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Impact Of Glomus Macrocarpum On The Chlorophyll Content And Antibacterial Activity Of Piper Betle

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ABSTRACT
Symbiotic association between arbuscular mycorrhizal fungi and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne pests, resistance to disease, resistance to drought, tolerance of heavy metals and better soil structure. However few plants have no chance of getting associated with mycorrhizal fungi by nature. In this study the plant, Piper betle was grown in the soil inoculated with mycorrhizal fungus (Glomus macrocarpum) and soil with different control were studied for their content of Chlorophyll A and B, and its antibacterial property were evaluated against few bacteria. The study revealed that the plant grown in soil inoculated with mycorrhizal fungus alone showed high level of chlorophyll A and Chlorophyll B content and better antibacterial property when compared with that of the plant grown in other control soils. The study emphasizes the importance of mycorrhizal fungi and its usage on agriculture plant as a biofertilizer after proper evaluation.
Keywords: *Mycorrhiza, Glomus macrocarpum, Biofertilizer, Antibacterial property, Chlorophyll content, Piper betle*

INTRODUCTİON

Frank in 1985 termed the association between the plant and fungi as 'Mycorrhiza' which has the meaning as root symbiosis of fungi. Mycorrhiza are found to have their impact on soil biology, soil chemistry and soil nutrition. The soil health in turn affects the plant growth and sustainability. However, the mycorrhiza function as a bridge between the plant and soil including nutrient fluxes. They majorly colonizes the plant root either as ectomycorrhiza or as endomycorrhiza depending upon their penetration of root cells. Among these mutualistic partner, the fungi are found to provide nutrients like potassium, nitrogen and phosphorus the major nutrients required for plants. In turn, the fungal partner gets Carbon as a nutrient source from the plants. The network of fungi away from the root of the host plant serve as an extended root system in adsorption of water and absorption of nutrients from soil.

The association between arbuscular mycorrhizal fungi and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, tolerance of heavy metals and better soil structure. It is known that nearly 80 % of the terrestrial plants are possesing mycorrhizal asociation (van der Heijden et al., 2015). Glomus macrocarpum is widely known mycorrhizal fungi belonging to the order Glomerales and the family Glomeraceae which is mostly popular for its pathogenicity inducing stunted growth. However, the benefit of the fungi as a mycorrhizal fertilizer is not widely reported except few.

It is known that enhanced plant growth and nutrient uptake are possible in relation with mycorrhizae. The relation with mycorrhizae provides an advantage of enhanced water adsorption, nutrient and mineral uptake, disease resistance, stress tolerance. Further they are also found to increase the secondary metabolites and medicinal property of the plants. Nearly 90 % of the medicinal plants are found to possess mycorrhizal association (Zhang et al., 2022). Although the reports are available stating nearly 90% of medicinal plants possess mycorrhizal association, the data pertaining to individual plants are not studied clearly. Specifically, there is no report available on one such plant Piper betle. The betel leaf is consumed as raw leaf as refreshener, digestive agent, stimulator and aphrodisiac in nature. They are also important for their aroma used by more than 2 billion people around the world (Guha et al., 2019). It was reported that VAM fungi enhances the ingredients of medicinal plants (Yang et al., 202). Thus, in this study an attempt was made to compare mycorrhizal rich soil and its impact on chlorophyll content and the antibacterial activity of the plant, Piper betle in comparison with other type of soils.

MATERİALS AND METHODS

Isolation of Mycorrhizal spores

The soils from different areas, i.e. Kovilambakam, Maduravoyal, Kovalam and Neelankarai were collected. Around 10gm of soil sample was dispersed in 100ml sterile distilled wter and agitated vigorously. After agitation, the samples are left unattended for 45 minutes. The spores floating on the surface of the supernatant was collected using a brush and examined under the microscope for their presence.

Preparation of Mycorrhiza amended soil

A sterile soil was inoculated with the mycorrhizal spores for the volume of 100 spores/ 500 gms of soil. Along with the mycorrhiza amended soil, different soil types like red soil, native soil where the plant were collected, non native soil (Soil collected from Punjab in India), Vermicompost amended soil were used in the study for comparison.

Growth of Piper betle plants

The plants of Piper betle were placed in the pots containing different types of soil. The measurement on shoot length and root length were measured initially. The same was repeated after the plant growth of 30 days duration.

Examination of roots for mycorrhizal infestation

The roots of the plant from mycorrhiza amended soil was collected and examined for the presence of mycorrhizal colonization. The young, fragile, soft roots were collected and cleared using KOH and examined under the microscope for the presence of mycelial growth and spore formation.

