Recent Research in Science and Technology 2009, 1(6): 259–263 ISSN: 2076-5061 www.recent-science.com

MICROBIOLOGY



# STUDIES ON THE GROWTH AND BIOCHEMICAL ACTIVITY OF COLEUS AROMATICUS BENTH. AS INFLUENCED BY AM FUNGI AND AZOSPIRILLUM

## P. Thamizhiniyan\*, M. Panneerselvam, M. Lenin

Department of Botany, Annamalai Univeristy, Annamalainagar - 608 002, Tamilnadu, India

### Abstract

Mycorrhizal fungi are obligated symbiotic soil fungi which colonize the roots of the majority of plants. These fungi help to ensure an opportunity for the utilization of the symbiosis and contribute to the success of sustainable medicinal plants. To a large degree, mycorrhizas seems to be symbiotic relationships, in which the fungus obtains at least some of its sugars from the plant, while the plant benefits from the efficient uptake of mineral nutrients by the fungal hyphae. *Coleus aromaticus* Benth. (Lamiaceae), commonly called Indian Borage, is a medicinal plant and several medicinal properties are attributed to this plant in the Indian system of medicine. The study was conduct to evaluate the morphological parameters such as root length, shoot length, fresh weight, dry weight, total leaf area and root nodules was measured. The biochemical viz., chlorophyll 'a' and 'b' total chlorophyll, protein, starch and amino acid contents were tabulated. The higher growth and biochemical content was observed the inoculation of AM fungi + *Azospirillum* applied plants when compared with control plants.

Key Words: AM Fungi; Azospirillum; Indian Borage; Growth parameters; Biochemical activity.

## Introduction

Mycorrhizae are symbiotic associations between plant roots and fungi that occur widely natural communities [1]. Arbuscular mycorrhizal fungal symbiosis is a highly dynamic interaction affecting many aspects of the host plant physiology, including an enhanced uptake of phosphorus (P) and nitrogen (N) [2], and increased photosynthetic capacity [3]. AM fungi are vital for uptake and accumulation of iron from soil and translocation to hosts because of their high metabolic rates and strategically diffuse distribution in upper soil layers. In fact the fungus serves as highly efficient extension of the host root system. The fungi derive most of their required organic matter from their symbiotic niches in roots and in turn, help their host plants in better growth by enhancing phytochrome levels in absorption of phosphorus and other mobile elements from soil, impart tolerance to heavy metals and afford protection against disease, salinity, drought and temperature extremes. Azospirillum is a free-living, plant growth promoting bacterium capable of affecting growth and yield of numerous plant species, many of which have agronomic and ecological significance. The leading theory concerning its growth promotion capacity lies in its ability to produce various phytohormones that improve root growth, absorption of water and minerals that eventually yield larger and in many cases more productive plants [4]. *Coleus aromaticus* Benth. (Lamiaceae), commonly called Indian Borage. This medicinal plant and several medicinal properties are attributed to this plant in the Indian system of medicine. In the present study, studies on the growth and biochemical activity of *Coleus aromaticus* Benth. As influenced by *Arbuscular mycorrhizal* fungi and *Azospirillum*.

### **Materials and Methods**

This experiment was conducted at the department of Botany in Botanical garden at Annamalai University. The medicinal plants of *Coleus aromaticus* Benth., were collected from Cuddalore district. Collected plants were used for pot culture experiment with AM species *Glomus fasciculatum* and *Azospirillum* was utilized for this study. The treatment details are Control, AM fungi, *Azospirillum* and VAM fungi + *Azospirillum*. AM inoculated and uninoculated plants were treated with water. Wetting of the filled pots were done for easy planting. Subsequently water was given every day and also when required to

<sup>\*</sup> Corresponding Author, Email: eniyanpt@gmail.com

keep the optimum moisture in the soil. The growth parameter and biochemical activity were measured within 30 days at pot culture experiment. The dry weight of plants was taken by using an electrical single pan balance after keeping the materials in a hot air oven at 80°C for 48h. Chlorophyll [5]; Protein [6]; Starch [7] and Amino acid [8] were estimated.

