates. It is the formation of mycorrhizae that in turn affects other physiological indexes of seedlings, such as increases the leaf chlorophyll contents, enhancing their photosynthesis [36], which are the basis of more carbohydrates been manufactured. The aerial parts of mycorrhizal plants grow better than those of non-mycorrhizal plants, and then lots of

carbohydrates manufactured via photosynthesis are translocated to roots, which may be a major cause of increase in soluble sugar levels. Thus, Mycorrhizal plants exhibit enhanced photosynthesis and assimilation of carbohydrates than those of non mycorrhizal plants [37]. Whereas, in the present analysis, highest total carbohydrate contents were recorded in *E. alba* and *A. aspera* inoculated with *G. aggregatum* and *G. fasciculatum* respectively (**Figure 3**). However, Wu *et al.*, [38] who observed that *G. mosseae* significantly increased soluble sugar concentrations of leaf and root in red tangerine. Selvaraj [32] reported increased soluble sugars in both leaves and roots in *G.fasciculatum* inoculated *P.juliflora*. Same *et al.*, [39] reported that the percentage of root infections by *G.fasciculatum* was closely correlated with concentrations of soluble carbohydrates in the inoculated roots.

The altered biosynthetic primary metabolite pathways have resulted in the variation of secondary metabolite contents since secondary metabolites are biosynthetically derived from primary metabolites. Phenols, flavonoids, alkaloids and terpenoids contents were more in all the selected plants inoculated with *A. bireticulata*, *G.aggregatum*, *G fasciculatum* and *G.mosseae* compared to other species. **Figures 4-7** summarizes the

contents of phenols, flavonoids, alkaloids and terpenoids in selected medicinal plants as a result of mycorrhization. Phenolics are known to be of major importance in pathogenic interactions between plant and fungi. Increased phenolic concentration in plant tissues following pathogenic attack is one of the important mechanisms by which pathogen activity may be limited or decreased [40]. Devi and Reddy [41] have reported that inoculation with *Glomus mosseae* induced not only qualitative but also quantitative changes in phenolic acids in groundnut plant tissues. Jasmonic acid concentration was nine times higher in mycorrhizal plants than non-mycorrhizal shoots [42]. The accumulation of phenols or their precursors protect the mycorrhizal plants from pathogens or prepares them to react faster by releasing defense phenolics [43]. In addition phenolics present in medicinal plants have received considerable attention because of their potential antioxidant activity as stated by Zheng *et al.*, [44]. Methanol and ethanol were proven as effective solvents to extract antioxidant phenolic compounds [45]. *E.alba* and *A.aspera* inoculated with *G. aggregatum* and *G.fasciculatum* showed the highest total phenolic content ($9.61 \pm 0.01\%$ dw), while all the tested medicinal plants inoculated with *Aculospora* species had the lowest value

ranging from 6.45% to 7.65% dw and very poor in uninoculated controls less than 3.00% dw. Similar accumulation of phenol in AM plants has been reported by Krishna and Bagyaraj [46] and experimental results suggested that phenolics may function as signals in plant development and in plant-microbe interactions [47].

Flavonoids are one of the most diverse and wide spread groups of natural products which are probably the most important natural phenolics. Several flavonoids have been reported to quench active oxygen species and inhibit *in vitro* oxidation of low-density lipoproteins [48, 49]. From the present analysis, it can be concluded that the selected plants inoculated with AMF are rich in flavonoids. The amount of flavonoids in the tested plants varies from 4.73% to 10.09 % dw. The highest flavonoids content was found in *A.aspera* (10.09 % dw) inoculated with *G.fasciculatum* and lowest amount was obtained in uninoculated control (4.73% dw). The flavonoid contents obtained from mycorrhizal plants was remarkably higher than the content in control. Steinkeller *et al.*, [50] have reviewed the role of flavonoids in pathogenic plant-fungus interactions and concluded that only limited information is available on flavonoids as signaling compounds for fungal pathogens of legumes.

