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Research Article

Enhanced Production of Secondary Metabolites and Synthesis of New Phenolic Compounds Due to VAM Fungus Inoculation in *Ocimum sanctum*

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Abstract

The value of medicinal plants is recognized by almost all people of earlier civilisations and cultures and they sought their help in alleviating human sufferings arising from different ailments. One of the earliest treatises on Indian medicine, the Charaka Samhita (2000 BC), records the use of over 340 drugs of vegetable origin. In Ayurveda, an Indian traditional system of medicine about 2,000 plant species are considered to have medicinal value, while the Chinese *Pharmacopoeia* lists over 5,700 traditional medicines, most of which are of plant origin. Many modern medicines are either directly derived from plants, or extracted from plants or artificially synthesized to copy plant chemical compounds. Today, plants and plant products hold a high promise as therapeutic agents and in total 13,000 plant species are known to have been used as drugs throughout the world. Inspite of phenomenal advances in the synthesis of new drugs and their production through microbial methods, nearly 25–30% of the drugs currently used are still of plant origin . A survey in USA during 1997 has shown that four out of ten persons used non-allopathic medicine. *Ocimum sanctum*, an important medicinal plant was tested for its growth response and production of secondary metabolites to a VAM fungus (*Glomus aggregatum*) inoculation by conducting a pot culture experiment using an unsterile soil. Its response was found to be positive and showed significantly increased VAM colonization in its root system when compared to control.

Keywords: VAM, Phenolic compounds, Secondary metabolites, Enhanced Synthesis, *Ocimum sanctum*

Introduction

Bacillary Plants generally owe their virtues as medicinal agents. Over 80% of the world population relies on plants for their health care needs , This is especially true in countries with developing economies where they offer local communities immediate access to safe and efficacious products to treat diseases through self medication (Akerele, 1991). Estimates suggest that as many as 3226 communities out of 4752 in India representing 70% of the population are dependent on traditional plant based medicine (Gadgil and Rao, 1998). A status report on ethnobiology in India undertaken by Ministry of Environment and Forests, Govt. of India has indicated that the tribal communities use over 7,500 species of plants for medicinal purposes (Pushpangadan, 1994).

Plants secondary metabolites exhibit both ecological and physiological significance. Their presence increases the chances of survival and fitness of species and act as feeding deterrents, allelopathic agent and protectants (against herbivores and from UV radiation) or provide defense against pathogens and inparts immunity to infectious agents at times by way of synthesis of phytoalexins (Bagyaraj and Manjunath,2008). Sometimes they are responsible in attracting pollinating agents (Harborne, 1988). They are also responsible for the medicinal properties of the plants (Basu and Srivastava,1998). Secondary metabolites include phenols, flavonoides, sterols, tannins, terpenes and lectins. It is essential to understand their nature, biosynthesis, their key

regulatory enzymes, storage in cells, etc (Bhagyaraj and Sreeramalu, 1982).

Plants generally owe their virtues as medical agents to these active principles (secondary metabolites) present in them and have contributed more than 7000 different compounds that are in use today as heart drugs, laxatives, anti-cancer agents, harmones, contraceptives, dieuritics, antibiotics, decongestants, analgesics, anaesthetics, ulcer treatments and antiparasitic compounds. Extensive screening of plants is being carried out now a day to identify chemical compounds that may provide new treatment for human diseases (Bisht and Khuble,1995, Eisenberg *et al.*,1998,Anandraj and Leela, 2008, Annapurna *et al.*,1999, Bakshu *et al.*, 2001, Baylis , 1967, Gehlot and Bohra, 1998, 1999,2000, Chitra and Kannabiran, 2001, Bowen, 1978). Of course, till today there is no other type of medicines other than herbal treatment for the cure of certain diseases like jaundice. Efforts are being made to discover still more potent plant drugs that can come to our rescue (Fuller, 1991). However, it should be stated in all fairness that our knowledge of the genetic and physiological make up of (Cooper, 1984) most of the medicinal plants is poor and we know still less about the biosynthetic pathways leading to the formation of active constituents for which these crops are valued.

