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MYCORRHIZAL IMPACT ON CHLOROPHYLL CONTENT AND ANTIBACTERIAL ACTIVITY OF *COLEUS AMBOINICUS*

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ABSTRACT

Mycorrhiza in general are the symbiotic fungi which grows in root favouring both the host and the colonizer. The host benefit by nutrient uptake specifically Potassium. The resistance to soil borne pathogens and pests are also reported due to the presence of mycorrhiza. Improved resistance to heavy metals and drought are the other benefits reported. In compensation the nutrient need, i.e. carbohydrate are compensated to the symbiotic fungi colonizing the roots of the host. The plant studied here are found to be the natural colonizer for the mycorrhiza among the plants belonging to Lamiaceae. In this study, the host plant is inoculated with the mycorrhizal fungi, *Glomus* sp. to learn its impact on the chlorophyll content and the antibacterial activity of the host plant, *Coleus amboinicus.* The plant was grown in soil inoculated with *Glomus macrocarpum* and control soil like a) soil without mycorrhiza b) Vermicompost as a biofertilizer c) Non native soil, i.e. soil from irrelevant land nature and d) Native soil without any amendment. The plant grown in these soil types were examined for the chlorophyll A, Chlrophyll B and Total Chlorophyll content. Further, the antibacterial potency of the host plant inoculated with mycorrhiza were studied for their antibacterial property againt, *Bacillus subtilis, Klebsiella pneumonia, Salmonella typhi* and *Staphylococcus aureus* using well diffusion method. The results showed that the plant possess more Chlorophyll content and recorded more antibacterial efficacy against the bacteria studied when compared with control soils. The study promotes the usage of mycorrhizal fungi on any of the agricultural plant for better property.

Keywords: Mycorrhizal fungi, *Glomus macrocarpum,* Chlorophyll content, Biofertilizer, Antibacterial property, *Coleus amboinicus*

INTRODUCTION

Mycorrhiza in general are the symbiotic fungi which grows in root of higher plants including orchids favouring both the host and the colonizer. Mycorrhiza in general defines the association between the fungi and the plant roots. It is a symbiotic association between the plant and fungi where both the partners get benefitted. The water and mineral sources are the benefit of host plant and the carbon and nitrogen rich compounds from the plants are for the other symbiont. The uptake of nutrients from soil is favored by the presence of mycorrhiza, resistance to soil borne pathogens and pests are also reported due to the presence of mycorrhiza. Improved resistance to heavy metals and drought are the other benefits reported. In compensation the nutrient need, i.e. carbohydrate is provided to the symbiotic fungi colonizing the roots of the host. Nearly 20 % of the carbon is provided to the fungi by the host. These mycorrhizae are classified in to endomycorrhiza and ectomycorrhiza based on their colonial ability of the root cells of the host plant. Similarly, the host plants are also classified as facultatively mycorrhizal, obligately mycorrhizal and non mycorrhizal plants (Brundrett, 2004). Lehnert et al., (2017) reported that nearly 72% of flowering plants are in association with mycorrhiza. Thus, the plant possessing mycorrhiza as their symbiotic partner is found to benefit and their sustenance to the environment is easy. Due to the agricultural challenges, nowadays mycorrhiza are physically added as a bio fertilizer to the soil to favour the growth of agricultural plants.

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. Further they are also found to increase the secondary metabolites and medicinal property of the plants. [10] Nearly 90 % of the medicinal plants are found to possess mycorrhizal association. [11] Although the reports are available stating nearly 90% of medicinal plants possess mycorrhizal association, the data pertaining to individual plants are not studied clearly.

Coleus amboinicus is an aromatic plant belonging to family Lamiaceae. The plant is used in culinary due to the aroma the plant possesses. This is also used in traditional medicine for cough and respiratory congestions. *Coleus amboinicus* is reported as antimicrobial, antioxidant and anti-inflammatory agent (Arumugam et al., 2016). In this study, the effect of mycorrhizal fungi, *Glomus macrocarpum* on the chlorophyll content and antibacterial activity of *Coleus amboinicus* was evaluated by inducing mycorrhizal colonization to the plant. The plant grown

in fungus different types of soil amended with the mycorrhizal fungus is evaluated for the content of Chlorophyll A, Chlorphyll B and total Chlorophyll. Further, the extracts of the plant is evaluated for its antibacterial ability against few of the bacterial species.

MATERIALS AND METHODS

Isolation of Mycorrhizal spores

The soils from different areas of Sathyamangalam forest in Erode District of the state of Tamil Nadu was collected and evaluated for the presence of mycorrhizal spores. The collected soil were allowed to dry to remove moisture visibly and sieved using a stainless steel sieve. From the sample, 100gm was dispersed in 1 litre of water and vigorously agitated. The set up was left out for a period of 45 minutes and 10ml of sample was taken in a watch glass and examined using a dissection microscope for the presence of mycorrhizal spores. The spores floating on the surface of the supernatant was collected using a brush and examined (Fig. 1) (Gerdemann and Nicolson, 1968).