The phytochemical estimation of the medicinal plants showed that they are rich in alkaloids and terpenoids. A variety of plant alkaloids have been shown to be anti-carcinogenic in several animal models [51, 52]. Colonisation of plant roots by symbiotic AMF has been shown to induce the accumulation of secondary metabolites arising from phenolic and terpenoid metabolism [53, 54, 55]. From present analysis, it can be concluded that total alkaloids was more in *E.hirta* (9.75%dw) and *L.aspera* (9.33%dw), inoculated with *G.mosseae* and *G.aggregatum* respectively, while terpenoids were high in *E.hirta* and *E.alba*, inoculated with *G.fasciculatum*, and *G.aggregatum* (4.81%dw and 4.66%dw) respectively than the non-mycorrhizal controls. The biological function of alkaloids is very important and is used in analgesic, antispasmodic and bacteroidal activities. It can be concluded that alkaloids were found to be very high in *E. hirta* as compared to what was obtained by Iqbal [56] in their study of selected medicinal herbs. The presence of high levels terpenoids in medicinal plants has been reported by Rahila *et al.*, [57] and Burkill [58]. The presence of high terpenoids in *E. hirta* and *E.alba* can be exploited in their usage as purgatives.

CONCLUSION

Earlier reports indicated that AM fungal association improves the physiological status and growth performance of mycorrhiza treated plants [59, 60]. AMF when associated with the roots of higher plants play an important role in improving the intake of water and nutrients from the rhizosphere which are otherwise of low mobility in soil solution, thereby enhancing the growth performance of mycorrhizal plants. While the present analysis clearly indicates that the selected medicinal plants which are considered as weeds have the potential to accumulate high levels of phytonutrients. Hence it can be concluded that all the selected medicinal plants can be grown in large scale in association with compatible AM fungal species to enhance their phytonutrients and have a high prospective in pharmaceuticals.

REFERENCES

- [1] Cox PA, The promise of Gerard's Herball: new drugs from old books, *Endeavour*, 22, 1998, 51-53.
- [2] MacLennan AH, Wilson DH and Taylor AW, The escalating cost and prevalence of alternative medicine, *Preventive Medicines*, 35, 2002, 166-173.
- [3] Duncan E, Food as medicine. *The Economist*, 369, 2003, 11-13.

- [4] O'Hara M, Kiefer D, Farrell K and Kemper K, A review of 12 commonly used medicinal herbs, Arch. of family Medicines, 7, 1998, 523-536.
- [5] Pubrick P, Medicinal Herbs, In: The new rural industries: A handbook for farmers and investors, RIRDC, 1998, 371-376.
- [6] Rai MK, Current advances in mycorrhization in micropropagation, In vitro Cell Dev. Biol. Plant, 37, 2001, 158-167.
- [7] Israelsen LD, Phytomedicines as a new crop opportunity, In: New crops, New York, Wiley and Sons Inc., 1993, 660-671.
- [8] Schutzendubel A and Polle A, Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization, J. Experimen. Bot., 53, 2002, 1351-1365.
- [9] Porcel R and Ruiz-Lozano JM, Arbuscular mucorrhizal influence on leaf water potential, solute accumulation and oxidative stress in soybean plant subjected to drought stress, J. Experimen. Bot., 55, 2004, 1743-1750.
- [10] Porcel R, Barea JM and Ruiz-Lozano JM, Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence, New Phytologist, 157, 2003, 135-143.
- [11] Haes, JH and Krikun J, Efficacy of endomycorrhizal-fungus isolates and inoculums quantities required for growth response, New Phytologist, 100 (4), 2006, 613-621.
- [12] Sofowara AE, Medicinal plants and traditional medicine in Africa, Vol. 2, Spectrum Books Ltd, Ibadan, 1993, 288.
- [13] Trease GE and Evans WC, Pharmacognosy 2nd Ed., Braille Tiridel and Macmillan Publishers, 1989.
- [14] Harborne JB, Methods of plant analysis, In: Phytochemical methods, Chapman and Hall, London, 1973.
- [15] Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ, Protein measurement with the folin-phenol reagent, J. Biol. Chem., 193, 1951, 263-275.
- [16] Yemm EW and Willis AJ, The estimation of carbohydrates in plant extracts by anthrone, Biochem., 57, 1954, 507-514.