Recent studies suggest that VAM associations may change the biochemical composition of host plant. A few studies have shown that considerable difference exist between mycorrhizal and non-mycorrhizal plants with respect to total carbohydrates (Krishna and Bagyaraj, 2012), amino acids (Nemec and Meridith, 1981), lipids (Nemec and Meridith, 1981; Selvaraj and Subramanian, 1990) and phenols (Krishna and Bagyaraj 1984; Selvaraj and Subramanian, 1990) Vertical distribution of a soil microbial community as affected by plant ecophysiological adaptation in a desert system (Barness *et al*.,2009).

VAM Associations in Medicinal Plants

The aim of mycorrhizal research is to utilize these fungi to accelerate the growth and productivity of plant in question with reduced need for the fertilizers and water. The growing necessity to understand the diversity of life contained within a gram of soil as well as intricacies of lining these living entities to terrestrial life in general, provided the impetus to work on VAM association in medicinal plants. The literature survey clearly indicates that, there is much to be digged out from the soil about VAMF, their associations and their role with reference to the productivity of medicinal plants (Nemec and Meridith, 1981,Azcon et al., 1982, Allen and Allen, 1980, Gerdemann, 1968). The available information is very meager on the use of VAMF inoculation to understand growth response, biomass production, uptake and changes in the chemical constituents in medicinal plants. Therefore, there is a need for a research in improving the quality and quantity of native medicinal plants, drugs in relatively shorter period and at lower expense by utilizing biological organisms like vesicular arbuscular mycorrhizal fungi.

Materials and Methods

Collection of native medicinal plants

a) Study area

For the collection of native medicinal plants the area presently surveyed is the Gulbarga University campus, Kalaburagi, Karnataka, India. For the collection of native medicinal plants the area presently surveyed is the Gulbarga University campus (about 840 acres). Most part of this area is still undisturbed and has grass lands with herbaceous vegetation with several wild species. Part of this area (10 acres) once under cultivation is now a botanical garden. Soon after monsoon rains, the herbaceous weed flora grow lush and wild during the months of August-September, which later get dominated by local grasses.

b) Collection and processing of root samples

Ocimum sanctum plant was collected by uprooting them without causing any damage to their root system and collected by uprooting them without causing any damage to their root system. Representative root samples, suspected to be mycorrhizal in nature, were brought to the laboratory in polythene covers. The roots samples were gently washed in running tap water to remove the adhering soil particles. They were, then cut into 1cm bits (segments) and fixed in standard FAA and then used for assessing the intensity of mycorrhizal associations (Powel and Bagyaraj,1984). The VA mycorrhizal association was observed by following root clearing and staining technique of Philips and Hayman (1970). Representative samples of root bits, washed with several changes of water, were taken in to test tubes and then cleared with 10% KOH solution by autoclaving at 15 lb pressure for 15 minutes. The KOH solution was poured off and then the root bits were washed thrice with tap water (in case of plant root bits that retained their pigmentation,

they were then treated with alkaline H_2O_2 solution (6% w/v) till the root segments were properly bleached. The roots were washed thrice again with tap water to remove H_2O_2) followed by treatment with 5N HCl for 3 to 5 minutes to neutralize the alkali effect. Later the roots were washed with tap water and stained by simmering in 0.5% trypan blue in lactophenol and by keeping in boiling water for 30–60 minutes. Then the root bits were mounted in lactophenol after removing the excess stain by putting then in lectophenol on a clean microscopic slides and observed under microscope for VAM fungal structures (i.e formation of vesicles and / or arbuscules).