### **Results and Discussion**

In our present research work growth and biochemical activity of Coleus aromaticus is maximum in AM + Azospirillum, when compared to all other treatments. The growth parameter such as shoot and root length, number of leaves, fresh and dry weight of Coleus aromaticus Benth., (Fig. 1). The shoot and root weight was increased in finger millet by inoculation of AM fungi Glomus fasciculatum in an unsterile soil which is lower in available phosphorus [9]. The inoculation of Glomus fasciculatum increased the growth of Onion [10]. AM inoculation induced maximum plant height and biomass of shoot in maize and soybean [11]. AM plants had a higher rate of root respiration, a higher shoot weight ratio and higher total P and N concentration in roots compared non-mycorrhizal Plantago with major species. Pletosperma plants shows with equal biomass and equal phosphorus concentration in the shoot.

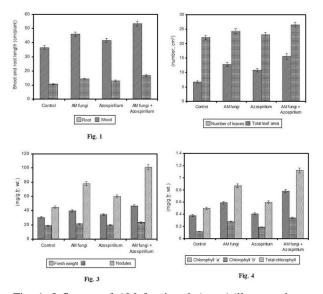


Fig. 1. Influence of AM fungi and *Azospirillum* on shoot length, Fig. 2. number of leaves and leaf area, Fig. 3. fresh, dry weight and root nodules and Fig. 4. chlorophyll 'a', chlorophyll 'b' and total chlorophyll of *Coleus aromaticus* Benth.

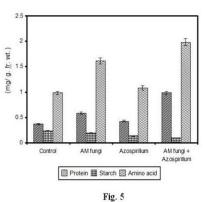
The AM plants shoot weight ratio was increased in concentration P and N and also decreased the

percentage of dry matter in the shoot. Species of AM fungi that can either directly or indirectly increased the plant growth by improving soil conditions [12, 13]. Direct benefits are usually related to the enhancement of phosphate uptake by the plant; however in some soils enhanced uptake such as zinc, copper and ammonium are also important [14]. The combined inoculation with *G. fasciculatum and B. magaterium* proved to have synergistic beneficial effects on the plant growth parameters, herbage and oil yield. VAM spores noticeably increased their growth parameters of colious plants shoot and root systems showed about increase, as plant grown in soil infected with AM + *Azospirillum*.

The enhanced height increment in peanut plants with mycorrhizal symbiosis as compared to the non inoculated plants. The mycorrhiza infection is known to enhance plant growth by increasing nutrients uptake. The height increment registered with inoculated plants could be as a result of enhanced inorganic nutrient absorption [15, 16] and greater rates of photosynthesis which obviously could have given rise to an increase in plant growth. The neem seedlings were inoculated with nine different VAM fungi, inoculated seedlings generally had greater plant height, stem girth, biomass, P content, Zn concentration, bio-volume index and guality index than uninoculated control plants, and this was reported by [17, 18]. Plant grown in soil inoculated with Glomus fasciculatum showed increased mycorrhizal colonization, fresh and dry shoot and root weight [19].

The biochemical changes of *Coleus aromaticus* Benth. were Fig. 2. Mycorrhizal infection by *Glomus fasciculatum* increased chlorophyll concentration in *Bouteloua gracilis* [20]. Total chlorophyll, chlorophyll 'a' and chlorophyll 'b' were maximum in AM inoculated cassava plants both under pot and field conditions [21]. Mycorrhizal plants have higher total chlorophyll and carotenoid contents observed [22].

Fig. 5. Influence of AM fungi and Azospirillum on protein, starch and amino acid of *Coleus aromaticus* Benth.