- [17] Rosen H, A modified ninhydrin colorimetric analysis for amino acids, Arch. Biochem. Biophys., 67, 1957, 10-15.
- [18] Malik EP and Singh MB, "Plant Enzymology and Hittoenzymology", (I Ed.), Kalyani Publishers: New Delhi, 1980, 286.
- [19] Mervat M, M El Far, Hanan A Taie, "Antioxidant activities, total anthroapines, phenolics and flavonoids contents of some sweet potato genotypes understress of different concentrations of sucrose and sorbitol", Aust. J. Basic Appl. Sci., 3, 2009, 3609-3616.
- [20] Shamsa F, Monsel H, Ghamooshi R and Verdian-rizi M "Sphectrophotometric determination of total alkaloids in some Iranian medicinal plants", Thai J. Pharma. Sci., 32, 2008, 17-20.
- [21] Ferguson N, A textbook of pharmacognosy, Max Millam Company, 1956, 191.
- [22] Tejavathi DH, Anitha P, Savitha M Murthy and Nijagunaiah R, Effect of AM fungal association with normal and micropropagated plants of *Andrographis paniculata* Nees on biomass, primary and secondary metabolites, Int. Res. J. Plant Sci., 2 (12), 2011, 338-348
- [23] Matsubara Y, Ishigaki T, Koshikawa K, Changes in free amino acid concentrations in mycorrhizal strawberry plants, Sci. Hort., 119, 2009, 392-396.
- [24] Barker SJ and Tagu D, The roles of auxins and cytokinins in mycorrhizal symbioses, J. Plant Growth Regulation, 19, 2000, 144-154.
- [25] Fester T, Schmidt D, Lohse S, Walter MH, Guiliano G, Bramley PM, Frase.PD, Hause B and Strack D, Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots, Planta, 216, 2002, 148-154.
- [26] Hause B, Maier W, Miersch O Kramell R and Strack D, Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots, Plant Physiol., 130, 2002, 1213-1220.
- [27] Zhu HH and Yao Q, Localized and systematic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanocearum*, J. Phytopathol., 152, 2004, 537-542.
- [28] Kapoor R, Giri B, and Mukerji KG, *Glomus macrocarpum*, potential bioinoculant to improve essential oil

- quality and concentration in dill (*Anethum graveolens* L.) and *carum* (*Trachyspermum ammi* L. Sprague). World J. Microbiol. and Biotechnol., 18, 2002, 459-463.
- [29] Kothamasi D, Kuhad RC and Babu CR, Arbuscular Mycorrhizae in plant survival strategies, Tropical Ecol., 4 (1), 2001, 1-13.
- [30] France R and Reid CPP, Interactions of nitrogen and carbon in the physiology of ectomycorrhizae, Can. J. Bot., 61, 1983, 964-984.
- [31] Fattah AGM and Mohamedin AH, Interactions between vesicular mycorrhizal fungus (*Glomus interadices*) and *Streptomyces coelicolor* and their effects on Sorghum plant grown in soil amended with chitin of brown scales, Biol. Fertil. Soils, 32, 2000, 401-409.
- [32] Selvaraj T, Studies on mycorrhizal and rhizobial symbioses on tolerance of tannery effluent treated *Prosopis juliflora*, Ph.D., Thesis, University of Madras, Chennai, India, 1998, 209.
- [33] Sadasivam S and Manickam A, Biochemical method, Iled., New Age International (P) Limited Publishers, New Delhi, 1996.
- [34] Arines J, Palma JM and Vilarino A, Comparison of protein patterns in non-mycorrhizal and vesicular-arbuscular mycorrhizal roots of red clover, New Phytol., 123, 1993, 763-768.
- [35] Thomsen S, Handen HS and Nyman V, Ribosome inhibiting proteins from in vitro cultures of *Phytolacca dodecandra*, Plant. Med., 57, 1991, 232-236.
- [36] Li YH, Zheng F, Ni DJ, Yang JF and Shi YT, Study on Physiological Characteristic of Tea Plant Inoculated by VA Mycorrhiza, J. Tea Sci., 31 (6), 2011, 504-512.
- [37] Ghorbanli M, Ebrahimzadeh H and Sharifi M, NaCl and mycorrhizal fungi on antioxidative enzymes in soybean, Biologia Plantarum ,48, 2004, 575-581.
- [38] Wu QS, Zou YN and He XH, Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress, Acta Physiol. Plant, 32, 2010, 29-304.
- [39] Same BI, Roboson AD and Abbott LK , Phosphorus, soluble carbohydrates and endomycorrhizal