c) Staining of treated root samples

The VA mycorrhizal association was observed by following root clearing and staining technique (Philips and Hayman, 1970). Representative samples of root bits, washed with several changes of water were taken in to test tubes and then cleared with 10% KOH solution by autoclaving at 15 lb pressure for 15 minutes. The KOH solution was poured off and then the root bits were washed thrice with tap water (in case of plant root bits that retained their pigmentation, they were then treated with alkaline H_2O_2 solution (6% w/v) till the root segments were properly bleached. The roots were washed thrice again with tap water to remove H_2O_2) followed by treatment with 5N HCl for 3 to 5 minutes to neutralize the alkali effect. Later the roots were washed with tap water and stained by immersing in 0.5% trypan blue in lactophenol and by keeping in boiling water for 30–60 minutes. Then the root bits were mounted in lactophenol on a clean microscopic slide and observed under microscope for VAM fungal structures (i.e. formation of vesicles and / or arbuscules).

d) Assessment of percent VAM colonization

The evaluation of endotrophic mycorrhizal association was done as per the methods of Giovannetti and Mosse (1980) and by following the formula given by Read *et al.*, (1976). About 50 segments were selected randomly and mounted in lactophenol on slides at the rate of 10 segments per slide and observed under microscope for the presence or absence of VAM fungal colonization in each root segment and expressed the results as percent VAM association / colonization.

$$\%\ \text{VAM colonization} = \frac{\text{Number of root bits positive for VAM colonization}}{\text{Total number of root bits observed}} \times 100$$

e) Selection of medicinal plant for the present study

In the present study one important medicinal plant *Ocimum sanctum is used* based on its natural occurrence and with scanty VAM associations were selected to study the effect of VAM fungus inoculation for the growth response and production of secondary metabolites.

f) Selection of VAM fungus for inoculation

In the present studies the VAM fungus *Glomus aggregatum* maintained in our laboratory is selected for the inoculation. It is the most prevalent, species found in campus soils. The method had been adapted as per the standard protocol. (Bagyaraj *et al.*,1989).

g) Effect of *Glomus aggregatum* inoculation on the growth and production of secondary metabolites

A pot culture experiment was conducted using sand and natural soil mixture (1:1). Five sets of 6 pots were taken and filled with 5kg unsterile soil in each pot and used. 3 pots in each set were treated as inoculated by adding VAM inoculum and the other three as control without the addition of inoculum. The inoculums having 50±5 spores /1g of soil was added at the rate of 500g/pot and thoroughly mixed and labeled as treated (Griffee and Metha 2000).

h) Estimation of dry weight

After 120 days of growth the pants thus harvested from all the sets (control and VAM treated). The roots and shoots were oven dried for 72h at 70^{o}C taking root and shoot separately. The dry weight of roots and shoots was recorded (Darade,2015).

i) Mycorrhizal dependency (MD)

Mycorrhizal dependency of all the 5 medicinal plants was determined as per the formula of Gerdemann (1975).

$$\text{Mycorrhizal dependency (MD)} = \frac{\text{Dry weight of inoculated plants}}{\text{Dry weight of uninoculated plants}} \times 100$$

j) Mycorrhizal efficiency (MEI)

Mycorrhizal efficiency of *Glomus aggregatum* in enhancing the growth was calculated by taking the total dry weights of the plants grown in unsterile control soil and VAM fungus inoculum treated soil using the formula of Singh and Tilak (1990) with modification.

$$\text{MEI} = 100 \times \left(1 - \frac{\text{Dry weight of plant grown in uninoculated soil}}{\text{Dry weight of plant grown in VAM fungus inoculated soil}}\right)$$

a. Quantitative estimation of secondary metabolites

Gulbarga University campus was still undisturbed and has grass lands with herbaceous vegetation with several wild species. Part of this area (10 acres) once under cultivation was now a botanical garden. Soon after monsoon rains, the herbaceous weed flora grows lush and wild during the months of August-September, which later get dominated by local grasses.

