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Beneficiary Effect of Arbuscular Mycorrhiza to *Trigonella Foenum-Graceum* in Contaminated Soil by Heavy Metal

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Abstract

Because of industrialization and urbanization, there is no much land is available for urban farming in and around Mumbai. Wherever the small lands are available as open space, unused lands, barren lands etc are contaminated by heavy metals which come through industrial waste disposal. Such lands can be mycoremediated by use of mycorrhizal fungi to a certain extent and can be utilized for urban farming of leafy vegetables. Present investigation was carried out in the form of pot experiment to check the response of Glomus mosseae to Trigonell foenum-graceum which was grown in soil contaminated with heavy metal Arsenic. During these experiments, soils with different concentrations of arsenic with and without mycorrhizal inoculums were tested in Trigonella foenum – graceum. The response of mycorrhiza in T. foenum-graceum was determined in terms of percentage germination of seeds, sustainability of seedlings, fresh weight and dry weight of plants etc. It was observed that in the pot with soil contaminated with arsenic and no mycorrhizal inoculum, performance was very bad in terms of all aspects of growth, whereas in the pot where mycorrhizal inoculum was added along with contaminated soil, the performance of the plant was better. The pot showing no contaminated soil with arsenic but the inoculum of mycorrhiza was showing best results in terms of percentage germination of seeds, sustainability of seedlings, fresh weight and dry weight of plants.

Keywords: Arbuscular mycorrhiza, glomus mosseae, trigonella foenum-graceum, heavy metal arsenic, fresh and dry weight.

Introduction

Soil contamination due to the disposal of industrial and urban wastes generated by human activities has become a major problem and an environmental concern. Controlled and uncontrolled disposal of wastes to agricultural soil are responsible for the migration of contaminants into non contaminated sites[1]. Because of industrialization and urbanization, there is no much land is available for urban farming in and around Mumbai. Wherever the small lands are available as open space, unused lands, barren lands etc are contaminated by heavy metals which come through industrial waste disposal.

Arbuscular mycorrhizal fungi are soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots. About 95 % of the plant species are mycorrhizal and potentially draw benefits of this mycorrhizal symbiosis in terms of nutrition and mineral uptake[2]. Arbuscular mycorrhizal fungi can contribute to plant growth, particularly in disturbed or heavy metal contaminated sites by increasing plant access to relatively immobile minerals as P[3].

Conflicting results are published by many researchers in relation to plants, mycorrhiza and heavy metals contamination[4,5]. In two pot experiments with maize in heavy metal contaminated soil, mycorrhizal colonization either increased plant biomass and decreased Cd, Cu, Zn and Mn concentrations in shoots and roots, or had no effect on growth and heavy metal uptake, depending on root density, plant growth conditions and mycorrhizal inoculum[6].

Present investigation was carried out in the form of pot experiment to check the response of *Glomus mosseae* to *Trigonell foenum-graceum* which was grown in soil contaminated with heavy metal Arsenic.

Material and Methods

In the present investigation pot culture method was used to set up the experiment. For the pot culture method, 10-liter capacity plastic pots were used in triplicates for each concentration of As. These pots were filled up with 9 kg garden soil which was sieved through 2 mm mesh sieve and autoclaved at 121^0C for 2 hours to eliminate any local mycorrhizal flora as well as any microorganisms present in it.

The pots were divided into groups as control pot, - As - M (no contaminated soil and no mycorrhizal culture), negative control, + As - M (contaminated soil but no mycorrhizal culture), positive control – As + M (no contaminated soil but with mycorrhizal culture) and experimental pots of varying concentrations of As (sodiun aresnite, $NaAsO_2$) contamination ranging 25, 50, 75, 100, 150 mg As Kg^{-1} with 20 g mycorrhizal inoculum of *Glomus mosseae*. In each pot, 20 viable seeds of *Trigonella foenum-graceum* were sown. Enough amount of soil moisture was maintained throughout the experimentation to avoid leaching of

heavy metal. The observations were recorded at 45th day of sowing. Various parameters of plant growth such as percentage germination of seeds, sustainability of seedlings in As contaminated soils, fresh and dry weights of plants were taken into consideration. At each interval, plants were carefully uprooted from pots. Rhizospheric soil was collected in clean petri-dishes and analysed for spore density. Fresh weight of each plant was noted and plants were kept in oven at 100^0 C overnight to get constant dry weight.

