

Research Article

Mycorrhizal association and influence on growth of Asian pigeonwings (*Clitoria ternatea* L.)

Mane S. R., Kumbhar V. R., Birajdar G. M., Naryankar R. S., Gavali M. T., U. N. Bhale*

Research Laboratory, Department of Botany, Arts, Science and Commerce College, Naldurg, Tq., Tuljapur, Dist. Osmanabad, 413602 (M.S.) India.

Received: 2017-05-22; Accepted: 2017-05-26

Available online: 1st June 2017

Abstract: *Clitoria ternatea* L. is an excellent herbal medicinal plant. Arbuscular Mycorrhizal fungal (AMF) colonization and biomass of three different tested conditions of *Clitoria ternatea* plant was investigated. Inoculums of indigenous AMF and *Trichoderma harzianum* was tested greenhouse experiment and compared with natural condition after 60th days of treatment. Percentage of Arbuscular mycorrhizal (AM) infection, number of resting spores and AM fungi species varies in different land. Among three different conditions, natural conditions showed maximum root colonization (75.89%) than treated one but minimum spore density (358.8/100g rhizosphere soil). Highest spore density (481.6/100g soil) was found in *T. harzianum* treated condition followed by indigenous AMF treatment *Acaulospora Glomus* and *Sclerocystis* these three genera were found frequently. AMF inoculums and *T. harzianum* treatments conditions were observed promising biomass data of 60th days after treatment (DAT). When AMF are more colonized to plants then enhanced the biomass productivity.

Keywords: *Clitoria ternatea*; *Trichoderma harzianum*; AM association; biomass production

Introduction

Clitoria ternatea is a plant species belonging to the Fabaceae family. In Southeast Asia the flower is used as a natural food colouring. This plant is native to tropical equatorial Asia (Indonesia and Malaysia), but has been introduced to Africa, Australia and America. In traditional Ayurvedic medicine, it is ascribed various qualities including memory enhancing, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing, and sedative properties. (Mukherjee *et al.*, 2008) In traditional Chinese medicine, due to its appearance similar to the female reproductive organ, and consistent with the Western concept of the doctrine of signatures, (Fantz, 1991) the plant has been ascribed properties affecting this organ. *C. ternatea* is one of four herbs traditionally used as *Shanka Pushpi*, an Ayurvedic medicine used to promote neurological health. It shows promise in animal models for its memory enhancing effects, and has a wide spectrum of neurological benefits (anti-depression, anxiolytic, anti-pyretic) yet for these latter claims preliminary evidence suggests it isn't overly potent. Some other preliminary evidence suggests that it might be healthy for the liver and circulating lipoproteins, as well as a possible benefit diabetics by inhibiting glucose uptake from the diet. However, these claims are much too early to guess their practical relevance on. In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer,

nootropic, anti-stress, anxiolytic, anti-depressant, anti-convulsant, tranquilizing and sedative agent. In Burmese and Thai cuisine the flowers are also dipped in batter and fried (Ghani, 1998). Piles are cleaned with the decoction and the paste of whole plant is applied over it. Leaf juice is used as nasal drops in headache.

The AM Fungi is ubiquitous group of fungi (Smith and Read, 1997). AM Fungi belongs to phylum Glomeromycota (Schenck and Perez, 1988). The general consensus is that AM fungi improve phosphate nutrition of legumes, which in turn enhances plant growth and nitrogen fixation (Cluett & Boucher, 1983). AMF have been shown to differentially colonize plant roots, causing a variety of effects on plant growth, biomass allocation, and photosynthesis (Fidelibus *et al.*, 2000). Increased access to low-mobility soil mineral nutrients has been considered to be the main beneficial effect of AMF on their host plants (Smith and Read, 1997). They are known to improve the plant growth through, better uptake of nutrients such as P, Zn, Cu, Ca, K, Br, Cr (Lambert, *et al.*, 1979; Brundrett, 2009) and water, resistance to drought and increased resistance to root pathogens. They also improve the activity of N fixing organisms in the root zone.