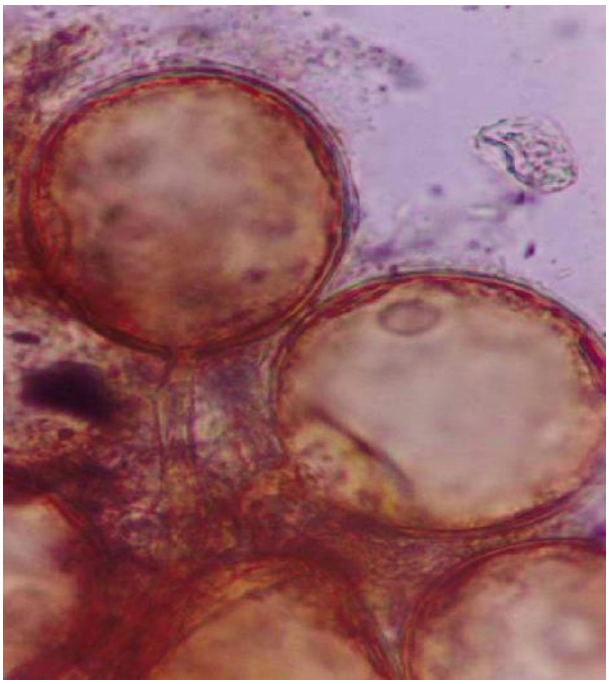

Figure -1: Lactophenol stained view of *Glomus aggregatum*

The biosynthesis of metabolites in plants although controlled genetically is affected to a large extent by various exogenous and endogenous factors. One such factor possibly altering the biochemical composition of the plant is VAM fungal associations (Selvaraj and Subramanian, 1990).

In the present investigations the effect of VAM inoculation on growth and production of secondary metabolites' include (Abbott and Robinson, 1982) the occurrence of VAM associations. Infectivity and efficacy of a local isolate of VAM fungus (*Glomus aggregatum*) on selected medicinal plant (*Ocimum sanctum)* grown in unsterile natural soil and its effect on the production of secondary metabolites is shown in Table-1. The study also shown after the addition of VAM inoculum and VAM inoculated plants extracts against certain common bacteria and fungi a satisfactory result as shown in Table-1.

Table-1 Effect of VAM (*Glomus aggregatum)* inoculation on the production of secondary metabolites in medicinal plant (*Ocimum sanctum*) grown in unsterile soil

Sl. No.	Secondary metabolite (mglg)	*Ocimum sanctum*	
		Control plant	VAM plant
1.	Phenols	**0.59**	**0.62***
2.	Flavonols	**0.35**	**0.49***
3.	Tannins	**0.54**	**0.57***
4.	Sterols	**0.10**	**0.17***
5.	Anthocynine	**0.80**	**0.84**

The results thus emerged are discussed in the light of the knowledge already gained in this regard. This fungus was also proved to be highly effective over the other species in its performance on local crops besides the root colonization and the ability to survive in the soil (Narayana Reddy and Rajender Sing, 1990).

Conclusion

In this current work the O*cimum sanctum* medicinal plant was screened for its secondary metabolites and were screened and evaluated for the occurrence of VAM association in their root system from Gulbarga University campus. and showed the formation of typical VAM fungal structures (vesicles and / or arbuscules) in their root cortices. Two plant species belongs to the families Amaranthaceae (*Digera aravensis*) and *Scrophulariacae* (*Russelia equisatiformis*) failed to harbour VAM association in their roots were selected and tested for their growth response to VAM fungus (*Glomus aggregatum*) inoculation by conducting a pot culture experiment using unsterile soil. All the 5 plant species following inoculation with VAM fungus responded positively and almost equally and showed significantly increased VAM association (showing 96-100%) and concurrently improved growth (dry matter production) as compared to control plants when they were harvested and assessed at the age level of 120 days (flowering stage).

Preliminary tests (qualitative analysis) as a prelude indicated the presence of secondary compounds such as phenols, flavonols, tannins, sterols and saponins in all the 5 species. When quantitatively estimated, a considerable increase in the content of these compounds was observed in VAM treated plants when compared to uninoculated controls.

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