Estimation of Chlorophyll (Amon 1949)

The leaves of 2 grams weight was collected and were treated with 80% acetone and crushed until paste is made. The extract was filtered and repeated until the extract becomes colorless. The extract of the leaves were studied using a spectrophotometer at 645nm and 663nm and OD value were recorded with acetone as blank. The conent of Chlrophyll a, Chlorophyll b and total chlorophyll were estimated using the following formulae:

Total chlorophyll: 20.2 (A645) + 8.02 (A663)

Chlorophyll A: 12.7 (A663) – 2.69 (A645)

Chlorophyll B: 22.9 (A645) – 4.68 (A663)

Antibacterial studies (Prakash et al., 2014)

The following bacteria, Bacillus subtilis (MTCC 121), Klebsiella pneumoniae (MTCC 109), Pseudomonas aeruginosa (MTCC 424), Salmonella typhi (MTCC 441) and Staphylococcus aureus (MTCC 96) were procured from Microbial Type Culture Collection centre, Chandigarh, India. The procured cultures were maintained in Nutrient Agar Slants for further analysis.

The antibacterial property of the leaf extracts of Piper betle were examined using Well diffusion method. The petridishes containing Mueller Hinton Agar were used for the evaluaton of antibacterial property. The aqueous extract of fresh leaves of Piper betle were used. The leaves of the plants grown in different soil types were examined with the concentration of 50ul / well. The diameter of the zone of inhibition in mm .was recorded after 24 hour period of incubtion. Streptomycin was used as a control.

RESULTS AND DİSCUSSİON

The symbiotic Mycorrhizal fungi are found to be beneficial not only for the plants but also for the soil health. They are involved in carbon cycle thus imparting transfer and storage of carbon in soil contributing to carbon sequestration. The adsorption of Nitrogen, Phosphorus and other nutrients from soil enhances the nutrient content of the host plant. However, the availability of mycorrhizal spores alone determines the colonization rate in plants. Thus determination of the count of mycorrhiza in any agricultural land is important and if it is found less applying mycorrhizal spores as a fertilizer is recommended. The soil samples collected from different areas for the isolation of mycorrhizal spores resulted in different number of spores/kg of soil. An average of 104 spores/kg was recorded. Among the sampling sites, Kovilambakkam recorded more number of spores when compared to all other sites. Soil of Neelankarai resulted in lesser number. The number of mycorrhizal spores recorded from different sampling sites is presented in Table 1 and few of the spores recorded are presented as Fig. 1.

The plant Piper betle belonging to the family Piperaceae is medicinally important. They are also found to possess different cultivars which differ with their phytochemical constituents and their biological activities. The study pertaining to the presence of total phenolic content, total flavonoid content and other antioxidant potency of six different cultivars of Piper betle was studied (Bhuvaneswari et al., 2014; Smila et al., 2014). Antibacterial and anticandidal activity of the plant was also reported. The soil conditions on the growth of Piper betle shows no difference among the native soil, red soil and mycorrhiza amended soil. However, lesser growth was observed in Non-native soil and it is revealed that the vermicompost soil too have not supported the growth of the plant, Piper betle. The shoot length and the root length of the plant while planting and after the growth of 30 days is presented in Table 2. The experimental set up is presented in Fig. 2. The root colonization by the mycorrhizal fungal spore and the spore of Glomus macrocarpum is presented in Fig. 3a and 3b.

The chlorophyll content of the plants grown in different soil conditions revealed that the plant grown in mycorrhiza rich soil is possessing high chlorophyll content. Both chlorophyll A and chlorophyll B are more in quantity when compared with the plants grown in different soil types. The least was recorded in native soil. Little increase was noticed when the plant is grown in red soil. Vermicompost enriched soil and non native soil collected from punjab showed difference in chlorophyll content A and B. However, total chlorophyll content is concerned no difference recorded. The contents of Chlrophyll A, Chlorophyll B and total chlorophyll is presented in Table 3.

The antimicrobial studies of the aqueous extract of the leaves of Piper betle grown in different soil types were evaluated. The leaf extract of the plant grown in mycorrhiza rich soil alone recorded the zone of inhibition for all the bacterial species studied. Although, the extract showed broad spectrum antibiotic activity the zone of inhibition recorded was less when compared to that of Streptomycin. The antibacterial activity showed by the mycorrhiza soil The plant grown in vermicompost amended soil showed antibacterial activity against the bacteria Bacillus subtilis and Pseudomonas aeruginosa. The zone of inhibition recorded against each bacteria for the plants grown in different soil types are presented in Table 4.

Although the study reveals that the biomass of the plant grown in mycorrhiza amended soil is not increased when compared with other type of soils, the chlorophyll content and their antimicrobial property are high in the plant Piper betle growing in the soil treated with mycorrhiza. The improvement in volatile oil content of Piper collasum after infection of Arbuscular Mycorrhiza and Rhizobacteria was reported (de Azevedo et al.,, 2024). The improvement in oil content of Anethum and Trachyspermum ammi due to the bioinoculant of G. macrocarpum was reported (Kapoor et al., 2002).

CONCLUSİON

The study emphasizes application of mycorrhiza for any plant of agricultural importance which enhances their metabolites. The study also highlights to conduct the study related to the impact of mycorrhiza amended soil on the secondary metabolites and other bioactivities on the other cultivars of the plant, Piper betle.

