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SYNERGISTIC EFFECT OF AM FUNGI AND *AZOSPIRILLUM* ON THE PHYTOCHEMICAL COMPOUNDS IN *Ocimum basilicum* L.

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ABSTRACT

Soil organisms particularly AM fungi are now being considered as biotic fertilizers and can be substituted for substantial quantities of chemical fertilizers. The biofertilizers may be of immense use in the cultivation of medicinal plants, as our resources diminish in near future and the costs of fertilizer sores, it is likely that these biofertilizers will definitely prove to be of economic advantage. The significance of AM fungi in medicinal crop production is widely appreciated and therefore the necessity of using AM fungi as a bioinoculant for improving crop production is increasingly realized, especially in medicinal plants. AM represents one of the nature's best gifts to mankind in the conservation of arid soil to productive and fertile soil. They help in the acquisition of immobile ions viz., P, Zn and Cu and mobile ions viz., N, K, Mg, Ca, Fe and Mn. The mycorrhizal fungi produce enzymes, auxins, cytokinins, vitamins and other substances that increase rootlet size and promote longevity. They absorb and translocate water to the host. This enables the host plants to withstand drought and salinity. Surprisingly only a little information of VAM association with medicinal plants is available. The synergistic effect of *Azospirillum lipoferum* and *Gigaspora margarita* on growth parameters and productivity of *Ocimum basilicum* L. under pot culturing method was carried out. The plant growth response and the enhancement of nutritional and phytochemical compounds of the synergistic effect of Azospirillum and AM fungi of medicinal plants are an untapped resource and, therefore, they merit study.

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INTRODUCTION

The mycorrhizal fungi produce enzymes, auxins, cytokinins, vitamins and other substances that increase rootlet size and promote longevity. They absorb and translocate water to the host. This enables the host plants to withstand drought and salinity. They physically and metabolically bind toxic heavy metals, thus enabling the host plants to survive in soil polluted with heavy metals (Raman and Mahadevan, 1996).At present considerable importance is being given to bio-inoculants because of the contemporary sensitivity to environmental pollution and health hazards resulting from the use of chemical fertilizers (Somani *et al.,* 1990). The significance of inoculation of efficient strains of nitrogen fixing bacteria into soil or to seed and application of VAM alone or together with a nitrogen fixing bacteria has been found beneficial to crop as well as medicinal plants growth and yield (Bagyaraj and Sreeramulu, 1982; Somani *et al.,* 1990). The plant growth response and the enhancement of nutritional and phytochemical compounds of the synergistic effect of *Azospirillum* and VAM fungi of medicinal plants are an untapped resource and, therefore, they merit study.

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Review of Literature

Importance of mycorrhizae

Arbuscular mycorrhizal fungi (AMF) are among the most important biofertilizers. Mycorrhizal fungi have a significant role in maintaining the ecological balance in the soil in addition to increasing yield (Weisany *et al.,* 2016). AMF are known as a main component of the plant's rhizosphere in natural systems, including several species of medicinal plants (Assis *et al.,* 2020).

Mycorrhizae are vital for uptake and accumulation of ions from soil and translocation to hosts because of their high metabolic rate and strategically diffuse distribution in the upper soil layers (Mukerji *et al.,* 1982). In fact, the fungus serves as a highly efficient extension of the host root system. Minerals like N, P, K, Ca, S, Zn, Cu and Mn absorbed from soils by the mycorrhizal fungi are translocated to the host plant (Smith *et al.,* 1994). Minerals more than 4 cm distant from the nearest host root can be absorbed by the fungal hyphae and translocated to roots in the mycorrhizal plants (Trappe, 1981).

The mycorrhizal fungi produce enzymes, auxins, vitamins, cytokinins and other substances that increase rootlet size and longevity (Gopinathan and Raman, 1991). They protect the rootlets from pathogens. They absorb and translocate water to the host (Mexal and Reid, 1973). VAM fungi alter the kinetic properties of the root, thereby enhancing its nutrient uptake abilities (Barea *et al.,* 1993). Hence it is clear that mycorrhizal fungi play a vital role in nutrient cycling and productivity of crops (Bansal and Mukerji, 1994).

Effect of dual inoculation with VAM and *Azospirillum* on plant growth

Dual inoculation with Glomus plus Azospirillum was at par with that of Gigaspora plus Azospirillum. Azospirillum alone in sterile soil enhanced nitrogen and phosphorus uptake in the plants has been reported by Konde et al. (1988). Nitrogen content of leaf tissues was higher in plants inoculated with A. brasilense alone while leaf phosphorus content was higher in plants inoculated with VAM alone than in dual inoculation as shown by Ratti and Janardhanan (1996).

Panwar (1991) reported that, wheat CV.ND 2428, inoculated with *Azospirillum brasilense* (seed inoculation) and *Glomus fasciculatum* (soil inoculation) showed that chlorophyll concentration, photosynthetic rate, nitrate reductase and glutamine synthetase activities and grain yield were highest in the dual-inoculated plants. *A. brasilense* mainly increased root growth while AM increased both root and shoot weights.

Padmavathy *et al.* (1991) found that the soil inoculation with *Glomus intraradices* resulted in a significant increase in spike weight, and levels of acid and alkaline phosphatases but not in plant growth of foxtail millet. A synergistic effect was noticed in plants that received *G. intraradices* and *Azospirillum lipoferum*. The biological interaction of dual inoculants resulted in significant increase in the mycorrhizal colonization and in uptake of mineral nutrients such as phosphorus, nitrogen, zinc, copper and iron in the shoots of foxtail millet.