- infection, *Soil Biol. Biochem.*, 15, 1983, 593-597.
- [40] Morandi D, Occurrence of phytoalexin and phenolic compounds in endomycorrhizal interactions and their potential role in biological control, *Plant Soil*, 185, 1996, 241-25.
- [41] Devi MC and Reddy MN, 'Phenolic acid metabolism of groundnut (*Arachis hypogaea* L.) plants inoculated with VAM fungus and Rhizobium, *Plant Growth Regulation*, 37, 2002, 151-156.
- [42] Kapoor R, Induced resistance in mycorrhizal tomato is correlated to concentration of Jasmonic acid, *Online J. Biol. Sci.*, 8, 2008, 49-56.
- [43] Morandi D, Bailey JA and Gianinazzi-Pearson V, Isoflavonoid accumulation in soybean roots infected with vesicular-arbuscular mycorrhizal fungi, *Physiological Plant Pathol.*, 24, 1984, 357-364.
- [44] Zheng X, Liu B, Li L, and Zhu Z, Micro-wave-assisted extraction and antioxidant activity of total phenolic compounds from pomegranate peel, *J. Med. Plants Res.*, 15, 2011, 1004-1011.
- [45] Esmaeili MA and Sonboli A, Antioxidant, free radical scavenging activities of *Salvia Brachyantha* and its protective effect against oxidative cardiac cell injury, *Food and Chemical Toxicol.*, 48, 2010, 846-853.
- [46] Krishna KR and Bagyaraj DJ, Phenols in mycorrhizal roots of *Arachis hypogaea*, *Experimentia*, 40, 1984, 85-86.
- [47] Lynn DG and Chang M, Phenolic signals in cohabitation: Implications for plant development, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 41, 1990, 497-526.
- [48] Leake D, Flavonoids and the oxidation of low-density lipoprotein, *Nutrition*, 17, 2001, 59-63.
- [49] Aviram M and Fuhrman B, Polyphenolics flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis, *Atherosclerosis*, 137, 1998, S45-S50.
- [50] Steinkeller S, Lenzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP and Vierheilig H, Flavonoids and Strigolactones in root exudates as signals in symbiosis and pathogenic plant-fungus interactions, *Molecules*, 12, 2007, 1290-1306.

- [51] Reddy L, Odhav B and Bhoola KD, Natural products for cancer prevention, A global perspective, *Pharmacol. and Therapeut.*, 99, 2003, 1-13.
- [52] Kandaswami C, Lee LT, Lee PP, Hwang JJ, Ke FC, Huang YT and Lee MT, The antitumour activities of flavonoids, *In Vivo*, 19, 2005, 895-909.
- [53] Peipp H, Maier W, Schmidt J, Wray V and Strack D, Arbuscular mycorrhizal fungus – induced changes in the accumulation of secondary compounds in Barley roots, *Phytochem.*, 44, 1997, 581-587.
- [54] Tang M, Chen H and Shang HS, Mechanism of vesicular-arbuscular mycorrhizal fungi enhanced the resistance of poplar to canker, *Scientia Silvae Sinica*, 36, 2000, 87-92.
- [55] Krishna H, Singh SK, Sharma RR, Khawale RN, Grover M and Patel VB, Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlet due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization, *Scientia Horticulturae*, 106, 2005, 554-567.
- [56] Iqbal H, Riaz U, Rooh U, Muhammad K, Naseem U, Abdul B, Farhat A, Muneeb R, Mohammed Z, Jehangir K and Naeem K, Phytochemical analysis of selected medicinal plants, *Afr. J. Biotechnol.*, 10 (38), 2001, 7487-7492.
- [57] Rahila T, Rukhsandra N and Zaidi A, Phytochemical screening of medicinal plants belonging to *Eubhorbiaceae*, *Pak. Vet. J.*, 14, 1994, 160-162.
- [58] Burkill H, The useful plants of West Tropical Africa families, A. D. Royal Botanical, 1994, 411-415.
- [59] Karthikeyan B, Joe MM and Jaleel CA, Response of some medicinal plants to VAM inoculations, *J. Sci. Res.*, 1 (1), 2009, 381-386.
- [60] Tejavathi DH and Jayashree DR, Effect of AM fungi association on the growth performance of selected medicinal herbs, *Indian J. Appl. Res.*, 3 (7), 2013, 12-15.