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Table 1. Number of mycorrhizal soil spores recorded from different sampling sites

Sl.No	**Name of the Area**	**No of Spores/ kg of soil**
a.	Kovilambakkam	122
b.	Maduravoyal	109
c.	Kovalam	96
b.	Neelankarai	88

Table 2. Shoot and Root length of Piper betle while planting and after 30 days of growth

Name	**When planting (shoot)**	**When planting (root)**	**After 30 days (shoot)**	**After 30 days (root)**
Red Soil	20.5 cm	9.8 cm	36 cm	13.3 cm
Native soil	18.7 cm	9.2 cm	34.1 cm	12.4 cm
Non-Native soil	18.1 cm	8.5 cm	31 cm	10.1 cm
Vermicompost soil	16 cm	7.3 cm	18 cm	8.2 cm
Mycorriza soil	14.3 cm	7.8 cm	30.2 cm	10.3 cm

Table 3. Chlorophyll A,B and Total chlorophyll content of Piper betle grown in different soil types

Plant Soil Names	**Chlorophyll A (g/ml)**	**Chlorophyll B (g/ml)**	**Total Chlorophyll (g/ml)**
Red soil	12.91	7.94	20.85
Vermicompost soil	25.67	21.43	47.08
Non native soil	18.89	26.33	45.22
Mycorrhiza soil	29.83	34.57	64.39
Native soil plant	7.67	11.19	18.86

Table 4. Zone of inhibition recorded for the leaf extract of Piper betle grown in different soil types.

Microorganism	**Zone of inhibition (In mm)**					
	Native soil	Mycorrhizal soil	Vermi-compost soil	Nonnative soil	Red soil	Antibiotic (Strepto-mycin)
Bacillus subtlis	-	15mm	13mm	-	-	19mm
Salmonella typhi	-	13mm	-	-	-	18mm
Klebsiella pneumoniae	-	11mm	-	-	-	12mm
Staphylococcus aureus	-	13mm	-	-	-	17mm
Pseudomonas aeruginosa	-	10mm	10mm	-	-	12mm

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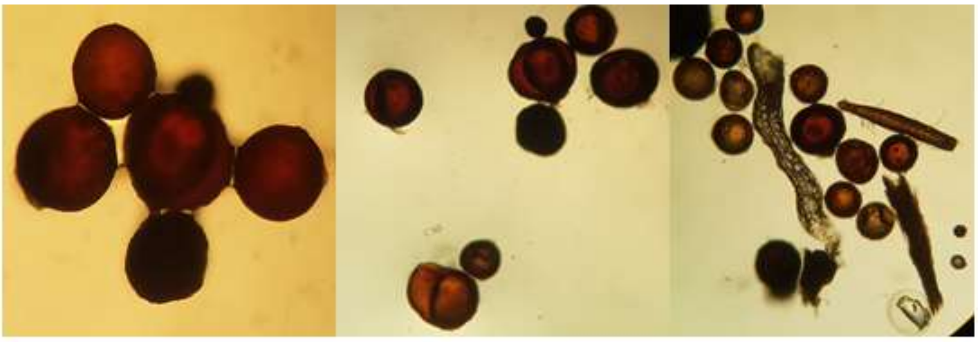

Fig. 1. Mycorrhizal spores isolated from soil samples

Fig. 2. Experimental set up of plants of Piper betle in different soils

Fig. 3a. Spore of Glomus macrocarpum

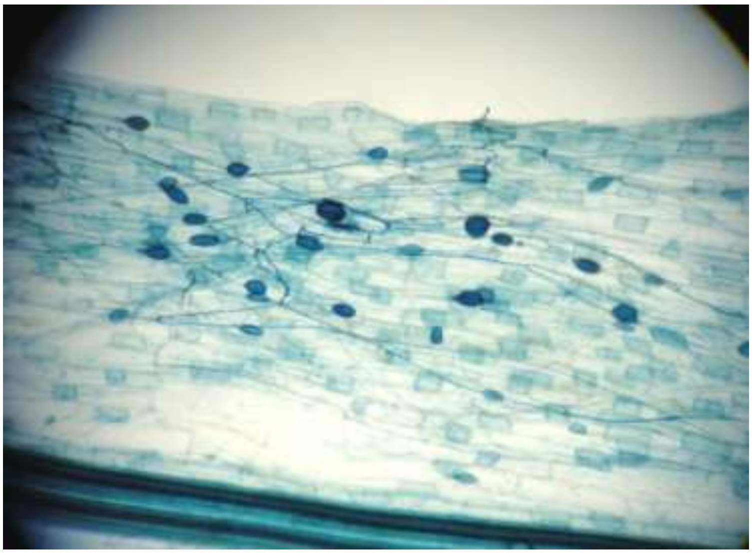

Fig. 3b. Root colonization by the spores of Glomus macrocarpum