Preparation of Mycorrhiza amended soil

The soil collected from Tiruvannamalai district (where the plant grows) was sterilized using an autoclave and the sterile soil was dried for loosing moisture. The dried soil is amended with the mycorrhizal spores (100 spores / 500 gms of soil) as an inoculum. This soil was used for the study as mycorrhiza amended soil. The plants grown in this soil is compared with other soil type where the plant is grown for the same period of time. The other soil types includes,

a) soil without mycorrhiza (Sterilized)
b) Vermicompost as a biofertilizer
c) Non native soil, i.e. soil from irrelevant land nature and
d) Native soil without any amendment.

Examination of roots for mycorrhizal infestation

The plant root of *Coleus amboinicus* growing in mycorrhiza amended soil was collected for the examination of mycorrhizal infestation. The young, tender roots of the pants were cleared with KOH and stained using Tryphan blue for the presence of mycorrhizal colonization. They were examined under the microscope for the mycelial growth and spore formation (Fig. 2).

Growth of *Coleus amboinicus* plants

The plants of *Coleus amboinicus* collected from Tiruvannamalai district of the state of Tamil Nadu were grown in containing different types of soil, i.e. a) soil without mycorrhiza b) Vermicompost as a biofertilizer c) Non native soil, i.e. soil from irrelevant land nature and d) Native soil without any amendment.along with the soil amended with the spores of mycorrhizal fungus, *Glomus macrocarpum* (Fig. 3).

Growth Measurement

The growth of the plant, *Coleus amboinicus* was measured for their shoot length and root length while planting and after the growth of 30 days duration.

Estimation of Chlorophyll

The chlorophyll content of the plant, *Coleus amboinicus* was estimated as per the methodology recommended by Kamble et al., (2015). A gram of the leaves of the plant grown in different soil types were cut in to pieces and grinded using mortar and pestle. To this 20ml of acetone along with 0.5g of Magnesium Carbonate was added and grinded and incubated in refrigerator for 3 hours time. This was centrifuged at 3000rpm for a period of 10 minutes and the supernatant was made upto 100ml further using 80% acetone. This was read in the spectrophotometer (Cyberlab, USA) at 645nm and 663nm respectively. The reading was made in triplicate and the average was taken into consideration for calculating the contents of chlorophyll. The chlorophyll a, chlorophyll b and totl chlorophyll were estimated using the formula recommended by Arnon (1945).

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

The bacterial strains of *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* were studied for their antibacterial susceptibility against the plant *Coleus amboinicus.* The antibacterial efficacy was studied using Well diffusion method (Prakash et al., 2014) using the concentration of 25µl / well. Streptomycin of

10ul concentration was used as a positive control. The diameter of the zone of inhibition in mm was recorded after 24 hours of incubation.

RESULTS AND DISCUSSION

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 plant *Coleus amboinicus* belongs to the family Lamiaceae is important for its aromatic nature. Although, the plant has the potency to naturally harbor the mycorrhizal fungi, the growth of the plant is found to increase when the soil is inoculated with mycorrhiza. The high growth is followed by the plant grown in non-native soil, and a native soil without inoculating mycorrhizal fungi. However, the plant growth is low in soil possessing vermicompost and the soil without mycorrhiza (sterile) (Table 1). Similar trend was followed with the content of Chlorophyll a, Chlorophyll b and total chlorophyll content (Table 2). This is also found to impact the antibacterial property of the plant in similar trend. The soil without mycorrhiza has shown no zone of inhibition against any of the bacteria studied. Against *Pseudomonas aeruginosa* none of the plant grown in different soil types showed any zone of inhibition (Table 3).

The colonization of mycorrhizal association with *Coleus amboinicus* was already reported (Aamila and Shahina, 2023). The distribution of Arbuscular mycorrhizal fungi on occurrence and distribution in the plant *Coleus aromaticus* from Cuddalore was reported (Panneerselvam and Thamizhiniyan, 2011). Similarly the impact of *Glomus fasiculatum* and *Gigaspora margirata* on the biomass and phytochemical compounds of *Coleus aromaticus* was evaluated by Pannerselvam (2017). However, the current study is the first report pertaining to evaluation of chlorophyll content and antibacterial efficacy of the plant, *Coleus amboinicus* inoculated with the mycorrhizal fungus, *Glomus macrocarpum.* The improvement in oil content of *Anethum* and *Trachyspermum ammi* due to the bioinoculant of *G. macrocarpum* was reported (Kapoor et al., 2002). Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria

promoted changes in plant metabolism and the volatile profile of *Piper callosum* was reported (de Azevedo et al., 2024). The study promotes the usage of mycorrhizal fungi on any of the agricultural plant for better property.