Mycorrhizal plants have higher chlorophyll contents than the non-mycorrhizal plants [23, 24]. The mycorhizal plants show a greater increase in the rate of photosynthesis than their controls which may be due to increase in the content of total chlorophyll [25, 26]. Mycorrhizal plants translocate higher amount of photosynthates from shoot and root than non mycorrhizal plants, without altering the leaf area and the AM fungi derive their carbon requirement from the host plants [27]. Plant grown in soil inoculated with Glomus fasciculatum showed increased chlorophyll content and phytochemical constituents [19, 28]. Inoculation of blackgram in an unsterile soil with Glomus epigaeum increased the chlorophyll content and N, P and K content [29]. The enhancement in chlorophyll 'a', 'b', total chlorophyll content can be attributed to the increase of absorption and translocation of essential metal ions, due to mycorrhizal infection. The higher increment registered with inoculated plants could be as a result of enhanced inorganic nutrient absorption and greater rates of photosynthesis [30, 31] observed higher protein content in AM fungi inoculated Nicotiana tobacum and Onion (Allium cepa) roots than the control [32] found that Glomus etunicatum inoculated Citrus limon leaves had higher total amino acids than control [33] found protein and amino acid increased in Glomus fasciculatum inoculated Arachis hypogaea roots. The interaction between AM fungus and plant cell takes place at both extracellular and intracellular level [34] showed high enzymatic activity in the interface between the cells of root and endomycorrhizal hyphae. According to [35] most metabolic processes occur at the interface between the fungus and plant cell. Higher levels of starch grains and insoluble protein have been found in root cap cells of finger millet with mycorrhiza inoculation [36].

The starch content in the leaves of *Coleus* species in the present study showed a decrease in mycorrhizal inoculation plants. Than non-mycorrhizal plants. The decrease in starch content may be due to the fact that the VAM fungi utilize 10-20% of net photosynthate in exchange for the transfer of nutrients to the host of lead a symbiotic life [21].

The decrease in starch may be due to the translocation of carbohydrate produced by the host to the fungal partner [37, 38] have found that since VAM fungi are obligate biotrophs, the host should have a substantial influence on the VAM fungi through the regulation of carbon supply [39-41]. In the present study, the morphological parameters such as root, shoot length, fresh weight and dry weight and biochemical contents chlorophyll 'a', 'b', total chlorophyll, protein, starch and amino acid are increased with the advancement age of the plant up to 30 days. AM inoculated plants shows the significant increased than the control plants. It can be

concluded that application of AM fungi *Azospirillum* in pot soils will result in improved growth of the plants.

## References

- Read, D.J, (2000). Mycorrhiza-the state of the art. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology, and biotechnology. Springer-Verlag Berlin Heidelberg, Germany, pp 3– 34.
- [2] Nowak, J. (2004). Effects of arbuscular mycorrhizal fungi and organic (eds) fertilization on growth, lowering, nutrient uptake, photosynthesis transpiration of geranium (*Pelargonium hortorum* L.H. Bailey '*Tango Orange*'). Symbiosis **37**: 259-266.
- [3] Borkowska, B. (2002). Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. *Acta Physiol Plant*, 24: 365–370
- [4] Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden P. Dutto, C. Labandera-Gonzalez, J. Caballero-Mellado, J.F. Aguirre, Y. Kapulnik, S. Brener, S. Burdman, D. Kadouri, S. Sarig, and Y. Okon, (2001). Responses of agronomically important crops to inoculation with *Azospirillum, Aust. J. Pl. Physiol.* **28**: 871-879.
- [5] Arnon, D. I., (1949). Copper enzymes in isolated choloroplast, Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1-15.
- [6] Lowry, O.H., N.J. Rose Brough, A.L. Farr and R.J. Randall, (1951). Protein estimation with folin-phenol reagent, *J. Biol. Chem.*, **193**: 265-275.
- [7] Clegg,. K.M. 1956. The application of the an throne reagent to the estimation of starch in cereals. *J. Sci. Food Agric.* **7:** 40-44.
- [8] Moore, S. and W.H. Stein, (1948). Photometric method for use in chromatography of amino acids. *J. Biol. Chem.*, **176:** 367-388.
- [9] Bagyaraj, D.J. and A. Manjunath, (1980). Response of crop plants to VA-mycorrhizal inoculation in an unsterile, *Indian Soil. New Phytol.*, 85: 33-36.
- [10] Hirrel, M.C. and J.W. Gerdemann, (1980). Improved growth of onion and bell pepper in saline soils by two vesicular arbuscular mycorrhizal fungi. *Soil Sci. Soc. Am. J.*, **44:** 654-655.
- [11] Thangaraju, M., R. Rajamanickam and D. Kandasamy (1986). Response of maize and

