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Response of mulberry cuttings to arbuscular mycorrhizal inoculation under protected cultivation

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Abstract
Mycorrhizal association with the plants has always proved to influence crop growth in a big way through increased availability of nutrients particularly phosphorus to them. Isolation of native AM fungi, their multiplication and re-inoculation in growing medium has further boosted the growth of various agricultural crops. Mulberry a plant from family *Moraceae* has been exploited for rearing of silkworm larvae by feeding them with its foliage. The other parts of plant are also being used for their economic importance and farmer friendly utility. The cuttings of mulberry plant were inoculated with native AM fungi and various growth parameters under polyhouse were observed to have significant impact upon them with increased quantitative parameters. Mean sprouting percentage of inoculated cuttings recorded by mid-April was 2.85% higher than un-inoculated cuttings where it was only 2.10%. The mean percentage increase from mid-April to ending May was observed to be 28.26% and in un-inoculated cuttings it was 20.41% and the mean pair difference between the two was 7.85%. There was also significant increase in the mean of survival by 9.70% between inoculated and un-inoculated cuttings. Similarly, the pair differences recorded for other growth parameters were: number of leaves per sapling, 1.35%; leaf area, 3.45 cm^2; fresh leaf weight per sapling, 1.15 g; dry leaf weight per sapling, 0.76 g; number of roots, 0.95; length of the longest root, 2.06 cm; total root length, 9.85 cm; root biomass, 0.23 g; and root volume, 0.06 cm^3. Overall, it was observed that the cuttings inoculated with mycorrhizal fungi indicated increased growth parameters.

Keywords: Mulberry, cuttings, inoculation, AM fungi, growth

Introduction

Sericulture, the art and science of silk production, has been an integral part of human culture for centuries providing succor to good number of families all over India. At the heart of this ancient industry lies the mulberry tree (*Morus* sp.), a keystone species whose leaf provides the food source for silkworm larvae (*Bombyx mori* L.). Mulberry trees, renowned for their vital role in sericulture, are not only a source of economic sustenance but also contribute significantly to ecological balance. The rising costs associated with chemical fertilizer production and the challenges in meeting their demand have prompted scientists to explore bio-fertilizers as an alternative or partial substitute for synthetic fertilizers (Vaxevanidou *et al*., 2015) [8]. Arbuscular Mycorrhizal fungi, along with potassium and phosphate solubilizing microbes, break down mineral and organic sources to release phosphorus (P) and potassium (K) for plant utilization (Rashida *et al*., 2016) [7]. Even application of fertilizers also results in movement of these ions into the food chain resulting in health issues. In view of growing health concerns, the increased application of organic sources of fertilizers is gaining more importance which has led to their excessive use in almost all agricultural crops which are mainly recognized by their activity at the soil level. Bio-fertilizers are also eco-friendly, cost-effective, non-toxic and easy to apply; thus, they help to maintain soil structure and biodiversity of the agricultural land. Thus, they serve as a good substitute for chemical fertilizers (Deepali *et al*., 2010) [2]. Arbuscular mycorrhizal fungi, along with potassium and phosphate solubilizing microbes, break down mineral and/or organic sources to release phosphorus (P) and potassium (K) for plant utilization (Bargaz *et al*., 2018) [1].

Arbuscular Mycorrhizal fungi establish symbioses with plant roots which help to improve nutrient uptake by the host plant and alter its physiology to withstand external abiotic factors and pathogens. The mulberry plant which is generally grown for its foliage also responds well to application of AM fungi.

Materials and Methods

This study was conducted under protected cultivation system. The soil based arbuscular mycorrhizal inoculum consisting of *Glomus* and *Acaluspora* species was applied to the mulberry cuttings of variety *Goshoerami* under polyhouse conditions. The media was placed in polybags and a small hole of the diameter of the cutting was made in medium. The mycorrhizal inoculum @ 10 g/cutting was placed in a hole and the cuttings were planted in such a way that contact was established between the inoculum and cutting. The polybags with cuttings inserted in them were placed under polyhouse in the month of March for three months till saplings grew and became ready for transplantation to main nursery (Fig-1). The experiment was repeated during two consecutive years, and the observations were recorded in the month of June each year. A control was also kept with saplings where no inoculum was placed. The observations were recorded on sprouting percentage and survival percentage, along with other quantitative parameters of saplings, including number of leaves per sapling, leaf area (cm^2), fresh leaf weight per sapling (g), dry leaf weight per sapling (g), number of roots per sapling, length of longest root (cm), total root length per sapling (cm), root biomass (g), root volume (cm^3), sapling height (cm), sapling thickness (cm), shoot biomass (g) and root/shoot ratio. A paired t test was performed for analysis of impact on inoculated cuttings over un-inoculated ones (control).