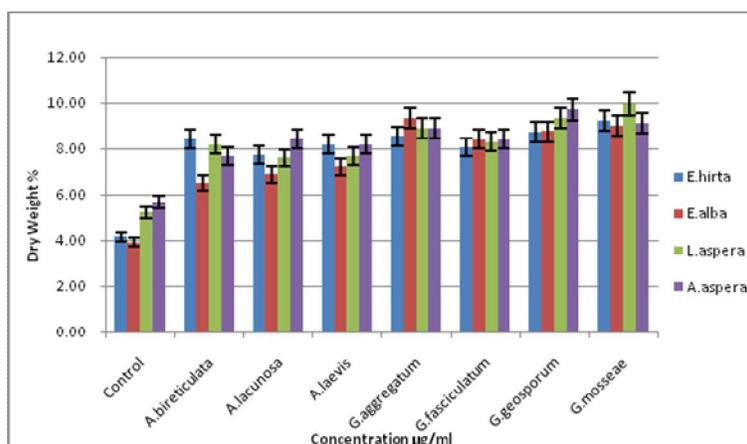


Figure 1: Estimation of Amino Acids in AMF Inoculated Selected Medicinal Herbs

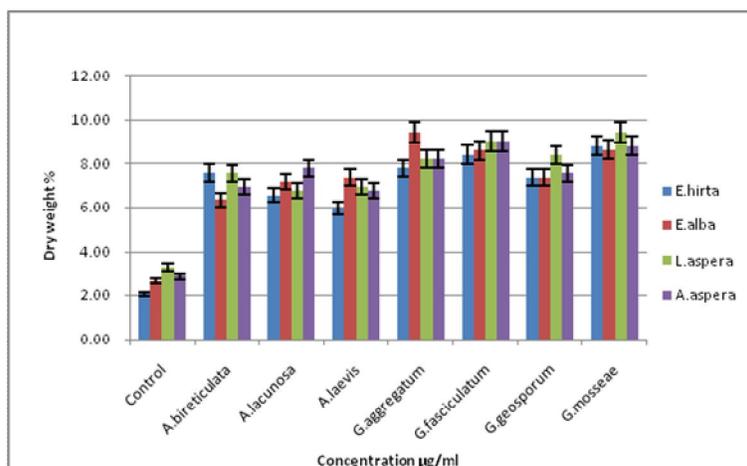


Figure 2: Estimation of Proteins in AMF Inoculated Selected Medicinal Herbs

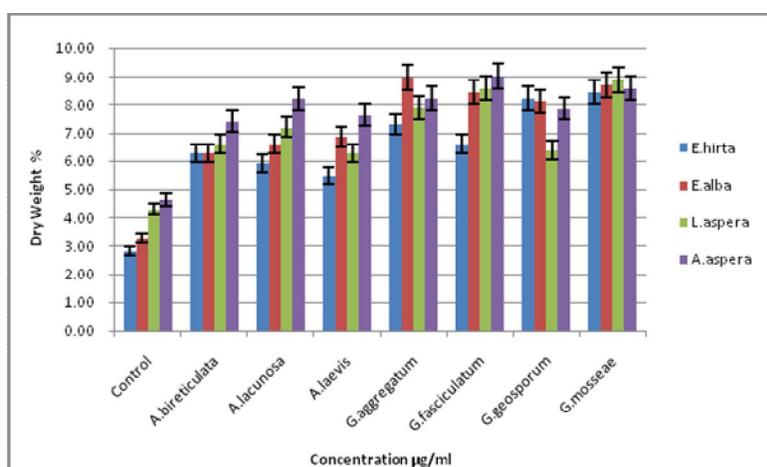


Figure 3: Estimation of Carbohydrates in AMF Inoculated Selected Medicinal Herbs

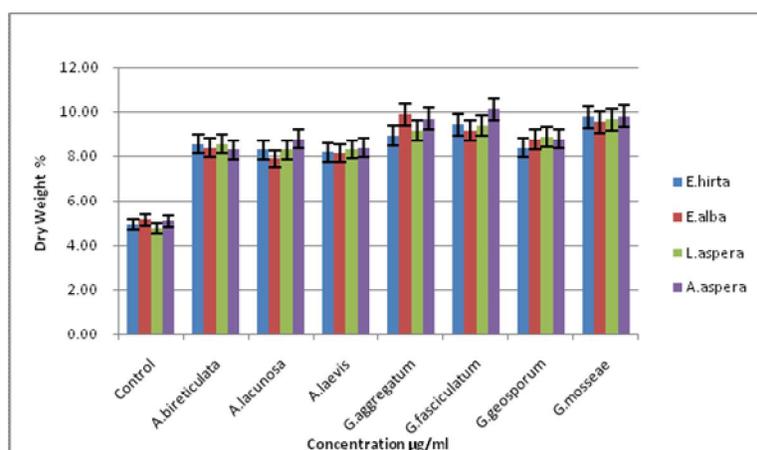