*Corresponding Author:

Dr. U. N. Bhale,
Associate Professor,
Research Laboratory, Department of Botany,
Arts, Science and Commerce College, Naldurg, Tq.
Tuljapur, Dist. Osmanabad, 413602 (M.S.) India.

E-mail: unbhale2007@rediffmail.com



Mycorrhiza is a symbiotic association between soils spread over all states of India; the major part of more than borne fungus and the roots of a plant (Kirk *et al.*, 2001). Therefore, the investigation was made % mycorrhizal association and influence on Growth of Asian pigeonwings (*Clitoria ternatea* L.).

Materials and Methods

Rhizospheric soil and roots of plant of *Clitoria ternatea* was collected from agricultural and treated lands. Naldurg (17.82°N 76.30°E) region of Osmanabad districts of Maharashtra. Soil type is deep loamy, alluvium, shallow to sandy and loamy brown to black. The summer temperature going up to 41°C or even more, while the winters are usually 9-24 °C. Naldurg place is located at an altitude of 566m and receives an average annual rainfall of 760 mm. in monsoon (June to September, 2015 & 2016) period. Seeds were collected from single mother growing plants in a natural condition and experiment was conducted in green house. Fresh carrier based culture of *T.harzianum* having 25 × 10⁵ Colony Forming Unit CFUs/g was treated @1 g/per seed. Inoculums of indigenous AMF maintained in green house with *Coleus* (*Plectranthus scutellarioides*) as host (containing 270-300 spores /100 g soil) was amended @ 05g per seed of *C.*

ternatea. Ten plants were sampled; roots were dug out& washed to remove soil and stored in FAA (Formalin Aceto Alcohol (5:5:90)) prior to staining. Rhizosphere soil samples of each land were enumerated of AM fungal spores. Root bits (size 1cm or 2cm) were boiled in 10% of KOH for 15-20 min washed in tap water and stained in 0.05% Trypan blue in lacto phenol (Phillips and Hayman, 1970) and percentage of AM colonization was estimated by the magnified intersection. The percent root colonization was measured by using the formula (Giovannetti and Mosse, 1980).AM fungal spores were improved by the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Spores were mounted in polyvinyl alcohol lacto phenol and identified using a manual (Schenck and Perez,1990) and INVAM (<http://www.invam.caf.wvu.edu>).Fresh weight of shoot and root samples were recorded. Shoots and roots were separated and oven dried at 60°C for 48 h for the determination of dry mass after recording their lengths (Muthukumar and Udaiyan, 2000).

Statistical analysis

Statistical analyses of the experiments were performed by using the book (Mungikar1997).

Table 1: Incidence and level of AM fungal colonization in plants of *Clitoria ternatea* from study sites (60 DAT)

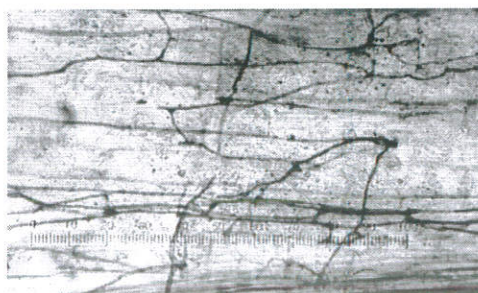
Sr. No	Conditions	% of Root Colonization			Total Root colonization (%)	Spore Density Per 100g rhizosphere soil	Types of AMF Genera
		Arbuscules	Vesicles	Hyphal			
1	Natural land	40.10	15.29	20.54	75.89±9.50	355.8±104.7	<i>Acaulospora</i> , <i>Glomus</i> , <i>Sclerocystis</i>
2	<i>Trichoderma harzianum</i> treated land	10.11	19.41	29.23	58.75±9.74	481.6±79.43	<i>Acaulospora</i> , <i>Glomus</i>
3	Indigenous AMF treated Land	8.00	15.00	35.12	58.12±12.62	374.2±147.64	<i>Acaulospora</i> , <i>Glomus</i> , <i>Sclerocystis</i>

Legend: Values are mean of 10 replicates, value after ± indicates standard deviation.