Conclusion

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 of *Coleus amboinicus.*

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Conflict of Interests

The authors declare that they have no conflict of interest

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Table 1. Shoot and Root length of *Coleus amboinicus* while planting and after 30 days of growth

Soil Type	When planting (shoot)	When planting (root)	After 30 days (shoot)	After 30 days (root)
Soil without mycorrhiza (sterile)	13 cm	4.5	20 cm	8.3 cm
Native soil	13 cm	4.5	24.1 cm	9.4 cm
Non-Native soil	13 cm	4.5	29.3 cm	11.1 cm
Vermicompost soil	13 cm	4.5	18 cm	8.2 cm
Mycorrhiza soil	13 cm	4.5	36.2 cm	14.3 cm

Table 2. Chlorophyll A, B and Total chlorophyll content of *Coleus amboinicus* grown in different soil types

Soil Type	Chlorophyll A (g/ml)	Chlorophyll B (g/ml)	Total Chlorophyll (g/ml)
Soil without mycorrhiza (sterile)	5.61	9.1	14.7
Native soil	5.94	9.42	5.3
Non-Native soil	6.58	11.1	17.1
Vermicompost soil	5.11	8.19	9.58
Mycorrhiza soil	10.0	14.	17.5

Table 3. Zone of inhibition recorded for the leaf extract of *Coleus amboinicus* grown in different soil types

Microorganism	Zone of inhibition (In mm)					
	Soil without mycorrhiza (sterile)	Native soil	Non-Native soil	Vermic ompost soil	Myco rrhiza soil	Antibiotic (Strepto-mycin)

Bacillus subtlis	-	6mm	8mm	-	10.2	19mm
Salmonella typhi	-	11mm	12mm	-	14.3	18mm
Klebsiella pneumoniae	-	7.2mm	8mm	-	11.3	12mm
Staphylococcus aureus	-	7.3mm	8mm	-	11.4	17mm
Pseudomonas aeruginosa	-	-	-	-	-	12mm

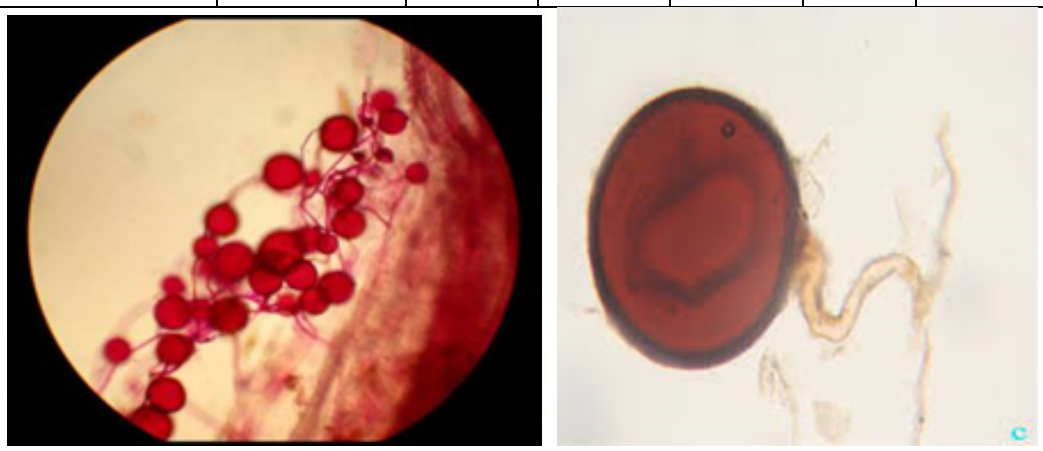

Fig. 1. Root infected with Arbuscular Mycorrhizal fungi and the spore of *Glomus macrocarpum*

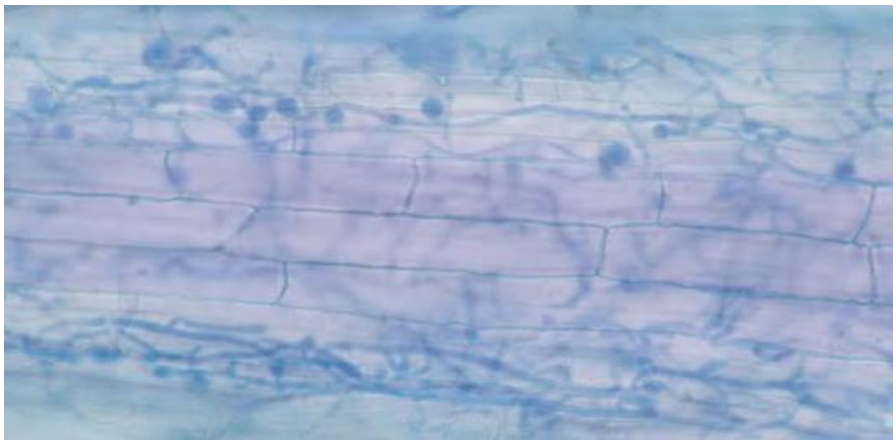

Fig.2. Presence of hyphal growth and spore formation of mycorrhiza in the roots of *Coleus amboinicus*

Fig. 3. Pot experiments of *Coleus amboinicus* in different soils