Materials and Methods

Quantitative phytochemical studies

Phytochemical studies were undertaken to find out the availability of total chlorophyll contents were extracted using acetone and its absorbancy was read using a spectrophotometer at 645 nm and 663 nm and against (80% acetone) blank solvent as followed by Arnon (1949).The content of total proteins was estimated by Lowry *et al.,(*1951), total amino acids (Troll and Canon 1953), total phenols (Farkas *et al.,* 1952), nitrate reductase activity (Stewart, 1979), acetylene reduction assay and glutamine synthetase activity (Stapiro and Statdman, 1962) acid and alkaline phosphatases (Torriani 1967) Synergistic effect of *Azospirillum lipoferum* and *Gigaspora margarita* on growth and productivity of *Ocimum basilicum* L.

Azospirillum lipoferum isolated from the soil was used to inoculate the sterilized potting medium before sowing the seeds. *Gigaspora margarita* was placed 3 cm below the soil surface before sowing the seeds. The four treatments in this study were:

1. Control (without AM and *A. lipoferum*)
2. *Azospirillum lipoferum* alone
3. *Gigaspora margarita* alone
4. *Azospirillum lipoferum* + *Gigaspora margarita*

Results and Discussion

Chlorophyll a, b total chlorophyll content in the leaves of *Ocimum basilicumis* presented in Table1. Chlorophyll a (Chl. a), chlorophyll b (Chl. b) and total chlorophyll content were increased in all the inoculated treatments. In AM fungi plus *Azospirillum* inoculation, Chl. a, Chl. b and total chlorophyll content were increased to the maximum level. It was followed by AM inoculation alone and *Azospirillum* alone (Table1). Total proteins, aminoacids, phenols, ortho di-hydroxy phenols, soluble sugars and reducing sugars in the leaves of the experimental plants were presented in the Table 1. The increased activity of acid and alkaline phosphatases in all the treatments of *Ocimum* observed in the present study could be an indication of active transport of phosphorus. Activity of both acid and alkaline phosphatases were found to be higher in the leaves of the plants inoculated with *Gigaspora* alone or in combination with *Azospirillum* treatment when compared to uninoculated control (Table1). Chlorophyll, protein, sugars on dry weight and all others on fresh weight basis.

Phytochemical constituents such as chlorophyll a, b, total chlorophyll, anthocyanin, carotenoids, total proteins, aminoacids, lipids, phenols, ortho-di-hydroxy phenols, soluble and reducing sugars, NR and GS activities, peroxidases, acid and alkaline phosphatases was found to be higher in the leaves of plant inoculated with VAM fungi and *Azospirillum* treatment compared to uninoculated control. Contents of total phenol and ortho di-hydroxy phenol were also increased in the leaves of the mycorrhizal and *Azospirillum* inoculated of medicinal plant. In dual inoculation, phenol content was much increased. Abdul Malik (2000) also reported an increase in total phenols and ortho dihydroxy phenol in leaves but not in roots of tobacco colonized by Glomus aggregatum.

Phytochemical content	Inoculation treatment			
	Control	*A. lipoferum* alone	*G. margarita* alone	*A. lipoferum* + *G. margarita*
Chlorophyll-a ($mg.g^{-1}$)	0.215	0.218	0.224	0.225
Chlorophyll-b ($mg.g^{-1}$)	0.483	0.492	0.495	0.498
Total chlorophyll ($mg.g^{-1}$)	0.698	0.712	0.719	0.723
Total soluble sugars ($\mu g.g^{-1}$)	180.0	195.0	260.5	275.5
Total reducing sugars ($\mu g.g^{-1}$)	135.0	142.5	160.5	162.5
Total proteins ($\mu g.g^{-1}$)	128.0	162.0	172.0	178.0
Total amino acids ($\mu g.g^{-1}$)	95.0	115.0	120.5	125.5
Total phenols ($\mu g.g^{-1}$)	152.5	162.5	165.5	170.5
Ortho di-hydroxy phenol ($\mu g.g^{-1}$)	65.5	72.5	75.0	75.5
Nitrate reductase ($\mu mol\ h^{-1}\ g^{-1}$) fresh weight)	0.18	0.22	0.29	0.31
Glutamine synthetase ($\mu mol\ h^{-1}\ g^{-1}$ fresh weight)	57.08	83.27	121.24	124.52
Acid phosphatase (μg^{-1})	233.33	243.33	316.67	485.52
Alkaline phosphatase (μg^{-1})	163.33	175.21	214.42	265.13

Table 1. Effect of inoculation with *Azospirillum lipoferum* and *Gigaspora margarita* on phytochemical content in the leaves of *Ocimum basilicum* L

Conclusion

Inoculation with AM fungi will enhance the growth and nutrition of medicinal plants. The interactions between AM fungi and rhizobacteria (beneficial free living N2 fixing bacteria, *Azospirillum*) could be a biotechnological tool for benefiting plant growth, nutrition, health and productivity of medicinal plants. From the present study it is concluded that the inoculation of *Ocimum* medicinal plant with indigenous *Gigaspora margarita* and *Azospirillum lipoferum* can become a great boon to the agriculturists in minimizing the requirements of costly nitrate and phosphate fertilizers in thismedicinal plant cultivation and also that *Gigaspora margarita* is a more efficient AM symbiont for this medicinal plant grown in Tamil Nadu soils.

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