Spore density of was estimated by wet sieving and decanting method[7]. 25 g of sundried rhizospheric soil of different soil of different plants collected at different localities was taken in separate beakers. Half a litre of water and a pinch of soap powder was added to this. The solution was stirred and was allowed to stand for half an hour. The soil solution was then filtered through sieves of 500, 250, 150, 105 and 55 mm mesh which were kept one above the other. The spores and soil particles which settled on the surface of 150, 105 and 55 mm mesh sieve were washed and collected in separate beakers. The water was again filtered through whatman filter no. 1. This paper was observed under stereoscopic binocular microscope to count the number of spores.

Percentage root colonization was calculated after staining procedure[8,9]. The roots of collected plants were washed thoroughly with running tap water for removal of alcohol which was used as preservative. The properly cleaned roots were subjected to staining procedure. Roots were subjected to 10 per cent of potassium hydroxide and heated in water bath at 90^0 C for one hour to clear the tissue. Such cleared roots were washed with distilled water several times to remove the traces of alkali. In case of dark pigmentation of roots, they were treated with three per cent hydrogen peroxide for bleaching. It took 5 to 30 minutes depends upon the degree of pigmentation. This step was eliminated in the case of roots which are white or non pigmented. Once again roots were washed thoroughly for several times with distilled water. Washed roots were kept in 1 per cent hydrochloric acid for about 18 hours to neutralize the effect of alkali used for cleaning the root tissue. Acidification was followed by washing several time the treated roots to remove traces of acid. The roots were then kept in acid glycerol containing 0.05 per cent aniline blue in test tune. Then it was autoclaved for 15 minutes under the pressure of 15 labs at 121^0 C. After that, excessive aniline blue was drained off and excessive stain was removed from stained roots by using acid glycerol. The stained roots were stored in amber coloured bottle in acid glycerol until further use to avoid destaining. The roots were cut into small pieces approximately 1 cm in length and mounted on micro slides using glycerine as mounting medium. After placing the cover slips, the slides were observed under low power of objective of 10 X magnification to confirm the presence of mycorrhizal hyphae and high power objective of 40 X magnification to observe the presence of vesicles, spores etc. the slides were sealed with nail polish. The per cent root colonization was calculated by using following formula[10].

$$\text{Per cent root colonization} = \frac{\text{No. of root pieces showing infection}}{\text{Total no. of root pieces observed}} \text{ X } 100$$

Results and Discussion

At each final harvest from various concentrations of As contaminated soil, mycorrhizal spore count and percentage root colonization were obtained table 1, figure 1. Mycorrhizal inoculum significantly increased the spore count of *Trigonella foenum-graceum*. Significant negative correlation was obtained in *Trigonella foenum-graceum* spore counts (Increase in concentration of heavy metal contamination leads to the reduction in spore count). Similar kind of trend was observed in percent root colonization and concentration of heavy metal contamination. In the pot where no As contamination was set, highest level of percent colonization and spore count was observed.

Germination percentage was too poor in the contaminated soil as overall as comparative to non contaminated soil. Contaminated soil showing mycorrhiza showed sustainability of seedlings in higher percentage table 2.

In the similar way, fresh and dry weight of plants was observed. Higher is the contamination of As in the soil, lower was the fresh and dry weight of plants table 3 and figure 2. But it was observed that the contaminated soil pots without mycorrhiza were unable to support the plant growth in all concentrations, hence plants were fail to sustain.

