

Response of *Phyllanthus reticulata* Poir. medicinal plant raised through stem cutting using native AM fungi

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SUMMARY

Sustainability in horticulture or medicinal plants requires balanced and functional microbial inoculation. Indigenous arbuscular mycorrhizal fungal strain *Glomus fasciculatus* was used as stem cuttings of *Phyllanthus reticulata* Poir. The result revealed mixed inoculum of *Glomus fasciculatum* significantly improved seedlings quality index biomass production of shoot and root. This ultimately influenced an increased plant height and decreased root/shoot ratio compare to non inoculated plants. An appropriate quality with native arbuscular mycorrhizal fungus could bring a good quality in the *Phyllanthus reticulata* Poir seedlings at nursery stages, with known quantity of AM spore population and encouraged root colonization of plants.

Key words : Arbuscular mycorrhizal fungi (AMF), *Phyllanthus reticulata* Poir, biomass production, Root/shoot ratio, seedling index

Rhizosphere is the portion of the soil with specialized niche and is the influence of the plant roots, which encompasses the root surface and adhering soil. The root exudates sloughed off cells and decaying roots, stimulate the microbes. The arbuscular mycorrhizal fungi are abundantly found in rhizospheric soil; these fungi are obligate symbionts and have not been cultured on nutrient media. These AM fungi are not host specific although evidence is growing that certain AM fungi may form preferential association with certain host plants (Powell *et al.*, 1985). Different species live together and hyphae of AM fungi inter connect the root system of adjacent plants changing the level of AM Fungal colonization. AMF hyphae can mediate nutrient transfer between plants. The plants benefit particularly through enhanced phosphorus, water, and mineral nutrient uptake, which often result the toxic effect of excessive concent ratios of heavy metals (Ocampo, 1986; Marschner, 1995; Lakshman, 2008).

The soil used to raise seedlings may not contain the optimum population of microbes needed for a healthy rising of seedlings. Bioagmulation with native arbuscular mycorrhizal fungi improves the quality of the seedlings in nurseries (Bagyaraj *et al.*, 1989; Muthukumar *et al.*, 2001).

Phyllanthus reticulata Poir is a perennial important medicinal shrub grows up to 3 feet height commonly

occurs in most part of Karnataka. The erect plant bears numbers branches with oval shaped leaves. The seeds are used in the preparation of Indian ink. Seed part is externally applied to skin disease and leprous sores. The leaf juice is considered to be a good refrigerant and antiscorbutic. A decoction prepared from the leaves is useful for treating bronchitis and gonorrhoea. Seeds are very minute and eaten by ants or insects. Therefore, it can be propagated through stem cuttings. An AM fungal study on this plant is not done as per the literature survey.

Therefore, the present investigation was undertaken to bioaugment the seedling quality of *Phyllanthus reticulata* Poir using native arbuscular mycorrhizal fungal isolates.

MATERIALS AND METHODS

Arbuscular mycorrhizal Spores from the rhizosphere of *Phyllanthus reticulata* Poir, were isolated using the wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). The isolated spores were mass-multiplied following the single spore culture technique on *Chloris gayana* Kunth. They were grown for two months. Shoots of the grass were severed and roots were air-dried for inoculation. An initial inoculum potential of 1250(N) infective propagule per bag, determined by the MPN (most probable number) method (Porter, 1979), having 2kg substrate was used different level of inoculum potential *i.e.*, 1/4N, 1/2N, 1N, and 4N were used along with a control, the inoculum was a mixture of three *Glomus fasciculatum*, *Glomus isolate 2*, and *Acaulspore scobiculata*.

Stem cuttings measuring 5cm of *Phyllanthus reticulata* Poir from the natural habitat brought where

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plants are growing in botanical garden. Cut stem samples were washed and surface-sterilized with 5% HgCl before they were planted for germination in sterilized sand. The seedlings were grown for 120 days. Stem cuttings with uniform height (5cm) were used for transplanting to earthen pots (20 x 20cm) in a completely randomized block design, plant height, number of leaves, collar diameter and leaf area of the seedlings were measured on the 120 day after transplantation, roots were properly washed to remove the adhering soil. Shoot and root biomass was then determined. The SQI (seedlings quality index) was calculated using the following formula (Dickson *et al.*, 1960).

$$SQI = \frac{\text{Total dry weight (g/plant)}}{\frac{\text{Height (cm)}}{\text{Root collar diameter (cm)}} + \frac{\text{Shoot dry weight (g/plant)}}{\text{Root dry weight (g/plant)}}}$$

The MIE (mycorrhizal inoculum effect) was calculated using the formula following method of Bagyaraj (1992).

$$SQI = \frac{\text{Dry weight of inoculum plants} - \text{Mean dry weight of uninoculated plants}}{\text{Dry weight of inoculated plants}}$$

Statistical analysis, that is, DMRT (Duncan's multiple range test) was done following the method given by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Growth parameter *viz.*, Plant height, leaf number, leaf area, root collar diameter, root biomass, were recorded on the 120 day after transplantation. The test plants showed a different growth with different levels of inoculum potential used. Maximum, plants height, leaf number, and total dry biomass were recorded in the treatment were four times higher than the normal

inoculum potential was used (Table 1).The growth parameters showed an increasing trend with the increase in inoculum potential. In general, application of mycorrhizal inoculum resulted in a significant increase in plant height, leaf number, and total dry biomass of the seedlings as compared to the control (Dickson *et al.*, 1960; Manjunath *et al.*, 1983; Rajan *et al.*, 2000).