soyabean to the inoculation of different isolates of VA-mycorrhizal fungi. Paper presented at *National Seminar on Microbial Ecology* held during Jan. 1986 at Tamil Nadu Agril. Univ., Coimbatore, *India, Abst.*, pp. 24.

- [12] Kapoor, A. and K.G. Mukerji, (1990). A strategy for selection and application of VAM fungi, *Current Trends in Mycorrhizal Research* (eds.) B. Jalali and H. Chand, Haryana Agricultural University, Hissar, pp. 139-140.
- [13] Saleh, AL., M. Garni, (2006). Influence of malathion and mancozab on mycorrhizal colonization and growth of *Zea mays* and *Vicia faba*.
- [14] Stribley, D.P., (1987). Mineral nutrition. In Ecophysiology of VA- Mycorrhizal Plant (eds.) G.R. Safir, C.R.C. Press, Boca Raton, F1. pp. 59-70.
- [15] Cooper, K.M., 1984. Physiology of VA mycorrhizal associations. In: VM mycorrhiza (Ed. By C.L. Powell and D.J. Bagyaraj), pp. 155-186. CRC Press, Inc., Bola Roton, Florida.
- [16] Marschner, H. and B. Dell, 1994. Nutrient uptake in mycorrhizal symbiosis plant and soil, 159: 89.
- [17] Sumana, D.A. and D.J. Bagyaraj, (1999). Selection of efficient VA-mycorrhizal fungi for inoculation Neem, In: *Proceedings of the National conference of Mychorrhiza*, (ed.) S. Singh, Bhopal, India).
- [18] Champawat, R.S. (1992). Effect of vesiculararbuscular mycorrhizal fungi on growth and nutrition of chickpea. *Madras Agric. J.*, **79(1)**: 91-93.
- [19] Selvaraj, T. (1989). Studies on Vesicular arbucular mycorrhizae of some crop and medicinal plants, Ph.D. Thesis, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India, p. 120.
- [20] Allen, M.F., W.K. Smith, T.S. Moore, and M. Christensen, (1981). Comparative water relations and photosynthesis of mycorrhizal and nonmycorrhizal *Bouteloua gracilis* H.B.K. Lag ex Steud. *Newphytol.*, 88: 683-643.
- [21] Ganesan, V. and A. Mahadevan, (1994). Effect of mycorrhizal inoculation of cassava, elephant foot yarn and taro. *J. Root Crops*, **20(1)**: 1-14.
- [22] Gemma, J.N., R.E. Koske, E.M. Roberts, N. Jackson and K. De Antonis, (1997). Mycorrhizal fungi improve drought resistance in creeping bentgrass. *J. Turfrass. Sci.*, **73**: 15-29.
- [23] Morte, A., C. Lovisola and A. Schubert, 2000. Effect of drought stress on growth and water relations of

the mycorrhizal association *Helianthemum almeriense* Terfezia clavery. *Mycorrhiza*, **10(3):** 115-119.