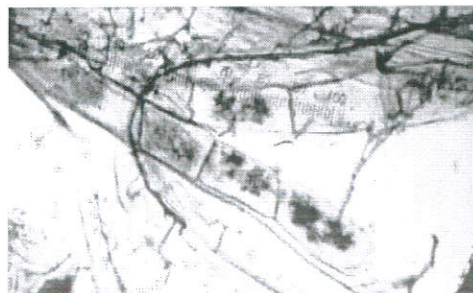
Table 2: Impact of biomass production in *Clitoria ternatea* from study sites.

Sr. No	Parameters	Study site condition		
		Natural land	<i>T. harzianum</i> treated Land	Indigenous AMF treated land
1	Height of stem(cm)	75.51±18.94	92.92±18.25	110.5±17.05*
2	Width of stem(cm)	1.52±0.38	1.56±0.30	1.74±0.31
3	Length of root(cm)	15.17±3.18	19.66±5.65	19.92±3.60
4	Number of leaves	220±69	276.6±89.85	316±97.11*
5	Number of leaflets	46.00±13.43	64.88±20.45	63.2±19.43
6	Fresh weight of shoot (g)	12.36±5.17	27.6±9.48	34.29±13.14
7	Fresh weight of root(g)	4.75±2.12*	3.36±1.95	4.21±2.87
8	Dry weight of shoot(g)	4.05±1.87	7.57±2.98	10.00±4.37*
9	Dry weight of root(g)	2.40±1.14	0.90±0.52	1.27±1.0
10	No of flowers	1.2±4.75	1.59±3.54	10.2±4.75
11	No of pods	2.2±4.03	5.00±4.75*	4.2±4.03

Legend: Values are mean of 10 replicates, value after ± indicates standard deviation, Data with * are significantly different at $P \leq 0.05$.



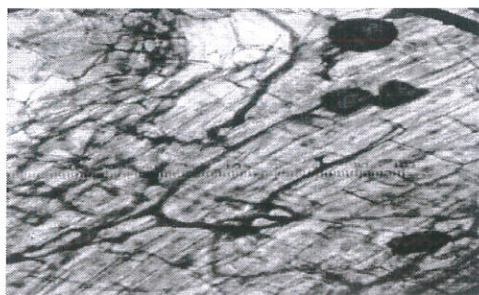
A-Hyphal



B-Arbuscles



C-Vesicles



D-Vesicles with Appresoria

Plate 1: Showing arbuscular mycorrhizal fungal root colonization types in softened root sections of *Clitoria ternatea*, A-Hyphal, B-Arbuscular, C-Vesicles, D-Vesicles with Appresoria (x 400).

Results

The experimental findings obtained from the present study have been discussed in the following heads.

Mycorrhizal Associations

Arbuscular Mycorrhizal Fungal (AMF) assessment of % root colonization and spore density was studied in *Clitoria ternatea* of 60th days after treatment (DAT) from three different conditions (Table 1; figure 1). Among them in natural conditions showed maximum root colonization (75.89%) than treated one but minimum spore density (358.8/100 g soil). The highest spore density (481.6/100 g rhizosphere soil l) was found in *Trichoderma harzianum* treated condition followed by indigenous AMF treatment. In root colonization, hyphal types of colonization were found maximum as compared to arbuscular and vesicular types whereas in natural condition, arbuscular type of root colonization was found more. Vesicular type of root colonization was increased in both treated conditions. Among AMF genera, *Acaulospora*, *Glomus* and *Sclerocystis* were found to be dominant.