Table-1
Relationship between *Glomus mosseae* spore density and percent root colonization in *Trigonella foenum-graceum*

Concentration of As (mg kg^{-1} of soil)	Number of spores per 100 g rhizospheric soil	% root colonization
- As - M	00	2.8
+ As – M	00	00
– As + M	457	78.4
25	321	65.3
50	234	34.2
75	123	22.9
100	55	10.8
150	N. A.	N. A.

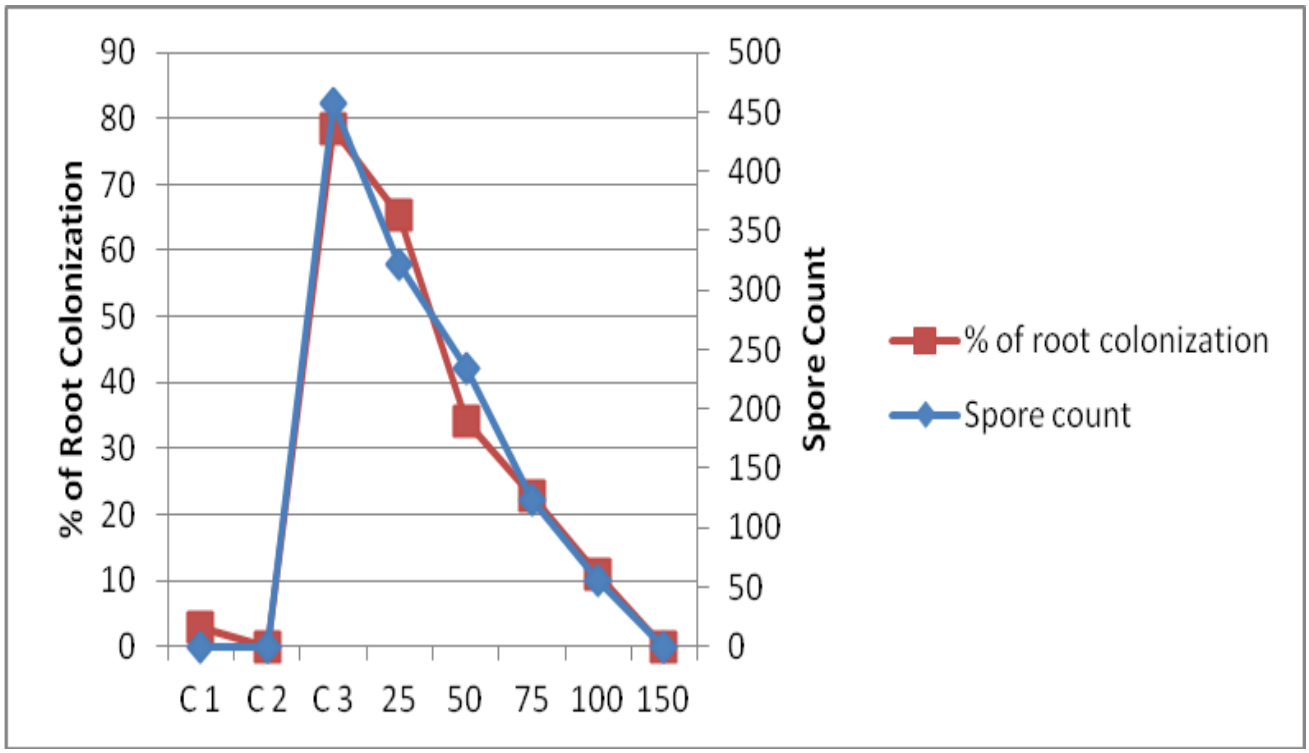
Table-2
Performance of experimental plant *Trigonella foenum-graceum* with various experimental conditions

Concentration of As (mg kg^{-1} of soil)	Percentage germination	Sustainability of plants
- As - M	+	+
+ As – M	---	---
– As + M	+++	+++
25	-	-
50	-	--
75	--	---
100	---	---
150	---	---