Fig-1 Placement of arbuscular mychorrhizal inoculum in a hole, plantation and establishment of mulberry cuttings under protected cultivation

Results and Discussion

The findings on the effect of arbuscular mycorrhizal (AM) fungi on the growth and yield of mulberry cuttings are given in Tables 1-6. The mulberry cuttings inoculated with AM fungi showed significant increase in the mean sprouting percentage, which was 2.85% higher than that of un-inoculated (2.10%) cuttings by the end of April. The percentage increase from mid-April to the end of April was 5.06% and 6.39% in un-inoculated cuttings, with a mean pair difference of 1.33. From mid-May to the end of May, the mean of inoculated cuttings was 8.30% higher during mid-May and 9.00% higher during the end of May. The percentage increase from mid-April to the end of May in inoculated cuttings was recorded as 28.26, and in un-inoculated cuttings, it was 20.41, with a mean pair difference between the two of 7.85, there was also a significant increase in the mean survival rate by 2.78%. Our findings are in conformity with those of Beevi *et al.* (2008) [5] who found that the survivability of the cuttings was increased in the nursery bed treated with VAM inoculation compared to control. Additionally, we recorded an increase in the number of leaves by 1.35, leaf area by 3.45 cm^2, fresh leaf weight by 1.35 g, dry leaf weight by 0.76 g, number of roots by 0.95, length of the longest root by 2.06 cm, total root length by 9.85 cm, root biomass by 0.23 g, root volume by 0.06 cm^3, sapling height by 0.77 cm, sapling thickness by 0.16 cm, shoot biomass by 0.58 g, and root/shoot ratio by 0.04 compared to the control (Fig-2). Nan lu *et al.* (2015) [4] indicated that *Morus alba* L. is an important tree species planted widely in China due to its economic value. During their investigation they investigated influence of two arbuscular mycorrhizal fungal (AMF) species, *Glomus mosseae* and *Glomus intraradices*, alone and together, on the growth of *M. alba* L. seedlings under greenhouse conditions. The growth parameters and physiological performance of *M. alba* L. seedlings were evaluated 90 days after colonization with the fungi. The growth and physiological performance of *M. alba* L. seedlings were significantly affected by the AMF species. The mycorrhizal seedlings were taller, had longer roots, more leaves and a greater biomass than the non-mycorrhizae-treated seedlings. Our results are also corroborated with the findings of Katiyar *et al.* (1995) [3], while working with Kanva-2 variety of mulberry, pre-inoculated with *Glomus fasciculatum* and *Glomus mosseae* at various doses of single super phosphate revealed that the effect of inoculation of mulberry with *Glomus mosseae* in combination with 30 kg P ha^{-1} yr^{-1} was similar for plant growth, leaf yield and leaf chemical constituents with the control, which received the full dose of phosphatic fertilizer (120 kg P ha^{-1} yr^{-1}) without inoculation.

Fig-2 Inoculated and un-inoculated mulberry cuttings

Table 1: Effect of Arbuscular Mycorrhizal Fungi (AMF) on sprouting percentage of mulberry cuttings

	Treatment	Mean	t-value	p-value
Mid-April	Inoculated	70.25	3.72	<0.05
	un-inoculated	67.40		
Ending April	Inoculated	73.80	2.83	<0.05
	un-inoculated	71.70		
% increase from Mid-April to ending April	Inoculated	5.06	4.75	<0.05
	un-inoculated	6.39		
Mid may	Inoculated	84.35	11.43	<0.01
	un-inoculated	76.05		
Ending may	Inoculated	90.10	12.34	<0.01
	un-inoculated	81.10		
% increase from Mid-April to ending may	Inoculated	28.26	9.51	<0.01
	un-inoculated	20.41		

Table 2: Effect of Arbuscular Mycorrhizal Fungi (AMF) on survival percentage of mulberry cuttings

Treatment	Mean	t-value	p-value
Inoculated	87.70	33.32	<0.01
un-inoculated	78.00		

Table 3: Effect of Arbuscular Mycorrhizal Fungi (AMF) on leaf parameters of mulberry cuttings

	Treatment	Mean	t-value	p-value
Leaf number	Inoculated	10.05	4.35	<0.05
	un-inoculated	8.70		
Leaf area(cm^{2})	Inoculated	66.15	2.46	<0.05
	un-inoculated	62.70		
Fresh leaf weight (g)	Inoculated	11.80	4.08	<0.05
	un-inoculated	10.64		
Dry leaf weight (g)	Inoculated	6.34	2.37	<0.05
	un-inoculated	5.57		

Table 4: Effect of Arbuscular Mycorrhizal Fungi (AMF) on root parameters of mulberry cuttings

	Treatment	Mean	t-value	p-value
Length of longest root (cm)	Inoculated	22.21	2.88	<0.05
	un-inoculated	20.14		
Total root length (cm)	Inoculated	180.95	2.83	<0.05
	un-inoculated	171.10		
Root biomass (g)	Inoculated	1.80	3.63	<0.05
	un-inoculated	1.56		
Number of roots	Inoculated	9.50	2.76	<0.05
	un-inoculated	8.55		
Root volume (cm^{3})	Inoculated	1.04	2.33	<0.05
	un-inoculated	0.97		

Table 5: Effect of Arbuscular Mycorrhizal Fungi (AMF) on shoot parameters of mulberry cuttings

	Treatment	Mean	t-value	p-value
Shoot length (cm)	Inoculated	17.16	2.23	<0.05
	un-inoculated	16.39		
Shoot thickness (cm)	Inoculated	1.55	2.88	<0.05
	un-inoculated	1.39		
Shoot biomass (g)	Inoculated	8.20	2.13	<0.05
	un-inoculated	7.62		

Table 6: Effect of Arbuscular Mycorrhizal Fungi (AMF) on root/shoot ratio of mulberry cuttings

	Treatment	Mean	t-value	p-value
Root/shoot ratio	Inoculated	0.39	3.25	<0.05
	un-inoculated	0.35		

Conclusion

Native endomycorrhizae has proved very effective in enhancing the sapling establishment through its positive impact on sprouting of mulberry cuttings, increasing growth parameters of root, shoot and leaves. The performance of arbuscular mycorrhizae with respect to plant growth has been found to improve when supplemented with beneficial bacterial consortium.

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