Biomass Production

The present study, eleven parameters of biomass productivity was studied in *C. ternatea* (Table 2). In this study, ten replications were used. AMF inoculums and *T.harzianum* treated conditions were observed promising data of 60th days after treatment (DAT). Height of stem (110.5cm) was observed more in case of AMF treatment followed by *T.harzianum* treated conditions. Width of stem

was observed almost equal in all three conditions but the length root was found more in case of both treated conditions. Number of leaves, number of leaflets, fresh weight of shoot (g), dry weight of shoot (g), number of flowers and number of pods were observed more in both treated conditions. But fresh and dry weight (4.75 & 2.40g) of roots was observed maximum in natural condition. Overall, treatment of AMF showed promising results followed by *T. harzianum* but *T. harzianum* showed more pods productivity.

Discussion

It is clearly evident from the present investigation on the effect of AM inoculation & biocontrol agent on mycorrhization and growth response in *C. ternatea* that plants have readily responded to AM colonization when grown with AM inoculum & *T.harzianum*. In the literature, there are several reports in which AMF inoculations decreased plant biomass but this decrease has often been found to be transient and reversed later, being followed by a positive growth response (Jones and Smith, 2004; Correa et al., 2006). The plants of *Clitoria ternatea* also readily responded to the endophytic colonization by VAM fungi; however, *Glomus aggregatum* inoculation had increased the % colonization significantly (Biradar and Reddy, 2007). Basu and Srivastava (1998) have earlier reported such enhanced growth in medicinal plants due to VAM fungal association. Similar improved growth response was also observed in 10 medicinal plants when inoculated with three VAM fungal species (*G. mosseae*, *G. fasciculatum*, and *G. monosporum*) for their efficacy by Kumar and Muruges (2002). All the 107-mycorrhizal species show hyphal presence in roots and more than 95 percent of these plants show presence of either hyphae and arbuscules or hyphae and vesicles or all the three structures individually (Koul et al., 2012). They suggested that mycorrhizal inoculation is more advantageous in obtaining healthy vigorous transplantable seedlings and results in higher biomass of medicinal plants that were found to grow better in the field. Similarly, most of the Leguminous plants growing in tropical soils with low phosphorus content in the soil could contribute the association of AM fungi in these Leguminous plants (Douds and Millner, 1999; Asghari, 2008). Percent of root colonization and spore number varied in the examined plants, however this process is not necessarily dependent on each other and previously published data are ambiguous (Abbott, 1982). Results also suggested that the extent of growth depression or reduction in *Pongamia pinnata* varied with inoculated AMF species, which can be related to the variations in carbon demand of different AMF species. Li et al. (2008) was suggested that such differences in growth response towards different AMF inoculants are directly related to the balance between benefits



and costs of the symbioses. It is relevant to mention that the possible synergistic effect would be the uptake by AM fungal hyphae and translocation into the plant of P released by PSB Phosphate solubilizing bacteria in soil (Garbage, 1991, Lakshman, 1999 & 2009). Recently, the results showed that the combined inoculation of PSB, AM fungi and rock phosphate produced vigorous plant growth of tree seedlings for quick planting in field (Jangandi et al., 2017). It is clearly evident from the data that almost all of the medicinal plants selected for the study were invariably found to harbour AM fungal association (Rajkumar and Sunilkumar, 2011).

Conclusion

It was concluded that biomass productivity of *C. ternatea* was enhanced when it treated with native AMF species and biological controlling agent i.e. *T.harzianum*. It is reported that the plant growth to mycorrhizal fungi depends on the level of soil fertility and accessibility of soil to inoculants. Therefore, the composition of AM population in an ecosystem will be an important determinant of plant growth response.

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Cite this article as:

Mane S. R., Kumbhar V. R., Birajdar G. M., Naryankar R. S., Gavali M. T., U. N. Bhale. Mycorrhizal association and influence on growth of Asian pigeonwings (*Clitoria ternatea* L.). *International Journal of Bioassays* 6.6 (2017) pp. 5415-5419.

DOI: <http://dx.doi.org/10.21746/ijbio.2017.06.006>

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Arts Science & Commerce College
Naldurg, Dist.Osmanabad-413602

Source of support: Nil.

Conflict of interest: None Declared