Figure 4: Estimation of Phenols in AMF Inoculated Selected Medicinal Herbs

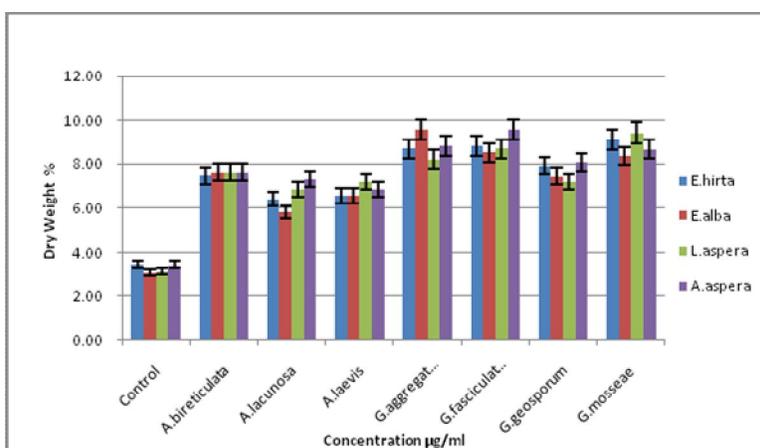


Figure 5: Estimation of Flavonoids in AMF Inoculated Selected Medicinal Herbs

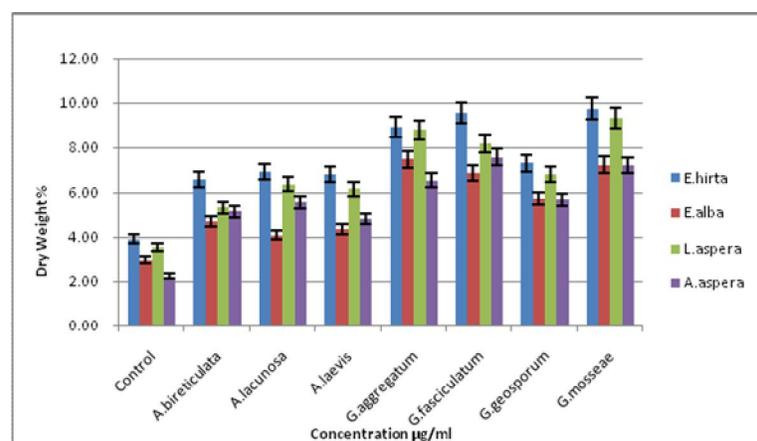


Figure 6: Estimation of Alkaloids in AMF inoculated Selected Medicinal Herbs

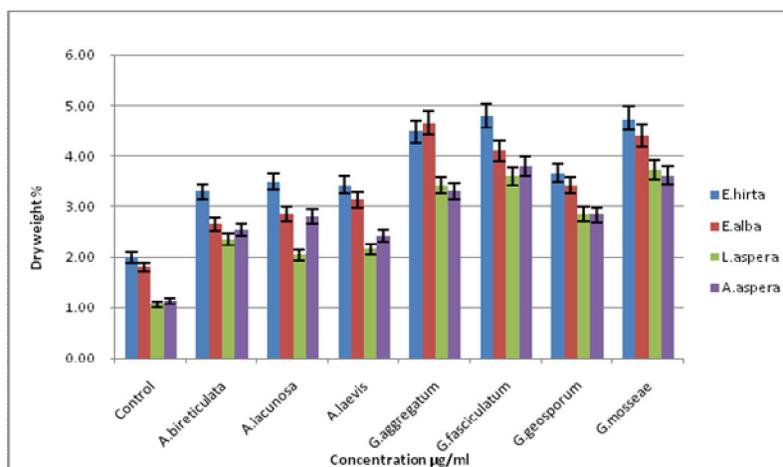


Figure 7: Estimation of Total Terpenoids in AMF inoculated Selected Medicinal Herbs