(+ indicates acceptable limits, +++ indicates highest range, -, --, --- indicates below acceptance from poor to very poor)

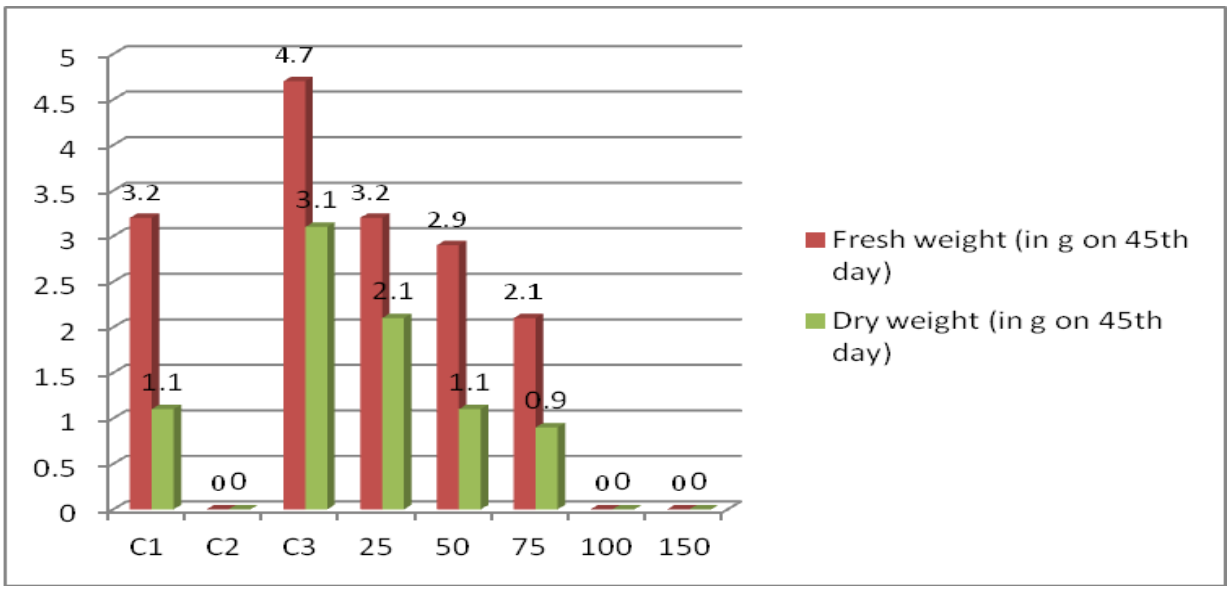
Table-3
Trigonella foenum-graceum as influenced by Glomus mosseae in contaminated soil with As

Concentration of As (mg kg^{-1} of soil)	Fresh weight (in g on 45th day)	Dry weight (in g on 45th day)
- As - M	3.2	1.1
+ As – M	N. A.	N. A.
– As + M	4.7	3.1
25	3.2	2.1
50	2.9	1.1
75	2.1	0.9
100	N. A.	N. A.
150	N. A.	N. A.



C 1 - As – M, C 2 + As – M C 3 – As + M

Figure-1
Relationship between *Glomus mosseae* spore density and percent root colonization in *Trigonella foenum-graceum*



C 1 - As – M, C 2 + As – M C 3 – As + M

Figure-2

Fresh and Dry weights of Trigonella foenum-graceum on 45th day with various treatments

Conclusion

In present investigation it was once again proved that mycorrhiza is a novel and ideal biofertilizer. It can act as a biofertilizer in optimum way in non polluted and stressed free conditions but its contribution as biofertilizer in heavy metal contaminated soil is also remarkable. From the data it is clearly reveal that mycorrhiza can be used as stress reducing agent in heavy metal contaminated soil and help plants to sustain in such stressed conditions.

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