- [24] Mathur, N. and A. Vyas, 2000. Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lan. Under water stress. *J. Arid. Environ.*, **45**: 191-195.
- [25] Shrestha, Y.H., T. Ishll and K. Kadoya, 1995. Effect of VAM fungi on the growth, photosynthesis, of transpiration and the distribution of photosynthates of bearing Satsuma mandarin tress. *J. Ipn. Soc. Hort. Sci.*, **64**: 517-525.
- [26] Wright, D.P., J.D. Scholes and D.J. Read, 1998. Effect of VAM colonization on photosynthesis and biomass production of *Trifolium repens* L. *Plant Cell Environ.*, 21: 209-216.
- [27] Harold, A. (1980). Regulation of photosynthesis by sink activity: The missing hink. *New Phytol.*, 86: 131-144.
- [28] Selvaraj, T. and P. Chellappan, 2006. Arbuscular mycorrhizae: A diverse personality. J. Cent. Europ. Agric., 7: 349-358.
- [29] Umadevi, G. and K. Sitaramaiah (1990). Influence of soil inoculation with endomycorrhizal fungi on growth and rhizosphere microflora on blackgram. 2nd Natl. Conf. Mycor., UAS, Bangalore. p. 6.
- [30] Manoharam P.T., M. Pandi, V. Shanmugaiah, S. Gomathinayagam and N. Balasubramanian (2008) Effect of vesicular *Arbuscular mycorrhizal* fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions.
- [31] Dumas, G.E., P. Guillaume, A.A. Tahiri, V. Gianinazzi-Pearson and S. Gianinazzi, (1994). Changes in polypeptide patterns in tobacco roots by *Glomus* species, *Mycorrhizae*, **4**: 215-221.
- [32] Nemec, S. and F.I. Meredith, (1981). Amino acid content of leaves in mycorrhizal and non-mycorrhizal *Citrus* root stocks, *Ann. Bot.*, **47**: 351-358.
- [33] Krishna, K.R. and D.J. Bagyaraj, (1983). Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut, *Arachis hypogaea, Can. J. Bot.*, 61(9): 2349-2351.
- [34] Jean Maire, C., J. Dexheimer and J. Gerard, (1988). Approch and *Niveau cellulaire*, du fonctionement des

endomycorrhizes a *Vesicules arbuscules*, *Cytologia*, **53**: 19-32.

- [35] Gianinazzi, S. (1991). Vesicular arbuscular (endo) mycorrhizae: cellular, biochemical and genetic aspects, Agric. *Ecosys. Environ.*, **35**: 105-119.
- [36] Doss, D.D., D.J. Bagyaraj and J. Syam Sundar, (1988). Anatomical and histochemical changes in the roots and leaves of finger millet colonized by VAmycorrhizae. *Indian J. Mycrobiol.*, 28: 276-280.
- [37] Fitter, A.H., 1991. Cost and benefits of mycorrhizas implications for functioning under natural conditions. *Experimentia*, **47**: 350-355.
- [38] Johnson, N.C., J.H. Graham and F.A. Smith, 1997. Functioning of mycorrhizal associations along the mutualism, parasitism and continuum. *New Phytol.*, 135: 575-586.
- [39] Kozlowski, T.T., 1992. Carbohydrate sources and sinks in woodly plants. *Bot. Rev.*, **58**: 107-122.
- [40] Hampp, R. and C. Schaeffer, 1995. Mycorrhiza carbohydrate and energy metabolism. *In:* mycorrhiza structure, function, molecular biology and biotechnology (eds.) Varm, A., Hock, B., Berlin, Germany, Springer Verlag, pp. 267-296.
- [41] Schaeffer C, Wallenda, T, Hamppr, Salzerp, Hager, A. (1997). Carbon allocation in mycorrhizal in: Treescontribution to modern tree physiology. (eds.) Renenberg H, Escherich W, Ziegler H. Leiden. The Netherlands. Buckhuya Publishers, pp. 393-407.