The root-shoot ratio varied significantly amongst the different treatments (Fig. 1).Treatments with half the

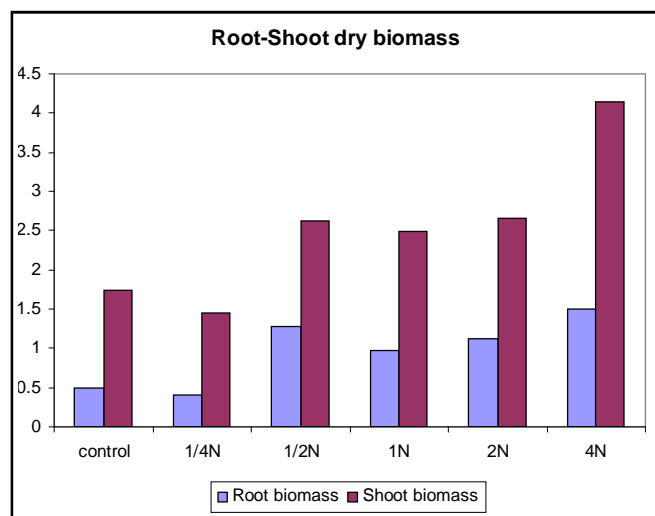


Fig. 1 : Root- shoot ratio in *Phyllanthus reticulata* Poir. as influenced by the mixed inoculum of mixed native arbuscular mycorrhizal fungi at different inoculum potentials

normal inoculum potential showed the height root-shoot ratio. Seedlings treated with four times the normal inoculum potential significantly.

Improved the quality by 221% as compared to the uninoculated control (Fig. 2). The mycorrhizal inoculum effect ranged between 25.85% and potential.

The present study reveals the importance of native arbuscular mycorrhizal fungi on the growth and development of *Phyllanthus reticulata* Poir seedlings. Though there were difference in response of the plant to

Table 1: Showing the seedling growth of *Phyllanthus reticulata* Poir., as influenced by the mixed inoculum of native arbuscular mycorrhizal fungi at different inoculum potentials

Inoculum densities	Height (cm)	Leaf number	Leaf area (cm ²)	Root collar diameter (cm)	Root biomass(g)	Shoot dry biomass(g)	Total biomass
Control	14.2 a	7.0 a	169.02 b	0.386 d	0.486 b	1.734 b	2.302 a
1/4N	18.11c	10.31 b	391.01 c	0.379 c	0.411 c	1.446 e	1.952 b
1/2N	8.05b	7.0 c	259.20 e	0.402 a	1.281 a	2.632 a	3.194 ab
1N	16.3 d	8.51 d	174.11 d	0.481 d	0.980 d	2.492 c	2.902 bc
2N	15.40e	8.32 b	184.02 c	0.576 e	1.132 e	2.651 d	3.583 d
4N	14.22b	11.02 a	284.13 a	0.613 b	1.494 b	4.136 a	4.674 e

Means sharing letter in columns are not significantly different according to Ducan's Test P<0.05.

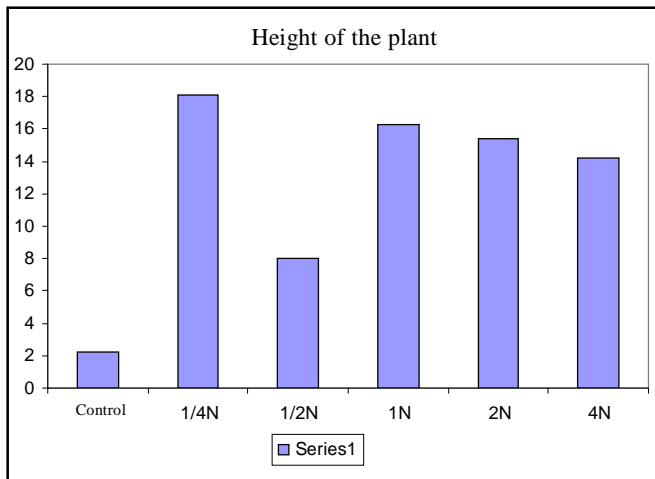


Fig. 2 : Mycorrhizal inoculum effect on plant height of *Phyllanthus reticulata* Poir. as influenced by various densities of mixed inoculum

different inoculum potential of the native arbuscular mycorrhizal fungi, there was always a positive response. An improvement in the quality of seedlings was observed in terms of growth parameter as compared to control. Arbuscular mycorrhizal fungi improve the plant growth and development of seedlings. (Ravikumar *et al.*, 1997). This view is further strengthened results of the present study in which overall quality, thus bioaugmenting the seedlings of the different treatments, the one with four-times the normal inoculum potential showed the best results, in terms of height, biomass, seedlings quality index, and mycorrhizal inoculation effect. This effect may be because of difference in spore number that might account for different infection rates (Munro *et al.*, 1999). In conclusion, the result confirms that appropriate quantity of arbuscular mycorrhizal fungal inoculum would help in production of quality seedlings for nursery practice. This may be because difference in spore number may accounts for different infection rate.

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